

Appendix

Glutaminolysis provides nucleotides and amino acids to regulate osteoclast differentiation in mice

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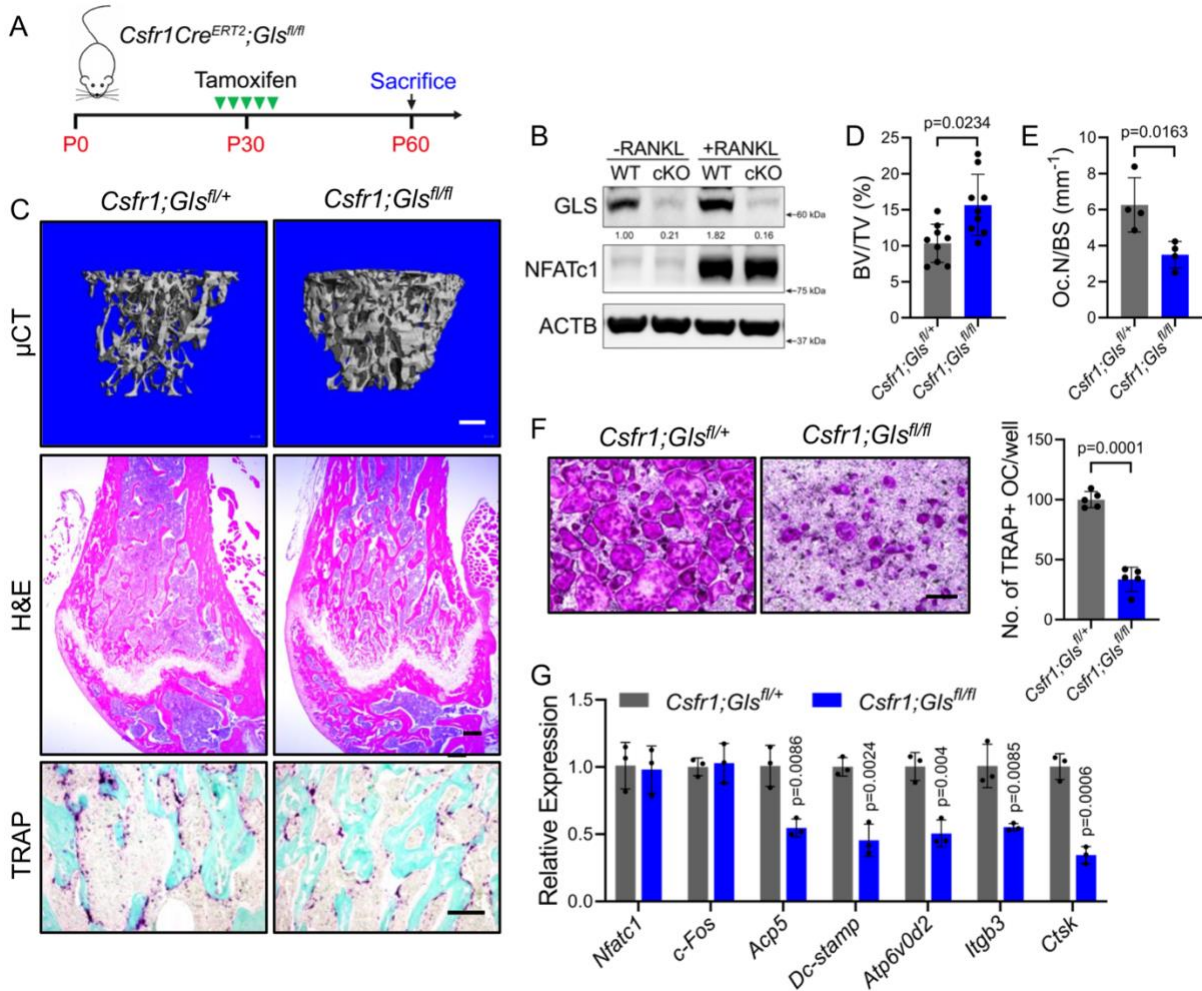
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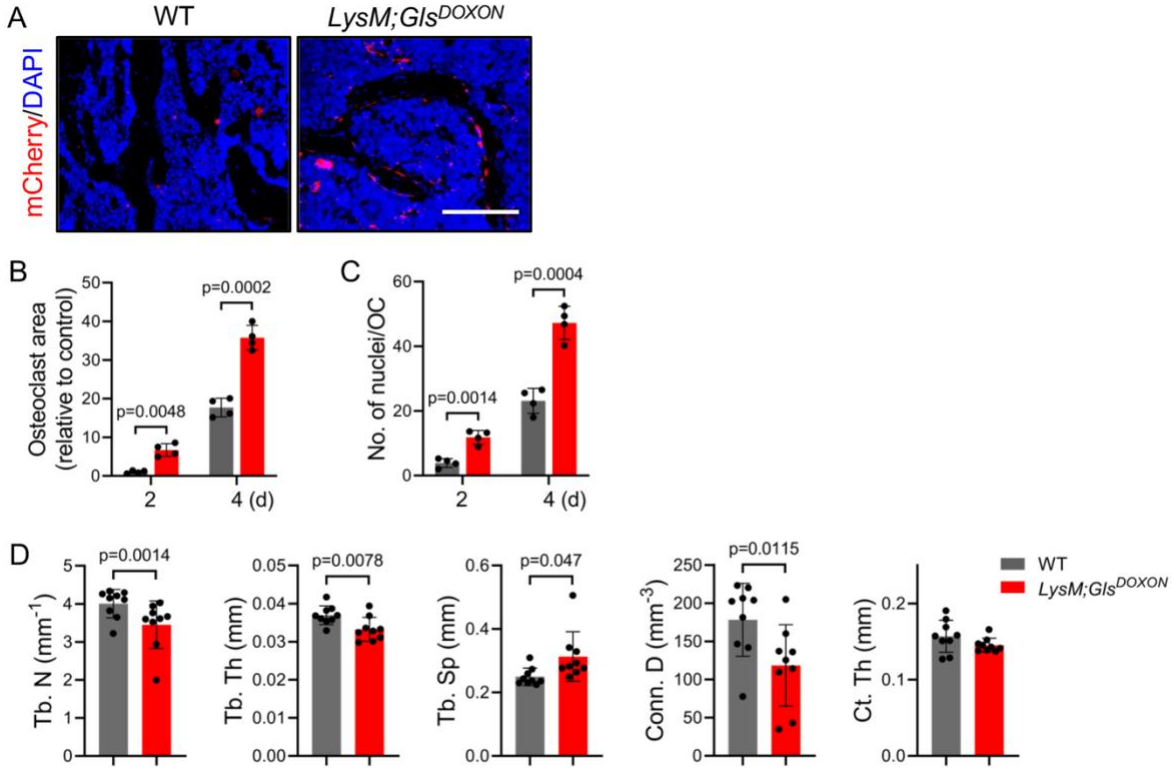
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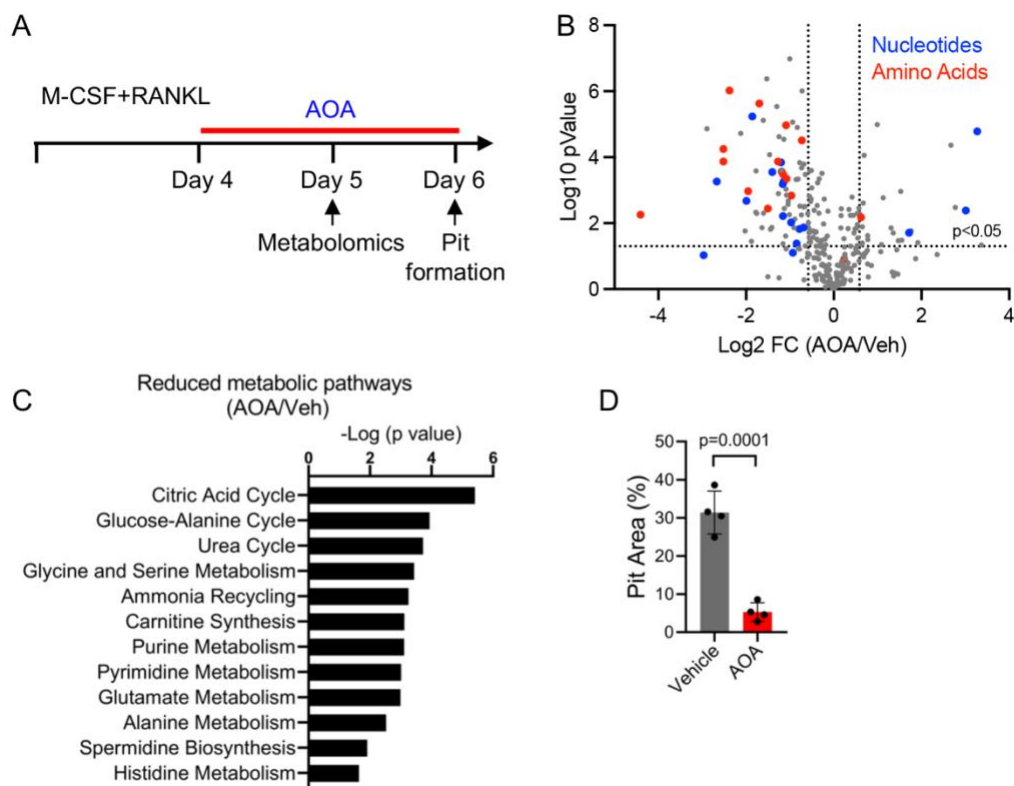
Appendix Figure S1. *Csfr1Cre^{ERT2};Gls^{fl/fl}* mice have increased bone mass due to reduced osteoclast numbers and bone resorption

(A) Schematic of the experimental design. (B) Western blot analysis of GLS and NFATc1 in BMMs derived from WT (*Csfr1Cre^{ERT2};Gls^{fl/+}*) and *Csfr1Cre^{ERT2};Gls^{fl/fl}* mice stimulated with or without RANKL for 72 hours ($n = 3$). (C) Representative μ CT ($n = 9$, scale bar: 200 μ m), H&E ($n = 4$, scale bar: 100 μ m), and TRAP staining ($n = 4$, scale bar: 100 μ m) performed on distal femur trabecular bone from 2-month-old WT and *Csfr1;Gls^{fl/fl}* female mice. (D) The calculated bone volume per tissue volume (BV/TV) ($n = 9$ mice). (E) Quantification of osteoclast number per bone surface (Oc.N/BS) ($n = 4$ mice). (F) TRAP staining (left panel) and quantification of TRAP-positive multi-nuclei cells (graph) of BMMs derived from WT or *Csfr1;Gls^{fl/fl}* female mice cultured in the presence of RANKL for 4 days ($n = 5$ independent experiments). Scale bar: 500 μ m. (G) qPCR analysis of osteoclast marker gene expression in BMMs derived from WT and *Csfr1;Gls^{fl/fl}* female mice cultured in the presence of RANKL for 4 days ($n = 3$ independent experiments). Data are shown as mean $\pm$ SD. 2-tailed Student's paired t test (D and E). 2-tailed Student's unpaired t test (F and G).



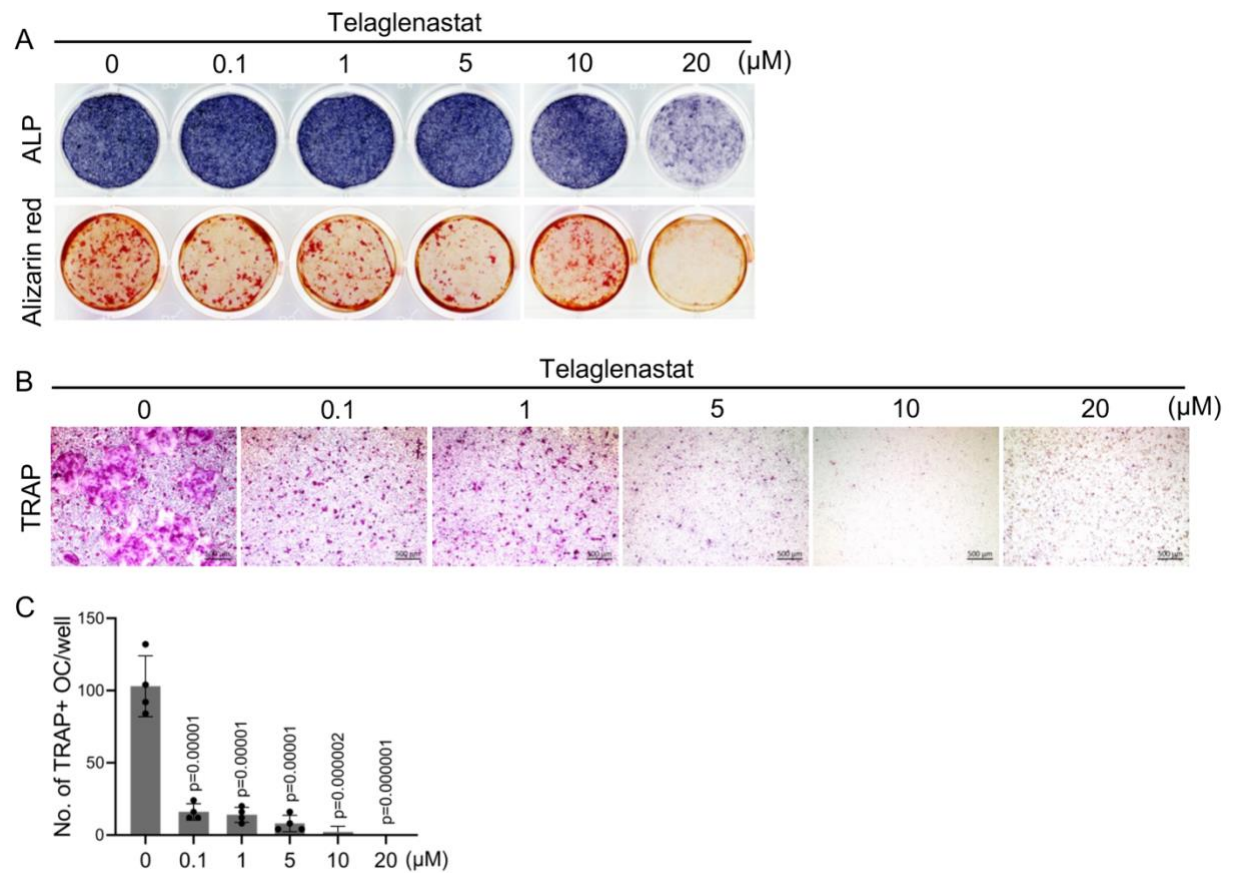
Appendix Figure S2. Confirmation of mCherry expression and microCT parameters of *LysM;Gls^{DOXON}* mice.

(A) mCherry immunofluorescence staining performed on distal femurs from 2-month-old WT and *LysM;Gls^{DOXON}* female mice ($n = 4$). Scale bar: 100 μm . (B-C) Quantification of TRAP+ osteoclast area (B) and the average number of nuclei per osteoclast (C) ($n = 4$ independent experiments). (D) μCT parameters of trabecular bone in the distal femurs of 2-month-old WT and *LysM;Gls^{DOXON}* female mice ($n = 9$ mice). Data are shown as mean $\pm$ SD. 2-tailed Student's unpaired t test (B and C). 2-tailed Student's paired t test (D).



Appendix Figure S3. Metabolic changes caused by pan-transaminase inhibition.

(A) Schematic depicting the experimental design used for metabolomics and pit formation assay. (B) Volcano plot showing metabolites that differed significantly between AOA (200 μ M)- and vehicle-treated mOC ($n = 4$). (C) Metabolic pathway analysis showing reduced pathways in AOA-treated mOC. (D) Quantification of the resorption pit area of Fig. 7K ($n = 4$ independent cultures). Data are shown as mean $\pm$ SD. 2-tailed Student's unpaired t test (B-D).



Appendix Figure S4. Sensitivity to CB-839 in osteoblast and osteoclast cultures.

(A) ALP staining and Alizarin red staining showing the effects of a gradient of CB-839 on osteoblast differentiation of primary calvarial osteoblast precursor cells ($n = 4$). (B) TRAP staining showing the effects of a gradient of CB-839 on the formation of multinucleated TRAP positive osteoclasts ($n = 4$). Scale bar: 500 μm . (C) Quantification of the number of TRAP-positive multinucleated osteoclasts from B ($n = 4$ independent cultures). Data are shown as mean $\pm$ SD. 1-way ANOVA (C).

Appendix Table S1. List of qPCR primers used in this study.

Gene symbol	Forward	Reverse
<i>Nfatc1</i>	GGAGCGGAGAACTTTGCG	GTGACACTAGGGGACACATAACT
<i>c-Fos</i>	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
<i>Acp5</i>	CACTCCCACCCTGAGATTTGT	CATCGTCTGCACGGTTCTG
<i>Dc-stamp</i>	TACGTGGAGAGAAGCAAGGAA	ACACTGAGACGTGGTTTAGGAAT
<i>Itgb3</i>	CCACACGAGGCGTGAACCTC	CTTCAGGTTACATCGGGGTGA
<i>Atp6v0d2</i>	CAGAGCTGTACTTCAATGTGGAC	AGGTCTCACACTGCACTAGGT
<i>Ctsk</i>	GAAGAAGACTCACCAGAAGCAG	TCCAGGTTATGGGCAGAGATT
<i>β-actin</i>	AGATGTGGATCAGCAAGCAG	GCGCAAGTTAGGTTTTGTCA