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The mechano-sensitive ion channel Piezo1 controls the cell fate determination of bone marrow mesenchymal stem cells

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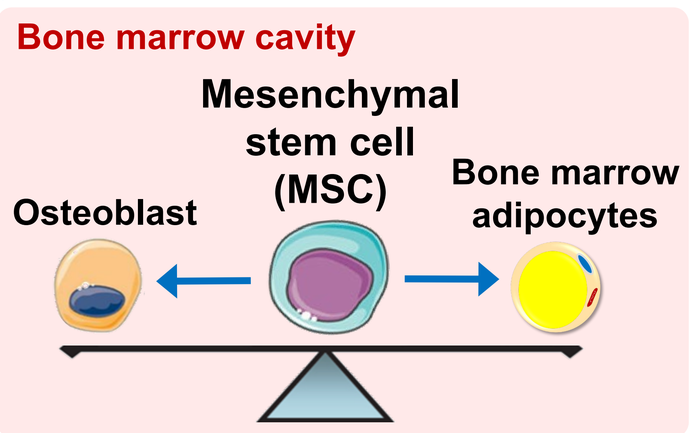
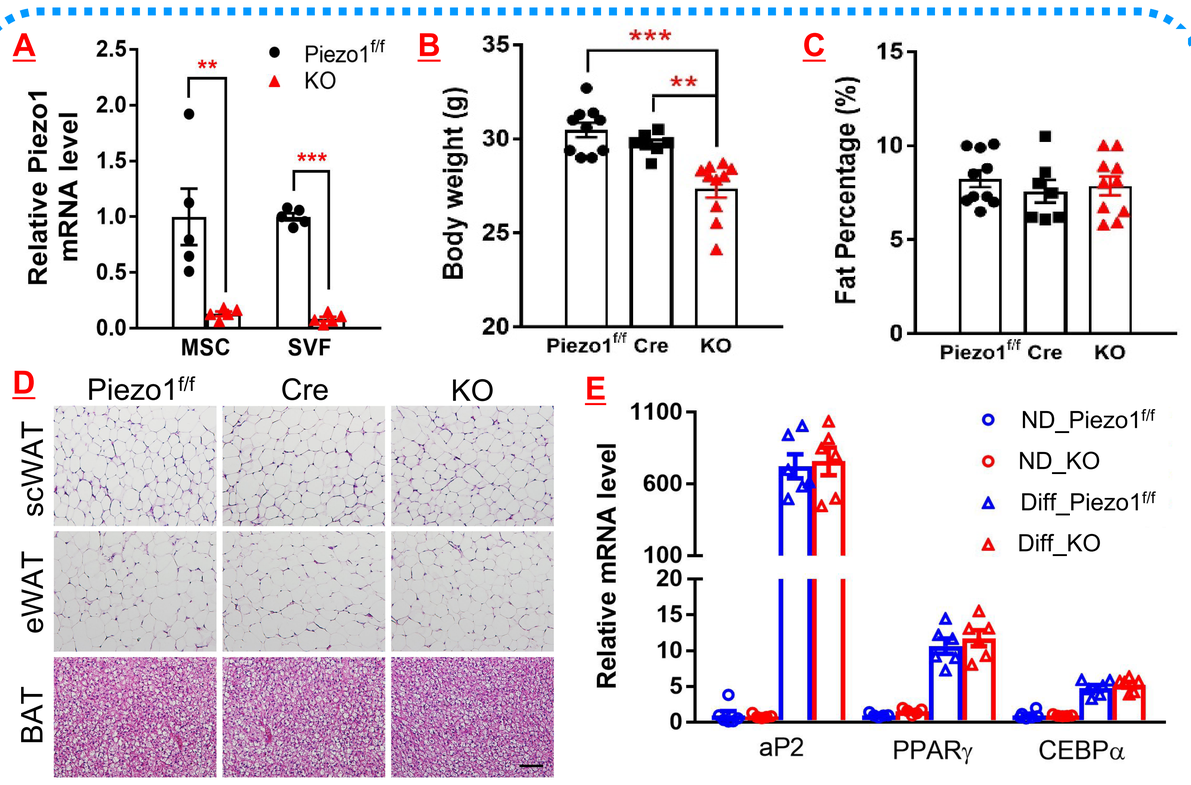
Introduction



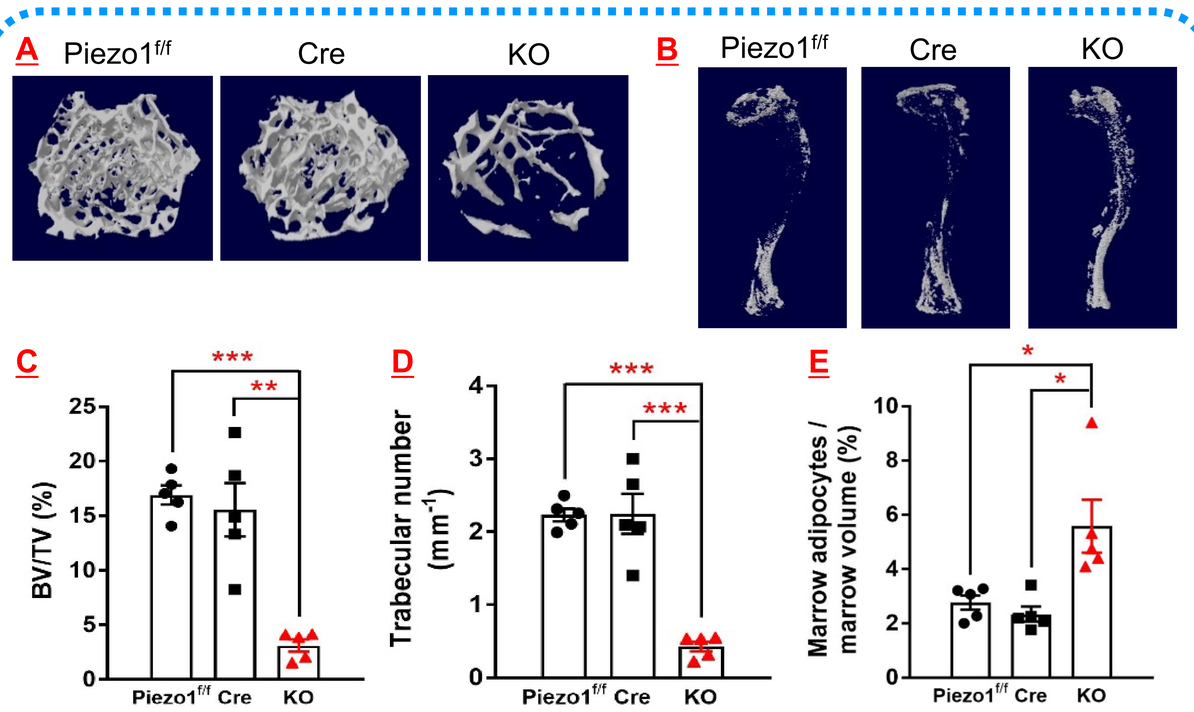
Methods and Results

Fig.1 PDGFRα-Piezo1 knockout (KO) mice have lower body weight but normal peripheral adipose tissues.



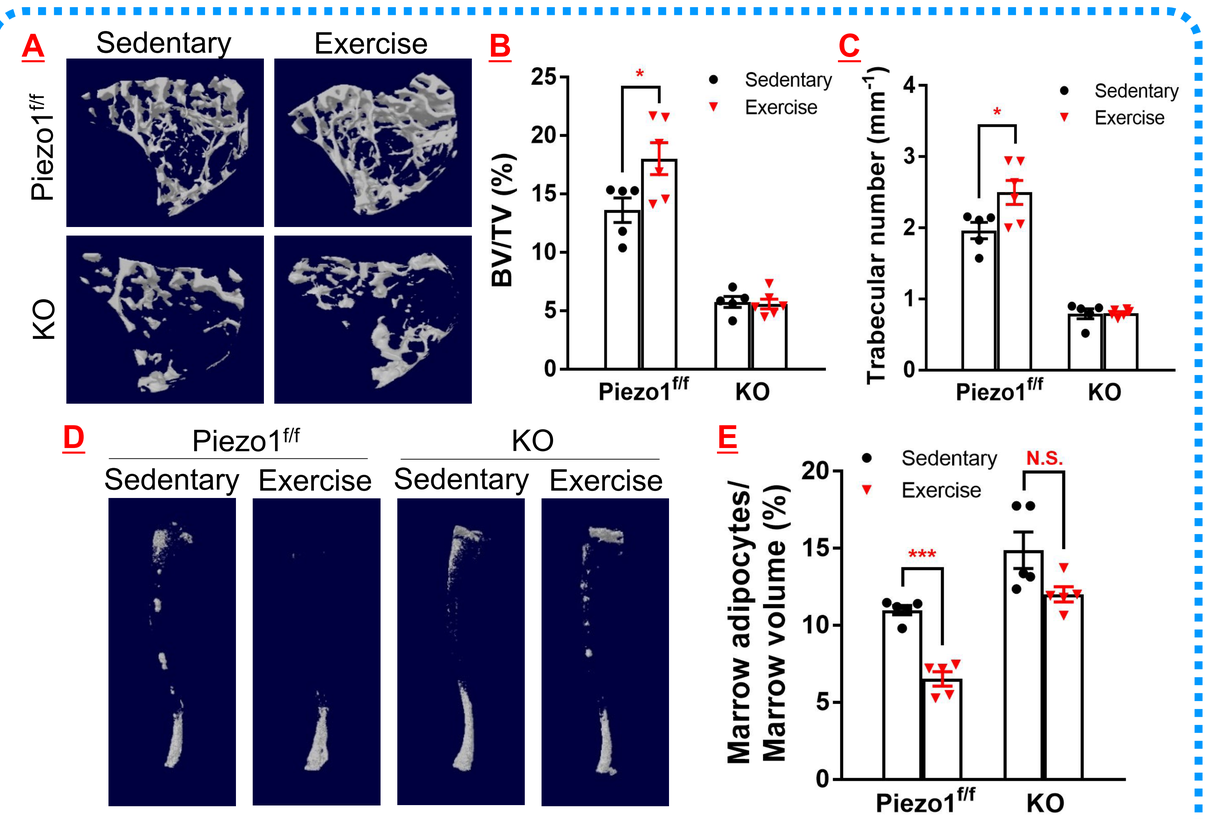
Piezo1-floxed mice (Piezo1$^{f/f}$) were crossed with PDGFRα-cre mice in C57BL/6J background for at least eight generations to generate PDGFRα-Piezo1 knockout (KO) mice. (A) Body weight, (B) body length of 18-week-old male PDGFRα-Piezo1 KO mice, Piezo1f/f and PDGFRα-Cre mice (both were used as wild-type controls). (C) Fat percentage was quantified by a NMR body composition analyzer. (D) H&E staining of scWAT, eWAT and BAT. (E) Stromal vascular fractions were isolated from 8-week-old PDGFRα-Piezo1 KO mice and Piezo1$^{f/f}$ control mice, followed by *in vitro* adipocyte differentiation. Gene expression of adipocyte markers was measured. Data are presented as mean ± SEM, ** p<0.01, ***p<0.001. n=6-10 in each group. Scale bar, 200 μm.

Fig.2 PDGFRα-Piezo1 KO mice exhibit markedly reduced bone volume, but increased bone marrow adipocytes.



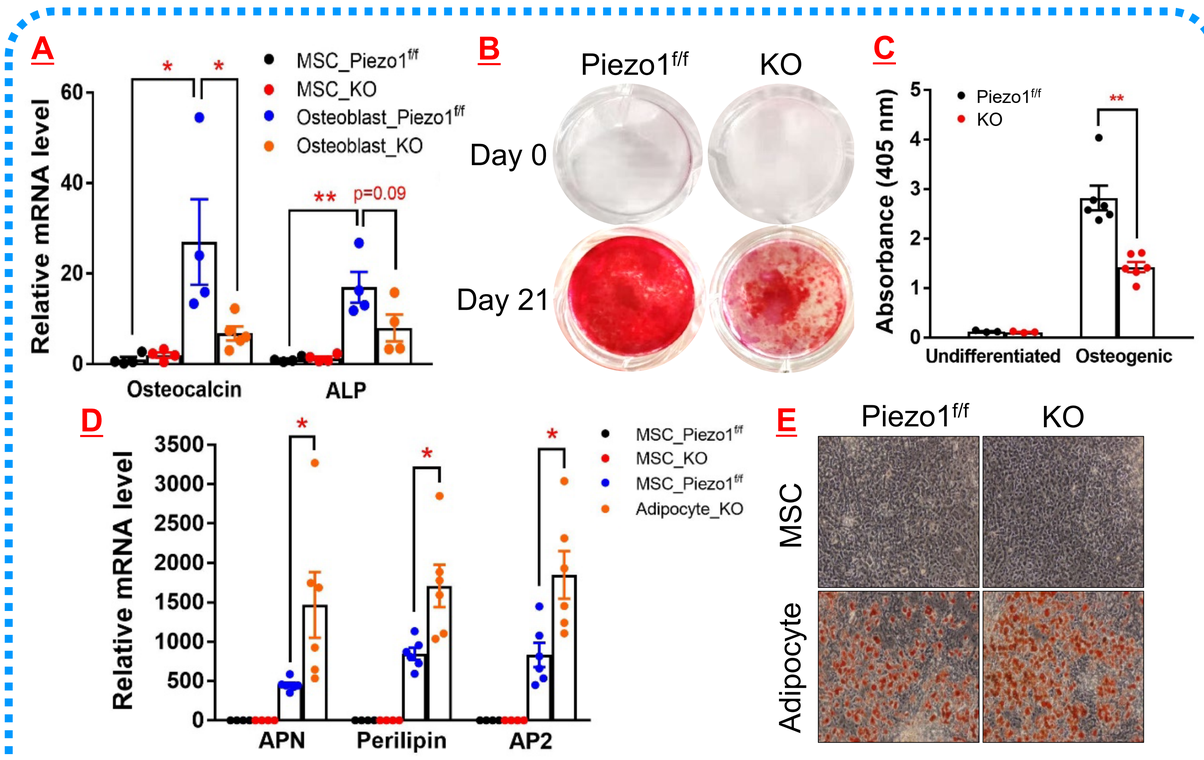
Femurs and tibias were collected from PDGFRα-Piezo1 KO mice and Piezo1 floxed mice. (A) Representative images showing three-dimensional trabecular architecture by micro-CT reconstruction at the distal femurs. (B) Representative images showing osmium-stained decalcified tibia scanned with micro-CT. (C-D) Micro-CT measurements for bone volume (BV/TV, C) and trabecular number (D) at the distal femurs. (E) Quantification of bone marrow adipocytes in tibia normalized to marrow volume. Data are presented as mean ± SEM, *p<0.05, **p<0.01, ***p<0.001. n=5 in each group.

Fig.3 Exercise-induced bone formation and loss of bone marrow adipocytes are impaired in PDGFRα-Piezo1 KO mice.



8-week-old PDGFRα-Piezo1 KO mice and Piezo1 floxed mice were subjected to treadmill exercise for 6 weeks (5 days per week, 30 min per day, at a speed of 15m/min). Another two age-matched groups without exercise were used as sedentary controls. (A) Representative images showing three-dimensional trabecular architecture and cortical bone by micro-CT reconstruction at the proximal tibias. (B-C) Micro-CT measurements for bone volume (BV/TV, B) and trabecular number (C) at the proximal tibias. (D) Representative images showing osmium-stained decalcified tibia scanned with micro-CT. (E) The quantification of bone marrow adipocytes in tibia normalized to marrow volume. Data are presented as mean ± SEM, *p<0.05, ***p<0.001. n=5-6 in each group.

Fig.4 Piezo1-deficient MSCs preferentially differentiate into bone marrow adipocytes rather than osteoblasts.



Bone marrow mesenchymal stem cells (MSCs) were isolated from femurs and tibias from 10-week-old male PDGFRα-Piezo1 KO mice and their Piezo1f/f controls. Isolated MSCs were then differentiated into osteoblasts or adipocytes by adding osteoblastogenesis- or adipogenesis-induction cocktail, respectively. (A) Real-time PCR analysis for the mRNA levels of osteoblast markers (Osteocalcin and ALP) at 21-day after osteogenic differentiation. (B) Alizarin red staining of osteoblasts at Day 0 and Day 21 after differentiation. (C) Quantitative analysis of alizarin red staining by determining OD405 absorbance values. (D) Real-time PCR analysis of adipocyte marker genes (Adiponectin (APN), Perilipin and AP2) after 8-day differentiation. (E) Oil red O staining of MSCs-derived adipocytes after 8-day differentiation and their undifferentiated controls. Data are presented as mean ± SEM, *p<0.05, **p<0.01. n=4-6.

Conclusion

Piezo1 is a critical controller that directs the differentiation of bone marrow mesenchymal stem cells into osteoblasts.

Acknowledgement

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