US 20140364338A1

(19) **United States**

(12) **Patent Application Publication**

Schaffer et al.

(10) **Pub. No.: US 2014/0364338 A1**

(43) **Pub. Date: Dec. 11, 2014**

(54) **ADENO-ASSOCIATED VIRUS VIRIONS WITH VARIANT CAPSID AND METHODS OF USE THEREOF**

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(21) Appl. No.: **14/444,375**

(22) Filed: **Jul. 28, 2014**

Related U.S. Application Data

(63) Continuation of application No. 14/113,205, filed on Jan. 22, 2014, filed as application No. PCT/US2012/034413 on Apr. 20, 2012.

(60) Provisional application No. 61/478,355, filed on Apr. 22, 2011.

Publication Classification

(51) **Int. Cl.**

C12N 7/00 (2006.01)

C12Q 1/70 (2006.01)

(52) **U.S. Cl.**

CPC .. ***C12N 7/00*** (2013.01); ***C12Q 1/70*** (2013.01); *C12N 2750/14122* (2013.01)

USPC **506/14**; 435/5; 506/26

(57) **ABSTRACT**

The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of retinal cells, when administered via intravitreal injection, compared to wild-type AAV. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

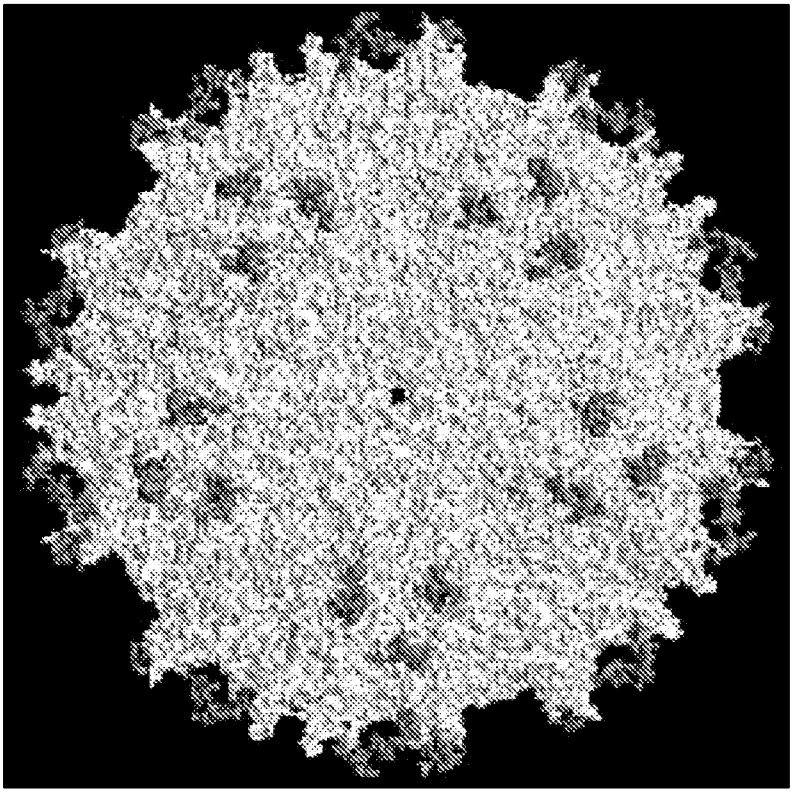
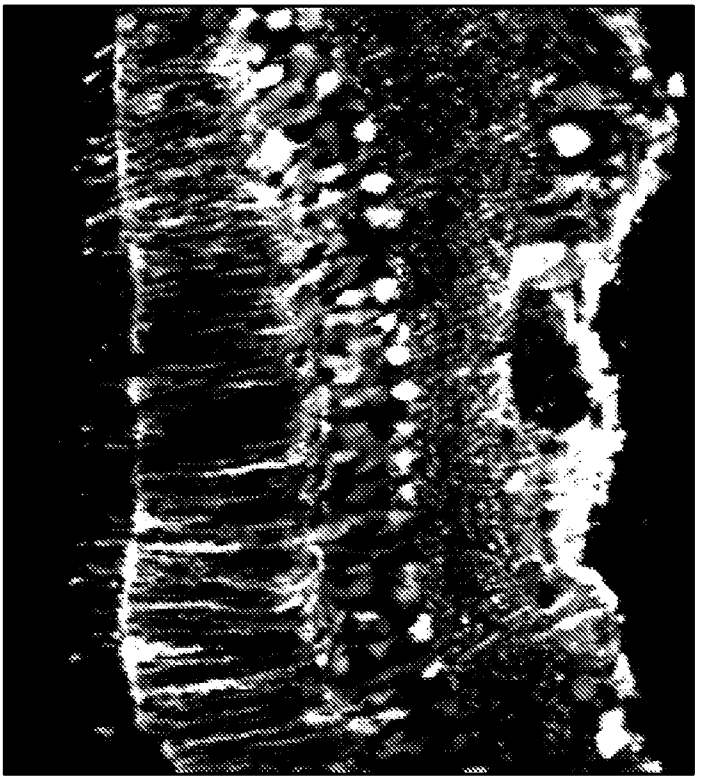
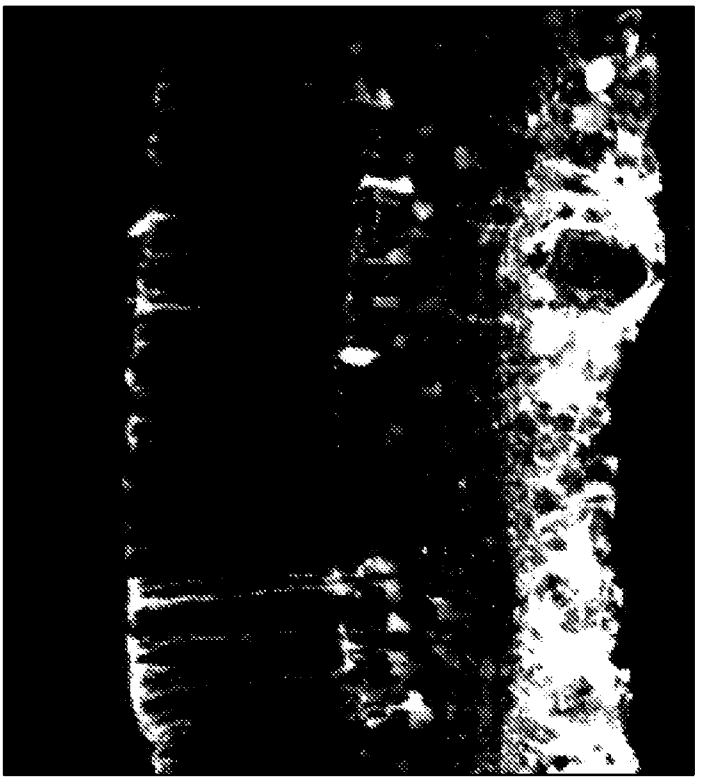


FIG. 1



7m8.CAG.GFP
(variant enhanced for PR transduction by directed evolution)



AAV2.CAG.GFP
(parental serotype)

FIG. 2

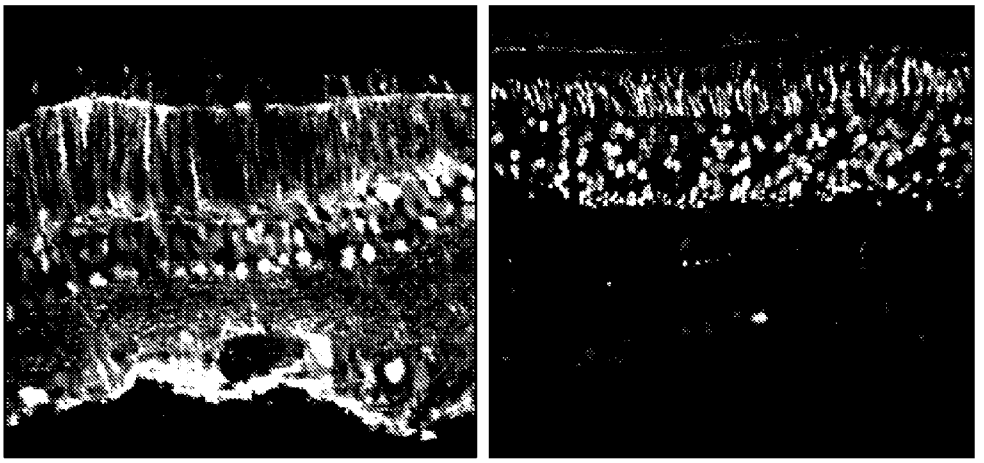

7m8.CAG.GFP
7m8.Rho.GFP

FIG. 3

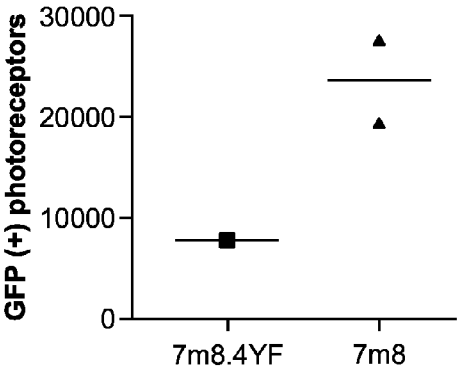

GFP (+) photoreceptors
30000
20000
10000
0
7m8.4YF
7m8

FIG. 4

AAV2 VP1 1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD

AAV2 VP1 61 KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ

AAV2 VP1 121 AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD

AAV2 VP1 181 SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI

AAV2 VP1 241 TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI

AAV2 VP1 301 NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG

AAV2 VP1 361 CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF

AAV2 VP1 421 HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG

AAV2 VP1 481 PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVL

AAV2 VP1 541 IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRG**NR**QAATADVNTQGV

AAV2 VP1 601 LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTT

AAV2 VP1 661 FSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVY

AAV2 VP1 721 SEPRPIGTRYLTR (SEQ ID NO:1)

FIG. 5

AAV-2	570	PVATEQYGSVSTNLQRG**NR**QAATADVNTQGVLPGMVWQDRDV	611 (SEQ ID NO:2)
AAV-1	571	PVATERFGTVAVNFQSSST**DP**ATGDVHAMGALPGMVWQDRDV	612 (SEQ ID NO:3)
AAV-5	560	RVAYNVGGQMATNNQ**SS**TTAPATGTYNLQEIVPGSVWMERDV	601 (SEQ ID NO:4)
AAV-6	571	PVATERFGTVAVNLQSSST**DP**ATGDVHVMGALPGMVWQDRDV	612 (SEQ ID NO:5)
AAV-7	572	PVATEEYGIVSSNLQAA**NT**AAQTQVVNNQGALPGMVWQNRDV	613 (SEQ ID NO:6)
AAV-8	573	PVATEEYGIVADNLQQQ**NT**APQIGTVNSQGALPGMVWQNRDV	614 (SEQ ID NO:7)
AAV-9	571	PVATESYGQVATNHQSA**QA**QAQTGWVQNQGILPGMVWQDRDV	612 (SEQ ID NO:8)
AAV-10	573	PVATEQYGVVADNLQ**QA**NTGPIVGNVNSQGALPGMVWQNRDV	614 (SEQ ID NO:9)

FIG. 6

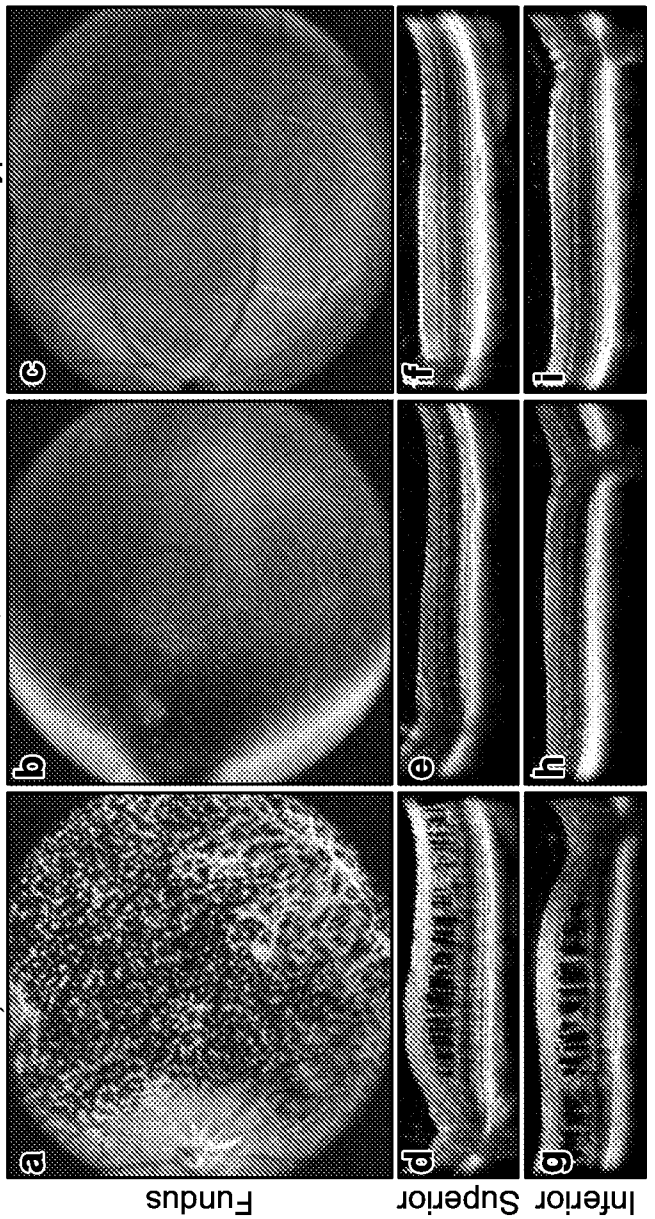

Rs1h-/-, 7m8-GFP
Rs1h-/-, 7m8-RS1
Wild-type
Fundus
Superior
Inferior
a
b
c
d
e
f
g
h
i

FIG. 7

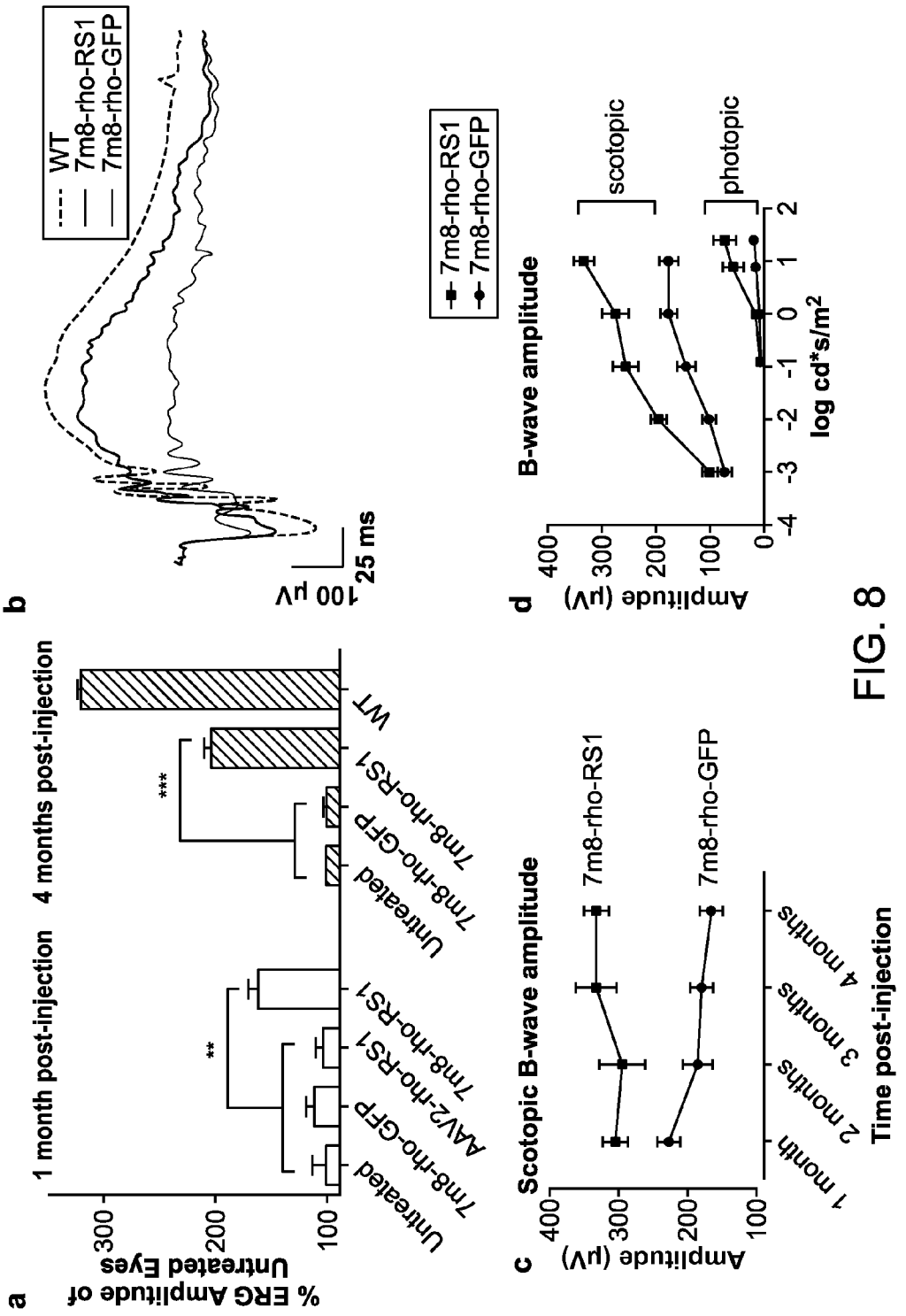

a
% ERG Amplitude of Untreated Eyes
1 month post-injection
4 months post-injection
Untreated
7m8-rho-GFP
AAV2-rho-RS1
7m8-rho-RS1
Untreated
7m8-rho-GFP
7m8-rho-RS1
WT
**
***
b
WT
7m8-rho-RS1
7m8-rho-GFP
100 µV
25 ms
c
Scotopic B-wave amplitude
Amplitude (µV)
7m8-rho-RS1
7m8-rho-GFP
1 month
2 months
3 months
4 months
Time post-injection
d
7m8-rho-RS1
7m8-rho-GFP
B-wave amplitude
scotopic
photopic
Amplitude (µV)
log cd*s/m2

FIG. 8

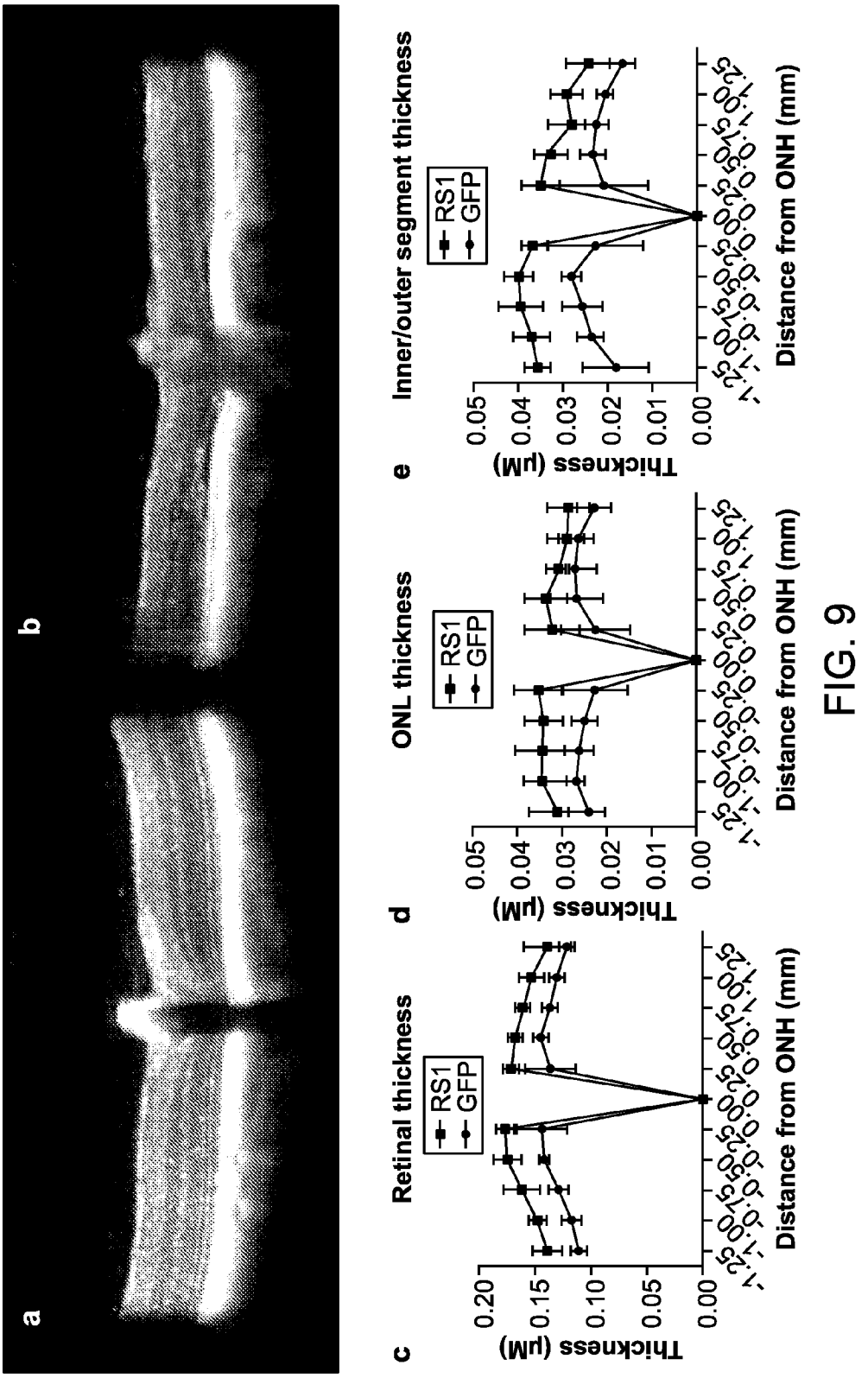

a
b
c
Retinal thickness
RS1
GFP
Thickness (μM)
0.20
0.15
0.10
0.05
0.00
Distance from ONH (mm)
d
ONL thickness
RS1
GFP
Thickness (μM)
0.05
0.04
0.03
0.02
0.01
0.00
Distance from ONH (mm)
e
Inner/outer segment thickness
RS1
GFP
Thickness (μM)
0.05
0.04
0.03
0.02
0.01
0.00
Distance from ONH (mm)

FIG. 9

Retinoschisin-1
Homo sapiens
GenBank CAI42483

```
  1 msrkiegfll lllfgyeatl glsstedege dpwyqkackc dcqggpnalw sagatsldci
 61 pecpyhkplg fesgevtpdq itcsnpeqyv gwysswtank arlnsqgfgc awlskfqdss
121 qwlqidlkei kvisgiltqg rcdidewmtk ysvqyrtder lnwiyykdqt gnnrvfygns
181 drtstvqnll rppiisrfir liplgwhvri airmellecv skca (SEQ ID NO:10)
```

FIG. 10

Brain-derived neurotrophic factor
Homo sapiens
GenBank CAA62632

```
  1 mtilfltmvi syfgcmkaap mkeanirgqg glaypgvrth gtlesvngpk agsrgltsla
 61 dtfehvieel ldedhkvrpn eennkdadly tsrvmlssqv pleppllfll eeyknyldaa
121 nmsmmvlrhs dparrgelsv cdsisewvta adkktavdms ggtvtvlekv pvskgqlkqy
181 fyetkcnpmg ytkegcrgid krhwnsqcrt tqsyvraltm dskkrigwrf iridtscvct
241 ltikrgr (SEQ ID NO:11)
```

FIG. 11

RPE65

Homo sapiens

GenBank AAC39660

```
  1 msiqvehpag gykklfetve elsspltahv tgriplwltg sllrcgpglf evgsepfyhl
 61 fdgqallhkf dfkeghvtyh rrfirtdayv ramtekrivi tefgtcafpd pcknifsrff
121 syfrgvevtd nalvnvypvg edyyactetn fitkinpetl etikqvdlcn yvsvngatah
181 phiendgtvy nigncfgknf siaynivkip plqadkedpi skseivvqfp csdrfkpsyv
241 hsfgltpnyi vfvetpvkin lfkflsswsl wganymdcfe snetmgvwlh iadkkrkkyl
301 nnkyrtspfn lfhhintyed ngflivdlcc wkgfefvyny lylanlrenw eevkknarka
361 pqpevrryvl plnidkadtg knlvtlpntt atailcsdet iwlepevlfs gprqafefpq
421 inyqkycgkp ytyayglgln hfvpdrlckl nvktketwvw qepdsypsep ifvshpdale
481 eddgvvlsvv vspgagqkpa yllilnakdl sevaraevei nipvtfhglf kks (SEQ ID NO:12)
```

FIG. 12

```
   1 agcttggatc caatcaacct ctggattaca aaatttgtga aagattgact ggtattctta
  61 actatgttgc tcctttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta
 121 ttgcttcccg tatggctttc attttctcct ccttgtataa atcctggttg ctgtctcttt
 181 atgaggagtt gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg
 241 caaccccac tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt
 301 tcccctccc tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag
 361 gggctcggct gttgggcact gacaattccg tggtgttgtc ggggaagctg acgtcctttc
 421 catggctgct cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc
 481 cttcggccct caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc
 541 ttccgcgtct tcgagatctg cctcgactgt gccttctagt tgccagccat ctgttgtttg
 601 ccctcccccc gtgccttcct tgaccctgga aggtgccact cccactgtcc ttcctaata
 661 aaatgaggaa attgcatcgc attgtctgag taggtgtcat tctattctgg ggggtggggt
 721 ggggcaggac agcaaggggg aggattggga agacaatagc aggcatgctg gggactcgag
 781 ttaagggcga attcccgatt aggatcttcc tagagcatgg ctacgtagat aagtagcatg
 841 gcgggttaat cattaactac aaggaacccc tagtgatgga gttggccact ccctctctgc
 901 gcgctcgctc gctcactgag gccgggcgac caaaggtcgc ccgacgcccg ggctttgccc
 961 gggcggcctc agtgagcgag cgagcgcgca gccttaatta acctaattca ctggccgtcg
1021 tttacaacg tcgtgactgg gaaaaccctg gcgttaccca acttaatcgc cttgcagcac
1081 atccccctt cgccagctgg cgtaatagcg aagaggcccg caccgatcgc ccttcccaac
1141 agttgcgcag cctgaatggc gaatgggacg cgccctgtag cggcgcatta agcgcggcgg
1201 gtgtggtggt tacgcgcagc gtgaccgcta cacttgccag cgccctagcg cccgctcctt
1261 tcgctttctt ccctcctttt ctcgccacgt tcgccggctt tccccgtcaa gctctaaatc
1321 ggggctccc tttagggttc cgatttagtg ctttacggca cctcgaccc aaaaaacttg
1381 attagggtga tggttcacgt agtgggccat cgccccgata gacggtttt cgccctttga
1441 cgctggagtt cacgttcctc aatagtggac tcttgttcca aactggaaca acactcaacc
1501 ctatctcggt ctatcttttt gatttataag ggattttcc gatttcggcc tattggttaa
1561 aaaatgagct gatttaacaa aaatttaacg cgaattttaa caaaatatta acgtttataa
1621 ttcaggtgg catctttcgg ggaaatgtgc gcggaacccc tatttgttta ttttctaaa
1681 tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt caataatatt
1741 gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc ttttttgcgg
1801 catttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa gatgctgaag
```

FIG. 13A

```
1861 atcagttggg tgcacgagtg ggttacatcg aactggatct caatagtggt aagatccttg
1921 agagttttcg ccccgaagaa cgtttttccaa tgatgagcac tttaaagttt ctgctatgtg
1981 gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc atacactatt
2041 ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga
2101 cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg gccaacttac
2161 ttctgacaac gatcggagga ccgaaggagc taaccgcttt ttgcacaac atggggatc
2221 atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca aacgacgagc
2281 gtgacaccac gatgcctgta gtaatggtaa caacgttgcg caaactatta actggcgaac
2341 tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat aaagttgcag
2401 gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa tctggagccg
2461 gtgagcgtgg gtctcgcggt atcattgcag cactgggggc agatggtaag ccctcccgta
2521 tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat agacagatcg
2581 ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt tactcatata
2641 tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg aagatccttt
2701 ttgataatct catgaccaaa atcccttaac gtgagttttc gttccactga gcgtcagacc
2761 ccgtagaaaa gatcaaagga tcttcttgag atccttttt tctgcgcgta atctgctgct
2821 tgcaaacaaa aaaccaccg ctaccagcgg tggtttgttt gccggatcaa gagctaccaa
2881 ctcttttcc gaaggtaact ggcttcagca gagcgcagat accaaatact gtccttctag
2941 tgtagccgta gttaggccac cacttcaaga actctgtagc accgcctaca tacctcgctc
3001 tgctaatcct gttaccagtg gctgctgcca gtggcgataa gtcgtgtctt accgggttgg
3061 actcaagacg atagttaccg gataaggcgc agcggtcggg ctgaacgggg ggttcgtgca
3121 cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag cgtgagctat
3181 gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta agcggcaggg
3241 tcggaacagg agagcgcacg agggagcttc cagggggaaa cgcctggtat ctttatagtc
3301 ctgtcgggtt tcgccacctc tgacttgagc gtcgatttt gtgatgctcg tcaggggggc
3361 ggagcctatg gaaaaacgcc agcaacgcgg cctttttacg gttcctggcc tttgctgcg
3421 gtttgctca catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg
3481 cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc gagtcagtga
3541 gcgaggaagc ggaagagcgc ccaatacgca aaccgcctct ccccgcgcgt tggccgattc
3601 attaatgcag ctggcacgac aggtttcccg actggaaagc gggcagtgag cgcaacgcaa
3661 ttaatgtgag ttagctcact cattaggcac cccaggcttt acactttatg cttccggctc
```

FIG. 13B

3721 gtatgttgtg tggaattgtg agcggataac aatttcacac aggaaacagc tatgaccatg
3781 attacgccag atttaattaa ggctgcgcgc tcgctcgctc actgaggccg cccgggcaaa
3841 gcccgggcgt cgggcgacct ttggtcgccc ggcctcagtg agcgagcgag cgcgcagaga
3901 gggagtggcc aactccatca ctaggggttc cttgtagtta atgattaacc cgccatgcta
3961 cttatctacg tagccatgct ctaggaagat cggaattcgc ccttaagcta gc**agatcttc**
4021 **cccacctagc cacctggcaa actgctcctt ctctcaaagg cccaaacatg gcctcccaga**
4081 **ctgcaacccc caggcagtca ggccctgtct ccacaacctc acagccaccc tggacggaat**
4141 **ctgcttcttc ccacatttga gtcctcctca gcccctgagc tcctctgggc agggctgttt**
4201 **ctttccatct ttgtattccc aggggcctgc aaataaatgt ttaatgaacg aacaagagag**
4261 **tgaattccaa ttccatgcaa caaggattgg gctcctgggc cctaggctat gtgtctggca**
4321 **ccagaaacgg aagctgcagg ttgcagcccc tgccctcatg gagctcctcc tgtcagagga**
4381 **gtgtggggac tggatgactc cagaggtaac ttgtggggga acgaacaggt aaggggctgt**
4441 **gtgacgagat gagagactgg gagaataaac cagaaagtct ctagctgtcc agaggacata**
4501 **gcacagaggc ccatggtccc tatttcaaac ccaggccacc agactgagct gggaccttgg**
4561 **gacagacaag tcatgcagaa gttaggggac cttctcctcc cttttcctgg atggatcctg**
4621 **agtaccttct cctccctgac ctcaggcttc ctcctagtgt caccttggcc cctcttagaa**
4681 **gccaattagg ccctcagttt ctgcagcggg gattaatatg attatgaaca cccccaatct**
4741 **cccagatgct gattcagcca ggagcttagg agggggaggt cactttataa gggtctgggg**
4801 **gggtcagaac ccagagtcat cccctgaatt ctgcagatat ccatcacact g**gcggccgcg
4861 ccacc**atg**tc acgcaagata gaaggctttt tgttattact tctctttggc tatgaagcca
4921 cattgggatt atcgtctacc gaggatgaag gcgaggaccc ctggtaccaa aaagcatgca
4981 agtgcgattg ccaaggagga cccaatgctc tgtggtctgc aggtgccacc tccttggact
5041 gtataccaga atgcccatat cacaagcctc tgggtttcga gtcaggggag gtcacaccgg
5101 accagatcac ctgctctaac ccggagcagt atgtgggctg gtattcttcg tggactgcaa
5161 acaaggcccg gctcaacagt caaggctttg ggtgtgcctg gctctccaag ttccaggaca
5221 gtagccagtg gttacagata gatctgaagg agatcaaagt gatttcaggg atcctcaccc
5281 aggggcgctg tgacatcgat gagtggatga ccaagtacag cgtgcagtac aggaccgatg
5341 agcgcctgaa ctggatttac tacaaggacc agactggaaa caaccgggtc ttctatggca
5401 actcggaccg cacctccacg gttcagaacc tgctgcggcc ccccatcatc tcccgcttca
5461 tccgcctcat cccgctgggc tggcacgtcc gcattgccat ccggatggag ctgctggagt
5521 gcgtcagcaa gtgtgcc**tga** a (SEQ ID NO:18)

FIG. 13C

Peripherin-2

```
  1 mallkvkfdq kkrvklaqgl wlmnwfsvla giiifslglf lkielrkrsd vmnnseshfv
 61 pnsligmgvl scvfnslagk icydaldpak yarwkpwlkp ylaicvlfni ilflvalccf
121 llrgslentl gqglkngmky yrdtdtpgrc fmkktidmlq iefkccgnng frdwfeiqwi
181 snryldfssk evkdriksnv dgrylvdgvp fsccnpsspr pciqyqitnn sahysydhqt
241 eelnlwvrgc raallsyyss lmnsmgvvtl liwlfevtit iglrylqtsl dgvsnpeese
301 sesqgwller svpetwkafl esvkklgkgn qveaegadag qapeag (SEQ ID NO:19)
```

FIG. 14

Peripherin

```
  1 mshhpsglra gfsstsyrrt fgpppslspg afsysssrf sssrllgsas psssvrlgsf
 61 rspragagal lrlpserldf smaealnqef latrsnekqe lqelndrfan fiekvrfleq
121 qnaalrgels qargqepara dqlcqqelre lrrelellgr erdrvqverd glaedlaalk
181 qrleeetrkr edaehnlvlf rkdvddatls rlelerkies lmdeieflkk lheeelrdlq
241 vsvesqqvqq veveatvkpe ltaalrdira qyesiaaknl qeaeewyksk yadlsdaanr
301 nhealrqakq emnesrrqiq sltcevdglr gtneallrql releeqfale aggyqagaar
361 leeelrqlke emarhlreyq ellnvkmald ieiatyrkll egeesrisvp vhsfaslnik
421 ttvpeveppq dshsrktvli ktietrngev vtesqkeqrs eldkssahsy (SEQ ID NO:20)
```

FIG. 15

RPGR-interacting protein-1

```
   1 mshlvdptsg dlpvrdidai plvlpaskgk nmktqpplsr mnreeledsf frlredhmlv
  61 kelswkqqde ikrlrttllr ltaagrdlrv aeeaaplset arrgqkagwr qrlsmhqrpq
 121 mhrlqghfhc vgpasprraq prvqvghrql htagapvpek pkrgprdrls ytappsfkeh
 181 atnenrgeva skpselvsgs nsiisfssvi smakpiglcm pnsahimasn tmqveeppks
 241 pekmwpkden feqrssleca qkaaelrasi kekvelirlk kllhernasl vmtkaqltev
 301 qeayetllqk nqgilsaahe allkqvnelr aelkeeskka vslksqledv silqmtlkef
 361 qervedleke rkllndnydk llesmldssd sssqphwsne liaeqlqqqv sqlqdqldae
 421 ledkrkvlle lsrekaqned lklevtnilq khkqevellq naatisqppd rqsepathpa
 481 vlqentqiep sepknqeekk lsqvlnelqv shaettlele ktrdmlilqr kinvcyqeel
 541 eammtkadnd nrdhkekler ltrlldlknn rikqlegilr shdlptseql kdvaygtrpl
 601 slcletlpah gdedkvdisl lhqgenlfel hihqafltsa alaqagdtqp ttfctysfyd
 661 fethctplsv gpqplydfts qyvmetdslf lhylqeasar ldihqamase hstlaagwic
 721 fdrvletvek vhglatliga ggeefgvley wmrlrfpikp slqacnkrkk aqvylstdvl
 781 ggrkaqeeef rseswepqne lwieitkccg lrsrwlgtqp spyavyrfft fsdhdtaiip
 841 asnnpyfrdq arfpvlvtsd ldhylrreal sihvfddedl epgsylgrar vpllplakne
 901 sikgdfnltd paekpngsiq vqldwkfpyi ppesflkpea qtkgkdtkds skisseeeka
 961 sfpsqdqmas pevpieagqy rskrkpphgg erkekehqvv sysrrkhgkr igvqgknrme
1021 ylslnilngn tpeqvnytew kfsetnsfig dgfknqheee emtlshsalk qkeplhpvnd
1081 kesseqgsev seaqttdsdd vivppmsqky pkadsekmci eivslafype aevmsdenik
1141 qvyveykfyd lplsetetpv slrkpragee ihfhfskvid ldpqeqqgrr rflfdmlngq
1201 dpdqghlkft vvsdpldeek keceevgyay lqlwqilesg rdileqeldi vspedlatpi
1261 grlkvslqaa avlhaiykem tedlfs (SEQ ID NO:21)
```

FIG. 16

```
AAV1            --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV6            --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV3            ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467
AAV2            ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466
AAV8            NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8.1          NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8 rh8         FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469
AAV10           NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469
AAV7            -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469
AAV9            -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV9.1          -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV5            NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453
                   *:* **:******:* **.* :* ***:***** :  *           : *

AAV1            PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV6            PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV3            PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527
AAV2            ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526
AAV8            PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8.1          PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8 rh8        S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527
AAV10           PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529
AAV7            PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529
AAV9            PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV9.1          PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV5            AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513
                .       :*::***  * *  .    *. *  .:.  .   *:* .    *     ::
```

FIG. 17A

```
AAV1            KDDEDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNF 584
AAV6            KDDKDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNL 584
AAV3            KDDEEKFFPMHGNLIFGK--EGTTASNAELD-NVMITDEEEIRTTNPVATEQYGTVANNL 584
AAV2            KDDEEKFFPQSGVLIFGK--QGSEKTNVDIE-KVMITDEEEIRTTNPVATEQYGSVSTNL 583
AAV8            KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8.1          KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8 rh8        KDDDDRFFPSSGVLIFGK--QGAGNDGVDYS-QVLITDEEEIKATNPVATEEYGAVAINN 584
AAV10           KDDEERFFPSSGVLMFGK--QGAGRDNVDYS-SVMLTSEEEIKTTNPVATEQYGVVADNL 586
AAV7            KDDEDRFFPSSGVLIFGK--TGAT-NKTTLE-NVLMTNEEEIRPTNPVATEEYGIVSSNL 585
AAV9            KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV9.1          KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV5            NLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNN 573
                :   .. :.  . ::*..   ..    .  . .:::*.*.* :..* ** :  * :: *

AAV1            QSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPP 644
AAV6            QSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV3            QSSNTAPTTGTVNHQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV2            QRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 643
AAV8            QQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV8.1          QGQRQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV8 rh8        QAANTQAQTGLVHNQGVIPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 644
AAV10           QQANTGPIVGNVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 646
AAV7            QAANTAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP 645
AAV9            QSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644
AAV9.1          QSGQAQAATGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP 644
AAV5            QSSTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP 633
                *     .     :    :** ** :**************.*..:***** *****:*:**
```

FIG. 17B

```
AAV1        PQILIK- 650 (SEQ ID NO:22)
AAV6        PQILIK- 650 (SEQ ID NO:23)
AAV3        PQIMIK- 650 (SEQ ID NO:24)
AAV2        PQILIKN 650 (SEQ ID NO:25)
AAV8        PQILIKN 653 (SEQ ID NO:26)
AAV8.1      PQILIKN 653 (SEQ ID NO:27)
AAV8 rh8    PQILIKN 651 (SEQ ID NO:28)
AAV10       PQILIKN 653 (SEQ ID NO:29)
AAV7        PQILIKN 652 (SEQ ID NO:30)
AAV9        PQILIK- 650 (SEQ ID NO:31)
AAV9.1      PQILIK- 650 (SEQ ID NO:32)
AAV5        PMMLIKN 640 (SEQ ID NO:33)
            * ::**
```

FIG. 17C

```
AAV1          --TFSYTFEEVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV6          --TFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ-NQSGSAQNKDLLFSRGS 467
AAV3          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAG 467
AAV2          ---FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN-TPSGTTTQSRLQFSQAG 466
AAV8          NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8.1        NFQFTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTT-GGTANTQTLGFSQGG 469
AAV8 rh8       FQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLVRTQTTGTGGTQTLAFSQAGPS 469
AAV10         NFEFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQST-GGTQGTQQLLFSQAG 469
AAV7          -FEFSYSFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGG 469
AAV9          -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV9.1        -FQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTI--NGSGQNQQTLKFSVAG 467
AAV5          NFEFTYNFEEVPFHSSFAPSQNLFKLANPLVDQYLYRFVSTN-------NTGGVQFNKNL 453

AAV1          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV6          PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASH 527
AAV3          PQSMSLQARNWLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASH 527
AAV2          ASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASH 526
AAV8          PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8.1        PNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATH 529
AAV8 rh8      S--MANQARNWVPGPCYRQQRVSTTTNQNNNSNFAWTGAAKFKLNGRDSLMNPGVAMASH 527
AAV10         PANMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATH 529
AAV7          PSTMAEQAKNWLPGPCFRQQRVSKTLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATH 529
AAV9          PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV9.1        PSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASH 527
AAV5          AGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTN 513
```

FIG. 18A

```
AAV1            KDDEDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNF 584
AAV6            KDDKDKFFPMSGVMIFGK--ESAGASNTALD-NVMITDEEEIKATNPVATERFGTVAVNL 584
AAV3            KDDEEKFFPMHGNLIFGK--EGTTASNAELD-NVMITDEEEIRTTNPVATEQYGTVANNL 584
AAV2            KDDEEKFFPQSGVLIFGK--QGSEKTNVDIE-KVMITDEEEIRTTNPVATEQYGSVSTNL 583
AAV8            KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8.1          KDDEERFFPSNGILIFGK--QNAARDNADYS-DVMLTSEEEIKTTNPVATEEYGIVADNL 586
AAV8 rh8        KDDDDRFFPSSGVLIFGK--QGAGNDGVDYS-QVLITDEEEIKATNPVATEEYGAVAINN 584
AAV10           KDDEERFFPSSGVLMFGK--QGAGRDNVDYS-SVMLTSEEEIKTTNPVATEQYGVVADNL 586
AAV7            KDDEDRFFPSSGVLIFGK--TGAT-NKTTLE-NVLMTNEEEIRPTNPVATEEYGIVSSNL 585
AAV9            KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV9.1          KEGEDRFFPLSGSLIFGK--QGTGRDNVDAD-KVMITNEEEIKTTNPVATESYGQVATNH 584
AAV5            NLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNN 573

AAV1            QSSSTDLALGETTRPAPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKNPP
AAV6            QSSSTDLALGETTRPAPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP
AAV3            QSSNTAPTTGTVNHQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP 644
AAV2            QRGNLALGETTRPARQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP
AAV8            QQQNLALGETTRPATAPQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV8.1          QGQRGLGETTRPAQAAQIGTVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV8 rh8        QAANLALGETTRPATQAQTGLVHNQGVIPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV10           QQLALGETTRPAANTGPIVGNVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV7            QAANLALGETTRPATAAQTQVVNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP
AAV9            QSAQLALGETTRPAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP
AAV9.1          QSGQAALGETTRPAQAATGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPP
AAV5            QSLALGETTRPASTTAPATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPP
```

FIG. 18B

AAV1	PQILIK-	(SEQ ID NO:34)
AAV6	PQILIK-	(SEQ ID NO:35)
AAV3	PQIMIK-	(SEQ ID NO:24)
AAV2	PQILIKN	(SEQ ID NO:36)
AAV8	PQILIKN	(SEQ ID NO:37)
AAV8.1	PQILIKN	(SEQ ID NO:38)
AAV8 rh8	PQILIKN	(SEQ ID NO:39)
AAV10	PQILIKN	(SEQ ID NO:40)
AAV7	PQILIKN	(SEQ ID NO:41)
AAV9	PQILIK-	(SEQ ID NO:42)
AAV9.1	PQILIK-	(SEQ ID NO:43)
AAV5	PMMLIKN	(SEQ ID NO:44)

FIG. 18C

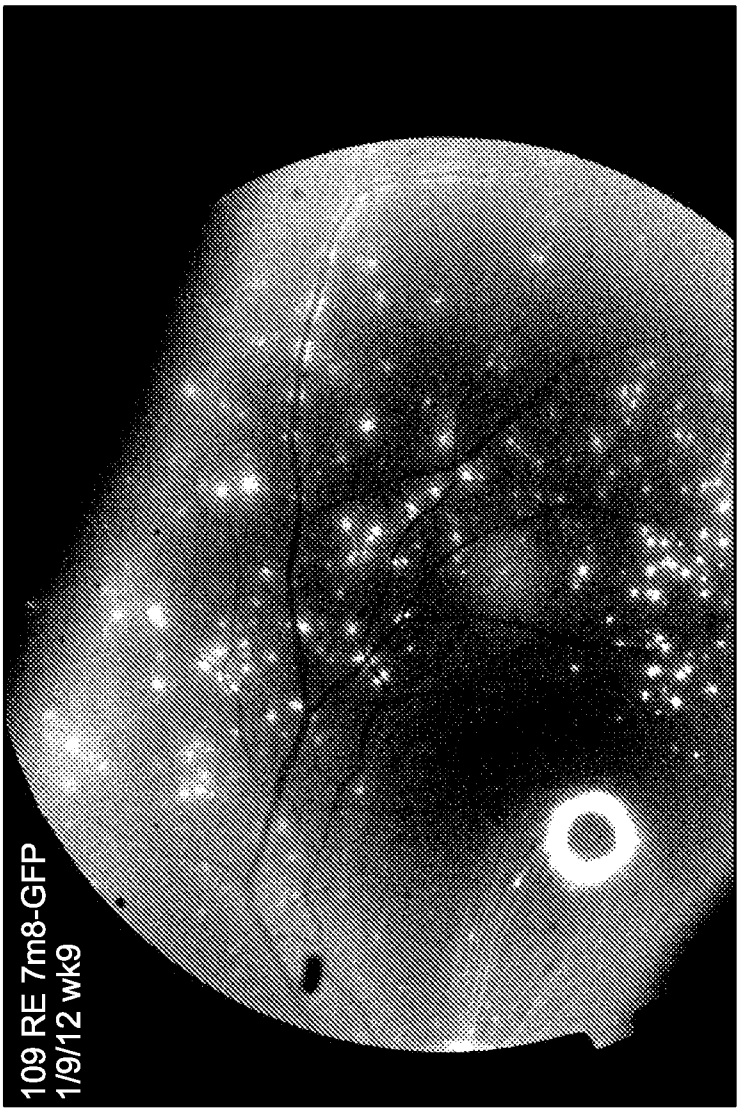


FIG. 19

ADENO-ASSOCIATED VIRUS VIRIONS WITH VARIANT CAPSID AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/478,355, filed Apr. 22, 2011, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant Nos. EY016994-02 and EY1018241 awarded by the National Eye Institute of the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Photoreceptors are the first neurons in the retina to receive and process visual information, converting visible electromagnetic radiation into hyperpolarized responses through phototransduction. The overwhelming majority of inherited retinal diseases result in the loss of these cells, either directly, such as in dominant mutations that affect rhodopsin protein folding, or indirectly, such as in recessive mutations that affect retinal recycling pathways in the retinal pigment epithelium (RPE).

[0004] AAV belongs to the Parvoviridae family and Dependovirus genus, whose members require co-infection with a helper virus such as adenovirus to promote replication, and AAV establishes a latent infection in the absence of a helper. Virions are composed of a 25 nm icosahedral capsid encompassing a 4.9 kb single-stranded DNA genome with two open reading frames: rep and cap. The non-structural rep gene encodes four regulatory proteins essential for viral replication, whereas cap encodes three structural proteins (VP1-3) that assemble into a 60-mer capsid shell. This viral capsid mediates the ability of AAV vectors to overcome many of the biological barriers of viral transduction—including cell surface receptor binding, endocytosis, intracellular trafficking, and unpackaging in the nucleus.

LITERATURE

[0005] U.S. Patent Publication No. 2005/0053922; U.S. Patent Publication No. 2009/0202490; Allocca et al. (2007) *J. Virol.* 81:11372; Boucas et al. (2009) *J. Gene Med.* 11:1103.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to wild-type AAV. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. **1** provides a representative three-dimensional model of AAV2 containing a random heptamer following amino acid 587.

[0008] FIG. **2** depicts greater levels of intravitreal transduction by AAV2 7M8 variant (right), relative to AAV2 (left).

[0009] FIG. **3** provides representative fluorescence images of retinal cryoslices showing green fluorescent protein (GFP) expression resulting from 7M8 carrying the GFP gene under the control of the ubiquitous CAG promoter (left) or a photoreceptor-specific Rho promoter (right).

[0010] FIG. **4** depicts GFP photoreceptor cells per million retinal cells as counted by flow cytometry, following transduction by 7M8 or by 7M8 bearing 4 tyrosine mutations (7m8.4YF).

[0011] FIG. **5** provides an amino acid sequence of AAV2 VP1 (SEQ ID NO:1).

[0012] FIG. **6** provides amino acid sequences corresponding to amino acids 570-610 of AAV2 (FIG. **5**) of AAV capsid protein VP1 of various AAV serotypes.

[0013] FIGS. **7**A-I depict structural improvements in the Rs1h−/− mouse retina after gene transfer.

[0014] FIGS. **8**A-D depict functional rescue of the electroretinogram A and B waves following RS1 gene delivery.

[0015] FIGS. **9**A-E depict sustained improvements in retinal thickness measured at 10 months post 7m8-rho-RS1 treatment.

[0016] FIG. **10** provides an amino acid sequence of retinoschisin.

[0017] FIG. **11** provides an amino acid sequence of brain derived neurotrophic factor.

[0018] FIG. **12** provides an amino acid sequence of RPE65.

[0019] FIGS. **13**A-C provide the nucleotide sequence of the 7m8-rho-RS1 construct.

[0020] FIG. **14** provides an amino acid sequence of peripherin-2.

[0021] FIG. **15** provides an amino acid sequence of peripherin.

[0022] FIG. **16** provides an amino acid sequence of retinitis pigmentosa GTPase regulator-interacting protein-1.

[0023] FIGS. **17**A-C provide an alignment of amino acid sequences of AAV capsid protein loop IV (GH loop) regions. Insertion sites are shown in bold and underlining.

[0024] FIGS. **18**A-C provide an alignment of amino acid sequences of AAV capsid protein GH loop regions, with heterologous peptide insertions.

[0025] FIG. **19** provides a fluorescence fundus image showing GFP expression in central primate retina 9 weeks after administration of 7m8 carrying GFP under the control of a connexin36 promoter.

DEFINITIONS

[0026] The term "retinal cell" can refer herein to any of the cell types that comprise the retina, such as retinal ganglion cells, amacrine cells, horizontal cells, bipolar cells, and photoreceptor cells including rods and cones, Müller glial cells, and retinal pigmented epithelium.

[0027] "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"). The term "AAV" includes AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. "Primate AAV" refers to AAV that infect primates, "non-primate AAV" refers

to AAV that infect non-primate mammals, "bovine AAV" refers to AAV that infect bovine mammals, etc.

[0028] The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077 (AAV-1), AF063497 (AAV-1), NC_001401 (AAV-2), AF043303 (AAV-2), NC_001729 (AAV-3), NC_001829 (AAV-4), U89790 (AAV-4), NC_006152 (AAV-5), AF513851 (AAV-7), AF513852 (AAV-8), and NC_006261 (AAV-8); the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g., Srivistava et al. (1983) *J. Virology* 45:555; Chiorini et al. (1998) *J. Virology* 71:6823; Chiorini et al. (1999) *J. Virology* 73:1309; Bantel-Schaal et al. (1999) *J. Virology* 73:939; Xiao et al. (1999) *J. Virology* 73:3994; Muramatsu et al. (1996) *Virology* 221:208; Shade et al., (1986) *J. Virol.* 58:921; Gao et al. (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris et al. (2004) *Virology* 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

[0029] An "rAAV vector" as used herein refers to an AAV vector comprising a polynucleotide sequence not of AAV origin (i.e., a polynucleotide heterologous to AAV), typically a sequence of interest for the genetic transformation of a cell. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. An rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV).

[0030] An "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and an encapsidated polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "rAAV vector particle" or simply an "rAAV vector". Thus, production of rAAV particle necessarily includes production of rAAV vector, as such a vector is contained within an rAAV particle.

[0031] "Packaging" refers to a series of intracellular events that result in the assembly and encapsidation of an AAV particle.

[0032] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV "packaging genes."

[0033] A "helper virus" for AAV refers to a virus that allows AAV (e.g. wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpesviruses and poxviruses such as vaccinia. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

[0034] "Helper virus function(s)" refers to function(s) encoded in a helper virus genome which allow AAV replication and packaging (in conjunction with other requirements for replication and packaging described herein). As described herein, "helper virus function" may be provided in a number of ways, including by providing helper virus or providing, for example, polynucleotide sequences encoding the requisite function(s) to a producer cell in trans. For example, a plasmid or other expression vector comprising nucleotide sequences encoding one or more adenoviral proteins is transfected into a producer cell along with an rAAV vector.

[0035] An "infectious" virus or viral particle is one that comprises a competently assembled viral capsid and is capable of delivering a polynucleotide component into a cell for which the viral species is tropic. The term does not necessarily imply any replication capacity of the virus. Assays for counting infectious viral particles are described elsewhere in this disclosure and in the art. Viral infectivity can be expressed as the ratio of infectious viral particles to total viral particles. Methods of determining the ratio of infectious viral particle to total viral particle are known in the art. See, e.g., Grainger et al. (2005) *Mol. Ther.* 11:S337 (describing a TCID50 infectious titer assay); and Zolotukhin et al. (1999) *Gene Ther.* 6:973. See also the Examples.

[0036] A "replication-competent" virus (e.g. a replication-competent AAV) refers to a phenotypically wild-type virus that is infectious, and is also capable of being replicated in an infected cell (i.e. in the presence of a helper virus or helper virus functions). In the case of AAV, replication competence generally requires the presence of functional AAV packaging genes. In general, rAAV vectors as described herein are replication-incompetent in mammalian cells (especially in human cells) by virtue of the lack of one or more AAV packaging genes. Typically, such rAAV vectors lack any AAV packaging gene sequences in order to minimize the possibility that replication competent AAV are generated by recombination between AAV packaging genes and an incoming rAAV vector. In many embodiments, rAAV vector preparations as described herein are those which contain few if any replication competent AAV (rcAAV, also referred to as RCA) (e.g., less than about 1 rcAAV per 10^2 rAAV particles, less than about 1 rcAAV per 10^4 rAAV particles, less than about 1 rcAAV per 10^8 rAAV particles, less than about 1 rcAAV per 10^{12} rAAV particles, or no rcAAV).

[0037] The term "polynucleotide" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0038] Nucleic acid hybridization reactions can be performed under conditions of different "stringency". Conditions that increase stringency of a hybridization reaction of

widely known and published in the art. See, e.g., Sambrook et al. *Molecular Cloning*, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, herein incorporated by reference. For example, see page 7.52 of Sambrook et al. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25° C., 37° C., 50° C. and 68° C.; buffer concentrations of 10×SSC, 6×SSC, 1×SSC, 0.1×SSC (where 1×SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6×SSC, 1×SSC, 0.1×SSC, or deionized water. An example of stringent hybridization conditions is hybridization at 50° C. or higher and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42° C. in a solution: 50% formamide, 1×SSC (150 mM NaCl, 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1×SSC at about 65° C. As another example, stringent hybridization conditions comprise: prehybridization for 8 hours to overnight at 65° C. in a solution comprising 6× single strength citrate (SSC) (1×SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5×Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C. in a solution containing 6×SSC, 1×Denhardt's solution, 100 μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C. for 1 h in a solution containing 0.2×SSC and 0.1% SDS (sodium dodecyl sulfate).

[0039] Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions. Other stringent hybridization conditions are known in the art and may also be employed to identify nucleic acids of this particular embodiment of the invention.

[0040] "T_m" is the temperature in degrees Celsius at which 50% of a polynucleotide duplex made of complementary strands hydrogen bonded in anti-parallel direction by Watson-Crick base pairing dissociates into single strands under conditions of the experiment. T_m may be predicted according to a standard formula, such as:

$$T_m = 81.5 + 16.6 \log [X^+] + 0.41(\% G/C) - 0.61(\% F) - 600/L$$

[0041] where $[X^+]$ is the cation concentration (usually sodium ion, Na^+) in mol/L; (% G/C) is the number of G and C residues as a percentage of total residues in the duplex; (% F) is the percent formamide in solution (wt/vol); and L is the number of nucleotides in each strand of the duplex.

[0042] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, Calif., USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970)

[0043] Of interest is the BestFit program using the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2: 482-489 (1981) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determined using the default parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wis., USA.

[0044] Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters:

Mismatch Penalty: 1.00;

Gap Penalty: 1.00;

Gap Size Penalty: 0.33; and

Joining Penalty: 30.0.

[0045] A "gene" refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated.

[0046] A "gene product" is a molecule resulting from expression of a particular gene. Gene products include, e.g., a polypeptide, an aptamer, an interfering RNA, an mRNA, and the like.

[0047] A "small interfering" or "short interfering RNA" or siRNA is a RNA duplex of nucleotides that is targeted to a gene interest (a "target gene"). An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of a RNA molecule. siRNA is "targeted" to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19-25 nucleotides in length. The RNA duplex portion of the siRNA can be part of a hairpin structure. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length.

[0048] A "short hairpin RNA," or shRNA, is a polynucleotide construct that can be made to express an interfering RNA such as siRNA.

[0049] "Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[0050] A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[0051] "Operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0052] An "expression vector" is a vector comprising a region which encodes a polypeptide of interest, and is used for effecting the expression of the protein in an intended target cell. An expression vector also comprises control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0053] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter. Thus, for example, an rAAV that includes a heterologous nucleic acid encoding a heterologous gene product is an rAAV that includes a nucleic acid not normally included in a naturally-occurring, wild-type AAV, and the encoded heterologous gene product is a gene product not normally encoded by a naturally-occurring, wild-type AAV.

[0054] The terms "genetic alteration" and "genetic modification" (and grammatical variants thereof), are used interchangeably herein to refer to a process wherein a genetic element (e.g., a polynucleotide) is introduced into a cell other than by mitosis or meiosis. The element may be heterologous to the cell, or it may be an additional copy or improved version of an element already present in the cell. Genetic alteration may be effected, for example, by transfecting a cell with a recombinant plasmid or other polynucleotide through any process known in the art, such as electroporation, calcium phosphate precipitation, or contacting with a polynucleotide-liposome complex. Genetic alteration may also be effected, for example, by transduction or infection with a DNA or RNA virus or viral vector. Generally, the genetic element is introduced into a chromosome or mini-chromosome in the cell; but any alteration that changes the phenotype and/or genotype of the cell and its progeny is included in this term.

[0055] A cell is said to be "stably" altered, transduced, genetically modified, or transformed with a genetic sequence if the sequence is available to perform its function during extended culture of the cell in vitro. Generally, such a cell is "heritably" altered (genetically modified) in that a genetic alteration is introduced which is also inheritable by progeny of the altered cell.

[0056] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component. Polypeptides such as anti-angiogenic polypeptides, neuroprotective polypeptides, and the like, when discussed in the context of delivering a gene product to a mammalian subject, and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof, which retains the desired biochemical function of the intact protein. Similarly, references to nucleic acids encoding anti-angiogenic polypeptides, nucleic acids encoding neuroprotective polypeptides, and other such nucleic acids for use in delivery of a gene product to a mammalian subject (which may be referred to as "transgenes" to be delivered to a recipient cell), include polynucleotides encoding the intact polypeptide or any fragment or genetically engineered derivative possessing the desired biochemical function.

[0057] An "isolated" plasmid, nucleic acid, vector, virus, virion, host cell, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more isolated. An isolated plasmid, nucleic acid, vector, virus, host cell, or other substance is in some embodiments purified, e.g., from about 80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

[0058] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease or at risk of

acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0059] The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, human and non-human primates, including simians and humans; mammalian sport animals (e.g., horses); mammalian farm animals (e.g., sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.).

[0060] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0061] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0062] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0063] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a recombinant AAV virion" includes a plurality of such virions and reference to "the photoreceptor cell" includes reference to one or more photoreceptor cells and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0064] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0065] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0066] The present disclosure provides adeno-associated virus (AAV) virions with altered capsid protein, where the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to wild-type AAV when administered via intravitreal injection. The present disclosure further provides methods of delivering a gene product to a retinal cell in an individual, and methods of treating ocular disease.

[0067] The retinal cell can be a photoreceptor (e.g., rods; cones), a retinal ganglion cell (RGC), a Müller cell (a Müller glial cell), a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium (RPE) cell.

Variant AAV Capsid Polypeptides

[0068] The present disclosure provides a variant AAV capsid protein, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in an insertion site in the capsid protein GH loop or loop IV, relative to a corresponding parental AAV capsid protein, and where the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. In some cases, the retinal cell is a photoreceptor cell (e.g., rods; cones). In other cases, the retinal cell is an RGC. In other cases, the retinal cell is an RPE cell. In other cases, the retinal cell is a Müller cell. Other retinal cells include amacrine cells, bipolar cells, and horizontal cells. An "insertion of from about 5 amino acids to about 11 amino acids" is also referred to herein as a "peptide insertion" (e.g., a heterologous peptide insertion). A "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same AAV serotype, without the peptide insertion.

[0069] The insertion site is in the GH loop, or loop IV, of the AAV capsid protein, e.g., in a solvent-accessible portion of the GH loop, or loop IV, of the AAV capsid protein. For the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) *Mol. Ther.* 14:809; Padron et al. (2005) *J. Virol.* 79:5047; and Shen et al. (2007) *Mol. Ther.* 15:1955. For example, the insertion site can be within amino acids 411-650 of an AAV capsid protein, as depicted in FIGS. **17**A and **17**B. For example, the insertion site can be within amino acids 570-611 of AAV2, within amino acids 571-612 of AAV1, within amino acids 560-601 of AAV5, within amino acids 571 to 612 of AAV6, within amino acids 572 to 613 of AAV7, within amino acids 573 to 614 of AAV8, within amino acids 571 to 612 of AAV9, or within amino acids 573 to 614 of AAV10, as depicted in FIG. **6**.

[0070] In some cases, from about 5 amino acids to about 11 amino acids are inserted in an insertion site in the GH loop or loop IV of the capsid protein relative to a corresponding parental AAV capsid protein. For example, the insertion site can be between amino acids 587 and 588 of AAV2, or the corresponding positions of the capsid subunit of another AAV serotype. It should be noted that the insertion site 587/588 is

based on an AAV2 capsid protein. From about 5 amino acids to about 11 amino acids can be inserted in a corresponding site in an AAV serotype other than AAV2 (e.g., AAV8, AAV9, etc.). Those skilled in the art would know, based on a comparison of the amino acid sequences of capsid proteins of various AAV serotypes, where an insertion site "corresponding to amino acids 587-588 of AAV2" would be in a capsid protein of any given AAV serotype. Sequences corresponding to amino acids 570-611 of capsid protein VP1 of AAV2 (see FIG. **5**) in various AAV serotypes are shown in FIG. **6**. See, e.g., GenBank Accession No. NP_049542 for AAV1; GenBank Accession No. AAD13756 for AAV5; GenBank Accession No. AAB95459 for AAV6; GenBank Accession No. YP_077178 for AAV7; GenBank Accession No. YP_077180 for AAV8; GenBank Accession No. AAS99264 for AAV9 and GenBank Accession No. AAT46337 for AAV10.

[0071] In some embodiments, the insertion site is a single insertion site between two adjacent amino acids located between amino acids 570-614 of VP1 of any AAV serotype, e.g., the insertion site is between two adjacent amino acids located in amino acids 570-610, amino acids 580-600, amino acids 570-575, amino acids 575-580, amino acids 580-585, amino acids 585-590, amino acids 590-600, or amino acids 600-614, of VP1 of any AAV serotype or variant. For example, the insertion site can be between amino acids 580 and 581, amino acids 581 and 582, amino acids 583 and 584, amino acids 584 and 585, amino acids 585 and 586, amino acids 586 and 587, amino acids 587 and 588, amino acids 588 and 589, or amino acids 589 and 590. The insertion site can be between amino acids 575 and 576, amino acids 576 and 577, amino acids 577 and 578, amino acids 578 and 579, or amino acids 579 and 580. The insertion site can be between amino acids 590 and 591, amino acids 591 and 592, amino acids 592 and 593, amino acids 593 and 594, amino acids 594 and 595, amino acids 595 and 596, amino acids 596 and 597, amino acids 597 and 598, amino acids 598 and 599, or amino acids 599 and 600.

[0072] For example, the insertion site can be between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 588 and 589 of AAV10.

[0073] As another example, the insertion site can be between amino acids 450 and 460 of an AAV capsid protein, as shown in FIG. **17**A. For example, the insertion site can be at (e.g., immediately N-terminal to) amino acid 453 of AAV2, at amino acid 454 of AAV1, at amino acid 454 of AAV6, at amino acid 456 of AAV7, at amino acid 456 of AAV8, at amino acid 454 of AAV9, or at amino acid 456 of AAV10, as shown in FIG. **17**A.

[0074] In some embodiments, a subject capsid protein includes a GH loop comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in FIG. **18**A-C.

Insertion Peptides

[0075] As noted above, a peptide of from about 5 amino acids to about 11 amino acids in length is inserted into the GH loop of an AAV capsid. The insertion peptide has a length of 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, or 11 amino acids.

[0076] The insertion peptide can comprise an amino acid sequence of any one of the formulas set forth below.

[0077] For example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula I:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
X_1, if present, is selected from Leu, Asn, and Lys;
X_2 is selected from Gly, Glu, Ala, and Asp;
X_3 is selected from Glu, Thr, Gly, and Pro;
X_4 is selected from Thr, Ile, Gln, and Lys;
X_5 is selected from Thr and Ala;
X_6 is selected from Arg, Asn, and Thr;
X_7, if present, is selected from Pro and Asn.

[0078] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIa:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
each of X_1-X_4 is any amino acid;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0079] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIb:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
X_1, if present, is selected from Leu and Asn;
X_2, if present, is selected from Gly and Glu;
X_3 is selected from Glu and Thr;
X_4 is selected from Thr and Ile;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0080] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula III:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is Lys;
X_2 is selected from Ala and Asp;
X_3 is selected from Gly and Pro;
X_4 is selected from Gln and Lys;
X_5 is selected from Thr and Ala;
X_6 is selected from Asn and Thr;
X_7, if present, is Asn.

[0081] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IV:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
X_1, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Leu, Asn, Arg, Ala, Ser, and Lys;
X_2 is a negatively charged amino acid or an uncharged amino acid; or is selected from Gly, Glu, Ala, Val, Thr, and Asp;
X_3 is a negatively charged amino acid or an uncharged amino acid; or is selected from Glu, Thr, Gly, Asp, or Pro;
X_4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;
X_5 is a polar amino acid, an alcohol (an amino acid having a free hydroxyl group), or a hydrophobic amino acid; or is selected from Thr, Ser, Val, and Ala;
X_6 is a positively charged amino acid or an uncharged amino acid; or is selected from Arg, Val, Lys, Pro, Thr, and Asn; and
X_7, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Pro, Gly, Phe, Asn, and Arg.

[0082] As non-limiting examples, the insertion peptide can comprise an amino acid sequence selected from LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60).

[0083] In some cases, the insertion peptide has from 1 to 4 spacer amino acids (Y_1-Y_4) at the amino terminus and/or at the carboxyl terminus of any one of LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60). Suitable spacer amino acids include, but are not limited to, leucine, alanine, glycine, and serine.

[0084] For example, in some cases, an insertion peptide has one of the following amino acid sequences: LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDTDTTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64). As another example, in some cases, an insertion peptide has one of the following amino acid sequences: AALGETTRPA (SEQ ID NO:49); AANETITRPA (SEQ ID NO:50), AAKAGQANNA (SEQ ID NO:51), and AAKDPKTTNA (SEQ ID NO:52). As yet another example, in some cases, an insertion peptide has one of the following amino acid sequences: GLGETTRPA (SEQ ID NO:53); GNETITRPA (SEQ ID NO:54), GKAGQANNA (SEQ ID NO:55), and GKDPKTTNA (SEQ ID NO:56). As another example, in some cases, an insertion peptide comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60), flanked on the C-terminus by AA and on the N-terminus by A; or comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60) flanked on the C-terminus by G and on the N-terminus by A.

[0085] In some embodiments, a subject variant AAV capsid does not include any other amino acid substitutions, insertions, or deletions, other than an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. In other embodiments, a subject variant AAV capsid includes from 1 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. For example, in some embodiments, a subject variant AAV capsid includes from 1 to about 5, from about 5 to about 10, from about 10 to about 15, from about 15 to about 20, or from about 20 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0086] In some embodiments, a subject variant capsid polypeptide does not include one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0087] In some embodiments, a subject variant capsid polypeptide comprises, in addition to an insertion peptide as described above, one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0088] In some embodiments, a variant AAV capsid polypeptide is a chimeric capsid, e.g., the capsid comprises a portion of an AAV capsid of a first AAV serotype and a portion of an AAV capsid of a second serotype; and comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0089] In some embodiments, a subject variant capsid protein comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the amino acid sequence provided in FIG. **5**; and an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0090] In some embodiments, a subject variant capsid protein is isolated, e.g., purified. In some cases, a subject variant capsid protein is included in an AAV vector, which is also provided. As described in detail below, a subject variant capsid protein can be included in a recombinant AAV virion.

Recombinant AAV Virion

[0091] The present disclosure provides a recombinant adeno-associated virus (rAAV) virion comprising: a) a variant AAV capsid protein, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in an insertion site in the capsid protein GH loop or loop IV, relative to a corresponding parental AAV capsid protein, and where the variant capsid protein confers increased infectivity of a retinal cell compared to the infec-

tivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein; and b) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some cases, the retinal cell is a photoreceptor cell (e.g., rods and/or cones). In other cases, the retinal cell is an RGC cell. In other cases, the retinal cell is an RPE cell. In other cases, the retinal cell is a Müller cell. In other cases, retinal cells may include amacrine cells, bipolar cells, and horizontal cells. An "insertion of from about 5 amino acids to about 11 amino acids" is also referred to herein as a "peptide insertion" (e.g., a heterologous peptide insertion). A "corresponding parental AAV capsid protein" refers to an AAV capsid protein of the same AAV serotype, without the peptide insertion.

[0092] The insertion site is in the GH loop, or loop IV, of the AAV capsid protein, e.g., in a solvent-accessible portion of the GH loop, or loop IV, of the AAV capsid protein. For the GH loop, see, e.g., van Vliet et al. (2006) *Mol. Ther.* 14:809; Padron et al. (2005) *J. Virol.* 79:5047; and Shen et al. (2007) *Mol. Ther.* 15:1955. For example, the insertion site is within amino acids 570-611 of AAV2, within amino acids 571-612 of AAV1, within amino acids 560-601 of AAV5, within amino acids 571 to 612 of AAV6, within amino acids 572 to 613 of AAV7, within amino acids 573 to 614 of AAV8, within amino acids 571 to 612 of AAV9, or within amino acids 573 to 614 of AAV10.

[0093] From about 5 amino acids to about 11 amino acids are inserted in an insertion site in the GH loop or loop IV of the capsid protein relative to a corresponding parental AAV capsid protein. For example, the insertion site can be between amino acids 587 and 588 of AAV2, or the corresponding positions of the capsid subunit of another AAV serotype. It should be noted that the insertion site 587/588 is based on an AAV2 capsid protein. From about 5 amino acids to about 11 amino acids can be inserted in a corresponding site in an AAV serotype other than AAV2 (e.g., AAV8, AAV9, etc.). Those skilled in the art would know, based on a comparison of the amino acid sequences of capsid proteins of various AAV serotypes, where an insertion site "corresponding to amino acids 587-588 of AAV2" would be in a capsid protein of any given AAV serotype. Sequences corresponding to amino acids 570-611 of capsid protein VP1 of AAV2 (see FIG. **5**) in various AAV serotypes are shown in FIG. **6**.

[0094] In some embodiments, the insertion site is a single insertion site between two adjacent amino acids located between amino acids 570-614 of VP1 of any AAV serotype, e.g., the insertion site is between two adjacent amino acids located in amino acids 570-614, amino acids 580-600, amino acids 570-575, amino acids 575-580, amino acids 580-585, amino acids 585-590, amino acids 590-600, or amino acids 600-610, of VP1 of any AAV serotype or variant. For example, the insertion site can be between amino acids 580 and 581, amino acids 581 and 582, amino acids 583 and 584, amino acids 584 and 585, amino acids 585 and 586, amino acids 586 and 587, amino acids 587 and 588, amino acids 588 and 589, or amino acids 589 and 590. The insertion site can be between amino acids 575 and 576, amino acids 576 and 577, amino acids 577 and 578, amino acids 578 and 579, or amino acids 579 and 580. The insertion site can be between amino acids 590 and 591, amino acids 591 and 592, amino acids 592 and 593, amino acids 593 and 594, amino acids 594 and 595, amino acids 595 and 596, amino acids 596 and 597, amino acids 597 and 598, amino acids 598 and 599, or amino acids 599 and 600.

[0095] For example, the insertion site can be between amino acids 587 and 588 of AAV2, between amino acids 590 and 591 of AAV1, between amino acids 575 and 576 of AAV5, between amino acids 590 and 591 of AAV6, between amino acids 589 and 590 of AAV7, between amino acids 590 and 591 of AAV8, between amino acids 588 and 589 of AAV9, or between amino acids 589 and 590 of AAV10.

Insertion Peptides

[0096] As noted above, a subject rAAV virion comprises a peptide of from about 5 amino acids to about 11 amino acids in length inserted into the GH loop of the AAV capsid. The insertion peptide has a length of 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, or 11 amino acids.

[0097] The insertion peptide can comprise an amino acid sequence of any one of the formulas set forth below.

[0098] For example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula I:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0099] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is selected from Leu, Asn, and Lys;

X_2 is selected from Gly, Glu, Ala, and Asp;

X_3 is selected from Glu, Thr, Gly, and Pro;

X_4 is selected from Thr, Ile, Gln, and Lys;

X_5 is selected from Thr and Ala;

X_6 is selected from Arg, Asn, and Thr;

X_7, if present, is selected from Pro and Asn.

[0100] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIa:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0101] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

each of X_1-X_4 is any amino acid;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0102] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIb:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0103] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is selected from Leu and Asn;

X_2, if present, is selected from Gly and Glu;

X_3 is selected from Glu and Thr;
X_4 is selected from Thr and Ile;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0104] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula III:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0105] where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
X_1, if present, is Lys;
X_2 is selected from Ala and Asp;
X_3 is selected from Gly and Pro;
X_4 is selected from Gln and Lys;
X_5 is selected from Thr and Ala;
X_6 is selected from Asn and Thr;
X_7, if present, is Asn.

[0106] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IV:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0107] where:
each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;
X_1, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Leu, Asn, Arg, Ala, Ser, and Lys;
X_2 is a negatively charged amino acid or an uncharged amino acid; or is selected from Gly, Glu, Ala, Val, Thr, and Asp;
X_3 is a negatively charged amino acid or an uncharged amino acid; or is selected from Glu, Thr, Gly, Asp, or Pro;
X_4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;
X_5 is a polar amino acid, an alcohol (an amino acid having a free hydroxyl group), or a hydrophobic amino acid; or is selected from Thr, Ser, Val, and Ala;
X_6 is a positively charged amino acid or an uncharged amino acid; or is selected from Arg, Val, Lys, Pro, Thr, and Asn; and
X_7, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Pro, Gly, Phe, Asn, and Arg.

[0108] As non-limiting examples, the insertion peptide can comprise an amino acid sequence selected from LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60).

[0109] In some cases, the insertion peptide has from 1 to 4 spacer amino acids (Y_1-Y_4) at the amino terminus and/or at the carboxyl terminus of any one of LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60). Suitable spacer amino acids include, but are not limited to, leucine, alanine, glycine, and serine.

[0110] For example, in some cases, an insertion peptide has one of the following amino acid sequences: LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDTDTTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64). As another example, in some cases, an insertion peptide has one of the following amino acid sequences: AALGETTRPA (SEQ ID NO:49); AANETITRPA (SEQ ID NO:50), AAKAGQANNA (SEQ ID NO:51), and AAKDPKTTNA (SEQ ID NO:52). As yet another example, in some cases, an insertion peptide has one of the following amino acid sequences: GLGETTRPA (SEQ ID NO:53); GNETITRPA (SEQ ID NO:54), GKAGQANNA (SEQ ID NO:55), and GKDPKTTNA (SEQ ID NO:56). As another example, in some cases, an insertion peptide comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60), flanked on the C-terminus by AA and on the N-terminus by A; or comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60) flanked on the C-terminus by G and on the N-terminus by A.

[0111] In some embodiments, a subject rAAV virion capsid does not include any other amino acid substitutions, insertions, or deletions, other than an insertion of from about 7 amino acids to about 10 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. In other embodiments, a subject rAAV virion capsid includes from 1 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 7 amino acids to about 10 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein. For example, in some embodiments, a subject rAAV virion capsid includes from 1 to about 5, from about 5 to about 10, from about 10 to about 15, from about 15 to about 20, or from about 20 to about 25 amino acid insertions, deletions, or substitutions, compared to the parental AAV capsid protein, in addition to an insertion of from about 7 amino acids to about 10 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0112] In some embodiments, a subject rAAV virion capsid does not include one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0113] In some embodiments, a subject variant capsid polypeptide comprises, in addition to an insertion peptide as described above, one, two, three, or four, of the following amino acid substitutions: Y273F, Y444F, Y500F, and Y730F.

[0114] In some embodiments, a subject rAAV virion capsid is a chimeric capsid, e.g., the capsid comprises a portion of an AAV capsid of a first AAV serotype and a portion of an AAV capsid of a second serotype; and comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0115] In some embodiments, a subject rAAV virion comprises a capsid protein comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid

sequence identity to the amino acid sequence provided in FIG. **5**; and an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein.

[0116] In some embodiments, a subject rAAV virion comprises a capsid protein that includes a GH loop comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in FIG. **18**A-C.

[0117] A subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a retinal cell, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0118] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a retinal cell, when administered via intravitreal injection, compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0119] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor (rod or cone) cell, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0120] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor (rod or cone) cell, when administered via intravitreal injection, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0121] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RGC, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein.

[0122] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RGC, when administered via intravitreal injection, compared to the infectivity of the RGC by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0123] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RPE cell, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0124] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an RPE cell, when administered via intravitreal injection, compared to the infectivity of the RPE cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0125] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a Müller cell, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0126] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a Müller cell, when administered via intravitreal injection, compared to the infectivity of the Müller cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0127] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a bipolar cell, compared to the infectivity of the bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0128] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a bipolar cell, when administered via intravitreal injection, compared to the infectivity of the bipolar cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0129] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an amacrine cell, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0130] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of an amacrine cell, when administered via intravitreal injection, compared to the infectivity of the amacrine cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0131] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a horizontal cell, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein.

[0132] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a horizontal cell, when administered via intravitreal injection, compared to the infectivity of the horizontal cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

[0133] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased ability to cross the internal limiting membrane (ILM), compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM.

[0134] In some cases, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased ability, when

administered via intravitreal injection, to cross the internal limiting membrane (ILM), compared to the ability of an AAV virion comprising the corresponding parental AAV capsid protein to cross the ILM when administered via intravitreal injection.

[0135] A subject rAAV virion can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells. For example, a subject rAAV virion, when administered via intravitreal injection, can cross the ILM, and can also traverse cell layers, including Müller cells, amacrine cells, etc., to reach the photoreceptor cells and or RPE cells.

[0136] In some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a retinal cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye. For example, in some embodiments, a subject rAAV virion selectively infects a retinal cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-retinal cell, e.g., a cell outside the eye.

[0137] In some embodiments, a subject rAAV virion selectively infects a photoreceptor cell, e.g., a subject rAAV virion infects a photoreceptor cell with 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, or more than 50-fold, specificity than a non-photoreceptor cell present in the eye, e.g., a retinal ganglion cell, a Müller cell, etc.

[0138] In some embodiments, a subject rAAV virion exhibits at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, or more than 50-fold, increased infectivity of a photoreceptor cell, when administered via intravitreal injection, compared to the infectivity of the photoreceptor cell by an AAV virion comprising the corresponding parental AAV capsid protein, when administered via intravitreal injection.

Gene Products

[0139] A subject rAAV virion comprises a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product. In some embodiments, the gene product is an interfering RNA. In some embodiments, the gene product is an aptamer. In some embodiments, the gene product is a polypeptide. In some embodiments, the gene product is a site-specific nuclease that provide for site-specific knockdown of gene function.

Interfering RNA

[0140] Where the gene product is an interfering RNA (RNAi), suitable RNAi include RNAi that decrease the level of an apoptotic or angiogenic factor in a cell. For example, an RNAi can be an shRNA or siRNA that reduces the level of a gene product that induces or promotes apoptosis in a cell. Genes whose gene products induce or promote apoptosis are referred to herein as "pro-apoptotic genes" and the products of those genes (mRNA; protein) are referred to as "pro-apoptotic gene products." Pro-apoptotic gene products include, e.g., Bax, Bid, Bak, and Bad gene products. See, e.g., U.S. Pat. No. 7,846,730.

[0141] Interfering RNAs could also be against an angiogenic product, for example VEGF (e.g., Cand5; see, e.g., U.S. Patent Publication No. 2011/0143400; U.S. Patent Publication No. 2008/0188437; and Reich et al. (2003) *Mol. Vis.* 9:210), VEGFR1 (e.g., Sirna-027; see, e.g., Kaiser et al. (2010) *Am. J. Ophthalmol.* 150:33; and Shen et al. (2006) *Gene Ther.* 13:225), or VEGFR2 (Kou et al. (2005) *Biochem.* 44:15064). See also, U.S. Pat. Nos. 6,649,596, 6,399,586, 5,661,135, 5,639,872, and 5,639,736; and U.S. Pat. Nos. 7,947,659 and 7,919,473.

Aptamers

[0142] Where the gene product is an aptamer, exemplary aptamers of interest include an aptamer against vascular endothelial growth factor (VEGF). See, e.g., Ng et al. (2006) *Nat. Rev. Drug Discovery* 5:123; and Lee et al. (2005) *Proc. Natl. Acad. Sci. USA* 102:18902. For example, a VEGF aptamer can comprise the nucleotide sequence 5'-cgcaaucagugaaugcuuauacauccg-3' (SEQ ID NO:17). Also suitable for use is a PDGF-specific aptamer, e.g., E10030; see, e.g., Ni and Hui (2009) *Ophthalmologica* 223:401; and Akiyama et al. (2006) *J. Cell Physiol.* 207:407).

Polypeptides

[0143] Where the gene product is a polypeptide, the polypeptide is generally a polypeptide that enhances function of a retinal cell, e.g., the function of a rod or cone photoreceptor cell, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelial cell. Exemplary polypeptides include neuroprotective polypeptides (e.g., GDNF, CNTF, NT4, NGF, and NTN); anti-angiogenic polypeptides (e.g., a soluble vascular endothelial growth factor (VEGF) receptor; a VEGF-binding antibody; a VEGF-binding antibody fragment (e.g., a single chain anti-VEGF antibody); endostatin; tumstatin; angiostatin; a soluble Flt polypeptide (Lai et al. (2005) *Mol. Ther.* 12:659); an Fc fusion protein comprising a soluble Flt polypeptide (see, e.g., Pechan et al. (2009) *Gene Ther.* 16:10); pigment epithelium-derived factor (PEDF); a soluble Tie-2 receptor; etc.); tissue inhibitor of metalloproteinases-3 (TIMP-3); a light-responsive opsin, e.g., a rhodopsin; anti-apoptotic polypeptides (e.g., Bcl-2, Bcl-X1); and the like. Suitable polypeptides include, but are not limited to, glial derived neurotrophic factor (GDNF); fibroblast growth factor 2; neurturin (NTN); ciliary neurotrophic factor (CNTF); nerve growth factor (NGF); neurotrophin-4 (NT4); brain derived neurotrophic factor (BDNF; e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in FIG. **11** (SEQ ID NO:11)); epidermal growth factor; rhodopsin; X-linked inhibitor of apoptosis; and Sonic hedgehog.

[0144] Suitable light-responsive opsins include, e.g., a light-responsive opsin as described in U.S. Patent Publication No. 2007/0261127 (e.g., ChR2; Chop2); U.S. Patent Publication No. 2001/0086421; U.S. Patent Publication No. 2010/0015095; and Diester et al. (2011) *Nat. Neurosci.* 14:387.

[0145] Suitable polypeptides also include retinoschisin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 224 amino acids of the amino acid sequence depicted in FIG. **10** (SEQ ID NO:10). Suitable polypeptides include, e.g., retinitis pigmentosa GTPase regulator (RGPR)-interacting protein-1 (see, e.g., GenBank Accession Nos. Q96KN7, Q9EPQ2, and

Q9GLM3) (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 1150 amino acids to about 1200 amino acids, or from about 1200 amino acids to 1286 amino acids, of the amino acid sequence depicted in FIG. **16** (SEQ ID NO:21); peripherin-2 (Prph2) (see, e.g., GenBank Accession No. NP_000313 (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to 346 amino acids of the amino acid sequence depicted in FIG. **14** (SEQ ID NO:19); and Travis et al. (1991) *Genomics* 10:733); peripherin (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids to about 470 amino acids of the amino acid sequence depicted in FIG. **15** (SEQ ID NO:20); a retinal pigment epithelium-specific protein (RPE65), (e.g., a polypeptide comprising an amino acid sequence having at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids to 247 amino acids of the amino acid sequence depicted in FIG. **12** (SEQ ID NO:12)) (see, e.g., GenBank AAC39660; and Morimura et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:3088); and the like.

[0146] Suitable polypeptides also include: CHM (choroideremia (Rab escort protein 1)), a polypeptide that, when defective or missing, causes choroideremia (see, e.g., Donnelly et al. (1994) *Hum. Mol. Genet.* 3:1017; and van Bokhoven et al. (1994) *Hum. Mol. Genet.* 3:1041); and Crumbs homolog 1 (CRB1), a polypeptide that, when defective or missing, causes Leber congenital amaurosis and retinitis pigmentosa (see, e.g., den Hollander et al. (1999) *Nat. Genet.* 23:217; and GenBank Accession No. CAM23328).

[0147] Suitable polypeptides also include polypeptides that, when defective or missing, lead to achromotopsia, where such polypeptides include, e.g., cone photoreceptor cGMP-gated channel subunit alpha (CNGA3) (see, e.g., GenBank Accession No. NP_001289; and Booij et al. (2011) *Ophthalmology* 118:160-167); cone photoreceptor cGMP-gated cation channel beta-subunit (CNGB3) (see, e.g., Kohl et al. (2005) *Eur J Hum Genet.* 13(3):302); guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2 (GNAT2) (ACHM4); and ACHM5; and polypeptides that, when defective or lacking, lead to various forms of color blindness (e.g., L-opsin, M-opsin, and S-opsin). See Mancuso et al. (2009) *Nature* 461(7265):784-787.

Site-Specific Endonucleases

[0148] In some cases, a gene product of interest is a site-specific endonuclease that provide for site-specific knockdown of gene function, e.g., where the endonuclease knocks out an allele associated with a retinal disease. For example, where a dominant allele encodes a defective copy of a gene that, when wild-type, is a retinal structural protein and/or provides for normal retinal function, a site-specific endonuclease can be targeted to the defective allele and knock out the defective allele.

[0149] In addition to knocking out a defective allele, a site-specific nuclease can also be used to stimulate homologous recombination with a donor DNA that encodes a functional copy of the protein encoded by the defective allele. Thus, e.g., a subject rAAV virion can be used to deliver both a site-specific endonuclease that knocks out a defective allele, and can be used to deliver a functional copy of the defective allele, resulting in repair of the defective allele, thereby providing for production of a functional retinal protein (e.g., functional retinoschisin, functional RPE65, functional peripherin, etc.). See, e.g., Li et al. (2011) *Nature* 475:217. In some embodiments, a subject rAAV virion comprises a heterologous nucleotide sequence that encodes a site-specific endonuclease; and a heterologous nucleotide sequence that encodes a functional copy of a defective allele, where the functional copy encodes a functional retinal protein. Functional retinal proteins include, e.g., retinoschisin, RPE65, retinitis pigmentosa GTPase regulator (RGPR)-interacting protein-1, peripherin, peripherin-2, and the like.

[0150] Site-specific endonucleases that are suitable for use include, e.g., zinc finger nucleases (ZFNs); and transcription activator-like effector nucleases (TALENs), where such site-specific endonucleases are non-naturally occurring and are modified to target a specific gene. Such site-specific nucleases can be engineered to cut specific locations within a genome, and non-homologous end joining can then repair the break while inserting or deleting several nucleotides. Such site-specific endonucleases (also referred to as "INDELs") then throw the protein out of frame and effectively knock out the gene. See, e.g., U.S. Patent Publication No. 2011/0301073.

Regulatory Sequences

[0151] In some embodiments, a nucleotide sequence encoding a gene product of interest is operably linked to a constitutive promoter. In other embodiments, a nucleotide sequence encoding a gene product of interest is operably linked to an inducible promoter. In some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a tissue-specific or cell type-specific regulatory element. For example, in some instances, a nucleotide sequence encoding a gene product of interest is operably linked to a photoreceptor-specific regulatory element (e.g., a photoreceptor-specific promoter), e.g., a regulatory element that confers selective expression of the operably linked gene in a photoreceptor cell. Suitable photoreceptor-specific regulatory elements include, e.g., a rhodopsin promoter; a rhodopsin kinase promoter (Young et al. (2003) *Ophthalmol. Vis. Sci.* 44:4076); a beta phosphodiesterase gene promoter (Nicoud et al. (2007) *J. Gene Med.* 9:1015); a retinitis pigmentosa gene promoter (Nicoud et al. (2007) supra); an interphotoreceptor retinoid-binding protein (IRBP) gene enhancer (Nicoud et al. (2007) supra); an IRBP gene promoter (Yokoyama et al. (1992) *Exp Eye Res.* 55:225).

Pharmaceutical Compositions

[0152] The present disclosure provides a pharmaceutical composition comprising: a) a subject rAAV virion, as described above; and b) a pharmaceutically acceptable carrier, diluent, excipient, or buffer. In some embodiments, the pharmaceutically acceptable carrier, diluent, excipient, or buffer is suitable for use in a human.

[0153] Such excipients, carriers, diluents, and buffers include any pharmaceutical agent that can be administered without undue toxicity. Pharmaceutically acceptable excipi-

ents include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

Methods of Delivering a Gene Product to a Retinal Cell and Treatment Methods

[0154] The present disclosure provides a method of delivering a gene product to a retinal cell in an individual, the method comprising administering to the individual a subject rAAV virion as described above. The gene product can be a polypeptide or an interfering RNA (e.g., an shRNA, an siRNA, and the like), an aptamer, or a site-specific endonuclease, as described above. Delivering a gene product to a retinal cell can provide for treatment of a retinal disease. The retinal cell can be a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelial cell. In some cases, the retinal cell is a photoreceptor cell, e.g., a rod or cone cell.

[0155] The present disclosure provides a method of treating a retinal disease, the method comprising administering to an individual in need thereof an effective amount of a subject rAAV virion as described above. A subject rAAV virion can be administered via intraocular injection, by intravitreal injection, or by any other convenient mode or route of administration. Other convenient modes or routes of administration include, e.g., intravenous, intranasal, etc.

[0156] A "therapeutically effective amount" will fall in a relatively broad range that can be determined through experimentation and/or clinical trials. For example, for in vivo injection, i.e., injection directly into the eye, a therapeutically effective dose will be on the order of from about 10^6 to about 10^{15} of the rAAV virions, e.g., from about 10^8 to 10^{12} rAAV virions. For in vitro transduction, an effective amount of rAAV virions to be delivered to cells will be on the order of from about 10^8 to about 10^{13} of the rAAV virions. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

[0157] In some embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., daily, weekly, monthly, yearly, etc.

[0158] Ocular diseases that can be treated using a subject method include, but are not limited to, acute macular neuroretinopathy; Behcet's disease; choroidal neovascularization; diabetic uveitis; histoplasmosis; macular degeneration, such as acute macular degeneration, non-exudative age related macular degeneration and exudative age related macular degeneration; edema, such as macular edema, cystoid macular edema and diabetic macular edema; multifocal choroiditis; ocular trauma which affects a posterior ocular site or location; ocular tumors; retinal disorders, such as central retinal vein occlusion, diabetic retinopathy (including proliferative diabetic retinopathy), proliferative vitreoretinopathy (PVR), retinal arterial occlusive disease, retinal detachment, uveitic retinal disease; sympathetic opthalmia; Vogt Koyanagi-Harada (VKH) syndrome; uveal diffusion; a posterior ocular condition caused by or influenced by an ocular laser treatment; posterior ocular conditions caused by or influenced by a photodynamic therapy; photocoagulation, radiation retinopathy; epiretinal membrane disorders; branch retinal vein occlusion; anterior ischemic optic neuropathy; non-retinopathy diabetic retinal dysfunction; retinoschisis; retinitis pigmentosa; glaucoma; Usher syndrome, cone-rod dystrophy; Stargardt disease (fundus flavimaculatus); inherited macular degeneration; chorioretinal degeneration; Leber congenital amaurosis; congenital stationary night blindness; choroideremia; Bardet-Biedl syndrome; macular telangiectasia; Leber's hereditary optic neuropathy; retinopathy of prematurity; and disorders of color vision, including achromatopsia, protanopia, deuteranopia, and tritanopia.

Nucleic Acids and Host Cells

[0159] The present disclosure provides an isolated nucleic acid comprising a nucleotide sequence that encodes a subject variant adeno-associated virus (AAV) capsid protein as described above, where the variant AAV capsid protein comprises an insertion of from about 5 amino acids to about 11 amino acids in the GH loop or loop IV relative to a corresponding parental AAV capsid protein, and where the variant capsid protein, when present in an AAV virion, provides for increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein. A subject isolated nucleic acid can be an AAV vector, e.g., a recombinant AAV vector.

Insertion Peptides

[0160] A variant AAV capsid protein encoded by a subject nucleic acid has an insertion peptide of from about 5 amino acids to about 11 amino acids in length is inserted into the GH loop of an AAV capsid. The insertion peptide has a length of 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, 10 amino acids, or 11 amino acids.

[0161] The insertion peptide can comprise an amino acid sequence of any one of the formulas set forth below.

[0162] For example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula I:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0163] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is selected from Leu, Asn, and Lys;

X_2 is selected from Gly, Glu, Ala, and Asp;

X_3 is selected from Glu, Thr, Gly, and Pro;

X_4 is selected from Thr, Ile, Gln, and Lys;

X_5 is selected from Thr and Ala;

X_6 is selected from Arg, Asn, and Thr;

X_7, if present, is selected from Pro and Asn.

[0164] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIa:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0165] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

each of X_1-X_4 is any amino acid;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0166] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IIb:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0167] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is selected from Leu and Asn;

X_2, if present, is selected from Gly and Glu;

X_3 is selected from Glu and Thr;

X_4 is selected from Thr and Ile;

X_5 is Thr;

X_6 is Arg; and

X_7 is Pro.

[0168] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula III:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0169] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is Lys;

X_2 is selected from Ala and Asp;

X_3 is selected from Gly and Pro;

X_4 is selected from Gln and Lys;

X_5 is selected from Thr and Ala;

X_6 is selected from Asn and Thr;

X_7, if present, is Asn.

[0170] As another example, an insertion peptide can be a peptide of from 5 to 11 amino acids in length, where the insertion peptide is of Formula IV:

$$Y_1Y_2X_1X_2X_3X_4X_5X_6X_7Y_3Y_4$$

[0171] where:

each of Y_1-Y_4, if present, is independently selected from Ala, Leu, Gly, Ser, and Thr;

X_1, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Leu, Asn, Arg, Ala, Ser, and Lys;

X_2 is a negatively charged amino acid or an uncharged amino acid; or is selected from Gly, Glu, Ala, Val, Thr, and Asp;

X_3 is a negatively charged amino acid or an uncharged amino acid; or is selected from Glu, Thr, Gly, Asp, or Pro;

X_4 is selected from Thr, Ile, Gly, Lys, Asp, and Gln;

X_5 is a polar amino acid, an alcohol (an amino acid having a free hydroxyl group), or a hydrophobic amino acid; or is selected from Thr, Ser, Val, and Ala;

X_6 is a positively charged amino acid or an uncharged amino acid; or is selected from Arg, Val, Lys, Pro, Thr, and Asn; and

X_7, if present, is a positively charged amino acid or an uncharged amino acid; or is selected from Pro, Gly, Phe, Asn, and Arg.

[0172] As non-limiting examples, the insertion peptide can comprise an amino acid sequence selected from LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60).

[0173] In some cases, the insertion peptide has from 1 to 4 spacer amino acids (Y_1-Y_4) at the amino terminus and/or at the carboxyl terminus of any one of LGETTRP (SEQ ID NO:13), NETITRP (SEQ ID NO:14), KAGQANN (SEQ ID NO:15), KDPKTTN (SEQ ID NO:16), KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60). Suitable spacer amino acids include, but are not limited to, leucine, alanine, glycine, and serine.

[0174] For example, in some cases, an insertion peptide has one of the following amino acid sequences: LALGETTRPA (SEQ ID NO:45); LANETITRPA (SEQ ID NO:46), LAKAGQANNA (SEQ ID NO:47), LAKDPKTTNA (SEQ ID NO:48), LAKDTDTTRA (SEQ ID NO:61), LARAGGSVGA (SEQ ID NO:62), LAAVDTTKFA (SEQ ID NO:63), and LASTGKVPNA (SEQ ID NO:64). As another example, in some cases, an insertion peptide has one of the following amino acid sequences: AALGETTRPA (SEQ ID NO:49); AANETITRPA (SEQ ID NO:50), AAKAGQANNA (SEQ ID NO:51), and AAKDPKTTNA (SEQ ID NO:52). As yet another example, in some cases, an insertion peptide has one of the following amino acid sequences: GLGETTRPA (SEQ ID NO:53); GNETITRPA (SEQ ID NO:54), GKAGQANNA (SEQ ID NO:55), and GKDPKTTNA (SEQ ID NO:56). As another example, in some cases, an insertion peptide comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60), flanked on the C-terminus by AA and on the N-terminus by A; or comprises one of KDTDTTR (SEQ ID NO:57), RAGGSVG (SEQ ID NO:58), AVDTTKF (SEQ ID NO:59), and STGKVPN (SEQ ID NO:60) flanked on the C-terminus by G and on the N-terminus by A.

[0175] A subject recombinant AAV vector can be used to generate a subject recombinant AAV virion, as described above. Thus, the present disclosure provides a recombinant AAV vector that, when introduced into a suitable cell, can provide for production of a subject recombinant AAV virion.

[0176] The present invention further provides host cells, e.g., isolated (genetically modified) host cells, comprising a subject nucleic acid. A subject host cell can be an isolated cell, e.g., a cell in in vitro culture. A subject host cell is useful for

producing a subject rAAV virion, as described below. Where a subject host cell is used to produce a subject rAAV virion, it is referred to as a "packaging cell." In some embodiments, a subject host cell is stably genetically modified with a subject nucleic acid. In other embodiments, a subject host cell is transiently genetically modified with a subject nucleic acid.

[0177] A subject nucleic acid is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, calcium phosphate precipitation, liposome-mediated transfection, and the like. For stable transformation, a subject nucleic acid will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, and the like.

[0178] A subject host cell is generated by introducing a subject nucleic acid into any of a variety of cells, e.g., mammalian cells, including, e.g., murine cells, and primate cells (e.g., human cells). Suitable mammalian cells include, but are not limited to, primary cells and cell lines, where suitable cell lines include, but are not limited to, 293 cells, COS cells, HeLa cells, Vero cells, 3T3 mouse fibroblasts, C3H10T1/2 fibroblasts, CHO cells, and the like. Non-limiting examples of suitable host cells include, e.g., HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like. A subject host cell can also be made using a baculovirus to infect insect cells such as Sf9 cells, which produce AAV (see, e.g., U.S. Pat. No. 7,271,002; U.S. patent application Ser. No. 12/297,958)

[0179] In some embodiments, a subject genetically modified host cell includes, in addition to a nucleic acid comprising a nucleotide sequence encoding a variant AAV capsid protein, as described above, a nucleic acid that comprises a nucleotide sequence encoding one or more AAV rep proteins. In other embodiments, a subject host cell further comprises an rAAV vector. An rAAV virion can be generated using a subject host cell. Methods of generating an rAAV virion are described in, e.g., U.S. Patent Publication No. 2005/0053922 and U.S. Patent Publication No. 2009/0202490.

EXAMPLES

[0180] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1

AAV Variant with Enhanced Transduction of Retinal Cells

[0181] The approach used was to create a peptide display library by introducing a unique AvrII site into the wild type AAV2 genome between amino acid 587 and 588 by polymerase chain reaction (PCR) mutagenesis. A random 21 nucleotide insert, 7mer For, was used to synthesize dsDNA inserts, along with the antisense primer 7mer Rev. The resulting dsDNA inserts were cloned into the AvrII site of the genome after digestion with NheI, producing a diverse 7mer display library which was then packaged (Perabo et al., 2003; Muller et al., 2003). The virus was generated such that each viral genome was packaged or encapsidated within the capsid protein variant that that genome encoded. In this respect, functional improvements identified through selection can be linked to the genome sequence encoding this improved function contained within the viral capsid.

[0182] This library was subjected to positive selection within rho-GFP mice (Wensel et al. (2005) *Vision Res.* 45:3445). Briefly, in one round of selection, adult rho-GFP mice were intravitreally injected with 2 μL of phosphate buffered saline (PBS)-dialyzed, iodixanol-purified library with a genomic titer of approximately 1×10^{12} viral genomes (vg)/mL. An ultrafine 30½-gauge disposable needle was passed through the sclera of the animal's eye, at the equator and next to the limbus, into the vitreous cavity. Injection of 2 μl of virus was made with direct observation of the needle in the center of the vitreous cavity. One week post-injection, eyes were enucleated and retinas dissociated using a light, papain protease treatment, followed by fluorescence activated cell sorter (FACS) isolation of photoreceptor populations. Successful virions were then PCR amplified from subsequent genomic extractions and further cloned and repackaged for injection.

[0183] Further iterations of this selection were performed, narrowing the pool of variants to a subset with the most permissive mutations. After three iterations, a round of error-prone PCR was performed to create a further generation of variants for selection. In total, this process was repeated for two generations. In this respect, this process of directed evolution created photoreceptor-permissive AAV variants through the application of positive selection and induced mutagenesis, similar to the process of natural evolution.

[0184] Subsequently, the cap genes of fifty variants were sequenced to determine the most prominent and successful variants to have permissive mutations for intravitreal photoreceptor transduction. Of the 50 clones, 46 gave readable sequences of a 7mer insert. Remarkably, nearly two thirds of clones contained the same distinct 7mer motif (~^{588}LGETTRP~; SEQ ID NO:13). Interestingly, the next most prominent variant (~^{588}NETITRP~; SEQ ID NO:14) also contained a similar flanking motif consisting of a positively-charged arginine residue in between a polar threonine and a nonpolar proline residue (TRP).

TABLE 1

Clone	Appr. Frequency (%)	Frequency
~^{588}LGETTRP~ (SEQ ID NO: 13)	64	31
~^{588}NETITRP~ (SEQ ID NO: 14)	12	5
~^{588}KAGQANN~ (SEQ ID NO: 15)	6	3
~^{588}KDPKTTN~ (SEQ ID NO: 16)	4	2
~^{588}KDTDTTR~ (SEQ ID NO: 57)		2
~^{588}RAGGSVG~ (SEQ ID NO: 58)		1
~^{588}AVDTTKF~ (SEQ ID NO: 59)		1
~^{588}STGKVPN~ (SEQ ID NO: 60)		1

[0185] Table 1 Sequencing of isolated variants from directed evolution reveals a high degree of convergence in viral libraries. All variants derived from the AAV2 7mer library, with approximately 64% of variants containing the same 7mer motif (~^{588}LGETTRP~(SEQ ID NO:13)).

[0186] Among the 7mer insert sequences, there were moderate preferences at particular positions, e.g., a positively charged amino acid at position 1; a negatively charged amino acid at position 2; an alcohol (e.g., an amino acid having an alcohol group (a free hydroxyl group), such as Thr or Ser) at position 5.

[0187] The 7mer inserts were flanked by spacers, as shown in Table 2:

Clone	Frequency
~^{588}LALGETTRPA~ (SEQ ID NO: 45)	31
~^{588}LANETITRPA~ (SEQ ID NO: 46)	5
~^{588}LAKAGQANNA~ (SEQ ID NO: 47)	3
~^{588}LAKDPKTTNA~ (SEQ ID NO: 48)	2
~^{588}LAKDTDTTRA~ (SEQ ID NO: 61)	2
~^{588}LARAGGSVGA~ (SEQ ID NO: 62)	1
~^{588}LAAVDTTKFA~ (SEQ ID NO: 63)	1
~^{588}LASTGKVPNA~ (SEQ ID NO: 64)	1

[0188] FIG. **1**. Representative three-dimensional capsid model of AAV2 containing a random heptamer (shown in orange) following amino acid 587. This area of the AAV2 capsid likely participates in cell-surface receptor binding.

[0189] In light of the high degree of library convergence from the above-described selection, a recombinant form of AAV2~^{588}LGETTRP~(SEQ ID NO:13; nick named 7M8) was cloned and packaged the vector with a scCAG-GFP transgene to visualize its transduction profile. Three weeks following intravitreal injection in adult mice, robust expression in numerous cell types, including retinal ganglion cells (RGCs) and Müller cells, was observed. Importantly, transduction of photoreceptors in retinas injected with 7M8, as seen by GFP expression in outer nuclear layer (ONL) nuclei (red arrows) and in outer segments (FIG. **2**, blue arrow), was observed, whereas AAV2 showed no discernable photoreceptor expression.

[0190] FIG. **2** AAV2 7M8 variant (right) demonstrates greater levels of intravitreal photoreceptor transduction relative to AAV2 (left). Confocal microscopy of transverse retinal sections three weeks after intravitreal injection of 2 μL of 1×10^{12} vg/mL of AAV2 7M8 and AAV2 scCAG GFP in adult mice. Red arrows (top) denote photoreceptor nuclei and blue arrow (top) denote photoreceptor outer segments.

[0191] In light of these gains in retinal cell transduction, an attempt was made to increase specificity in expression through the use of a ssRho-eGFP transgene containing a photoreceptor-specific rhodopsin promoter to better resolve transduction efficiencies specifically in photoreceptors (FIG. **3**). Indeed the use of a photoreceptor specific Rho promoter limited the GFP expression to the photoreceptors. An attempt was made to improve 7M8 transduction efficiency by combining a rational design approach to the previous directed evolution approach. Therefore, four surface exposed tyrosine residues were mutagenized to phenylalanines on the 7M8 capsid (Y273F, Y444F, Y500F, and Y730F) which has previous been shown to increase photoreceptor infectivity (Petrs-Silva et al., 2009). Interestingly, the addition of mutations decreased number of photoreceptors transduced compared to the original virus as show by FACs sorting of the GFP(+) photoreceptors from 7m8 or 7m8-4YF infected retinas (FIG. **4**).

[0192] FIG. **3**. Representative fluorescence images of retinal cryoslices showing GFP expression resulting from 7m8 carrying the GFP gene under the control of the ubiquitous CAG promoter (left) or a photoreceptor specific Rho promoter (right).

[0193] FIG. **4**. GFP(+) photoreceptor cells per million retinal cells as counted by flow cytometry. 7m8 transduces more than 2× the amount of photoreceptors compared 7m8 bearing 4 tyrosine mutations (top).

Example 2

Treatment of Retinoschisis

[0194] Using the expression construct 7m8-rho-RS1, a functional retinoschisin (RS1) protein was delivered to retinoschisin-deficient mice (Rs1h-deficient mice; Rs1h is the mouse homolog of human RS1). The vector comprises a nucleotide sequence encoding a functional retinoschisin protein under transcriptional control of a rhodopsin promoter. See FIGS. **13**A-C, where the bold and underlined nucleotide sequence (nucleotides 4013-4851) are the rhodopsin promoter; and nucleotides 4866-5540 (with the start atg and stop tga sequences shown in bold) encode a human RS1 protein.

[0195] The 7m8-rho-RS1 construct was administered intravitreally to Rs1h−/− mice at P15. Rs1h−/− mice were generated through targeted disruption of exon 3 of the Rs1h gene, as described (Weber et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:6222). The Rs1h−/− mice are deficient in the Rs1h protein product, have an electronegative ERG (e.g., a reduced b-wave with relative preservation of the a-wave) and splitting of the layers of the retina, similar to what is seen in human retinoschisis patients. Injection of the 7m8-rho-RS1 vector into the Rs1h−/− led to high levels of panretinal RS1 expression from photoreceptors in the retina. RS1 expression led to improved retinal morphology with a decrease in the number and size of cavities in the retina as seen in spectral-domain optical coherence tomography (SD-OCT) imaging (FIGS. **7**A-I), a rescue of the ERG b-wave (FIGS. **8**A-D), and long-term structural preservation of the retina (FIGS. **9**A-E).

[0196] FIGS. **7**A-I. Representative high-resolution SD-OCT images of retinas injected with 7m8-rho-GFP (left column), 7m8-rho-RS1 (middle column), or uninjected WT animals (right column). Fundus images were taken through the inner nuclear layer of the superior retina and exclude other layers (a-c). Transverse images of the superior (d-f) and inferior (g-i) retina were taken using the optic nerve head as a landmark.

[0197] The untreated RS1 retina increases in overall thickness when measured from the inner limiting membrane (ILM) to the photoreceptors, as the pathology progresses due to the schisis splitting the inner retina. This process is distinct from that observed in most retinal degenerative diseases (RDD) which do not form schisis, but exhibit progressive photoreceptor cell death in the INL and concomitant retinal thinning and loss of ERG amplitude. In RS1, the ONL thins as photoreceptors die from the disease, but this is distinct from the overall retinal thickness change. It is generally thought that a successful therapy for RS1 would return the overall retinal thickness to the wildtype and ameliorate the photoreceptor loss in the ONL. In most RDD other than Rs1, the loss of photoreceptors, marked by ONL thinning, is paralleled by a decrease in retinal physiological output as measured by the ERG amplitude. RS1 is one of the very few examples of a retinal disease in which the pathology increases the retinal thickness with concomitant erg amplitude loss. In summary, restoring the RS1 gene product, an extracellular retinal "glue; —thins the retina back to the wildtype thickness and the erg amplitude returns to near normal levels as the schisis resolves.

[0198] FIG. **8***a* shows a comparison of functional rescue of untreated Rs1−/− eyes to AAV2-rho-RS1, 7m8-rho-GFP, and 7m8-rho-RS1 injected eyes both one month (left) and 4 months (right) after injection. One month post-injection, 7m8-rho-RS1 led to considerable rescue of the ERG b-wave amplitude, whereas AAV2-rho.RS1 was statistically indistinguishable from untreated eyes.

[0199] After 4 months, the 7m8-rho-RS1 amplitude further increases toward the wild-type amplitude (right). FIG. **8***b* shows representative ERG traces from 7m8-rho-RS1-injected eyes show improved amplitude of the a-wave and b-wave and a waveform closer to wild-type eyes, compared to 7m8-rho-GFP-injected eyes. FIG. **8***c* shows the amplitude of the full-field scotopic b-wave resulting from a high intensity (1 log cd×s/m2) stimulus was recorded on a monthly basis beginning one month after injection at P15 for each condition. Three responses were recorded and averaged for each eye at each time point.

[0200] Mean ERG b-wave amplitudes were plotted as a function of time post-injection. n=7 was used for both conditions. FIG. **8***d* shows an analysis of ERG responses under scotopic (upper traces, stimulus range from −3 to 1 log cd×s/m2) and photopic (lower traces, range from −0.9 to 1.4 log cd×s/m2) conditions indicates improved rod and cone function over a range of stimuli intensities.

[0201] FIGS. **9**A-E. Sustained improvements in retinal thickness measured at 10 months post 7m8-rho-RS1 treatment. Representative transverse SD-OCT images of a) 7m8-rho-RS1 or b) or 7m8-rho-GFP treated retinas 10 months post-injection centered on the optic nerve head. Measurements of c) retinal thickness, d) ONL thickness, and e) and inner and outer segment thickness are plotted as a function of distance from the optic nerve head.

Example 3

AAV Variant Used to Deliver a Protein to Retinal Cells in the Macaque

[0202] A recombinant AAV2 virion (7m8 carrying GFP under the control of a connexin36 promoter) was generated. The recombinant AAV2 virion included an AAV2 capsid variant with an insertion of LALGETTRPA peptide between amino acids 587 and 588 of AAV2 capsid, and GFP under transcriptional control of a connexin36 promoter, which is expressed in interneurons. The rAAV2 virion was injected intravitreally into the eye of a macaque. The data are shown in FIG. **18**.

[0203] FIG. **18** provides a fluorescence fundus image showing GFP expression at the back of the retina 9 weeks after administration of 7m8 carrying GFP under the control of a connexin36 promoter. Compared to the parental AAV2 serotype (Yin et al, IOVS 52(5); 2775), a higher level of expression was seen in the foveal ring, and visible fluorescence was seen in the central retina outside the fovea.

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[0220] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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            20                  25                  30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145                 150                 155                 160

Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
            180                 185                 190
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Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly
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Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
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Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
            260                 265                 270

Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
        275                 280                 285

Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
    290                 295                 300

Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305                 310                 315                 320

Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
                325                 330                 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
            340                 345                 350

Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
        355                 360                 365

Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
    370                 375                 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385                 390                 395                 400

Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
                405                 410                 415

Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
            420                 425                 430

Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr
        435                 440                 445

Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser Arg Leu Gln Phe Ser Gln
    450                 455                 460

Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly
465                 470                 475                 480

Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp Asn Asn
                485                 490                 495

Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly
            500                 505                 510

Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
        515                 520                 525

Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys
    530                 535                 540

Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
545                 550                 555                 560

Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr
                565                 570                 575

Gly Ser Val Ser Thr Asn Leu Gln Arg Gly Asn Arg Gln Ala Ala Thr
            580                 585                 590

Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
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        595                 600                 605

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
    610                 615                 620

Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
625                 630                 635                 640

His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn
                645                 650                 655

Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln
            660                 665                 670

Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys
        675                 680                 685

Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr
    690                 695                 700

Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr
705                 710                 715                 720

Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
                725                 730


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Gly Asn Arg Gln Ala Ala Thr Ala Asp Val Asn Thr Gln Gly Val Leu
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        35                  40


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Pro Gly Ser Val Trp Met Glu Arg Asp Val
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Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ser Ser Asn Leu Gln Ala
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Trp Tyr Gln Lys Ala Cys Lys Cys Asp Cys Gln Gly Gly Pro Asn Ala
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Leu Trp Ser Ala Gly Ala Thr Ser Leu Asp Cys Ile Pro Glu Cys Pro
    50                  55                  60

Tyr His Lys Pro Leu Gly Phe Glu Ser Gly Glu Val Thr Pro Asp Gln
65                  70                  75                  80

Ile Thr Cys Ser Asn Pro Glu Gln Tyr Val Gly Trp Tyr Ser Ser Trp
                85                  90                  95

Thr Ala Asn Lys Ala Arg Leu Asn Ser Gln Gly Phe Gly Cys Ala Trp
            100                 105                 110

Leu Ser Lys Phe Gln Asp Ser Ser Gln Trp Leu Gln Ile Asp Leu Lys
        115                 120                 125

Glu Ile Lys Val Ile Ser Gly Ile Leu Thr Gln Gly Arg Cys Asp Ile
    130                 135                 140

Asp Glu Trp Met Thr Lys Tyr Ser Val Gln Tyr Arg Thr Asp Glu Arg
145                 150                 155                 160

Leu Asn Trp Ile Tyr Tyr Lys Asp Gln Thr Gly Asn Asn Arg Val Phe
                165                 170                 175

Tyr Gly Asn Ser Asp Arg Thr Ser Thr Val Gln Asn Leu Leu Arg Pro
            180                 185                 190

Pro Ile Ile Ser Arg Phe Ile Arg Leu Ile Pro Leu Gly Trp His Val
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Ala Tyr Pro Gly Val Arg Thr His Gly Thr Leu Glu Ser Val Asn Gly
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His Val Ile Glu Glu Leu Leu Asp Glu Asp His Lys Val Arg Pro Asn
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Glu Glu Asn Asn Lys Asp Ala Asp Leu Tyr Thr Ser Arg Val Met Leu
                85                  90                  95

Ser Ser Gln Val Pro Leu Glu Pro Pro Leu Leu Phe Leu Leu Glu Glu
            100                 105                 110

Tyr Lys Asn Tyr Leu Asp Ala Ala Asn Met Ser Met Met Val Leu Arg
        115                 120                 125

His Ser Asp Pro Ala Arg Arg Gly Glu Leu Ser Val Cys Asp Ser Ile
    130                 135                 140

Ser Glu Trp Val Thr Ala Ala Asp Lys Lys Thr Ala Val Asp Met Ser
145                 150                 155                 160

Gly Gly Thr Val Thr Val Leu Glu Lys Val Pro Val Ser Lys Gly Gln
                165                 170                 175

Leu Lys Gln Tyr Phe Tyr Glu Thr Lys Cys Asn Pro Met Gly Tyr Thr
            180                 185                 190

Lys Glu Gly Cys Arg Gly Ile Asp Lys Arg His Trp Asn Ser Gln Cys
        195                 200                 205

Arg Thr Thr Gln Ser Tyr Val Arg Ala Leu Thr Met Asp Ser Lys Lys
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Arg Ile Gly Trp Arg Phe Ile Arg Ile Asp Thr Ser Cys Val Cys Thr
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Leu Thr Ile Lys Arg Gly Arg
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            20                  25                  30

Arg Ile Pro Leu Trp Leu Thr Gly Ser Leu Leu Arg Cys Gly Pro Gly
        35                  40                  45

Leu Phe Glu Val Gly Ser Glu Pro Phe Tyr His Leu Phe Asp Gly Gln
    50                  55                  60

Ala Leu Leu His Lys Phe Asp Phe Lys Glu Gly His Val Thr Tyr His
65                  70                  75                  80

Arg Arg Phe Ile Arg Thr Asp Ala Tyr Val Arg Ala Met Thr Glu Lys
                85                  90                  95

Arg Ile Val Ile Thr Glu Phe Gly Thr Cys Ala Phe Pro Asp Pro Cys
            100                 105                 110

Lys Asn Ile Phe Ser Arg Phe Phe Ser Tyr Phe Arg Gly Val Glu Val
        115                 120                 125

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Ala Thr Ala His Pro His Ile Glu Asn Asp Gly Thr Val Tyr Asn Ile
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Gly Asn Cys Phe Gly Lys Asn Phe Ser Ile Ala Tyr Asn Ile Val Lys
        195                 200                 205

Ile Pro Pro Leu Gln Ala Asp Lys Glu Asp Pro Ile Ser Lys Ser Glu
    210                 215                 220

Ile Val Val Gln Phe Pro Cys Ser Asp Arg Phe Lys Pro Ser Tyr Val
225                 230                 235                 240

His Ser Phe Gly Leu Thr Pro Asn Tyr Ile Val Phe Val Glu Thr Pro
                245                 250                 255

Val Lys Ile Asn Leu Phe Lys Phe Leu Ser Ser Trp Ser Leu Trp Gly
            260                 265                 270

Ala Asn Tyr Met Asp Cys Phe Glu Ser Asn Glu Thr Met Gly Val Trp
        275                 280                 285

Leu His Ile Ala Asp Lys Lys Arg Lys Lys Tyr Leu Asn Asn Lys Tyr
    290                 295                 300

Arg Thr Ser Pro Phe Asn Leu Phe His His Ile Asn Thr Tyr Glu Asp
305                 310                 315                 320

Asn Gly Phe Leu Ile Val Asp Leu Cys Cys Trp Lys Gly Phe Glu Phe
                325                 330                 335

Val Tyr Asn Tyr Leu Tyr Leu Ala Asn Leu Arg Glu Asn Trp Glu Glu
            340                 345                 350

Val Lys Lys Asn Ala Arg Lys Ala Pro Gln Pro Glu Val Arg Arg Tyr
        355                 360                 365

Val Leu Pro Leu Asn Ile Asp Lys Ala Asp Thr Gly Lys Asn Leu Val
    370                 375                 380

Thr Leu Pro Asn Thr Thr Ala Thr Ala Ile Leu Cys Ser Asp Glu Thr
385                 390                 395                 400

Ile Trp Leu Glu Pro Glu Val Leu Phe Ser Gly Pro Arg Gln Ala Phe
                405                 410                 415

Glu Phe Pro Gln Ile Asn Tyr Gln Lys Tyr Cys Gly Lys Pro Tyr Thr
            420                 425                 430

Tyr Ala Tyr Gly Leu Gly Leu Asn His Phe Val Pro Asp Arg Leu Cys
        435                 440                 445

Lys Leu Asn Val Lys Thr Lys Glu Thr Trp Val Trp Gln Glu Pro Asp
    450                 455                 460

Ser Tyr Pro Ser Glu Pro Ile Phe Val Ser His Pro Asp Ala Leu Glu
465                 470                 475                 480

Glu Asp Asp Gly Val Val Leu Ser Val Val Val Ser Pro Gly Ala Gly
                485                 490                 495

Gln Lys Pro Ala Tyr Leu Leu Ile Leu Asn Ala Lys Asp Leu Ser Glu
            500                 505                 510

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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 14

Asn Glu Thr Ile Thr Arg Pro
1 5

<210> SEQ ID NO 15
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 15

Lys Ala Gly Gln Ala Asn Asn
1 5

<210> SEQ ID NO 16
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 16

Lys Asp Pro Lys Thr Thr Asn
1 5

<210> SEQ ID NO 17
<211> LENGTH: 27
<212> TYPE: RNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 17

cgcaaucagu gaaugcuuau acauccg 27

<210> SEQ ID NO 18
<211> LENGTH: 5541
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 18

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actatgttgc tccttttacg ctatgtggat acgctgcttt aatgcctttg tatcatgcta 120

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ttgcttcccg tatggctttc attttctcct ccttgtataa atcctggttg ctgtctcttt 180

atgaggagtt gtggcccgtt gtcaggcaac gtggcgtggt gtgcactgtg tttgctgacg 240

caacccccac tggttggggc attgccacca cctgtcagct cctttccggg actttcgctt 300

tccccctccc tattgccacg gcggaactca tcgccgcctg ccttgcccgc tgctggacag 360

gggctcggct gttgggcact gacaattccg tggtgttgtc ggggaagctg acgtcctttc 420

catggctgct cgcctgtgtt gccacctgga ttctgcgcgg gacgtccttc tgctacgtcc 480

cttcggccct caatccagcg gaccttcctt cccgcggcct gctgccggct ctgcggcctc 540

ttccgcgtct tcgagatctg cctcgactgt gccttctagt tgccagccat ctgttgtttg 600

cccctccccc gtgccttcct tgaccctgga aggtgccact cccactgtcc tttcctaata 660

aaatgaggaa attgcatcgc attgtctgag taggtgtcat tctattctgg ggggtggggt 720

ggggcaggac agcaaggggg aggattggga agacaatagc aggcatgctg gggactcgag 780

ttaagggcga attcccgatt aggatcttcc tagagcatgg ctacgtagat aagtagcatg 840

gcgggttaat cattaactac aaggaacccc tagtgatgga gttggccact ccctctctgc 900

gcgctcgctc gctcactgag gccgggcgac caaaggtcgc ccgacgcccg ggctttgccc 960

gggcggcctc agtgagcgag cgagcgcgca gccttaatta acctaattca ctggccgtcg 1020

ttttacaacg tcgtgactgg gaaaaccctg gcgttaccca acttaatcgc cttgcagcac 1080

atccccctttt cgccagctgg cgtaatagcg aagaggcccg caccgatcgc ccttcccaac 1140

agttgcgcag cctgaatggc gaatgggacg cgccctgtag cggcgcatta agcgcggcgg 1200

gtgtggtggt tacgcgcagc gtgaccgcta cacttgccag cgccctagcg cccgctcctt 1260

tcgctttctt cccttccttt ctcgccacgt tcgccggctt tccccgtcaa gctctaaatc 1320

gggggctccc tttagggttc cgatttagtg ctttacggca cctcgacccc aaaaaacttg 1380

attagggtga tggttcacgt agtgggccat cgccccgata gacggttttt cgccctttga 1440

cgctggagtt cacgttcctc aatagtggac tcttgttcca aactggaaca acactcaacc 1500

ctatctcggt ctattctttt gatttataag ggatttttcc gatttcggcc tattggttaa 1560

aaaatgagct gatttaacaa aaatttaacg cgaattttaa caaaatatta acgtttataa 1620

tttcaggtgg catctttcgg ggaaatgtgc gcggaacccc tatttgttta tttttctaaa 1680

tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt caataatatt 1740

gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc ttttttgcgg 1800

cattttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa gatgctgaag 1860

atcagttggg tgcacgagtg ggttacatcg aactggatct caatagtggt aagatccttg 1920

agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt ctgctatgtg 1980

gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc atacactatt 2040

ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg gatggcatga 2100

cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg gccaacttac 2160

ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac atgggggatc 2220

atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca aacgacgagc 2280

gtgacaccac gatgcctgta gtaatggtaa caacgttgcg caaactatta actggcgaac 2340

tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat aaagttgcag 2400

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gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa tctggagccg 2460

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ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt tactcatata 2640

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ccgtagaaaa gatcaaagga tcttcttgag atccttttt tctgcgcgta atctgctgct 2820

tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgttt gccggatcaa gagctaccaa 2880

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gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta agcggcaggg 3240

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gttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg 3480

cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc gagtcagtga 3540

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attaatgcag ctggcacgac aggtttcccg actggaaagc gggcagtgag cgcaacgcaa 3660

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ctgcttcttc ccacatttga gtcctcctca gcccctgagc tcctctgggc agggctgttt 4200

ctttccatct ttgtattccc aggggcctgc aaataaatgt ttaatgaacg aacaagagag 4260

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ccagaaacgg aagctgcagg ttgcagcccc tgccctcatg gagctcctcc tgtcagagga 4380

gtgtggggac tggatgactc cagaggtaac ttgtgggggа acgaacaggt aaggggctgt 4440

gtgacgagat gagagactgg gagaataaac cagaaagtct ctagctgtcc agaggacata 4500

gcacagaggc ccatggtccc tatttcaaac ccaggccacc agactgagct gggaccttgg 4560

gacagacaag tcatgcagaa gttaggggac cttctcctcc cttttcctgg atggatcctg 4620

agtaccttct cctccctgac ctcaggcttc ctcctagtgt caccttggcc cctcttagaa 4680

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gggtcagaac ccagagtcat cccctgaatt ctgcagatat ccatcacact ggcggccgcg   4860

ccaccatgtc acgcaagata gaaggctttt tgttattact tctctttggc tatgaagcca   4920

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aggggcgctg tgacatcgat gagtggatga ccaagtacag cgtgcagtac aggaccgatg   5340

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gcgtcagcaa gtgtgcctga a                                             5541
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<210> SEQ ID NO 19
<211> LENGTH: 346
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

```
Met Ala Leu Leu Lys Val Lys Phe Asp Gln Lys Lys Arg Val Lys Leu
1               5                   10                  15

Ala Gln Gly Leu Trp Leu Met Asn Trp Phe Ser Val Leu Ala Gly Ile
            20                  25                  30

Ile Ile Phe Ser Leu Gly Leu Phe Leu Lys Ile Glu Leu Arg Lys Arg
        35                  40                  45

Ser Asp Val Met Asn Asn Ser Glu Ser His Phe Val Pro Asn Ser Leu
    50                  55                  60

Ile Gly Met Gly Val Leu Ser Cys Val Phe Asn Ser Leu Ala Gly Lys
65                  70                  75                  80

Ile Cys Tyr Asp Ala Leu Asp Pro Ala Lys Tyr Ala Arg Trp Lys Pro
                85                  90                  95

Trp Leu Lys Pro Tyr Leu Ala Ile Cys Val Leu Phe Asn Ile Ile Leu
            100                 105                 110

Phe Leu Val Ala Leu Cys Cys Phe Leu Leu Arg Gly Ser Leu Glu Asn
        115                 120                 125

Thr Leu Gly Gln Gly Leu Lys Asn Gly Met Lys Tyr Tyr Arg Asp Thr
    130                 135                 140

Asp Thr Pro Gly Arg Cys Phe Met Lys Lys Thr Ile Asp Met Leu Gln
145                 150                 155                 160

Ile Glu Phe Lys Cys Cys Gly Asn Asn Gly Phe Arg Asp Trp Phe Glu
                165                 170                 175

Ile Gln Trp Ile Ser Asn Arg Tyr Leu Asp Phe Ser Ser Lys Glu Val
            180                 185                 190

Lys Asp Arg Ile Lys Ser Asn Val Asp Gly Arg Tyr Leu Val Asp Gly
        195                 200                 205
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Val Pro Phe Ser Cys Cys Asn Pro Ser Ser Pro Arg Pro Cys Ile Gln
    210                 215                 220

Tyr Gln Ile Thr Asn Asn Ser Ala His Tyr Ser Tyr Asp His Gln Thr
225                 230                 235                 240

Glu Glu Leu Asn Leu Trp Val Arg Gly Cys Arg Ala Ala Leu Leu Ser
                245                 250                 255

Tyr Tyr Ser Ser Leu Met Asn Ser Met Gly Val Val Thr Leu Leu Ile
            260                 265                 270

Trp Leu Phe Glu Val Thr Ile Thr Ile Gly Leu Arg Tyr Leu Gln Thr
        275                 280                 285

Ser Leu Asp Gly Val Ser Asn Pro Glu Glu Ser Glu Ser Glu Ser Gln
    290                 295                 300

Gly Trp Leu Leu Glu Arg Ser Val Pro Glu Thr Trp Lys Ala Phe Leu
305                 310                 315                 320

Glu Ser Val Lys Lys Leu Gly Lys Gly Asn Gln Val Glu Ala Glu Gly
                325                 330                 335

Ala Asp Ala Gly Gln Ala Pro Glu Ala Gly
            340                 345


<210> SEQ ID NO 20
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Ser His His Pro Ser Gly Leu Arg Ala Gly Phe Ser Ser Thr Ser
1               5                   10                  15

Tyr Arg Arg Thr Phe Gly Pro Pro Pro Ser Leu Ser Pro Gly Ala Phe
            20                  25                  30

Ser Tyr Ser Ser Ser Ser Arg Phe Ser Ser Ser Arg Leu Leu Gly Ser
        35                  40                  45

Ala Ser Pro Ser Ser Ser Val Arg Leu Gly Ser Phe Arg Ser Pro Arg
    50                  55                  60

Ala Gly Ala Gly Ala Leu Leu Arg Leu Pro Ser Glu Arg Leu Asp Phe
65                  70                  75                  80

Ser Met Ala Glu Ala Leu Asn Gln Glu Phe Leu Ala Thr Arg Ser Asn
                85                  90                  95

Glu Lys Gln Glu Leu Gln Glu Leu Asn Asp Arg Phe Ala Asn Phe Ile
            100                 105                 110

Glu Lys Val Arg Phe Leu Glu Gln Gln Asn Ala Ala Leu Arg Gly Glu
        115                 120                 125

Leu Ser Gln Ala Arg Gly Gln Glu Pro Ala Arg Ala Asp Gln Leu Cys
    130                 135                 140

Gln Gln Glu Leu Arg Glu Leu Arg Arg Glu Leu Glu Leu Leu Gly Arg
145                 150                 155                 160

Glu Arg Asp Arg Val Gln Val Glu Arg Asp Gly Leu Ala Glu Asp Leu
                165                 170                 175

Ala Ala Leu Lys Gln Arg Leu Glu Glu Glu Thr Arg Lys Arg Glu Asp
            180                 185                 190

Ala Glu His Asn Leu Val Leu Phe Arg Lys Asp Val Asp Asp Ala Thr
        195                 200                 205

Leu Ser Arg Leu Glu Leu Glu Arg Lys Ile Glu Ser Leu Met Asp Glu
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    210                 215                 220

Ile Glu Phe Leu Lys Lys Leu His Glu Glu Glu Leu Arg Asp Leu Gln
225                 230                 235                 240

Val Ser Val Glu Ser Gln Gln Val Gln Gln Val Glu Val Glu Ala Thr
                245                 250                 255

Val Lys Pro Glu Leu Thr Ala Ala Leu Arg Asp Ile Arg Ala Gln Tyr
            260                 265                 270

Glu Ser Ile Ala Ala Lys Asn Leu Gln Glu Ala Glu Glu Trp Tyr Lys
        275                 280                 285

Ser Lys Tyr Ala Asp Leu Ser Asp Ala Ala Asn Arg Asn His Glu Ala
    290                 295                 300

Leu Arg Gln Ala Lys Gln Glu Met Asn Glu Ser Arg Arg Gln Ile Gln
305                 310                 315                 320

Ser Leu Thr Cys Glu Val Asp Gly Leu Arg Gly Thr Asn Glu Ala Leu
                325                 330                 335

Leu Arg Gln Leu Arg Glu Leu Glu Glu Gln Phe Ala Leu Glu Ala Gly
            340                 345                 350

Gly Tyr Gln Ala Gly Ala Ala Arg Leu Glu Glu Glu Leu Arg Gln Leu
        355                 360                 365

Lys Glu Glu Met Ala Arg His Leu Arg Glu Tyr Gln Glu Leu Leu Asn
    370                 375                 380

Val Lys Met Ala Leu Asp Ile Glu Ile Ala Thr Tyr Arg Lys Leu Leu
385                 390                 395                 400

Glu Gly Glu Glu Ser Arg Ile Ser Val Pro Val His Ser Phe Ala Ser
                405                 410                 415

Leu Asn Ile Lys Thr Thr Val Pro Glu Val Glu Pro Pro Gln Asp Ser
            420                 425                 430

His Ser Arg Lys Thr Val Leu Ile Lys Thr Ile Glu Thr Arg Asn Gly
        435                 440                 445

Glu Val Val Thr Glu Ser Gln Lys Glu Gln Arg Ser Glu Leu Asp Lys
    450                 455                 460

Ser Ser Ala His Ser Tyr
465                 470


<210> SEQ ID NO 21
<211> LENGTH: 1286
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Met Ser His Leu Val Asp Pro Thr Ser Gly Asp Leu Pro Val Arg Asp
1               5                   10                  15

Ile Asp Ala Ile Pro Leu Val Leu Pro Ala Ser Lys Gly Lys Asn Met
            20                  25                  30

Lys Thr Gln Pro Pro Leu Ser Arg Met Asn Arg Glu Glu Leu Glu Asp
        35                  40                  45

Ser Phe Phe Arg Leu Arg Glu Asp His Met Leu Val Lys Glu Leu Ser
    50                  55                  60

Trp Lys Gln Gln Asp Glu Ile Lys Arg Leu Arg Thr Thr Leu Leu Arg
65                  70                  75                  80

Leu Thr Ala Ala Gly Arg Asp Leu Arg Val Ala Glu Glu Ala Ala Pro
                85                  90                  95
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Leu Ser Glu Thr Ala Arg Arg Gly Gln Lys Ala Gly Trp Arg Gln Arg
            100                 105                 110

Leu Ser Met His Gln Arg Pro Gln Met His Arg Leu Gln Gly His Phe
        115                 120                 125

His Cys Val Gly Pro Ala Ser Pro Arg Arg Ala Gln Pro Arg Val Gln
    130                 135                 140

Val Gly His Arg Gln Leu His Thr Ala Gly Ala Pro Val Pro Glu Lys
145                 150                 155                 160

Pro Lys Arg Gly Pro Arg Asp Arg Leu Ser Tyr Thr Ala Pro Pro Ser
                165                 170                 175

Phe Lys Glu His Ala Thr Asn Glu Asn Arg Gly Glu Val Ala Ser Lys
            180                 185                 190

Pro Ser Glu Leu Val Ser Gly Ser Asn Ser Ile Ile Ser Phe Ser Ser
        195                 200                 205

Val Ile Ser Met Ala Lys Pro Ile Gly Leu Cys Met Pro Asn Ser Ala
    210                 215                 220

His Ile Met Ala Ser Asn Thr Met Gln Val Glu Glu Pro Pro Lys Ser
225                 230                 235                 240

Pro Glu Lys Met Trp Pro Lys Asp Glu Asn Phe Glu Gln Arg Ser Ser
                245                 250                 255

Leu Glu Cys Ala Gln Lys Ala Ala Glu Leu Arg Ala Ser Ile Lys Glu
            260                 265                 270

Lys Val Glu Leu Ile Arg Leu Lys Lys Leu Leu His Glu Arg Asn Ala
        275                 280                 285

Ser Leu Val Met Thr Lys Ala Gln Leu Thr Glu Val Gln Glu Ala Tyr
    290                 295                 300

Glu Thr Leu Leu Gln Lys Asn Gln Gly Ile Leu Ser Ala Ala His Glu
305                 310                 315                 320

Ala Leu Leu Lys Gln Val Asn Glu Leu Arg Ala Glu Leu Lys Glu Glu
                325                 330                 335

Ser Lys Lys Ala Val Ser Leu Lys Ser Gln Leu Glu Asp Val Ser Ile
            340                 345                 350

Leu Gln Met Thr Leu Lys Glu Phe Gln Glu Arg Val Glu Asp Leu Glu
        355                 360                 365

Lys Glu Arg Lys Leu Leu Asn Asp Asn Tyr Asp Lys Leu Leu Glu Ser
    370                 375                 380

Met Leu Asp Ser Ser Asp Ser Ser Ser Gln Pro His Trp Ser Asn Glu
385                 390                 395                 400

Leu Ile Ala Glu Gln Leu Gln Gln Gln Val Ser Gln Leu Gln Asp Gln
                405                 410                 415

Leu Asp Ala Glu Leu Glu Asp Lys Arg Lys Val Leu Leu Glu Leu Ser
            420                 425                 430

Arg Glu Lys Ala Gln Asn Glu Asp Leu Lys Leu Glu Val Thr Asn Ile
        435                 440                 445

Leu Gln Lys His Lys Gln Glu Val Glu Leu Leu Gln Asn Ala Ala Thr
    450                 455                 460

Ile Ser Gln Pro Pro Asp Arg Gln Ser Glu Pro Ala Thr His Pro Ala
465                 470                 475                 480

Val Leu Gln Glu Asn Thr Gln Ile Glu Pro Ser Glu Pro Lys Asn Gln
                485                 490                 495

Glu Glu Lys Lys Leu Ser Gln Val Leu Asn Glu Leu Gln Val Ser His
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            500                 505                 510

Ala Glu Thr Thr Leu Glu Leu Glu Lys Thr Arg Asp Met Leu Ile Leu
        515                 520                 525

Gln Arg Lys Ile Asn Val Cys Tyr Gln Glu Glu Leu Glu Ala Met Met
    530                 535                 540

Thr Lys Ala Asp Asn Asp Asn Arg Asp His Lys Glu Lys Leu Glu Arg
545                 550                 555                 560

Leu Thr Arg Leu Leu Asp Leu Lys Asn Asn Arg Ile Lys Gln Leu Glu
                565                 570                 575

Gly Ile Leu Arg Ser His Asp Leu Pro Thr Ser Glu Gln Leu Lys Asp
            580                 585                 590

Val Ala Tyr Gly Thr Arg Pro Leu Ser Leu Cys Leu Glu Thr Leu Pro
        595                 600                 605

Ala His Gly Asp Glu Asp Lys Val Asp Ile Ser Leu Leu His Gln Gly
    610                 615                 620

Glu Asn Leu Phe Glu Leu His Ile His Gln Ala Phe Leu Thr Ser Ala
625                 630                 635                 640

Ala Leu Ala Gln Ala Gly Asp Thr Gln Pro Thr Thr Phe Cys Thr Tyr
                645                 650                 655

Ser Phe Tyr Asp Phe Glu Thr His Cys Thr Pro Leu Ser Val Gly Pro
            660                 665                 670

Gln Pro Leu Tyr Asp Phe Thr Ser Gln Tyr Val Met Glu Thr Asp Ser
        675                 680                 685

Leu Phe Leu His Tyr Leu Gln Glu Ala Ser Ala Arg Leu Asp Ile His
    690                 695                 700

Gln Ala Met Ala Ser Glu His Ser Thr Leu Ala Ala Gly Trp Ile Cys
705                 710                 715                 720

Phe Asp Arg Val Leu Glu Thr Val Glu Lys Val His Gly Leu Ala Thr
                725                 730                 735

Leu Ile Gly Ala Gly Gly Glu Glu Phe Gly Val Leu Glu Tyr Trp Met
            740                 745                 750

Arg Leu Arg Phe Pro Ile Lys Pro Ser Leu Gln Ala Cys Asn Lys Arg
        755                 760                 765

Lys Lys Ala Gln Val Tyr Leu Ser Thr Asp Val Leu Gly Gly Arg Lys
    770                 775                 780

Ala Gln Glu Glu Glu Phe Arg Ser Glu Ser Trp Glu Pro Gln Asn Glu
785                 790                 795                 800

Leu Trp Ile Glu Ile Thr Lys Cys Cys Gly Leu Arg Ser Arg Trp Leu
                805                 810                 815

Gly Thr Gln Pro Ser Pro Tyr Ala Val Tyr Arg Phe Phe Thr Phe Ser
            820                 825                 830

Asp His Asp Thr Ala Ile Ile Pro Ala Ser Asn Asn Pro Tyr Phe Arg
        835                 840                 845

Asp Gln Ala Arg Phe Pro Val Leu Val Thr Ser Asp Leu Asp His Tyr
    850                 855                 860

Leu Arg Arg Glu Ala Leu Ser Ile His Val Phe Asp Asp Glu Asp Leu
865                 870                 875                 880

Glu Pro Gly Ser Tyr Leu Gly Arg Ala Arg Val Pro Leu Leu Pro Leu
                885                 890                 895

Ala Lys Asn Glu Ser Ile Lys Gly Asp Phe Asn Leu Thr Asp Pro Ala
            900                 905                 910
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Glu Lys Pro Asn Gly Ser Ile Gln Val Gln Leu Asp Trp Lys Phe Pro
        915                 920                 925

Tyr Ile Pro Pro Glu Ser Phe Leu Lys Pro Glu Ala Gln Thr Lys Gly
    930                 935                 940

Lys Asp Thr Lys Asp Ser Ser Lys Ile Ser Ser Glu Glu Glu Lys Ala
945                 950                 955                 960

Ser Phe Pro Ser Gln Asp Gln Met Ala Ser Pro Glu Val Pro Ile Glu
                965                 970                 975

Ala Gly Gln Tyr Arg Ser Lys Arg Lys Pro Pro His Gly Gly Glu Arg
            980                 985                 990

Lys Glu Lys Glu His Gln Val Val  Ser Tyr Ser Arg Arg  Lys His Gly
        995                 1000                1005

Lys Arg  Ile Gly Val Gln Gly  Lys Asn Arg Met Glu  Tyr Leu Ser
    1010                1015                1020

Leu Asn  Ile Leu Asn Gly Asn  Thr Pro Glu Gln Val  Asn Tyr Thr
    1025                1030                1035

Glu Trp  Lys Phe Ser Glu Thr  Asn Ser Phe Ile Gly  Asp Gly Phe
    1040                1045                1050

Lys Asn  Gln His Glu Glu Glu  Glu Met Thr Leu Ser  His Ser Ala
    1055                1060                1065

Leu Lys  Gln Lys Glu Pro Leu  His Pro Val Asn Asp  Lys Glu Ser
    1070                1075                1080

Ser Glu  Gln Gly Ser Glu Val  Ser Glu Ala Gln Thr  Thr Asp Ser
    1085                1090                1095

Asp Asp  Val Ile Val Pro Pro  Met Ser Gln Lys Tyr  Pro Lys Ala
    1100                1105                1110

Asp Ser  Glu Lys Met Cys Ile  Glu Ile Val Ser Leu  Ala Phe Tyr
    1115                1120                1125

Pro Glu  Ala Glu Val Met Ser  Asp Glu Asn Ile Lys  Gln Val Tyr
    1130                1135                1140

Val Glu  Tyr Lys Phe Tyr Asp  Leu Pro Leu Ser Glu  Thr Glu Thr
    1145                1150                1155

Pro Val  Ser Leu Arg Lys Pro  Arg Ala Gly Glu Glu  Ile His Phe
    1160                1165                1170

His Phe  Ser Lys Val Ile Asp  Leu Asp Pro Gln Glu  Gln Gln Gly
    1175                1180                1185

Arg Arg  Arg Phe Leu Phe Asp  Met Leu Asn Gly Gln  Asp Pro Asp
    1190                1195                1200

Gln Gly  His Leu Lys Phe Thr  Val Val Ser Asp Pro  Leu Asp Glu
    1205                1210                1215

Glu Lys  Lys Glu Cys Glu Glu  Val Gly Tyr Ala Tyr  Leu Gln Leu
    1220                1225                1230

Trp Gln  Ile Leu Glu Ser Gly  Arg Asp Ile Leu Glu  Gln Glu Leu
    1235                1240                1245

Asp Ile  Val Ser Pro Glu Asp  Leu Ala Thr Pro Ile  Gly Arg Leu
    1250                1255                1260

Lys Val  Ser Leu Gln Ala Ala  Ala Val Leu His Ala  Ile Tyr Lys
    1265                1270                1275

Glu Met  Thr Glu Asp Leu Phe  Ser
    1280                1285
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<210> SEQ ID NO 22
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-1

<400> SEQUENCE: 22

Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr Ala
1 5 10 15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
20 25 30

Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
35 40 45

Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
50 55 60

Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65 70 75 80

Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
85 90 95

Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
100 105 110

Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met Ser
115 120 125

Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
130 135 140

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145 150 155 160

Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln Ser
165 170 175

Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Ala Met Gly Ala Leu
180 185 190

Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
195 200 205

Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu
210 215 220

Met Gly Gly Phe Gly Leu Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys
225 230 235 240

<210> SEQ ID NO 23
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-6

<400> SEQUENCE: 23

Thr Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala
1 5 10 15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
20 25 30

Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
35 40 45

Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
50 55 60

Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65 70 75 80

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Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
                85                  90                  95

Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Lys Asp Lys Phe Phe Pro Met Ser
        115                 120                 125

Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
    130                 135                 140

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Leu Gln Ser
                165                 170                 175

Ser Ser Thr Asp Pro Ala Thr Gly Asp Val His Val Met Gly Ala Leu
            180                 185                 190

Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
        195                 200                 205

Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu
    210                 215                 220

Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys
225                 230                 235                 240


<210> SEQ ID NO 24
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-3

<400> SEQUENCE: 24

Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His
1               5                   10                  15

Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu
            20                  25                  30

Tyr Tyr Leu Asn Arg Thr Gln Gly Thr Thr Ser Gly Thr Thr Asn Gln
        35                  40                  45

Ser Arg Leu Leu Phe Ser Gln Ala Gly Pro Gln Ser Met Ser Leu Gln
    50                  55                  60

Ala Arg Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Leu Ser
65                  70                  75                  80

Lys Thr Ala Asn Asp Asn Asn Asn Ser Asn Phe Pro Trp Thr Ala Ala
                85                  90                  95

Ser Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Pro
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Glu Glu Lys Phe Phe Pro Met His
        115                 120                 125

Gly Asn Leu Ile Phe Gly Lys Glu Gly Thr Thr Ala Ser Asn Ala Glu
    130                 135                 140

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Gln Tyr Gly Thr Val Ala Asn Asn Leu Gln Ser
                165                 170                 175

Ser Asn Thr Ala Pro Thr Thr Gly Thr Val Asn His Gln Gly Ala Leu
            180                 185                 190

Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
        195                 200                 205
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Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu
    210                 215                 220

Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Met Ile Lys
225                 230                 235                 240


<210> SEQ ID NO 25
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-2

<400> SEQUENCE: 25

Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His
1               5                   10                  15

Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu
            20                  25                  30

Tyr Tyr Leu Ser Arg Thr Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser
        35                  40                  45

Arg Leu Gln Phe Ser Gln Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser
    50                  55                  60

Arg Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys
65                  70                  75                  80

Thr Ser Ala Asp Asn Asn Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr
                85                  90                  95

Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala
            100                 105                 110

Met Ala Ser His Lys Asp Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly
        115                 120                 125

Val Leu Ile Phe Gly Lys Gln Gly Ser Glu Lys Thr Asn Val Asp Ile
    130                 135                 140

Glu Lys Val Met Ile Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro
145                 150                 155                 160

Val Ala Thr Glu Gln Tyr Gly Ser Val Ser Thr Asn Leu Gln Arg Gly
                165                 170                 175

Asn Arg Gln Ala Ala Thr Ala Asp Val Asn Thr Gln Gly Val Leu Pro
            180                 185                 190

Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
        195                 200                 205

Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu Met
    210                 215                 220

Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
225                 230                 235                 240


<210> SEQ ID NO 26
<211> LENGTH: 243
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-8

<400> SEQUENCE: 26

Asn Phe Gln Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1               5                   10                  15

Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
            20                  25                  30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala
        35                  40                  45
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```
Asn Thr Gln Thr Leu Gly Phe Ser Gln Gly Gly Pro Asn Thr Met Ala
    50                  55                  60

Asn Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65                  70                  75                  80

Val Ser Thr Thr Thr Gly Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Ala Gly Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro
            100                 105                 110

Gly Ile Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
        115                 120                 125

Ser Asn Gly Ile Leu Ile Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn
    130                 135                 140

Ala Asp Tyr Ser Asp Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145                 150                 155                 160

Thr Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu
                165                 170                 175

Gln Gln Gln Asn Thr Ala Pro Gln Ile Gly Thr Val Asn Ser Gln Gly
            180                 185                 190

Ala Leu Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly
        195                 200                 205

Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser
    210                 215                 220

Pro Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu
225                 230                 235                 240

Ile Lys Asn


<210> SEQ ID NO 27
<211> LENGTH: 243
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 27

Asn Phe Gln Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1               5                   10                  15

Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
            20                  25                  30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala
        35                  40                  45

Asn Thr Gln Thr Leu Gly Phe Ser Gln Gly Gly Pro Asn Thr Met Ala
    50                  55                  60

Asn Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65                  70                  75                  80

Val Ser Thr Thr Thr Gly Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Ala Gly Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro
            100                 105                 110

Gly Ile Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
        115                 120                 125

Ser Asn Gly Ile Leu Ile Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn
    130                 135                 140
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```
Ala Asp Tyr Ser Asp Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145                 150                 155                 160

Thr Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu
                165                 170                 175

Gln Gly Gln Arg Gln Ala Ala Gln Ile Gly Thr Val Asn Ser Gln Gly
            180                 185                 190

Ala Leu Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly
        195                 200                 205

Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser
    210                 215                 220

Pro Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu
225                 230                 235                 240

Ile Lys Asn


<210> SEQ ID NO 28
<211> LENGTH: 241
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-rh8

<400> SEQUENCE: 28

Phe Gln Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr
1               5                   10                  15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
            20                  25                  30

Tyr Leu Tyr Tyr Leu Val Arg Thr Gln Thr Thr Gly Thr Gly Gly Thr
        35                  40                  45

Gln Thr Leu Ala Phe Ser Gln Ala Gly Pro Ser Ser Met Ala Asn Gln
    50                  55                  60

Ala Arg Asn Trp Val Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65                  70                  75                  80

Thr Thr Thr Asn Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala
                85                  90                  95

Ala Lys Phe Lys Leu Asn Gly Arg Asp Ser Leu Met Asn Pro Gly Val
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Asp Asp Arg Phe Phe Pro Ser Ser
        115                 120                 125

Gly Val Leu Ile Phe Gly Lys Gln Gly Ala Gly Asn Asp Gly Val Asp
    130                 135                 140

Tyr Ser Gln Val Leu Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Glu Tyr Gly Ala Val Ala Ile Asn Asn Gln Ala
                165                 170                 175

Ala Asn Thr Gln Ala Gln Thr Gly Leu Val His Asn Gln Gly Val Ile
            180                 185                 190

Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile
        195                 200                 205

Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu
    210                 215                 220

Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys
225                 230                 235                 240

Asn
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<210> SEQ ID NO 29
<211> LENGTH: 243
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-10

<400> SEQUENCE: 29

Asn Phe Glu Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1 5 10 15

Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
20 25 30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Ser Thr Gly Gly Thr Gln
35 40 45

Gly Thr Gln Gln Leu Leu Phe Ser Gln Ala Gly Pro Ala Asn Met Ser
50 55 60

Ala Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65 70 75 80

Val Ser Thr Thr Leu Ser Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
85 90 95

Gly Ala Thr Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro
100 105 110

Gly Val Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
115 120 125

Ser Ser Gly Val Leu Met Phe Gly Lys Gln Gly Ala Gly Arg Asp Asn
130 135 140

Val Asp Tyr Ser Ser Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145 150 155 160

Thr Asn Pro Val Ala Thr Glu Gln Tyr Gly Val Val Ala Asp Asn Leu
165 170 175

Gln Gln Ala Asn Thr Gly Pro Ile Val Gly Asn Val Asn Ser Gln Gly
180 185 190

Ala Leu Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly
195 200 205

Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser
210 215 220

Pro Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu
225 230 235 240

Ile Lys Asn

<210> SEQ ID NO 30
<211> LENGTH: 242
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-7

<400> SEQUENCE: 30

Phe Glu Phe Ser Tyr Ser Phe Glu Asp Val Pro Phe His Ser Ser Tyr
1 5 10 15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
20 25 30

Tyr Leu Tyr Tyr Leu Ala Arg Thr Gln Ser Asn Pro Gly Gly Thr Ala
35 40 45

Gly Asn Arg Glu Leu Gln Phe Tyr Gln Gly Gly Pro Ser Thr Met Ala
50 55 60

Glu Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Phe Arg Gln Gln Arg
65 70 75 80

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```
Val Ser Lys Thr Leu Asp Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Gly Ala Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Val Asn Pro
            100                 105                 110

Gly Val Ala Met Ala Thr His Lys Asp Asp Glu Asp Arg Phe Phe Pro
        115                 120                 125

Ser Ser Gly Val Leu Ile Phe Gly Lys Thr Gly Ala Thr Asn Lys Thr
    130                 135                 140

Thr Leu Glu Asn Val Leu Met Thr Asn Glu Glu Glu Ile Arg Pro Thr
145                 150                 155                 160

Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ser Ser Asn Leu Gln
                165                 170                 175

Ala Ala Asn Thr Ala Ala Gln Thr Gln Val Val Asn Asn Gln Gly Ala
            180                 185                 190

Leu Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro
        195                 200                 205

Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro
    210                 215                 220

Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile
225                 230                 235                 240

Lys Asn


<210> SEQ ID NO 31
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-9

<400> SEQUENCE: 31

Phe Gln Phe Ser Tyr Glu Phe Glu Asn Val Pro Phe His Ser Ser Tyr
1               5                   10                  15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
            20                  25                  30

Tyr Leu Tyr Tyr Leu Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln
        35                  40                  45

Gln Thr Leu Lys Phe Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln
    50                  55                  60

Gly Arg Asn Tyr Ile Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser
65                  70                  75                  80

Thr Thr Val Thr Gln Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala
                85                  90                  95

Ser Ser Trp Ala Leu Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro
            100                 105                 110

Ala Met Ala Ser His Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser
        115                 120                 125

Gly Ser Leu Ile Phe Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp
    130                 135                 140

Ala Asp Lys Val Met Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser
                165                 170                 175

Ala Gln Ala Gln Ala Gln Thr Gly Trp Val Gln Asn Gln Gly Ile Leu
            180                 185                 190
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```
Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile
        195                 200                 205

Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu
    210                 215                 220

Met Gly Gly Phe Gly Met Lys His Pro Pro Pro Gln Ile Leu Ile Lys
225                 230                 235                 240
```

<210> SEQ ID NO 32
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 32

```
Gln Phe Ser Tyr Glu Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala
1               5                   10                  15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
            20                  25                  30

Leu Tyr Tyr Leu Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln
        35                  40                  45

Thr Leu Lys Phe Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly
    50                  55                  60

Arg Asn Tyr Ile Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr
65                  70                  75                  80

Thr Val Thr Gln Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser
                85                  90                  95

Ser Trp Ala Leu Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala
            100                 105                 110

Met Ala Ser His Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly
        115                 120                 125

Ser Leu Ile Phe Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala
    130                 135                 140

Asp Lys Val Met Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro
145                 150                 155                 160

Val Ala Thr Glu Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser Gly
                165                 170                 175

Gln Ala Gln Ala Ala Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro
            180                 185                 190

Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
        195                 200                 205

Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met
    210                 215                 220

Gly Gly Phe Gly Met Lys His Pro Pro Pro Gln Ile Leu Ile Lys
225                 230                 235
```

<210> SEQ ID NO 33
<211> LENGTH: 240
<212> TYPE: PRT
<213> ORGANISM: Adeno-associated virus-5

<400> SEQUENCE: 33

```
Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser Ser
1               5                   10                  15
```

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```
Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val Asp
            20                  25                  30

Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val Gln
        35                  40                  45

Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn Trp
    50                  55                  60

Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser Gly
65                  70                  75                  80

Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met Glu
                85                  90                  95

Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met Thr
            100                 105                 110

Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met Ile
        115                 120                 125

Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu Glu
    130                 135                 140

Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn Arg
145                 150                 155                 160

Val Ala Tyr Asn Val Gly Gly Gln Met Ala Thr Asn Asn Gln Ser Ser
                165                 170                 175

Thr Thr Ala Pro Ala Thr Gly Thr Tyr Asn Leu Gln Glu Ile Val Pro
            180                 185                 190

Gly Ser Val Trp Met Glu Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
        195                 200                 205

Ala Lys Ile Pro Glu Thr Gly Ala His Phe His Pro Ser Pro Ala Met
    210                 215                 220

Gly Gly Phe Gly Leu Lys His Pro Pro Pro Met Met Leu Ile Lys Asn
225                 230                 235                 240


<210> SEQ ID NO 34
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 34

Thr Phe Ser Tyr Thr Phe Glu Glu Val Pro Phe His Ser Ser Tyr Ala
1               5                   10                  15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
            20                  25                  30

Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
        35                  40                  45

Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
    50                  55                  60

Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65                  70                  75                  80

Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
                85                  90                  95

Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Glu Asp Lys Phe Phe Pro Met Ser
        115                 120                 125
```

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```
Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
    130                 135                 140

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Phe Gln Ser
                165                 170                 175

Ser Ser Thr Asp Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Pro Ala
            180                 185                 190

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
        195                 200                 205

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    210                 215                 220

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
225                 230                 235                 240

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys
                245                 250


<210> SEQ ID NO 35
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 35

Thr Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala
1               5                   10                  15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
            20                  25                  30

Leu Tyr Tyr Leu Asn Arg Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn
        35                  40                  45

Lys Asp Leu Leu Phe Ser Arg Gly Ser Pro Ala Gly Met Ser Val Gln
    50                  55                  60

Pro Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65                  70                  75                  80

Lys Thr Lys Thr Asp Asn Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala
                85                  90                  95

Ser Lys Tyr Asn Leu Asn Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Lys Asp Lys Phe Phe Pro Met Ser
        115                 120                 125

Gly Val Met Ile Phe Gly Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala
    130                 135                 140

Leu Asp Asn Val Met Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Arg Phe Gly Thr Val Ala Val Asn Leu Gln Ser
                165                 170                 175

Ser Ser Thr Asp Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Pro Ala
            180                 185                 190

Thr Gly Asp Val His Val Met Gly Ala Leu Pro Gly Met Val Trp Gln
        195                 200                 205

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    210                 215                 220
```

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```
Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
225                 230                 235                 240

Lys His Pro Pro Pro Gln Ile Leu Ile Lys
                245                 250


<210> SEQ ID NO 36
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 36

Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His
1               5                   10                  15

Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu
            20                  25                  30

Tyr Tyr Leu Ser Arg Thr Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser
        35                  40                  45

Arg Leu Gln Phe Ser Gln Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser
    50                  55                  60

Arg Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys
65                  70                  75                  80

Thr Ser Ala Asp Asn Asn Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr
                85                  90                  95

Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala
            100                 105                 110

Met Ala Ser His Lys Asp Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly
        115                 120                 125

Val Leu Ile Phe Gly Lys Gln Gly Ser Glu Lys Thr Asn Val Asp Ile
    130                 135                 140

Glu Lys Val Met Ile Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro
145                 150                 155                 160

Val Ala Thr Glu Gln Tyr Gly Ser Val Ser Thr Asn Leu Gln Arg Gly
                165                 170                 175

Asn Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Arg Gln Ala Ala Thr
            180                 185                 190

Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
        195                 200                 205

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
    210                 215                 220

Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
225                 230                 235                 240

His Pro Pro Pro Gln Ile Leu Ile Lys Asn
                245                 250


<210> SEQ ID NO 37
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 37

Asn Phe Gln Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1               5                   10                  15
```

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```
Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
            20                  25                  30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala
        35                  40                  45

Asn Thr Gln Thr Leu Gly Phe Ser Gln Gly Gly Pro Asn Thr Met Ala
    50                  55                  60

Asn Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65                  70                  75                  80

Val Ser Thr Thr Thr Gly Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Ala Gly Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro
            100                 105                 110

Gly Ile Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
        115                 120                 125

Ser Asn Gly Ile Leu Ile Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn
    130                 135                 140

Ala Asp Tyr Ser Asp Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145                 150                 155                 160

Thr Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu
                165                 170                 175

Gln Gln Gln Asn Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Thr Ala
            180                 185                 190

Pro Gln Ile Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
        195                 200                 205

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    210                 215                 220

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
225                 230                 235                 240

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
                245                 250


<210> SEQ ID NO 38
<211> LENGTH: 252
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 38

Asn Phe Gln Phe Thr Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1               5                   10                  15

Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
            20                  25                  30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala
        35                  40                  45

Asn Thr Gln Thr Leu Gly Phe Ser Gln Gly Gly Pro Asn Thr Met Ala
    50                  55                  60

Asn Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65                  70                  75                  80

Val Ser Thr Thr Thr Gly Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Ala Gly Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro
            100                 105                 110
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Gly Ile Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
        115                 120                 125

Ser Asn Gly Ile Leu Ile Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn
    130                 135                 140

Ala Asp Tyr Ser Asp Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145                 150                 155                 160

Thr Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu
                165                 170                 175

Gln Gly Gln Arg Gly Leu Gly Glu Thr Thr Arg Pro Ala Gln Ala Ala
            180                 185                 190

Gln Ile Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp
        195                 200                 205

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    210                 215                 220

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
225                 230                 235                 240

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
                245                 250


<210> SEQ ID NO 39
<211> LENGTH: 251
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 39

Phe Gln Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr
1               5                   10                  15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
            20                  25                  30

Tyr Leu Tyr Tyr Leu Val Arg Thr Gln Thr Thr Gly Thr Gly Gly Thr
        35                  40                  45

Gln Thr Leu Ala Phe Ser Gln Ala Gly Pro Ser Ser Met Ala Asn Gln
    50                  55                  60

Ala Arg Asn Trp Val Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser
65                  70                  75                  80

Thr Thr Thr Asn Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala
                85                  90                  95

Ala Lys Phe Lys Leu Asn Gly Arg Asp Ser Leu Met Asn Pro Gly Val
            100                 105                 110

Ala Met Ala Ser His Lys Asp Asp Asp Asp Arg Phe Phe Pro Ser Ser
        115                 120                 125

Gly Val Leu Ile Phe Gly Lys Gln Gly Ala Gly Asn Asp Gly Val Asp
    130                 135                 140

Tyr Ser Gln Val Leu Ile Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Glu Tyr Gly Ala Val Ala Ile Asn Asn Gln Ala
                165                 170                 175

Ala Asn Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Thr Gln Ala Gln
            180                 185                 190

Thr Gly Leu Val His Asn Gln Gly Val Ile Pro Gly Met Val Trp Gln
        195                 200                 205
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Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    210                 215                 220

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
225                 230                 235                 240

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
                245                 250


<210> SEQ ID NO 40
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 40

Asn Phe Glu Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser
1               5                   10                  15

Tyr Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp
            20                  25                  30

Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr Gln Ser Thr Gly Gly Thr Gln
        35                  40                  45

Gly Thr Gln Gln Leu Leu Phe Ser Gln Ala Gly Pro Ala Asn Met Ser
    50                  55                  60

Ala Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg
65                  70                  75                  80

Val Ser Thr Thr Leu Ser Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
                85                  90                  95

Gly Ala Thr Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro
            100                 105                 110

Gly Val Ala Met Ala Thr His Lys Asp Asp Glu Glu Arg Phe Phe Pro
        115                 120                 125

Ser Ser Gly Val Leu Met Phe Gly Lys Gln Gly Ala Gly Arg Asp Asn
    130                 135                 140

Val Asp Tyr Ser Ser Val Met Leu Thr Ser Glu Glu Glu Ile Lys Thr
145                 150                 155                 160

Thr Asn Pro Val Ala Thr Glu Gln Tyr Gly Val Val Ala Asp Asn Leu
                165                 170                 175

Gln Gln Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Ala Asn Thr Gly
            180                 185                 190

Pro Ile Val Gly Asn Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
        195                 200                 205

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    210                 215                 220

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
225                 230                 235                 240

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
                245                 250


<210> SEQ ID NO 41
<211> LENGTH: 252
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide
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<400> SEQUENCE: 41

Phe Glu Phe Ser Tyr Ser Phe Glu Asp Val Pro Phe His Ser Ser Tyr
1 5 10 15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
20 25 30

Tyr Leu Tyr Tyr Leu Ala Arg Thr Gln Ser Asn Pro Gly Gly Thr Ala
35 40 45

Gly Asn Arg Glu Leu Gln Phe Tyr Gln Gly Gly Pro Ser Thr Met Ala
50 55 60

Glu Gln Ala Lys Asn Trp Leu Pro Gly Pro Cys Phe Arg Gln Gln Arg
65 70 75 80

Val Ser Lys Thr Leu Asp Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr
85 90 95

Gly Ala Thr Lys Tyr His Leu Asn Gly Arg Asn Ser Leu Val Asn Pro
100 105 110

Gly Val Ala Met Ala Thr His Lys Asp Asp Glu Asp Arg Phe Phe Pro
115 120 125

Ser Ser Gly Val Leu Ile Phe Gly Lys Thr Gly Ala Thr Asn Lys Thr
130 135 140

Thr Leu Glu Asn Val Leu Met Thr Asn Glu Glu Glu Ile Arg Pro Thr
145 150 155 160

Asn Pro Val Ala Thr Glu Glu Tyr Gly Ile Val Ser Ser Asn Leu Gln
165 170 175

Ala Ala Asn Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Thr Ala Ala
180 185 190

Gln Thr Gln Val Val Asn Asn Gln Gly Ala Leu Pro Gly Met Val Trp
195 200 205

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
210 215 220

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
225 230 235 240

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
245 250

<210> SEQ ID NO 42
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 42

Phe Gln Phe Ser Tyr Glu Phe Glu Asn Val Pro Phe His Ser Ser Tyr
1 5 10 15

Ala His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln
20 25 30

Tyr Leu Tyr Tyr Leu Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln
35 40 45

Gln Thr Leu Lys Phe Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln
50 55 60

Gly Arg Asn Tyr Ile Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser
65 70 75 80

Thr Thr Val Thr Gln Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala

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                85                  90                  95

Ser Ser Trp Ala Leu Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro
            100                 105                 110

Ala Met Ala Ser His Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser
        115                 120                 125

Gly Ser Leu Ile Phe Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp
    130                 135                 140

Ala Asp Lys Val Met Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn
145                 150                 155                 160

Pro Val Ala Thr Glu Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser
                165                 170                 175

Ala Gln Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala Gln Ala Gln Thr
            180                 185                 190

Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp Gln Asp
        195                 200                 205

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
    210                 215                 220

Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Met Lys
225                 230                 235                 240

His Pro Pro Pro Gln Ile Leu Ile Lys
                245


<210> SEQ ID NO 43
<211> LENGTH: 248
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 43

Gln Phe Ser Tyr Glu Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala
1               5                   10                  15

His Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr
            20                  25                  30

Leu Tyr Tyr Leu Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln
        35                  40                  45

Thr Leu Lys Phe Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly
    50                  55                  60

Arg Asn Tyr Ile Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr
65                  70                  75                  80

Thr Val Thr Gln Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser
                85                  90                  95

Ser Trp Ala Leu Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala
            100                 105                 110

Met Ala Ser His Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly
        115                 120                 125

Ser Leu Ile Phe Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala
    130                 135                 140

Asp Lys Val Met Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro
145                 150                 155                 160

Val Ala Thr Glu Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser Gly
                165                 170                 175

Gln Ala Ala Leu Gly Glu Thr Thr Arg Pro Ala Gln Ala Ala Thr Gly
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            180                 185                 190

Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp Gln Asp Arg
        195                 200                 205

Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr Asp
    210                 215                 220

Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Met Lys His
225                 230                 235                 240

Pro Pro Pro Gln Ile Leu Ile Lys
                245
```

<210> SEQ ID NO 44
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 44

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Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser Ser
1               5                   10                  15

Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val Asp
            20                  25                  30

Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val Gln
        35                  40                  45

Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn Trp
    50                  55                  60

Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser Gly
65                  70                  75                  80

Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met Glu
                85                  90                  95

Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met Thr
            100                 105                 110

Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met Ile
        115                 120                 125

Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu Glu
    130                 135                 140

Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn Arg
145                 150                 155                 160

Val Ala Tyr Asn Val Gly Gly Gln Met Ala Thr Asn Asn Gln Ser Leu
                165                 170                 175

Ala Leu Gly Glu Thr Thr Arg Pro Ala Ser Thr Thr Ala Pro Ala Thr
            180                 185                 190

Gly Thr Tyr Asn Leu Gln Glu Ile Val Pro Gly Ser Val Trp Met Glu
        195                 200                 205

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro Glu Thr
    210                 215                 220

Gly Ala His Phe His Pro Ser Pro Ala Met Gly Gly Phe Gly Leu Lys
225                 230                 235                 240

His Pro Pro Pro Met Met Leu Ile Lys Asn
                245                 250
```

<210> SEQ ID NO 45
<211> LENGTH: 10
<212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 45

Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala
1 5 10

<210> SEQ ID NO 46
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 46

Leu Ala Asn Glu Thr Ile Thr Arg Pro Ala
1 5 10

<210> SEQ ID NO 47
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 47

Leu Ala Lys Ala Gly Gln Ala Asn Asn Ala
1 5 10

<210> SEQ ID NO 48
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 48

Leu Ala Lys Asp Pro Lys Thr Thr Asn Ala
1 5 10

<210> SEQ ID NO 49
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 49

Ala Ala Leu Gly Glu Thr Thr Arg Pro Ala
1 5 10

<210> SEQ ID NO 50
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 50

Ala Ala Asn Glu Thr Ile Thr Arg Pro Ala
1 5 10

<210> SEQ ID NO 51

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<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 51

Ala Ala Lys Ala Gly Gln Ala Asn Asn Ala
1 5 10

<210> SEQ ID NO 52
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 52

Ala Ala Lys Asp Pro Lys Thr Thr Asn Ala
1 5 10

<210> SEQ ID NO 53
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 53

Gly Leu Gly Glu Thr Thr Arg Pro Ala
1 5

<210> SEQ ID NO 54
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 54

Gly Asn Glu Thr Ile Thr Arg Pro Ala
1 5

<210> SEQ ID NO 55
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 55

Gly Lys Ala Gly Gln Ala Asn Asn Ala
1 5

<210> SEQ ID NO 56
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 56

Gly Lys Asp Pro Lys Thr Thr Asn Ala
1 5

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<210> SEQ ID NO 57
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 57

Lys Asp Thr Asp Thr Thr Arg
1 5

<210> SEQ ID NO 58
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 58

Arg Ala Gly Gly Ser Val Gly
1 5

<210> SEQ ID NO 59
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 59

Ala Val Asp Thr Thr Lys Phe
1 5

<210> SEQ ID NO 60
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 60

Ser Thr Gly Lys Val Pro Asn
1 5

<210> SEQ ID NO 61
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 61

Leu Ala Lys Asp Thr Asp Thr Thr Arg Ala
1 5 10

<210> SEQ ID NO 62
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 62

Leu Ala Arg Ala Gly Gly Ser Val Gly Ala

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1               5                   10


<210> SEQ ID NO 63
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 63

Leu Ala Ala Val Asp Thr Thr Lys Phe Ala
1               5                   10


<210> SEQ ID NO 64
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic polypeptide

<400> SEQUENCE: 64

Leu Ala Ser Thr Gly Lys Val Pro Asn Ala
1               5                   10
```

1-27. (canceled)

28. A method of identifying a variant recombinant adeno-associated virus (rAAV) virion having greater infectivity of a cell as compared to the corresponding parental AAV virion, the method comprising:

polymerase chain reaction (PCR) amplifying virion capsid DNA from cells contacted in vivo with a library of variant rAAV virions comprising variant AAV virion capsid protein and variant AAV virion capsid DNA encoding the variant AAV capsid protein.

29. The method according to claim **28**, wherein the variant AAV virion capsid protein comprises a peptide inserted into the GH loop of the corresponding parental AAV capsid protein.

30. The method according to claim **29**, wherein the peptide consists of five to eleven amino acids.

31. The method according to claim **29**, wherein the peptide is located between two adjacent amino acids within amino acids 570-614 of the corresponding parental AAV capsid protein.

32. The method according to claim **28**, wherein the variant AAV virion capsid protein comprises 1 to 25 amino acid substitutions.

33. The method according to claim **28**, wherein the variant AAV virion capsid protein is a chimeric capsid protein.

34. The method according to claim **28**, further comprising, following said PCR amplification, sequencing the variant AAV virion capsid DNA.

35. The method according to claim **28**, further comprising, following said PCR amplification,

cloning the variant AAV virion capsid DNA, and

packaging variant AAV virions from the cloned variant AAV virion capsid DNA.

36. The method according to claim **35**, wherein the PCR amplification comprises error-prone PCR.

37. The method according to claim **28**, wherein the cell is a retinal cell.

38. The method according to claim **28**, wherein the increased infectivity is a greater infectivity of said cells following administration of the variant rAAV virion in vivo as compared to the infectivity of an AAV virion comprising the corresponding parental AAV capsid protein when administered in vivo.

39. A method of screening a library of variant recombinant adeno-associated virus (rAAV) virions to identify a variant rAAV virion having greater infectivity of a cell as compared to a corresponding parental AAV virion, the method comprising:

contacting cells in vivo with a library of variant rAAV virions comprising variant AAV capsid protein and variant AAV virion capsid DNA encoding the variant AAV capsid protein.

40. The method according to claim **39**, wherein the variant AAV virion capsid protein comprises a peptide inserted into the GH loop of the corresponding parental AAV capsid protein.

41. The method according to claim **40**, wherein the peptide consists of five to eleven amino acids.

42. The method according to claim **40**, wherein the peptide is located between two adjacent amino acids within amino acids 570-614 of the corresponding parental AAV capsid protein.

43. The method according to claim **40**, wherein the variant AAV virion capsid protein comprises 1 to 25 amino acid substitutions.

44. The method according to claim **40**, wherein the variant AAV virion capsid protein is a chimeric capsid protein.

45. The method according to claim **39**, further comprising:

sequencing the variant AAV virion capsid DNA.

46. The method according to claim **39**, further comprising:

polymerase chain reaction (PCR) amplifying the variant AAV virion capsid DNA from the cells.

47. The method according to claim **46**, further comprising, following said PCR amplification,

cloning the variant rAAV virion capsid DNA, and

packaging variant AAV virions from the cloned variant rAAV virion capsid DNA.

48. The method according to claim **47**, wherein the PCR amplification comprises error-prone PCR.

49. The method according to claim **39**, wherein the cell is a retinal cell.

50. The method according to claim **39**, wherein the increased infectivity is a greater infectivity of said cells following administration of the variant rAAV virion in vivo as compared to the infectivity of an AAV virion comprising the corresponding parental AAV capsid protein when administered in vivo.

51. A method of generating a library of variant AAV virions, each virion comprising a variant AAV capsid comprising at least one amino acid substitution, the method comprising:

producing a library of AAV cap gene variants comprising a nucleic acid sequence that encodes for a peptide inserted into the GH loop of the encoded variant AAV capsid protein;

cloning said library of AAV cap gene variants; and

producing a library of variant rAAV virions comprising variant AAV capsid protein encoded by said AAV cap gene variants.

52. The method according to claim **51**, wherein the encoded peptide is located between two adjacent amino acids located between amino acids 570-614 of the corresponding parental AAV capsid protein.

53. The method according to claim **51**, wherein the encoded variant AAV capsid proteins further comprise 1 to 25 amino acid substitutions.

54. The method according to claim **51**, wherein the variant AAV virion capsid protein is a chimeric capsid protein.

55. A library of rAAV virions generated by the method according to claim **51**.

* * * * *