Supplemental figure 1. Dys-regulated signal pathways in MSCs from TNF-Tg mice. 965 dys-regulated genes were uploaded to IPA and David bioinformatics Resources software. The top 53 and top 11 dys-regulated pathways were identified by IPA (A) and David bioinformatics Resources (B) analysis according to P value. The ratio is the number of genes found in our data set divided by the referenced genes in pathways of software data base.

Supplemental figure 2. Expression of Notch genes in MSCs of TNF-Tg mice via RNAseq. mRNA levels of Notch-related factors in CD45-/Scal1+/CD105+ MSCs from TNF-Tg mice and WT littermates by RNA-sequencing. Values are means ± SD of 3 mice.

Supplemental figure 3. Characterization of 3rd passage of bone-derived MSCs. Bone-derived cells from WT mice were cultured in basal medium and passaged 3 times. (A) The expression profile of MSC surface markers was examined by FACS. MSC surface markers are CD45-/Sca1+/CD105+/CD44+/CD31-/CD11b-/CD117-. (B) The differentiation potential to osteoblasts, adipocytes and chondrocytes were examined by culturing cells in the appropriate inducing media for 2-4 weeks.

Supplemental figure 4. The effect of long-term DAPT treatment on expression levels of Notch target genes. TNF-Tg mice were treated with DAPT or vehicle by daily gavage for 3 months. Total RNA was extracted from popliteal lymph nodes and spleens, and expression levels of Notch-related genes were examined by qPCR. Values are means and SD of 3 samples. *$p<0.05$ vs vehicle-treated mice.

Supplemental figure 5. Characterization of CFU colony cells. BM cells from WT mice were used. Red blood cells were lysed first to obtain BM mononuclear cells. Cells were cultured in basal condition for 3 weeks. Low cell density (10E4/10 cm dish)

was used in CFU colony assay to generate colony cells (A). High cell density (10E6/ml) was used to generate BM stromal cells (B). Media were changed every 5 days. Primary BM mononuclear cells were isolated freshly from a separate WT mouse (C). Cells were stained with anti-CD45, Scal1 and CD105 antibodies and subjected to FACS analyses.

Supplemental figure 6. In vivo bone repair model. Mice received bone scaffold alone (A) or bone scaffold plus WT CFU cells (B) were sacrificed 6 weeks post surgery. H&E-stained sections show the bone defect area (black dash line) and newly formed bone (sold black line). The defects that were filled with bone scaffold alone have significantly lesser bone volume compared to those that were filled with bone scaffold plus CFU cells (C). N= 5 mice/group. *p<0.05 vs no cell group.

Supplemental figure 7. The effect of long-term DAPT treatment on morphology of internal organs. WT mice were treated with DAPT or vehicle by daily gavage for 3 months. Internal organs were stained with H&E. Representative sections were shown.

Supplemental figure 8. Thapsigargin reduces Hes1 expression and revises decreased osteoblast differentiation of MSCs in TNF-Tg mice. TNF-Tg mice and WT littermates were i.p. injected with the new Notch inhibitor Thapsigargin (0.4mg/kg/time) or vehicle daily for 4 days. (A) The inhibitory effect of Thapsigargin on Notch activation (*Hes1* mRNA) was confirmed in the popliteal lymph nodes (positive Ctl) and in CD45- MSC-enriched cells by qPCR. (B) Representative photos and # of CFU-ALP+ colonies in BM stromal cells from Thapsigargin- or vehicle-treated mice. TNF-Tg mice and WT littermates were i.p. injected with Thapsigargin (0.4mg/kg/time, 3 time/week) or vehicle for 2 months. (C) The expression levels of Hey1 in CFU cells from Thapsigargin- or PBS-treated mice were examined by qPCR. *p<0.05 vs vehicle-treated mice, #p<0.05 vs WT mice.

Supplemental figure 9. Activation status of Notch signaling in cells at different

confluence. Expression levels of Hes1 and NICD (Notch-2) in C3H10T1/2 cells at different confluence levels were examined by Western blotting using anti-NICD (Notch-2) antibody.

Supplemental figure 10. Correlation of gene expression levels in CD45- MSCs from BM and peripheral blood mononuclear cells. CD45- cells were isolated form BM mononuclear cells (BMMCs) and peripheral blood mononuclear cells (PBMCs) of TNF-Tg mice and WT littermates. The expression levels of Hes1 and Runx2 from BMMCs and paired PBMCs were determined by qPCR. Values were calculated based the equation= ½ CT(gene of interest) – CT(actin).

Supplemental figure 1A

Ingenuity Canonical Pathways	p-value
HGF Signaling	0.000676
Acute Myeloid Leukemia Signaling	0.001
SAPK/JNK Signaling	0.001148
B Cell Receptor Signaling	0.00263
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	0.00302
Xenobiotic Metabolism Signaling	0.00309
Molecular Mechanisms of Cancer	0.003236
PI3K/AKT Signaling	0.003548
p53 Signaling	0.004365
LPS/IL-1 Mediated Inhibition of RXR Function	0.005248
Assembly of RNA Polymerase I Complex	0.006457
Phospholipid Degradation	0.006761
Insulin Receptor Signaling	0.006918
Notch Signaling	0.007079
Type II Diabetes Mellitus Signaling	0.007762
Huntington's Disease Signaling	0.007762
Glioblastoma Multiforme Signaling	0.007943
IL-15 Production	0.008128
CD27 Signaling in Lymphocytes	0.008128
Pentose and Glucuronate Interconversions	0.008128
NF-κB signaling	0.008511
JAK/Stat Signaling	0.009333
Histidine Metabolism	0.01
Pancreatic Adenocarcinoma Signaling	0.010471
PPARα/RXRα Activation	0.01122
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.012303

Continued Supplemental figure 1A

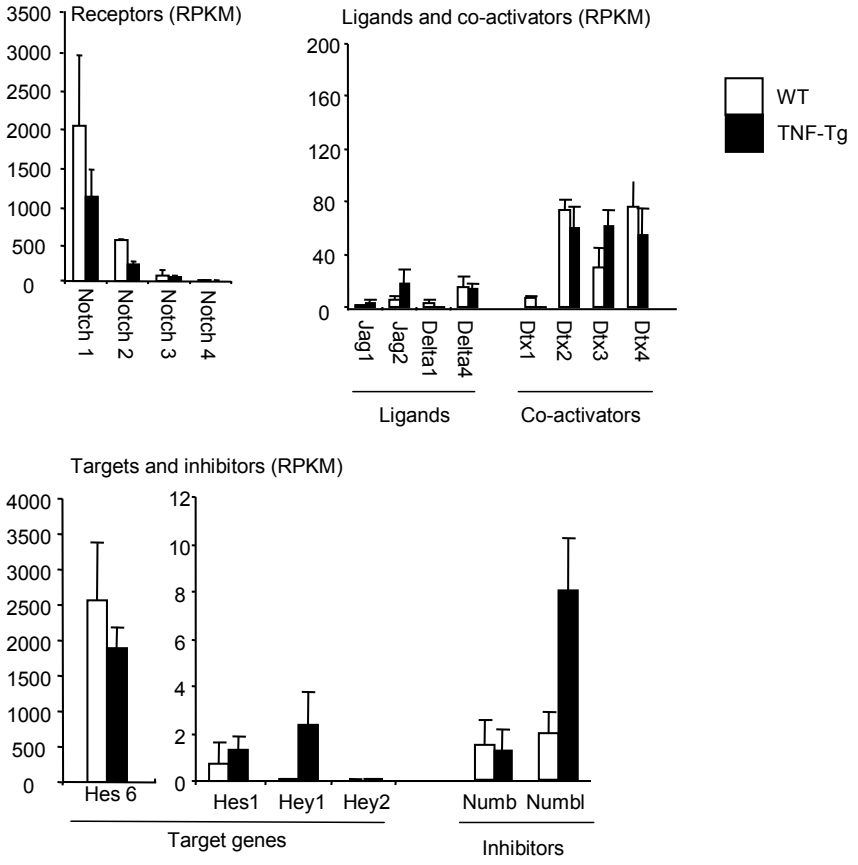
Ingenuity Canonical Pathways	p-value
Acute Phase Response Signaling	0.012589
Lymphotoxin β Receptor Signaling	0.014791
PDGF Signaling	0.015488
FGF Signaling	0.016596
Atherosclerosis Signaling	0.016982
MSP-RON Signaling Pathway	0.017783
Factors Promoting Cardiogenesis in Vertebrates	0.017783
Glycerophospholipid Metabolism	0.017783
IL-9 Signaling	0.018197
Glycolysis/Gluconeogenesis	0.019498
Human Embryonic Stem Cell Pluripotency	0.020893
Androgen and Estrogen Metabolism	0.021878
Leptin Signaling in Obesity	0.022387
Assembly of RNA Polymerase II Complex	0.025704
Colorectal Cancer Metastasis Signaling	0.026915
Type I Diabetes Mellitus Signaling	0.027542
GM-CSF Signaling	0.028184
Estrogen Receptor Signaling	0.032359
Chronic Myeloid Leukemia Signaling	0.033113
Glioma Signaling	0.037154
PKC θ Signaling in T Lymphocytes	0.038019
Prostate Cancer Signaling	0.038019
Tyrosine Metabolism	0.038019
Docosahexaenoic Acid (DHA) Signaling	0.038019
Thrombopoietin Signaling	0.038905
PXR/RXR Activation	0.042658
Glucocorticoid Receptor Signaling	0.044668

Supplemental figure 1B

Sublist	Category	Term	P-Value
☐	KEGG_PATHWAY	p53 signaling pathway	3.8E-3
☐	KEGG_PATHWAY	Hematopoietic cell lineage	1.3E-2
☐	KEGG_PATHWAY	Pentose and glucuronate interconversions	3.9E-2
☐	KEGG_PATHWAY	Prostate cancer	5.1E-2
☐	KEGG_PATHWAY	MAPK signaling pathway	5.5E-2
☐	KEGG_PATHWAY	Chemokine signaling pathway	7.0E-2
☐	KEGG_PATHWAY	Ether lipid metabolism	7.3E-2
☐	KEGG_PATHWAY	Notch signaling pathway	7.5E-2
☐	KEGG_PATHWAY	Primary immunodeficiency	7.9E-2
☐	KEGG_PATHWAY	Adipocytokine signaling pathway	8.3E-2
☐	KEGG_PATHWAY	Cytokine-cytokine receptor interaction	8.8E-2

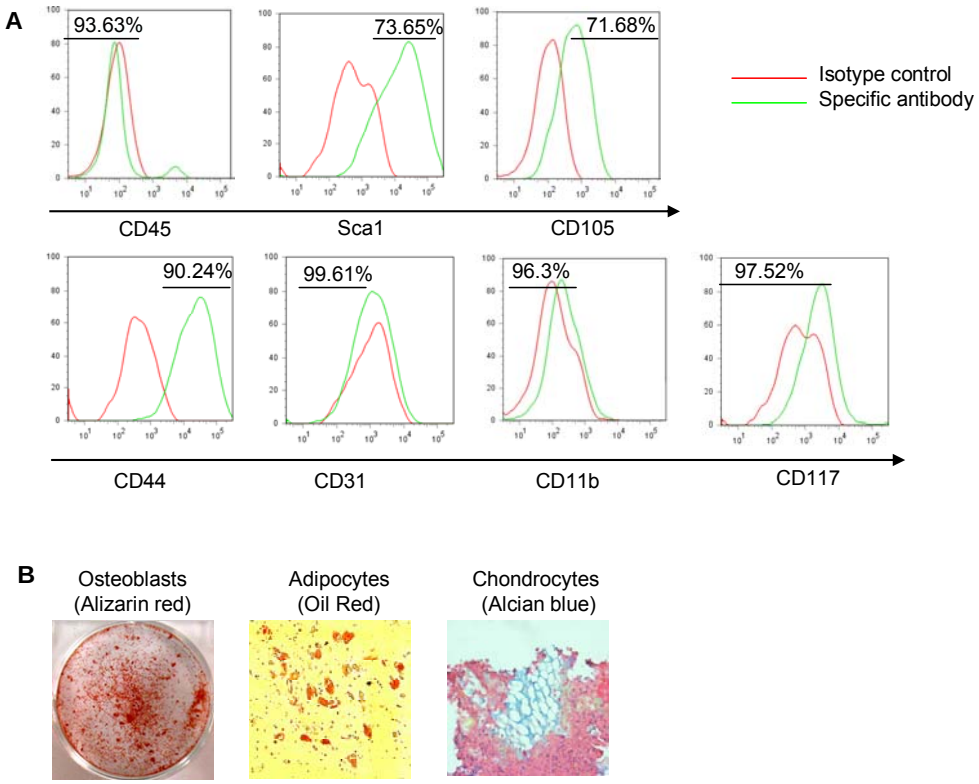
Supplemental figure 1. Dys-regulated signal pathways in MSCs from TNF-Tg mice

Supplemental figure 2



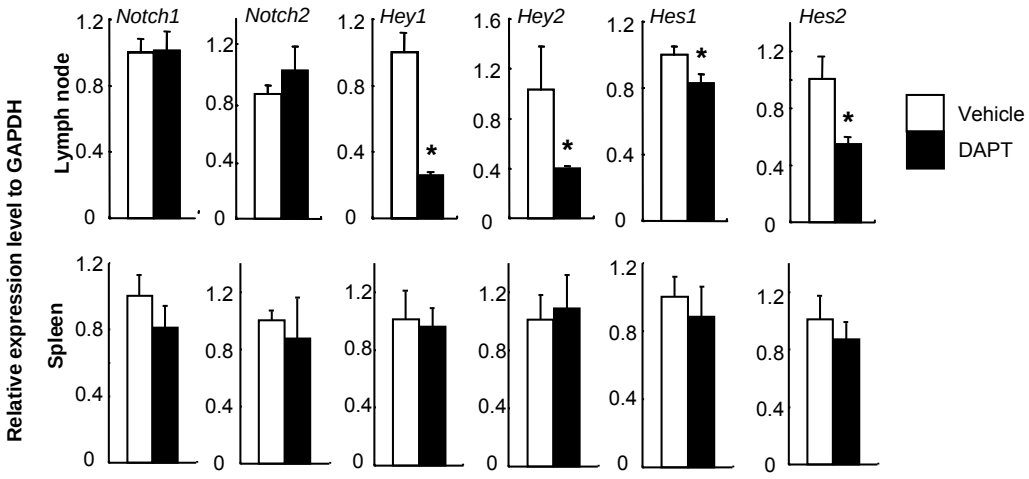
Supplemental figure 2. Expression of Notch genes in MSCs of TNF-Tg mice via RNAseq.

Supplemental figure 3



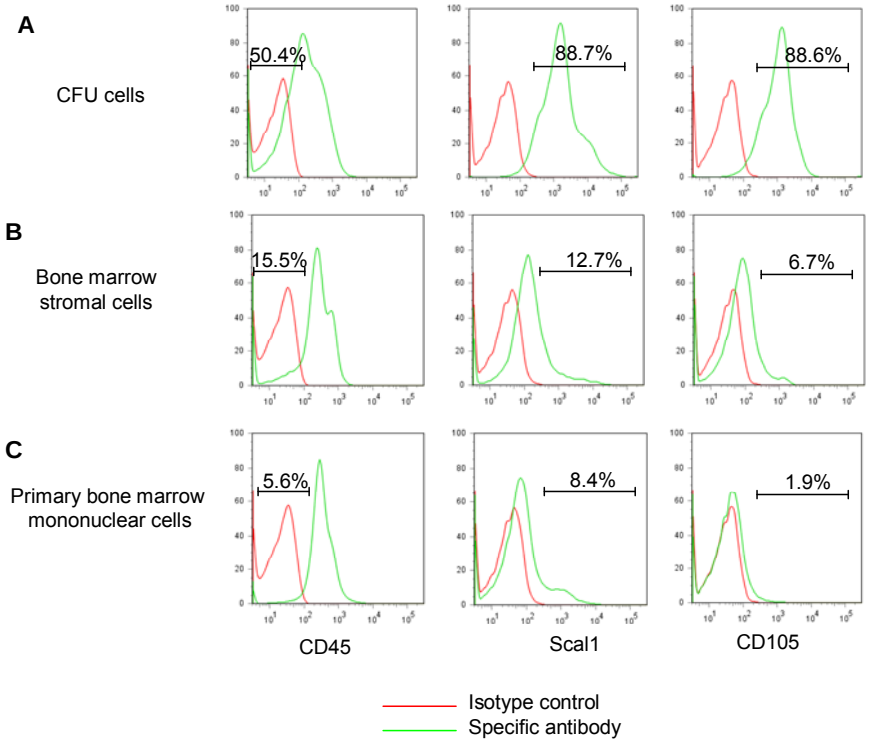
Supplemental figure 3. Characterization of 3rd passage of bone-derived MSCs.

Supplemental figure 4



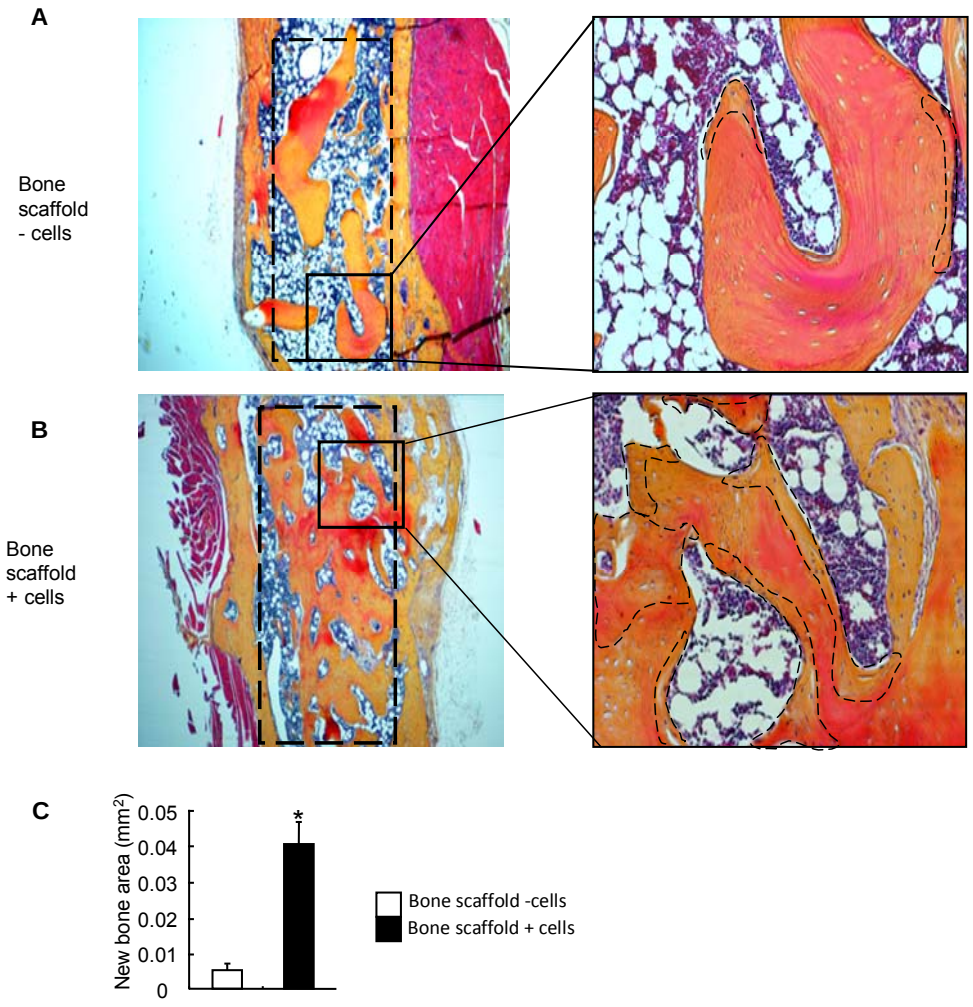
Supplemental figure 4. The effect of long-term DAPT treatment on expression levels of Notch target genes.

Supplemental figure 5



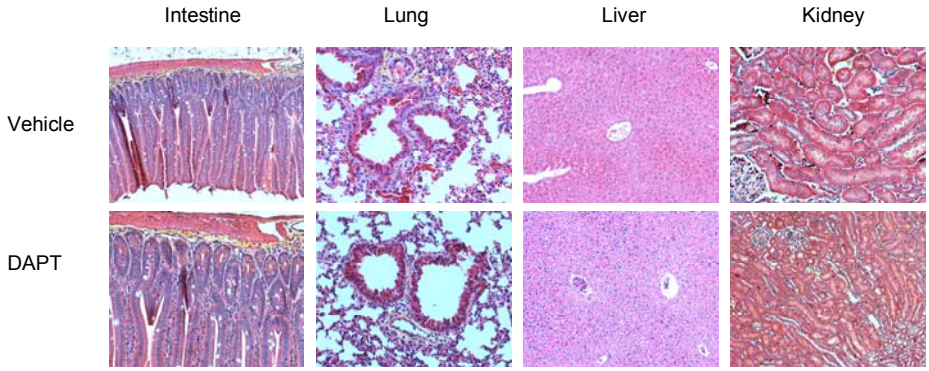
Supplemental figure 5. Characterization of CFU colony cells.

Supplemental figure 6



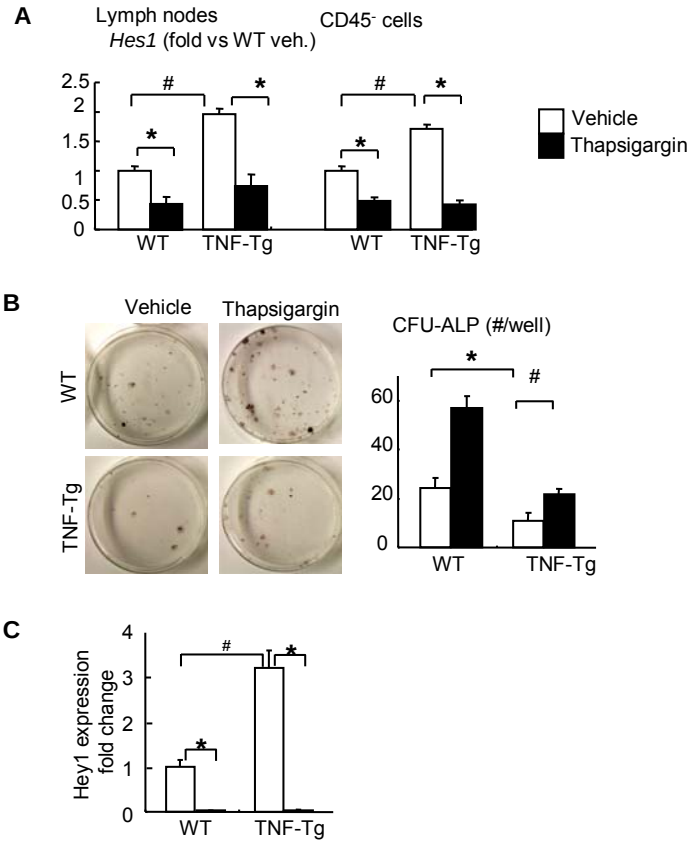
Supplemental figure 6. In vivo bone repair model.

Supplemental figure 7



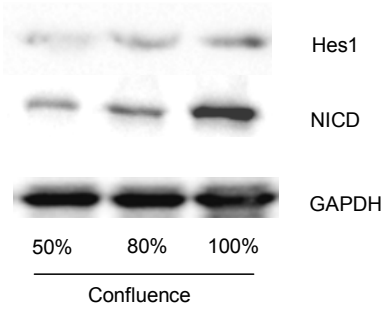
Supplemental figure 7. The effect of long-term DAPT treatment on morphology of internal organs.

Supplemental figure 8



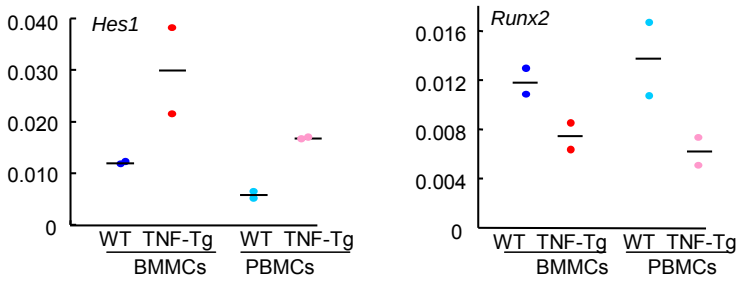
Supplemental figure 8. Thapsigargin reduces Hes1 expression and revises decreased osteoblast differentiation of MSCs in TNF-Tg mice.

Supplemental figure 9



Supplemental figure 9. Activation status of Notch signaling in cells at different confluence.

Supplemental figure 10



Supplemental figure 10. Correlation of gene expression levels in CD45- MSCs from BM and peripheral blood mononuclear cells.

Supplemental table

A. Murine primer sequences

Name	F / R	Sequences
mHes1	F	GACCCAGATCAACGCCATGA
	R	TGGAAGCCGCCAAAAACCTT
mHes2	F	CTGAAGGGTCTCGTATTGCCG
	R	CGCAGGTGCTCTAGTAGGC
mHey1	F	GCGCGGACGAGAATGGAAA
	R	TCAGGTGATCCACAGTCATCTG
mHey2	F	AAGCGCCCTTGTGAGGAAAC
	R	GGTAGTTGTCGGTGAATTGGAC
mNotch1	F	CCCTTGCTCTGCCTAACGC
	R	GGAGTCCTGGCATCGTTGG
mNotch2	F	ATGTGGACGAGTGTCTGTTGC
	R	GGAAGCATAGGCACAGTCATC
mALP	F	CTTGCTGGTGGAAGGAGGCAGG
	R	CACGTCTTCTCCACCGTGGGTC
mRunx2	F	CAAGAAGGCTCTGGCGTTTA
	R	TGCAGCCTTAAATGACTCGG
mGAPDH	F	GGTCGGTGTGAACGGATTTG
	R	ATGAGCCCTTCCACAATG

B. Human primer sequences

Name	F / R	Sequences
hHes1	F	TGAGCCAGCTGAAAACACTG
	R	GTGCGCACCTCGGTATTAAC
hHey1	F	GTTCGGCTCTAGGTTCCATGT
	R	CGTCGGCGCTTCTCAATTATTC
hp52	F	ATGGAGAGTTGCTACAACCCA
	R	CTGTTCCACGATCACCAGGTA
hRelB	F	CCATTGAGCGGAAGATTCAACT
	R	CTGCTGGTCCCGATATGAGG
hALP	F	ACCACCACGAGAGTGAACCA
	R	CGTTGTCTGAGTACCAGTCCC
hRunx2	F	TCAACGATCTGAGATTTGTGGG
	R	GGTCAAGGTGAAACTCTTGCC
hGAPDH	F	AAGGTGAAGGTCGGAGTCAAC
	R	GGGGTCATTGATGGCAACAATA