Major vault protein (MVP) negatively regulates osteoclastogenesis via calcineurin-NFATc1 pathway inhibition

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Supplemental Material and Figures

Supplemental Material

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Mvp	TCCCTCTGGACCAAAATGAG	CCTTTTCCCACAGGACTTCA
Nfatc1	CGAGTTCACATCCCACAG	GACAGCACCATCTTCTTCC
Pu.1	GTAGCGCAAGAGATTTATGCAAAC	CCGTTTCTTCTGCGCTCATAC
c-Fos	CACTCTGGTCTCCTCCGT	ATTCTCCGTTTCTCTTCCTC
Ctsk	CCCATCTCTGTGTCCATC	AGTGCTTGCTTCCCTTCT
Trap	CAGCAGCCAAGGAGGACTAC	ACATAGCCCACACCGTTCTC
Mmp9	TCACTTTCCCTTCACCTTC	ATTTGCCGTCCTTATCGT
Calcineurin	CTCCCAGTTCAGCGTCAA	ATCGCCATCCTTATCCAG

Table S1. List of primer sequences used for the qRT-PCR analysis

Supplementary Figures

Figure S1.



Figure S1. Global MVP deletion results in an osteoporosis phenotype in mice.

(A) 3D microstructural analysis of femurs in wide-type (WT) and $Mvp^{-/-}$ (KO) male mice aged 24-week-old. Scale bar: 500 µm. (B) Micro-CT analysis of heads in WT and KO male mice aged 3, 8, and 24 weeks. Scale bar: 1 mm. (C) H&E staining of femurs from 3-week-old WT and KO mice. Scale bar: 200 µm. (D) Quantification of H&E staining. BV/TV, bone volume/tissue volume. (E) Goldner's trichrome staining of femurs from WT and KO mice. Scale bar: 50µm. (F) Quantification of osteoblast numbers in Figure S1E; N.Ob/B.Pm, osteoblast number/bone perimeter. All experiments were repeated three times. **p < 0.01; ns, not significant, as determined by Student's t-test.

Figure S2.



Figure S2. Specific deletion of MVP in osteoclast precursors also induces an osteoporosis phenotype in mice.

(A) Immunohistochemical staining of MVP in femurs of 2-week-old Mvp^{ff} (f/f) and $Mvp^{ff}Lyz^2$ -*Cre* (f/f/ \triangle) mice. Scale bar: 50 µm. (B) Quantification of MVP positive cells per view. (C) Micro-CT analysis of heads in f/f and f/f/ \triangle male mice aged 3, 8, and 24 weeks. Scale bar: 1 mm. (D) Goldner's trichrome staining of femurs from f/f and f/f/ \triangle mice. Scale bar: 50 µm. (E) Quantification of osteoblast numbers in Figure S2D; N.Ob/B.Pm, osteoblast number/bone perimeter. All experiments were repeated three times. **p < 0.01; ns, not significant, as determined by Student's t-test.

Figure S3.



Figure S3. Expression of MVP overexpressing lentivirus.

(A) Green fluorescence intensity was observed 72 hours post-transfection with control or MVPoverexpressing lentiviruses. (B) Bright field of cells in Figure S3A. All experiments were repeated three times.

Figure S4.



Figure S4. MVP protects mice from pathologic bone loss.

(A) Body weight after ovariectomy (OVX) or sham surgery in female mice. (B) Green fluorescence intensity of calvarial sections injecting with PBS, AAV-GFP or AAV-Mvp. Scale bar: 100 μ m. (C) Quantitative analysis of calvaria in Figure 7D; TV/BV, tissue volume/bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation. (D) Quantification of TRAP staining in Figure 7F. N.Oc/B.Pm, osteoclast number/bone perimete. (E) Quantitative analysis of calvaria in Figure 7G. (F) Quantification of TRAP staining in Figure 7I. All experiments were repeated three times. *p < 0.05; **p < 0.01; ns, not significant, as determined by Student's t-test.