Supplementary Information

RANKL-responsive epigenetic mechanism reprograms macrophage to bone resorbing osteoclasts

Seyeon Bae, Kibyeong Kim, Keunsoo Kang, Heamin Kim, Minjoon Lee, Brian Oh, Kaichi Kaneko, Sungkook Ma, Jae Hoon Choi, Hojoong Kwak, Eun Young Lee, Sung Ho Park, and Kyung-Hyun Park-Min

Online supplementary figure legend

Online supplementary figure 1. Mapping of RANKL-sensitive SEs in human osteoclasts. (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. (b) Gene ontology for down-regulated SEs with corresponding adjusted p-value.

Online supplementary figure 2. 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.

Online supplementary figure 3. Transcription Factors at RANKL-sensitive SEs. (a) 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 NF-kB 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 red 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).

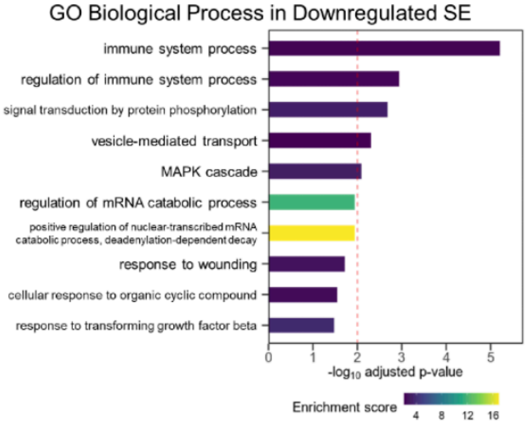
Online supplementary figure 4. RANKL-induced SE-eRNA expression in human osteoclasts. Representative tracks of pro-seq (upper tracks) and RNA-seq (bottom tracks, RA: RA synovial OCPs, CTRL: disease control) at PRDM-associated (a), MYC-associated (b) SE domain in the indicated conditions. Up-regulated dREG peaks of pro-seq (brown) are shown.

Online supplementary figure 5. 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).

a

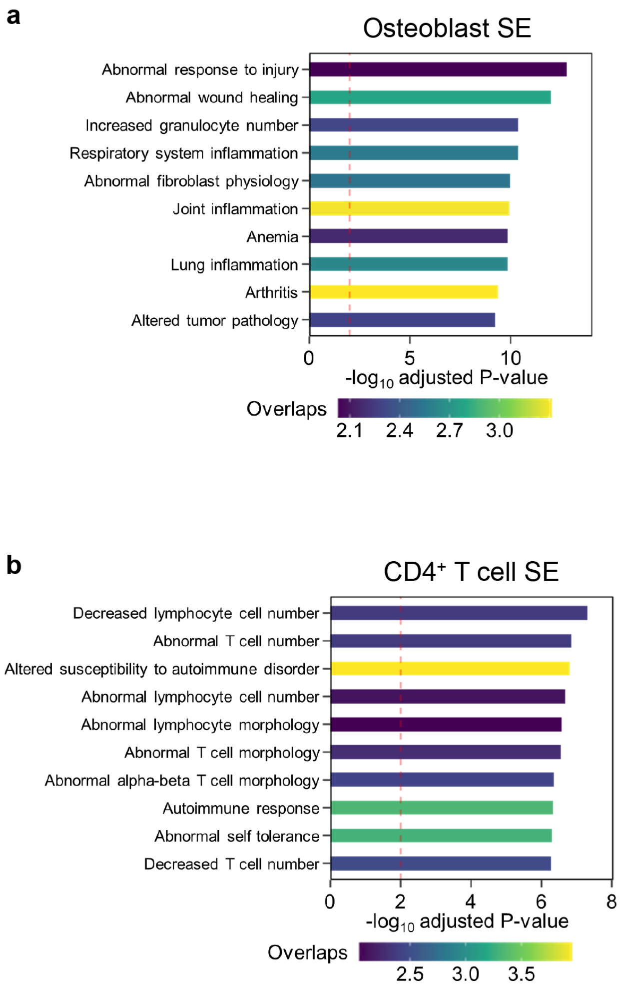
	H3K27ac_CTRL_1	H3K27ac_CTRL_2	
H3K27ac_CTRL_1	1	0.9492	
H3K27ac_CTRL_2	0.9492		
	H3K27ac_RANKL_1	1	
H3K27ac_RANKL_1	1	0.9134	
H3K27ac_RANKL_2	0.9134	1	
	ATAC_CTRL_1	ATAC_CTRL_2	ATAC_CTRL_3
ATAC_CTRL_1	1	0.9994	0.9964
ATAC_CTRL_2	0.9994	1	0.9981
ATAC_CTRL_3	0.9964	0.9981	1
	ATAC_RANKL_1	ATAC_RANKL_2	ATAC_RANKL_3
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	

b

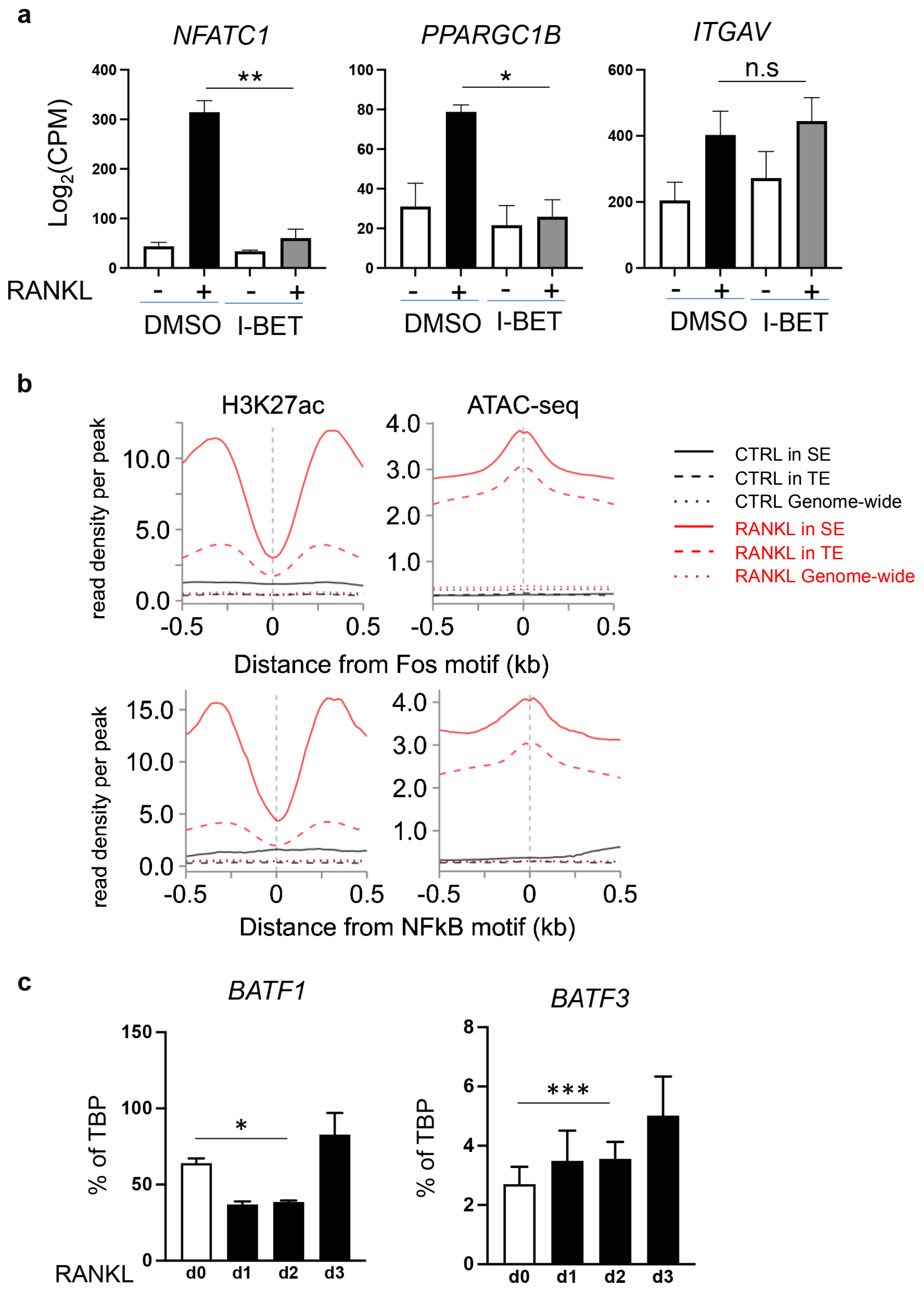


GO Biological Process in Downregulated SE

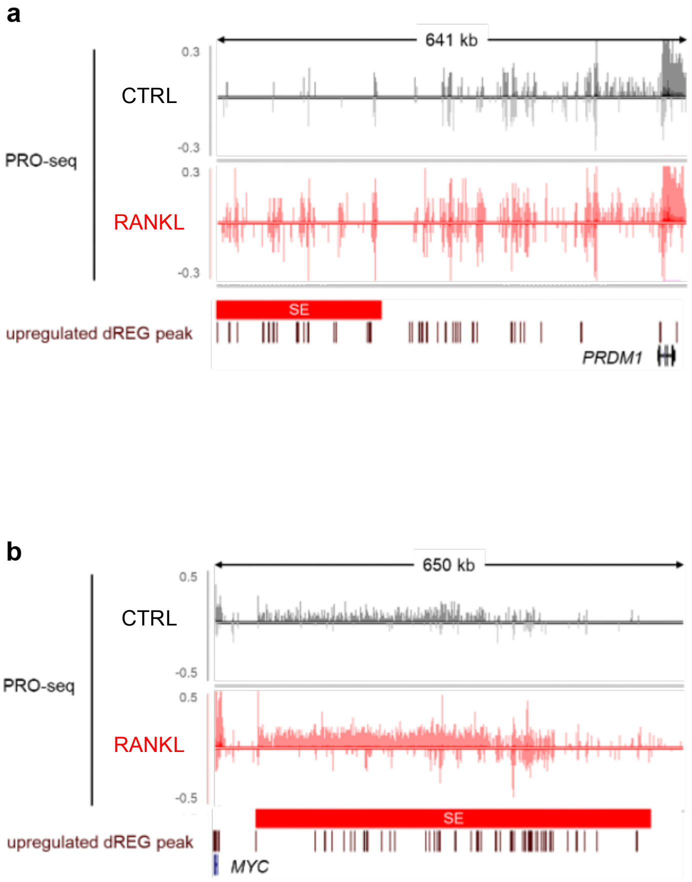
Supplementary figure 1



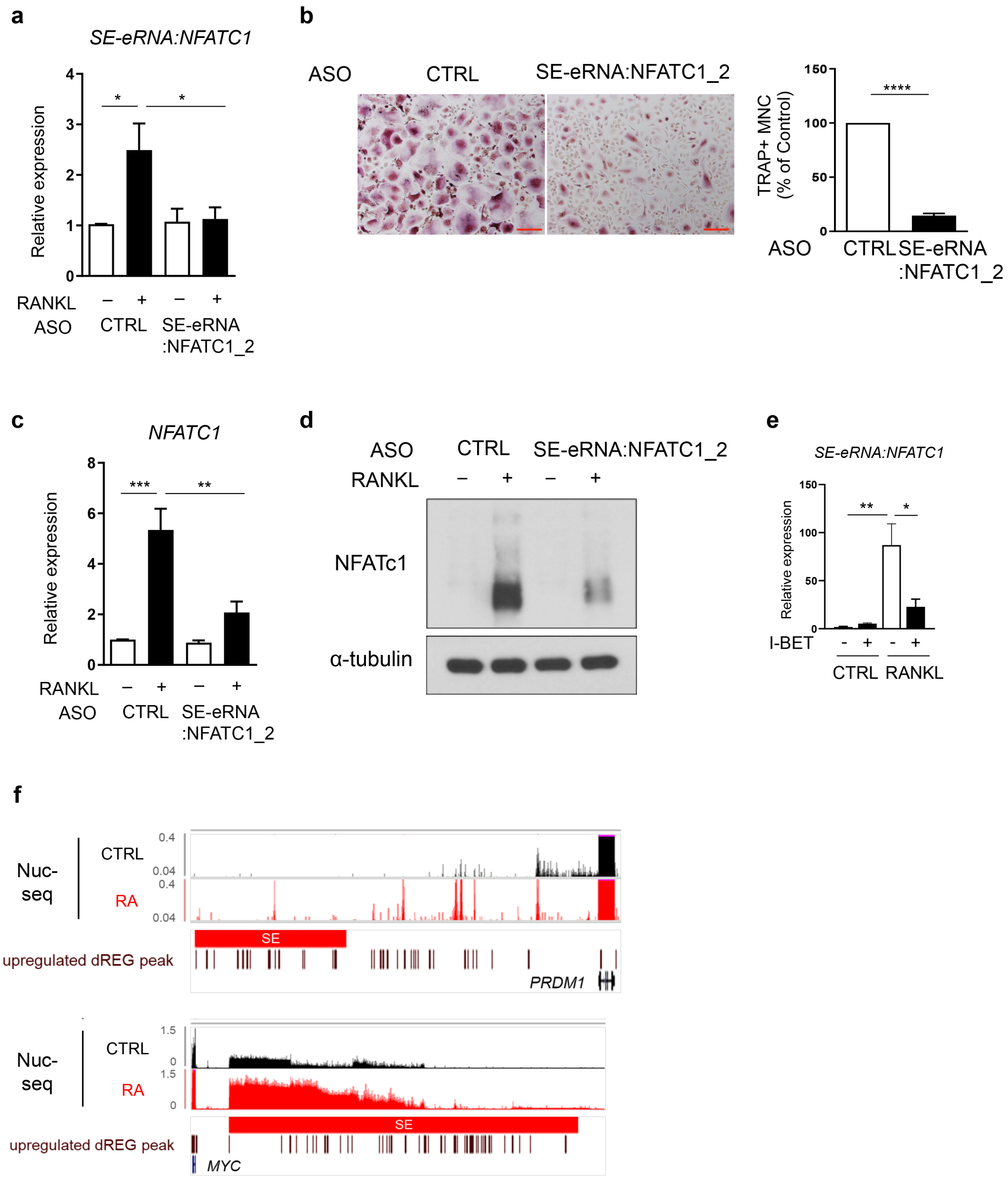
Supplementary figure 2



Supplementary figure 3



Supplementary figure 4



Supplementary figure 5

Supplementary Table 1. A list of primers used in this study.

Gene Symbol	Sequence
Human TBP	CACCACAGCTCTTCCACTA GGGGAGGGATACAGTGGAGT
Human NFATc1	CTTCTTCCAGTATTCCACCTAT TTGCCCTAATTACCTGTTGAAG
SE-eRNA:NFATc1	CAATCCCATGAAACAAACGCTCA CCAGGCCCTTCGCGATT
Human BATF1	AAATCGTATTGCCGCCCAG TAGAGCCGCGTTCTGTTTCT
Human BATF3	ATGAGAGCCTGGAGCAAGAA TTCAGTGCCTCTGTCAGGTG

Appendix; A list of acronyms and abbreviations

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
eRNA	Enhancer RNA
Elf4	E74 Like ETS Transcription Factor 4
FBS	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