Mechanical sensing protein PIEZO1 regulates bone homeostasis via osteoblast-osteoclast crosstalk

Wolff's law and the Utah Paradigm of skeletal physiology state that bone architecture adapts to mechanical loads. These models predict the existence of a mechanostat that links strain induced by mechanical forces to skeletal remodeling. However, how the mechanostat influences bone remodeling remains elusive. Here, we find that Piezo1 deficiency in osteoblastic cells leads to loss of bone mass and spontaneous fractures with increased bone resorption. Furthermore, Piezo1-deficient mice are resistant to further bone loss and bone resorption induced by hind limb unloading, demonstrating that PIEZO1 can affect osteoblast-osteoclast crosstalk in response to mechanical forces. At the mechanistic level, in response to mechanical loads, PIEZO1 in osteoblastic cells controls the YAP-dependent expression of type II and IX collagens. In turn, these collagen isoforms regulate osteoclast differentiation. Taken together, our data identify PIEZO1 as the major skeletal mechanosensor that tunes bone homeostasis.
Bone remodeling involves the removal of old or damaged bone by osteoclasts (bone resorption) and subsequent replacement of new bone formed by osteoblasts (bone formation). One of the major functions of bone remodeling is to adapt bone material and structural properties to the mechanical demands that are placed on the skeleton, including mechanical loading and weight bearing. Increased loading stimuli, e.g., through exercise and vigorous muscular activity, will enhance bone mass and bone strength. Alternatively, lack of mechanical loading in the setting of prolonged bed rest or exposure to long-term microgravity environment in space leads to a rapid reduction in bone mass and bone strength. This mechanical force-bone interplay has been recognized as Julius Wolff’s law since the 19th century. Understanding how mechanical loading regulates the balance between bone formation and resorption would lead to the therapeutic strategies for disuse osteoporosis.

It has been reported that osteoblast lineage cells, including bone mesenchymal stem cells (BMSCs), osteoblast progenitor cells, osteoblasts, and osteocytes can respond to mechanical loading in bone, TRPV4 mediates fluid shear stress induced calcium signaling and early osteogenic gene expression in BMSCs. The opening of Connexin43 (Cx43) hemichannel depends on interaction with integrin in response to shear stress in osteocytes. However, Cx43 deficient mice showed an enhanced anabolic response to mechanical load, YAP and TAZ can sense a broad range of mechanical cues and transduce them into transcriptional response in a manner that is specific for each type of cell and mechanical stress, including ECM rigidity and topology, stretching and tensional forces. The above molecules are important transducers of mechanical signals in skeletal cells and regulate the function of osteoblast lineage cells. While mechanical loading promotes osteoblast differentiation, it also inhibits osteoclast formation, migration, and adhesion in vivo. However, how mechanical loading coordinates bone remodeling remains incompletely understood.

Mechanically activated cation channel activity has been recorded in many cells, though the responsible sensing molecules were mysterious until recently. Expression profiling and RNA interference knockdown of candidate genes in a mouse neuroblastoma cell line identified PIEZO proteins (PIEZO1 and PIEZO2) as nonselective Ca\(^{2+}\)-permeable channel proteins. PIEZO proteins are emerging as important mediators of various aspects of mechanotransduction. PIEZO1 is expressed by non-sensory tissues and could sense various mechanical stresses, including static pressure, shear stress, and membrane stretch. PIEZO1 has been reported to mediate the function of hydrostatic pressure on cell fate determination of mesenchymal stem cells. However, it is unknown whether PIEZO1 can regulate bone remodeling responding to mechanical load. In this study, we have generated Piezo1 conditional-knockout mice, and find that mice with Piezo1-deficiency in BMSCs and their progeny cells display increased bone resorption and multiple spontaneous fractures after weight bearing. Piezo1-deficient mice are resistant to further bone loss and osteoclast accumulation induced by mechanical unloading. Mechanistically, Piezo1 deficiency impairs COL2 and COL9 production through decreasing YAP nuclear localization, causing increased osteoclast activity. All of the above data support the notion that PIEZO1 can function as the mechanostat that directly senses mechanical loading to coordinate the osteoblast–osteoclast crosstalk in skeleton. Our study advances understanding and stimulates targeted therapeutic approaches for disuse osteoporosis.

Results

Osteoblastic Piezo1 deficiency resulted in osteoporosis. As PIEZO proteins are components of mechanically activated cation channels, we hypothesize that PIEZO channels could function as the long sought mechanostat that directly senses mechanical loading in skeletal cells. If true, we predict that PIEZO channel deficiency would regulate bone mass and strength in vivo. qPCR confirmed that Piezo1 was highly expressed in bone and skeletal cells (Supplementary Fig. 1a–c), while Piezo2 was highly expressed in the dorsal root ganglia neurons instead of the whole bone and primary osteoblasts as reported. These results suggest that PIEZO1 could have a crucial role in osteoblasts. To further elucidate the function of PIEZO1 in the bone homeostasis, we generated a PIEZO1 conditional-knockout mouse model (Supplementary Fig. 1d, e) by crossing Piezo1\(^{+/−}\) mice with Prx1\(^{Cre}\) mice, which expressed Cre recombinase in osteoblast progenitors that form the limbs and parts of the skull, but not the spine or other organs in vivo. qPCR confirmed a reduction of Piezo1 mRNA in bone cells from Piezo1\(^{−/−}\) mice (Supplementary Fig. 1f). Immunofluorescence confirmed the deletion of PIEZO1 in osteoblast progenitors (Supplementary Fig. 1g). Yoda1 has been identified as a specific agonist for PIEZO1 but not PIEZO2. Yoda1 stimulated calcium influx in wild-type, but not in Piezo1-deficient osteoblastic cells (Supplementary Fig. 1h). These data demonstrate functional deletion of PIEZO1 in osteoblast lineage cells in Piezo1\(^{−/−}\) mice.

To determine in vivo effects of PIEZO1 within the skeletal system, we performed quantitative computed tomography (μ-QCT) analysis. Trabecular bone mass was significantly reduced in male Piezo1\(^{−/−}\) mice compared to WT controls (Fig. 1a), as confirmed by decreased bone mineral density (BMD, Fig. 1b), trabecular bone volume (BV/TV, Fig. 1c), trabecular number (Tb. N, Fig. 1d) and increased trabecular spacing (Tb.Sp, Fig. 1f). Trabecular thickness (Tb.Th, Fig. 1e) was not changed significantly. In addition, cortical thickness was decreased in male Piezo1\(^{−/−}\) mice (Ct.Th) (Fig. 1a, g). Female WT and Piezo1\(^{−/−}\) mice were also analyzed by μ-QCT. The difference between WT and Piezo1-deficient female mice was comparable to male (Supplementary Fig. 2a–f), indicating that the function of Piezo1 is independent of gender. Furthermore, the long bones of Piezo1\(^{−/−}\) mice were smaller than WT mice (Supplementary Fig. 2g). We also analyzed the bone surfaces of WT and Piezo1\(^{−/−}\) mice by μ-QCT. Both cortical and trabecular bone surfaces of Piezo1\(^{−/−}\) mice were significantly decreased, compared with WT mice (Supplementary Fig. 2h, i). Notably, we observed multiple bone fractures in the Piezo1\(^{−/−}\) mice in weight-bearing appendicular bones (Fig. 1h). The fractures first occurred between P0 and P3, with no significant differences observed between wild-type and Piezo1-deficient mice at embryonic day 17.5 (Supplementary Fig. 3a) and postnatal day 0 (Supplementary Fig. 3b). However, multiple bone fractures occurred in 3-day-old Piezo1\(^{−/−}\) mice (Fig. 1i, Supplementary Fig. 3c). The femurs of 3-day-old Piezo1-deficient mice exhibited increased cortical porosity compared to WT control mice by μ-CT analysis (Fig. 1j, bottom panel). However, neonatal P0 mice showed only subtle differences between Piezo1\(^{−/−}\) mice and WT controls (Fig. 1j, top panel). Embryonic mice are surrounded by amniotic fluid, and therefore bones are not weight bearing. Therefore, we hypothesize that the absence of mechanical loading makes the effects of Piezo1 deficiency unseen at this stage. Overall the kinetics of phenotypic onset is consistent with PIEZO1 functioning downstream of postnatal mechanical loading. In addition, calvarial bones from WT and Piezo1\(^{−/−}\) mice were indistinguishable (Supplementary Fig. 4a, b), compared to more dramatic differences in the distal femurs, perhaps due to the fact that the skull is relatively under-loaded compared to long bones. Collectively, these data support that Piezo1 deficiency impairs the response of osteoblastic cells to...
Mechanical loading, leading to decreased bone mass and giving rise to bone fractures soon after birth.

Osteoblastic Piezo1 deficiency promoted bone resorption. We next sought to understand the relative contributions of osteoblast and osteoclast activity to the bone loss seen in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice. Osteoblasts originate from SSCs (skeletal stem cells), Pre-BCSPs (pre-bone, cartilage and stromal progenitors), and BCSPs (bone, cartilage and stromal progenitors), whose expansion and self-renewal must be tightly controlled<sup>23</sup>. Firstly, we analyzed these three cell types in Prx1<sup>Cre</sup>, Piezo1<sup>fl/fl</sup> mice. Consistently, these stem cells and progenitors were not significantly affected by PIEZO1 deficiency (Fig. 2a, b). We further speculated if PIEZO1 would impair osteoblast differentiation. To test this hypothesis, we performed histomorphometric analysis of WT and Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice to evaluate static and dynamic parameters of bone formation and resorption. Consistent with the µ-QCT data, histomorphometric analysis showed that Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice had a significant decrease in both BV/TV and Tb.N (Fig. 2c–e). Interestingly, the number of osteoblasts per bone perimeter (N.Ob/B.Pm) (Fig. 2f) and osteoblast surface per bone surface (Ob.S/BS) (Fig. 2g) were not significantly changed in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice compared to WT controls. Consistently, the bone mineral apposition rate and bone formation rate were not significantly changed in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice either (Fig. 2h–k). Furthermore, the bone formation marker PINP was not significantly changed in the serum of Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice (Fig. 2l). However, there was significantly increased bone resorption activity in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice compared to WT controls, demonstrated by increased eroded surface normalized to bone surface (ES/BS) (Fig. 2m), osteoclast number (N.Oc/Bpm) (Fig. 2n), osteoclast surface per bone surface (Oc.S/BS) (Fig. 2o) and serum CTX-I level (Fig. 2p) in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice. Tartrate-resistant acid phosphatase (TRAP) staining confirmed the increase of osteoclasts along the surface of trabecular bone in Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice (Fig. 2q). Also, Piezo1 deficiency did not impact osteoblast differentiation as determined by ALP activity and Alizarin red S staining in vitro (Supplementary Fig. 5a, b). Consistently, the expression of a series of osteoblastic marker genes showed no significant difference between control and Piezo1-deficient cells (Supplementary Fig. 5c).

**Fig. 1 Loss of Piezo1 in skeletal cells resulted in severe osteoporosis.** a 3D µ-CT images of trabecular bones of distal femurs isolated from 6-week-old male WT and Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice. b–g µ-CT analysis of distal femurs from WT (open bars) and Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> (filled bars) mice. Representative images for 3 independent samples. h Whole mount skeleton staining of 3-day-old WT and Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice by Alcian blue and Alizarin red S. Scale bar = 5 mm. j 3D µ-CT images of femurs isolated from 0-day-old and 3-day-old WT and Prx1<sup>Cre</sup>; Piezo1<sup>fl/fl</sup> mice. Source data are provided in the Source Data File.
Fig. 2 Loss of Piezo1 in osteoblastic cells resulted in increased bone resorption. a, b FACS analysis of mSSCs, mBSCPs and mPre-BSCPs of bone marrow cells of 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Data are mean ± SD, WT, n = 4, KO, n = 6. c Representative images of Von kossa staining of 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Scale bar = 500 μm. d-g Histomorphometric analysis of proximal tibias from 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice for bone volume per tissue volume (BV/TV) (d), trabecular number (Tb.N) (e), number of osteoblasts per bone perimeter (N.Ob/B. Pm) (f), osteoblast surface per bone surface (Ob.S/BS) (g). Data are mean ± SD, WT, n = 4, KO, n = 3. h Representative images of dual Calcein-Alizarin red S labeling of proximal tibias from 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Scale bar = 20 μm, n = 6. i-k Quantification of mineral apposition rate (MAR) (i), bone formation rate normalized to bone surface (BFR/BS) (j) and mineralizing surface normalized to bone surface (MS/BS) (k) of endocortical and trabecular bones of proximal tibias from 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Data are mean ± SD, n = 6. l Serum PINP levels in 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Data are mean ± SD, WT, n = 6, KO, n = 8. m-o Histomorphometric analysis of the images from (c) for eroded surface per bone surface (ES/BS) (m), number of osteoclasts per bone perimeter (N.Oc/B.Pm) (n) and osteoclast surface per bone surface (Oc.S/BS) (o). Data are mean ± SD, WT, n = 4, KO, n = 3. p Serum CTX-I levels in 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Data are mean ± SD, WT, n = 6, KO, n = 8. q Tartrate-resistant acid phosphatase (TRAP) staining of femurs isolated from 6-week-old male WT and Prx1Cre, Piezo1fl/fl mice. Scale bar = 50 μm. *P < 0.05; **P < 0.01. Two-tailed Student’s t test. Source data are provided in the Source Data File.

Osteocytes, bone cells embedded in the bone matrix, are thought to be critical mechanosensory cells in bone. Dmp1Cre; Piezo1fl/fl mice, which deleted PIEZO1 in osteocytes, exhibited decreased bone mass in both trabecular and cortical bones, compared with control mice (Supplementary Fig. 6a–g). In addition, osteoclast number was also increased in Dmp1Cre; Piezo1fl/fl mice (Supplementary Fig. 6h, i). However, different from Prx1Cre; Piezo1fl/fl mice, no spontaneous fractures occurred in Dmp1Cre; Piezo1fl/fl mice (Supplementary Fig. 6j), indicating that not only osteocytes but also earlier osteoblastic cells are essential for mechanical sensing in bone.

Osteoclastic Piezo1 deficiency did not affect bone mass. In order to test if PIEZO1 affects the osteoclast function, we crossed Piezo1fl/fl mice with CtskCre mice to delete Piezo1 in osteoclasts. CtskCre; Piezo1fl/fl mice exhibited normal bone mass (Fig. 3a–c) and unaffected bone resorption (Fig. 3d, e), compared with
control mice. These data indicate that PIEZO1 acts primarily in osteoblastic cells to orchestrate bone resorption by osteoclasts in a non-cell autonomous manner.

**PIEZO1 sensed the weight-bearing induced mechanical stress.** Although bone resorption was strongly increased in the long bones of Prx1Cre; Piezo1fl/fl mice, TRAP positive osteoclasts were not significantly changed in skull whole mounts of Prx1Cre; Piezo1fl/fl mice (Supplementary Fig. 7a). Moreover, TRAP positive osteoclasts in Prx1Cre; Piezo1fl/fl mice were only modestly increased at P0, but were more substantially expanded on postnatal day 3 (Supplementary Fig. 7b–e), consistent with the kinetics of postnatal weight bearing.

To further determine if PIEZO1 mediates skeletal mechanical loading, tail suspension experiments were performed to remove body weight-induced mechanical loading from hind limbs, mimicking the bone loss due to microgravity or disuse.24,25 As expected, tail suspension induced bone loss in the distal femurs of WT mice (Fig. 4a, left panel). Strikingly, Prx1Cre; Piezo1fl/fl mice did not demonstrate tail suspension induced bone loss (Fig. 4a–f). Consistently, tail suspension treatment increased TRAP positive cells in WT but not in Prx1Cre; Piezo1fl/fl mice (Fig. 4g, h). These data support that PIEZO1 in osteoblastic cells is required to sense mechanical loading and accordingly regulate bone mass by modulating the bone resorption activity of osteoclasts.

**Collagens mediated inhibition of bone resorption by PIEZO1.** We next investigated the molecular mechanisms through which Piezo1 deficiency in osteoblast lineage cells controls bone homeostasis. BMSCs-derived osteoblasts from Prx1Cre; Piezo1fl/fl mice showed an enhanced ability to support osteoclastogenesis, as indicated by the increased number of giant multinucleated cells (Fig. 5a) and TRAP activity of culture supernatants (Fig. 5b) in an osteoblast–osteoclast co-culture system. To determine whether osteoclasts are more active or whether there are more osteoclasts with similar activity, we performed pit resorption assay in osteoblast–osteoclast co-culture system. Osteoclasts in the Piezo1-deficient osteoblastic cells co-culture system are increased in number and more active in resorption, compared with control osteoblastic cells (Fig. 5c, d). These data demonstrate that increased bone resorption in Piezo1-deficient mice results from both increased osteoclast number and activity.

We next screened the key factors secreted by osteoblasts to tune the activity of osteoclasts, including RankL, Opg, Sost, Pthlh, Mcsf and Sema3a.26 However, none of these factors showed a significant difference in the cortical bones of WT and Prx1Cre; Piezo1fl/fl mice (Fig. 5e). Therefore, we performed RNA-Seq using RNA from tibial and femoral cortical bones of WT and Prx1Cre; Piezo1fl/fl mice. As shown in Supplementary Figure 8a, the global transcriptome was changed between wild-type and Piezo1-deficient mice. Among a total of 19,201 genes expressed, 560 genes were up-regulated, and 297 genes were down-regulated (fold change > 1.5, P value < 0.05) in Piezo1-depleted cortical bones. We performed GO analysis and found that the genes of proteinaceous extracellular matrix were significantly down-regulated (Supplementary Fig. 8b). Among these matrix proteins, COL2a1, COL9a1/2 and COL10a1 were decreased substantially in Prx1Cre; Piezo1fl/fl mice (Supplemental Fig. 8c). However, there was no significant difference in type 1 collagen. QPCR further validated the changes of these genes in the cortical bones of WT and Prx1Cre; Piezo1fl/fl mice (Fig. 5f). Immunofluorescence analysis confirmed the changes of protein levels of different collagens including COL2a1 (Fig. 5g) and COL9a2 (Fig. 5h). These data indicate that Piezo1 deficiency changes the integrity of the bone matrix, especially of multiple collagens.

To directly examine if PIEZO1 acts as downstream of mechanical stimulation to regulate collagen expression, BMSCs-derived osteoblasts from Prx1Cre; Piezo1fl/fl and WT mice were cultured in the Flexcell Compression System. The expression...
Piezo1 deficiency resisted to bone loss induced by unloading. a 3D μ-CT images of trabecular bones of distal femurs isolated from male WT and Piezo1fl/fl mice, subjected with tail-suspended for one week trial or ground control, respectively. Scale bar = 50 μm. b-f μ-CT analysis of distal femurs for bone mineral density (BMD) (b), bone volume per tissue volume (BV/TV) (c), trabecular number (Tb.N) (d), trabecular thickness (Tb.Th) (e) and trabecular spacing (Tb.Sp) (f). Data are mean ± SD, n ≥ 5. g TRAP staining of the femurs isolated from male WT and Piezo1fl/fl mice, subjected with tail-suspended trial or ground control, respectively. Scale bar = 50 μm. h Quantification of the TRAP staining by osteoclast surface per bone surface (Oc.S/BS). Data are mean ± SD, n = 3. *P < 0.05; **P < 0.01. Ordinary one-way ANOVA. Source data are provided in the Source Data File.

PIEZO1 promoted Col2α1 and Col9α2 expression through YAP. We then investigated how PIEZO1-linked mechanical signaling could modulate bone remodeling in osteoclast lineage cells thereby affecting osteoclastogenesis.

Inducible osteoblastic Piezo1 deletion decreased bone mass. To mimicking sudden loss of gravity or weight-bearing in a space flight or long-term bedridden, we utilized tamoxifen-inducible osteoblast lineage cells expressing Cre, Col1creert2 to induce Piezo1 deletion at 8-week-old for 2 weeks (Fig. 7a). To determine the in vivo effects of Piezo1 in this system, we performed μ-QCT analysis and found that trabecular bone mass was significantly reduced in Col1creert2; Piezo1fl/fl mice compared to WT controls (Fig. 7b), as confirmed by decreased trabecular bone volume (BV/TV, Fig. 7c), trabecular number (Tb.N, Fig. 7d), trabecular thickness (Tb.Th, Fig. 7e) and increased trabecular spacing (Tb.Sp, Fig. 7f). Also, cortical thickness (Ct.Th.) was decreased in Col1creert2; Piezo1fl/fl mice (Fig. 7b, g). Consistent with our previous findings in Prx1Cre, Piezo1fl/fl mice, TRAP positive osteoclasts were increased in Col1creert2; Piezo1fl/fl mice (Fig. 7h, i). Also, collagen expression was compromised in this sudden Piezo1-loss model (Fig. 7j). In conclusion, our data demonstrate that sudden loss of the mechanosensor Piezo1 in osteoblast lineage cells can modulate bone remodeling and mechanotransduction by PIEZO1 is essential for maintaining the bone homeostasis.

Discussion
Our current study found that PIEZO1 acts in osteoblast lineage cells as a regulator of bone remodeling via sensing mechanical loading. First, Piezo1-deficient osteoblastic cells promote bone
resorption by osteoclasts, accounting for the decreased bone mass of Piezo1-deficient mice. This phenotype is similar to the one of mechanical unloading with the increased bone resorption29. Secondly, our mechanism study demonstrates that PIEZO1 regulates YAP expression that in turn regulates bone resorption28. Type II collagen is encoded by COL2 gene, and synthesized as a pro-collagen form with extension pro-peptides (NH2-pro-peptide and COOH-pro-peptide) that are removed by specific proteinases before the mature molecules are incorporated into fibrils in matrix34. The NH2-pro-peptide of type II collagen (PIIBNP) putatively acts on bone to bone homeostasis5,6. Interestingly, an atlas of genetic in SNPs of PIEZO1 associated with osteoporosis and fractures35, indicating that PIEZO1 could also regulate bone mass and bone strength in humans in vivo.

Our study found that PIEZO1 regulates YAP-dependent expression of collagens that in turn regulate bone resorption. Type II collagen is encoded by Col2a1 gene, and synthesized as a pro-collagen form with extension pro-peptides (NH2-pro-peptide and COOH-pro-peptide) that are removed by specific proteinases before the mature molecules are incorporated into fibrils in matrix34. The NH2-pro-peptide of type II collagen (PIIBNP) putatively acts on bone to bone homeostasis5,6. Interestingly, an atlas of genetic in SNPs of PIEZO1 associated with osteoporosis and fractures35, indicating that PIEZO1 could also regulate bone mass and bone strength in humans in vivo.
osteolects responding to these type II and IX collagen ‘ligands’ remain to be defined. Integrins are heterodimeric adhesion receptors that mediate cell–matrix and cell–cell interactions. Integrin αvβ3 and α2β1 are two integrin receptors highly expressed on osteoclasts and can respond to RGD-containing ligands in the bone matrix to regulate osteoclast differentiation and function. We proposed that integrin could mediate the repression of collagen on osteoclastogenesis. To test this hypothesis, we enriched the COL2α1 and COL9α2 proteins from the supernatants of Col2α1 and Col9α2 overexpressing 293T cells and treated the bone marrow monocytes during osteoclast differentiation.

Recent discoveries are converging on a model in which multiple types of mechanical inputs in a variety of cellular settings rely on the regulation of two transcriptional regulators, YAP and TAZ. YAP/TAZ activity is regulated by the conformation and tension of the F-actin cytoskeleton, which in turn depends primarily on the substrate to which cells adhere. In addition, YAP nucleo–cytoplasmic localization is influenced by PIEZO1 in human neural stem/progenitor cells. Combinatorial YAP/TAZ deletion from skeletal lineage cells caused an osteogenesis imperfecta-like phenotype through regulation of osteoblast activity, matrix quality, and osteoclastic remodeling. In our study, decreased YAP nuclear localization was observed in Piezo1-deficient mice, resulting in impaired collagen II and IX expression which leads to increased bone resorption. YAP/TAZ and PIEZO1 serve as downstream effectors of Wnt5a-mediated mechanotransduction-mediated mediated bone remodeling.

The skeletal system provides the mechanical framework for the whole body. The reduction of bone mass due to aging or hormone related osteoporosis could be treated by different strategies including anabolic treatments to increase bone formation and catabolic treatments to decrease bone resorption. Indeed, astronauts typically lose more bone mass during one month than postmenopausal women on Earth lose in 1 year, at approximately 1–2% per month, with an even greater decrease in bone strength. However, bone loss due to microgravity or the absence of mechanical forces cannot be effectively suppressed, indicating that the molecular mechanisms underlying bone loss from...
Fig. 7 Sudden loss of Piezo1 in osteoblast lineage cells decreased the bone mass. a Scheme of the experiment: male WT and Col1Creert2, Piezo1fl/fl mice were injected with tamoxifen (0.1g/kg) at 8-week-old and sacrificed for analysis at 10-week-old. b 3D µ-CT images of trabecular bones of distal femurs. c-g µ-CT analysis of distal femurs for bone volume per tissue volume (BV/TV) (c), trabecular number (Tb.N) (d), trabecular thickness (Tb.Th) (e), trabecular spacing (Tb.Sp) (f) and cortical thickness (Ct.Th) of middle shaft of femurs (g). Data are mean ± SD, WT, n = 3, CKO, n = 4. h TRAP staining of the femurs isolated from 10-week-old male WT and Col1Creert2, Piezo1fl/fl mice. Scale bar = 50 μm. i Quantification of the TRAP staining by osteoclast surface per bone surface (Oc.S/BS). Data are mean ± SD, n = 3. j Immunofluorescence assay of COL2α1 of 10-week-old male WT and Col1Creert2, Piezo1fl/fl mice. Scale bar = 50 μm. *P < 0.05; **P < 0.01. Two-tailed Student’s t test. Source data are provided in the Source Data File.

Histological staining. Tissues were fixed in 4% paraformaldehyde for 48 h and incubated in 15% DEPC-EDTA (pH 7.8) for decalcification. Then specimens were embedded in paraffin and sectioned at 6 μm. Tissue sections were used for TRAP staining according to the manufacturer’s instructions (Sigma, 387A-1KT). Images were captured using a microscope (Olympus BX51, Tokyo, Japan).

Immunofluorescence. Freshly dissected bones were fixed in 4% paraformaldehyde for 48 h and incubated in 15% DEPC-EDTA (pH 7.8) for decalcification. Then specimens were embedded in paraffin and sectioned at 6 μm. Sections were blocked in PBS with 10% horse serum for 1 h and then stained overnight with rabbit-anti-Collagen IX alpha 2, rabbit-anti-Collagen II alpha 1 or rabbit-anti-YAP. Donkey-anti-rabbit Cy3 was used as secondary antibody. DAPI (sigma, D8417) was used for counterstaining. For cellular immunofluorescence studies, the indicated cells were seeded on glass slides and then fixed with 4% paraformaldehyde for 20 min. Subsequent steps were identical to those used for tissue immunofluorescence. Slides were mounted with anti-fade fluorescence mounting medium (Dako, S3023) and images were acquired with microscope (Olympus BX51, Tokyo, Japan) or confocal microscope (SP8, Leica).

Calcein-Alizarin red S labeling. Mice were injected intraperitoneally with 20 mg/kg Calcein (1 mg/ml in 2% NaHCO3 solution) and 40 mg/kg Alizarin red S (2 mg/ml in H2O) on day 0 and day 4 separately. On day 7, the mice were sacrificed and the bones were fixed and dehydrated and embedded with Embed-812 (Electron Microscopy Sciences). The samples were sectioned as 5 μm with hard tissue cutter. Consecutive sections were stained with silver nitrate to measure the bone morphology. The histomorphometric analysis was carried out semi-automatically with the OsteoMeasure image analyzer (OsteoMetric) according to recommended guidelines.

Micro-quantitative computed tomography analysis. The mouse femurs were skinned and fixed in 70% ethanol. Scanning was performed with the instrument micro CT system SkyScan1276 (Bruker, Karztuerwexweg, Belgium) at a 9 μm resolution for quantitative analysis. The region from −40 to −240 slides below the growth plate was analyzed for trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), bone volume per tissue volume (BV/TV) and bone mineral density (BMD) with CTAn. The region from −400 to −456 slides below
the growth plate was analyzed for cortical thickness (CL.Th) with CT.An. Three-dimensional reconstructions were created by stacking the two-dimensional images from the indicated regions with CT Vox.

**RNA-seq and analysis.** Raw reads were mapped to mm9 using the TopHat version 1.4.1 program. We assigned FPKM (fragment per kilo base per million) as an expression value for each gene using Cufflinks version 1.3.0 software. The Cuffdiff software was used to identify differentially expressed genes between treatment and control groups. Differentially expressed genes were clustered by k-means clustering using the Euclidean distance as the distance and visualized using the Java TreeView software. The data were deposited into the GEO repository (GSE135328).

**Skeletal preparation and staining.** Mice were sacrificed by CO2 and then removed skin and muscle. The remaining samples were transferred into acetone for 48 h, then fixed with formaldehyde in 95% ethanol. Specimens were then transferred into 10% Alcian blue (Sigma, A5268) and 0.1% Alizarin red S (Sigma, A5533) solution (70% ethanol, 5% acetic acid)\(^{22}\). Specimens were incubated in 1% KOH until tissue was completely cleared and then kept in glycerol for pictures by Canon Camera.

**Calcium imaging.** Cells, isolated from WT and Pieszol-deficient mice, were seeded into 24-well cell slides (NEST), and incubated overnight at 37 °C and 5% CO2. For Ca\(^{2+}\) imaging, these cells were loaded with Fluo-4 AM ester (2 μM, Thermo Fisher Scientific) for 30 min, washed with HBSS (ThermoFisher, 14025076) for 3 times, and then rest for 15 min to allow dye-de-esterification\(^{23}\). Then, the cells were exposed to Yoda1 (10 μM) using a flow chamber. The fluorescence intensity of Fluo-4 AM was monitored by live-cell imaging (UltraView ULS-Auto; PerkinElmer). Time-lapse fluorescence intensity measurements were analyzed using Image J.

**Flow cytometry.** Bone marrow cells were extracted from femurs and tibias of three WT and six CKO mice at 6-week. Marrow plugs were flushed with a 1-ml syringe using ice-cold DPBS (Corning, 21-031-CMR) containing 2% fetal bovine serum (FBS). These plugs were then dispelled into single cell and the red blood cells were removed by RBC lysis buffer for 5 min (Beyotime, C3702). Wash the cells twice by using ice-cold DPBS (Corning, 21031-CMR) containing 2% fetal bovine serum. Table 1. Primers used for real-time reverse transcription RT-PCR reaction was performed with the Bio-Rad CFX System. The primer sets used were listed: mBglap-qF: GCACCGACAGTCTCAGAATAG; mBglap-qR: GGGCCAATAAGTGAAGCAG; mPhl-h: CTACAGCTCATGCTAGA CAAAG; mPhl-h: GTGGTTTTTT GTGGTTGGAAG; mMCSF-qF: GCTTGGTCGGTTGTAG; mMCSF-qR: GAGGAGTCGGAGCTACC; mSema3a-qF: AACAGAGCTTATGATGGC; mSema3a-qR: CGATAGGAAGACGCTCAGG; mPiezo1-qF: CTTCACGTTGCTTGTGG; mPiezo1-qR: CACTCATGATGAGCCGGAAACT; mPiet-2-qF: GCTTGGTGCAATGGTGTAC; mPiet-2-qR: CTCTCTGTCAGCTTAC; mACACCAAGAGTTCGACCATG; mACACCAAGAGTTCGACCATG; mAOC2a1-qF: AACGGGAGAGGAAGGACGG; mAOC2a1-qR: GCCGGACTG GTACTCGGATAA; mCol1α1-qF: CGGGACTG GTACTCGGATAA; mCol1α1-qR: GCTCACGTCTCGTTTTCATC. The real-time reverse transcription RT-PCR reaction was performed according to the manufacturer’s protocol. A definite condition of 1% intensity, 0.5 Hz, 4 h was applied to the cells to simulate moderate forces. In parallel as a control, cells were cultured on compression plates with the same conditions but without mechanical stress.

**Cell differentiation.** BMSCs were extracted from femurs and tibias of 4- to 6-week-old mice. Marrow plugs were flushed with a 1-ml syringe using ice-cold DPBS (Corning, 21-031-CMR) (supplemented with 10% FBS, 1% Penicillin-Streptomycin). These plugs were then dispelled into 10-dish with a 1-cm diameter of a dish (Corning, 10-022-CVR) (supplemented with 10% FBS, 1% Penicillin-Streptomycin). The non-adherent cells were used for BMSCs-derived osteoblastic cells.

For osteoblast differentiation, cells were cultured on a biomimetic synthetic surface was treated with 10% NaClO solution after the co-culture. Resorption area was quantified using Image J.

**Mechanical compression devices.** For mechanical compression system, BMSCs-derived osteoblastic cells were seeded into hydrogel (2 × 10^6 cells per well) with a control sample. Differentially expressed gene heat maps were clustered by k-means using the Java TreeView software. The data were deposited into the GEO repository (GEO: GSE135328).

**Real-time RT-PCR analysis.** Total RNA was prepared using TRIzol (Sigma, T9424) and then was reverse transcribed into cDNA with the PrimeScript RT Reagent Kit (Takara, PR037A). The real-time reverse transcription RT-PCR reaction was performed according to the manufacturer’s instructions. Each group, unless otherwise stated. The data are presented as mean ± SD. Two-tailed Student’s t test was used to compare the effects of two groups, and ordinary one-way ANOVA was used to compare the effects of more than two groups. Statistical significant differences are indicated as follows: * for p < 0.05 and ** for p < 0.01.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Antibodies.** Antibody (492) was purchased from CST. Rabbit polyclonal to PIEZO1 (ab128245) and rabbit polyclonal to Collagen II alpha 1 (ab34712) antibodies were purchased from Abcam. Collagen IX alpha 2 antibody (NB2-30450) was purchased from Novus Biologicals.

**Statistics.** Statistical analysis was performed using the GraphPad Prism 8 software (GraphPad Software). Cell-based experiments were performed at least twice. Animals were randomized into different groups and at least 3 mice were used for each group, unless otherwise stated. The data are presented as mean ± SD. Two-tailed Student’s t test was used to compare the effects of two groups, and ordinary one-way ANOVA was used to compare the effects of more than two groups.
Data availability
Source data for Figs. 1–7 and Supplementary Figure 1, 2, 5, 6, 7, 9, 10 have been provided in the Source Data File. The RNA sequencing data have been deposited in the Gene Expression Omnibus (GEO) under accession GSE135282. All data and genetic material used for this paper are available from the authors on request.

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Author contributions
L.W. and W.Z. conceived the study and wrote the manuscript. L.W. and X.Y. designed and performed experiments about the phenotype analysis of different mice and analyzed data. S.L. performed and analyzed the histomorphometry of Prx1Cre; Piezo1fl/fl mice. L.Z. performed the compression system. N.W. and W.Z. contributed ideas and reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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