Supplemental Data

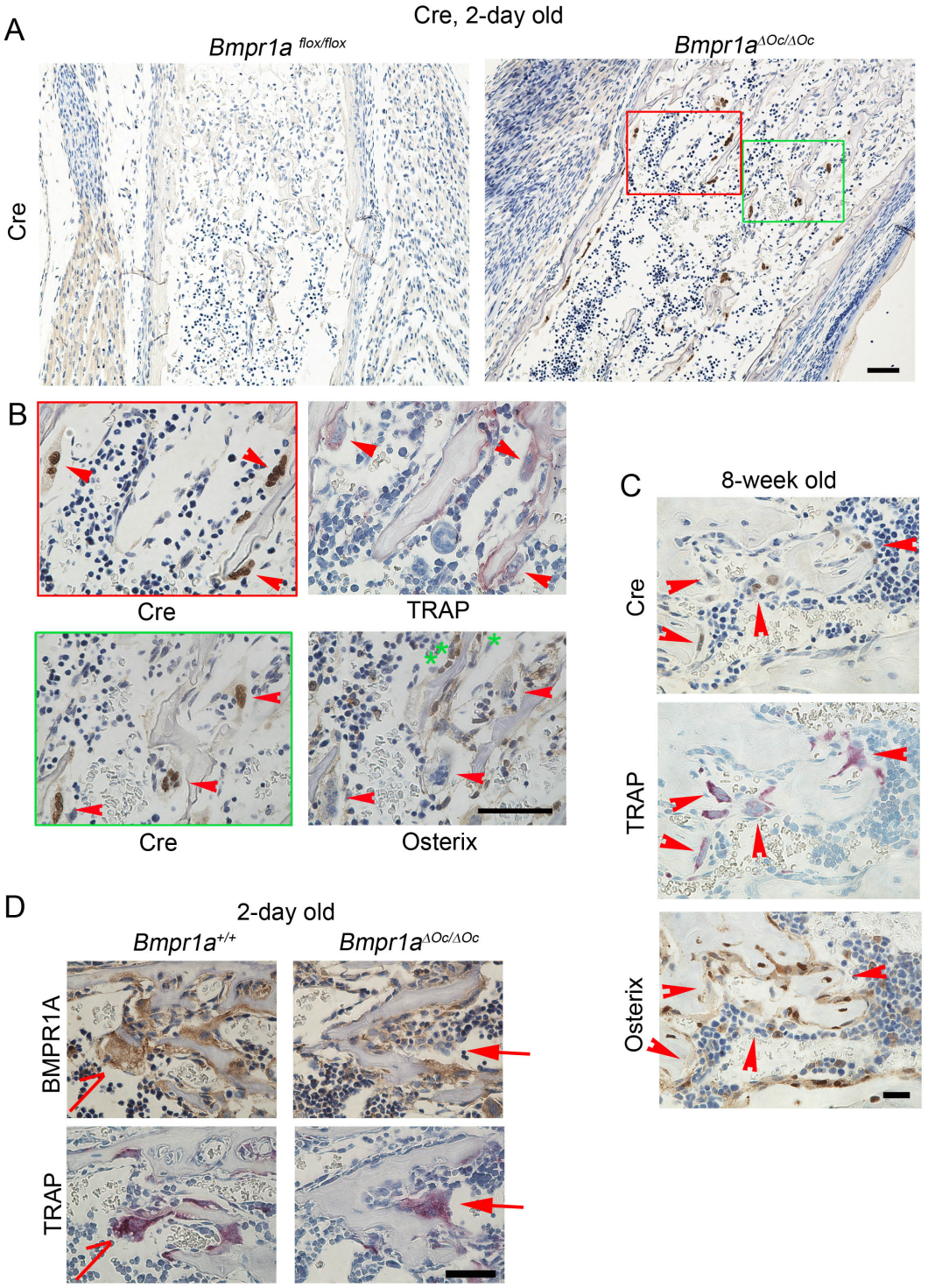
Conditional deletion of *Bmpr1a* in differentiated osteoclasts increases osteoblastic bone formation, increasing volume of remodeling bone in mice

Mina Okamoto, Junko Murai, Yuuki Imai, Daisuke Ikegami, Nobuhiro Kamiya, Shigeaki Kato, Yuji Mishina, Hideki Yoshikawa, and Noriyuki Tsumaki

Supplemental Table 1. Primers used in real-time RT-PCR.

Primer	Sequence
Trap S	GGCCGGCCACTACCCCATCT
*Trap*AS	CACCGTAGCGACAAGCAGGACTCT
Mmp9 S	GCCCTACAGCGCCCCCTACT
Mmp9 AS	AGACACGCCCCTTGCTGAACA
Rank S	TTCGGCGTTTACTACAGGA
Rank AS	CATTGACCCAATTCCACAAA
Gapdh S	GAGATGATGACCCTTTTGGCT
Gapdh AS	TCAAGGCCGAGAATGGGAAG
Cathepsin K S	AGGGCCAACTCAAGAAGAAAACT
Cathepsin K AS	TGCCATAGCCCACCACCAACACT
Nfatc1 S	CAACGCCCTGACCACCGATA
Nfatc1 AS	GGCTGCCTTCCGTCTCATAG

Supplemental Figure 1



Supplemental Fig. 1. Expression patterns of Cre and BMPR1A in tibia.

(A) Histological sections of tibia of *Bmpr1a*$^{flox/flox}$ and *Bmpr1a*$^{\Delta Oc/\Delta Oc}$ mice at age 2 days were immunostained using anti-Cre antibodies.

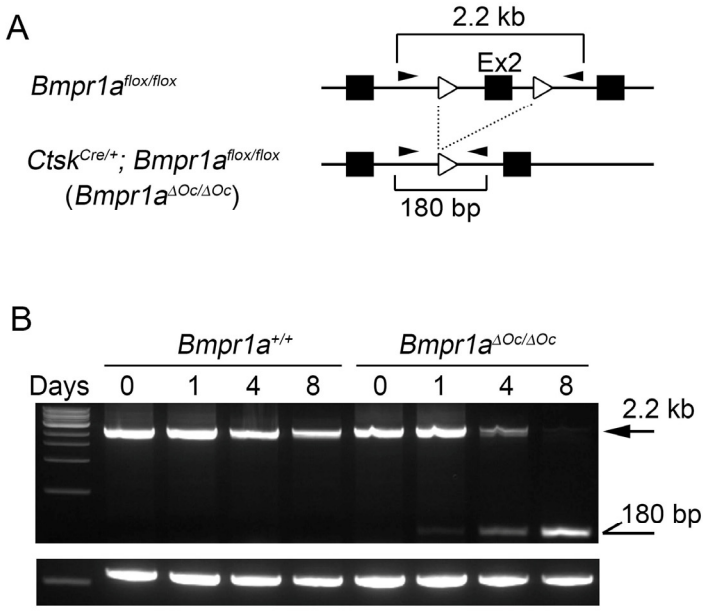
(B) *Top*, Magnification of red boxed region in (A) is shown (*left*) and serial sections were subjected to TRAP staining (*right*). *Bottom*, Magnification of green boxed region in (A) is shown (*left*) and serial sections were subjected to immunostaining with anti-Osterix antibody (*right*). Arrowheads indicate Cre-expressing TRAP-positive cells. Asterisks indicate Osterix-expressing cells.

(C) Serial sections of trabecular bone of proximal tibia of *Bmpr1a*$^{\Delta Oc/\Delta Oc}$ mice at 8 weeks of age were immunostained with anti-Cre and anti-Osterix antibodies, and subjected to TRAP staining. Arrowheads indicate Cre-expressing TRAP-positive cells.

(D) Serial sections of trabecular bone of proximal tibia of *Bmpr1a*$^{+/+}$ and *Bmpr1a*$^{\Delta Oc/\Delta Oc}$ mice at age 2 days were immunostained with anti-BMPR1A antibody and subjected to TRAP staining. Half-arrows and arrows indicate multinucleated osteoclasts.

Scale bars: 50 μm

Supplemental Figure 2



Supplemental Fig. 2. Genomic PCR analysis of recombinations at loxP sites during induction of osteoclasts *in vitro*. Bone marrow macrophage cultures prepared from *Bmpr1a*$^{+/+}$ and *Bmpr1a*$^{\Delta Oc/\Delta Oc}$ mice with M-CSF were further cultured in the presence of M-CSF and RANKL for the indicated number of days. Genomic DNAs were extracted and subjected to PCR amplification.

(A) Primer locations for amplification of *Bmpr1a* gene fragments. *Closed boxes*, exons; *Ex2*, 2nd exon; *open triangles*, loxP sequences; *arrowheads*, primer pair fx1 and fx4.

(B) PCR products separated on the agarose gel. *Top*, PCR products with sizes around 2.2 kb (arrow) were amplified from *Bmpr1a*$^{+/+}$ and *Bmpr1a*$^{flox/flox}$ genomic DNA. After excision by Cre recombinase, PCR products of 180 bp (half-arrow) were amplified from *Ctsk*$^{Cre/+}$;*Bmpr1a*$^{flox/flox}$ DNA (*Bmpr1a*$^{\Delta Oc/\Delta Oc}$). *Bottom*, RAPSYN gene fragment (590 bp) was amplified as an internal control.