

Supporting information

Piezo1 in hypertrophic chondrocytes regulates osteoclastogenesis during bone development and fracture healing

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Table S1. Primer pairs used for quantitative real-time PCRs in murine ATDC5 cells and human C28/I2 cells.

Gene	forward primer sequence (5' to 3')	reverse primer sequence (3' to 5')
murine <i>GAPDH</i>	ACCCAGAAGACTGTGGATGG	GGATGCAGGGATGATGTTCT
murine <i>OPG</i>	CTGCCTGGGAAGAAGATCAG	TTGTGAAGCTGTGCAGGAAC
human <i>GAPDH</i>	GAAGGTGAAGGTCGGAGTC	GAAGATGGGATGGGATTTTC
human <i>RANKL</i>	CCAGCATCAAAATCCCAAGT	CCCCAAAGTATGTTGCATCCTG
human <i>OPG</i>	AGGAAATGCAACCAACGACA	TACTTTGGTGCCAGGCAAAT
murine p53	GGAAATTTGTATCCCGAGTATCTG	GTCTTCCAGTGTGATGATGGTAA
murine Piezo1	CTCCGACTTCCTCGAGTGGT	CTAGGTGGGCTGACCTTGTC

Supplementary Figures

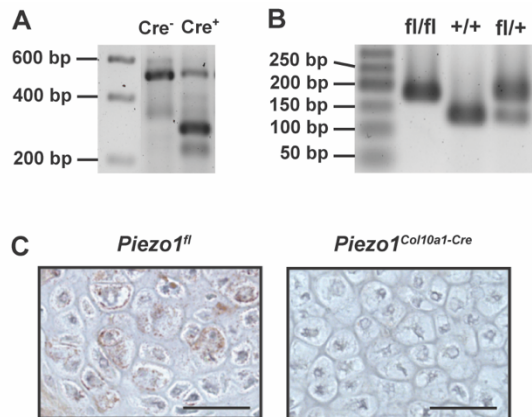


Figure S1. Gel documentation of (A) the wildtype (500 bp) and *Col10a1-Cre* PCR product (300 bp) and (B) the floxed (fl, 200 bp) and wildtype (+, 150 bp) *Piezo1* allele PCR product. (C) Immunohistochemical detection of the Piezo1 extracellular domain on fracture callus sections of *Piezo1^{fl}* and *Piezo1^{Col10a1-Cre}* mice. Scale bars represent 50 μm.

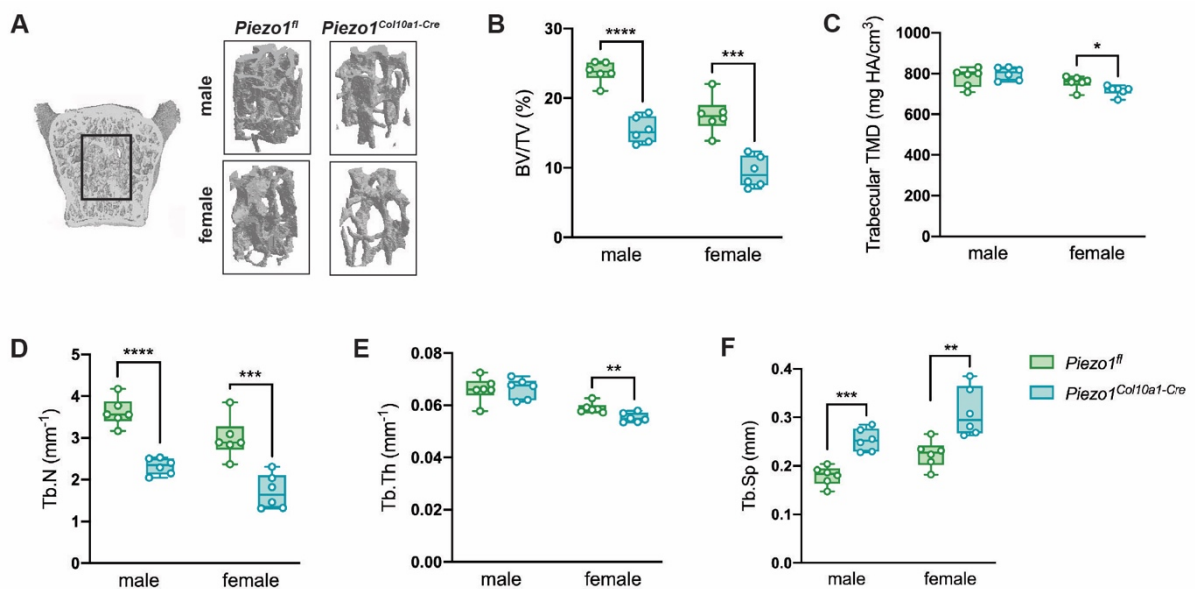


Figure S2. (A) Representative images of analyzed ROIs in lumbar vertebra L4, (B) bone volume/tissue volume ratio (BV/TV), (C) trabecular tissue mineral density (TMD), (D) trabecular number (Tb.N), (E) trabecular thickness (Tb.Th) and (F) trabecular separation (Tb.Sp) of spines of 12-week-old male and female *Piezo1^{fl}* and *Piezo1^{Col10a1-Cre}* mice. N = 6, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

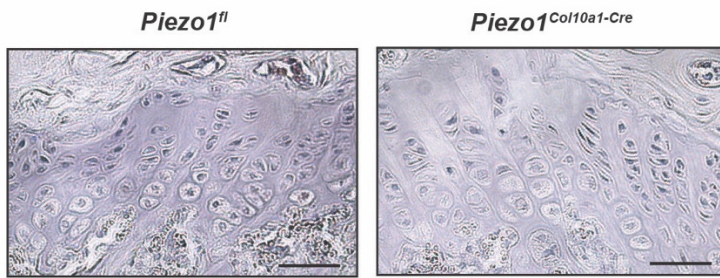


Figure S3. Immunohistochemical detection of OPG in growth plates of *Piezo1^{fl}* and *Piezo1^{Col10a1-Cre}* mice. Scale bars represent 50 μm.

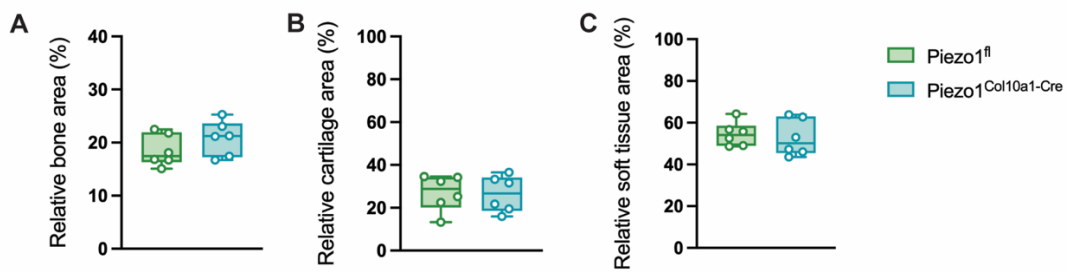


Figure S4. Relative (A) bone, (B) cartilage and (C) soft tissue area in fracture calli of *Piezo1^{fl}* and *Piezo1^{Col10a1-Cre}* mice 14 days post-fracture. N = 6.

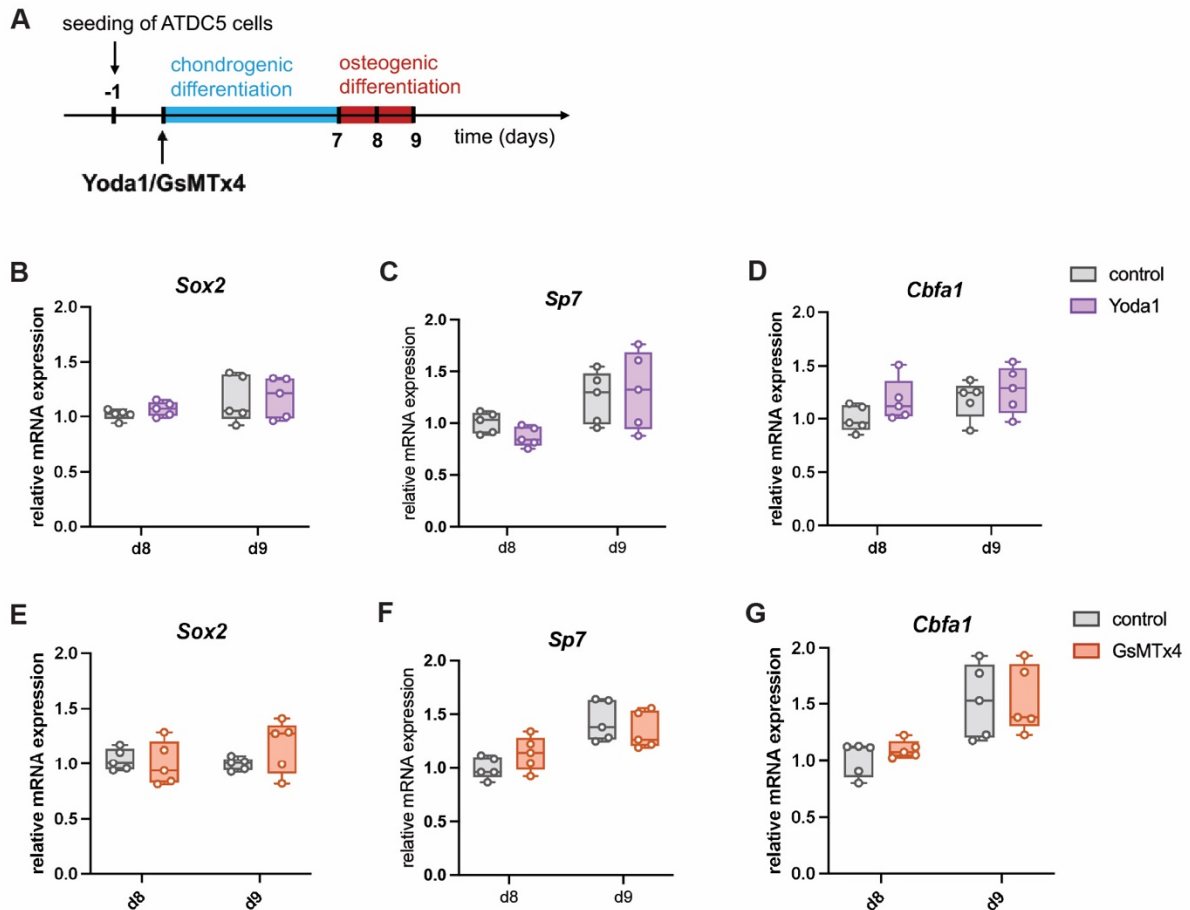


Figure S5. (A) Experimental setup of testing the effect of Yoda1 and GsMTx4 on the transdifferentiation of chondrogenic ATDC5 cells. Relative mRNA expression of (B, E) *Sox2*, (C, F) *Sp7*, (D, G) *Cbfa1* in transdifferentiating ATDC5 cells treated with Yoda1 or GsMTx4. N = 5, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

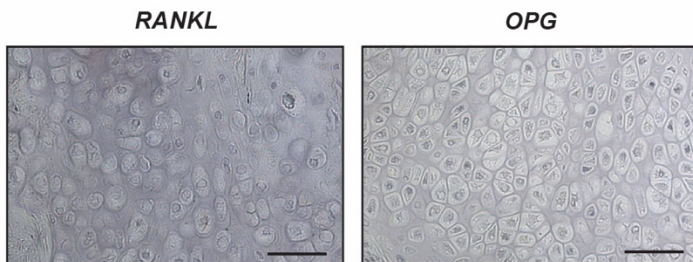


Figure S6. Representative negative control stainings for RANKL and OPG in the fracture callus.