Supplemental Information for:

The collection of NFATc1-dependent transcripts in the osteoclast includes numerous genes non-essential to physiologic bone resorption

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Supplemental Figure Legends

Supplemental Fig. 1 – Supplemental analysis of *Nhedc2$^{GT/GT}$* mice. (A) Genotyping PCR reactions for the *Nhedc2^{WT}* (left gel) and *Nhedc2^{GT}* (right gel) alleles on tail DNA from *Nhedc2$^{+/+}$* (lane 2), *Nhedc2$^{GT/+}$* (lane 3) and *Nhedc2$^{GT/GT}$* (lane 4) mice. A 100 bp DNA ladder is shown in lane 1. **(B)** Micro-CT quantification of femoral metaphyseal trabecular parameters in 12-14 week old female and male *Nhedc2$^{+/+}$* (n=9, female; n=11, male) and *Nhedc2$^{GT/GT}$* (n=9, female; n=11, male) mice. (n.s., not significant).

Supplemental Fig. 2 – Supplemental analysis of *Serpind1$^{-/-}$* mice. (A) Micro-CT quantification of femoral metaphyseal trabecular parameters in 12-week-old female and male *Serpind1$^{?/+}$* (female, n=7; male, n=6) and *Serpind1$^{-/-}$* (female, n=4; male, n=3). **(B)** Real time PCR analysis for osteoclast marker genes in samples from *Serpind1$^{?/+}$* (n=2) and *Serpind1$^{-/-}$* (n=4) OCPs stimulated with MCSF and RANKL to induce osteoclast differentiation. **(C, D)** Real time PCR analysis using primers targeting *Serpind1* exons 2 (forward) and 3 (reverse) on **(C)** *Serpind1$^{+/+}$* and *Serpind1$^{-/-}$* liver or **(D)** WT BMMs cultured with MCSF alone or MCSF and RANKL to induce osteoclast differentiation. **(E)** Real time PCR analysis for *Serpin* homologs in samples from *Serpind1$^{?/+}$* (n=2) and *Serpind1$^{-/-}$* (n=4) OCPs stimulated with MCSF and RANKL to induce osteoclast differentiation. (n.s., not significant; N.D., not detectable).

Supplemental Fig. 3 – Supplemental analysis of *Adcy3$^{-/-}$* mice. (A) Micro-CT quantification of femoral metaphyseal trabecular parameters in 8-week-old female and male *Adcy3$^{?/+}$* (female, n=6; male, n=5) and *Adcy3$^{-/-}$* (female, n=7; male, n=8) mice. **(B)** Body weight of 8-week old female and male *Adcy3$^{?/+}$* and *Adcy3$^{-/-}$* mice used for micro-CT analysis in Fig. 4 A,B and Supplemental Fig. 3A. **(C)** Real time PCR analysis for osteoclast marker genes expression in *Adcy3$^{+/+}$* (n=3) and *Adcy3$^{-/-}$* (n=4) OCPs stimulated with MCSF and RANKL to induce osteoclast differentiation. Data in (C) is normalized to WT littermates. **(D)** Real time PCR analysis for *Adcy3* expression in *Adcy3$^{+/+}$* (n=2) and *Adcy3$^{-/-}$* (n=3) OCPs stimulated with MCSF and RANKL to induce osteoclast differentiation. (n.s., not significant).

Supplemental Fig. 4 – Supplemental analysis of *Rhoc$^{-/-}$* mice. (A) Micro-CT quantification of femoral metaphyseal trabecular parameters in 8-10 week old female and male *Rhoc*$^{?/+}$ (female, n=7; male, n=2) and *Rhoc*$^{-/-}$ (female, n=5; male, n=2). **(B)** Real time PCR analysis for *Rhoc* expression in *Rhoc*$^{?/+}$ (n=2) and *Rhoc*$^{-/-}$ (n=2) OCPs stimulated with MCSF and RANKL to induce osteoclast differentiation.

Supplemental Fig. 5 – Supplemental analysis of *Rab38*$^{cht/cht}$ mice. (A) Micro-CT quantification of femoral metaphyseal trabecular parameters in 8-week-old female and male *Rab38*$^{+/+}$ (female, n=2; male, n=4) and *Rab38*$^{cht/cht}$ (female, n=3; male, n=3) mice. **(B)** Real time PCR analysis for osteoclast marker genes expression in *Rab38*$^{+/+}$ (n=2) and *Rab38*$^{cht/cht}$ (n=1) OCPs stimulated with MCSF alone or MCSF and RANKL to induce osteoclast differentiation. (n.s., not significant; *, $p<0.05$)

Supplemental Table 1 – Quantitative Real-time PCR primers used in this study

Primer Set	Forward Primers (5'-3')	Reverse Primers (5'-3')	Source
Adcy3	CCTGAGTCCTTGGAGAACCT	CCACGTAGCAGTCAAAGAGG	A
Hmbs	ATGAGGGTGATTCGAGTGGG	CAAACTGTATGCCAGGGTACAA	B
Hprt	GTTAAGCAGTACAGCCCCAAA	AGGGCATATCCAACAACAAACTT	C
Nfatc1	TGCCTTTTGCGAGCAGTATCT	CAGGCAAGGATGGGCTCATAT	D
Nhedc1	CCACTGGGCAGATGGTATTT	GCAGCATGCCAAGAAGA	E
Nhedc2	TTGTTCCATCACCGGAGGTAA	TTGGTGTTGAGTGCTTGTCC	E
Rhoc	ACTCCATGCTGCCAGTTTCT	AGGCCAGAGGGACTAAGAGC	E
Rab38	CCAAGGGAAGGATGTGCTTA	GTGAGATGGGGCTTCACAAT	E
Serpind1 (ex2-3)	GGCAAACAACCACATTCTG	TCTCTCATTCAGCCGGAAGT	E
Serpind1 (ex3-4)	GAGTACGTAGGGGGCATCAG	GGACCTCCACCAGGTTGTAA	E
Serpinb1a	*ACATCCATTCACGCTTCCAAA*	*GGCCAAGTCAGCACCATACAT*	*C*
Serpinb2	*GTGCTGGGGGTAACACTGAAC*	*GCGAAATCACAGCCACTGAAG*	*C*
Serpine2	*CACATGGGATCGCGTCCATC*	*CAGCACTTTACCAACTCCGTTTA*	*C*
Serpinh1	*GCCGAGGTGAAGAAACCCC*	*CATCGCCTGATATAGGCTGAAG*	*C*

A – Designed with the on-line GenScript Real Time PCR (TaqMan) Primer Design program.

B – Gift of Dr. Kevin McHugh, College of Dentistry, University of Florida

C - The Center for Comparative and Integrative Biology Primer Bank, Harvard Medical School. Primerbank ID: *Hprt* (7305155a2), *Serpinb1a* (13384828a1), *Serpinb2* (6755098a1), *Serpine2* (6678099a1), *Serpinh1* (6753304a1).

D - A.O. Aliprantis, Y. Ueki, R. Sulyanto, A. Park, K.S. Sigrist, S.M. Sharma, M.C. Ostrowski, B.R. Olsen, L.H. Glimcher, NFATc1 in mice represses osteoprotegerin during osteoclastogenesis and dissociates systemic osteopenia from inflammation in cherubism, J Clin Invest. 118 (2008) 3775-89.

E - Designed with the on-line Primer 3 tool. Steve Rozen and Helen J. Skaletsky (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386.

Supplemental Table 2 – Raw microarray data for the genes depicted on the heat map in Fig. 1A.

Heatmap.order	Probeset	Gene Symbol	p-value	FDR	log2(FC) KOvWT
98	1417700_at	Rab38	0.005108	0.063074	-3.71
97	1420575_at	Mt3	0.004283	0.060491	-4.51
96	1437308_s_at	F2r	0.003823	0.059292	-4.82
95	1451710_at	Oscar	0.002111	0.052424	-4.88
94	1433474_at	Edil3	0.004097	0.060422	-4.28
93	1448605_at	Rhoc	0.022969	0.110736	-2.87
92	1426951_at	Crim1	0.020935	0.106308	-3.20
91	1460197_a_at	Steap4	0.015549	0.093885	-6.51
90	1420461_at	Mst1r	0.011264	0.082114	-4.90
89	1431711_a_at	Kazn	0.013887	0.089020	-3.22
88	1421959_s_at	Adcy3	0.009205	0.076730	-2.66
87	1425829_a_at	Steap4	0.009882	0.078992	-3.83
86	1452646_at	Trp53inp2	0.011529	0.082658	-4.30
85	1426869_at	Boc	0.007521	0.071389	-2.92
84	1450625_at	Col5a2	0.016968	0.097514	-2.83
83	1422809_at	Rims2	0.015804	0.094740	-3.29
82	1437226_x_at	Marcksl1	0.000028	0.044454	-2.82
81	1422619_at	Ppap2a	0.000004	0.044454	-4.07
80	1424133_at	Tmem98	0.000042	0.044454	-5.57
79	1418569_at	Fblim1	0.000068	0.044454	-3.46
78	1417089_a_at	Ckmt1	0.000133	0.044454	-2.98
77	1450429_at	Capn6	0.000069	0.044454	-2.70
76	1450843_a_at	Serpinh1	0.000033	0.044454	-4.39

75	1434588_x_at	Tbca	0.001101	0.046468	-2.68
74	1421791_at		0.000757	0.044454	-3.07
73	1425702_a_at	Enpp5	0.000312	0.044454	-2.90
72	1425339_at	Plcb4	0.000146	0.044454	-4.77
71	1455235_x_at	Ldhb	0.000055	0.044454	-3.62
70	1425622_at	Edil3	0.000145	0.044454	-4.18
69	1421511_at	Itgb3	0.000092	0.044454	-6.33
68	1448237_x_at	Ldhb	0.000107	0.044454	-3.06
67	1422620_s_at	Ppap2a	0.000103	0.044454	-4.05
66	1416666_at	Serpine2	0.000070	0.044454	-5.04
65	1422967_a_at	Tfrc	0.000281	0.044454	-2.79
64	1452257_at	Bdh1	0.000490	0.044454	-2.71
63	1448942_at	Gng11	0.000355	0.044454	-2.83
62	1419136_at	Akr1c18	0.000436	0.044454	-4.28
61	1417730_at	Ext1	0.000294	0.044454	-3.06
60	1422515_at	Pate4	0.000105	0.044454	-3.43
59	1415874_at	Spry1	0.000112	0.044454	-2.74
58	1430233_a_at	Nhedc1	0.000064	0.044454	-2.86
57	1451596_a_at	Sphk1	0.000103	0.044454	-2.90
56	1439995_at	Nhedc2	0.002112	0.052424	-4.95
55	1435463_s_at	Myo1d	0.002468	0.053783	-4.18
54	1423892_at	Apbb1	0.001599	0.049710	-3.05
53	1425442_at	Oscar	0.001743	0.050429	-4.25
52	1418680_at	Serpind1	0.001835	0.051020	-3.35
51	1454114_a_at	Nhedc1	0.001183	0.046674	-4.84
50	1449141_at	Fblim1	0.001226	0.047183	-2.89

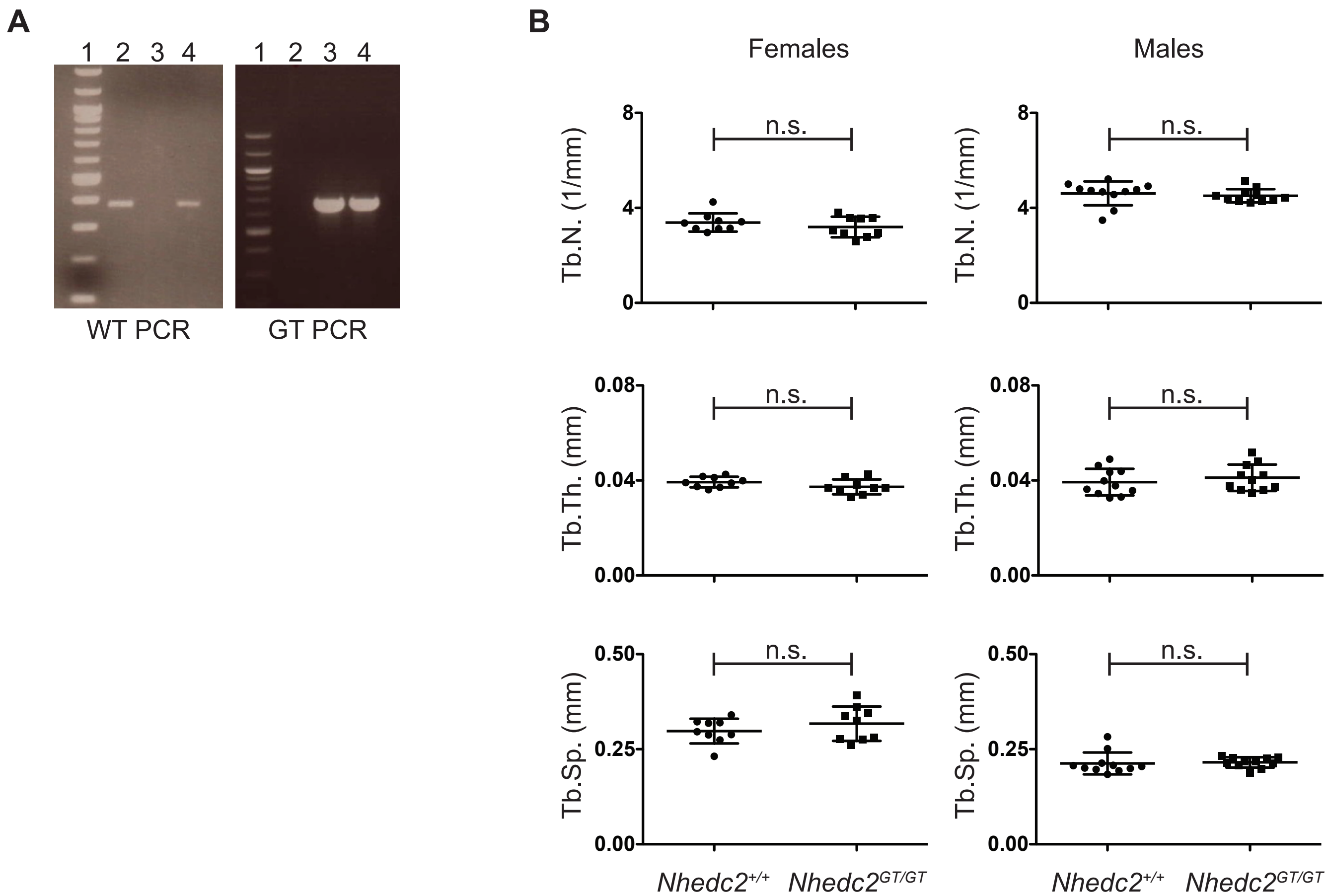
49	1450852_s_at	F2r	0.000737	0.044454	-4.72
48	1451601_a_at	Spns2	0.000813	0.045119	-3.20
47	1427891_at	Gimap6	0.000683	0.044454	-5.31
46	1448502_at	Slc16a7	0.001020	0.046468	-3.79
45	1434499_a_at	Ldhb	0.000500	0.044454	-4.13
44	1418688_at	Calcr	0.000394	0.044454	-4.80
43	1426959_at	Bdh1	0.000220	0.044454	-3.55
42	1421425_a_at	Rcan2	0.000292	0.044454	-3.13
41	1425250_a_at	Slc14a2	0.000940	0.046404	-2.94
40	1449945_at	Ppargc1b	0.001283	0.047286	-2.64
39	1423893_x_at	Apbb1	0.000328	0.044454	-2.62
38	1416601_a_at	Rcan1	0.000481	0.044454	-2.63
37	1425842_at	Edil3	0.000503	0.044454	-3.72
36	1422090_a_at	Pfkfb2	0.000861	0.045901	-4.12
35	1452492_a_at	Slc37a2	0.001084	0.046468	-2.61
34	1419082_at	Serpinb2	0.037980	0.143389	-2.59
33	1436929_x_at	Adcy3	0.031294	0.129404	-3.09
32	1449943_at	Lfng	0.023573	0.112131	-2.72
31	1428895_at	Rftn2	0.005551	0.065208	-4.22
30	1450932_s_at	Dock9	0.007990	0.073392	-3.91
29	1452405_x_at	Gm10889	0.004160	0.060422	-3.33
28	1426168_a_at		0.003382	0.057404	-3.54
27	1423952_a_at	Krt7	0.002774	0.054990	-3.65
26	1422875_at	Cd84	0.000035	0.044454	-2.91
25	1434624_x_at	Rps9	0.000177	0.044454	-2.85
24	1416007_at	Satb1	0.000082	0.044454	-2.94

23	1428372_at	St5	0.001109	0.046468	-4.79
22	1419621_at	Ankrd2	0.000473	0.044454	-4.66
21	1425444_a_at	Tgfbr2	0.000379	0.044454	-4.60
20	1425934_a_at	B4galt4	0.000027	0.044454	2.79
19	1448301_s_at	Serpinb1a	0.000049	0.044454	3.10
18	1453223_s_at	Dppa2	0.000044	0.044454	3.87
17	1416444_at	Elovl2	0.000086	0.044454	3.65
16	1416318_at	Serpinb1a	0.000336	0.044454	3.03
15	1427760_s_at		0.000177	0.044454	4.90
14	1437693_at	D1Pas1	0.000583	0.044454	4.02
13	1434334_at	Prkd2	0.000568	0.044454	3.79
12	1449033_at	Tnfrsf11b	0.000033	0.044454	5.44
11	1427231_at	Robo1	0.001623	0.050016	3.15
10	1422368_at	Vmn1r43	0.001813	0.051020	3.47
9	1437275_at	Ctnna1	0.000322	0.044454	4.40
8	1452426_x_at		0.015491	0.093772	4.93
7	1439243_x_at	Cops5	0.016630	0.096553	4.22
6	1450922_a_at	Tgfb2	0.009969	0.079513	3.45
5	1415780_a_at	Armcx2	0.020081	0.103541	3.34
4	AFFX-DapX-5_at		0.009520	0.078134	2.70
3	1449782_at	AA517650	0.010090	0.079789	2.79
2	1419521_at	Zfp94	0.038739	0.144595	3.22
1	1450930_at	Hpca	0.040907	0.148765	2.76

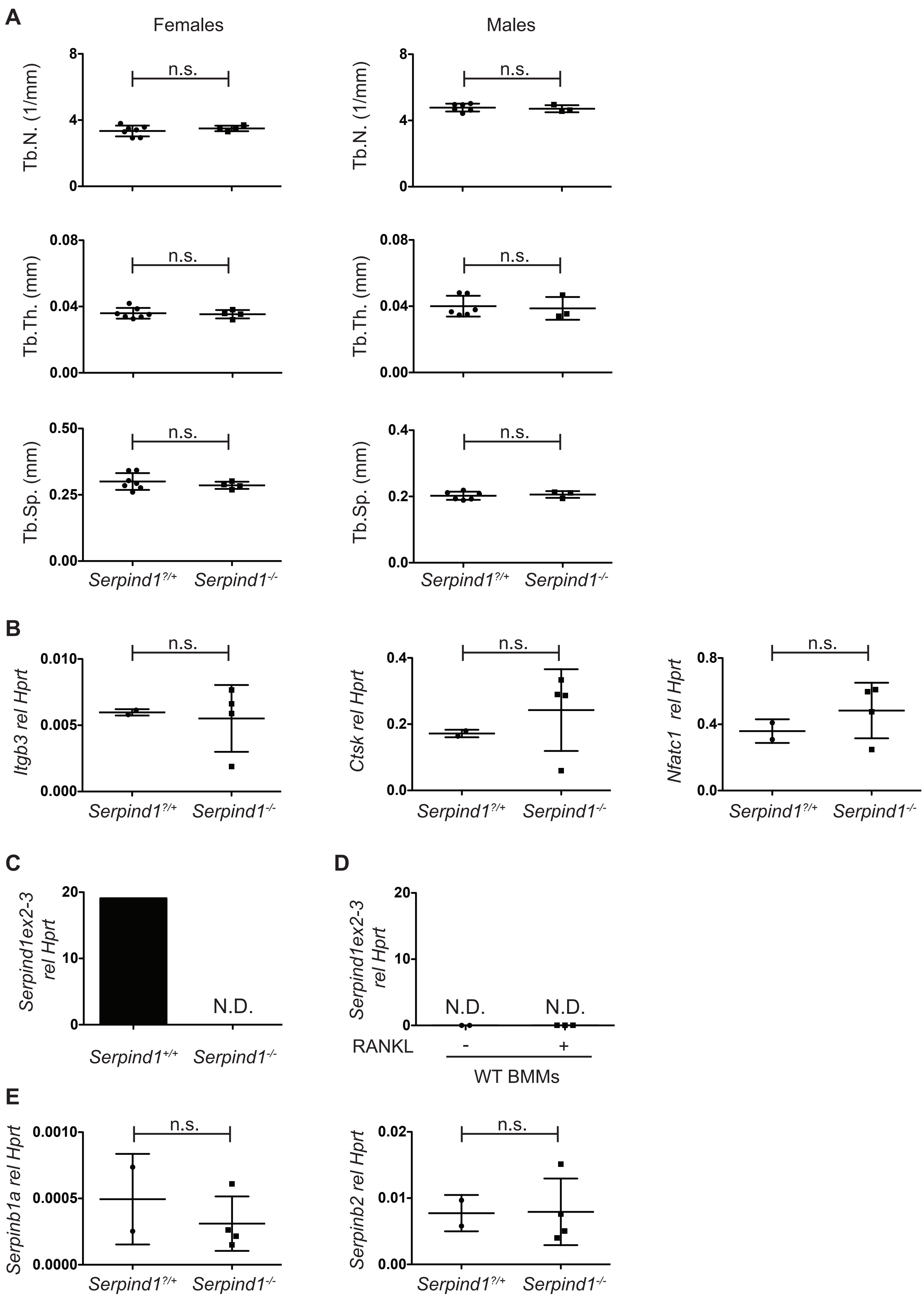
Supplemental Table 3 – Histomorphometric analysis of *Rhoc*$^{-/-}$ mice.

Histomorphometric analysis of the tibia of 8 week of *Rhoc*$^{+/+}$ or *Rhoc*$^{-/-}$ female mice. p-value determined by 2-tailed student's t-test.

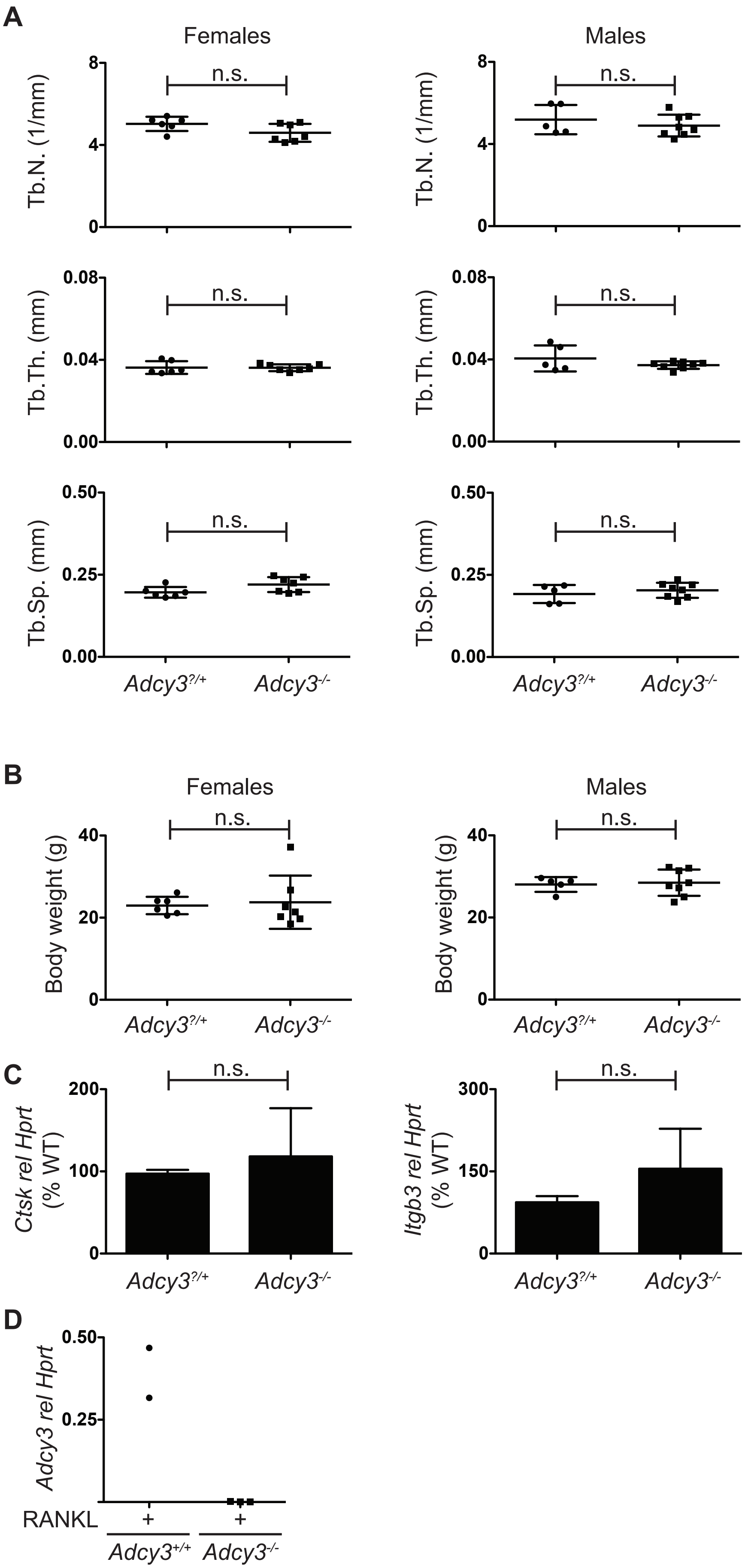
	Rhoc$^{+/+}$ (n=5)		*Rhoc*$^{-/-}$ (n=5)		
	Avg.	St.Dev.	**Avg.**	St.Dev	p-value
BV/TV	**20.5**	3.9	**17.2**	2.3	0.157
TbTh	**29.2**	1.1	**31.6**	4.4	0.355
TbN	**7.0**	1.3	**5.5**	0.6	0.057
TbSp	**117.5**	28.5	**153.2**	16.8	0.054
BS/BV	**68.6**	2.6	**64.1**	8.4	0.369
OTh	**2.7**	0.3	**2.8**	0.2	0.748
OV/TV	**1.7**	0.4	**1.4**	0.2	0.188
OV/BV	**8.4**	2.3	**8.2**	2.0	0.901
OS/BS	**41.9**	7.1	**43.5**	5.5	0.715
NOb/TAR	**339.7**	45.2	**296.0**	51.5	0.230
NOb/BPm	**31.8**	7.2	**35.1**	8.4	0.545
NOb/Opm	**75.1**	6.3	**79.8**	10.0	0.454
Nob/Obpm	**111.0**	6.9	**110.5**	3.2	0.893
ObS/BS	**28.5**	6.0	**31.8**	7.3	0.503
ObS/OS	**67.7**	3.2	**72.2**	8.6	0.379
NOc/TAR	**26.9**	4.6	**24.4**	3.6	0.386
NOc/BPm	**2.5**	0.6	**2.9**	0.6	0.413
OcS/BS	**7.6**	1.8	**8.9**	2.5	0.418



Supplemental Figure 1



Supplemental Figure 2



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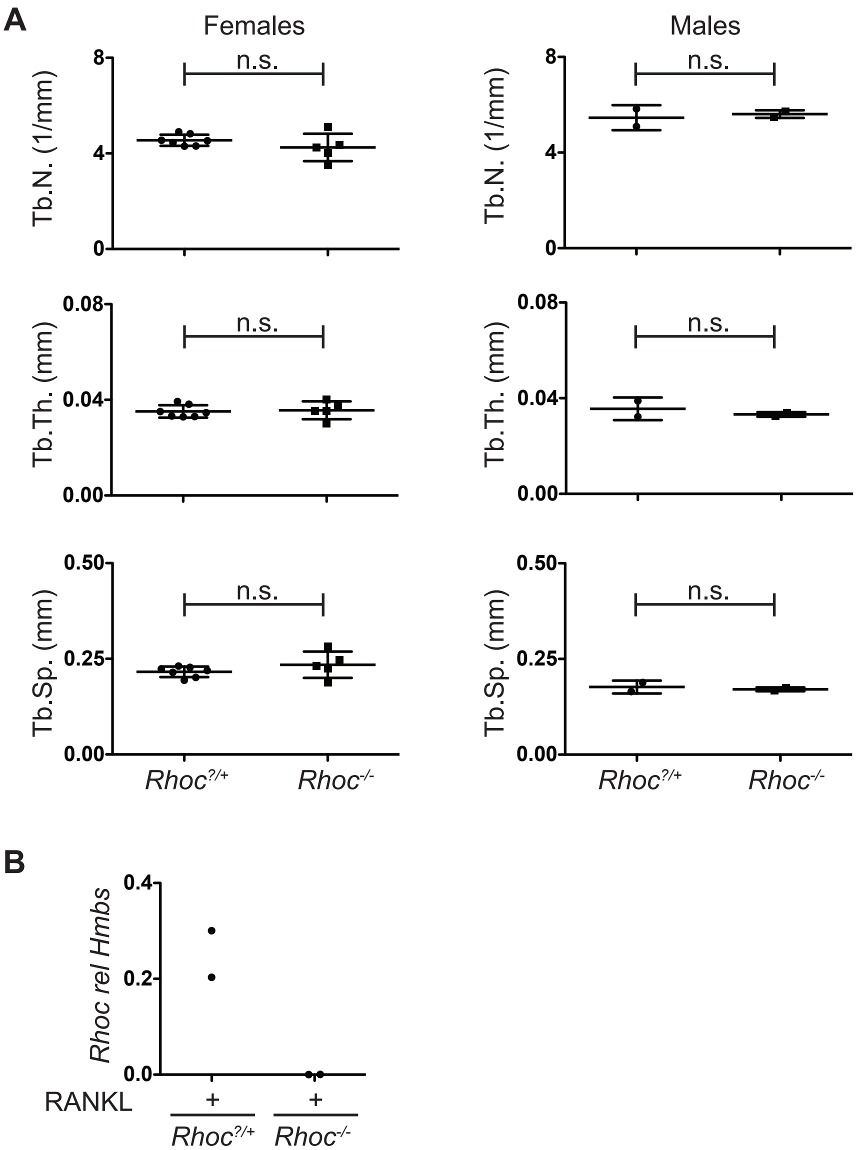

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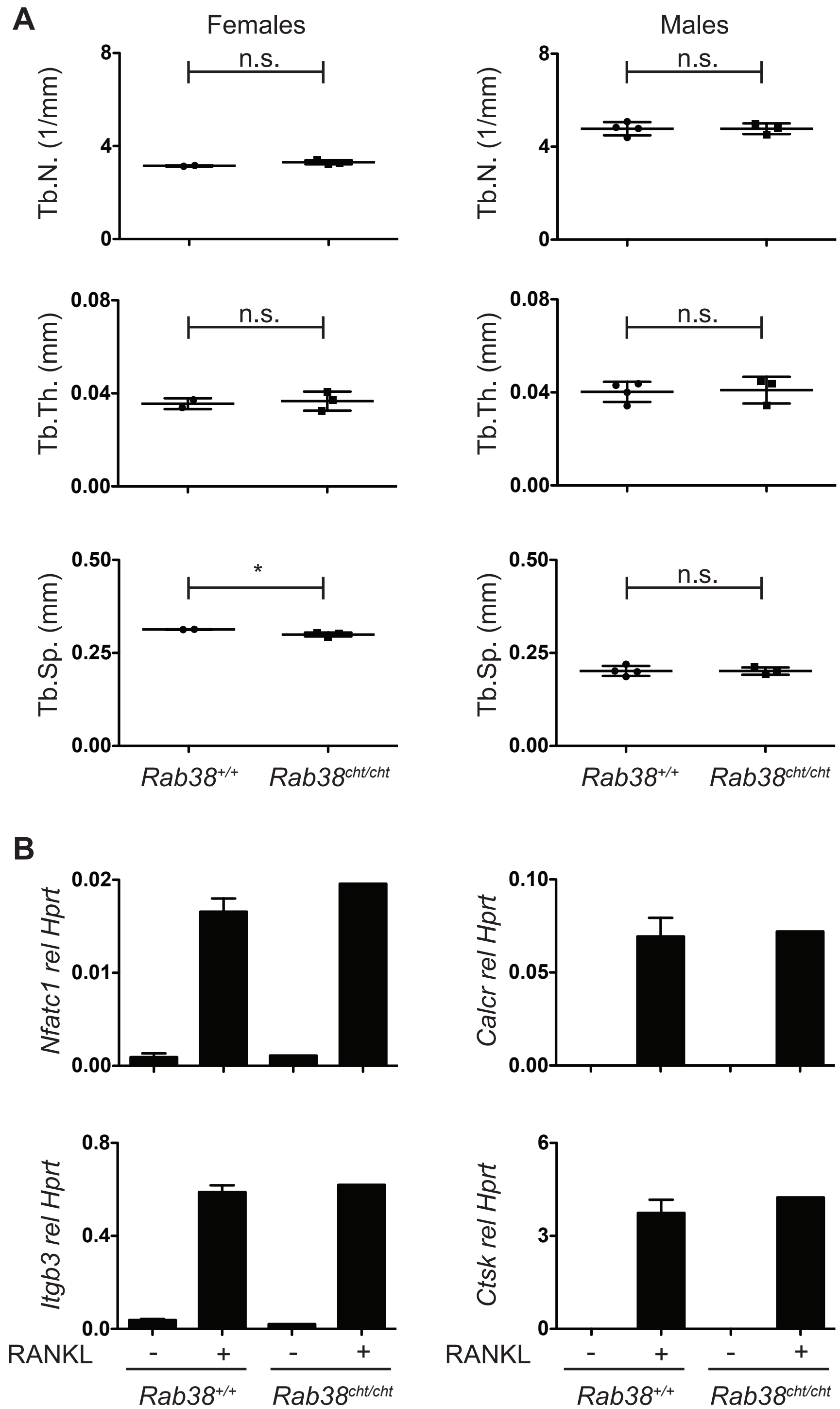

Supplemental Figure 5