Mechanosensitive Ion Channel Piezo1 Regulates Osteoclast Differentiation by Activating the Metalloproteinases ADAM10 and ADAM17

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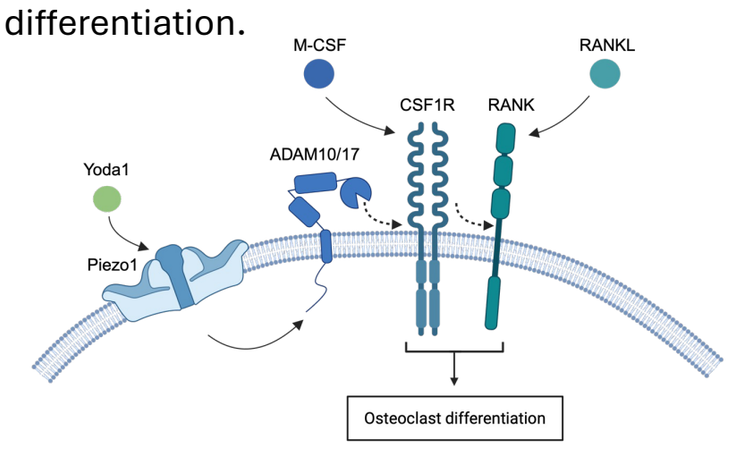
BACKGROUND

Bone adapts to mechanical loading through continuous remodeling, as described by Wolff's law. While mechanosensation in osteoblasts and osteocytes is well characterized, its role in osteoclasts remains poorly understood. Emerging evidence implicates Piezo1 in osteoclast mechanotransduction, but the underlying signaling pathways are largely unknown. In other cellular systems, Piezo1 activation has been shown to activate the metalloproteinases ADAM10 and ADAM17, which mediate the shedding of numerous membrane-bound signaling molecules. Whether a similar Piezo1–ADAM axis exists in osteoclasts and contributes to bone remodeling remains unknown.

AIM

→ To investigate the role of Piezo1-mediated ADAM10/17 activation in osteoclast differentiation.



RESULTS

Piezo1-mediated ADAM activation regulates osteoclast differentiation in RAW264.7 cells

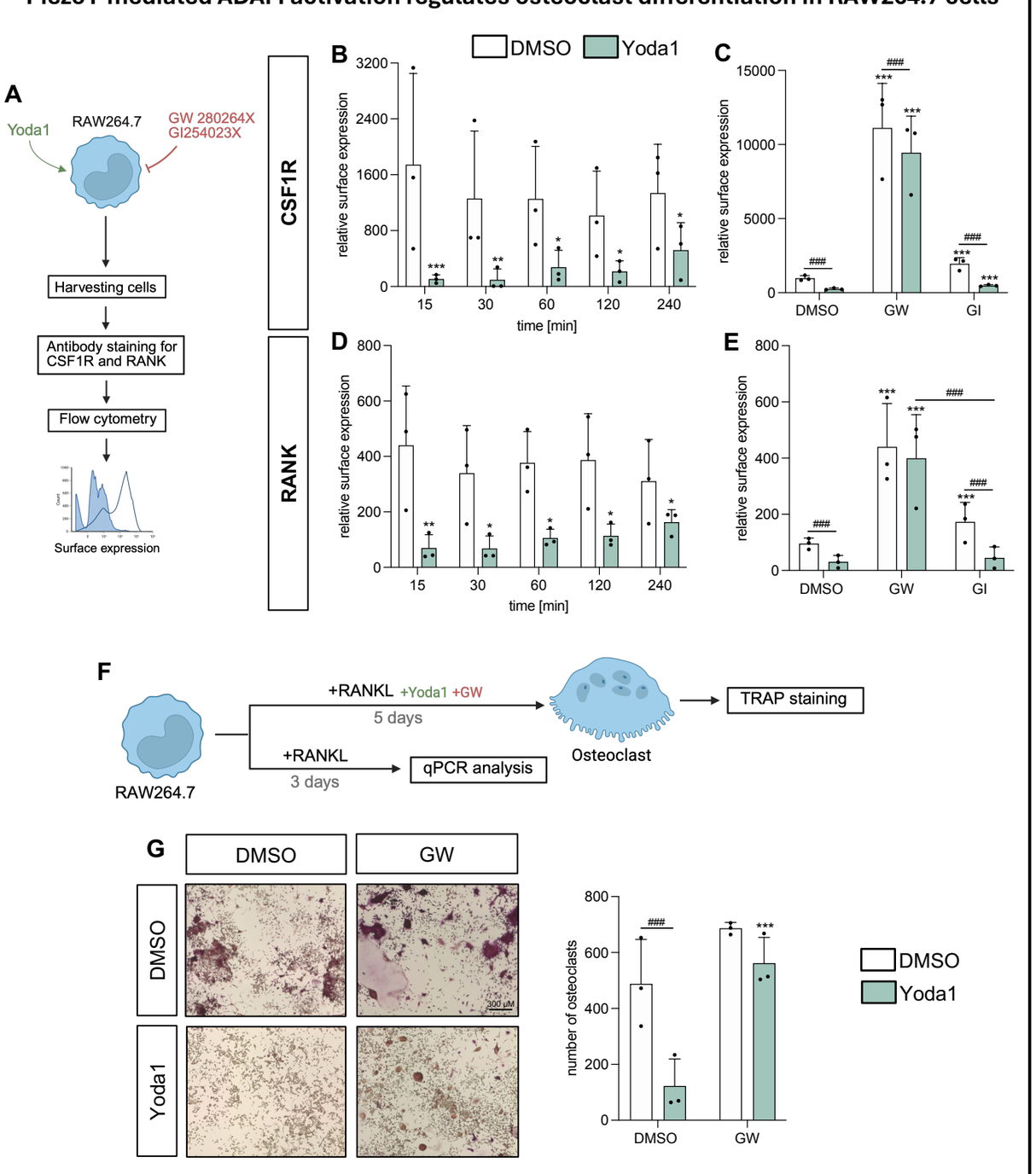


Figure 1: Piezo1-mediated ADAM activation regulates osteoclast differentiation in RAW264.7 cells. (A) Experimental workflow for the analysis of CSF1R and RANK surface expression in RAW264.7 cells following Piezo1 activation with Yoda1 (3 µM) and inhibition of ADAM10/17 using GW 280264X or ADAM10 using GI254023X (10 µM each). (B–E) Time-dependent changes in CSF1R (B, C) and RANK (D, E) surface expression upon Piezo1 activation and ADAM10/17 inhibition, assessed by flow cytometry. (F) Experimental design of osteoclast differentiation assays in RAW264.7 cells treated with RANKL (100 ng/ml) , Yoda1 (3 µM) , and GW 280264X (5 µM) for 5 days. (G) Representative TRAP staining images and quantification of osteoclast formation. (H–J) Relative mRNA expression of *Adam10*, *Adam17*, and *Rhbdf2* in control and RANKL-stimulated cells after 3 days.

ADAM17 deficiency rescues Piezo1-impaired osteoclast differentiation in BMDMCs

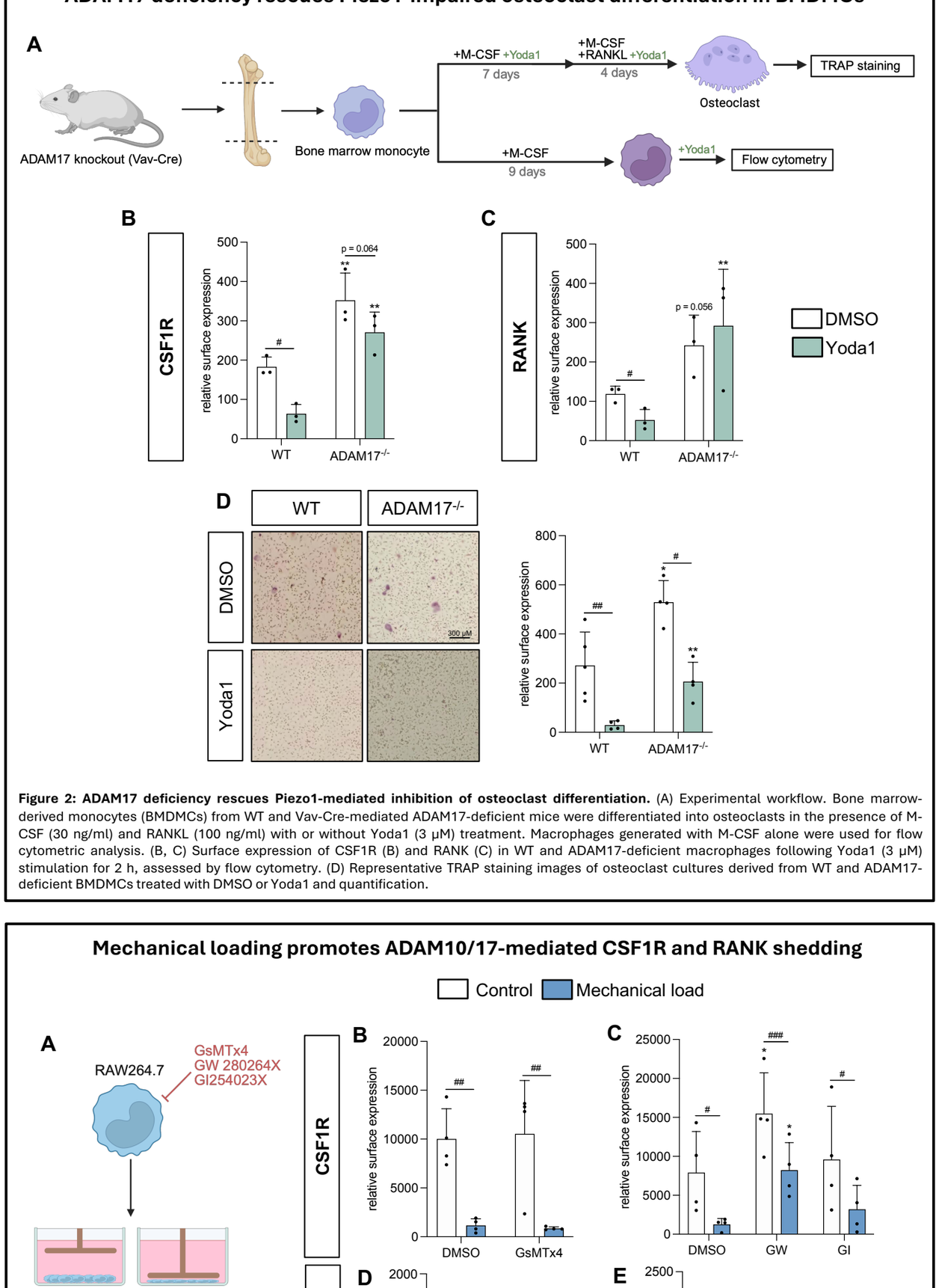


Figure 2: ADAM17 deficiency rescues Piezo1-mediated inhibition of osteoclast differentiation. (A) Experimental workflow. Bone marrow-derived monocytes (BMDMCs) from WT and Vav-Cre-mediated ADAM17-deficient mice were differentiated into osteoclasts in the presence of M-CSF (30 ng/ml) and RANKL (100 ng/ml) with or without Yoda1 (3 µM) treatment. Macrophages generated with M-CSF alone were used for flow cytometric analysis. (B, C) Surface expression of CSF1R (B) and RANK (C) in WT and ADAM17-deficient macrophages following Yoda1 (3 µM) stimulation for 2 h, assessed by flow cytometry. (D) Representative TRAP staining images of osteoclast cultures derived from WT and ADAM17-deficient BMDMCs treated with DMSO or Yoda1 and quantification.

Mechanical loading promotes ADAM10/17-mediated CSF1R and RANK shedding

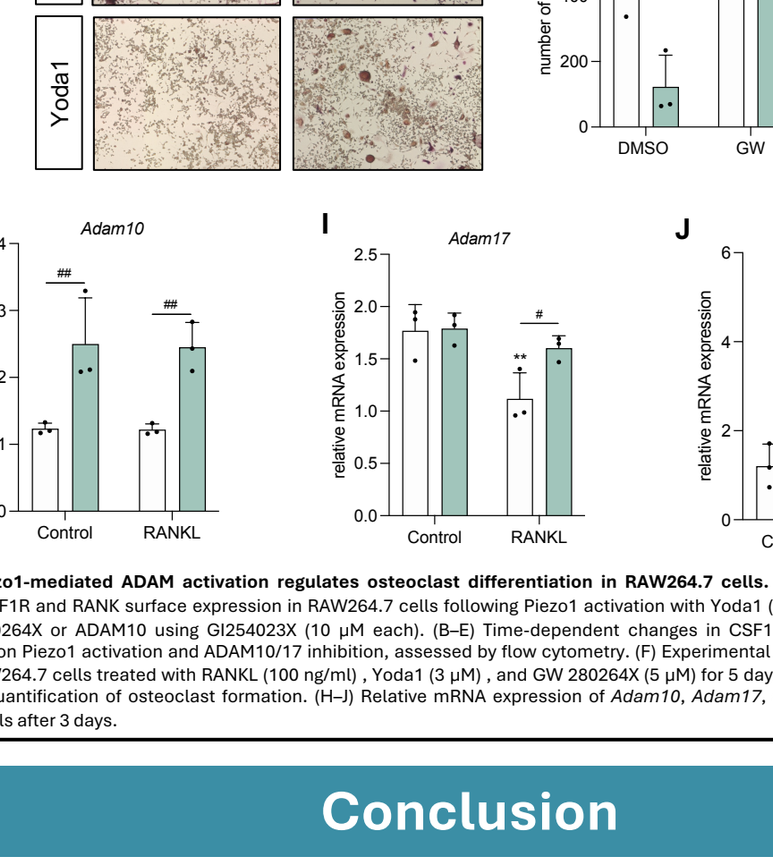
Figure 3: Mechanical loading promotes ADAM10/17-mediated CSF1R and RANK shedding. (A) Experimental workflow. RAW264.7 cells were subjected to 2 h of mechanical loading in the presence of vehicle (DMSO), the Piezo1 inhibitor GsMTx4 (10 µM), GW280264X (GW) or GI254023X (GI) (10 µM each), followed by flow cytometric analysis. (B, C) Surface expression of CSF1R following mechanical loading in the presence or absence of GsMTx4 (B) or GW and GI (C). (D, E) Surface expression of RANK following mechanical loading in the presence or absence of GsMTx4 (D) or GW and GI (E).

Conclusion

→ Piezo1 activates ADAM10 and ADAM17 in osteoclast precursors.

→ ADAM17-dependent shedding of CSF1R and RANK inhibits osteoclast differentiation.

→ Mechanical loading induces CSF1R and RANK shedding.



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