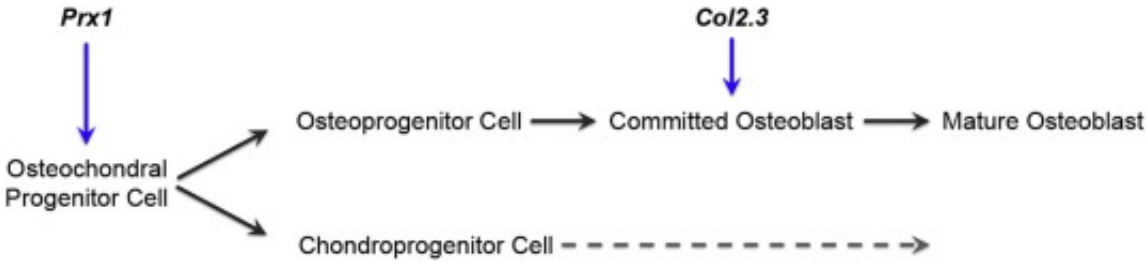

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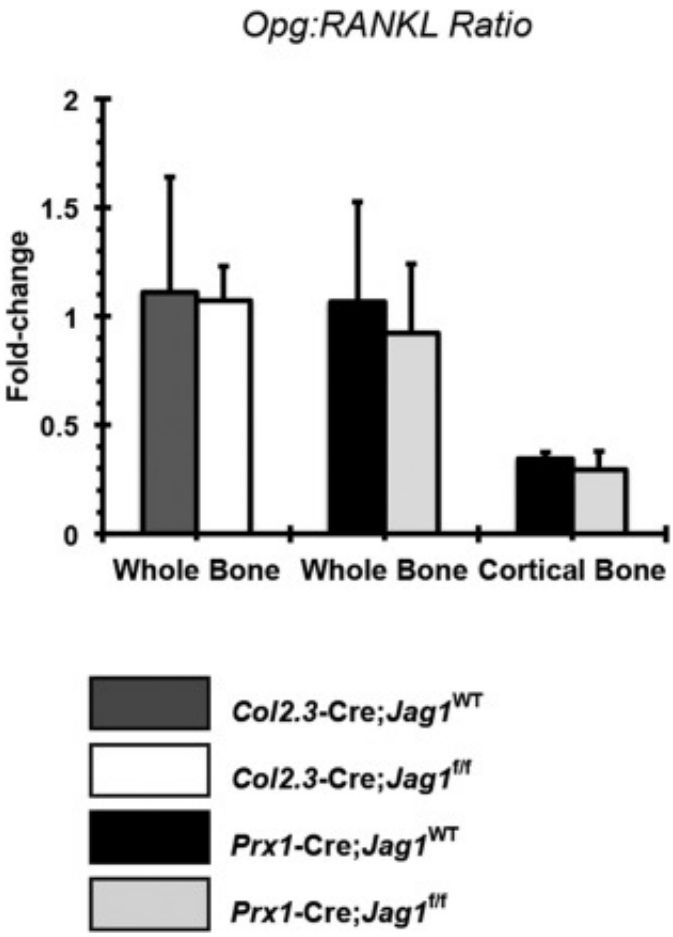
Supplemental Fig. 1. Schematic depicting *Prx1* and *Col2.3* expression as markers of osteochondral lineage differentiation. *Prx1* is first expressed in undifferentiated osteochondral progenitor cells. Cre will be expressed in cells of the early mesenchyme, and all of their progeny, including osteoblasts and osteocytes, will lack *Jag1*. *Col2.3* is first expressed in committed osteoblasts, so osteoblasts and osteocytes will have *Jag1* disrupted.



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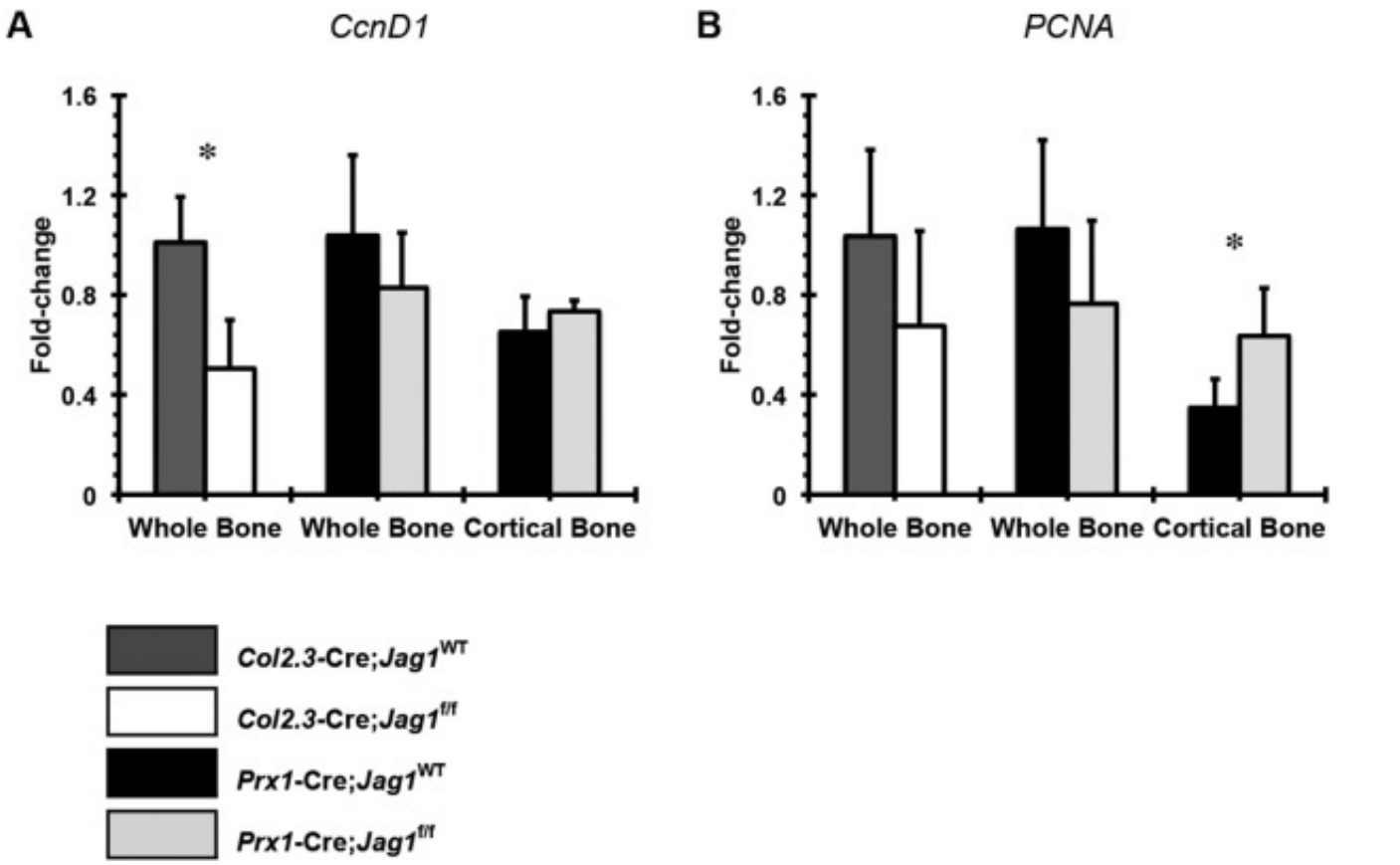
Supplemental Fig. 2. Gene expression of proliferation markers cyclin D1 (*CcnD1*) and proliferating cell nuclear antigen (*PCNA*). Data are presented as fold change expression to each genotype's respective whole bone (*Prx1*-Cre;*Jag1*$^{f/f}$ or *Col2.3*-Cre;*Jag1*$^{f/f}$) calculated using the formula $2^{-\Delta\Delta C(t)}$ (*p < 0.050,

#p < 0.100).



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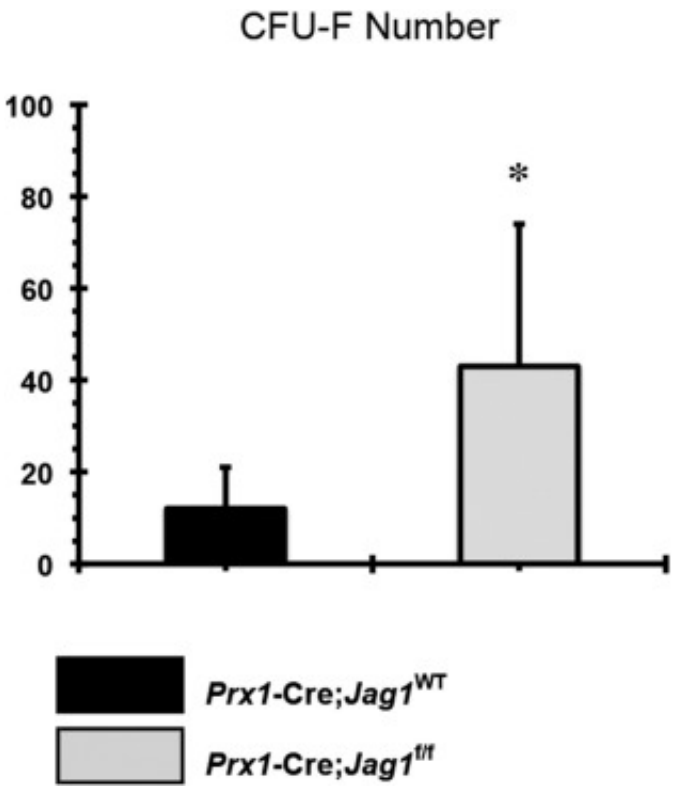
Supplemental Fig. 3. Results of CFU-F assays for marrow-derived cells from WT and *Prx1*-Cre;*Jag1*$^{f/f}$ mice. Marrow-derived cells from *Prx1*-Cre;*Jag1*$^{f/f}$ mice form more colonies *in vitro* (n = 11, *p < 0.050).



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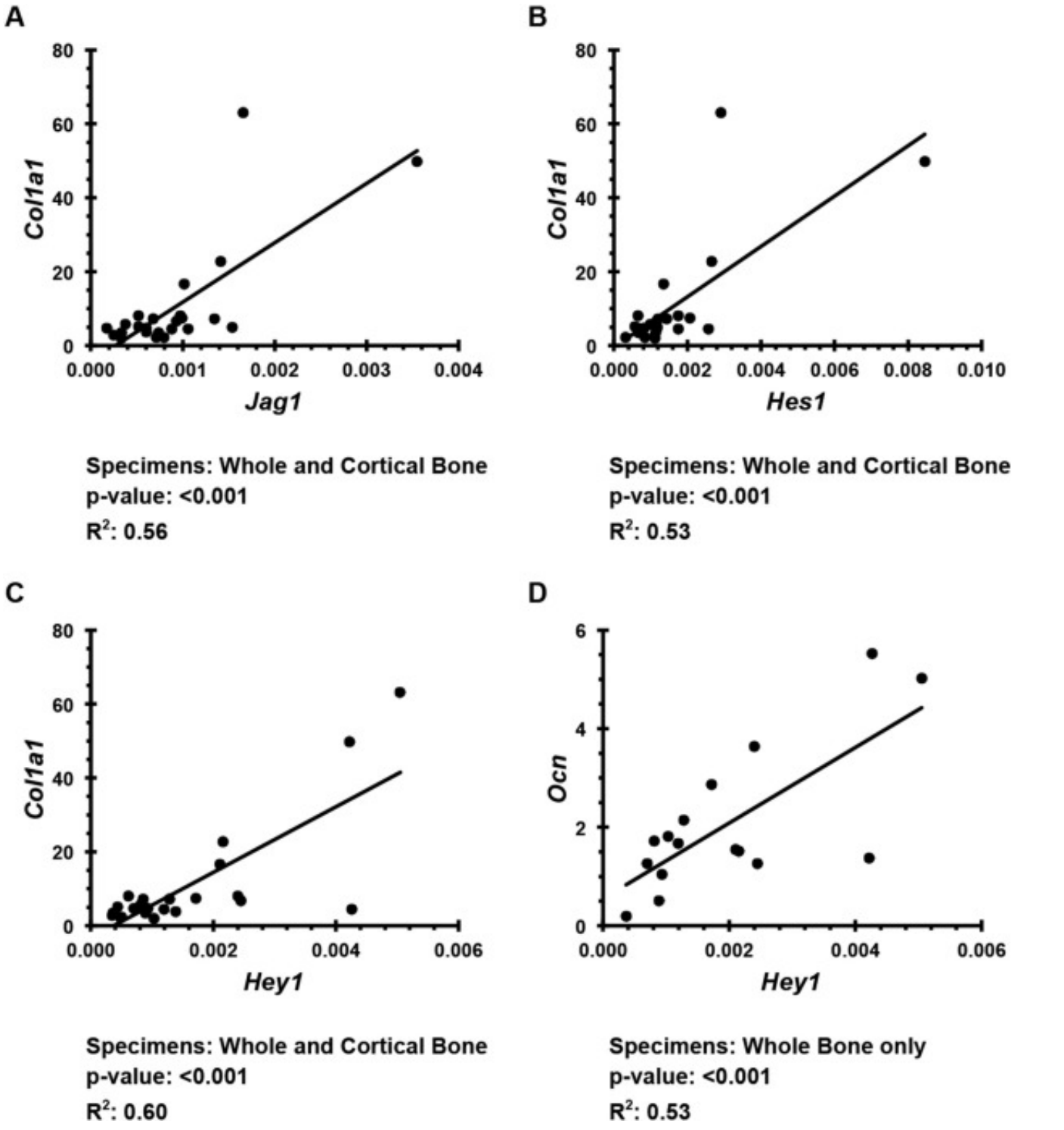
Supplemental Fig. 4. Ratio of relative changes in gene expression of osteoblast mediators of osteoclast activity *RANKL* and *Opg* show no significant differences.



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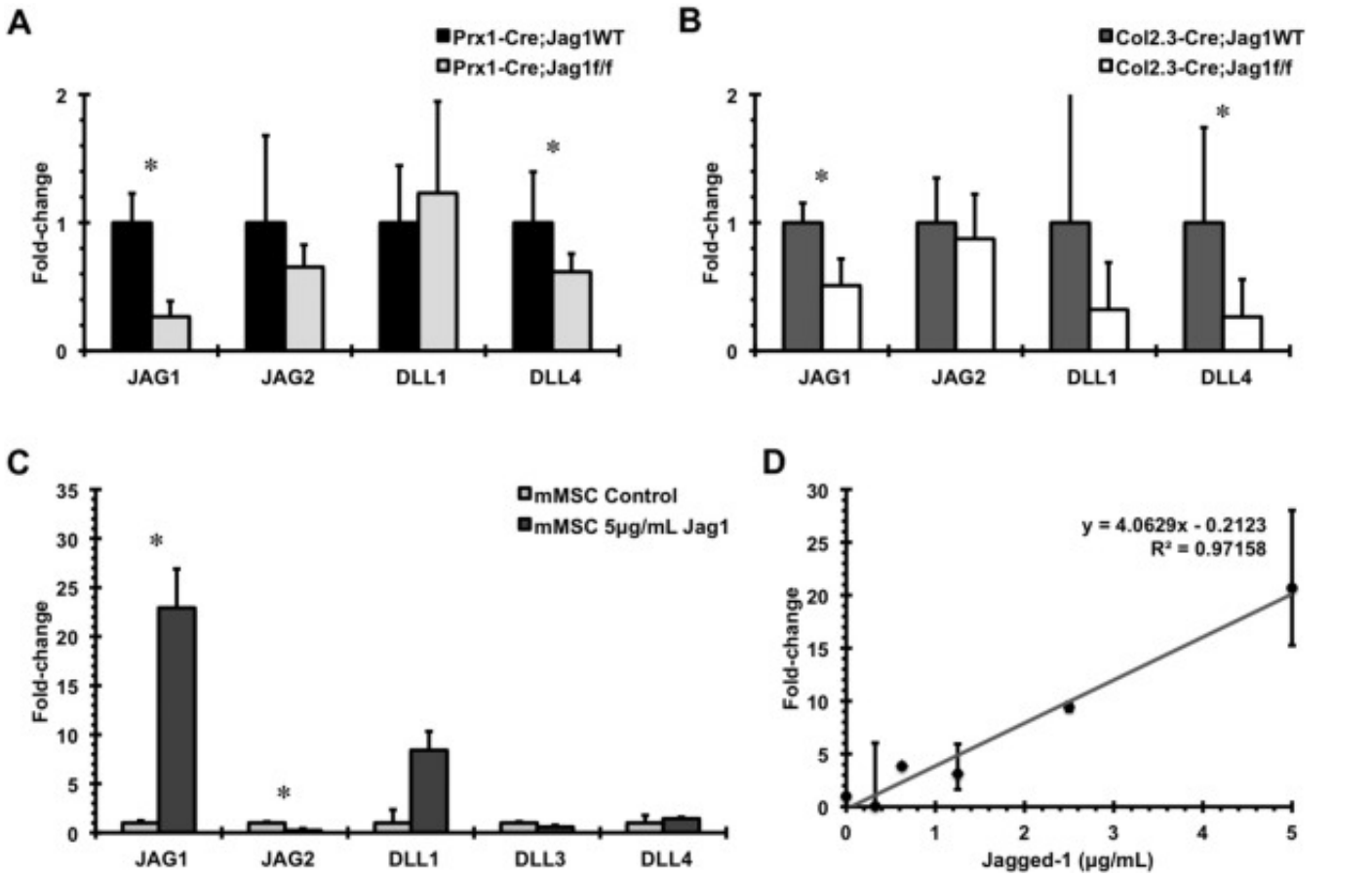
Supplemental Fig. 5. Linear correlation of Notch components (*Jag1*, *Hes1*, *Hey1*) with osteogenic markers (*Col1a1*, *Ocn*). Data are presented as relative expression to β-actin calculated using the formula $2^{-\Delta\Delta C(t)}$.



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Supplemental Fig. 6. Whole-bone gene expression of Notch ligands following Jag1 knockout and mMSC gene expression following activation with Jag1. *DLL4* expression is co-downregulated with *Jag1* 0.62 ± 0.14-fold in *Prx1*-Cre;*Jag1*$^{f/f}$ mice ($p = 0.048$) and 0.26 ± 0.29-fold in *Col2.3*-Cre;*Jag1*$^{f/f}$ mice ($p = 0.041$) *in vivo*. Expression of other ligands is unchanged (*DLL3* not detected). Jag1-stimulated mMSCs demonstrate a dose-dependent upregulation of *Jag1*. At a dose of 5 µg/mL, *Jag1* is upregulated 22.9-fold, while Jag2 is downregulated 0.24-fold. Statistical analysis was not performed on this pilot mMSC data, which represents a single pool of cells per group (error bars represent values from technical replicates). All data are presented as relative expression to β-actin calculated using the formula $2^{-\Delta\Delta C(t)}$ (*$p < 0.050$).