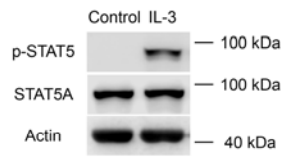
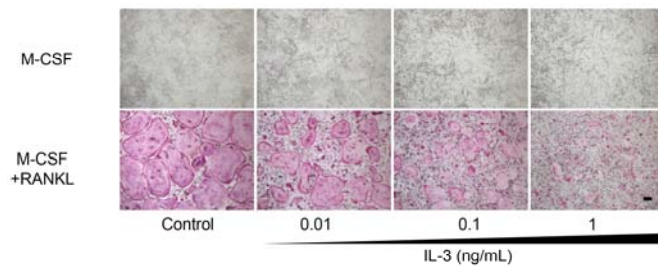
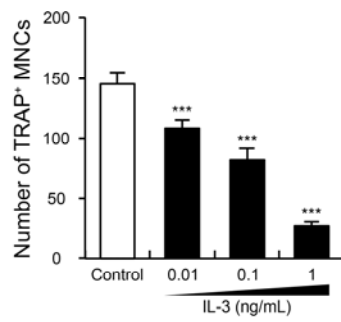
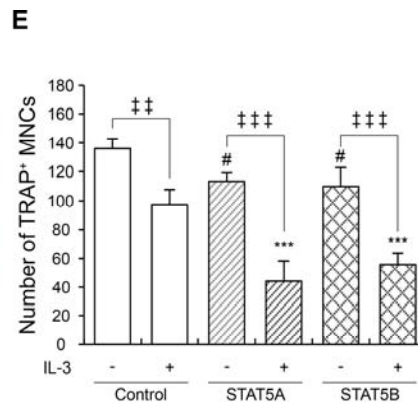
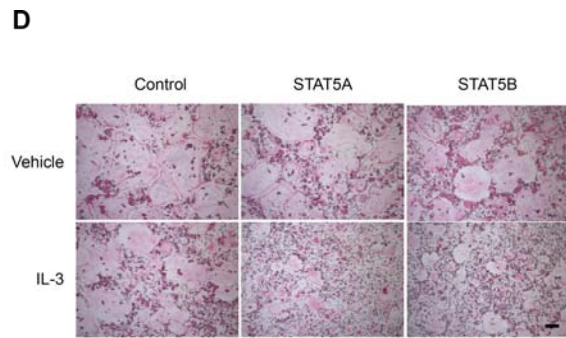
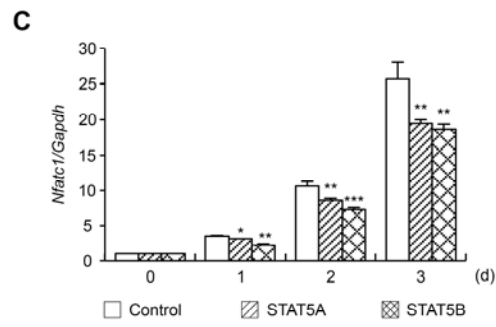
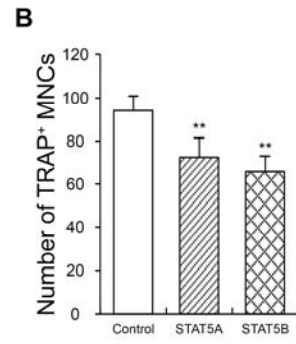
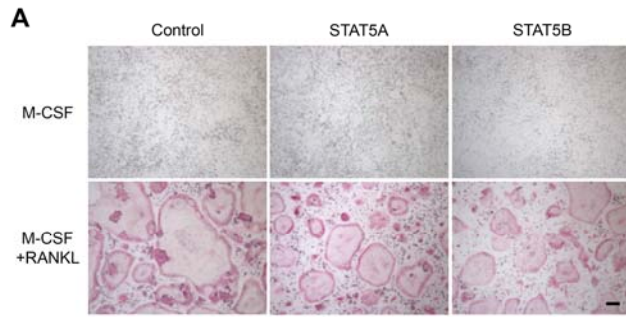


**STAT5 is a key transcription factor for IL-3-mediated inhibition of
RANKL-induced osteoclastogenesis**

Jongwon Lee^{1,5}, Semun Seong^{1,2,5}, Jung Ha Kim¹, Kabsun Kim¹, Inyoung Kim¹, Byung-chul
Jeong^{1,2}, Kwang-Il Nam³, Kyung Keun Kim¹, Lothar Hennighausen⁴, Nacksung Kim^{1,2,*}

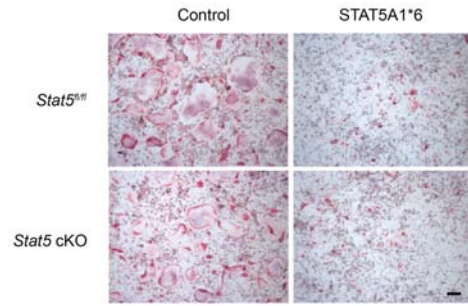
A**B****C**

Supplementary Figure 1. IL-3 inhibits RANKL-mediated osteoclastogenesis. (A) BMMs were cultured for one day with IL-3. Whole cell lysates were harvested from cultured cells and were immunoblotted with the indicated antibodies. (B-C) BMMs were cultured for three days with M-CSF and RANKL in the presence of various concentrations of IL-3 as indicated. (B) Cultured cells were stained for TRAP. (C) The number of TRAP-positive MNCs per well was counted. Data are represented as the mean \pm SD. ***, $P < 0.001$ vs. control; $n = 4$. Bar: 100 μm . All results are representative of at least three independent experiments. Statistical analyses were performed using ANOVA.

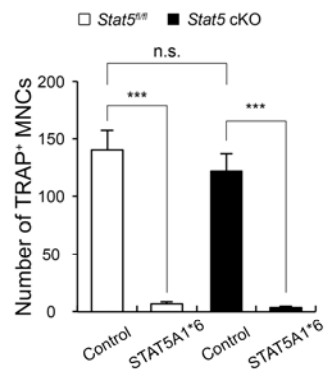


Supplementary Figure 2. Redundant roles of STAT5A and STAT5B in RANKL-induced osteoclastogenesis. (A-B) BMMs were transduced with pMX-IRES-EGFP (control), STAT5A, or STAT5B retrovirus and cultured with M-CSF for three days in the presence or absence of RANKL as indicated. (A) Cultured cells were stained for TRAP. (B) The number of TRAP-positive MNCs per well was counted. Data are represented as the mean \pm SD. **, $P < 0.01$ vs. control; $n = 4$. (C) BMMs were transduced with pMX-IRES-EGFP (control), STAT5A, or STAT5B retrovirus and cultured with M-CSF and RANKL for the indicated times. mRNA levels of *Naftc1* were assessed by quantitative real-time PCR. Data represent mean \pm SD of triplicate samples. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; vs. control. (D-E) BMMs were transduced with pMX-IRES-EGFP (control), STAT5A, or STAT5B retrovirus and cultured with M-CSF and RANKL for three days in the presence or absence of IL-3 as indicated. (D) Cultured cells were stained for TRAP. (E) The number of TRAP-positive MNCs per well was counted. Data are represented as the mean \pm SD. #, $P < 0.05$; vs. IL-3-untreated control, ***, $P < 0.001$ vs. IL-3-treated control; ††, $P < 0.01$; †††, $P < 0.001$ versus the indicated lines; $n = 4$. Bars: (A and D) 100 μ m. All results are representative of at least three independent experiments. All statistical analyses were performed using ANOVA.

A



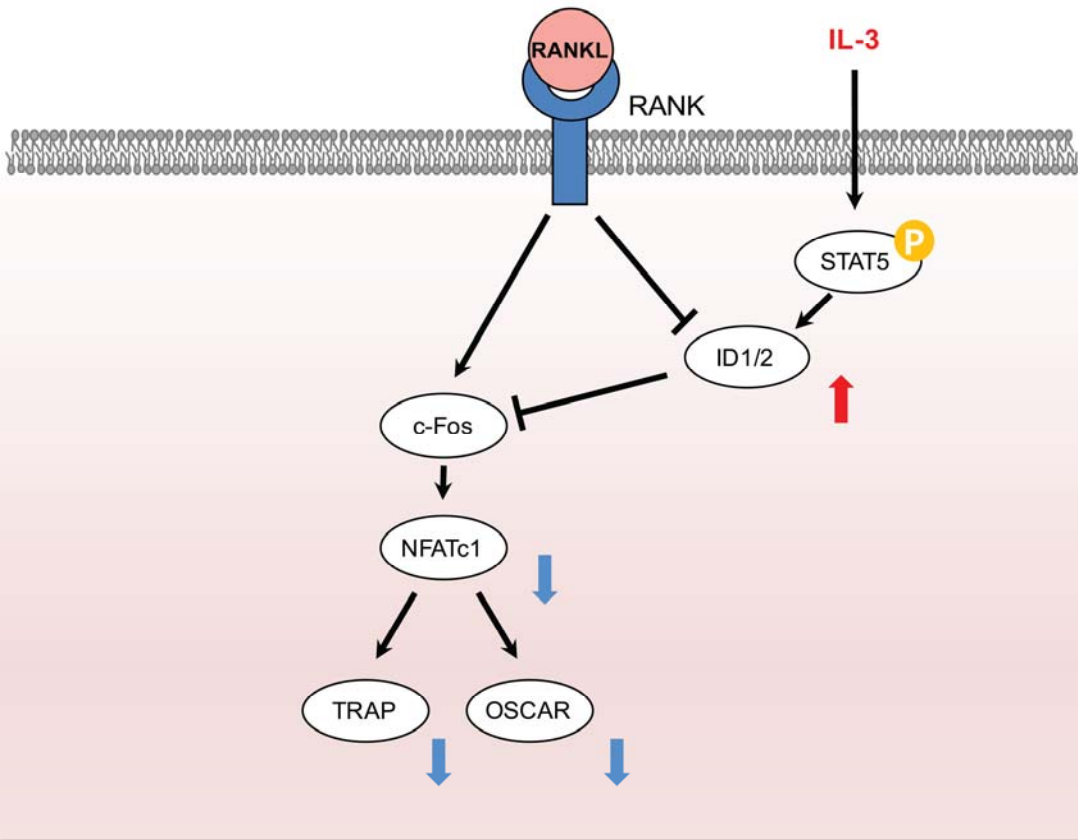
B



Supplementary Figure 3. STAT5A activation is sufficient to inhibit osteoclastogenesis independent of STAT5B. (A-B) Bone marrow cells were harvested from the long bones of *Stat5* cKO. BMMs were transduced with pMX-IRES-EGFP (control) or constitutively active STAT5A (STAT5A1*6) retrovirus and cultured with M-CSF and RANKL for three days. (A) Cultured cells were stained for TRAP. (B) The number of TRAP-positive MNCs per well was counted. Data are represented as the mean \pm SD. ***, $P < 0.001$ vs. control; n.s., not significant; $n = 4$. Bar: 100 μ m. All results are representative of at least three independent experiments. Statistical analyses were performed using ANOVA.

Supplementary Figure 4. Conversion of cell fate to dendritic cells by STAT5 activation.

BMMs transduced with pMX-IRES-EGFP (control) or STAT5A1*6 retrovirus were cultured with M-CSF in the presence or absence of RANKL for three days and collected. The collected cells were stained with anti-CD80, anti-CD86, anti-MHC class II, and anti-CD11c antibodies and analyzed by fluorescence-activated cell sorting. Results are representative of at least three independent experiments.



Supplementary Figure 5. Proposed model for the role of STAT5 in osteoclastogenesis.

Summary of RANKL-induced osteoclast differentiation and the mechanism of IL-3-induced inhibition of osteoclast differentiation via STAT5-Id. Under normal physiological conditions, binding of RANKL to RANK induces c-Fos, which upregulates NFATc1, a master regulator of osteoclastogenesis, and its downstream signals such as *TRAP*, *OSCAR*, *etc.* This signaling pathway is interrupted when IL-3 phosphorylates STAT5 and the activated STAT5 induces ID1/2, which in turn downregulates c-Fos and its downstream signals, which ultimately results in attenuated osteoclastogenesis.

Supplementary Table 1. Gene expression changes in RANKL-mediated osteoclast differentiation and IL-3-mediated inhibition of osteoclast differentiation.

Category	Gene_symbol	Gene expression fold change		
		WT-M-CSF-RANKL vs. WT-M-CSF	WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL	KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3
WT-M-CSF-RANKL vs. WT-M-CSF (down) and WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (up) and KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (down)	AA467197	-3.204772	12.343473	-4.30564
	Alox5	-3.078875	4.268578	-3.558701
	Ang	-10.194463	3.650933	-3.570706
	Anxa3	-7.258497	7.393438	-3.915549
	Arg1	-7.277787	109.872002	-35.973683
	Arhgap25	-4.475555	5.153716	-3.801109
	Atf3	-9.616502	9.709122	-7.180086
	Atp6v0a1	-7.591318	11.568932	-5.660032
	Atp6v0a1	-5.363165	7.559801	-4.396774
	AU022793	-4.439623	21.839085	-10.29531
	B430306N03Rik	-7.39276	7.154513	-3.776136
	Bcl2a1b	-3.794083	19.245227	-21.721051
	Bcl2l1	-11.122434	8.210381	-4.482407
	Bcl2l1	-7.265129	8.66144	-4.158217
	C1qa	-15.040143	7.474966	-3.748695
	C1qb	-10.313852	4.929246	-6.239563
	C1qc	-10.231077	4.31271	-3.513921
	Ccl12	-22.223777	242.879248	-9.819019
	Ccl2	-18.075896	48.901042	-5.832743
	Ccl3	-3.749232	34.452754	-10.053547
	Ccl4	-19.927549	99.107158	-11.82831
	Ccl6	-11.64122	210.069574	-41.536871
	Ccl7	-31.820448	167.568698	-7.840487
	Ccr2	-5.553874	5.145877	-5.661242
	Ccr5	-5.765497	8.743835	-6.095364
	Cd200r1	-4.068562	8.353642	-5.847812
	Cd300lf	-11.746858	31.272067	-8.688648
	Cd300lf	-3.92102	9.421184	-6.913499

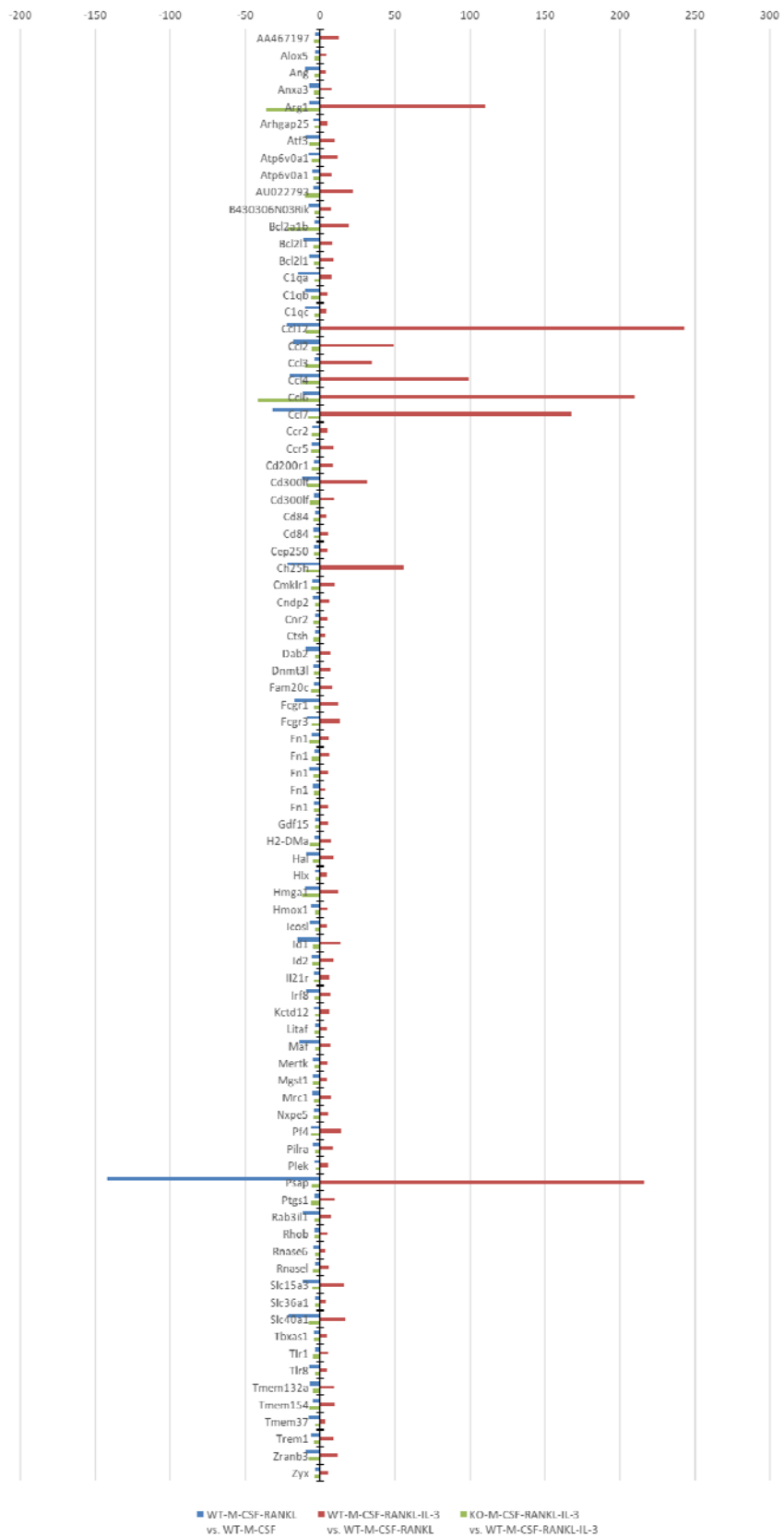
Cd84	-3.410261	4.323763	-4.672291
Cd84	-4.728823	5.625843	-4.144187
Cep250	-4.258908	4.975471	-4.261925
Ch25h	-21.620895	55.58655	-9.64555
Cmklr1	-5.197711	9.885982	-6.128512
Cndp2	-4.764804	6.412249	-3.08357
Cnr2	-3.056071	4.968549	-4.435651
Ctsh	-3.219848	3.460654	-4.48648
Dab2	-9.529592	6.91415	-3.056564
Dnmt3l	-4.611788	6.849288	-4.209885
Fam20c	-4.029736	8.110167	-6.146261
Fcgr1	-17.128146	11.836058	-4.039028
Fcgr3	-8.976882	13.13273	-5.916132
Fn1	-5.767593	5.898017	-7.024515
Fn1	-3.477186	6.380904	-5.934579
Fn1	-7.06525	5.197604	-4.593518
Fn1	-4.860589	3.220314	-4.172795
Fn1	-3.929027	5.326355	-4.112473
Gdf15	-3.123662	5.359159	-3.419765
H2-DMa	-3.484855	7.263071	-6.552643
Hal	-9.458547	9.027426	-4.848071
Hlx	-3.109511	4.429881	-3.01627
Hmga1	-10.023472	11.778641	-12.018328
Hmxo1	-6.475946	5.084216	-3.143732
Icosl	-6.749219	4.463312	-3.103888
Id1	-15.145906	13.539643	-4.997802
Id2	-5.797508	8.816544	-5.219676
Il21r	-4.137183	6.138872	-4.157938
Irf8	-9.328849	6.539895	-3.679706
Kctd12	-4.057421	6.302394	-3.322494
Litaf	-3.175425	4.610565	-3.620559
Maf	-14.06373	6.633199	-3.309991
Mertk	-5.174899	4.870496	-3.500171
Mgst1	-5.063172	4.664832	-5.001095
Mrc1	-5.35804	7.238886	-3.990227
Nxpe5	-4.010395	5.358019	-4.344138
Pf4	-6.063321	14.184867	-6.431475
Pilra	-5.078432	8.439752	-3.362338
Plek	-3.821644	5.455437	-3.00209

	Psap	-141.941266	215.840179	-5.744513
	Ptgs1	-3.681428	9.812489	-6.384626
	Rab3il1	-11.431955	7.209147	-3.72051
	Rhob	-3.837444	4.962606	-3.662089
	Rnase6	-4.37949	3.235004	-3.047195
	Rnase1	-3.32473	5.928657	-4.867796
	Slc15a3	-11.630782	15.919331	-5.335479
	Slc36a1	-3.053709	3.839491	-3.15042
	Slc40a1	-20.945115	16.528046	-7.577392
	Tbxas1	-4.306787	4.338054	-4.146963
	Tlr1	-3.200308	5.423097	-5.013597
	Tlr8	-7.31868	4.726743	-3.224056
	Tmem132a	-6.544304	9.374055	-4.865452
	Tmem154	-4.984457	9.923224	-7.001715
	Tmem37	-7.724976	3.205014	-3.092214
	Trem1	-6.133213	9.081651	-3.933309
	Zranb3	-9.780071	11.305611	-7.562593
	Zyx	-3.145585	5.469068	-3.70631
	WT-M-CSF-RANKL vs. WT-M-CSF (up) and WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (down) and KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (up)	Acp5	72.542687	-23.308944
Avpi1		3.904183	-3.807859	3.261959
Bbs9		8.022205	-3.888928	3.566136
Bdh2		3.166562	-5.521662	3.284488
Cav1		9.111685	-5.883492	3.200653
Cav1		11.990228	-5.400171	3.150264
Cd82		3.533061	-3.650205	4.674451
Ctsk		75.454743	-12.80813	3.851024
Dusp9		5.5049	-5.913806	3.457609
Fam83g		5.089386	-4.276312	3.260021
Gfi1b		5.56785	-4.007287	3.579352
Gfra2		13.724001	-10.860133	6.551509
Gfra2		5.637937	-3.772141	3.284024
Gja1		3.778619	-6.978433	4.616275
Gm11545		3.519081	-4.332652	4.313536
Gtf3c5		6.60216	-3.923702	3.214448
Igsf11		3.760873	-3.558916	3.011648
Jdp2		32.548581	-12.319083	7.114027
Jdp2		18.431914	-9.464058	6.51116
Lamc2		5.095735	-4.490866	3.243346
Lphn2		4.862458	-3.092864	3.395416

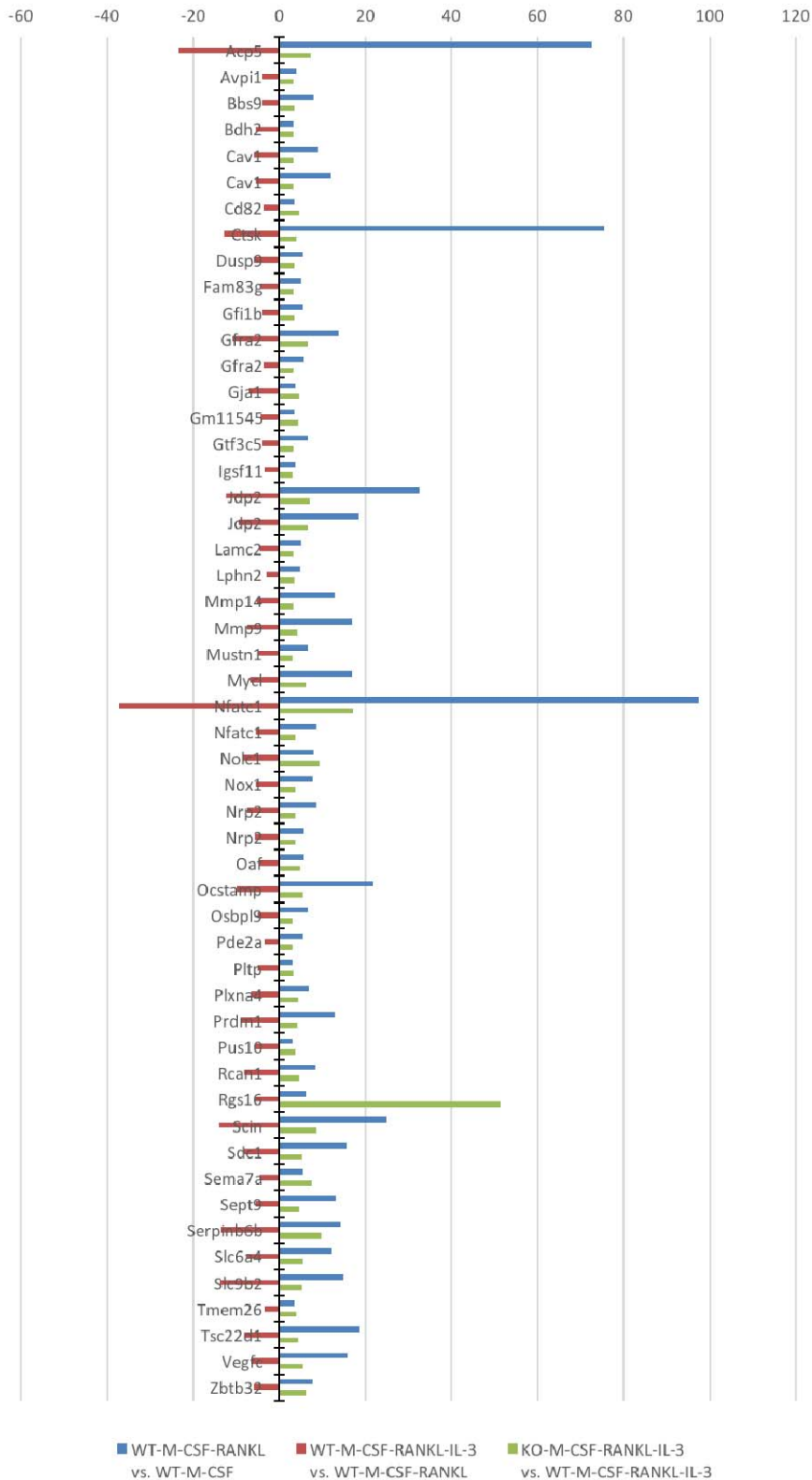
	Mmp14	12.89212	-5.249274	3.30948
	Mmp9	16.84516	-7.494702	4.071386
	Mustn1	6.606894	-4.886056	3.015609
	Mycl	16.905582	-6.923258	6.113919
	Nfatc1	97.372702	-37.164703	17.161056
	Nfatc1	8.535158	-5.425237	3.648573
	Nolc1	8.036622	-8.427702	9.31993
	Nox1	7.688506	-5.489285	3.776971
	Nrp2	8.551599	-7.50853	3.778248
	Nrp2	5.628992	-5.603937	3.638669
	Oaf	5.732848	-4.553084	4.792864
	Ocstamp	21.820044	-9.998031	5.50078
	Osbp19	6.643192	-5.038208	3.099599
	Pde2a	5.531907	-3.553674	3.06715
	Pltp	3.018255	-5.121849	3.321738
	Plxna4	6.780524	-6.559659	4.438787
	Prdm1	12.782004	-9.138299	4.147898
	Pus10	3.098	-5.78979	3.700373
	Rcan1	8.290801	-8.117266	4.500374
	Rgs16	6.23316	-5.700509	51.281159
	Scin	25.013124	-13.967793	8.682338
	Sdc1	15.629892	-8.269206	5.258236
	Sema7a	5.372466	-4.519331	7.588241
	Sept9	13.08068	-5.534157	4.690844
	Serpina6b	14.300244	-13.475148	9.80544
	Slc6a4	12.109968	-7.685788	5.530507
	Slc9b2	14.825507	-13.688978	5.257152
	Tmem26	3.573558	-3.452836	3.97436
	Tsc22d1	18.64694	-8.216998	4.434437
	Vegfc	15.72597	-6.670281	5.49483
Zbtb32	7.81553	-5.929728	6.157381	
and WT-M-CSF- RANKL-IL-3 vs. WT-M- CSF-RANKL (up)	Arrb2	3.263433	2.311626	-7.586969

	Cd274	4.004952	2.099275	-3.169258
	Il1rn	6.946865	2.708472	-5.262722
	Socs3	5.847861	2.582227	-2.508697
WT-M-CSF-RANKL vs. WT-M-CSF (down) and WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (down) and KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (up)	Cx3cr1	-4.508868	-3.572496	4.553183

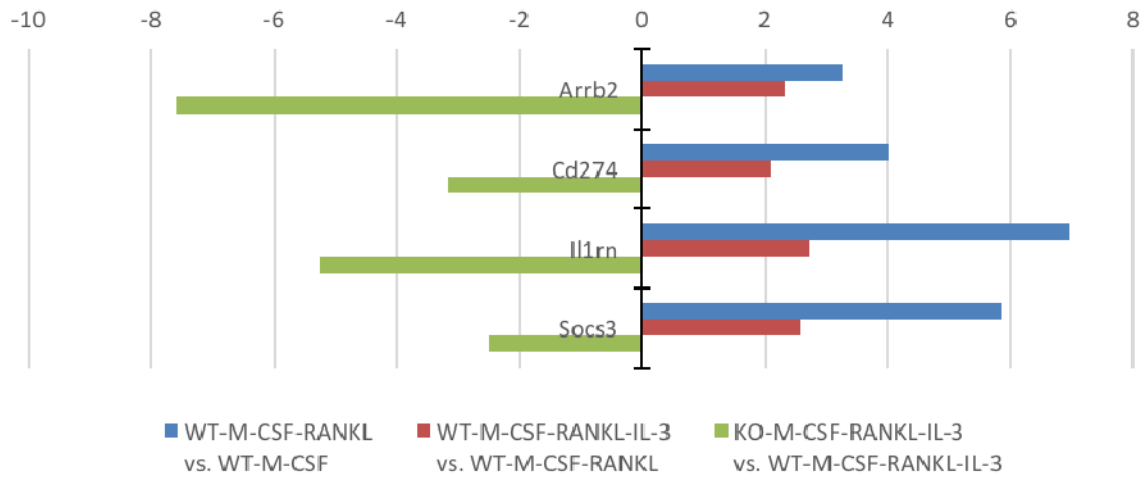
WT-M-CSF-RANKL vs. WT-M-CSF (down)
 and
 WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (up)
 and
 KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (down)



WT-M-CSF-RANKL vs. WT-M-CSF (up)
 and
 WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (down)
 and
 KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (up)



WT-M-CSF-RANKL vs. WT-M-CSF (up)
 and
 WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (up)
 and
 KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (down)



WT-M-CSF-RANKL vs. WT-M-CSF (down)
 and
 WT-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL (down)
 and
 KO-M-CSF-RANKL-IL-3 vs. WT-M-CSF-RANKL-IL-3 (up)

