

**Fig. S1 SMAD1/5/8 and SMAD2/3 in bones of *Bmpr2*-cKO mice.** **A:** Western blots for phosphorylated isoforms of SMAD1/5/8 (pS1/5/8) and SMAD2/3 (pS2/3) relative to total SMAD1 or SMAD2. Lysates are from marrow-free femora of male mice at 4 weeks of age. Densitometry are normalized to *Bmpr2*-floxed from n=3 each genotype. Vertical bars indicate removal of intervening lane(s) so that samples most representative of group mean are shown. **B:** Western blot for SMAD1/5/8 activation in bones of *Bmpr2*-cKO mice at 9 weeks of age showing the intervening lane that was cropped so that samples most representative of group mean could be shown in Fig. 1D. **C-D:** SMAD1 and SMAD2 expression levels in bones of *Bmpr2*-cKO mice at 4 and 9 weeks of age. Western blots and densitometry for total SMAD1 or SMAD2 relative to  $\beta$ -actin. Lysates are from marrow-free femora of male mice at 4 weeks of age (C) and female mice at 9 weeks of age (D). Densitometry are normalized to *Bmpr2*-floxed from n $\geq$ 3 each genotype. **E:** SMAD1 and SMAD2 expression levels in bones of ACVR2B-Fc treated mice at 9 weeks of age. Western blots and densitometry for total SMAD1 or SMAD2 relative to  $\beta$ -actin. Lysates are from marrow-free femora of *Bmpr2*-floxed mice two days after ACVR2B-Fc administration. Densitometry are normalized to untreated *Bmpr2*-floxed from n $\geq$ 2 each genotype.

**Fig. S2 A:** Quantitative RT-PCR for Activin subunits (*Inhba* and *Inhbb*) and antagonists (*Inhibin* (*Inha*) and *Follistatin*) in *Bmpr2*-cKO osteoblasts. n=4 per genotype for all analyses. No statistically significant change in expression level of the queried genes was observed. **B:** Activin-responsiveness in *Bmpr2*-cKO osteoblasts. Western blot for phosphorylated isoforms of SMAD2/3 (pS2/3) relative to total SMAD2 in *Bmpr2*-floxed (Control) and *Bmpr2*-cKO (CMV-Cre) osteoblasts +/- 100 ng/ml Activin A treatment for 1 hour.  $\beta$ -actin expression serves a loading control.

**Fig. S3 A:** Quantitative RT-PCR for bone marker expression in marrow-free humerii of 4-week-old *Bmpr2*-floxed and *Bmpr2*-cKO mice. n $\geq$ 4 per genotype for all analyses. No statistically significant change in expression level of the queried genes was observed. **B:** Quantitative RT-PCR for Activin ligands, BMP ligands, and antagonists in marrow-free humerii of 15-week-old *Bmpr2*-floxed and *Bmpr2*-cKO mice. n $\geq$ 4 per genotype for all analyses. No statistically significant change in expression level of the queried genes was observed.

**Fig. S4 Effects of Activin receptor decoy administration in *Bmpr2*-floxed mice. A-B.** Western blots for phosphorylated isoforms of SMAD2/3 (A, pS2/3) and SMAD1/5/8 (B, pS1/5/8) relative to total SMAD2 or SMAD1. Lysates are from marrow-free femora of *Bmpr2*-floxed mice two days after ACVR2B-Fc administration. Vertical bar in B indicates removal of intervening lane so that samples most representative of group mean are shown. **C:** Rate of body weight gain over 5-week ACVR2B-Fc treatment period in *Bmpr2*-floxed mice expressed as mean percent change from subject body weight on study day 0. **D-F:** Effect of Activin sequestration on dry weight of pectoralis major (D) and tibia trabecular bone volume (E) and bone mineral density (F) in *Bmpr2*-floxed mice. Data for untreated cohorts in E-F are also reported in Table 1.  $n \geq 2$  for all groups.

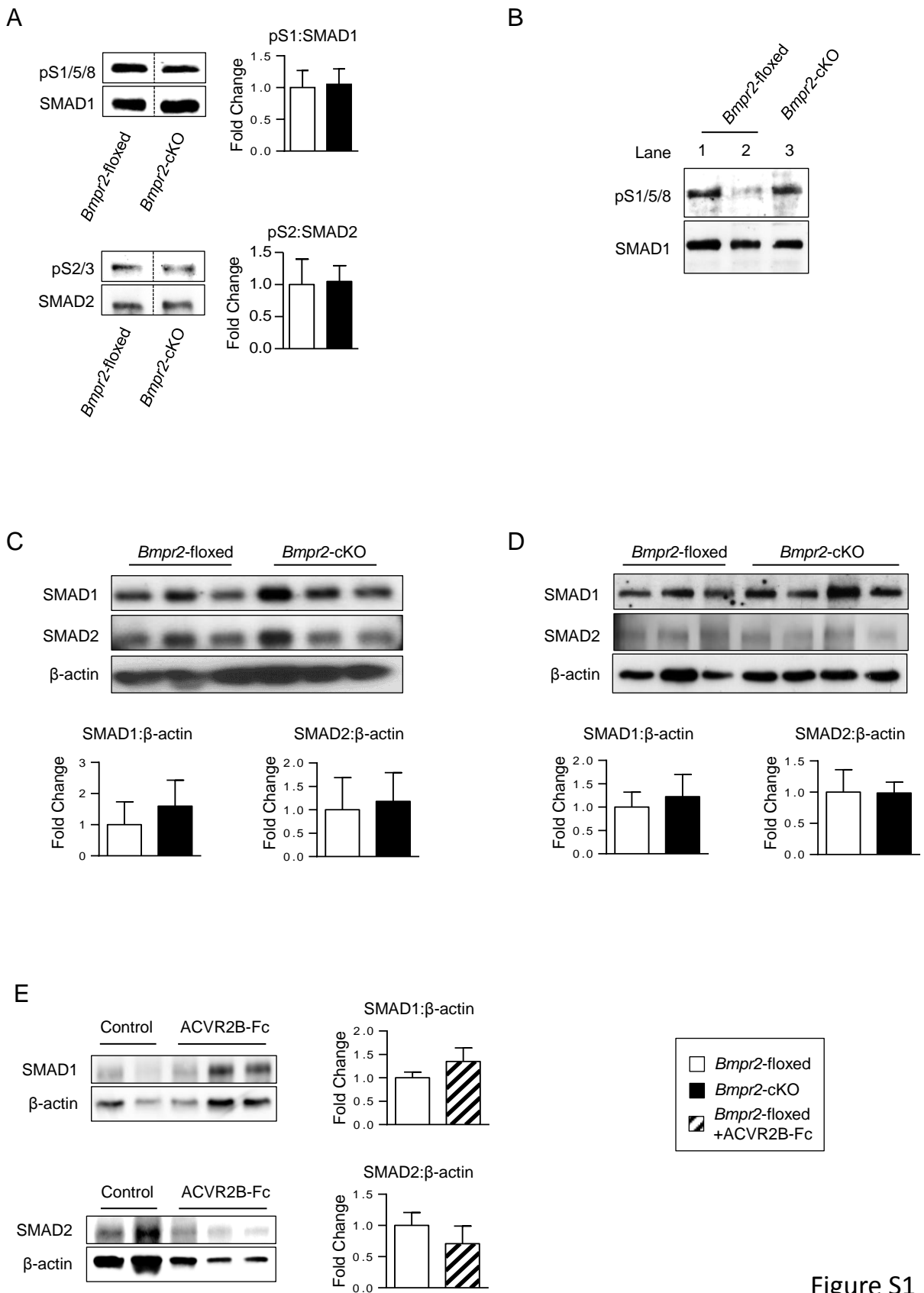


Figure S1

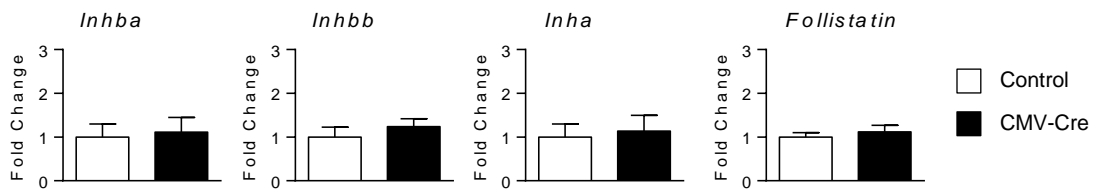
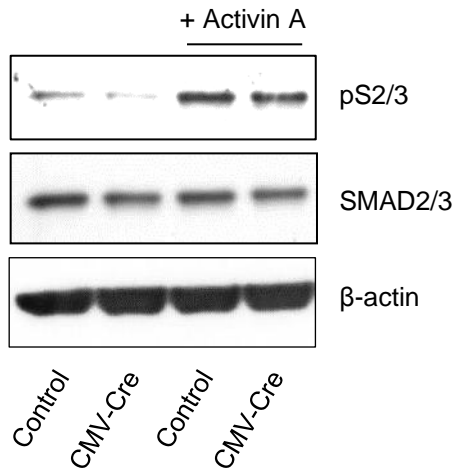
**A****B**

Figure S2

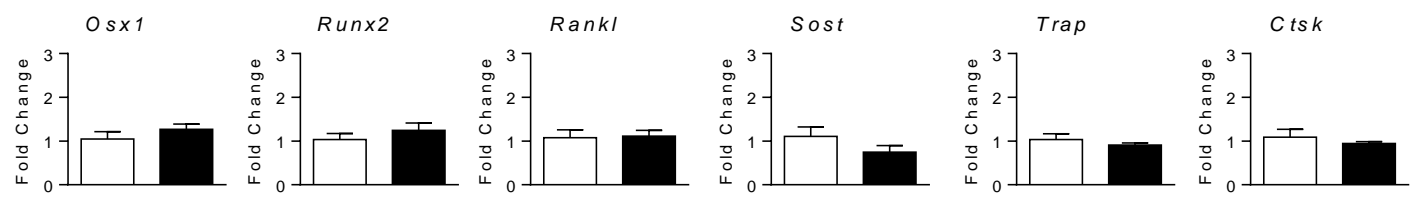
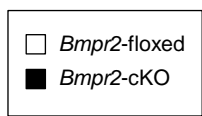
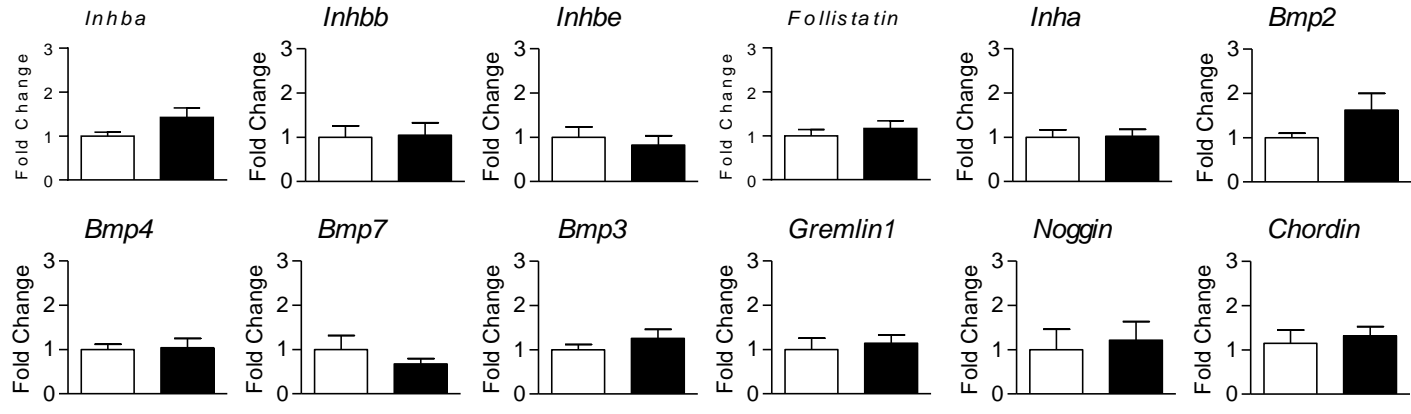
**A****B**

Figure S3

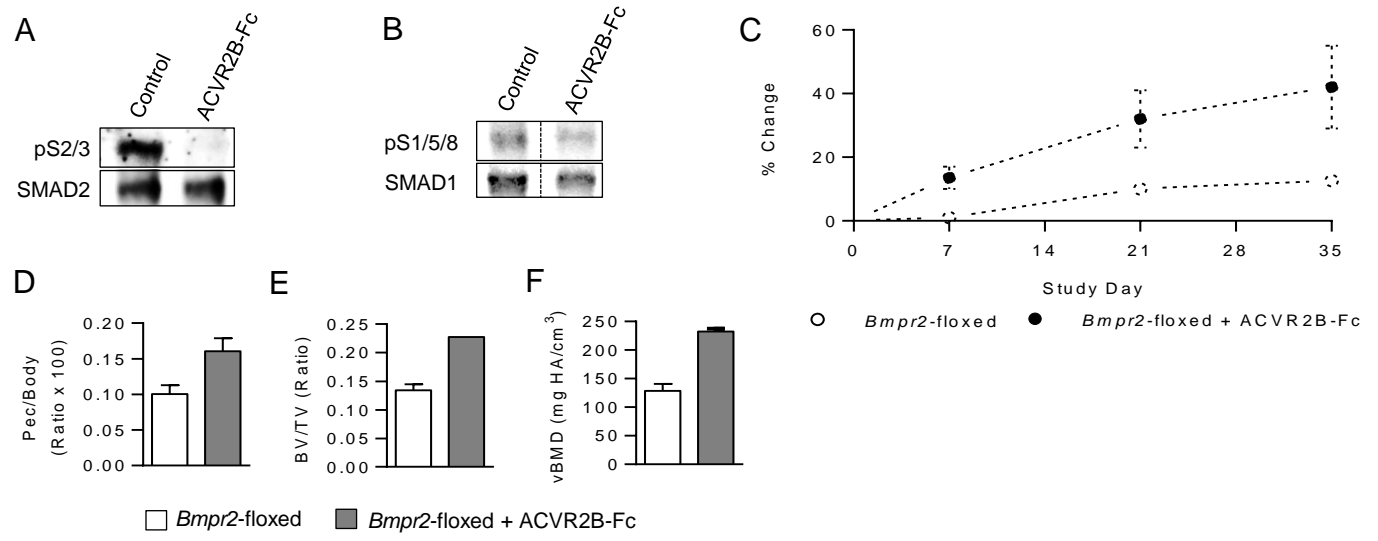


Figure S4

**Table S1.**  $\mu$ CT analyses of L5 vertebrae from 9-week-old *Bmpr2*-floxed and *Bmpr2*-cKO mice.

|            |        | <i>Bmpr2</i> -floxed    | <i>Bmpr2</i> -cKO       | <i>p</i> value |
|------------|--------|-------------------------|-------------------------|----------------|
| BV/TV      | Male   | 0.2598±0.01333 (n=6)    | 0.2294±0.01572 (n=6)    | 0.1702         |
|            | Female | 0.2209±0.01348 (n=3)    | 0.2362±0.00892 (n=5)    | 0.3591         |
| Tb.Th (mm) | Male   | 0.04597±0.0006535 (n=6) | 0.04357±0.001912 (n=6)  | 0.2624         |
|            | Female | 0.04707±0.002202 (n=3)  | 0.04706±0.0004007 (n=5) | 0.9970         |

Number of animals for each group indicated by (n). BV: bone volume, TV: tissue volume, Tb.Th: trabecular thickness.

**Table S2.** Sequences of primers used for RT-PCR analyses.

| Target mRNA        | Forward Primer           | Reverse Primer           |
|--------------------|--------------------------|--------------------------|
| <i>Acvr2a</i>      | GCAATGGCTTCAACCCTAGT     | CCCTCCTGTACTTGTTCCTACTCA |
| <i>Acvr2b</i>      | GGCCATGTACCGTCTGGT       | TGGCTGTTCGGTTTGAGC       |
| <i>Bmpr2</i>       | GAGCCCTCCCTTGACCTG       | GTATCGACCCCGTCCAATC      |
| <i>Bmp2</i>        | AGATCTGTACCGCAGGCACT     | GTTCTCCACGGCTTCTTC       |
| <i>Bmp4</i>        | GAGGAGTTTCCATCACGAAGA    | GCTCTGCCGAGGAGATCA       |
| <i>Bmp7</i>        | CGAGACCTTCCAGATCACAGT    | CAGCAAGAAGAGGTCCGACT     |
| <i>Bmp3</i>        | TCTCCCAAGTCATTTGATGCT    | GCGTGATTTGATGGTTTCAA     |
| <i>Chordin</i>     | TCACTGCCACCTCCTTG        | GATCTTTTACCACGCCCTGA     |
| <i>Ctsk</i>        | AGCGAACAGATTCTCAACAGC    | AGACAGAGCAAAGCTCACCAT    |
| <i>Follistatin</i> | AAGCATTCTGGATCTTGCAACT   | GATAGGAAAGCTGTAGTCCTGGTC |
| <i>Hprt</i>        | CCTGCTGGATTACATTAAGCACTG | GTCAAGGGCATATCCAACAACAAC |
| <i>Inha</i>        | GGAAGATGTCTCCAGGCTA      | TGGCTGGTCCTCACAGGT       |
| <i>Inhba</i>       | ATCATCACCTTTGCCGAGTC     | TCACTGCCTTCCTTGGAAT      |
| <i>Inhbb</i>       | GATCATCAGCTTTGCAGAGACA   | TGCCTTCATTAGAGACGAAGAA   |
| <i>Inhbe</i>       | CATCAGCTTTGCTACCATCATAGA | AGGTGGTGGGACCAAAGAG      |
| <i>Noggin</i>      | TGATGGATCCCCACCAAC       | CGCTAGAGGGTGGTGAAACT     |
| <i>Osx1</i>        | AGAGATCTGAGCTGGGTAGAGG   | AAGAGAGCCTGGCAAGAGG      |
| <i>Phex</i>        | AGCGCTATGATTCCCCAGT      | TTCAAGTGTGGGTAGAGTCTGG   |
| <i>Rankl</i>       | AGCCATTTGCACACCTCAC      | CGTGGTACCAAGAGGACAGAGT   |
| <i>Runx2</i>       | CCACAAGGACAGAGTCAGATTACA | TGGCTCAGATAGGAGGGGTA     |
| <i>Sost</i>        | TCCTGAGAACAACCAGACCA     | GCAGCTGTACTCGGACACATC    |
| <i>Trap</i>        | CGTCTCTGCACAGATTGCAT     | AAGCGCAAACGGTAGTAAGG     |