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(54) Title: COMPOSITIONS FOR TREATING OSTEOCLASTOGENESIS DISORDERS AND/OR RHEUMATOID ARTHRITIS

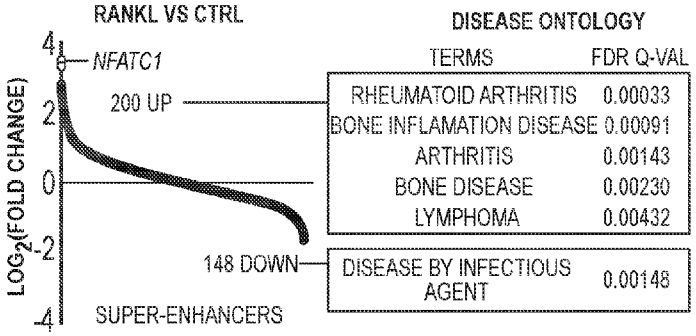

FIG. 1C

(57) Abstract: Compositions and uses thereof are provided for treating an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis. The compositions comprise an agent which specifically binding to a human ostcoclast-spccific NFATcl regulatory region.

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COMPOSITIONS FOR TREATING OSTEOCLASTOGENESIS DISORDERS AND/OR RHEUMATOID ARTHRITIS

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

The contents of the electronic sequence listing (HSS-2022-009.PCT_Seq-Listing.xml; Size: 13,481 bytes; and Date of Creation: November 6, 2023) is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Osteoclasts are large, multinucleated cells derived from myeloid lineage cells and are the primary cells responsible for bone resorption and bone homeostasis[1-3]. Dysregulated osteoclast differentiation and function lead to bone destruction in pathological conditions, including rheumatoid arthritis (RA)[4-6]. Macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) are key regulators of osteoclast differentiation, survival, and activity of osteoclasts. RANKL binds to its receptor, RANK[2]. RANKL activates the canonical osteoclastogenic signaling pathways and induces osteoclastogenic transcriptional and epigenetic program during osteoclastogenesis[7-11]. RANKL- or RANK-deficient mice exhibit severe osteopetrosis with impaired osteoclast activity[12-14]. The mechanisms by which RANKL induces osteoclast differentiation have been extensively studied, and the central transcription factors that are involved in initiating and maintaining the osteoclastogenic program are well defined. Among them, Nuclear Factor of Activated T cells, c1 (NFATc1) is known as a master regulator of osteoclastogenesis[15], and NFATc1 deficiency also leads to an osteoporotic phenotype in mice[16-18]. However, NFATc1 and other key factors driving osteoclastogenesis also express and function in other cell types, and the cell-specific regulatory mechanisms controlling the expression of such factors in osteoclasts remain poorly characterized.

Environmental stimuli trigger immediate and/or chronic changes in gene transcription, leading to the differentiation of cells into various subsets with a distinct function and phenotype. Epigenetic regulation plays a critical role in cell-type specific gene expression that establishes physiological phenotypes of cells but also contributes to human diseases[11,19,20]. There have been considerable efforts to understand epigenetic regulation in

osteoclasts[7-11,21]. Nishikawa et al. demonstrated that RANKL-induced S-adenosylmethionine (SAM) production mediates DNA methylation by de novo DNA methyltransferase 3a (Dnmt3a) during osteoclastogenesis and that inhibiting Dnmt3a regulates osteoclastogenesis and ovariectomy-induced bone loss[21]. Recent studies have also shown that targeting bromodomain and extraterminal (BET) proteins, a chromatin reader, using small molecule inhibitors suppresses osteoclastogenesis and bone destruction in inflammatory arthritis[22,23]. However, targeting epigenetic regulators broadly affects many cell types since most cells utilize similar chromatin regulators to regulate epigenetic mechanisms. Thus, unraveling the osteoclast-specific regulatory mechanism is important for understanding the epigenetic network mediating lineage control of osteoclast formation and activity and for identifying a safe therapeutic target.

Enhancers are *cis* regulatory elements and contain transcription factor-binding motifs[24]. Enhancers derive cell-type specific transcriptional activation or suppression independent of orientation or distance[24]. Super-enhancers (SEs) are clusters of enhancers and underlie cell identity and lineage commitment by driving high-level expression of genes encoding key regulators of cell identity and cell fate[25-29]. SEs serve as hubs that recruit large protein complexes including the transcriptional apparatus, transcription factors, co-activators and chromatin regulators, and promote the expression of cell-fate decision genes[30]. Histone 3 acetylation at lysine 27 (H3K27ac), an active enhancer mark, is highly enriched in SEs[31]. SEs are also sensitive to the dynamic changes of pioneer master regulators during differentiation[32]. A recent study identified SEs in mouse osteoclasts using ChIP-seq of PU.1, a myeloid pioneer transcription factor[33]. However, human osteoclast-specific SEs have not yet been defined. Moreover, recent data has revealed that enhancers produce non-coding RNA species, called enhancer RNAs (eRNAs)[34-37]. eRNAs are transcribed from active tissue-specific enhancers including super-enhancers, and their biological function has been discovered in many different cells[38-51]. However, the function and mechanism of super-enhancers as well as SE-associated eRNAs in RANKL-stimulated human osteoclasts are yet to be firmly established.

There remains a need for osteoclast-specific specific therapies for treatment of bone and joint destruction and/or rheumatoid arthritis.

SUMMARY OF THE INVENTION

The inventor has identified a novel region within the regulatory region for a Nfatc1 gene which is osteoclast-specific. The identification of this region has enabled the design of various osteoclast-specific therapies, including antisense oligonucleotide (ASO) therapy. Other suitable agents may be additionally or alternatively targeted to this region.

In one embodiment, the invention provides a composition comprising:

a pharmaceutically acceptable delivery vehicle, carrier, and/or diluent, and at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:

(a)

TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising a sequence having at least 95% complementarity to SEQ ID NO: 1 or 2, or a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or (d) combinations of (a), (b), or (c).

The composition comprises at least one of these antisense oligonucleotides comprising 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1. In certain embodiments, the at least one antisense oligonucleotide comprises 18 to 30 nucleotides in length which comprise at least 15 consecutive nucleotides of SEQ ID NO: 2. In certain embodiments, the composition comprises an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 2. In certain embodiments, the composition comprises an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2. In certain embodiments, the composition comprises an antisense oligonucleotide having at least one modified internucleoside linkage, sugar moiety, or nucleobase. In certain embodiments, the composition comprises a non-viral vector comprising the oligo is a lipid nanoparticle, lipidoid, or liposome.

In certain embodiments, a method is provided for treating an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis,

and/or rheumatoid arthritis, said method comprising delivering an agent which specifically binding to a human osteoclast-specific NFATc1 regulatory region in chromosome 18. In certain embodiments, the agent is an anti-sense oligonucleotide, an RNAi, siRNA, or combinations thereof. In certain embodiments, the agent is delivered via a lipid nanoparticle, lipidoid, or liposome. In certain embodiments, the composition is delivered intravenously, via site-specific administration to a joint, via targeted delivery to the bone, or intrathecally.

In certain embodiments, use of at least one antisense oligonucleotide (ASO) of 15 to 50 nucleotides in length in preparing a medicament is provided. In certain embodiments, a composition is provided which is suitable for treatment of an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis which comprises an agent which modulates the regulatory region of the osteoclast-specific Nfatc1 gene.

These and other aspects and embodiments of the invention will be apparent from the specification.

BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A-1F provide the identification of RANKL-sensitive super enhancers (SEs) in human osteoclasts. Human $CD14^+$ monocytes cultured overnight with M-CSF (20 ng ml^{-1}) and then were treated with M-CSF (CTRL) or M-CSF with RANKL (40 ng ml^{-1}, RANKL) for three days (FIG 1A) or one day (FIGS 1B-1F). FIG 1A: Schematic showing the differentiation of osteoclasts. FIGs 1Bb-F H3K27ac ChIP-seq was performed. (FIG 1B) Distribution of H3K27ac ChIP-seq enrichment scores in the indicated conditions. Enhancer regions are plotted in an increasing order based on their input-normalized H3K27ac ChIP-seq signal. SEs are defined as the population of enhancers above the inflection point of the curve. (FIG 1C) RANKL-sensitive SE was defined as a > 1.5-fold change in H3K27ac read density by RANKL (left panel). Disease ontology for RANKL-sensitive SE-associated genes with corresponding q-value (right panel) (FIG 1D; control in black, R) Read density plot of H3K27ac ChIP-seq across typical enhancers (TEs) and SEs domains. (FIG 1E) Graphs showing the differential H3K27ac ChIP-seq signals between RANKL and CTRL across enhancer domains. Increased (left) or decreased (right) H3K27ac ChIP-seq signals in RANKL are shown. Dark dots indicate the signals from SE regions and gray dots indicate the signals from TE regions. Boxplots of the change in H3K27ac ChIP-seq signals in the indicated conditions. *;$p<0.01$ by Kruskal-Wallis test with the Bonferroni correction.

Representative SEs are highlighted along with their associated genes. (f) Representative tracks of H3K27ac ChIP-seq at NFATc1, PRDM1, MYC, IRF8, and KLF2 loci in the indicated conditions. Gray or black bar indicates up-regulated or down-regulated SEs, respectively.

FIGs. 2A-2D show RANKL-sensitive SEs exhibit osteoclast-specificity. (a) H3K27ac ChIP-seq distribution for osteoclast, osteoblast, CD34+ progenitor and CD4+ T cell within RANKL-induced osteoclast SEs. Each color indicates a different cell type. (b) Box plots showing H3K27ac ChIP-seq signals across SE domains in the indicated cell type. ***; $p<0.001$, n.s.: not significant by Kruskal-Wallis test with the Bonferroni correction. (c) Chow-Ruskey diagram of SE- or TE-associated genes in osteoclast, osteoblast, CD34+ progenitor and CD4+ T cell. Lighter shades represent the overlap of fewer cell types. Area of each intersection is proportional to number of genes within the intersection. (d) Representative tracks for H3K27ac ChIP-seq of the indicated cell types in vicinity of SEs of osteoclast, osteoblast, CD34+ progenitor or CD4+ T cells.

FIGs. 3A-3F illustrate dynamic chromatin accessibility at RANKL-sensitive SEs. (a) Heatmaps of differential chromatin accessibility (ATAC-seq) upon RANKL (40 ng ml^{-1}) treatment; left panel: increased ATAC-seq peaks in RANKL (a black box indicates highly inducible ATAC-seq peaks), right panel: decreased ATAC-seq peaks in RANKL. (b) Read density plot of ATAC-seq across TE and SEs domains in the indicated conditions. (c) Box plots of ATAC-seq read density at TEs and SEs in the indicated conditions. ***; $p<0.01$ by Kruskal-Wallis test with the Bonferroni correction. (d) Representative tracks of ATAC-seq in the vicinity of NFATc1, PRDM1 and MYC loci. Gray boxes indicate the RANKL-induced SE regions (left). Enlarged ATAC-seq tracks and peaks at SE domains are shown (right). (e, f) Motif analysis of RANKL-regulated ATAC-seq peaks within SE regions.

FIGs. 4A-4I show sensitivity to iBET differentially regulates SE-eRNA expression. FIG4A is a Box plot displaying read densities of H3K27ac ChIP-seq in RANKL-sensitive SE domains in presence or absence of I-BET151 (500 nM). (b) Distribution of H3K27ac ChIP-seq signals within all RANKL-induced SEs (left, n=200) and I-BET151-sensitive SEs that underwent 1.5-fold H3K27ac enrichment changes from 200 RANKL-sensitive SEs (right, n=49). (c) Box plots showing H3K27ac ChIP-seq signals across SE domains in the indicated conditions. (d) Representative tracks of IBET-sensitive or -insensitive SEs in the indicated conditions. Normalized read counts of H3K27ac ChIP-seq are shown. (e, f) Motif enrichment analysis of I-BET151-sensitive or insensitive SEs. (g) Overall sequencing

coverage of H3K27ac and ATAC-seq around BATF binding motifs. Dotted line, dashed line, and solid lines represent coverage around motifs genome-wide, motifs within TEs, and motifs within SEs, respectively. The black line represents the control group (CTRL) and the gray line represents the RANKL-treated group. (h, i) Osteoclastogenesis assay for human OCPs transfected with control or BATF1/3-specific siRNA and treated with RANKL (40 ng ml^{-1}) (n=3). Scale bar: 200 μm. (h) BATF1/3 knock-down efficiency in human OCPs was measured by RT-qPCR. (i) Graph shows the percentage of TRAP-positive multinuclear cells (MNCs: more than three nuclei) per control, normalized relative to the number of osteoclasts formed in control siRNA conditions. ***; $p<0.001$, **; $p< 0.01$, or *; $p< 0.05$ by Kruskal-Wallis test with the Bonferroni correction (c) or by student *t*- test (h,i).

FIGs. 5A-5H demonstrate active RNA Pol II recruitment and transcription within RANKL-sensitive super-enhancers in human osteoclasts. (a) Heatmaps show the enrichment of RNA Pol II ChIP-seq signals in RANKL-sensitive enhancer regions. (b) Distribution of RNA Pol II ChIP-seq signals across RANKL-sensitive TEs and SE domains in the indicated conditions. (c) The box plots depict quantitation of normalized tag counts of RNA pol II ChIP-seq signals in TE and SE domains in the indicated conditions. (d) The box plots depict the quantitation of nascent transcripts of nuclear RNA-seq signals in TE and SE domains in the indicated conditions. (e) Scatter plot for transcript levels of the genes associated with RANKL-sensitive SEs depending on whether those are upregulated or downregulated. Dark dots indicate the genes related to RANKL-induced SEs, while blue dots indicate the genes related to RANKL-suppressed SEs. (g) Volcano plot of RNA-seq analysis of differentially expressed SE-associated genes. Light dots show genes associated with up-regulated SEs and dark dots show genes associated with down-regulated SEs. (g, h) Representative tracks of RNA Pol II ChIP-seq (g) and RNA-seq (h) in the vicinity of NFATc1, PRDM1, and MYC loci in the indicated conditions. Gray boxes depict the RANKL-sensitive SE region. ***; $p<0.001$, n.s.: not significant by Kruskal-Wallis test with the Bonferroni correction (c, d).

FIGs. 6A-6G show identification of dREG peaks in human osteoclasts. (a) Representative tracks of pro-seq at NFATc1-associated SE domain in the indicated conditions (CTRL: controls and RANKL: RANKL treated conditions). Up-regulated dREG peaks of pro-seq are shown. (b) A pie chart showing the genomic location of RANKL-inducible dREG peaks from pro-seq data. A bar graph is depicted to number of dREG peaks at TE and SE domains. (c) Aggregate plots showing mean Pro-seq, H3K27ac, Pol II ChIP-seq, and ATAC-seq signals centered on up-regulated dREG peaks in the indicated

conditions. (d) Box plots showing Pro-seq, H3K27ac, Pol II ChIP-seq, and ATAC-seq signals centered on up-regulated dREG peaks in the indicated conditions. ***; $p<0.001$ by Kruskal-Wallis test with the Bonferroni correction. (e, f) Motif analysis of RANKL-induced (e) and RANKL-suppressed (f) dREG peaks. (g) Box plots showing Pro-seq signals across RANKL-induced SE domains in the indicated conditions.

FIGs. 7A-7K show RANKL-induced SE-eRNAs are enriched in synovial CD14+ cells from RA patients. (a) Disease ontology for dREG peak-associated genes with corresponding adjusted p-value. (b, c) RT-qPCR analysis of NFATc1 during osteoclastogenesis (b) or RA synovial CD14+ cells (c), normalized by TBP mRNA. (d) Applications of fluorescence in situ hybridization (FISH) in human osteoclasts. DAPI and signals for SE-eRNA:NFATc1 are shown. Scale bar: 50 μm. (e-h) The expression of SE-eRNA:NFATc1 is knocked down by electroporation with antisense-LNA GapmeR in human OCPs. (n=3) (e) The expression of SE-eRNA:NFATc1 was measured by RT-qPCR in the indicated conditions. (f) Osteoclastogenesis assay. Cells were subsequently cultured with M-CSF and RANKL for three days. Left panel shows representative images of TRAP-stained cells. Right panel shows the percentage of TRAP-positive multinuclear cells (MNCs: more than three nuclei) per control. Scale bar: 200 μm. (g) NFATc1 mRNA were measured by RT-qPCR in the indicated conditions. (h) Immunoblot analysis of NFATc1 expression by human CD14+ cells transfected with control or SE-eRNA:NFATc1-specific siRNA and treated for 24 h with RANKL. α-tubulin was served as a loading control. (i) Box plots showing pro-seq signals within RANKL-induced SE derived from 2 different RA synovial CD14+ cells (RA-1, RA-2) and CD14+ cells from healthy control (CTRL). (j) Representative tracks for nuclear seq of RA synovial CD14+ cells in vicinity of NFATc1 (RA: RA synovial OCPs, CTRL: disease control). (k) SE-eRNA:NFATc1 was measured using RT-qPCR (see Methods). The data are shown as means ± SEM. *; $p< 0.05$, **; $p< 0.01$, ****; $p< 0.0001$, n.s.: not significant by one way ANOVA (b, e, g) or by student *t*-test (c, f, k) or by Kruskal-Wallis test with the Bonferroni correction (i).

FIGs. 8A – 8B provides a mapping of RANKL-sensitive SEs in human osteoclasts. FIG. 8A is a Table showing the correlation between biological replicates for H3K27ac, Pol II ChIP-seq, ATAC-seq experiments. The Pearson correlation coefficient was estimated based on the average read densities in 4kb regions around the TSS of RefSeq. FIG 8B shows gene ontology for downregulated SEs with corresponding adjusted p-value.

FIGs. 9A – 9B provides Cell-type specific SEs in osteoblast and CD4+ T cell. Gene ontology for osteoblast-specific (a) and CD4+ T cell-specific (b) SEs with corresponding adjusted p-value.

FIGs. 10A-10C provide Transcription Factors at RANKL-sensitive SEs. FIG 10A mRNA expression of NFATc1, PPARGC1B, and ITGAV obtained from publicly available data SRP047069. (b) Overall sequencing coverage of H3K27ac and ATAC-seq around Fos and NFkB binding motifs. Dotted line, dashed line, and solid lines represent coverage around motifs genome-wide, motifs within TEs, and motifs within SEs, respectively. The black line represents the control group (CTRL) and the gray line represents the RANKL-treated group. The solid line represents super-enhancer and the dotted line represents typical enhancer (TE). (c) Human BATF1 or 3 expressions are measured by real time-qPCR. Cells were cultured with RANKL for the indicated times. *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$, n.s.: not significant by Student's *t*-test (a) or one way ANOVA with *post-tukey* test (c).

FIG. 11 provides RANKL-induced SE-eRNA expression in human osteoclasts. Representative tracks of pro-seq (upper tracks) and RNA-seq (bottom tracks, RA: RA synovial OCPs, CTRL: disease control) at PRDM-associated (a), MYC-associated (b) SE domain in the indicated conditions. Up-regulated dREG peaks of pro-seq (brown) are shown.

FIGs. 12A-F demonstrate the role of SE-eRNA:NFATc1 in human osteoclasts. The expression of SE-eRNA:NFATc1 was knocked down by electroporation with ASO-LNA in human OCPs. Cells were subsequently cultured with M-CSF (20ng/ml) and RANKL (40ng/ml), n=4. (a) The expression of SE-eRNA:NFATc1 was measured by RT-qPCR in the indicated conditions after RANKL stimulation for 24 h. (b) Osteoclastogenesis assay. Left panel shows representative images of TRAP-stained cells. Right panel shows the percentage of TRAP positive multinuclear cells (MNCs: more than three nuclei) per control. Scale bar: 200 μm. (c) NFATc1 mRNA were measured by RT-qPCR in the indicated conditions after RANKL stimulation for 24 h. (d) Immunoblot analysis of NFATc1 expression in the indicated conditions after RANKL stimulation for 24 h. α-tubulin served as a loading control. Representative results from three donors are shown. (e) SE-eRNA:NFATc1 was measured using real-time PCR in presence or absence of I-BET151 (500 nM) after RANKL stimulation for 24 h. (f) Representative tracks for nuclear seq of RA synovial CD14+ cells in vicinity of PRDM1 and MYC (RA: RA synovial OCPs, CTRL: disease control). *: $p< 0.05$, **$p< 0.01$, ***: $p< 0.001$, ****: $p< 0.0001$ by one way ANOVA with *post-tukey* test (a,c,e) or by Student's *t*-test (b).

DETAILED DESCRIPTION OF THE INVENTION

The compositions provided herein are useful in therapies for treating rheumatoid arthritis, osteoporosis, bone and joint destruction associated disorders, and other disorders associated with osteoclastogenesis.

As described in the examples below, novel RANKL-responsive human osteoclast-specific super enhancers (SEs) and SE-associated enhancer RNAs (SE-eRNAs) have been identified using an integrated analysis of ChIP-seq, ATAC-seq, nuclear RNA-seq and Pro-seq. RANKL induced 200 SEs, large clusters of enhancers, while suppressing 148 SEs compared to macrophages. RANKL-responsive SEs are strongly correlated with genes in the osteoclastogenic program and are increased selectively in human osteoclasts but are marginally presented in osteoblasts, CD4+ T cells, or CD34+ cells. In addition to the known major transcription factors in osteoclasts, BATF binding motifs were found to be highly enriched in RANKL-responsive SEs. The depletion of BATF1/3 inhibited RANKL-induced osteoclast differentiation. Furthermore, increased chromatin accessibility was found within SE regions, where RNA polymerase II was significantly recruited to induce extragenic transcription of SE-eRNAs in human osteoclasts. Knockdown of SE-eRNAs in the vicinity of NFATc1 gene diminished expression of NFATc1, a major regulator of osteoclasts, and osteoclastogenesis. Inhibiting BET proteins suppressed the formation of RANKL-responsive SEs and NFATc1-associated SEs and the expression of SE-eRNA:NFATc1. Moreover, SE-eRNA:NFATc1 was highly expressed in synovial macrophages of rheumatoid arthritis patients exhibiting high-osteoclastogenic potential. The genome-wide analysis described herein identified RANKL-inducible SE-eRNAs as an osteoclast-specific signature, permitting the development of osteoclast-specific therapeutic intervention.

In certain embodiments, the DNA sense (positive (+)) strand or its complementary strand (-), or a transcript thereof (an RNA), may be targeted by an agent as provided herein which interferes with binding to the osteoclast-specific Nuclear Factor of Activated T cells, c1 (NFATc1) regulatory region. In certain embodiments, osteoclast-specific NFATc1 refers to a sequence which, when targeted, modulates the expression of NFATc1-associated SEs, the expression of SE-eRNA:NFATc1, and/or RANKL-inducible SE-eRNAs in osteoclasts. Suitably, the knockdown of the targeted region defined herein avoids undesirable off-target modulation of one or more of osteoblasts, CD4+ T cells and/or CD34+ cells.

In certain embodiments, the osteoclast-specific NFATc1 regulatory region in human chromosome 18 (e.g., 18q23) is targeted by an agent which interferences with its function. In certain embodiments, the target sequence is located in one or more of: chr18: position 8966290 to 9255580; chr18 position 59836471 to 60067649; and/or chr18 position 77322750 to 77406946, or a transcript thereof.

In certain embodiments, the agent is an ASO, RNAi, small interfering RNA (siRNA), microRNA (miRNA), or another interfering sequence which targets the osteoclast-specific regulatory region. In certain embodiments, the ASOs are designed as gapmer ASO's.

"Gapmer" means an ASO comprising an internal region having a plurality of nucleosides that support RNase H cleavage positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings." In certain embodiments, at least one antisense oligonucleotide in a composition of the invention is a gapmer.

In certain embodiments, the ASOs and other therapeutic agents (e.g., RNAi) described herein are targeted to sequences which interfere the osteoclast-specific Nuclear Factor of Activated T cells, c1 (NFATc1) regulatory region. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of at least 12 consecutive nucleotides of SEQ ID NO: 1 or 2, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of at least 18 consecutive nucleotides of SEQ ID NO: 1, or comprising at least 12 or 15 consecutive nucleotides of SEQ ID NO: 2, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of a nucleic acid sequence of at least 15, 20, 25, 30, 35, 40, 45, 50, consecutive nucleotides of SEQ ID NO: 1 or values therebetween, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain

embodiments, an ASO is selected which has a sequence (5' to 3') of a nucleic acid sequence of at least 15, 20, 25, 30, 35, 40, 45, 50, consecutive nucleotides of SEQ ID NO: 2 or values therebetween, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of a nucleic acid sequence comprising at least 18 to 50, or about 20, about 25, about 30, about 35, about 40, about 45, or up to 50, consecutive nucleotides comprising SEQ ID NO: 1, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof. In certain embodiments, an ASO is selected which has a sequence (5' to 3') of a nucleic acid sequence comprising at least 18 to 50, or about 20, about 25, about 30, about 35, about 40, about 45, or up to 50, consecutive nucleotides comprising SEQ ID NO: 2, a sequence at least 99% identical thereto, a sequence having at least 95% complementarity thereto, or a pharmaceutically acceptable salt thereof.

In certain embodiments, combinations of two or more different ASOs targeted to one, two or all three of the positions identified above provided.

In certain embodiments, the at least one agent comprises an ASO of 15 to 30 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising a sequence having at least 95% complementarity to SEQ ID NO: 1 or 2, or a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof.

In certain embodiments, a composition and/or a therapeutic regimen comprises an ASO of 15 to 30 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising: combinations of a sequence having at least 95% complementarity to SEQ ID NO: 1 or 2, or a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof.

In certain embodiments, a composition comprises a combination of an ASO having 100% complementarity to SEQ ID NO: 1 and an ASO having 100% complementarity to SEQ ID NO: 2.

In certain embodiments, a composition comprises combinations of ASOs, combinations of one or more different ASOs with another agent having therapeutic effect, and/or combinations of the ASOs or another interfering agent as provided herein with gene replacement therapy and/or other therapies useful for treating osteoclastogenesis disorders, osteoporosis, joint or bone destruction, rheumatoid arthritis, or other disorders.

As described herein, an ASO or another moiety may be in the form of a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salts" includes salts of the active compounds (agents, e.g., ASOs) that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When the compounds contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolyl- sulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, e.g., Berge et al., Journal of Pharmaceutical Science 66: 1 - 19 (1977)). Certain specific compounds contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. Other pharmaceutically acceptable carriers known to those of skill in the art are suitable. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder that is combined with buffer prior to use.

Provided herein are additionally or alternatively, other therapeutic agents. The agent may be any suitable genetic element or chemical moiety including, e.g., an anti-sense oligonucleotide (ASO), an RNAi, or combinations thereof. In certain embodiments, the agent is engineered to be delivered via a viral vector or another genetic element. Suitable viral vectors may include, e.g., selected from a recombinant parvovirus, a recombinant lentivirus, or non-viral vector. Additionally or alternatively, a non-viral vector may be selected which

comprises one or more agent(s). In certain embodiments, the non-viral vector is a lipid nanoparticle, lipidoid, or liposome.

As used herein, an "antisense oligonucleotide" or "ASO" means an oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid or region or segment thereof. An antisense oligonucleotide is specifically hybridizable to a target nucleic acid or region or segment thereof, the hybridization of which results in RNase H mediated cleavage of the target nucleic acid.

As used herein, enhancer RNAs (eRNAs) are relatively long (about 500 bp to about 2000 bp in length) non-coding RNA (ncRNA) molecules transcribed from the DNA sequence of enhancer regions. These eRNA may function independently of orientation. The term "SE" refers to super enhancers. The term SE-eRNAs refers to super enhancer-associated eRNAs.

"Contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other in a sequence.

"Portion" refers to a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an oligomeric compound.

The term "complementary" is used to describe the relationship between nucleotide bases and/or polynucleotides that are capable of hybridizing to one another, e.g., the nucleotide sequence of such polynucleotides or one or more regions thereof matches the nucleotide sequence of another polynucleotide or one or more regions thereof when the two nucleotide sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, include the following pairs: adenine (A) with thymine (T), adenine (A) with uracil (U), cytosine (C) with guanine (G), and 5-methyl cytosine (^{m}C) with guanine (G). Complementary polynucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. Accordingly, the present disclosure also includes isolated polynucleotides that are complementary to sequences as disclosed or used herein as well as those substantially similar nucleic acid sequences. The degree to which two polynucleotides have matching nucleobases can be expressed in terms of "percent complementarity" or

"percent complementary." In some embodiments, a polynucleotide has 70%, at least 70%, 75%, at least 75%, 80%, at least 80%, 85%, at least 85%, 90%, at least 90%, 95%, at least 95%, 97%, at least 97%, 98%, at least 98%, 99%, or at least 99% or 100% complementarity with another polynucleotide or a target nucleic acid provided herein. In embodiments wherein two polynucleotides or a polynucleotide and a target nucleic acid are "fully complementary" or "100% complementary," such polynucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches. Unless otherwise indicated, percent complementarity is the percent of the nucleobases of the shorter sequence that are complementary to the longer sequence.

An ASO or RNA agent may contain one or more mismatches to the target sequence. In one aspect, the sequence as described herein contains no more than 3 mismatches. If the sequence contains mismatches to a target sequence, in some aspects, the area of mismatch is not located in the center of the region of complementarity. If the oligonucleotide contains mismatches to the target sequence, in some embodiments, the mismatch should be restricted to be within the last 5 nucleotides from either the 5′- or 3′-end of the region of complementarity.

"Specifically hybridizable" refers to a polynucleotide having a sufficient degree of complementarity between the polynucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids. In certain embodiments, specific hybridization occurs under physiological conditions.

"Specifically interfering" refers to an agent which blocks binding of a protein to its native target, while having minimal or no effect on non-target nucleic acids.

"Mismatch" or "non-complementary" means a nucleobase of a first polynucleotide that is not complementary to the corresponding nucleobase of a second polynucleotide or target nucleic acid when the first and second polynucleotides are aligned. For example, nucleobases including but not limited to a universal nucleobase, inosine, and hypoxanthine, are capable of hybridizing with at least one nucleobase but are still mismatched or non-complementary with respect to nucleobase to which it hybridized. As another example, a nucleobase of a first polynucleotide that is not capable of hybridizing to the corresponding nucleobase of a second polynucleotide or target nucleic acid when the first and second polynucleotides are aligned is a mismatch or non-complementary nucleobase.

In certain embodiments, the agent comprises is an antisense oligonucleotide having at least one modified internucleoside linkage, sugar moiety, or nucleobase.

In certain embodiments, one or more ASO is a chimeric oligonucleotide having a gap segment positioned between 5′ and 3′ wing segments. In certain embodiments, the gap segment of the chimeric oligonucleotide is comprised of 2′-deoxynucleotides and the wing segments are comprised of nucleotides having modified sugar moieties. In certain embodiments, the gap segment of the chimeric oligonucleotide consists of ten 2′-deoxynucleotides and each wing segment consists of five 2′-O-methoxyethyl-modified nucleotides.

In certain embodiments, one or more ASO comprises a modified sugar moiety is 2′-OMe or a bicyclic nucleic acid.

An oligonucleotide, or pharmaceutically acceptable salt thereof, can be chemically synthesized. An oligonucleotide, or pharmaceutically acceptable salt thereof, can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

An oligonucleotide, or pharmaceutically acceptable salt thereof, compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide, or pharmaceutically acceptable salt thereof, comprising unnatural or alternative nucleotides can be easily prepared. A single-stranded oligonucleotide, or pharmaceutically acceptable salt thereof, can be prepared using solution-phase or solid-phase organic synthesis or both.

In some embodiments, the oligonucleotide, or contiguous nucleotide region thereof, has a gapmer design or structure also referred herein merely as “gapmer.” In a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5′-flanking sequence (also known as a 5′-wing), a DNA core sequence (also known as a gap) and a 3′-flanking sequence (also known as a 3′-wing), in ‘5->3’ orientation. In this design, the 5′ and 3′ flanking sequences comprise at least one alternative nucleoside which is adjacent to a DNA core sequence, and can, in some aspects, comprise a contiguous stretch of 2 to 7 alternative nucleosides, or a contiguous stretch of alternative and DNA nucleosides (mixed flanking sequences comprising both alternative and DNA nucleosides).

The length of the 5′-flanking sequence region can be at least two nucleosides in length (e.g., at least at least 2, at least 3, at least 4, at least 5, at least 6, or more nucleosides in length). The length of the 3′-flanking sequence region can be at least two nucleosides in length (e.g., at least 2, at least 3, at least at least 4, at least 5, at least 6, or more nucleosides

in length). The 5′ and 3′ flanking sequences can be symmetrical or asymmetrical with respect to the number of nucleosides they comprise. In some aspects, the DNA core sequence comprises about 10 nucleosides flanked by a 5′ and a 3′ flanking sequence each comprising about 5 nucleosides. In some aspects, the DNA core sequence comprises about 11 nucleosides flanked by a 5′ and a 3′ flanking sequence each comprising about 5 or about 6 nucleosides. In some aspects, the DNA core sequence comprises about 12 nucleosides flanked by a 5′ sequence comprising about 5 nucleosides, and a 3′ flanking sequence comprising about 6 nucleosides. In some aspects, the DNA core sequence comprises about 12 nucleosides flanked by a 5′ sequence comprising about 6 nucleosides, and a 3′ flanking sequence comprising about 5 nucleosides. In some aspects, the DNA core sequence comprises about 12 nucleosides flanked by a 5′ and a 3′ flanking sequence each comprising about 6 nucleosides.

"2′-deoxyfuranosyl sugar moiety" or "2′-deoxyfuranosyl sugar" means a furanosyl sugar moiety having two hydrogens at the 2′-position. 2′-deoxyfuranosyl sugar moieties may be unmodified or modified and may be substituted at positions other than the 2′-position or unsubstituted. A β-D-2′-deoxyribosyl sugar moiety in the context of an oligonucleotide is an unsubstituted, unmodified 2′-deoxyfuranosyl and is found in naturally occurring deoxyribonucleic acids (DNA).

"2′-deoxynucleoside" means a nucleoside comprising 2′-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In certain embodiments, a 2′-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

"2′-O-methoxyethyl" (also 2′-MOE) refers to a 2′-O$(CH_2)_2$—OCH_3) in the place of the 2′—OH group of a ribosyl ring. A 2′-O-methoxyethyl modified sugar is a modified sugar.

"2′-MOE nucleoside" (also 2′-O-methoxyethyl nucleoside) means a nucleoside comprising a 2′-MOE modified sugar moiety.

"2′-substituted nucleoside" or "2-modified nucleoside" means a nucleoside comprising a 2′-substituted or 2′-modified sugar moiety. As used herein, "2′-substituted" or "2-modified" in reference to a sugar moiety means a sugar moiety comprising at least one 2′-substituent group other than H or OH.

"5-methylcytosine" means a cytosine with a methyl group attached to the 5 position. A 5-methyl cytosine is a modified nucleobase.

"Overhanging nucleosides" refers to unpaired nucleotides at either or both ends of a duplex formed by hybridization of an antisense RNAi oligonucleotide and a sense RNAi oligonucleotide.

The 5′ and 3′ flanking sequences, flanking the 5′ and 3′ ends of an ASO core sequence (e.g., SEQ ID NO: 1, 2, 3 or 4), can comprise one or more affinity enhancing alternative nucleosides. In some aspects, the 5′ and/or 3′ flanking sequence comprises at least one 2′-O-methoxyethyl (MOE) nucleoside. In some aspects, the 5′ and/or 3′ flanking sequences, contain at least two MOE nucleosides. In some aspects, the 5′ flanking sequence comprises at least one, at least two, at least three, at least four, at least five, or at least six or more MOE nucleosides. In some aspects, the 5′ flanking sequence comprises at least one, at least two, at least three, at least four, at least five, or at least six or more MOE nucleosides. In some aspects, both the 5′ and 3′ flanking sequence comprise a MOE nucleoside. In some aspects, all the nucleosides in the flanking sequences are MOE nucleosides. In other aspects, the flanking sequence can comprise both MOE nucleosides and other nucleosides (mixed flanking sequence), such as DNA nucleosides and/or non-MOE alternative nucleosides, such as bicyclic nucleosides (BNAs) (e.g., LNA nucleosides (e.g., A-LNA, 5mC L-NA, G-LNA, T-LNA) or cET nucleosides), or other 2′ substituted nucleosides. In this case the DNA core sequence is defined as a contiguous sequence of at least 5 RNase H recruiting nucleosides (such as 5 to 16 DNA nucleosides or gapmers) flanked at the 5′ and 3′ end by an affinity enhancing alternative nucleoside, such as an MOE nucleoside.

In certain embodiments, the 5′ and/or 3′ flanking sequence comprises at least one BNA (e.g., at least one LNA nucleoside (e.g., A-LNA, 5mC L-NA, G-LNA, T-LNA) or cET nucleoside). In some embodiments, 5′ and/or 3′ flanking sequence comprises at least 2 bicyclic nucleosides. In some embodiments, the 5′ flanking sequence comprises at least one BNA. In some embodiments, both the 5′ and 3′ flanking sequence comprise a BNA. In some aspects, all the nucleosides in the flanking sequences are BNAs. In other aspects, the flanking sequence can comprise both BNAs and other nucleosides (mixed flanking sequences), such as DNA nucleosides and/or non-BNA alternative nucleosides, such as 2′ substituted nucleosides. In this case the DNA core sequence is defined as a contiguous sequence of at least five RNase H recruiting nucleosides (such as 5-16 DNA nucleosides) flanked at the 5′ and 3′ end by an affinity enhancing alternative nucleoside, such as a BNA, such as an LNA, such as beta-D-oxy-LNA.

The 5′ flank attached to the 5′ end of the DNA core sequence comprises, contains, or consists of at least one alternative sugar moiety (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative sugar moieties). In some aspects, the flanking sequence comprises or consists of from 1 to 7 alternative nucleobases, such as from 2 to 6 alternative nucleobases, such as from 2 to 5 alternative nucleobases, such as from 2 to 4 alternative nucleobases, such as from 1 to 3 alternative nucleobases, such as one, two, three or four alternative nucleobases. In some aspects, the flanking sequence comprises or consists of at least one alternative internucleoside linkage (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

The 3′ flank attached to the 3′ end of the DNA core sequence comprises, contains, or consists of at least one alternative sugar moiety (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative sugar moieties). In some aspects, the flanking sequence comprises or consists of from 1 to 7 alternative nucleobases, such as from 2 to 6 alternative nucleobases, such as from 2 to 5 alternative nucleobases, such as from 2 to 4 alternative nucleobases, such as from 1 to 3 alternative nucleobases, such as one, two, three, or four alternative nucleobases. In some aspects, the flanking sequence comprises or consists of at least one alternative internucleoside linkage (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

In an aspect, one or more or all of the alternative sugar moieties in the flanking sequence are 2′ alternative sugar moieties.

In a further aspect, one or more of the 2′ alternative sugar moieties in the wing regions are selected from 2′-O-alkyl-sugar moieties, 2′-O-methyl-sugar moieties, 2′-amino-sugar moieties, 2′-fluoro-sugar moieties, 2′-alkoxy-sugar moieties, MOE sugar moieties, LNA sugar moieties, arabino nucleic acid (ANA) sugar moieties, and 2′-fluoro-ANA sugar moieties.

In one aspect, all the alternative nucleosides in the flanking sequences are bicyclic nucleosides. In a further aspect, the bicyclic nucleosides in the flanking sequences are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET, and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof.

In some aspects, the one or more alternative internucleoside linkages in the flanking sequences are phosphorothioate internucleoside linkages. In some aspects, the phosphorothioate linkages are stereochemically pure phosphorothioate linkages. In some aspects, the phosphorothioate linkages are Sp phosphorothioate linkages. In other aspects,

the phosphorothioate linkages are Rp phosphorothioate linkages. In some aspects, the alternative internucleoside linkages are 2′-alkoxy internucleoside linkages. In other aspects, the alternative internucleoside linkages are alkyl phosphate internucleoside linkages.

In certain embodiments, ASOs or RNA are chemically linked or encapsulated in one or more ligands, moieties, or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, an aliphatic chain, e.g., dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or the like.

The oligonucleotides used in the conjugates can be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art can additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In certain embodiments, compositions provided herein comprise polynucleotides (e.g., ASOs or RNA transcripts, e.g., siRNA, RNAi, or miRNA) linked or encapsulated in a lipid or lipid-like particle, polymer, or other non-viral delivery system. For example, sequences may be encapsulated in a lipid nanoparticle (LNP). As used herein, the phrase "lipid nanoparticle" refers to a transfer vehicle comprising one or more lipids (e.g., cationic lipids, non- cationic lipids, and PEG-modified lipids). Preferably, the lipid nanoparticles are formulated to deliver one or more ASOs and/or siRNA or RNAi to one or more target cells. Examples of suitable lipids include, for example, the phosphatidyl compounds (e.g., phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides). Also contemplated is the use of polymers as transfer vehicles, whether alone or in combination with other transfer vehicles. Suitable polymers may include, for example, polyacrylates, polyalkycyanoacrylates, polylactide, polylactide- polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins, dendrimers and polyethylenimine. In one embodiment, the transfer vehicle is selected based upon its ability to facilitate the transfection of a target cell. Useful lipid nanoparticles for a cationic lipid to encapsulate and/or enhance the delivery of an siRNA (RNAi, or ASO) into the target cell. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH. The contemplated lipid

nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing one or more cationic lipids, non-cationic lipids and PEG- modified lipids. Several cationic lipids have been described in the literature, many of which are commercially available. See, e.g., WO2014/089486, US 2018/0353616A1, and US 8,853,377B2, which are incorporated by reference. In certain embodiments, LNP formulation is performed using routine procedures comprising cholesterol, ionizable lipid, helper lipid, PEG-lipid and polymer forming a lipid bilayer around encapsulated mRNA (Kowalski et al., 2019, Mol. Ther. 27(4):710-728). In some embodiments, LNP comprises a cationic lipids (i.e. N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)) with helper lipid DOPE. In some embodiments, LNP comprises an ionizable lipid Dlin-MC3-DMA ionizable lipids, or diketopiperazine-based ionizable lipids (cKK-E12). In some embodiments, polymer comprises a polyethyleneimine (PEI), or a poly(β-amino)esters (PBAEs). See, e.g., WO2014/089486, US 2018/0353616A1, US2013/0037977A1, WO2015/074085A1, US9670152B2, and US 8,853,377B2, which are incorporated by reference.

In certain embodiment, a non-viral vector is used for delivery of ASO. In some embodiments, the ASO and/or RNAi is delivered at an amount greater than about 0.5 mg/kg (e.g., greater than about 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 4.0 mg/kg, 5.0 mg/kg, 6.0 mg/kg, 7.0 mg/kg, 8.0 mg/kg, 9.0 mg/kg, or 10.0 mg/kg) body weight per dose. In some embodiments, the ASO and/or RNAi is delivered at an amount ranging from about 0.1-100 mg/kg (e.g., about 0.1-90 mg/kg, 0.1-80 mg/kg, 0.1-70 mg/kg, 0.1-60 mg/kg, 0.1-50 mg/kg, 0.1-40 mg/kg, 0.1-30 mg/kg, 0.1-20 mg/kg, 0.1-10 mg/kg) body weight per dose. In some embodiments, the ASO and/or RNAi is delivered at an amount of or greater than about 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, or 500 mg per dose.

In certain embodiments, ASOs and/or RNAi are encapsulated in a lipid nanoparticle (LNP). As used herein, the phrase "lipid nanoparticle" refers to a transfer vehicle comprising one or more lipids (e.g., cationic lipids, non- cationic lipids, and PEG-modified lipids). Preferably, the lipid nanoparticles are formulated to deliver one or more miRNA to one or more target cells (e.g., osteoclasts or other cell types identified above in the bone, joint, CNS). Examples of suitable lipids include, for example, the phosphatidyl compounds (e.g., phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine,

sphingolipids, cerebrosides, and gangliosides). Also contemplated is the use of polymers as transfer vehicles, whether alone or in combination with other transfer vehicles. Suitable polymers may include, for example, polyacrylates, polyalkycyanoacrylates, polylactide, polylactide- polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins, dendrimers and polyethylenimine. In one embodiment, the transfer vehicle is selected based upon its ability to facilitate the transfection of an agent (e.g., ASO, miRNA, and/or siRNA) to a target cell. Useful lipid nanoparticles for and agent comprise a cationic lipid to encapsulate and/or enhance the delivery of the agent into the target cell that will act as a depot for protein production. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH. The contemplated lipid nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing one or more cationic lipids, non-cationic lipids and PEG- modified lipids. Several cationic lipids have been described in the literature, many of which are commercially available. See, e.g., WO2014/089486, US 2018/0353616A1, and US 8,853,377B2, which are incorporated by reference. In certain embodiments, LNP formulation is performed using routine procedures comprising cholesterol, ionizable lipid, helper lipid, PEG-lipid and polymer forming a lipid bilayer around encapsulated mRNA (Kowalski et al., 2019, Mol. Ther. 27(4):710-728). In some embodiments, LNP comprises a cationic lipids (i.e. N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), or 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)) with helper lipid DOPE. In some embodiments, LNP comprises an ionizable lipid Dlin-MC3-DMA ionizable lipids, or diketopiperazine-based ionizable lipids (cKK-E12). In some embodiments, polymer comprises a polyethyleneimine (PEI), or a poly(β-amino)esters (PBAEs). See, e.g., WO2014/089486, US 2018/0353616A1, US2013/0037977A1, WO2015/074085A1, US9670152B2, and US 8,853,377B2, which are incorporated by reference.

Recombinant Viral Vectors

In some embodiments, one or more nucleic acid sequences provided herein may be delivered via a recombinant, replication-defective viral vector (e.g, a lentivirus, adenovirus, adeno-associated virus). In certain embodiments, a viral vector may further comprise one or more additional sequences for delivery to a subject.

In certain embodiments, an agent (e.g., an ASO or RNAi) is expressed from a recombinant adeno-associated virus, which has an AAV capsid and a vector genome packaged in the AAV capsid. In certain embodiments, a "vector genome" refers to the nucleic acid sequence packaged inside a parvovirus (e.g., rAAV) capsid which forms a viral particle. Such a nucleic acid sequence contains AAV inverted terminal repeat sequences (ITRs). In the examples herein, a vector genome contains, at a minimum, from 5' to 3', an AAV 5' ITR, coding sequence(s), and an AAV 3' ITR. ITRs from AAV2, a different source AAV than the capsid, or other than full-length ITRs may be selected. In certain embodiments, the ITRs are from the same AAV source as the AAV which provides the rep function during production or a transcomplementing AAV. Further, other ITRs, e.g., self-complementary (scAAV) ITRs, may be used. Further, the vector genome contains regulatory sequences which direct expression of the gene products. Suitable components of a vector genome are discussed in more detail herein.

Construction of vectors for expression of polynucleotides can be accomplished using conventional techniques. For generation of efficient expression vectors, it is necessary to have regulatory sequences that control the expression of the polynucleotide. These regulatory sequences include, e.g., at least one promoter, a poly A signal, and various other vector elements (e.g., one or more of each, an enhancer, an intron, a post-translational regulatory element) and are influenced by specific cellular factors that interact with these sequences, and are well known in the art.

As used herein, an "expression cassette" refers to a nucleic acid molecule which comprises a coding sequence, promoter, and may include other regulatory sequences therefor. In certain embodiments, a vector genome may contain two or more expression cassettes. In other embodiments, the term "transgene" may be used interchangeably with "expression cassette".

Dosages of the vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of viral vector is generally in the range of from about 25 to about 1000 microliters to about 100 mL of solution containing concentrations of from about 1 x 10^9 to 1 x 10^{16} genomes virus vector (to treat an average subject of 70 kg in body weight) including all integers or fractional amounts within the range, and preferably 1.0 x 10^{12} GC to 1.0 x 10^{14} GC for a human patient. In one embodiment, the compositions are formulated to contain at least 1x10^9, 2x10^9, 3x10^9, 4x10^9,

$5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, or $9x10^9$ GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, or $9x10^{10}$ GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least $1x10^{11}$, $2x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $6x10^{11}$, $7x10^{11}$, $8x10^{11}$, or $9x10^{11}$ GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least $1x10^{12}$, $2x10^{12}$, $3x10^{12}$, $4x10^{12}$, $5x10^{12}$, $6x10^{12}$, $7x10^{12}$, $8x10^{12}$, or $9x10^{12}$ GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least $1x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $7x10^{13}$, $8x10^{13}$, or $9x10^{13}$ GC per dose including all integers or fractional amounts within the range. In one embodiment, for human application the dose can range from $1x10^9$ to about $1x10^{13}$ GC per dose including all integers or fractional amounts within the range.

In certain embodiments, the vector is administered to a subject in a single dose. In certain embodiments, vector may be delivered via multiple injections (for example 2 doses) if desired.

The composition for delivery may contain a buffered saline aqueous solution. In certain embodiments, the composition does not comprise sodium bicarbonate. Examples of suitable buffered saline aqueous solutions comprising one or more of sodium phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium chloride and mixtures thereof, in water, such as a Harvard's buffer. The aqueous solution may further contain Kolliphor® P188, a poloxamer which is commercially available from BASF which was formerly sold under the trade name Lutrol® F68. The aqueous solution may have a pH of 7.2 or a pH of 7.4. In another embodiment, the formulation may contain a buffered saline aqueous solution comprising 1 mM Sodium Phosphate (Na3PO4), 150 mM sodium chloride (NaCl), 3mM potassium chloride (KCl), 1.4 mM calcium chloride (CaCl2), 0.8 mM magnesium chloride (MgCl2), and 0.001% Kolliphor® 188. See, e.g., harvardapparatus.com/harvard-apparatus-perfusion-fluid.html. In certain embodiments, Harvard's buffer is preferred. In other embodiments, the formulation may contain one or more permeation enhancers. Examples of suitable permeation enhancers may include, e.g., mannitol, sodium glycocholate, sodium taurocholate, sodium deoxycholate, sodium

salicylate, sodium caprylate, sodium caprate, sodium lauryl sulfate, polyoxyethylene-9-laurel ether, or EDTA.

In another embodiment, the composition includes a carrier, diluent, excipient and/or adjuvant. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The buffer/carrier should include a component that prevents the rAAV, from sticking to the infusion tubing but does not interfere with the rAAV binding activity in vivo.

Optionally, the compositions may contain, in addition to the vector and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host. Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present invention into suitable host cells. In particular, the rAAV vector delivered transgenes may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

In one embodiment, a composition includes a final formulation suitable for delivery to a subject, e.g., is an aqueous liquid suspension buffered to a physiologically compatible pH and salt concentration. Optionally, one or more surfactants are present in the formulation. In another embodiment, the composition may be transported as a concentrate which is diluted for administration to a subject. In other embodiments, the composition may be lyophilized and reconstituted at the time of administration.

In certain embodiments, a method for treating a human with an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis is provided. In certain embodiments, use of a composition as described herein in preparing a medicament is provided. In certain embodiments, a composition suitable for treating an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis is provided.

In certain embodiments, the composition(s) comprises use of at least one agent which specifically binding to a human osteoclast-specific NFATc1 regulatory region (e.g., the target sequence is located in one or more of: chr18: position 8966290 to 9255580; chr18 position 59836471 to 60067649; and/or chr18 position 77322750 to 77406946, or a transcript thereof). In certain embodiments, the at least one agent is at least one anti-sense oligonucleotide, at least one RNAi, at least one siRNA, or combinations thereof. In certain embodiments, the agent is delivered via a viral vector (e.g., AAV) or a non-viral vector or other carrier, e.g., a lipid nanoparticle, lipidoid, or liposome.

Various of these agents are described herein. For example, in certain embodiments, the at least one agent is at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising: (a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof; (b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof; (c) a sequence comprising a sequence having at least 95% complementarity to SEQ ID NO: 1 or 2, or a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or (d) combinations of (a), (b), or (c). In certain embodiments, the method comprises use of at least one antisense oligonucleotide comprises 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1. In certain embodiments, the method comprises use of at least one antisense oligonucleotide comprises 18 to 30 nucleotides in length which comprises SEQ ID NO: 2. In certain embodiments, the method comprises use of an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID

NO: 2. In certain embodiments, method comprises use of an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In certain embodiments, a composition as provided herein is delivered intravenously, via site-specific administration to a joint, via targeted delivery to the bone, or intrathecally.

As used herein, the term "operably linked" refers to both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

The term "heterologous" when used with reference to a protein or a nucleic acid indicates that the protein or the nucleic acid comprises two or more sequences or subsequences which are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid. For example, in one embodiment, the nucleic acid has a promoter from one gene arranged to direct the expression of a coding sequence from a different gene. Thus, with reference to the coding sequence, the promoter is heterologous.

Identity or similarity with respect to a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (i.e., same residue) or similar (i.e., amino acid residue from the same group based on common side-chain properties, see below) with the peptide and polypeptide regions provided herein, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Percent (%) identity is a measure of the relationship between two polynucleotides or two polypeptides, as determined by comparing their nucleotide or amino acid sequences, respectively. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. The alignment of the two sequences is examined and the number of positions giving an exact amino acid or nucleotide correspondence between the two sequences determined, divided by the total length of the alignment and multiplied by 100 to give a % identity figure. This % identity figure may be determined over the whole length of the sequences to be compared, which is particularly suitable for sequences of the same or very similar length and which are highly homologous, or over shorter defined lengths, which is more suitable for sequences of unequal length or which have a lower level of homology. There are a number of algorithms, and computer programs based thereon, which are available to be used the literature and/or publicly or commercially available for

performing alignments and percent identity. The selection of the algorithm or program is not a limitation.

Examples of suitable alignment programs including, e.g., the software CLUSTALW under Unix and then be imported into the Bioedit program (Hall, T. A. 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98); the Clustal Omega available from EMBL-EBI (Sievers, Fabian, et al. "Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega." Molecular systems biology 7.1 (2011): 539 and Goujon, Mickael, et al. "A new bioinformatics analysis tools framework at EMBL–EBI." Nucleic acids research 38.suppl 2 (2010): W695-W699); the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J. et al., Nucleic Acids Res., 12:387-395, 1984, available from Genetics Computer Group, Madison, Wis., USA). The programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity between two polypeptide sequences.

Other programs for determining identity and/or similarity between sequences include, e.g, the BLAST family of programs available from the National Center for Biotechnology Information (NCB), Bethesda, Md., USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov), the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used; and FASTA (Pearson W. R. and Lipman D. J., Proc. Natl. Acad. Sci. USA, 85:2444-8, 1988, available as part of the Wisconsin Sequence Analysis Package). SeqWeb Software (a web-based interface to the GCG Wisconsin Package

The term "a" or "an" refers to one or more. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

The term "about" encompasses a variation within and including ± 10%, unless otherwise specified.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

Specific embodiments

1. A composition comprising:

a pharmaceutically acceptable delivery vehicle, carrier, and/or diluent, and

at least one nucleic acid of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:

(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or

(d) combinations of (a), (b), or (c).

2. The composition according to embodiment 1, wherein at least one nucleic acid comprises 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1.

3. The composition according to embodiment 1, wherein at least one nucleic acid comprises 18 to 30 nucleotides in length which comprise at least 15 consecutive nucleotides of SEQ ID NO: 2.

4. The composition of embodiment 1 or 2, wherein said composition comprises an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 2.

5. The composition of embodiment 1 or 2, wherein said composition comprises an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

6. The composition of any one of embodiments 1 to 5 wherein the nucleic acid is an antisense oligonucleotide having at least one modified internucleoside linkage, sugar moiety, or nucleobase.

7. The composition of any one of embodiments 1 to 5, wherein the nucleic acid is a chimeric oligonucleotide having a gap segment positioned between 5′ and 3′ wing segments.

8. The composition of embodiment 6, wherein the gap segment of the chimeric oligonucleotide is comprised of 2′-deoxynucleotides and the wing segments are comprised of nucleotides having modified sugar moieties.

9. The composition according to any one of embodiments 1 to 8, wherein the composition comprises a non-viral vector comprising the nucleic acid which is a lipid nanoparticle, lipidoid, or liposome.

10. A method for treating an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis, said method comprising delivering a nucleic acid that specifically binds to a human osteoclast-specific NFATc1 regulatory region in chromosome 18.

11. The method according to embodiment 10, wherein the nucleic acid is an anti-sense oligonucleotide, an RNAi, siRNA, or combinations thereof.

12. The method according to embodiment 10 or 11, wherein the nucleic acid is delivered via a lipid nanoparticle, lipidoid, or liposome.

13. The method according to any one of embodiments 10 to 12, wherein the nucleic acid comprises at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:
(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or
(d) combinations of (a), (b), or (c).

14. The method of embodiment 13, wherein at least one antisense oligonucleotide comprises 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1.

15. The method of embodiment 13, wherein at least one antisense oligonucleotide comprises 18 to 30 nucleotides in length which comprises SEQ ID NO: 2.

16. The method of embodiment 13, wherein said composition comprises an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 2.

17. The method of embodiment 13, wherein said composition comprises an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

18. The method of embodiment 13, wherein the composition is delivered intravenously, via site-specific administration to a joint, via targeted delivery to the bone, or intrathecally.

19. Use of at least one antisense oligonucleotide (ASO) of 15 to 50 nucleotides in length in preparing a medicament, wherein the ASO comprises at least 15 consecutive nucleotides of a sequence comprising:

(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or

(d) combinations of (a), (b), or (c).

20. A composition suitable for treatment of an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis, said composition comprising at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:

(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or

(d) combinations of (a), (b), or (c).

EXAMPLES:

The following examples investigated the super enhancer (SE) landscape during human osteoclastogenesis and defined RANKL-responsive SEs and SE-associated eRNAs in human osteoclasts. Along with key osteoclastogenic transcription factors enriched in RANKL-responsive SEs, we found Basic Leucine Zipper ATF-Like Transcription Factor (BATF) binding motifs in RANKL-responsive SEs. Moreover, we discovered BATF1/3 as new positive regulators of osteoclasts. We also identified eRNAs transcribed from super-enhancers (named *SE-eRNAs*) of human osteoclasts. Furthermore, RANKL-responsive SE-eRNAs were found in RA synovial macrophages that are prone to form osteoclasts. Accordingly, knock-down of SE-eRNA in the vicinity *NFATc1* gene suppressed NFATc1 expression and RANKL-induced osteoclastogenesis. This study offers the first evidence of the potential role of RANKL-responsive SE-eRNAs in reprograming the differentiation of myeloid cells toward human osteoclasts and provides insights into the development of hyperactive osteoclasts in the pathological setting.

AP-1 Activator protein-1

ASO-LNA Antisense oligonucleotides

ATAC-seq Transposase-Accessible Chromatin with high-throughput sequencing

ATF3 Activating Transcription Factor 3 BATF Basic Leucine Zipper

ATF-Like Transcription Factor BET Bromodomain and extraterminal

BRD4 Bromodomain-containing protein 4

CAGE Cap-analysis of gene expression

ChIP-seq Chromatin immunoprecipitation (ChIP)-sequencing

Dnmt3a DNA methyltransferase 3a

dREG Regulatory-element detection from gro-seq

Elf4 Enhancer RNA E74 Like ETS Transcription Factor 4

FBS Feta1 Fetal bovine serum

Fra1 Fos-related antigen 1

H3K27ac Histone 3 acetylation at lysine 27

H3K4me1 Histone 3 mono-methylation at lysine 4

IRF8 Interferon Regulatory Factor 8

ITGAV Integrin Subunit Alpha V

KLF2 Kruppel Like Factor 2

MAFA MAF BZIP Transcription Factor A

MCSF Macrophage colony stimulating factor

MMP9 Matrix metalloproteinase-9

NFATc1 Nuclear factor of activated T cells, c1

Nuc-seq Nuclear RNA sequencing

OVX Ovariectomy

PBMC Peripheral blood mononuclear cells

Pol II RNA polymerase II

POSTN Periostin

PPARGC1B Peroxisome proliferator-activated receptor gamma coactivator 1-beta PRDM1

PR/SET Domain 1 Pro-seq Precision nuclear run-on and sequencing

RA Rheumatoid arthritis

RANKL Receptor activator of NF-κB ligand

ROSE algorithm The rank ordering of super-enhancer algorithm

RUNX2 Runt-related transcription factor 2

SAM S-adenosylmethionine

SE Super-enhancer

SE-eRNA Super-enhancer-associated enhancer RNA

SF Human synovial fluid

siRNAs Small interfering RNAs

TE Typical enhancers

TF Transcription factors

TNFRSF1B TNF receptor superfamily member 1B

TRAP Tartrate-resistant acid phosphatase

EXAMPLE 1: Materials and methods

Human studies Human synovial fluid (SF) samples were collected from RA patients as previously described[52]. The protocol was approved by the Ethics Committee of Seoul National University Hospital Institutional Review Board (1511-094-721) and by the Hospital for Special Surgery Institutional Review Board (2016-957, 2016-958, and 2016-139). The diagnosis of RA was based on the 1987 revised criteria of the American College of Rheumatology[53]. There was limited information about patients' medications, and correlation of the following findings with therapy was not possible.

Cell culture. Peripheral blood mononuclear cells (PBMCs) from blood leukocyte preparations purchased from the New York Blood Center or mononuclear cells from SF of RA patients were isolated by density gradient centrifugation with Ficoll (Invitrogen, Carlsbad, CA). $CD14^+$ cells were obtained by isolation using anti-CD14 magnetic beads, as recommended by the manufacturer (Miltenyi Biotec, CA). Human $CD14^+$ cells were cultured in α-MEM medium (Invitrogen) supplemented with 10 % fetal bovine serum (FBS, Hyclone; SH30070.03) and 1% L-glutamin with 20 ng/ml of M-CSF for 24 hours to generate osteoclast precursor cells (OCPs) The purity of monocytes was >97%, as verified by flow cytometric analysis [22].

For human osteoclastogenesis assays, cells were added to 96 well plates in triplicate at a seeding density of $5x10^4$ cells per well. Osteoclast precursors were incubated with 20 ng/ml of M-CSF and 40 ng/ml of human soluble RANKL (Peprotech, Rocky Hill, NJ) up to 5 days in α-MEM supplemented with 10 % FBS and 1% L-glutamine. Cytokines were replenished every 3 days. On each day, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using the Acid Phosphatase Leukocyte diagnostic kit (Sigma; 387A) as recommended by the manufacturer. Multinucleated (greater than 3 nuclei), TRAP-positive osteoclasts were counted in triplicate wells. All cell-cultures were performed by a modification of previously published method[22].

Gene expression analysis. For real-time qPCR, DNA free RNA was obtained using the RNeasy Mini Kit from QIAGEN with DNase treatment, and 0.5 mg of total RNA was reverse transcribed using a First Strand cDNA Synthesis kit (Fermentas, Hanover, MD). Real-time PCR was performed in triplicate using the iCycler iQ thermal cycler and detection system (Applied Biosystems, Carlsbad, CA) following the manufacturer's protocols. Expression of the tested gene was normalized relative to the levels of TBP. The primer sequences are listed in the following Table.

Gene Symbol	Sequence	SEQ ID NO:
Human TBP	CACCACAGCTCTTCCACTA	3
	GGGGAGGGATACAGTGGAGT	4
Human NFATc1	CTTCTTCCAGTATTCCACCTAT	5
	TTGCCCTAATTACCTGTTGAAG	6
SE-eRNA:NFATc1	CAATCCCATGAAACAAACGCTCA	7
	CCAGGCCCTTCGCGATT	8
Human BATF1	AAATCGTATTGCCGCCCAG	9
	TAGAGCCGCGTTCTGTTTCT	10
Human BATF3	ATGAGAGCCTGGAGCAAGAA	11

	TTCAGTGCCTCTGTCAGGTG	12

Immunoblot Whole cell extracts were prepared by lysis in buffer containing 1x Lamin sample buffer (Bio-rad) and 2-Mercaptoethanol (Sigma). Proteins were separated on 7.5 % SDS-PAGE gels, transferred to polyvinylidene difluoride membranes (PVDF, Millipore; ISEQ00010), and detected by antibodies as listed in the figure legends. NFATc1(556602, BD Pharmagen) and α-tubulin (T9026, Sigma Aldrich).

TRAP staining Cells were fixed and stained for TRAP using the Acid Phosphatase Leukocyte diagnostic kit (Sigma) as recommended by the manufacturer. Nuclei was stained with methyl green. Multinucleated (more than three nuclei), TRAP-positive osteoclasts were counted in triplicate wells.

RNA Interference 0.2 nmol of antisense LNAs (LNA™ GapmeR) or siRNAs (BATF1 and BATF3) were designed and synthesized by Qiagen (Exiqon) and Ambion, respectively. Knock down of human Se-RNA; NFATc1 or control siRNA were transfected into primary human $CD14^+$ monocytes with the Amaxa Nucleofector device set to program Y-001 using the Human Monocyte Nucleofector kit (Amaxa), as previously described[54]. Custom antisense-LNA GapmeR for SE-RNA:NFATc1_1: 5'TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT3' (SEQ ID NO: 1; Figure 7); SE-RNA:NFATc1_2: 5'ATTCAGCTCCACTTA3' (SEQ ID NO: 2; FIG 8); Pre-designed siRNA for BATF1 (Ambion, siRNA ID# 3851): 5'GGGAACGGUUAUUUUUCUAtt3' (SEQ ID NO: 13); Pre-designed siRNA BATF3 (Ambion, siRNA ID# 116229): 5'GCUAGUAGGUUCUGCUGUUtt3' (SEQ ID NO: 14) (Figure 3).

Fluorescence *in situ* hybridization Fluorescence-conjugated *Se-RNA:NFATc1* probes for RNA–FISH were generated according to protocols of Biosearch Technologies. Hybridization was carried according to the protocol of Biosearch Technologies. The stained cells were imaged using a Zeiss Axioplan microscope (Zeiss) with an attached Leica DC 200 digital camera (Leica).

ChIP-sequencing Cells were crosslinked for 5 min at room temperature by the addition of one tenth of the volume of 11% formaldehyde solution (11% formaldehyde, 50 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM EDTA, pH 8.0, and 0.5 mM EGTA, pH 8.0) to the growth medium, followed by 5 min of quenching with 100 mM glycine. Cells were pelleted at 4 °C and washed with ice-cold PBS. The crosslinked cells were lysed with lysis

buffer (50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton X-100) with protease inhibitors on ice for 10 min and were washed with washing buffer (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA) for 10 min. The lysis samples were resuspended and sonicated in sonication buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate and 0.5% N-lauroylsarcosine) using a Bioruptor (Diagenode) with 30 s on and 30 s off on a high-power output for 12 cycles. After sonication, samples were centrifuged at 12,000 r.p.m. for 10 min at 4 °C, and 1% of sonicated cell extracts was saved as input. Precleared extracts were then incubated with 5 mg of H3K27ac antibody (ab4729) and RNA polymerase II (pol II, MMS-126R) from abcam overnight at 4 °C. After overnight incubation, antibody-bound agarose beads were washed twice with sonication buffer, once with sonication buffer with 500 mM NaCl, once with LiCl wash buffer (10 mM Tris- HCl, pH 8.0, 1 mM EDTA, 250 mM LiCl and 1% NP-40) and once with TE with 50 mM NaCl. After washing, DNA was eluted in freshly prepared elution buffer (1% SDS and 0.1 M NaHCO3). Cross-links were reversed by overnight incubation at 65 °C. RNA and protein were digested using RNase A and proteinase K, respectively, and DNA was purified with ChIP DNA Clean & Concentrator (Zymo Research). 10 ng of purified immunoprecipitated DNA per sample was ligated with adaptors, and 100–300 bp DNA fragments were purified to prepare DNA libraries using an Illumina TruSeq ChIP Library Prep Kit following the manufacturer's instructions. ChIP libraries were sequenced (50 bp single end reads) using an Illumina HiSeq 2500 Sequencer at the Weill Cornell Medicine Epigenomic Core Facility per the manufacturer's recommended protocol.

ATAC-sequencing ATAC-seq was performed as previously described. To prepare nuclei, we spun 50,000 cells at 500*g* for 5 min, which was followed by a wash using 50 mL of cold 1× PBS and centrifugation at 500*g* for 5 min. Cells were lysed using cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2 and 0.1% IGEPAL CA-630). Immediately after lysis, nuclei were spun at 500*g* for 10 min in a refrigerated centrifuge. Immediately following the nuclei prep, the pellet was resuspended in the transposase reaction mix (Illumina). The transposition reaction was carried out for 30 min at 37 °C. Directly following transposition, the sample was purified using a Qiagen MinElute kit. Then, we amplified library fragments using 1× NEB next PCR master mix and custom Nextera PCR primers as previously described, using the following PCR conditions: 72 °C for 5 min; 98 °C for 30 s; and thermocycling at 98 °C for 10s, 63 °C for 30s and 72 °C for 1min.The

libraries were purified using a Qiagen PCR cleanup kit yielding a final library concentration of ~30 nM in 20 ml. Libraries were amplified for a total of 10–13 cycles and were subjected to high-throughput sequencing using the Illumina HiSeq 2500 Sequencer (single end).

ChIP-seq and ATAC-seq analysis. Quality of reads was assessed with FastQC (v0.11.9). Low-quality reads were filtered with skewer (v.0.2.2). Reads were then mapped to reference genome GRCh37 (hg19) with bowtie2 (v2.3.5.1) with "--very-sensitive" parameters. Ambiguous alignments were filtered out with samtools (v1.10) "view -q 10", then the alignments were sorted according to the coordinates with samtools sort. Definition of super- or typical enhancers was performed with HOMER "findPeaks -style super -typical". For visualization of average coverages of the peaks, HOMER annotatePeaks.pl was run with "-hist -size given -d" options. For building genomic coverage tracks, the alignment files were processed to bedGraph files with bedtools (v2.27.1) genomecov, then converted to bigWig format with bedSort and bedGraphToBigWig (UCSC tools), then uploaded to UCSC genome browser. HOMER annotatePeaks.pl was used to assign each interval to its nearest gene, whose list is then presented as a Chow-Ruskey plot with R "vennerable" package. ChIP-seq read count at enhancer regions is measured with bedtools multicov, then normalized to RPKM with sequencing depth and interval length. Enhancers' "sensitivity to IBET" was determined with HOMER getDifferentialPeaks with -F 1.5 option.

RNA-sequencing Nuclear RNA was first extracted using RNeasy mini kit (Qiagen). True-seq sample preparation kits (Illumina) were then used to generate libraries with multiplexed barcode adaptors. All samples passed quality control analysis on a Bioanalyzer 2100 (Agilent). Paired-end reads were obtained on an Illumina HiSeq 2500/1500 in the Weill Cornell Medical College Genomics Resources Core Facility. The reads were aligned to hg19 using STAR aligner (v2.7.3a) with default parameters. We used bedtools multicov to count reads that are mapped within the intervals, then the counts were normalized to FPKM considering both sequencing depth and interval length. The read count matrix is further analyzed with DESeq2 to find differential transcription.

PRO-sequencing Cells were incubated in the nuclear run-on reaction condition (5 mM Tris-HCl pH 8.0, 2.5 mM $MgCl_2$, 0.5 mM DTT, 150 mM KCl, 0.5% Sarkosyl, 0.4 units / μl of RNase inhibitor) with biotin-NTPs and rNTPs supplied (18.75 μM rATP, 18.75 μM rGTP, 1.875 μM biotin-11-CTP, 1.875 μM biotin-11-UTP for uPRO; 18.75 μM rATP, 18.75 μM rGTP, 18.75 μM rUTP, 0.75 μM CTP, 7.5 μM biotin-11-CTP for pChRO) for 5 min at 37°C. Run-On RNA was extracted using TRIzol, and fragmented under 0.2 N NaOH for 15

min on ice. Fragmented RNA was neutralized, and buffer exchanged by passing through P-30 columns (Biorad). 3′ RNA adaptor is ligated at 5 μM concentration for 1 hours at room temperature using T4 RNA ligase (NEB), followed by 2 consecutive streptavidin bead bindings and extractions. Extracted RNA is converted to cDNA using template switch reverse transcription with 1 μM RP1-short RT primer, 3.75 μM RTP-Template Switch Oligo, 1x Template Switch Enzyme and Buffer (NEB) at 42°C for 30 min. After a SPRI bead clean-up, the cDNA is PCR amplified up to 20 cycles using primers compatible with Illumina Small RNA sequencing. Low quality reads along with Illumina small RNA 3' adapter were filtered. PRO-seq reads were aligned to hg19 genome with STAR aligner with default options. 5'-end positions of the alignments upon each strand were summarized to bedGraph tracks with bedtools genomecov -5 -strand + (or -), then converted to bigWig formatted files. The bigWig files, along with pre-trained support vector matrix (SVM) that is provided with the program, were run as inputs to the dREG program. Among resulting dREG peaks, ones with peak score greater than 0.7 were selected for further analysis. The remaining dREG peaks were then run in dREG-HD. "Relaxed" dREG-HD result peaks from all samples were merged with cat command, bedSort and bedtools merge -d 100. Read counts of each merged dREG-HD site extended by 250 bases from the center were assessed with bedtools multicov. Differential expression analysis was done with DESeq2, and differentially expressed dREG peak was defined as ones with adjusted p-value smaller than 0.01.

Data Accessions H3K27ac ChIP-seq data for osteoblasts and CD4+ T cells are available in ENCODE project (www_encodeproject_org), under entry codes ENCFF000CVS and ENCFF000CVU for osteoblasts and ENCFF017MGJ and ENCFF416ZFL for CD4+ T cells. Data for this project have been deposited at NCBI's Gene Expression Omnibus (GEO) and GSE number is GSE203587. Next-generation sequencing experiments used in this study are listed in FIG. 8a. All data are from two (ChIP-seq and Nuc-seq) or three (ATAC-seq and Pro-seq) replicate experiments.

Statistical Analyses All statistical analyses were performed with GraphPad Prism 8, or R (v4.1.0) software. Detailed information about statistical analysis, including tests and values used is provided in the figure legends. Shapiro-Wilk normality tests were performed; for data that fell within a Gaussian distribution, we performed appropriate parametric statistical tests and for those that did not fall within an equal variance-Gaussian distribution, we performed non-parametric statistical tests. The non-parametric Kruskal-Wallis test was

performed with the Bonferroni correction for comparisons for read density in each region. A p value less than 0.05 is considered statistically significant.

EXAMPLE 2: RANKL regulates SEs in the vicinity of master osteoclast regulators in human osteoclast

RANKL is a key driver to induce human osteoclast differentiation[22]. Human CD14+ monocytes differentiated into tartrate-resistant acid phosphatase (TRAP)-positive multinuclear osteoclasts with characteristic actin ring formation under M-CSF and RANKL stimulation (FIG.1a). Osteoclasts are defined as TRAP-positive multinuclear cells (> 3 nuclei). Phalloidin staining showed actin ring formation of osteoclasts. These studies were designed to identify cell-specific programs in human osteoclasts. Reprogramming of super-enhancers (SEs) by micro-environmental stimuli contributes to a distinct cell-specific phenotype in health and disease[26]. The hypothesis that osteoclast-specific SEs are established early by RANKL stimulation and contribute to osteoclast-specific gene expression was tested. Super-enhancers exhibit higher enrichment of histone 3 lysine 27 acetylation (H3K27ac) and DNase I hypersensitive regions than typical enhancers[25]. ChIP-seq was performed against H3K27ac after differentiating CD14+ cells with or without RANKL stimulation (FIG. 8A) to catalog SEs. Bioinformatic analysis using the rank ordering of super-enhancer (ROSE) algorithm[25] defined 808 SEs in CD14+ monocytes (CTRL) and 962 SEs in RANKL-treated OCPs (RANKL) (FIG. 8B). Among SEs in osteoclasts, 200 Ses were induced by RANKL relative to control and were closely correlated with gene sets related to bone disease showing pathological bone resorption such as RA and bone inflammation diseases (FIG.1C). 148 Ses that were suppressed by RANKL are associated with disease by infectious agents and regulation of immune system processes (FIG. 1C, FIG. 8B). The 200 upregulated SEs exhibited higher enrichment of H3K27ac compared to typical enhancers (TEs) (FIG. 1d). In total SEs and TEs, basal H3K27ac levels were higher in SEs compared to in TEs, and RANKL treatment increased or decreased H3K27ac enrichment in both SEs and TEs (FIG. 1E). It has been shown that SEs are strongly associated with the expression of master transcription factors (TFs) that control cell identity and state[25,27]. Accordingly, RANKL-induced SEs were located in the vicinity of NFATC1, a master regulator of osteoclastogenesis[16], and key regulators of osteoclast differentiation such as MYC[55], PR/SET Domain 1 (PRDM1)[56], and matrix metalloproteinase-9 (MMP9)[57] (FIGs.1E-F). In contrast, H3K27ac read density near negative regulators such as interferon regulatory factor 8

(IRF8)[58], Kruppel Like factor 2 (KLF2)[59], and TNF receptor superfamily member 1B (TNFRSF1B)[60] was decreased in response to RANKL (FIGs.1e-f). These results suggest that RANKL-regulated SEs are strongly associated with both positive and negative TFs to promote osteoclast differentiation.

EXAMPLE 3: RANKL-induced SEs are osteoclast-specific and mark osteoclast identity genes

To further examine whether defined SEs in osteoclast are cell-type specific, the study of RANKL-regulated SEs was extended to other cell types which are related to bone inflammation and RA.H3K27ac ChIP-seq datasets of osteoblasts, CD34+ progenitors and CD4+ T cells were retrieved from the ENCODE project and identified SEs in each cell type. In the defined RANKL-induced 200 SEs, H3K27ac read density was exclusively enriched in human osteoclasts, but not in other cell types (FIG. 2a). A set of SEs from osteoblasts, CD34+ progenitors and CD4+ T cells revealed that H3K27ac was highly enriched in cell-type specific SEs (FIG. 2b). Chow-Ruskey diagrams of TE-associated and SE-associated genes in osteoclasts, osteoblasts, CD34+ progenitor cells, and T cells revealed that TEs shared genes that are active in all four cell types (FIG. 2c, the circle in the middle), while SE elements represented the overlap of a few cell types and spanned domains that were almost cell-type specific (FIG. 2c). For example, osteoclast-specific SEs, but not SEs from other cell types, were associated with the gene encoding NFATC1 and TNFRSF11A, which are well-defined osteoclast genes (FIG. 2d). In contrast, osteoblast-specific SEs were associated with the genes encoding runt-related transcription factor 2 (RUNX2) and periostin (POSTN), which are required for osteoblast differentiation (FIG. 2d). Gene ontology analysis using the set of genes associated with SEs in osteoblasts and CD4+ T cells showed that significant biological process terms were strongly descriptive of cell-type specific function (FIG. 9). This result suggests that the genes associated with RANKL-induced SEs are highly cell-type specific relative to TE-associated genes.

EXAMPLE 4: Chromatin accessibility is dynamically regulated in osteoclast SEs

Active enhancers are cis-regulatory elements that modulate the quality and quantity of gene expression[24]. These regions are characterized primarily by specific combinations of posttranslational modifications of histone proteins (H3K27ac and H3K4me1) and "open" chromatins where major TFs can bind[31].Assay for Transposase-Accessible Chromatin with

high-throughput sequencing (ATAC-seq) was performed to investigate chromatin changes during osteoclast differentiation. The ATAC-Seq peaks were binned according to their respective fold changes in response to RANKL (FIG. 3A). Increased chromatin accessibility showed both higher fold change (FIG. 3A, black box in left panel) and at least within two-fold change in response to RANKL, but decreased ATAC-seq peaks were uniformly distributed within two-fold change (FIG. 3A, right panel). Highly opened chromatin regions (a black box from FIG. 3A, n=5,899) were correlated with increased read densities of H3K27ac, suggesting that the enhancer regulation during osteoclast differentiation is closely related to local chromatin accessibility. Consistently, the most regulated peaks were located in the potential enhancer regions such as the distal, intragenic, or intergenic regions rather than the promoter. Chromatin accessibility increased in both TE and SE in response to RANKL but was significantly increased in TE with a relatively narrow width (> 500 bp) (FIGs. 3B-C). These suggest that chromatin accessibility is regulated in several specific regions within SE (FIGs. 3B-C), which has a very wide width (> 12.5 kb) defined by H3K27ac (FIG. 1C). Accordingly, relatively narrow ATAC-seq peaks, in contrast to H3K27ac, was upregulated within RANKL-sensitive SEs near genes of NFATC1, PRDM1, and MYC (FIG. 3D). To gain insight into the mechanisms involved in SE regulation, TF binding motifs were investigated in open chromatin within RANKL-regulated SEs. Open chromatin within RANKL-induced SEs were significantly enriched for TF motifs bound by BATF, activator protein-1 (AP-1), NFAT:AP-1, NFAT, and NF-kB, which has shown to be an important TF for osteoclast differentiation[2] (FIG. 3E). Sequence motifs bound by TFs regulating osteoclast differentiation, such as PU.1:IRF8, MAF BZIP transcription factor A (MAFA), and IRF8, were enriched in down-regulated ATAC-seq peaks within SEs (FIG. 3F). These results suggest that RANKL-sensitive SEs may be formed as a consequence of the binding of RANKL-dependent signaling TFs to specific open chromatin regions.

EXAMPLE 5: BET protein inhibition differentially regulates SE-eRNA expression

BET protein inhibition effectively suppresses osteoclastogenesis and arthritic bone erosion[22]. Bromodomain-containing protein 4 (BRD4) occupancy at SEs in other cell types is highly sensitive to BET inhibition[26], I-BET151-mediated suppression of osteoclastogenesis was assessed to determine if it results from disturbing SE formation which drives target gene expression in human osteoclasts. H3K27ac ChIP-seq was performed in the presence of I-BET151 and examined intensity of H3K27ac signals in pre-defined osteoclast-specific SEs.

I-BET treatment significantly decreased H3K27ac signals in RANKL-induced SEs (FIG.4a). Of note, I-BET151 treatment was found to not cause a decrease of H3K27ac in all RANKL-induced SEs, whereas significant changes in 49 out of 200 SEs contributed to an overall decrease of H3K27ac read densities in RANKL-induced SEs (FIGs.4b-c). For instance, SEs in the vicinity of NFATC1 and peroxisome proliferator-activated receptor gamma coactivator 1-beta (PPARGC1B) genes were highly sensitive to I-BET151, while SEs near integrin subunit alpha V (ITGAV) were relatively resistant to inhibitors (FIG.4d). Concomitantly, the expression of NFATc1 and PPARGC1B were suppressed by I-BET151 treatment while I-BET151 had a minimal effect on ITGAV (FIG.10a). Motif analysis further showed that IBET-sensitive H3K27ac peaks within RANKL-induced SEs were enriched for TF motifs bound by AP-1, activating transcription factor 3 (ATF3), and BATF (FIG.4e), which are similar to the results of motif enrichment derived from chromatin accessibility (FIG.3g). IBET-insensitive H3K27ac peaks were also enriched for PU.1 family motifs (FIG.4e). Identified I-BET sensitive SEs were associated with AP-1 family transcription factors that play a key role in osteoclastogenesis[61]. BATF was consistently found in SE regions (FIGs. 3g and 4e). However, the role of BATF in osteoclasts remains unclear. The chromatin landscape was examined near BATF, Fos and NF-kB motif sites. As with key osteoclastogenic transcription factors, Fos and NF-kB motif sites (FIG. 10g and FIG.10b), BATF motif sites within RANKL-induced SEs are surrounded by robust signals of H3K27ac and ATAC-seq relative to TEs, indicating that the BATF binding site is in active SEs (FIG.4g). BATF belongs to the AP-1 family and consists of three members: BATF, BATF2, and BATF3[62,63]. BATF1 was diminished initially and increased later, while BATF3 was induced by RANKL during osteoclastogenesis (FIG.10c). However, BATF2 was not detected (data not shown). It has been shown that BATF1 and BATF3 compensate each other[64]. To test the role of BATF in osteoclastogenesis, both BATF1 and BATF3 were knocked-down using small interfering RNAs (siRNAs) in CD14+ cells. KD cells were cultured with M-CSF and RANKL to differentiate into osteoclasts. Intriguingly, decreased BATF1/3 expression suppressed osteoclastogenesis (FIGs.4h-i), suggesting that BATF1/3 is a positive regulator of osteoclast differentiation. Taken together, these results suggest that BET proteins modulate osteoclast differentiation by selectively regulating RANKL-induced SE formation.

EXAMPLE 6: RNA Polymerase II is recruited to osteoclast SE regions to promote enhancer RNA transcription

ChIP-seq and RNA-seq were performed to investigate how Pol II recruitment and eRNA expression are associated with SEs and TEs across the human osteoclast genome. As measured by ChIP-seq reads, Pol II was found to be enriched in overall enhancers in human osteoclasts (FIG.5a). However, it is notable that RANKL-induced SEs, relative to TEs, were highly occupied by a large portion of Pol II (FIGs.5b-c). The evidence that the high level of Pol II is occupied within RANKL-induced SEs suggests that these large *cis*-element domains may promote transcription of eRNA which drive high-level expression of SE-associated genes. Thus, to measure eRNA expression in osteoclasts, nuclear RNA and examined nascent transcripts were isolated by Nuclear RNA (Nuc)-seq, as previously described[67]. The presence of higher levels of eRNAs transcribed from SEs than from TEs and RANKL treatment further increased expression of eRNA from SEs (FIG.5d) were observed. Expression of eRNA was increased in 83 - 84 % RANKL-induced SEs, while 85 - 88 % down-regulated SEs showed decreased eRNA expression (FIG.5e). Correlated expression of SE and eRNAs included a set of gene encoding major TFs important for osteoclasts such as NFATC1 and MYC (FIG.5f). These studies also found that both recruitment of Pol II and eRNA expression were enriched in SEs regions in proximity to key osteoclast regulators such as NFATC1, PRDM1, and MYC (FIGs.5g-h), suggesting that osteoclast-specific SE activities may be inferred from eRNA expression and signals.

EXAMPLE 7: Global identification and characteristics of SE-eRNA in human osteoclast

To obtain a better resolution map of eRNA at RANKL-sensitive SEs in osteoclasts, a precision nuclear run-on and sequencing (PRO-seq) assay[68] were performed which offers single nucleotide resolution of nascent RNA 3' ends. Similar to Nuc-seq (FIG.5h), nascent transcripts were found to be highly enriched at NFATC1-associated SE as well as PRDM1- and MYC-associated SE (FIG.6a, FIG. 11). Using discriminative regulatory-element detection from gro-seq (dREG) analysis [69], a sensitive machine learning method that predicts active transcriptional regulatory elements and eRNA loci, most dREG peaks were found to be distributed in potential enhancer regions (intergenic: 26 %, intragenic 48.5%), while promoter regions included 19.1% dREG peaks (FIGs.6a-b). The 1,308 dREG peaks were found in the SE region and the 1,569 dREG peaks were located in the TE, suggesting that a

significant amount of eRNAs is transcribed in the SE region (FIG.6b). Predicted eRNA loci derived from dREG analysis were highly occupied by several marks of active enhancer including H3K27ac, open chromatin (ATAC-seq), and Pol II (FIGs.6c-d). RANKL stimulation dramatically increased signaling intensities of H3K27ac, Pol II ChIP-seq, and ATAC-seq, indicating that dREG analysis successfully predicted eRNA loci in both SE and TE (FIGs.6c-d). dREG was also found within RANKL-induced enhancers were significantly enriched for TF motifs bound by AP-1 family such as ATF3, Fos, Fra1, and BATF (FIG.6e). In contrast, down-regulated dREG regions were enriched for TF motifs bound by PU.1 family TFs such as ETS1, Elf4, and PU.1 (FIG.6f). RANKL significantly increased the expression of eRNAs in both I-BET-sensitive and -insensitive SEs. However, the abundance of RANKL-induced eRNAs in I-BET-sensitive SEs was comparatively higher than in I-BET insensitive SEs (FIG.6g). This result suggests that BET proteins may associate with SE-eRNA transcription by interacting with SEs during osteoclastogenesis. Taken together, these data suggest that RANKL-regulated signaling input is associated with SE-eRNA expression.

EXAMPLE 8: SE-eRNA regulates expression of SE-associated TF in human osteoclast

Consistent with H3K27ac ChIP-seq data (FIG. 1c), gene ontology analysis revealed that dREG peaks were strongly associated with osteoclast and bone-related diseases, including rheumatoid arthritis (RA) (FIG. 7a). NFATc1 is a master regulator of osteoclasts[15]. During osteoclastogenesis, *NFATc1* mRNA expression was induced by RANKL in a time-dependent manner (FIG. 7b). Freshly isolated synovial CD14+ cells from RA patients have a higher osteoclastogenic potential and express NFATc1 protein without RANKL stimulation[70]. Consistently, quantitative RT-PCR analysis showed increased expression of NFATc1 in RA synovial CD14+ cells relative to disease control CD14+ cells from healthy donors (FIG. 7c). To evaluate the functional role of SE-eRNA in human osteoclasts, NFATC1-associated SE-eRNA (SE-eRNA:NFATC1) were examined using fluorescent in situ hybridization (FISH) with antisense probes. SE-eRNA:NFATC1 was presented only in RANKL-treated cells (FIG. 7d). Subsequently, knockdown of SE-eRNA:NFATC1 was observed to be mediated by antisense oligonucleotides (ASO-LNA) suppressed the differentiation of human OCPs into multi-nucleated TRAP-positive cells (FIGs. 7e-f, FIGs. 12a-b, see Example 1 for details of experiment). Moreover, knockdown of SE-eRNA:NFATC1 abrogated RANKL-induced expression of NFATc1 mRNA (FIG. 7g, FIG.

12c) and protein (FIG. 7h, FIG. 12d) in human OCPs. To investigate whether RANKL-responsive SE-eRNAs would be associated with a high osteoclastogenic potential of synovial CD14+ cells from RA patients, we assessed the expression of SE-eRNAs profile in RA synovial CD14+ cells. Nuc-seq data derived from synovial CD14+ cells from RA patients or disease control revealed that some of RANKL-sensitive SE-eRNAs were highly upregulated in synovial CD14+ cells of RA patients (FIG. 7i). Among them, nascent RNA expression within NFATC1-associated SE (SE-eRNA:NFATC1) was increased in synovial CD14+ cells from RA patients (FIG. 7j). Consistently, quantitative RT-PCR analysis showed increased expression of eRNAs in NFATC1-associated SE in RA synovial CD14+ cells relative to disease control CD14+ cells from healthy donors (FIG. 7k). The previous study showed that administration of I-BET151 suppressed arthritic bone erosion and NFATc1 expression[22]. I-BET151 treatment also suppressed the expression of SE-eRNA:NFATC1 (FIG.12e), suggesting that I-BET-mediated inhibition of NFATc1 expression might be mediated, in part, by decreased expression of SE-eRNA:NFATC1. SE-eRNA:PRDM1 and SE-eRNA:MYC were also found to be significantly increased in RA synovial OCPs (FIG. 12f). Taken together, these data suggest that eRNAs transcribed from NFATC1-associated SE are indispensable for the transcriptional activation of NFATC1 and osteoclast differentiation, and the presence of RANKL-responsive SE-eRNAs in RA synovial CD14+ cells may contribute to the high osteoclastogenic potential of RA synovial macrophages.

Discussion

Dysregulation of osteoclast differentiation and activity was linked to pathological bone diseases and arthritic bone destruction[2]. The major transcription factors and key molecules driving osteoclastogenesis have been extensively studied, and their global or conditional knock-out mice exhibited defects in bone homeostasis and pathological bone destruction[2]. However, globally targeting these key factors is often accompanied with non-specific effects, and conditional deletion approaches may not be available for the treatment of human patients. Identifying cell-specific signatures in human osteoclasts is fundamental to developing osteoclast-specific therapeutic interventions for pathological bone resorption of humans. Here, this study defined 348 RANKL-responsive SEs and SE-associated eRNAs in human early osteoclasts as osteoclast-specific epigenetic programs. RANKL-responsive SEs were enriched by BATF-motifs and BET proteins, which are important regulators of osteoclasts. Targeting SE-eRNAs in the proximity of the NFATc1 gene suppressed *NFATc1*

expression and osteoclastogenesis. Moreover, RANKL-responsive SE-eRNAs were found to be present in synovial macrophages from RA patients. This study provides better understanding of the cell-specific programs driving osteoclastogenesis and insights into the control of pathological bone resorption and suggests that manipulating SE-eRNAs offers a novel, osteoclast-specific therapeutic strategy for treating osteoclast-mediated bone diseases.

Epigenetic mechanisms are increasingly appreciated for their critical roles in transcriptional control in human health and disease. Among them, super enhancers play a crucial role in regulating genes associated with cell-identity and cell-fate decision[25-29]. this study identified RANKL-responsive SEs in human osteoclasts. Interestingly, the majority of RANKL-responsive SEs were found to be osteoclast-specific, as they are not detected in osteoblasts, CD4+ T cells, or CD34+ progenitor cells. In this analysis, the specificity of SEs in CD34+ progenitors were found to be ambiguous. CD34+ progenitor cells can differentiate into different cell types including monocytes, osteoclasts, T cells, and even osteoprogenitor cells[72-74]. Thus, it is possible that CD34+ progenitor cells may share common features in the histone code with other cells, which can be retained in subsequently differentiated cells. However, the importance of shared features between progenitors and differentiated cells remains unknown. Human and mouse osteoclasts share key transcription factors that drive osteoclastogenesis including NFATc1[2]. RANKL transduces the same signaling pathways, including the NF-kB and MAPK pathways, in both human and mouse osteoclasts. Although genes proximal to SEs are important for tissue or cell identity, SEs among different species do not exhibit sequence conservation[75], and the overall sequence similarity in non-coding areas between different species is widely variable[76,77]. This study suggests the possibility that SEs of osteoclast-identity genes may vary among different species. For example, while this study identified that super enhancers in the vicinity of the NFATc1 gene were located at the intergenic region of C-terminal of the NFATc1 gene in human osteoclasts, Carey et al. showed NFATc1 super-enhancer loci in mouse osteoclasts by specifically focusing on two intronic enhancers of NFATc1[33]. However, it remains unresolved whether SE-associated genes are modulated by the same regulatory regions in human and mouse cells, which is an area for the future research.

SEs are occupied with large numbers of transcription factor binding sites, and master transcription factors and BET proteins contribute to SE formation[26]. In this analysis, NFATc1, AP-1, BATF and NF-kB binding motifs are top-ranked motifs in RANKL-responsive SEs. Among them, the crucial contribution of NFATc1, AP-1, and NF-kB to

osteoclasts is well known. Carey et al. showed that PU.1 and MITF coordinated the expression of essential transcription factors for mouse bone marrow derived osteoclasts and the PU.1-Eomes-MITF complex is enriched in two intronic enhancer of NFATc1 in mouse osteoclasts[10,33]. In this study, the PU.1 motif was not in the top-ranked TF motifs in RANKL-responsive SEs. However, PU.1-binding motifs were found to be pre-existing in both induced and repressed RANKL-responsive SEs in control conditions (data not shown), which is consistent with the previous report showing that PU.1 can reshape the chromatin landscape by occupying all open regulatory regions in multipotent myeloid precursor cells, including closed sites[78-80]. However, PU.1 is able to activate or repress transcription by cooperative action with other TFs. Izawa et al. showed that osteoclast-specific PU.1 binding sites overlapped with NFATc1 binding sites[81]. In addition to the existing regulators of osteoclastogenesis, the BATF motif was found to be highly enriched in RANKL-responsive SEs, suggesting that BATF may be required for SE formation in human osteoclasts. However, the role of BATF proteins in osteoclasts has not been characterized yet. BATF deficiency was found attenuated osteoclast differentiation in human cells and identified BATF as a new regulator of osteoclastogenesis. Defining the mechanistic link between BATF and SE formation requires more investigation, but consistent with the observations provided herein, the BATF3 module has been found in SEs of Anaplastic large cell lymphoma[82]. The role of key TFs in osteoclasts is extensively studied with a specific focus on their function for activating the promoter. These data suggest that TFs might regulate osteoclastogenesis by controlling SE formation in addition to their well-defined functions. It has been shown that BET proteins are also highly enriched in SEs[25]. Indeed, targeting BRD4, a BET protein, with small molecule inhibitors, including JQ1 and I-BET, suppressed osteoclast differentiation and joint inflammation and bone erosion in murine models of inflammatory arthritis[22,83]. This study also showed that RANKL-responsive SEs are preferentially lost in the presence of a BET inhibitor. Given previous findings on the positive role of BET proteins in NFATc1 expression and osteoclasts, these data suggest that BET proteins might regulate gene expression and osteoclastogenesis, in part, by controlling SE formation.

Non-coding RNAs contribute significantly to cellular functions and differentiation[84,85]. The expression and role of non-coding RNAs such as micro-RNAs, long non-coding (linc) RNAs, and intronic eRNAs in osteoclasts and in osteoporosis have been documented[86-88]. Although several non-coding RNA species have been identified in mouse

and human osteoclasts, SE-eRNAs and their function in osteoclastogenesis and bone resorption are largely unexplored in human cells. eRNAs were found to be transcribed from SEs in human osteoclasts. Sakaguchi et al. identified 87 putative eRNAs from mouse osteoclasts using cap-analysis of gene expression (CAGE) and deleting the region containing intronic eRNAs in Nfatc1 genes suppressed Nfatc1 gene expression[88]. Thus, this suggests that the location of eRNAs for the same gene differs between mouse osteoclasts and human osteoclasts, supporting the importance of studies with human cells. Advances in gene editing technology such as CRISPR-Cas9 have enabled direct manipulation of SEs in human cells[90,91]. However, directly targeting super-enhancer regions in humans may disrupt the chromatin landscape and thus remains questionable as an approach. eRNAs are nascent RNA transcripts which can be easily targetable. Knock-down of SE-eRNA have been shown in the vicinity of NFATc1 gene suppressed *NFATC1* mRNA expression and osteoclastogenesis. Thus, the identification of osteoclast-specific SE-eRNAs provides a target for safe and effective therapeutics for bone diseases. Further comprehensive functional analysis of other SE-eRNAs and characterization of the mechanism of action of RANKL-responsive SEs would be required. Taken together, this study provides the first evidence of the link between SE-eRNAs and human osteoclast differentiation.

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints, along with various body systems[92]. Bone erosion is one of the key clinical features of RA [6], leading to joint destruction and impaired mobility[93-96]. Osteoclasts are the major cell type responsible for bone erosion of RA and are found at sites of structural bone damage in inflammatory arthritis[97]. Increased number and activity of osteoclasts play crucial roles in rapid bone erosion in RA[98,99]. Recent advances in genome wide sequencing and high-dimensional molecular profiling provide insights into RA pathogenesis and the contribution of cell types and pathways to its pathogenesis. In addition, tissues from RA patients have been thoroughly analyzed by various techniques including genome-wide sequencing, histological analysis, and flow cytometry, which provide patient-specific pathogenesis of RA. Notably, RA synovial CD14+ cells also have high osteoclastogenic potential and high levels of NFATc1 expression[70]. This shows that nascent eRNAs in RA synovial CD14+ cells were overlapped with RANKL-responsive SE-eRNAs, suggesting that high-levels of osteoclast SE-eRNAs may be associated with a higher osteoclastogenic potential of RA synovial macrophages. In summary, this study is the first study in human osteoclasts to

define SEs and SE-eRNAs and will provide a better understanding of human osteoclast biology and open new therapeutic avenues for human pathological bone destruction.

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All patents, patent applications, and publications, and references to GenBank or another publicly available sequences database cited throughout the disclosure, are expressly incorporated herein by reference in its entirety. In addition, US Provisional Patent Application No. 63/382,669 is also incorporated in its entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention are devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims include such embodiments and equivalents.

CLAIMS:

1. A composition comprising:

a pharmaceutically acceptable delivery vehicle, carrier, and/or diluent, and

at least one nucleic acid of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:

(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or

(d) combinations of (a), (b), or (c).

2. The composition according to claim 1, wherein at least one nucleic acid comprises 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1.

3. The composition according to claim 1, wherein at least one nucleic acid comprises 18 to 30 nucleotides in length which comprise at least 15 consecutive nucleotides of SEQ ID NO: 2.

4. The composition of claim 1 or 2, wherein said composition comprises an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 2.

5. The composition of claim 1 or 2, wherein said composition comprises an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

6. The composition of any one of claims 1 to 5 wherein the nucleic acid is an antisense oligonucleotide having at least one modified internucleoside linkage, sugar moiety, or nucleobase.

7. The composition of any one of claims 1 to 5, wherein the nucleic acid is a chimeric oligonucleotide having a gap segment positioned between 5′ and 3′ wing segments.

8. The composition of claim 6, wherein the gap segment of the chimeric oligonucleotide is comprised of 2′-deoxynucleotides and the wing segments are comprised of nucleotides having modified sugar moieties.

9. The composition according to any one of claims 1 to 8, wherein the composition comprises a non-viral vector comprising the nucleic acid which is a lipid nanoparticle, lipidoid, or liposome.

10. A method for treating an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis, said method comprising delivering a nucleic acid that specifically binds to a human osteoclast-specific NFATc1 regulatory region in chromosome 18.

11. The method according to claim 10, wherein the nucleic acid is an anti-sense oligonucleotide, an RNAi, siRNA, or combinations thereof.

12. The method according to claim 10 or 11, wherein the nucleic acid is delivered via a lipid nanoparticle, lipidoid, or liposome.

13. The method according to any one of claims 10 to 12, wherein the nucleic acid comprises at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:
(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or
(d) combinations of (a), (b), or (c).

14. The method of claim 13, wherein at least one antisense oligonucleotide comprises 18 to 45, 20 to 40, 25 to 35, or about 30 consecutive nucleotides of SEQ ID NO: 1.

15. The method of claim 13, wherein at least one antisense oligonucleotide comprises 18 to 30 nucleotides in length which comprises SEQ ID NO: 2.

16. The method of claim 13, wherein said composition comprises an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 1 or an antisense oligonucleotide having 100% complementarity to one of SEQ ID NO: 2.

17. The method of claim 13, wherein said composition comprises an antisense oligonucleotide of SEQ ID NO: 1 and/or SEQ ID NO: 2.

18. The method of claim 13, wherein the composition is delivered intravenously, via site-specific administration to a joint, via targeted delivery to the bone, or intrathecally.

19. Use of at least one antisense oligonucleotide (ASO) of 15 to 50 nucleotides in length in preparing a medicament, wherein the ASO comprises at least 15 consecutive nucleotides of a sequence comprising:
(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;

(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or
(d) combinations of (a), (b), or (c).

20. A composition suitable for treatment of an osteoclastogenesis-related disease, bone or joint destruction, bone-related impaired mobility, osteoporosis, and/or rheumatoid arthritis, said composition comprising at least one antisense oligonucleotide of 15 to 50 nucleotides in length comprising at least 15 consecutive nucleotides of a sequence comprising:
(a) TGTTTACACGCTAACAGGATGACAGCAGACACTGTGTGAAATCAGTCAGT (SEQ ID NO: 1), a sequence at least 99% identical to SEQ ID NO: 1, a sequence having at least 95% complementarity to SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(b) ATTCAGCTCCACTTA (SEQ ID NO: 2), a sequence at least 99% identical to SEQ ID NO: 2, a sequence having at least 95% complementarity to SEQ ID NO: 2, or a pharmaceutically acceptable salt thereof, or combinations thereof;
(c) a sequence comprising at least 15 consecutive nucleotides of SEQ ID NO: 1 or 2, or a pharmaceutically acceptable salt thereof, or combinations thereof, or
(d) combinations of (a), (b), or (c).

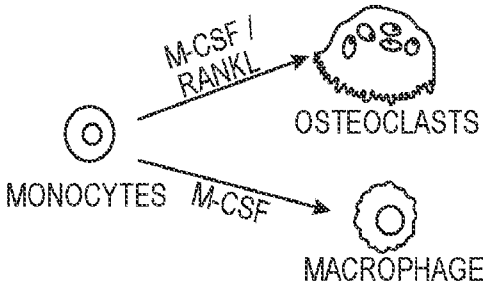


FIG. 1A

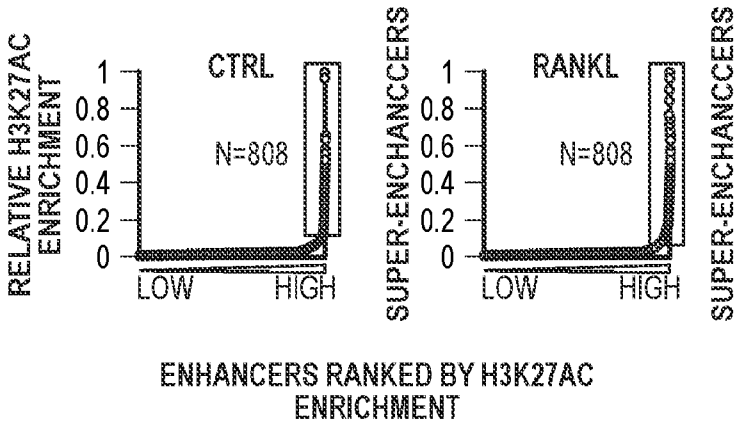


FIG. 1B

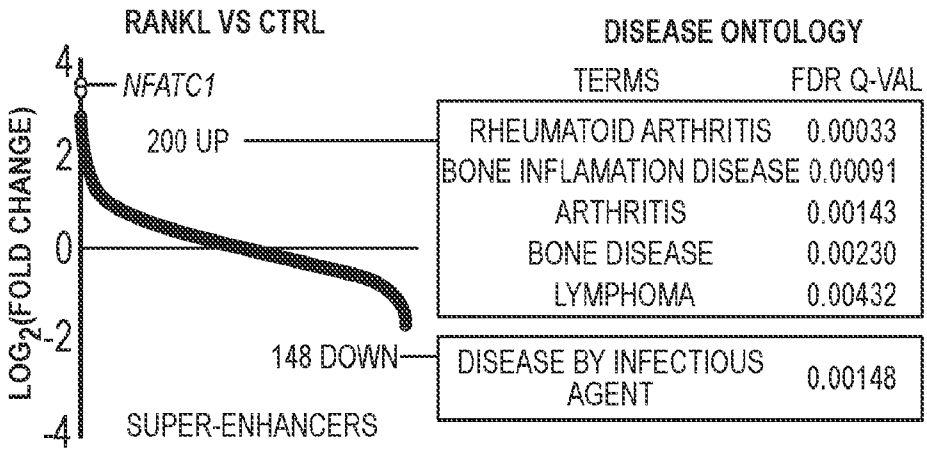


FIG. 1C

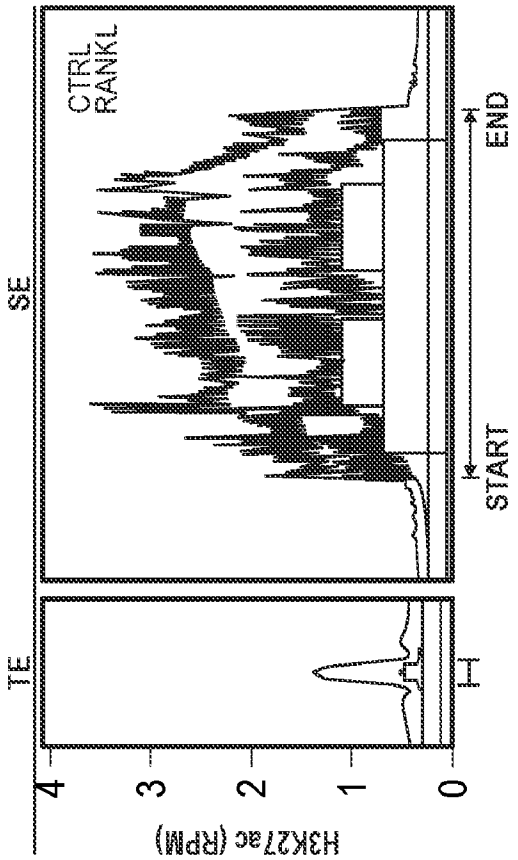

SE
TE
CTRL
RANKL
H3K27ac (RPM)
4
3
2
1
0
START
END

FIG. 1D

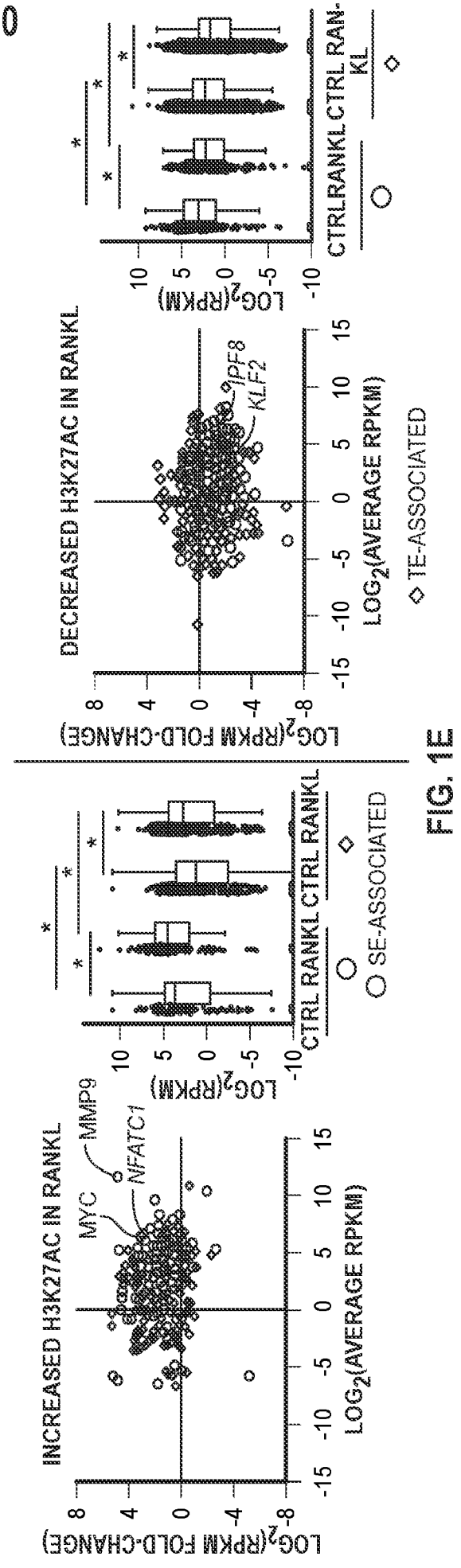

DECREASED H3K27AC IN RANKL
LOG2(RPKM FOLD-CHANGE)
LOG2(AVERAGE RPKM)
IPF8
KLF2
TE-ASSOCIATED
LOG2(RPKM)
CTRL RANKL CTRL RANKL
INCREASED H3K27AC IN RANKL
MMP9
MYC
NFATC1
SE-ASSOCIATED

FIG. 1E

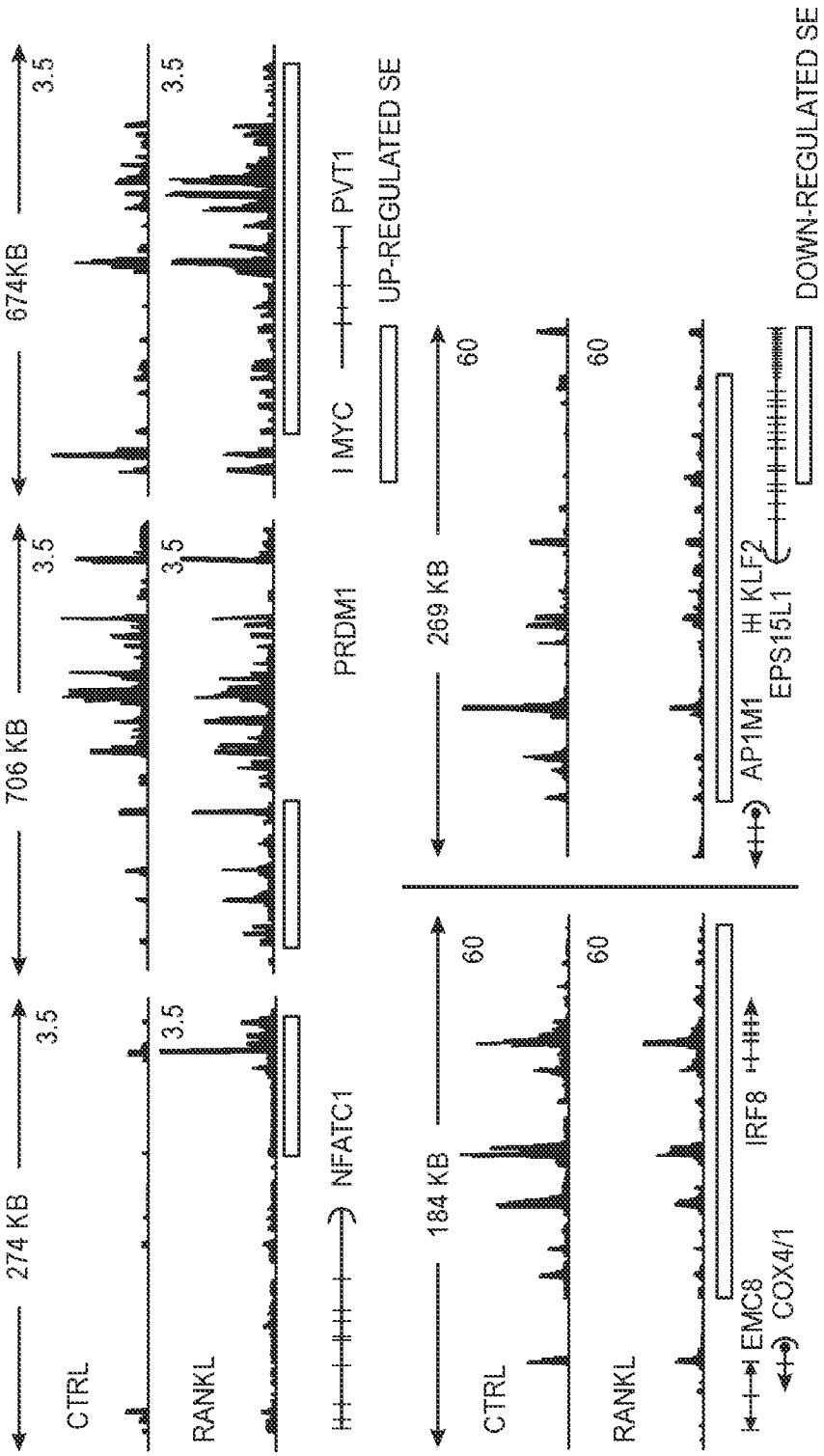

274 KB
706 KB
674KB
3.5
CTRL
RANKL
NFATC1
PRDM1
MYC
PVT1
UP-REGULATED SE
184 KB
269 KB
60
EMC8
COX4I1
IRF8
AP1M1
HH KLF2
EPS15L1
DOWN-REGULATED SE

FIG. 1F

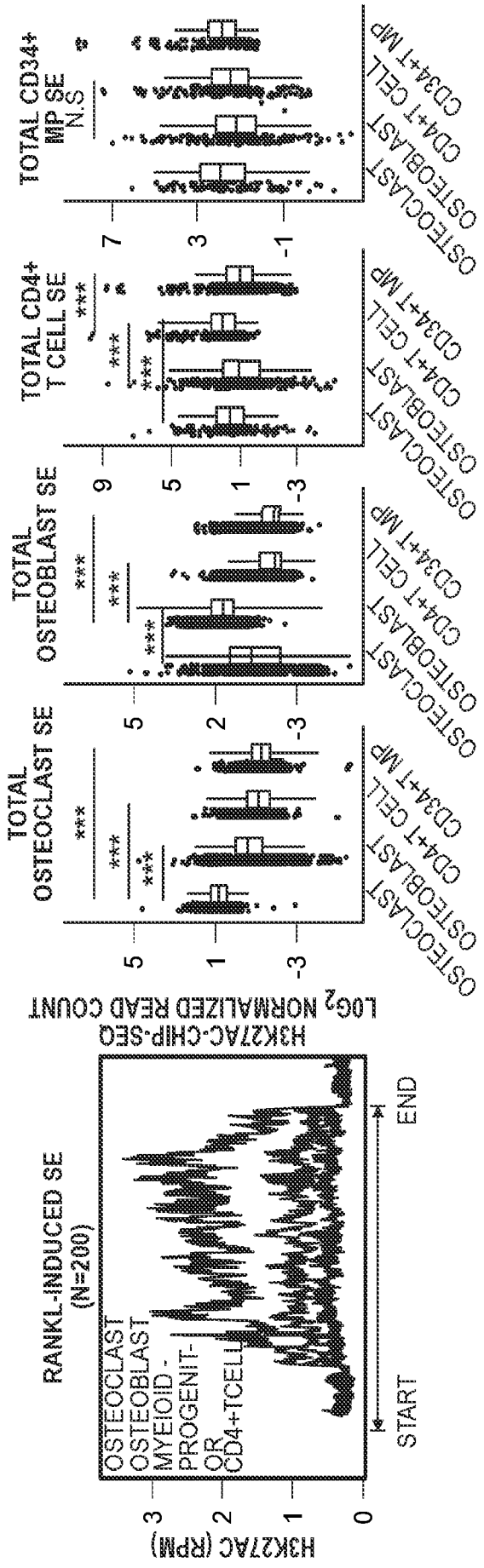

H3K27AC (RPM)
RANKL-INDUCED SE (N=200)
OSTEOCLAST
OSTEOBLAST
MYEIOID-PROGENITOR
CD4+TCELL
START
END
H3K27AC-CHIP-SEQ LOG2 NORMALIZED READ COUNT
TOTAL OSTEOCLAST SE
TOTAL OSTEOBLAST SE
TOTAL CD4+ T CELL SE
TOTAL CD34+ MP SE
N.S
***
OSTEOCLAST
OSTEOBLAST
CD4+T CELL
CD34+T MP

FIG. 2A

FIG. 2B

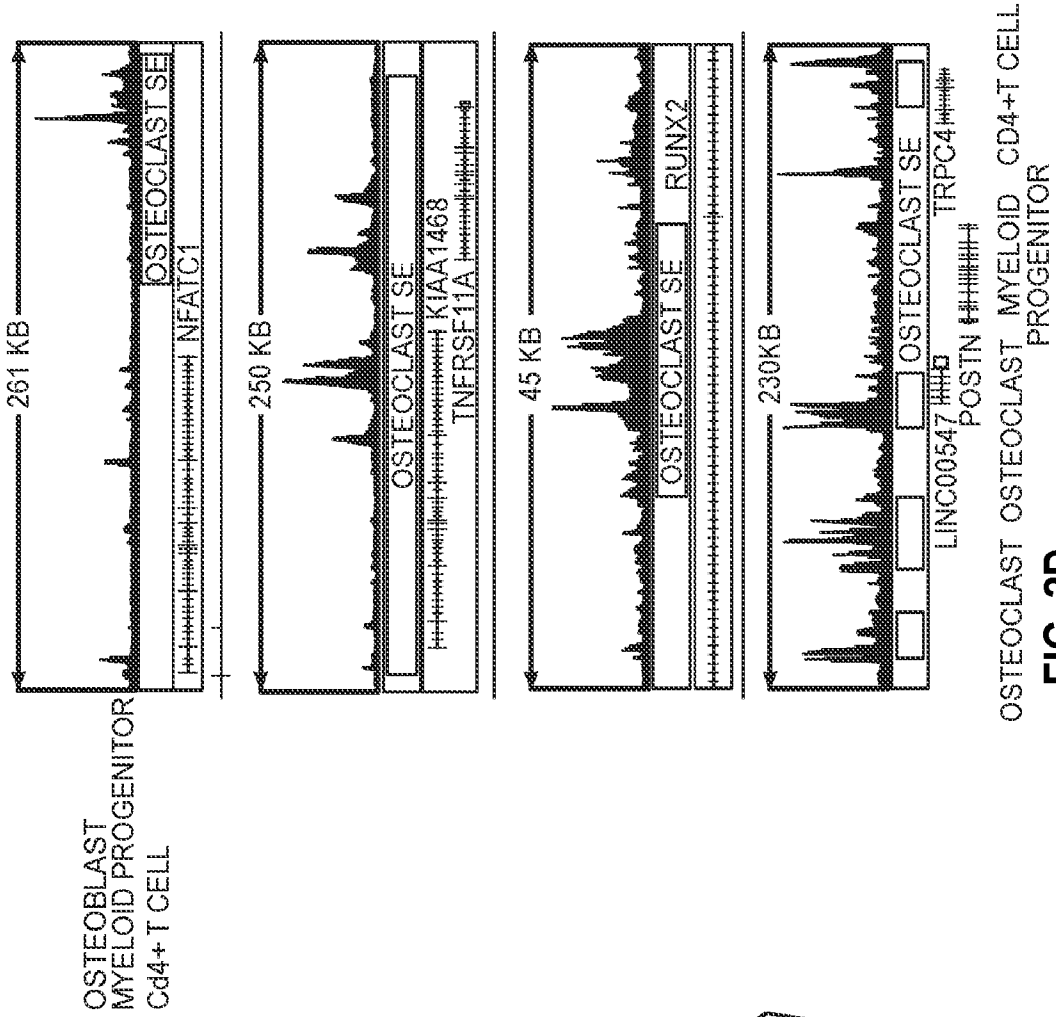

OSTEOBLAST
MYELOID PROGENITOR
Cd4+ T CELL
261 KB
OSTEOCLAST SE
NFATC1
250 KB
OSTEOCLAST SE
KIAA1468
TNFRSF11A
45 KB
OSTEOCLAST SE
RUNX2
230KB
OSTEOCLAST SE
LINC00547
POSTN
TRPC4
OSTEOCLAST OSTEOCLAST MYELOID CD4+T CELL
PROGENITOR

FIG. 2D

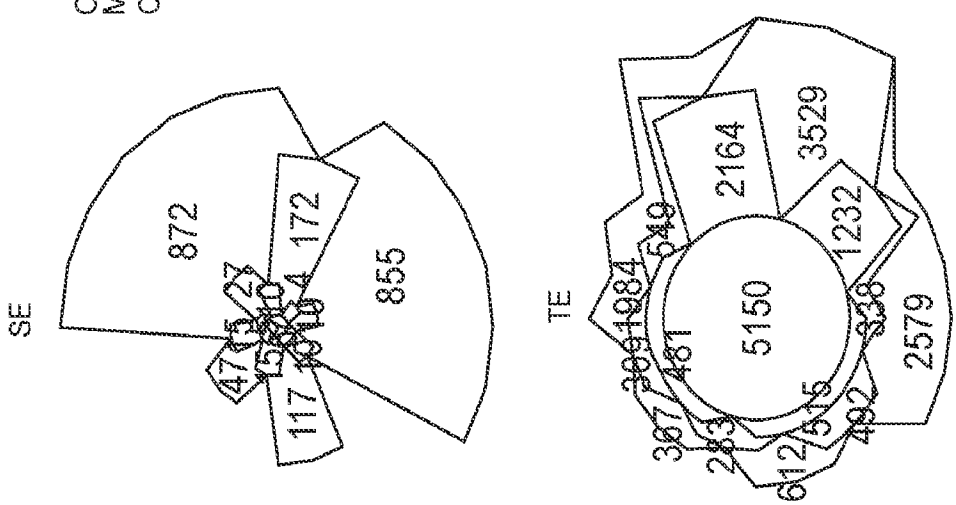

SE
872
172
855
117
TE
2164
3529
1232
5150
2579
549
481
367
283
612
515
492
338

FIG. 2C

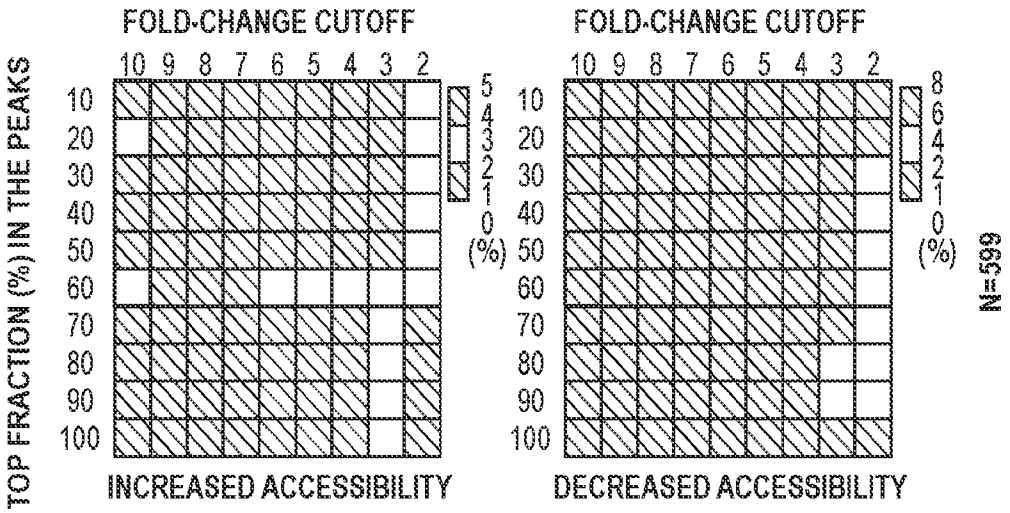


FIG. 3A

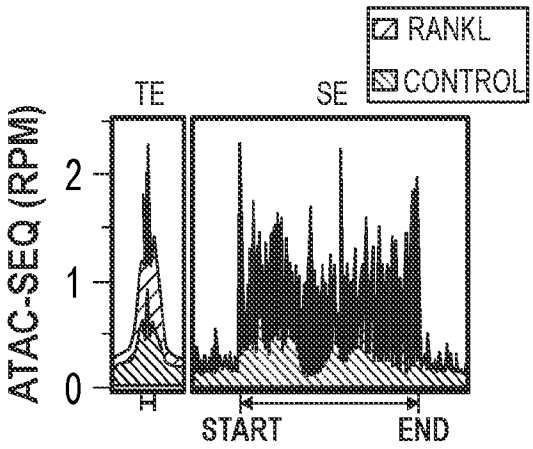


FIG. 3B

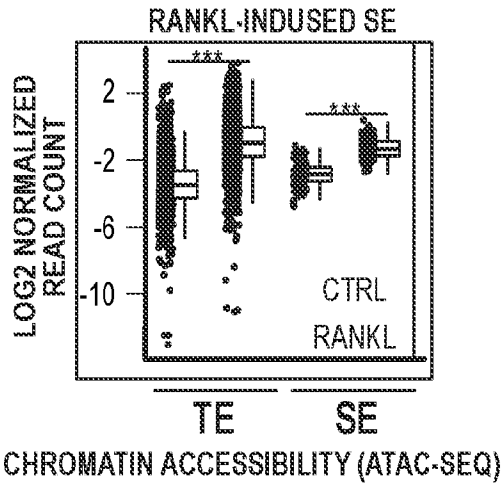


FIG. 3C

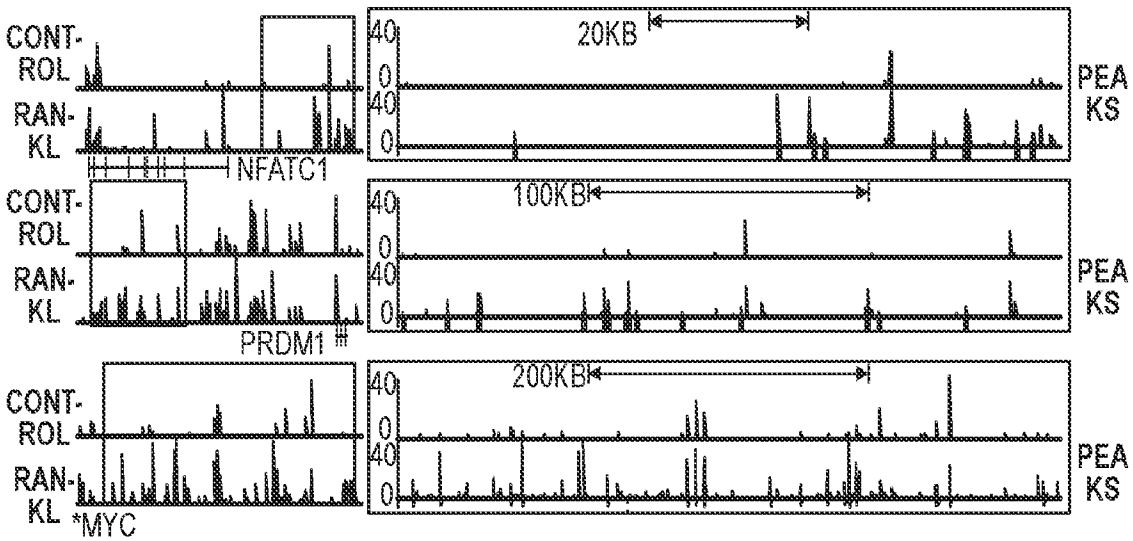


FIG. 3D

UPREGULATED ATAC-SEQ PEAKS (N = 359)

TF	MOTIF	P-VALUE
BATF		1E-75
AP 1		1E-71
NFAT:IAP 1		1E-40
NFAT		1E-32
NFKB		1E-10

FIG. 3E

DOWNREGULATED ATAC-SEQ PEAKS (N = 255)

TF	MOTIF	P-VALUE
PU.1:IRF8		1E-37
MAFA		1E-35
ELF4		1E-34
ETS1		1E-32
IRF8		1E-25

FIG. 3F

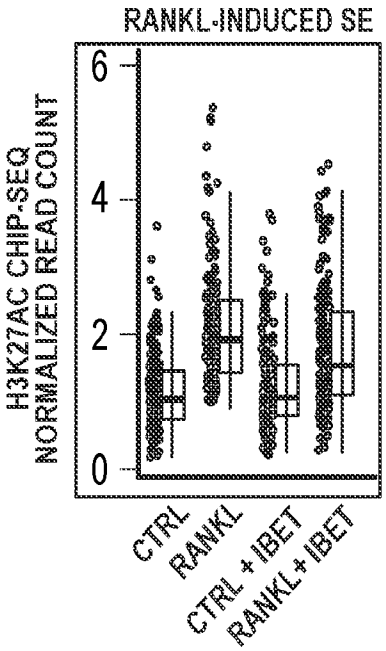


FIG. 4A

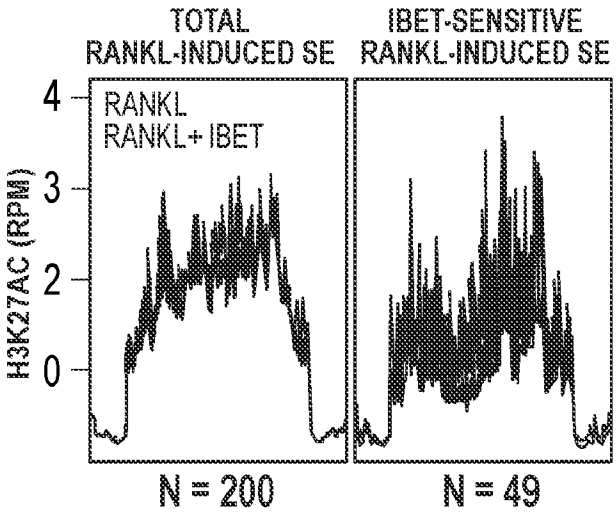


FIG. 4B

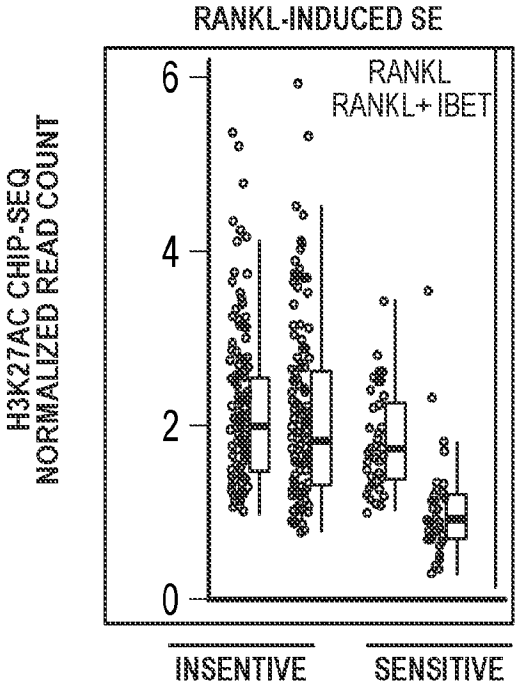


FIG. 4C

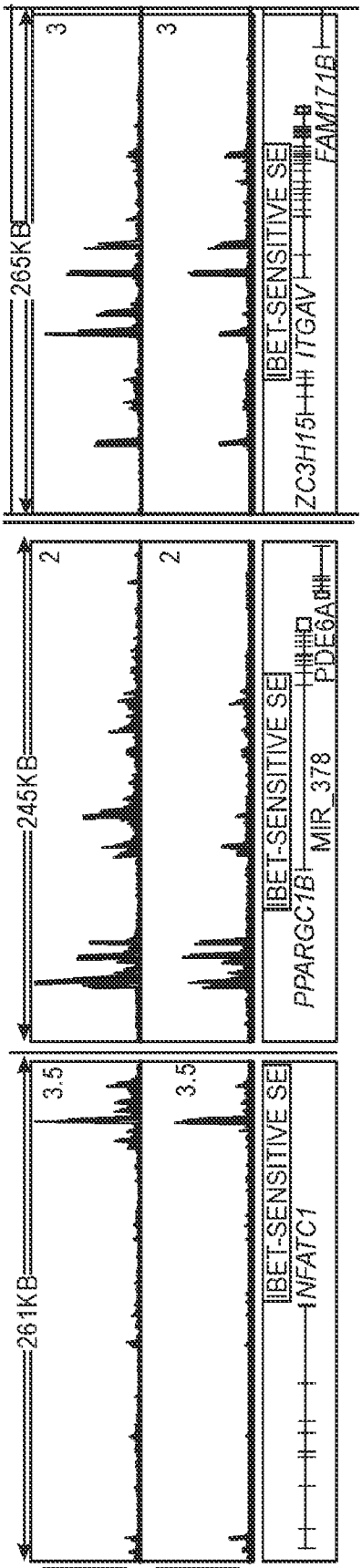


FIG. 4D

IBET-SENSITIVE H3K27AC PEAKS WITHIN SE

TF	MOTIF	P-VALUE
AP-1		1E-9
ATF3		1E-9
BATF		1E-9
FRA2		1E-8
FRA1		1E-8
FOSL2		1E-7
JUNB		1E-7

FIG. 4E

IBET-SENSITIVE H3K27AC PEAKS WITHIN SE

TF	MOTIF	P-VALUE
ELF4		1E-9
ERG		1E-7
ELF5		1E-6
ETV1		1E-6
PU.1:IRF8		1E-6
ELF3		1E-6
SPIB		1E-5

FIG. 4F

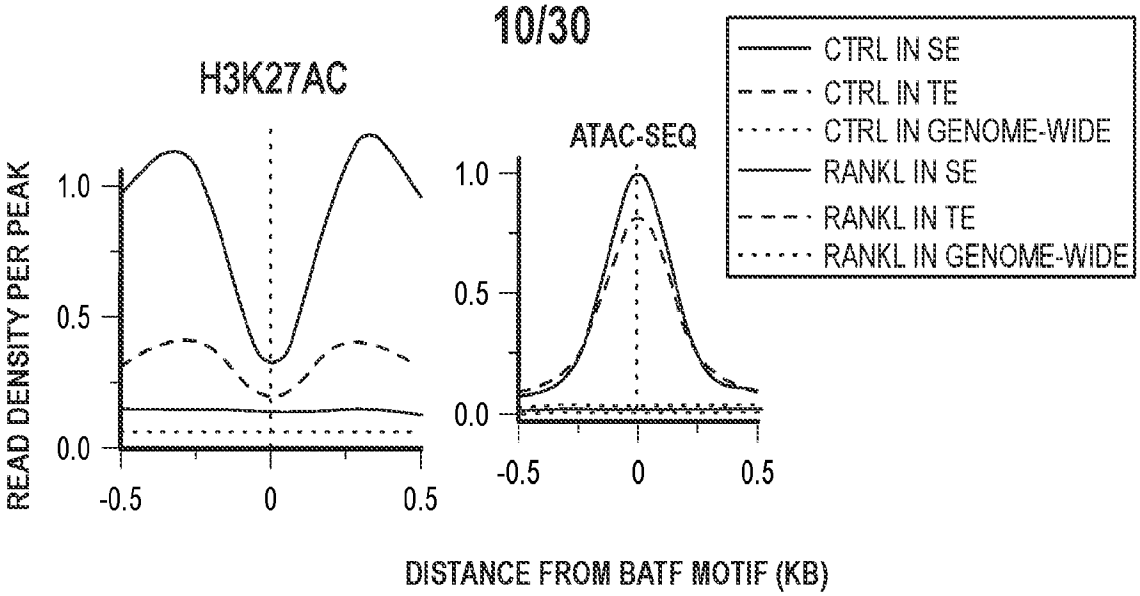


FIG. 4G

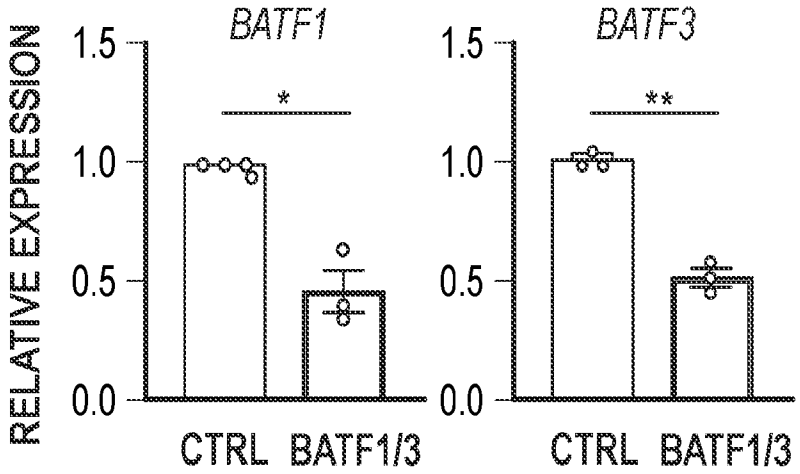


FIG. 4H

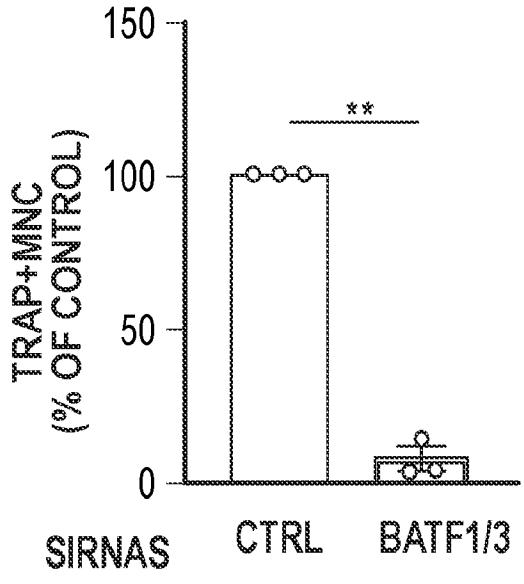


FIG. 4I

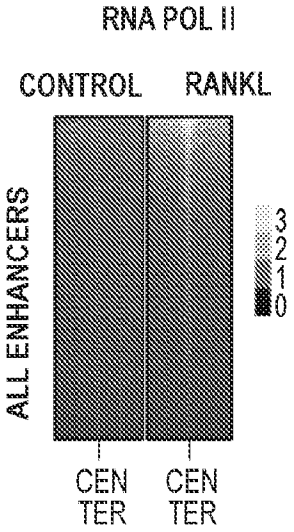


FIG. 5A

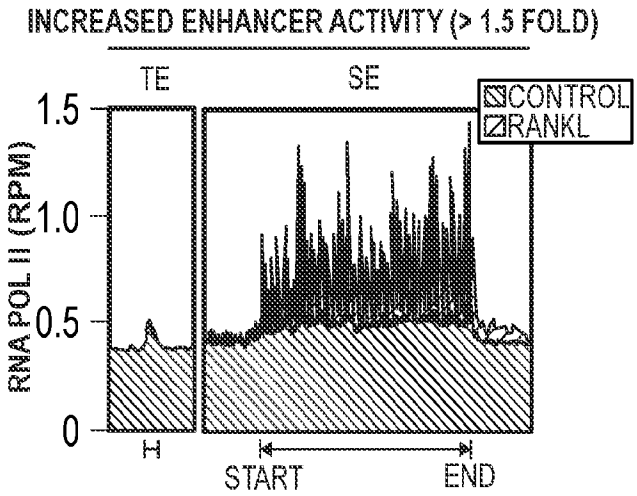


FIG. 5B

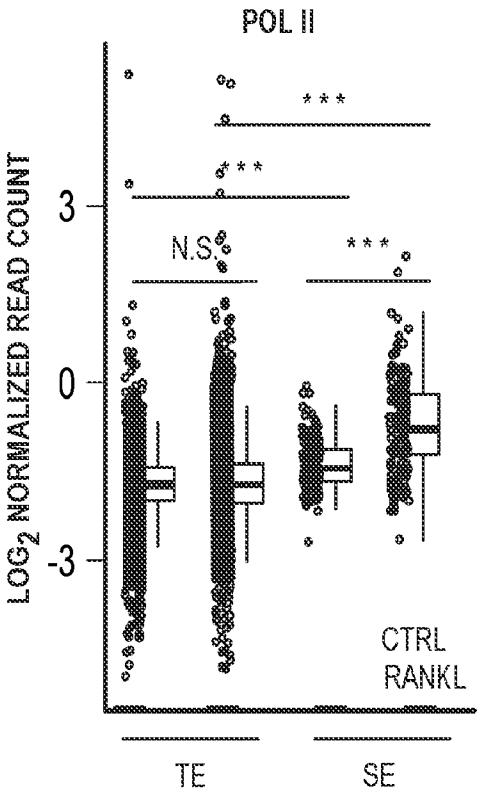


FIG. 5C

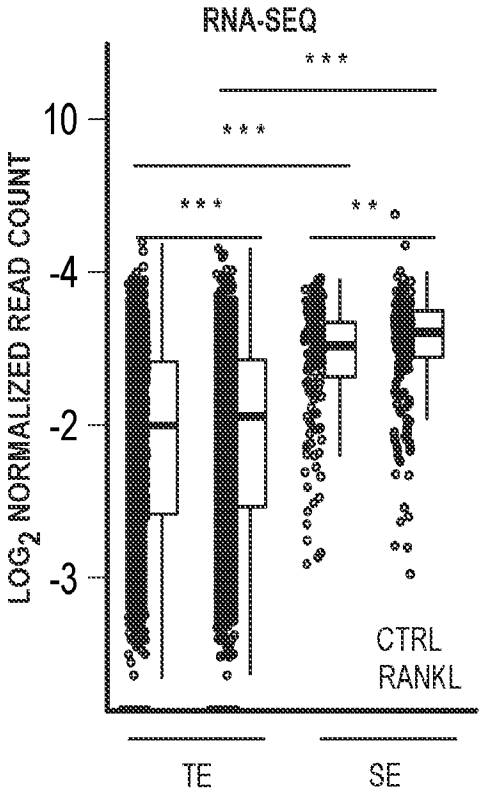


FIG. 5D

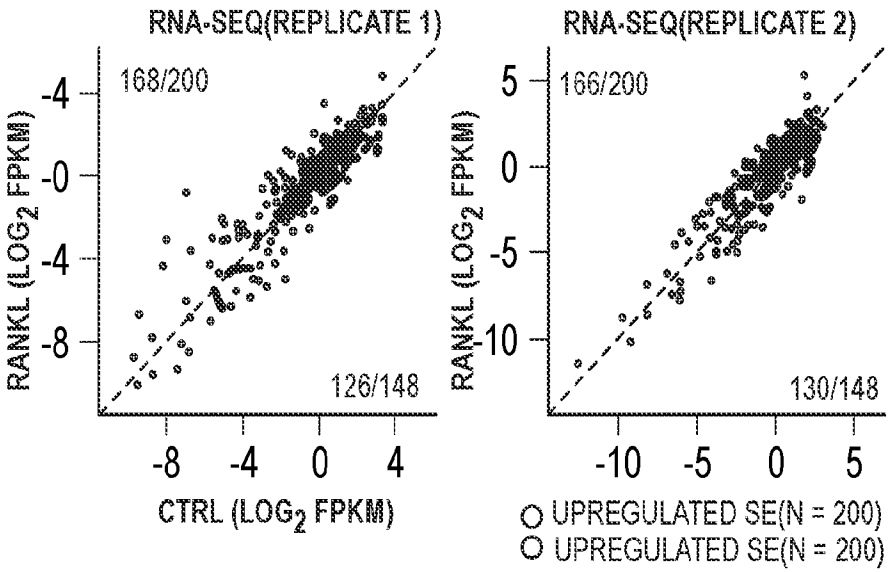

RNA-SEQ(REPLICATE 1)
168/200
126/148
RANKL (LOG2 FPKM)
CTRL (LOG2 FPKM)
-4
-0
-4
-8
-8
-4
0
4
RNA-SEQ(REPLICATE 2)
166/200
130/148
5
0
-5
-10
-10
-5
0
5
UPREGULATED SE(N = 200)
UPREGULATED SE(N = 200)

FIG. 5E

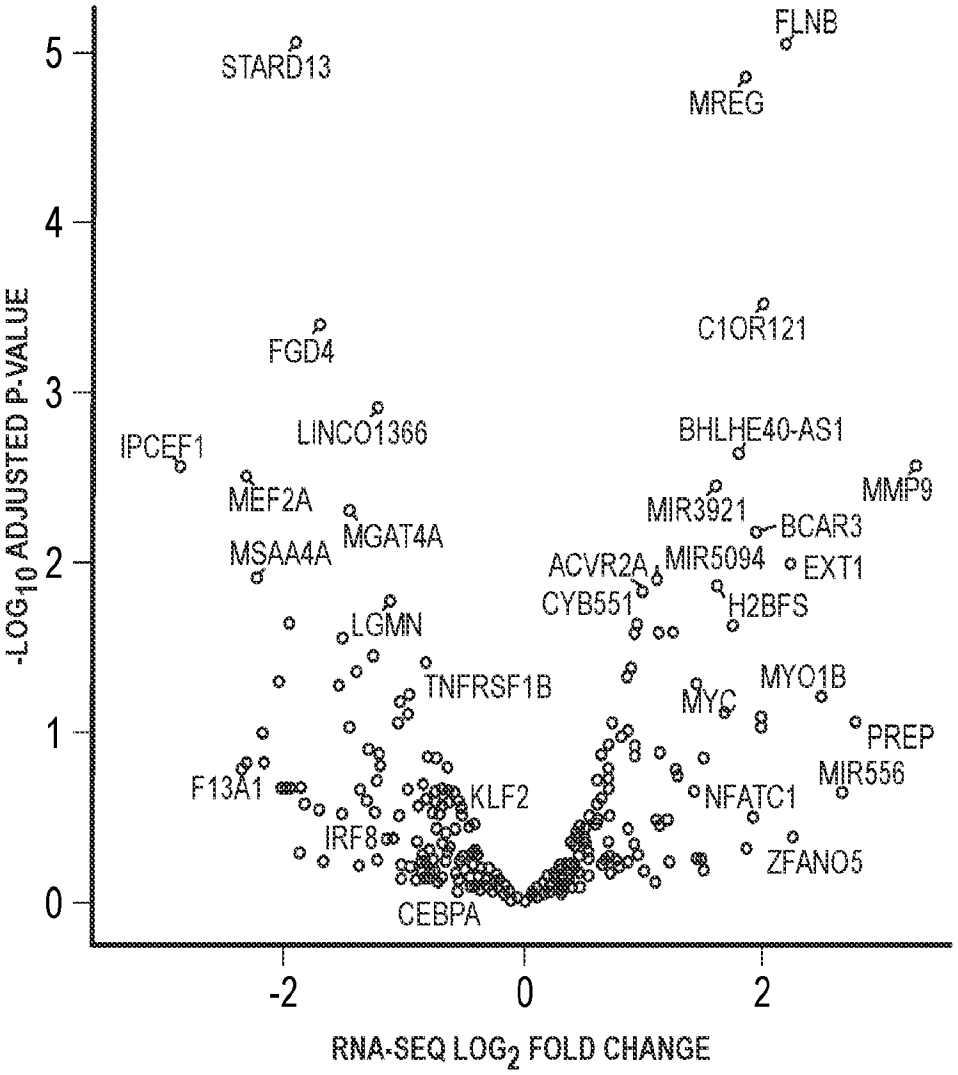

FLNB
STARD13
MREG
C1OR121
FGD4
LINC01366
BHLHE40-AS1
IPCEF1
MMP9
MEF2A
MIR3921
BCAR3
MGAT4A
MSAA4A
ACVR2A
MIR5094
EXT1
CYB551
H2BFS
LGMN
TNFRSF1B
MYO1B
MYC
PREP
MIR556
F13A1
KLF2
NFATC1
IRF8
ZFAND5
CEBPA
5
4
3
2
1
0
-2
0
2
-LOG10 ADJUSTED P-VALUE
RNA-SEQ LOG2 FOLD CHANGE

FIG. 5F

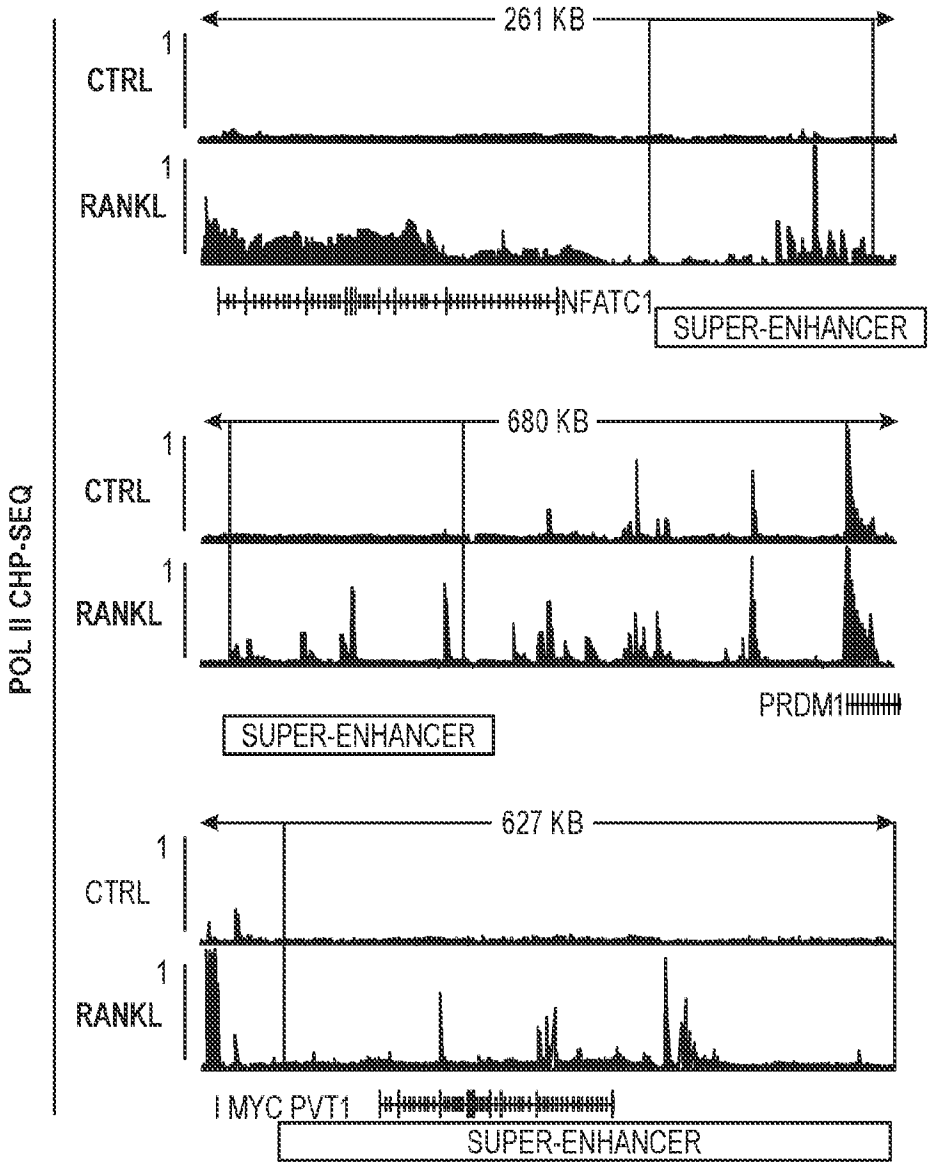

POL II CHP-SEQ
261 KB
CTRL
RANKL
1
1
NFATC1
SUPER-ENHANCER
680 KB
CTRL
RANKL
1
1
PRDM1
SUPER-ENHANCER
627 KB
CTRL
RANKL
1
1
MYC PVT1
SUPER-ENHANCER

FIG. 5G

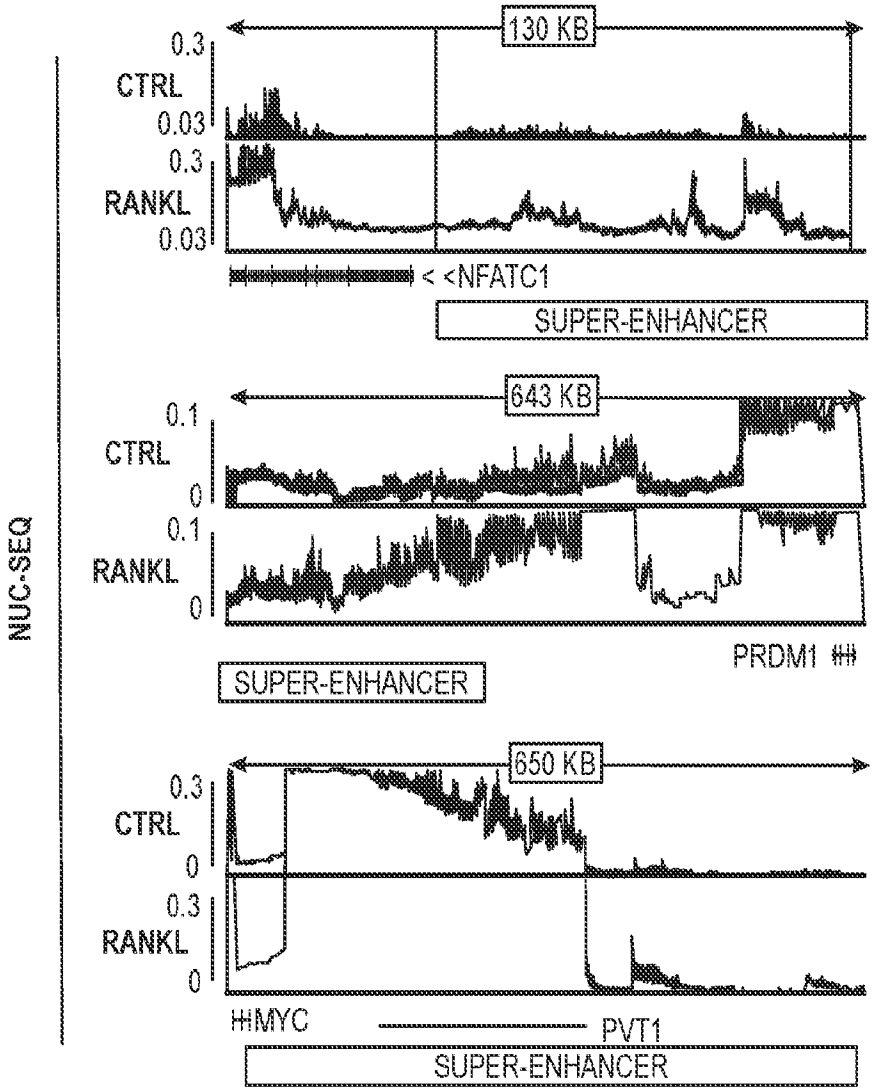

NUC-SEQ
130 KB
0.3
CTRL
0.03
0.3
RANKL
0.03
< <NFATC1
SUPER-ENHANCER
643 KB
0.1
CTRL
0
0.1
RANKL
0
PRDM1
SUPER-ENHANCER
650 KB
0.3
CTRL
0
0.3
RANKL
0
MYC
PVT1
SUPER-ENHANCER

FIG. 5H

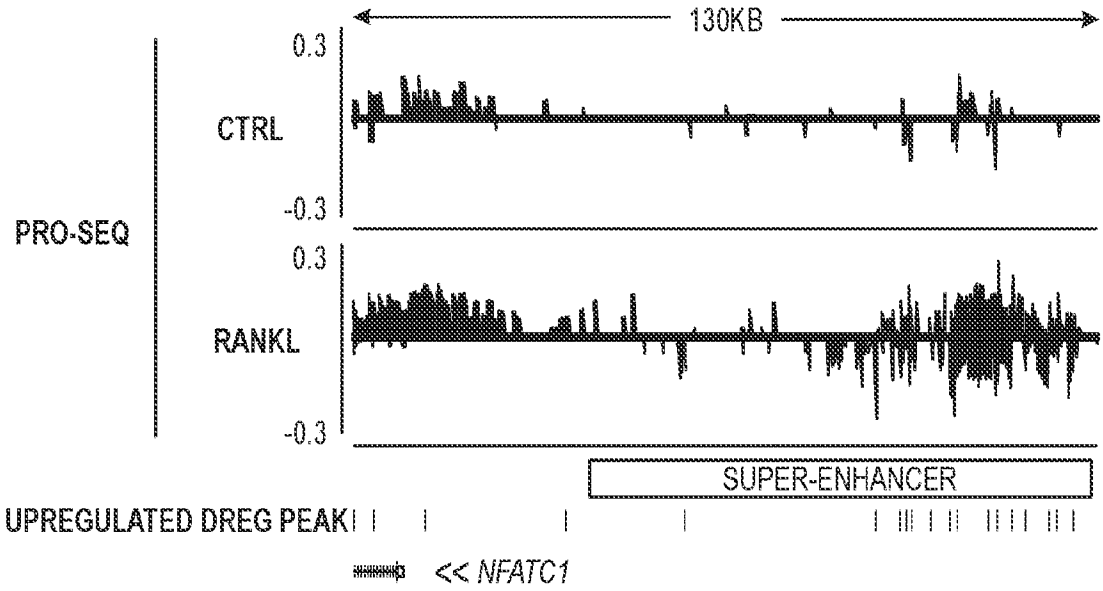

130KB
PRO-SEQ
CTRL
RANKL
0.3
-0.3
0.3
-0.3
SUPER-ENHANCER
UPREGULATED DREG PEAK
<< NFATC1

FIG.6A

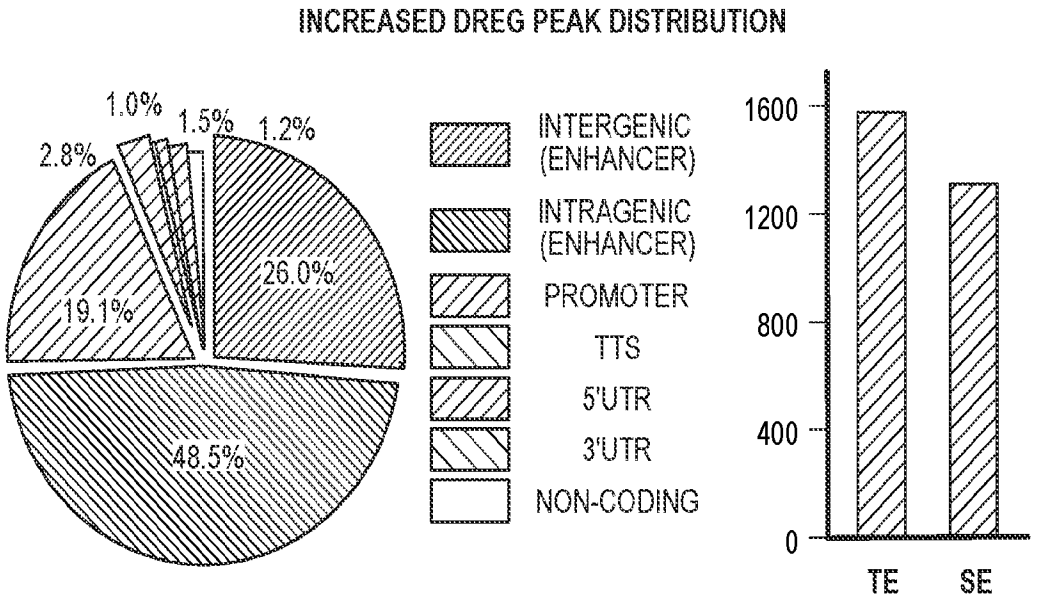

INCREASED DREG PEAK DISTRIBUTION
1.0%
1.5%
1.2%
2.8%
26.0%
19.1%
48.5%
INTERGENIC (ENHANCER)
INTRAGENIC (ENHANCER)
PROMOTER
TTS
5'UTR
3'UTR
NON-CODING
1600
1200
800
400
0
TE
SE

FIG.6B

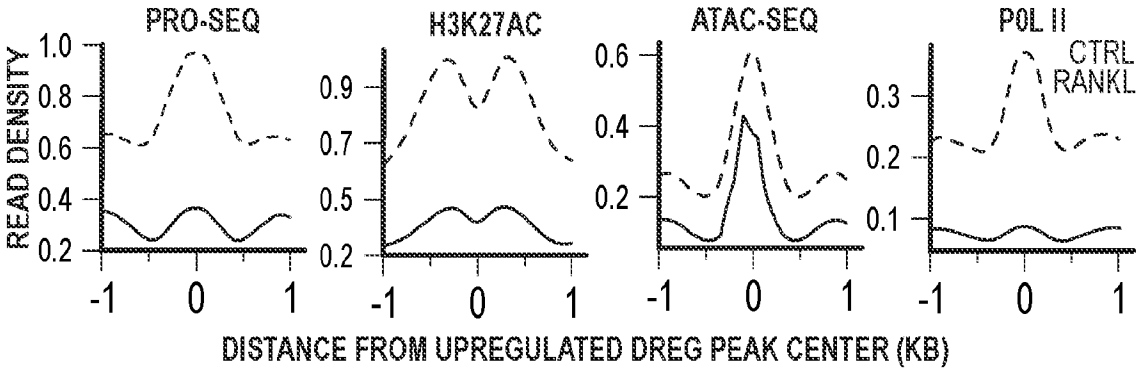


FIG. 6C

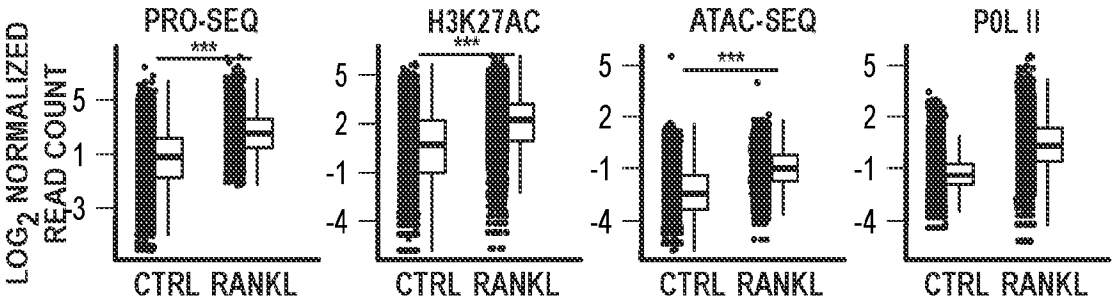


FIG. 6D

UPREGULATED DREG PEAKS

TF	MOTIF	P-VALUE
ATF3		1E-188
FOS		1E-184
FRA1		1E-182
BATF		1E-177
AP-1		1E-167

FIG. 6E

DOWNREGULATED DREG PEAKS

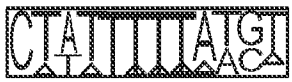
TF	MOTIF	P-VALUE
ETS1		1E-149
ELF4		1E-145
PU.1		1E-142
ELF5		1E-134
ETV1		1E-133

FIG. 6F

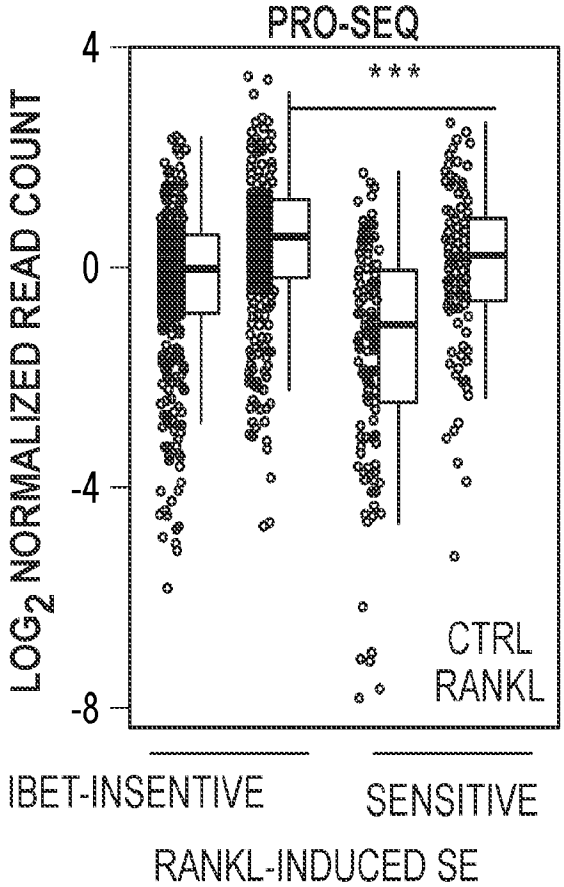


FIG. 6G

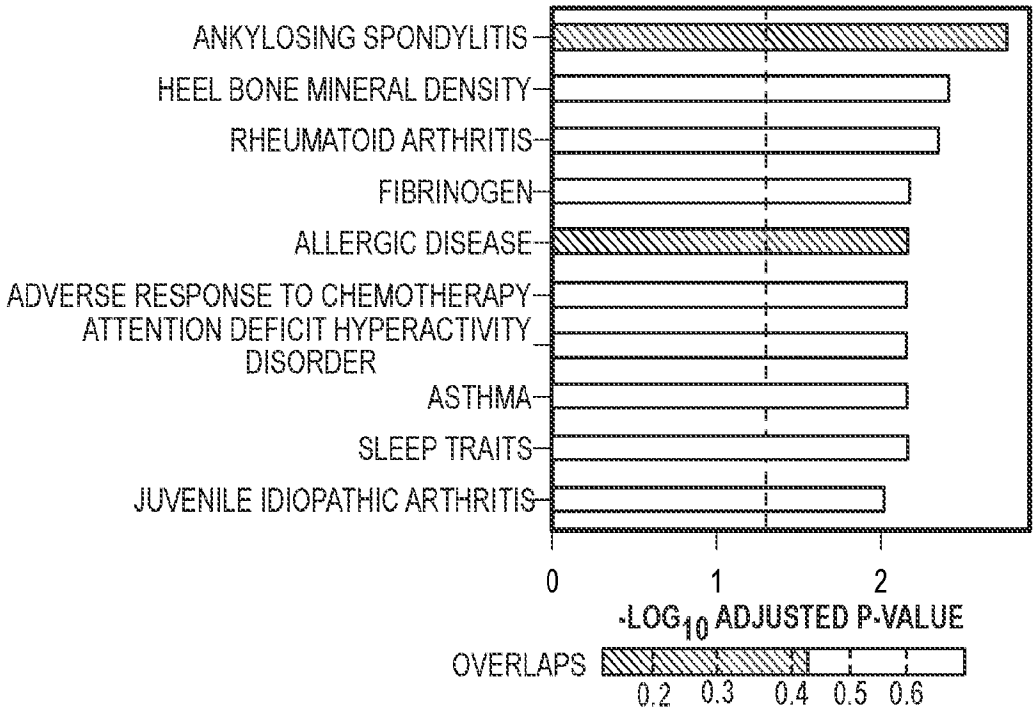


FIG.7A

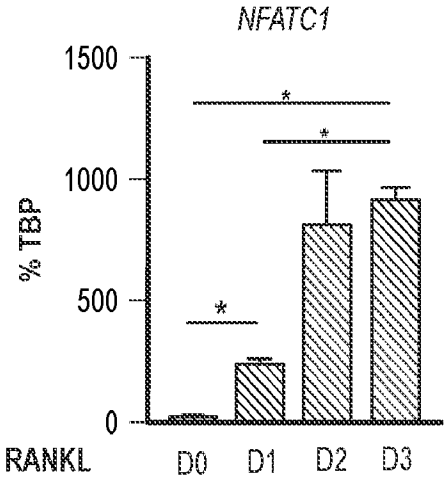


FIG.7B

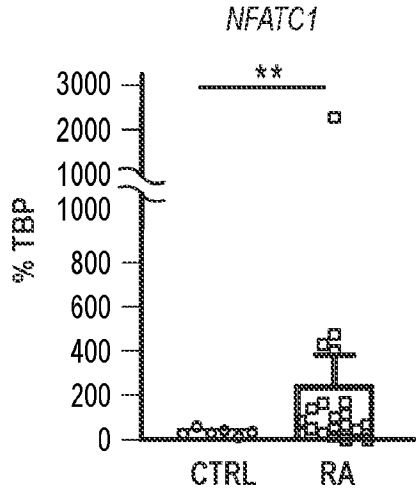


FIG.7C

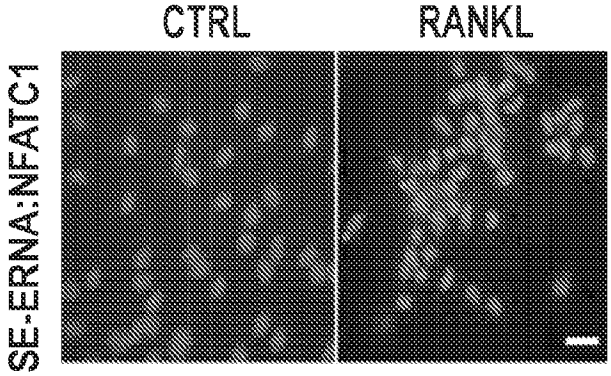


FIG.7D

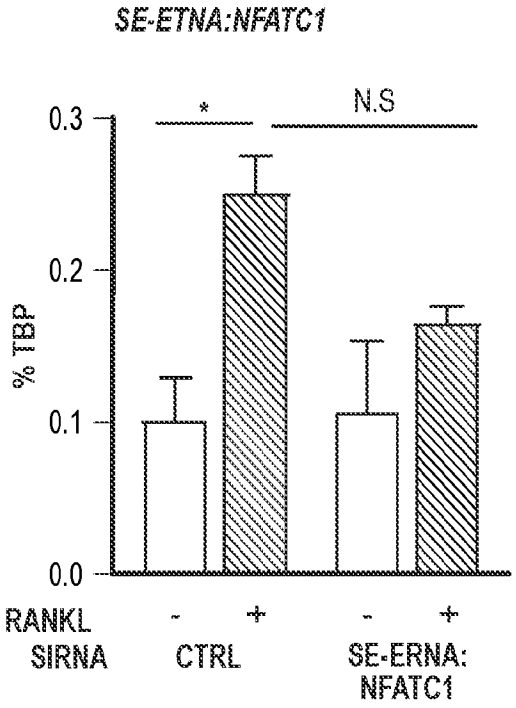


FIG.7E

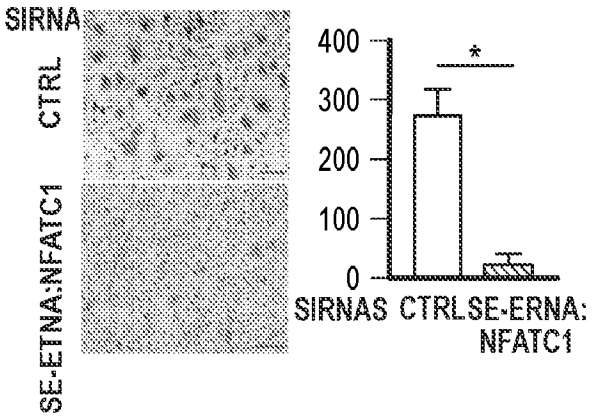


FIG.7F

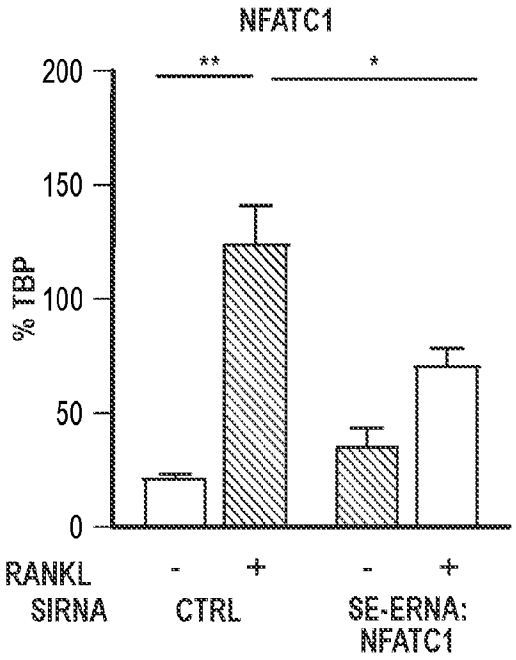


FIG.7G

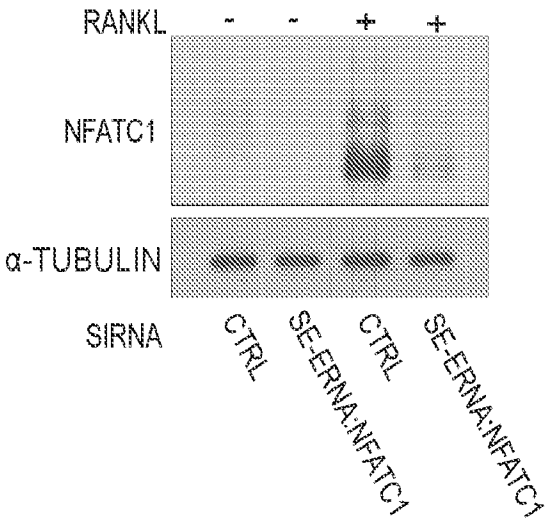


FIG.7H

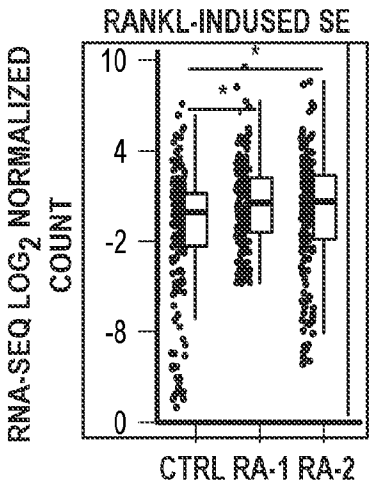
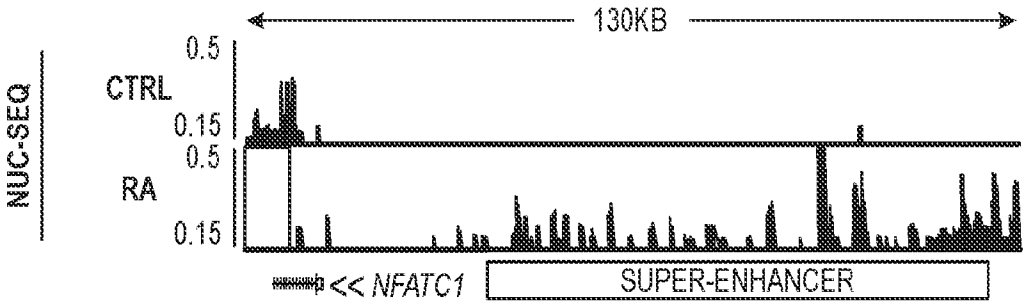


FIG.7I



SE-ERNA:NFATC1

FIG.7J

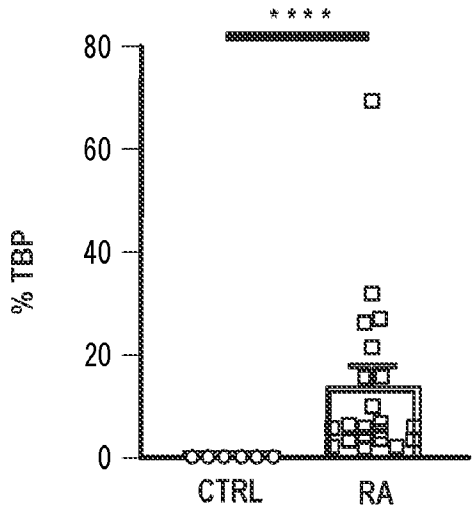


FIG.7K

	H3K27AC_CTRL_1	H3K27AC_CTRL_2	
H3K27AC_CTRL_1	1	0.9492	
H3K27AC_CTRL_2	0.9492		
	H3K27AC_RANKL_1	1	
H3K27AC_RANKL_1	1	0.9134	
H3K27AC_RANKL_2	0.9134	1	
	ATAC_CTRL_1	ATAC_CTRL_2	ATAC_CTRL_3
ATAC_CTRL_1	1	1	0.9964
ATAC_CTRL_2	0.9994	0.9981	0.9981
ATAC_CTRL_3	0.9964	1	1
	ATAC_RANKL_1	ATAC_RANKL_2	ATAC_RANKL_3
ATAC_RANKL_1	1	0.998	0.9978
ATAC_RANKL_2	0.998	1	0.9935
ATAC_RANKL_3	0.9978	0.9935	1
	POLII_CTRL_1	POLII_CTRL_2	
POLII_CTRL_1	1	0.8165	
POLII_CTRL_2	0.8165	1	
	POLII_RANKL_1	POLII_RANKL_2	
POLII_RANKL_1	1	0.8012	
POLII_RANKL_2	0.8012	1	

FIG. 8A

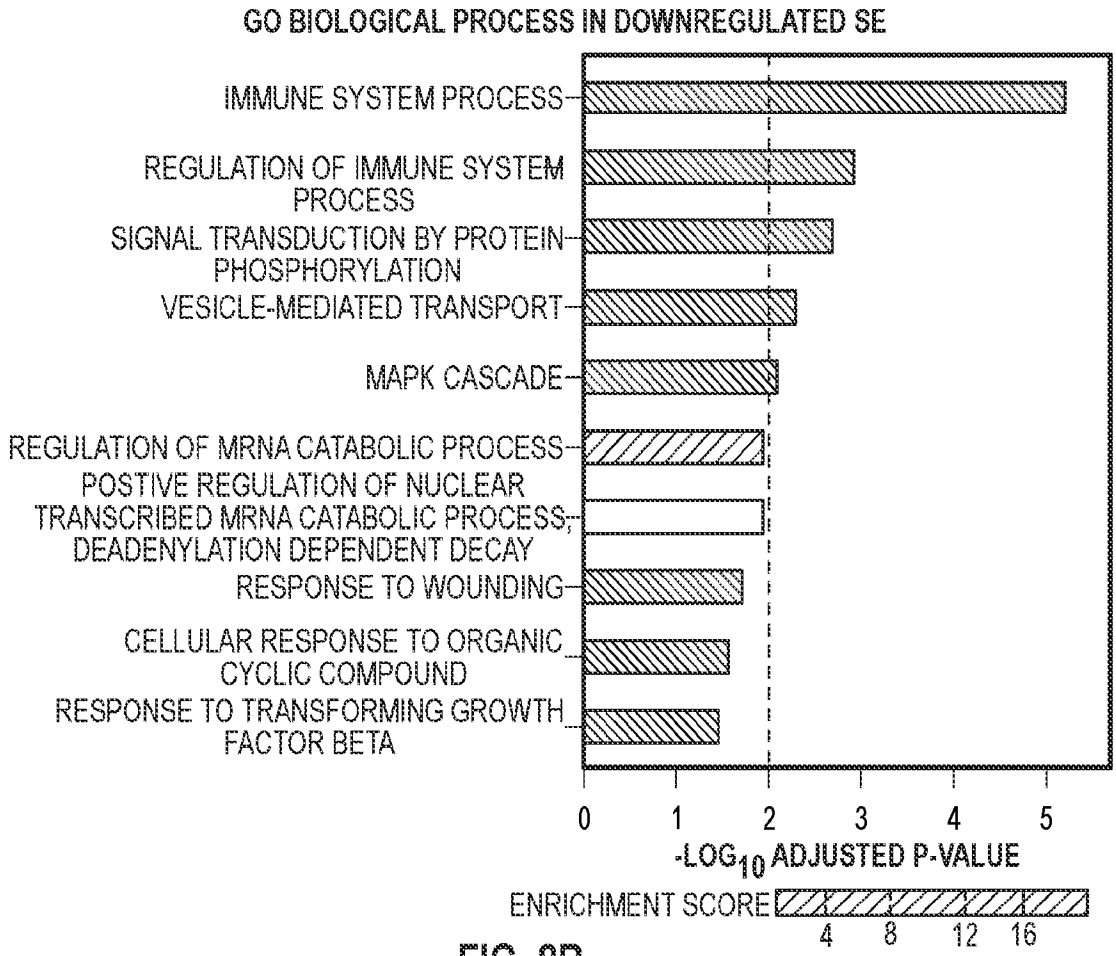


FIG. 8B

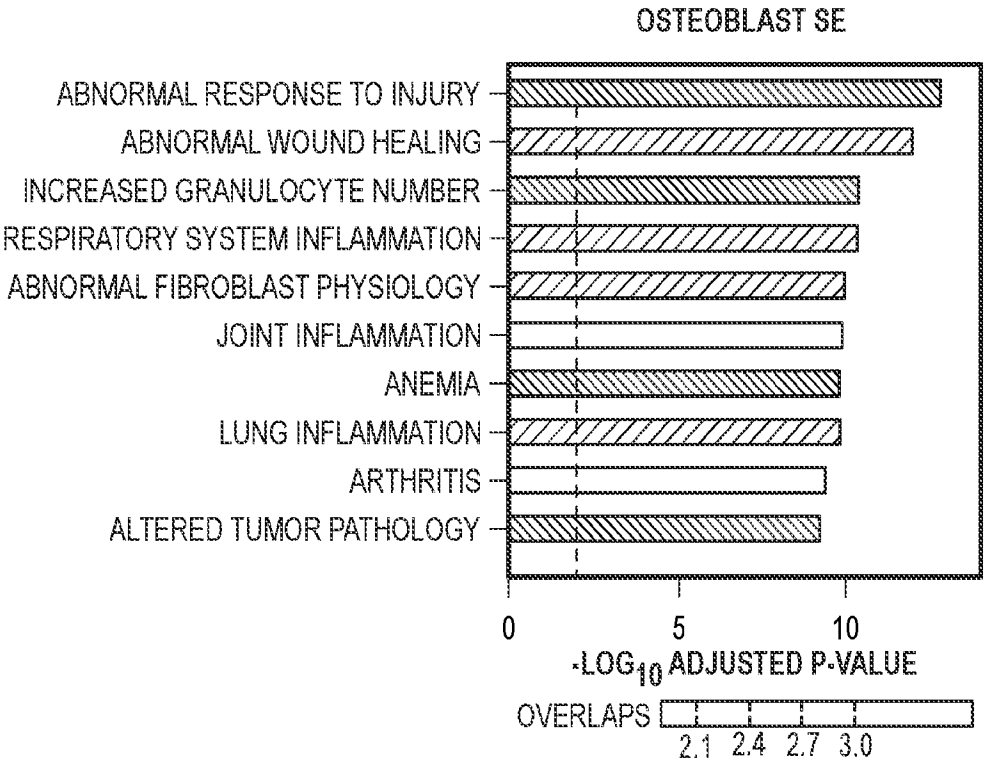

OSTEOBLAST SE
ABNORMAL RESPONSE TO INJURY
ABNORMAL WOUND HEALING
INCREASED GRANULOCYTE NUMBER
RESPIRATORY SYSTEM INFLAMMATION
ABNORMAL FIBROBLAST PHYSIOLOGY
JOINT INFLAMMATION
ANEMIA
LUNG INFLAMMATION
ARTHRITIS
ALTERED TUMOR PATHOLOGY
0
5
10
-LOG10 ADJUSTED P-VALUE
OVERLAPS
2.1 2.4 2.7 3.0

FIG.9A

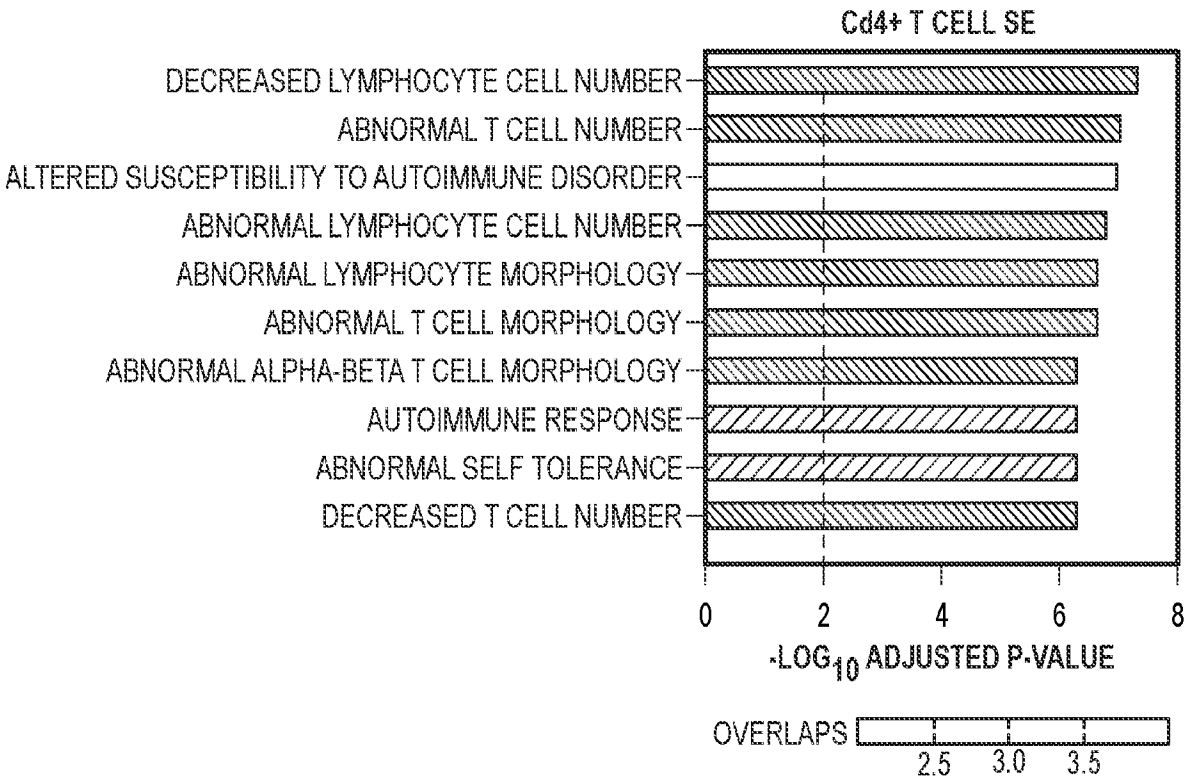

Cd4+ T CELL SE
DECREASED LYMPHOCYTE CELL NUMBER
ABNORMAL T CELL NUMBER
ALTERED SUSCEPTIBILITY TO AUTOIMMUNE DISORDER
ABNORMAL LYMPHOCYTE CELL NUMBER
ABNORMAL LYMPHOCYTE MORPHOLOGY
ABNORMAL T CELL MORPHOLOGY
ABNORMAL ALPHA-BETA T CELL MORPHOLOGY
AUTOIMMUNE RESPONSE
ABNORMAL SELF TOLERANCE
DECREASED T CELL NUMBER
0
2
4
6
8
-LOG10 ADJUSTED P-VALUE
OVERLAPS
2.5 3.0 3.5

FIG.9B

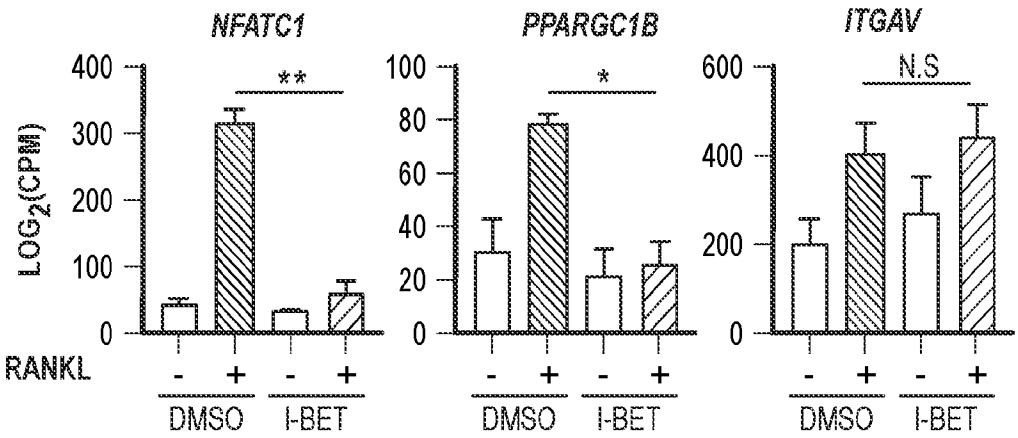


FIG. 10A

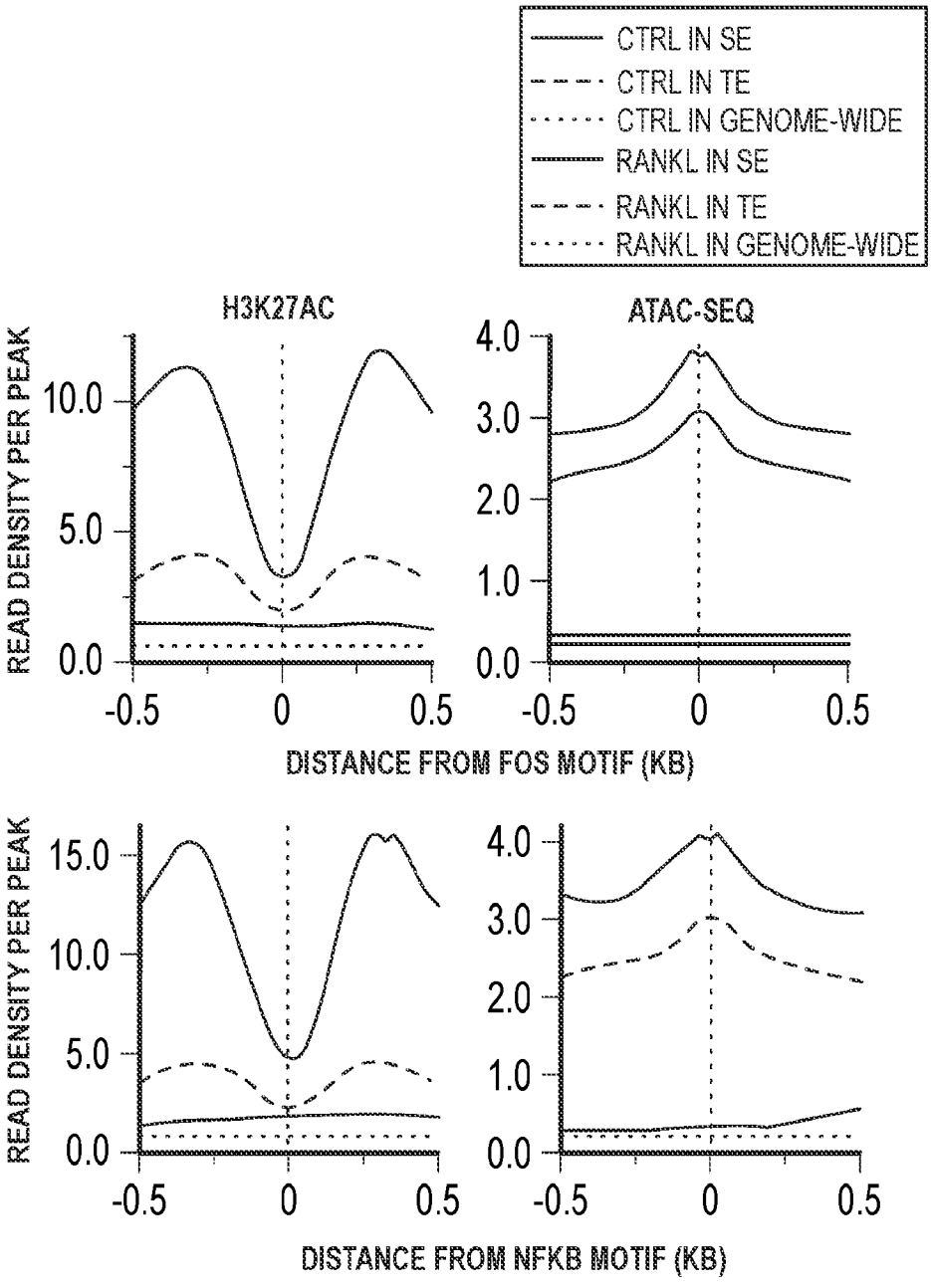


FIG. 10B

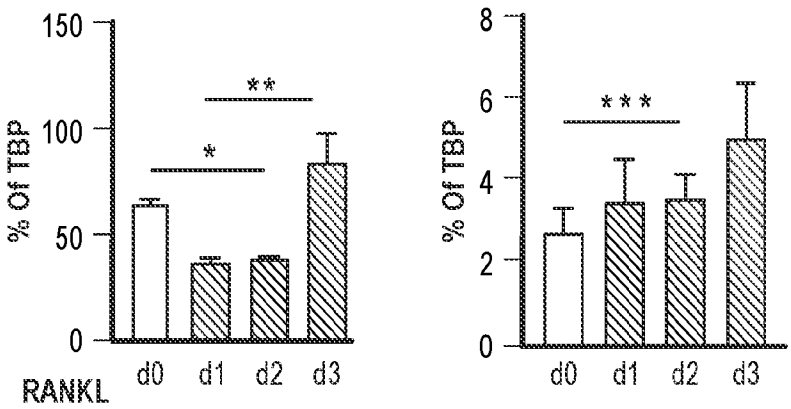


FIG. 10C

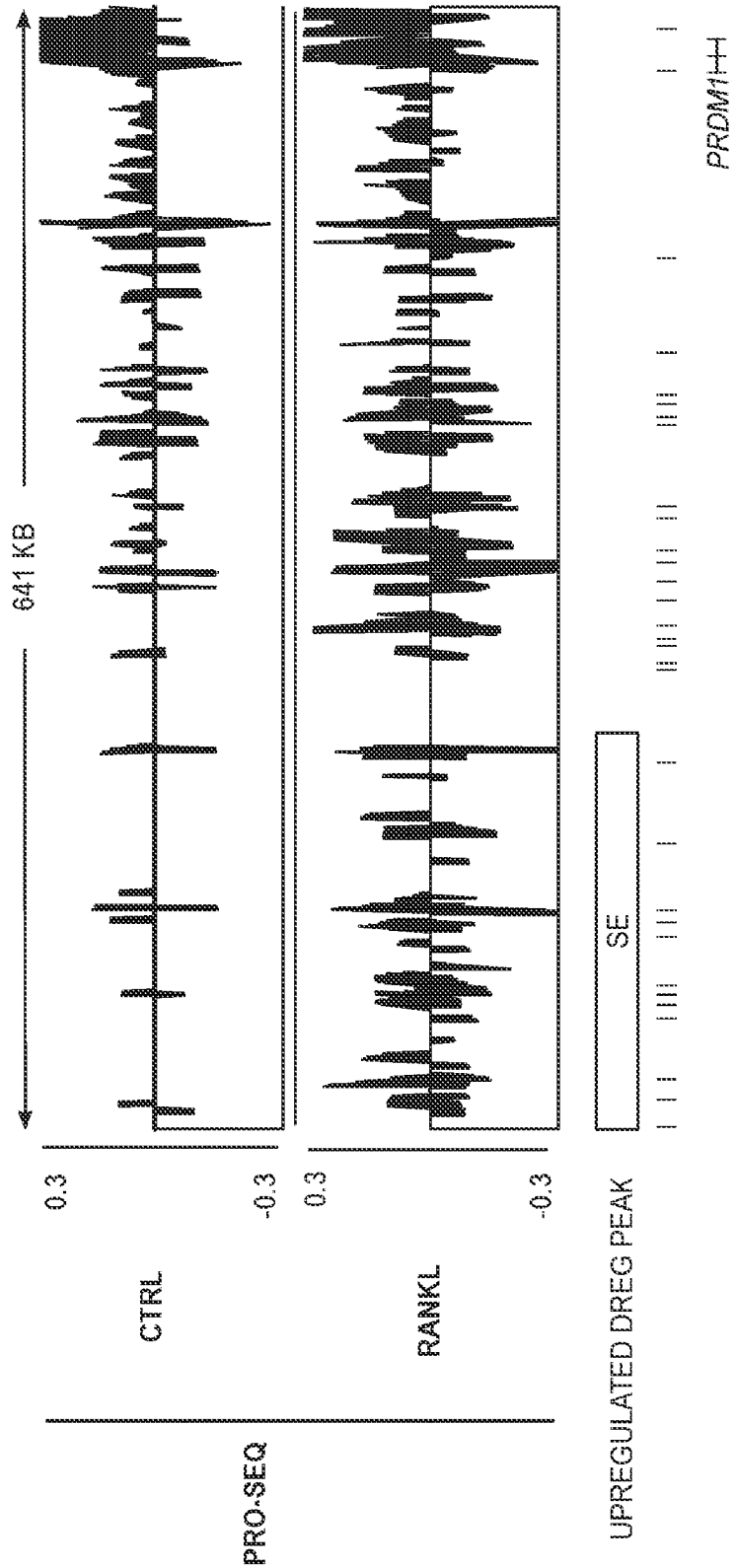

641 KB
PRDM1-H
PRO-SEQ
CTRL
0.3
-0.3
RANKL
0.3
-0.3
UPREGULATED DREG PEAK
SE

FIG. 11A

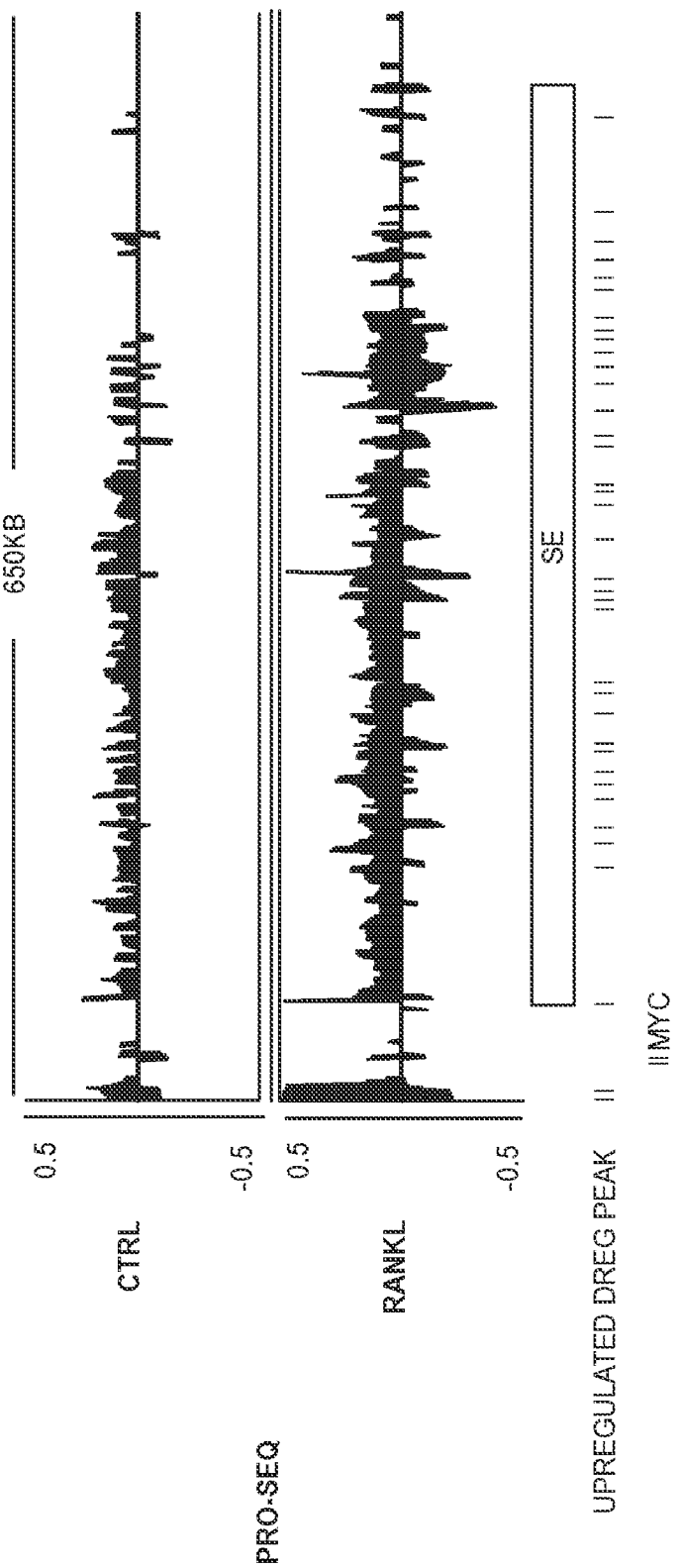

650KB
PRO-SEQ
CTRL
RANKL
0.5
-0.5
0.5
-0.5
SE
UPREGULATED DREG PEAK
MYC

FIG. 11B

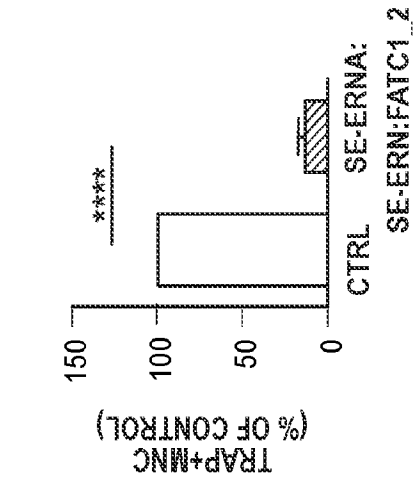
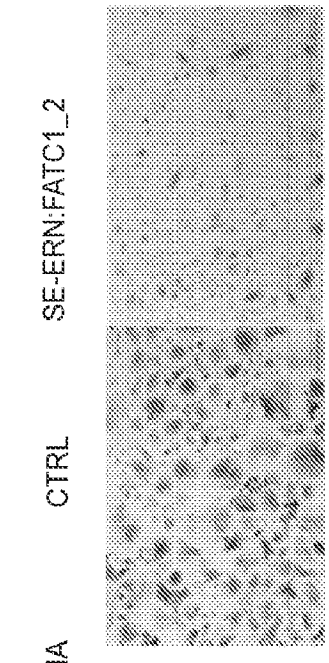


FIG. 12B



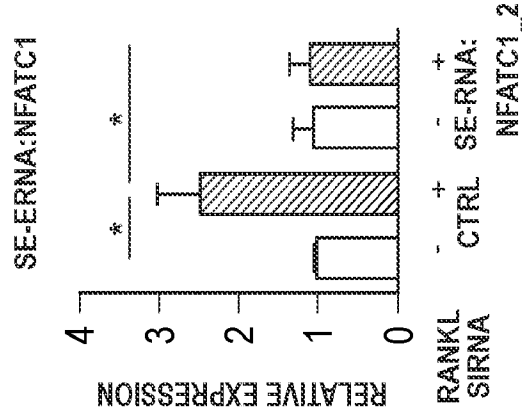


FIG. 12A

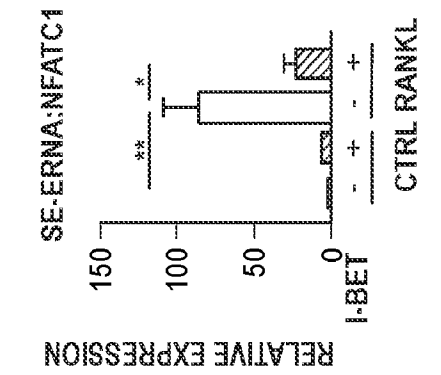
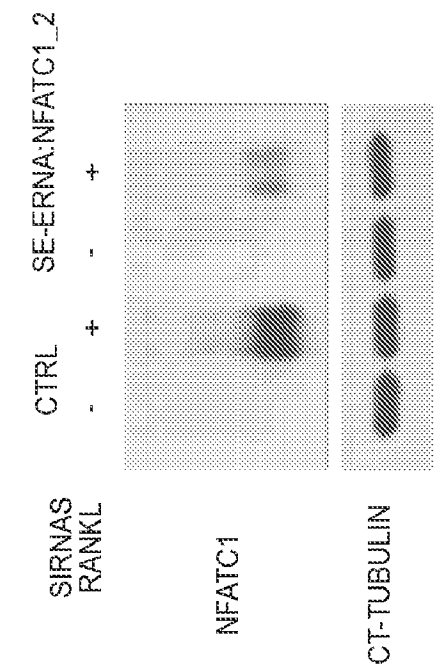


FIG. 12D



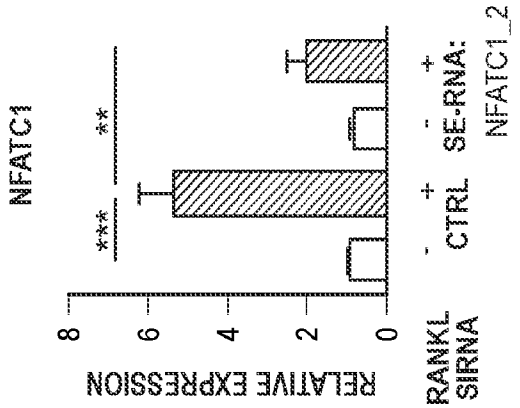


FIG. 12C

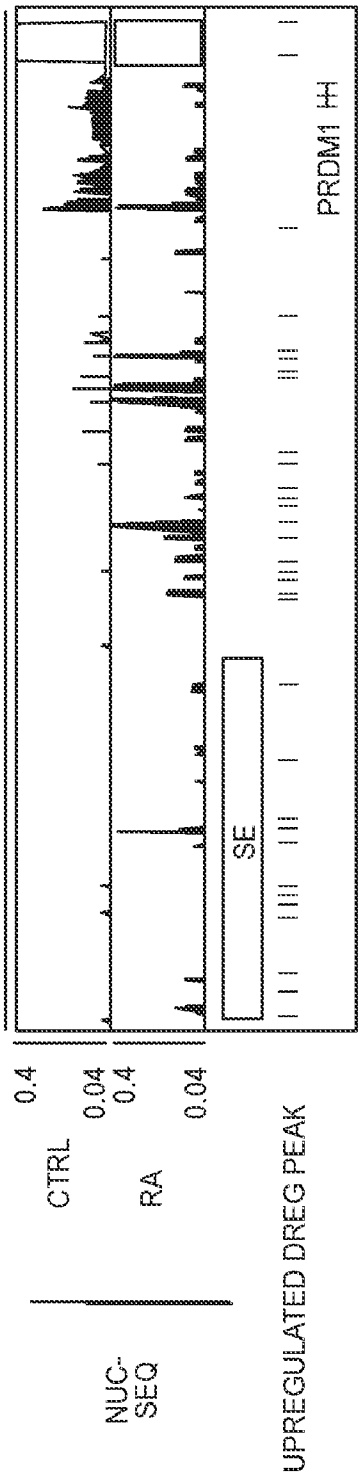


FIG. 12E

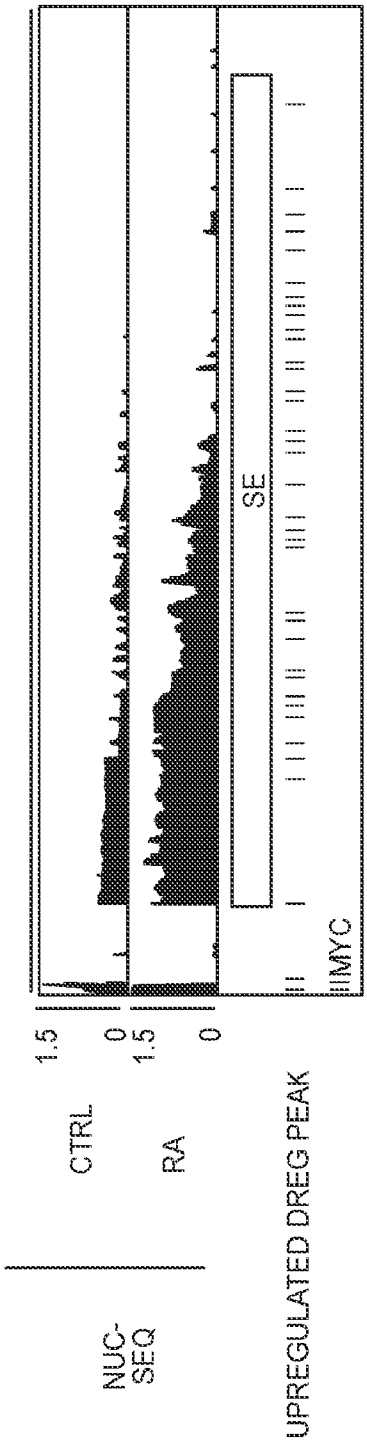


FIG. 12F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/78886

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/113; A61K 9/127; A61P 19/10; C12N 15/86 (2023.01)
ADD.

CPC - INV. C12N 15/113; A61K 9/127; A61K 48/005; A61P 19/10; C12N 15/86; C12N 15/907

ADD. C12N 2510/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0242599 A1 (ZAYZAFOON MAJD) 02 October 2008; [0081], [0094], [0128], [0133], [0135]	10-12
A	WO 2006/074061 A2 (SEQUENOM INC.) 13 July 2006; claim [34]; [0018], [0046], [0118], [0175]	1-5, 19-20
A	US 2008/0125583 A1 (RIGOUTSOS ISIDORE) 29 May 2008; [0049]	1-5, 19-20
A	US 2019/0032136 A1 (CELERA CORPORATION) 31 January 2019; [0049]	1-5, 19-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 February 2024 (23.02.2024)	APR 09 2024
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/78886

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/78886

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-9, 13-18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.