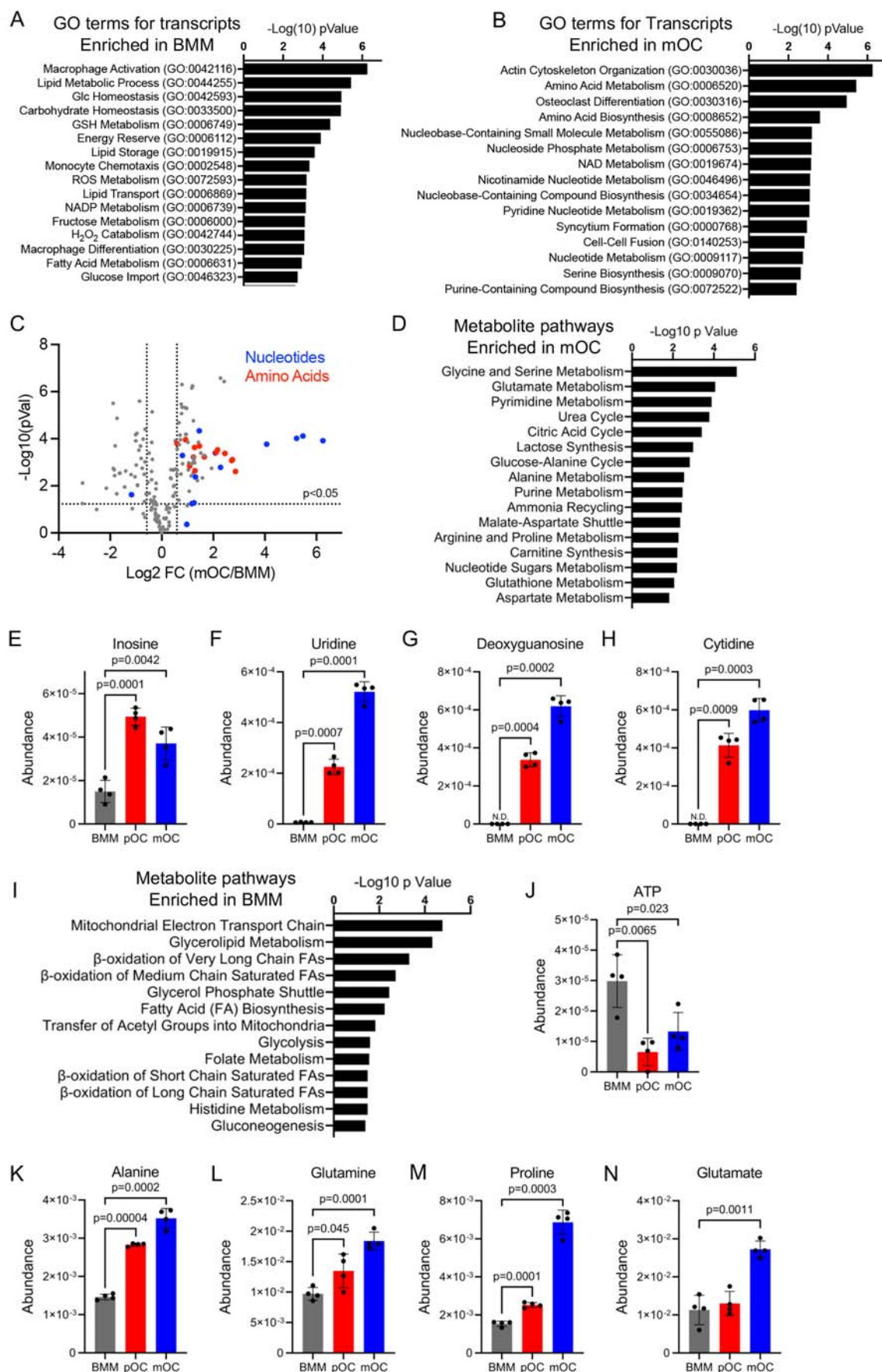


Expanded View Figures

Figure EV1. The transcriptomic and metabolic changes during osteoclastogenesis.

(A, B) Gene Ontology pathway analysis of transcripts enriched in BMM (A) or mOC (B). (C) Volcano plots showing metabolites that differed significantly between BMM and mature osteoclast (mOC) ($n = 4$). Amino acids are colored red, and nucleotides are colored blue. (D) Metabolic pathway analysis (based on the Human Metabolome Database, HMDB) showing pathways enriched in mOC. (E-H) Graphical depiction of the intracellular abundance of select nucleotides in BMM, pOC, and mOC as determined by mass spectrometry ($n = 4$ independent experiments). (I) Metabolic pathways enriched in BMM. (J) Graphical depiction of the intracellular abundance of ATP ($n = 4$ independent experiments). (K-N) Graphical depiction of the intracellular abundance of select amino acids (alanine, glutamine, proline, and glutamate) in BMM, pOC, and mOC as determined by mass spectrometry ($n = 4$ independent experiments). Data are shown as mean $\pm$ SD. Two-tailed Student's unpaired t test (A-D, and I). 1-way ANOVA (E-H, J, K-N).



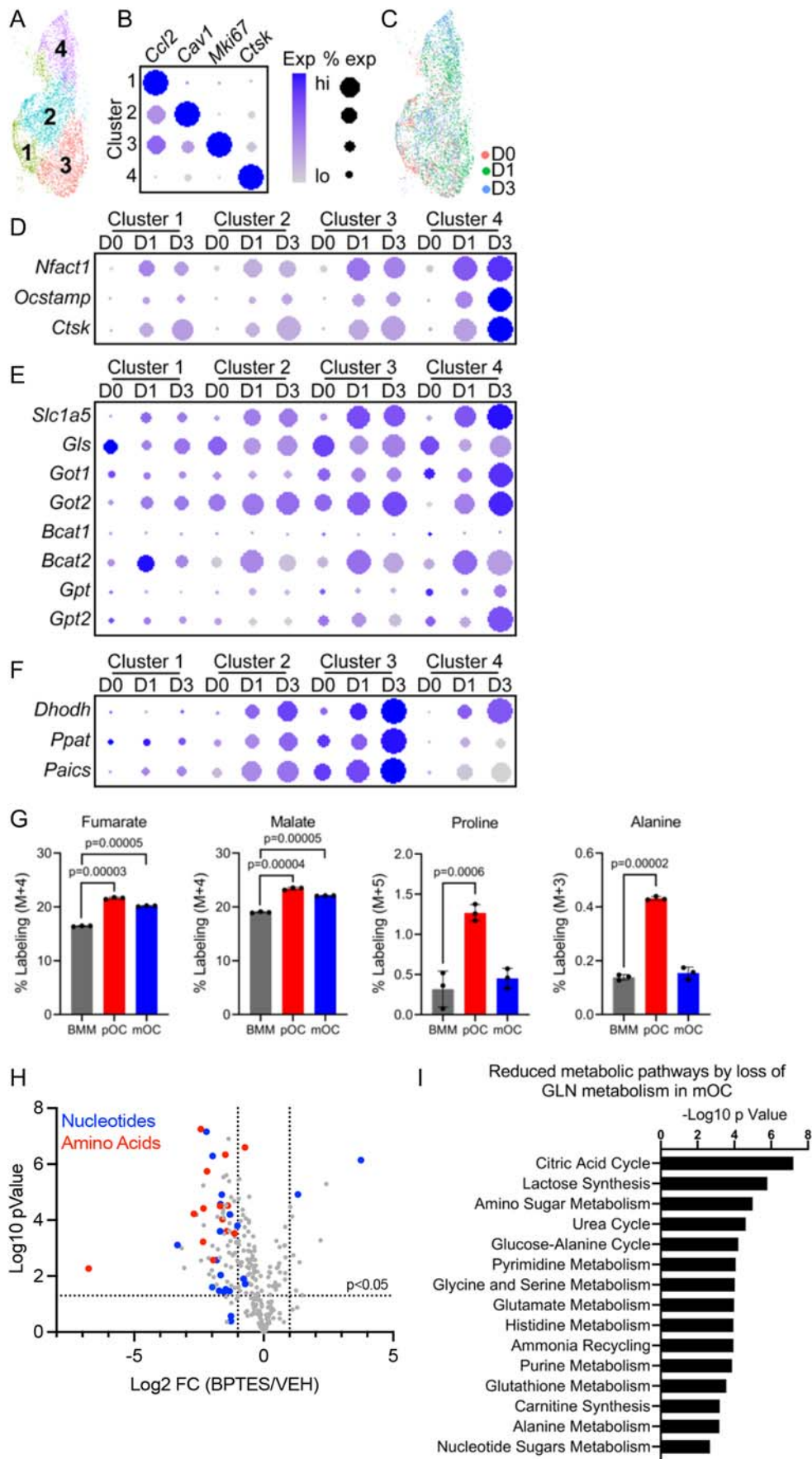


Figure EV2. Glutaminolysis is activated during osteoclastogenesis to support metabolic reprogramming.

(A) UMAP visualization of 4 clusters during osteoclastogenesis in the osteoclast culture system (Day 0–3 with RANKL). (B) Dot plot showing cluster marker genes. (C) UMAP visualization colored by days post RANKL stimulation. (D) Average osteoclast marker gene expression in 4 clusters. (E) The expression of genes associated with glutaminolysis in 4 clusters. (F) The expression of genes associated with nucleotide biosynthesis in 4 clusters. (G) Graphical depiction showing the percent labeling of fumarate, malate, proline, and alanine from [U-¹³C]-glutamine in BMM, pOC, and mOC ($n = 3$ independent experiments). (H) Volcano plot showing metabolites that differed significantly between BPES (10 μ M)- and vehicle-treated mOC ($n = 4$). (I) Metabolic pathway analysis showing reduced pathways in BPES-treated mOC. Data are shown as mean $\pm$ SD. One-way ANOVA (G). Two-tailed Student's unpaired t test (H, I).

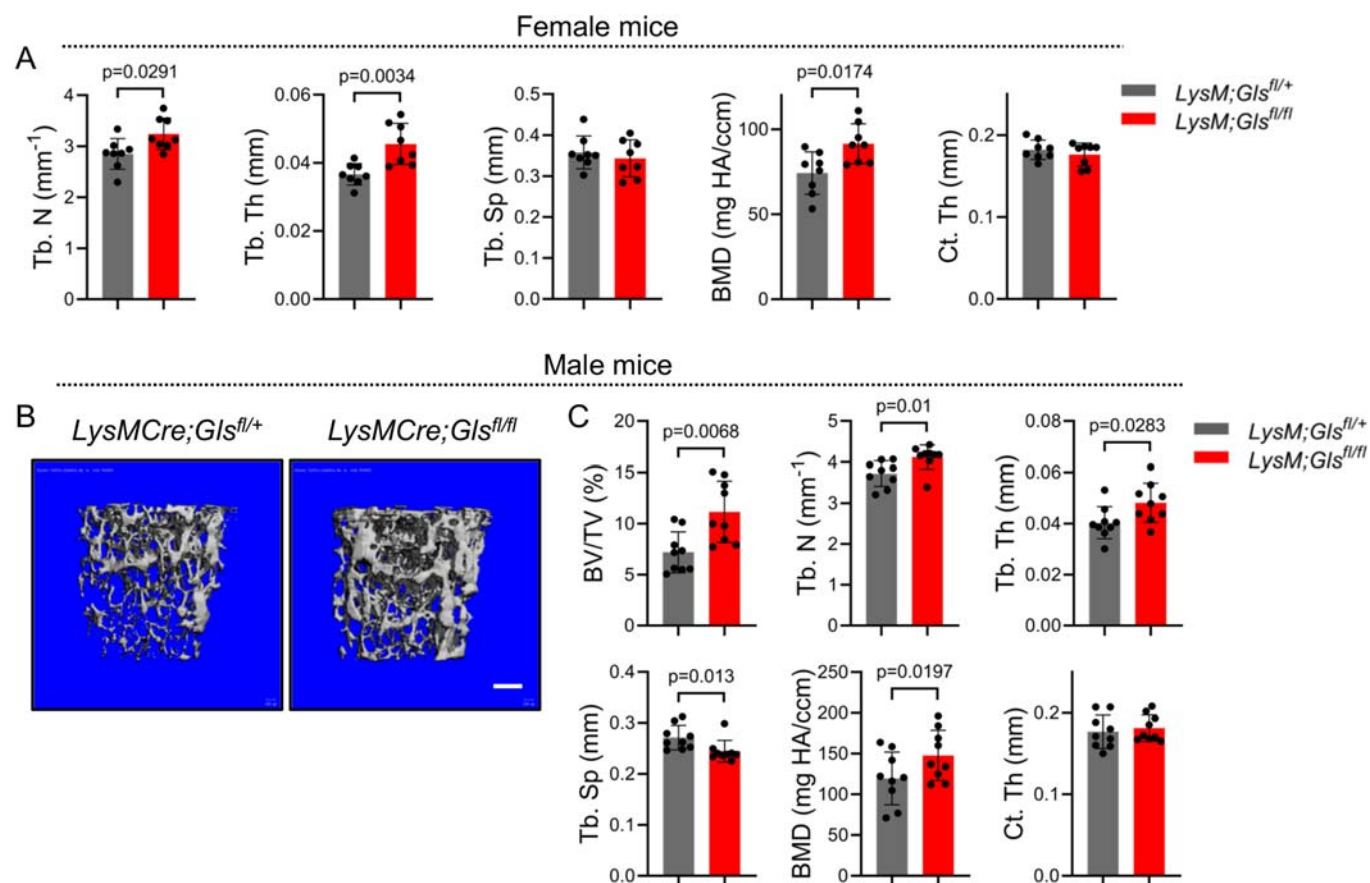
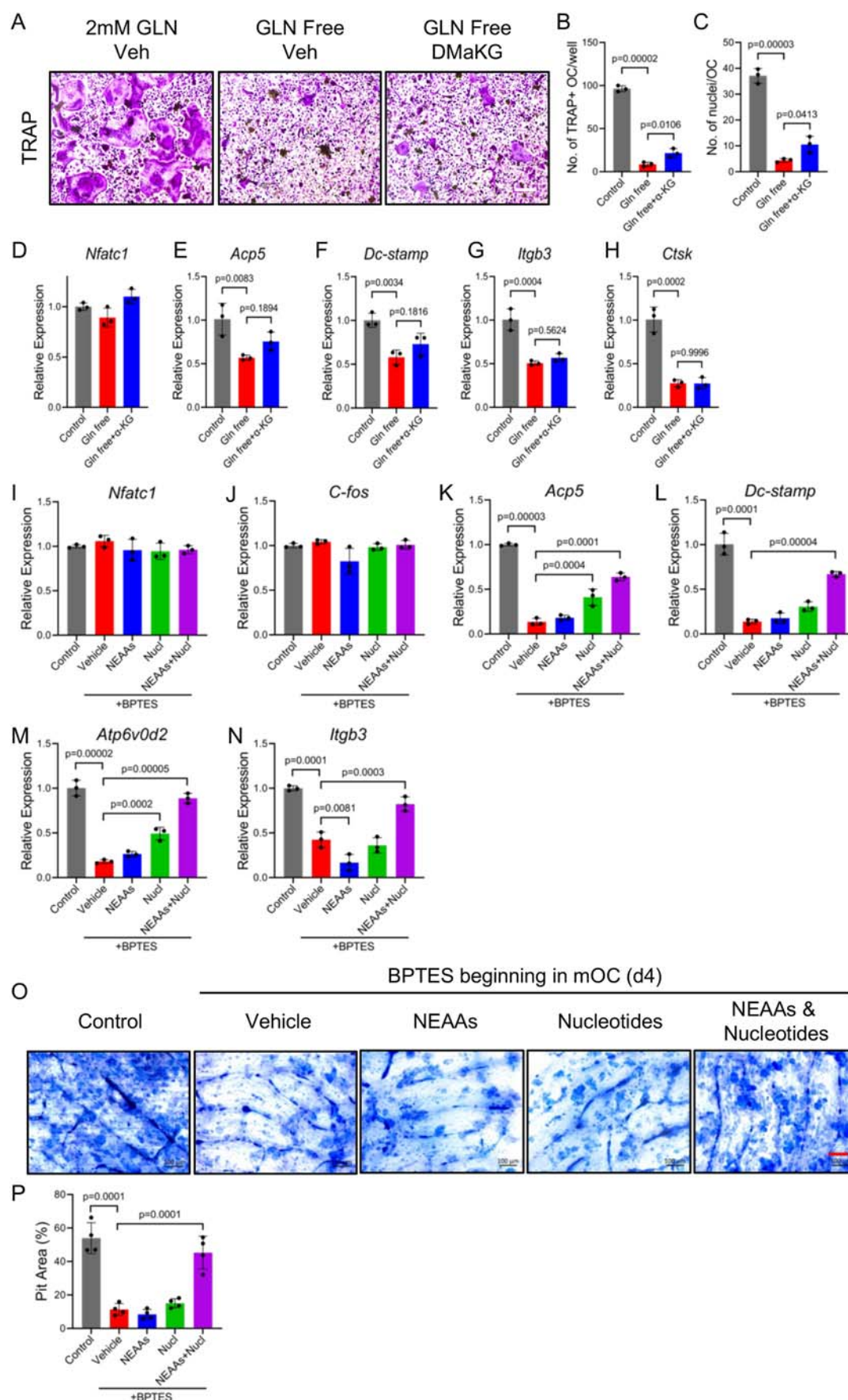


Figure EV3. *Gls* ablation in myeloid lineage cells increases bone mass in both male and female mice.

(A) μ CT parameters of trabecular bone in the distal femurs of 4-month-old WT and *LysM;Gls^{fl/fl}* female mice ($n = 8$ mice). (B, C) Representative μ CT images (B) and μ CT parameters of trabecular bone in the distal femurs of 4-month-old WT and *LysM;Gls^{fl/fl}* male mice ($n = 9$ mice). Scale bar: 200 μm . Data are shown as mean $\pm$ SD. Two-tailed Student's paired t test (A and C).



◀ **Figure EV4. Glutaminolysis provides amino acids and nucleotides, but not α KG, to regulate osteoclastogenesis.**

(A–H) The effect of supplementing dimethyl- α -ketoglutarate (DM α KG) on osteoclast differentiation as measured by TRAP staining (A, $n = 5$, scale bar: 500 μ m) or qPCR analysis ($n = 3$ independent experiments) (E–H). (C) Quantification of TRAP-positive multi-nuclei cells from (A). (D) Quantification of the number of nuclei per TRAP-positive osteoclast from (A). (I–P) The effect of supplementing non-essential amino acids (NEAAs) and/or nucleotides (Nucl) on osteoclast differentiation and bone resorption as measured by qPCR analysis of osteoclast marker gene mRNA expression ($n = 3$ independent experiments) (I–N). (O–P) The effect of supplementing non-essential amino acids (NEAA) and/or nucleotides beginning at day 4 on bone resorption as measured by pit assay. Scale bar: 100 μ m. (P) Quantification of the resorption pit area from O ($n = 4$ independent experiments). Data are shown as mean $\pm$ SD. One-way ANOVA (B–H), two-way ANOVA (I–N, P).

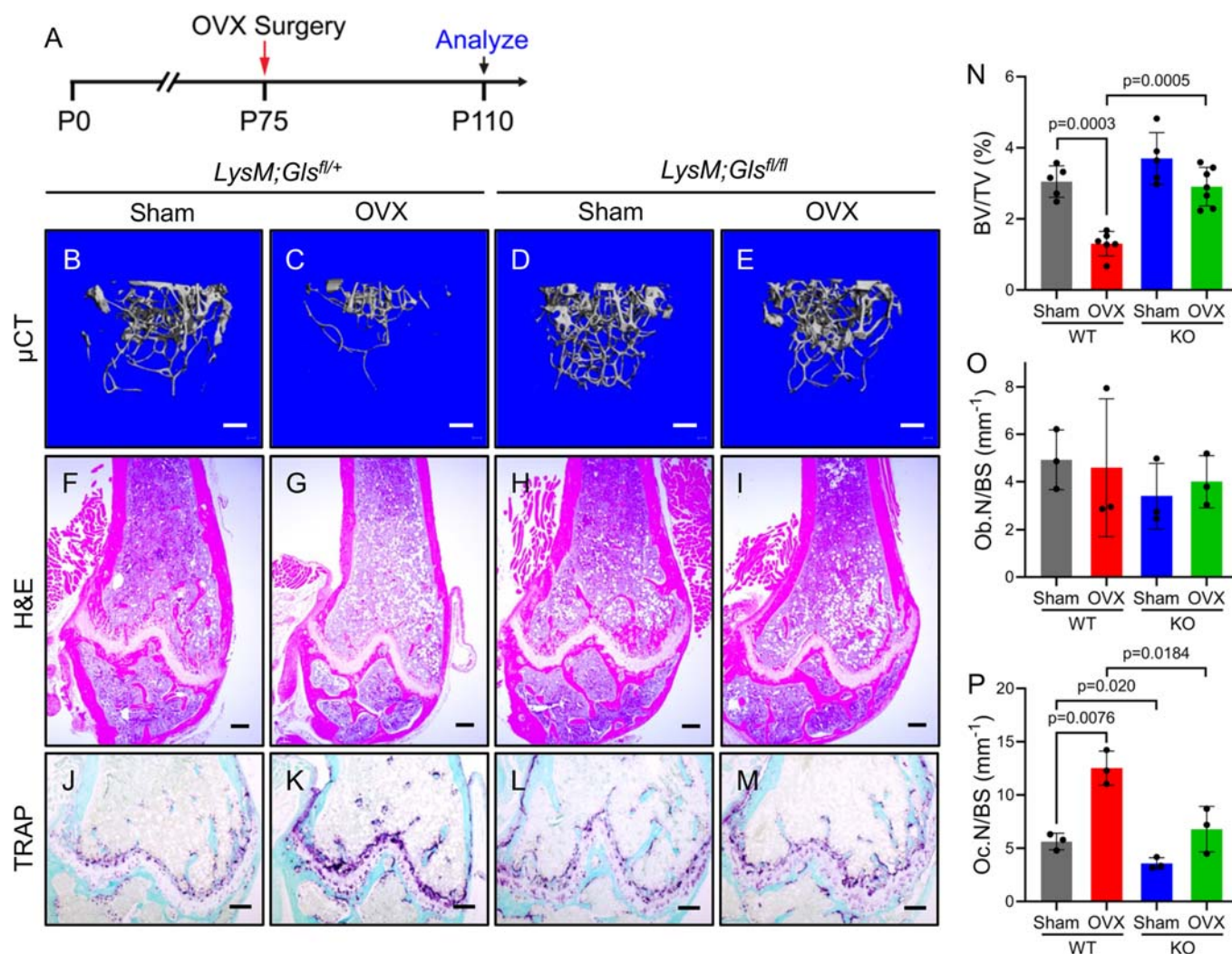


Figure EV5. Genetically targeting glutaminolysis prevents OVX-induced bone loss.

(A) Schematic showing the experimental design. (B–M) Representative images of μ CT (B–E, scale bar: 200 μ m), H&E staining (F–I, scale bar: 100 μ m), and TRAP staining (J–M, scale bar: 100 μ m) of trabecular bone in the distal femurs of WT and *LysM;Gls^{fl/fl}* female mice subjected to OVX or sham surgery ($n = 5-7$). (N) The calculated BV/TV from μ CT images ($n = 5-7$ mice). (O) Osteoblast number per bone surface (Ob.N/BS) quantified from H&E stains ($n = 3$ mice). (P) Osteoclast number per bone surface (Oc.N/BS) quantified from TRAP stains ($n = 3$ mice). Data are shown as mean $\pm$ SD. Two-way ANOVA (N–P).