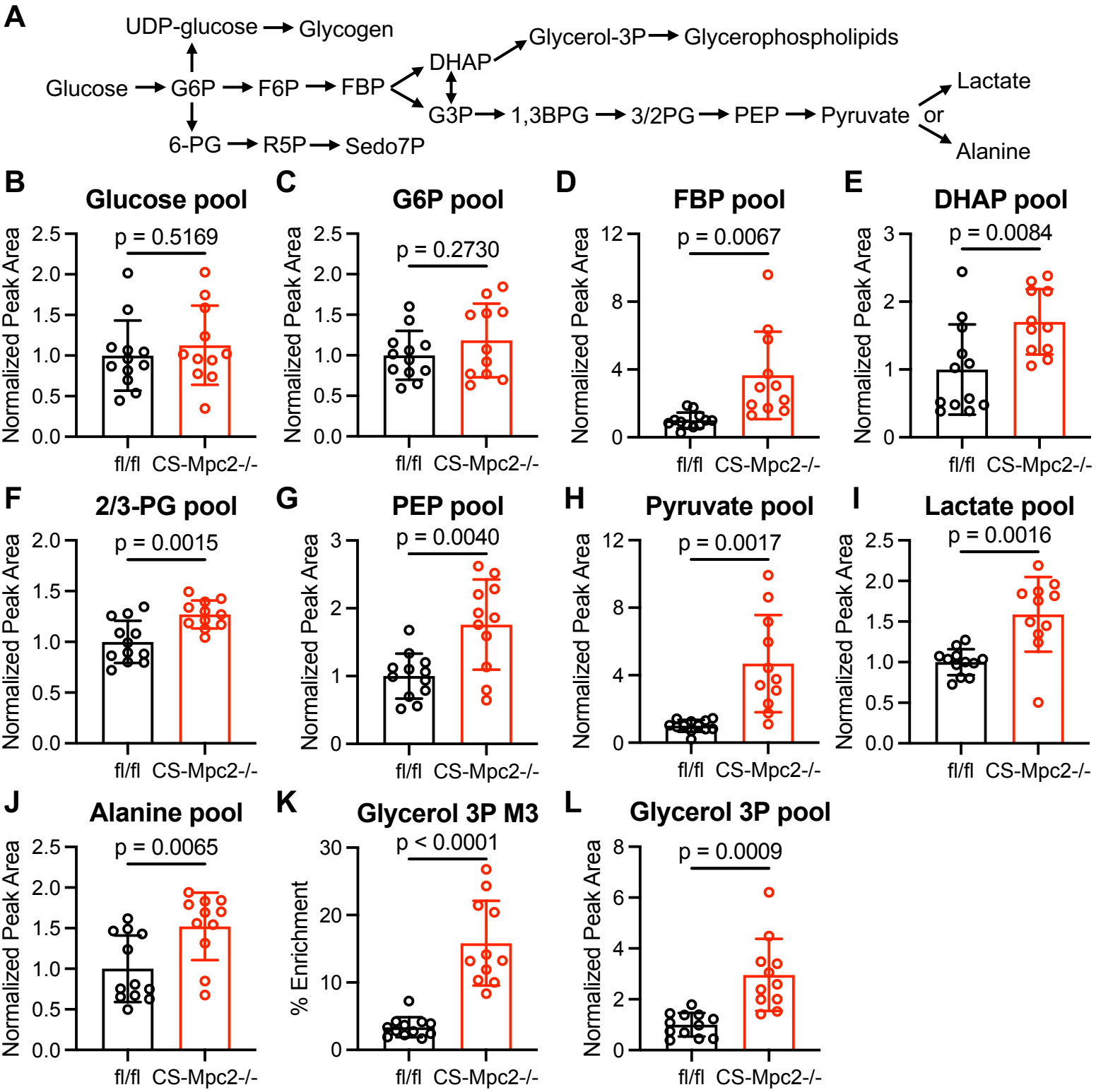
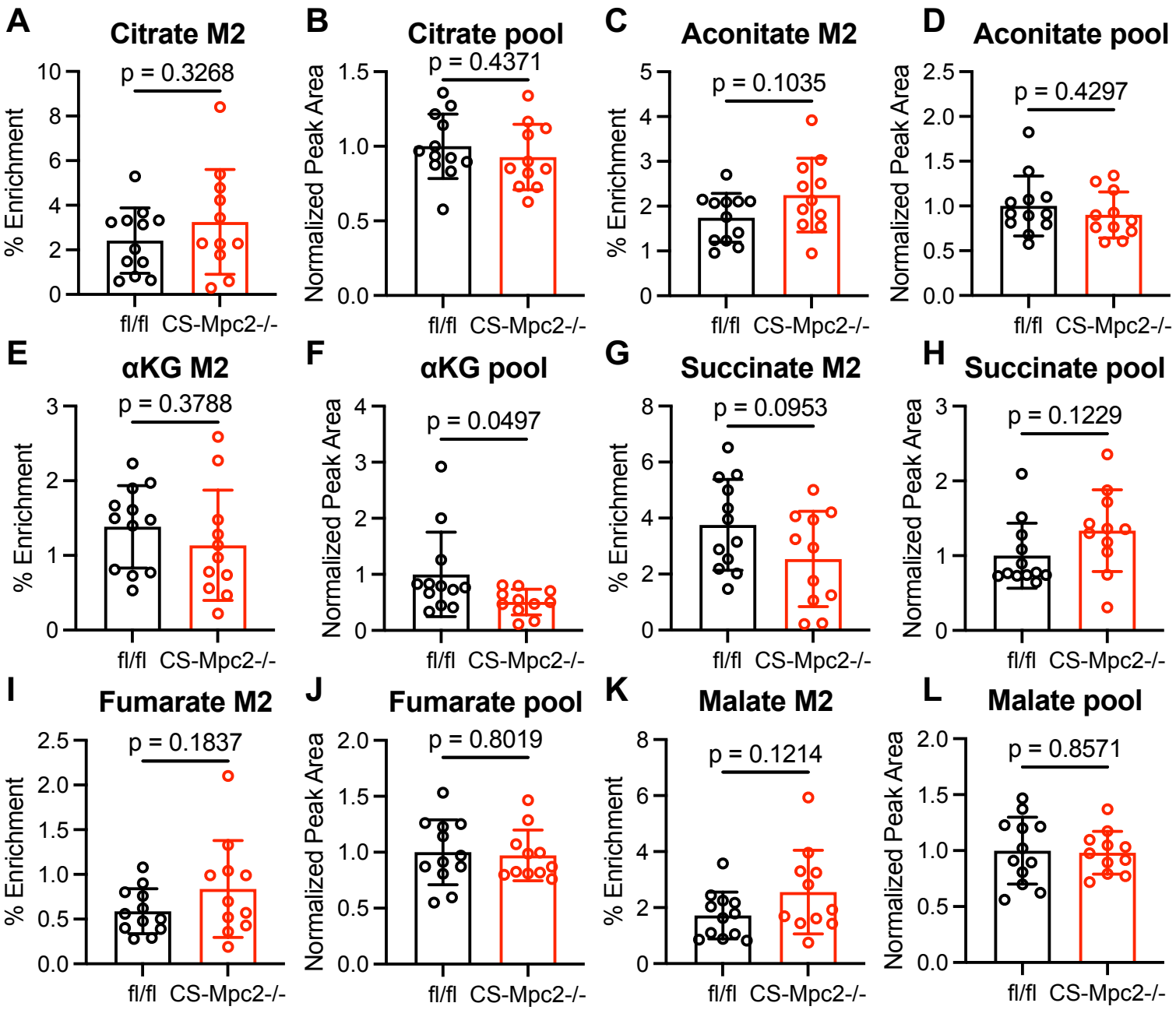
Supplemental Fig. 1. Glycolytic pool sizes in failing MPC-/- hearts.



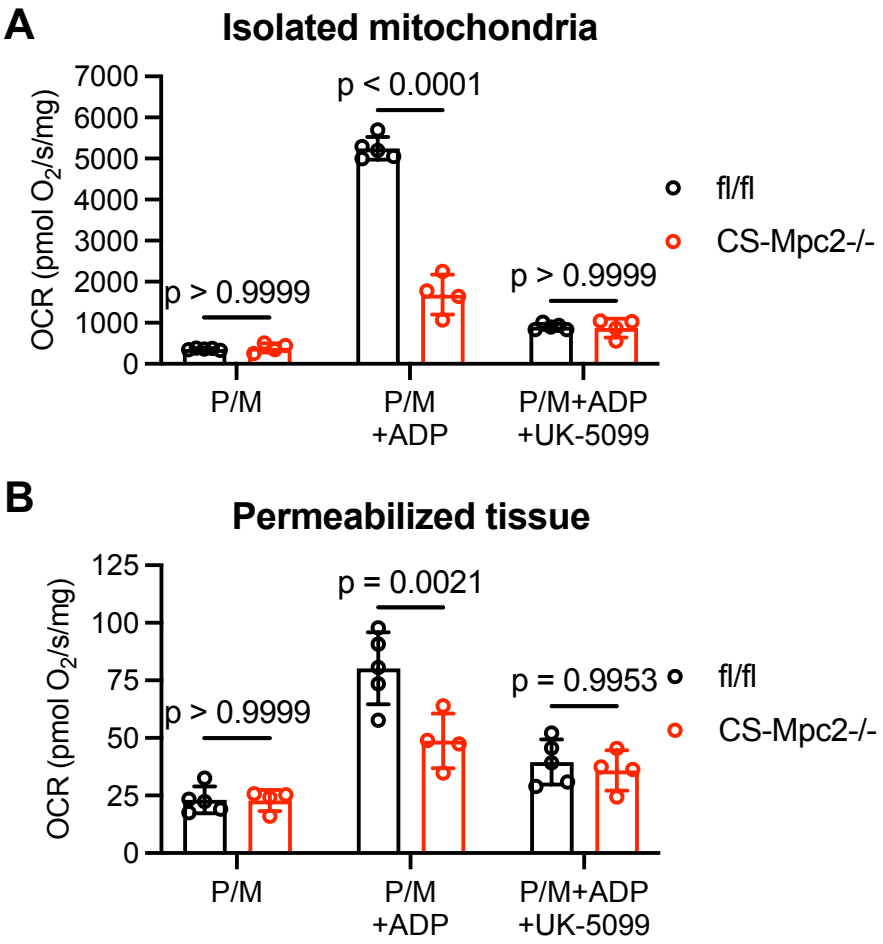
Supplemental Figure 1. Glycolytic pool sizes in failing MPC-/- hearts. A, Schematic pathway of glycolysis and accessory glucose pathways. **B** through **L**, Normalized pool size of glucose **(B)**, glucose-6-phosphate (G6P) **(C)**, fructose bisphosphate (FBP) **(D)**, dihydroxyacetone phosphate (DHAP) **(E)**, 2/3-phosphoglycerate (PG) **(F)**, phosphoenolpyruvate (PEP) **(G)**, pyruvate **(H)**, lactate **(I)**, alanine **(J)**, and the ^{13}C % enrichment of glycerol 3-phosphate (Glycerol 3P) (**K**) and glycerol 3P pool **(L)** of failing CS-Mpc2-/- hearts and fl/fl littermates injected with U-^{13}C-glucose, n=11-12. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

Supplemental Fig. 2. TCA cycle enrichment and pool sizes in failing hearts



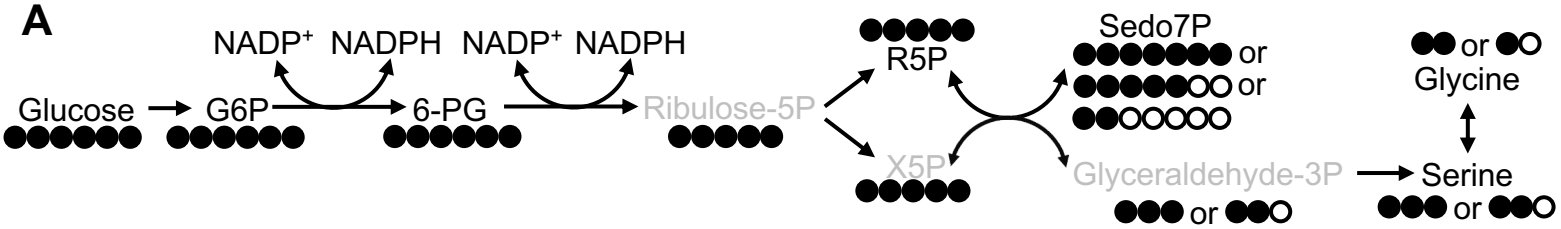
Supplemental Figure 2. TCA cycle enrichment and pool sizes in failing CS-Mpc2-/- hearts. A through **L**, the ^{13}C % enrichment and pool sizes of citrate **(A-B)**, aconitate **(C-D)**, α-ketoglutarate (KG) **(E-F)**, succinate **(G-H)**, fumarate **(I-J)**, and malate **(K-L)** in fl/fl and failing CS-Mpc2-/- hearts of mice injected i.p. with U-^{13}C-glucose, n=11-12. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

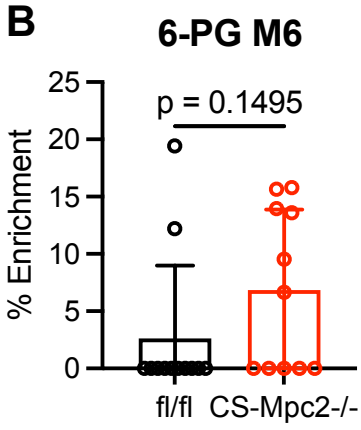
Supplemental Fig. 3 - MPC deletion reduces pyruvate oxidation

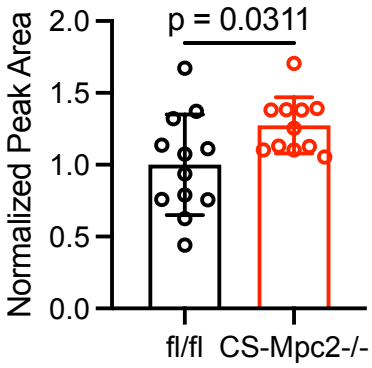


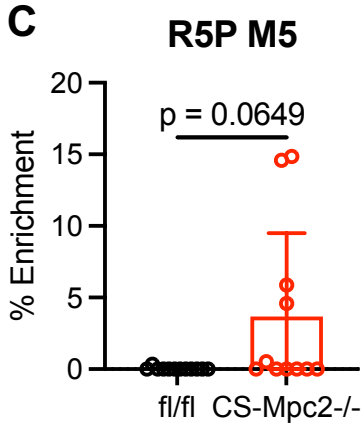
Supplemental Figure 3. MPC deletion reduces pyruvate oxidation. A and **B,** Oxygen consumption rates (OCR) measured from isolated cardiac mitochondria (**A**) and permeabilized cardiac muscle fibers (**B**) from hearts of fl/fl and CS-Mpc2-/- littermates stimulated with pyruvate/malate (P/M), P/M plus adenosine diphosphate (ADP), and P/M, ADP, and the MPC inhibitor UK-5099 (5 μM), n=4-5. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

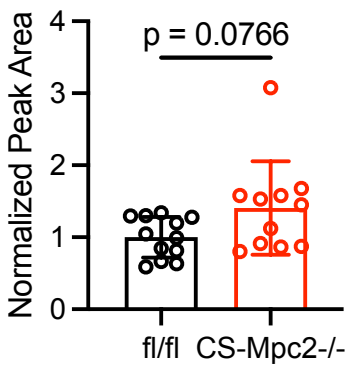
Supplemental Fig. 4 No major changes in the pentose phosphate pathway in failing MPC-/- hearts

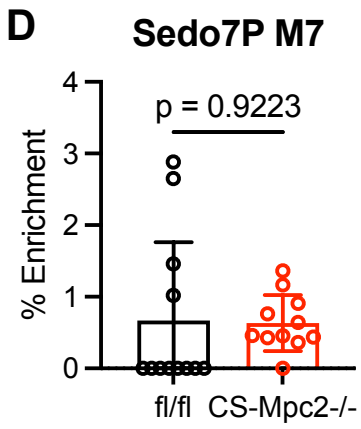


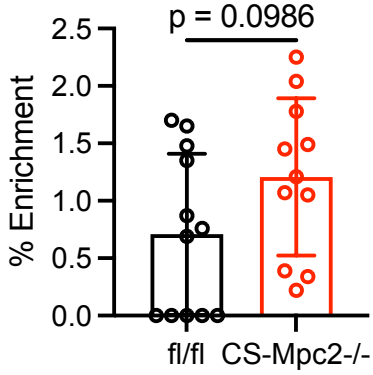


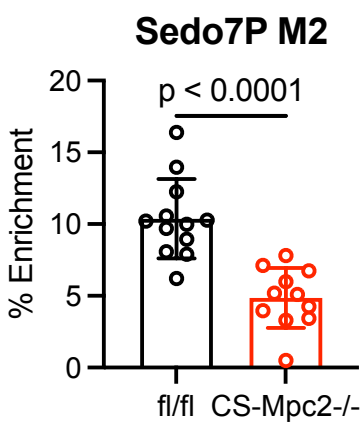


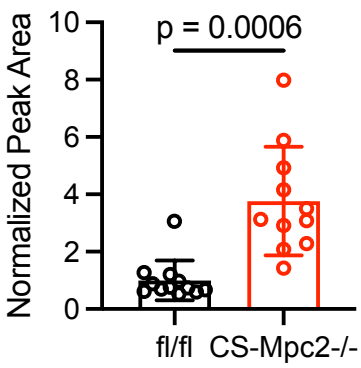


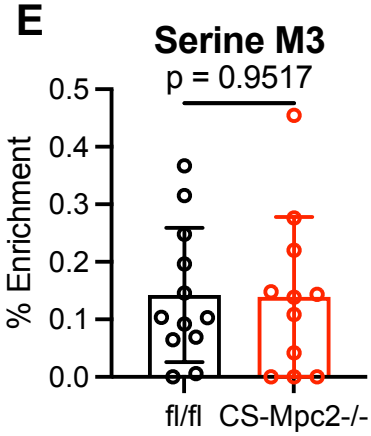


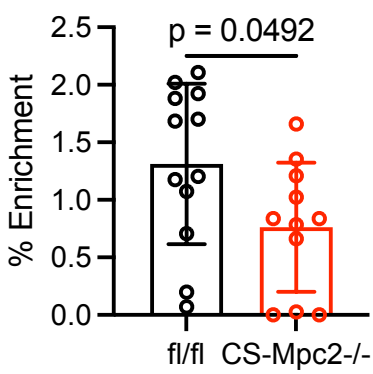


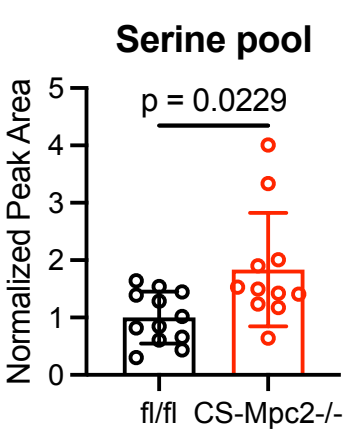


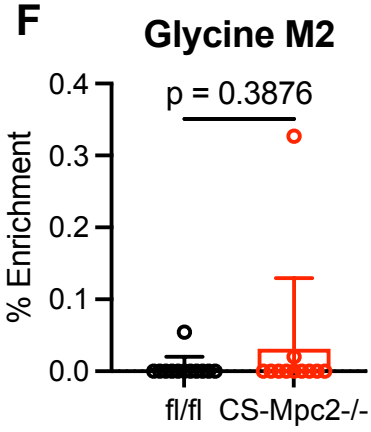


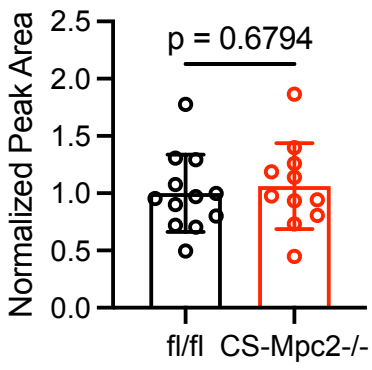






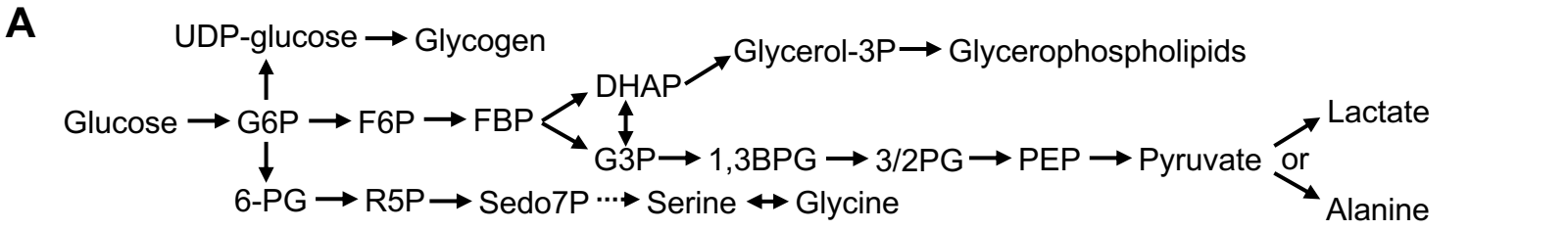


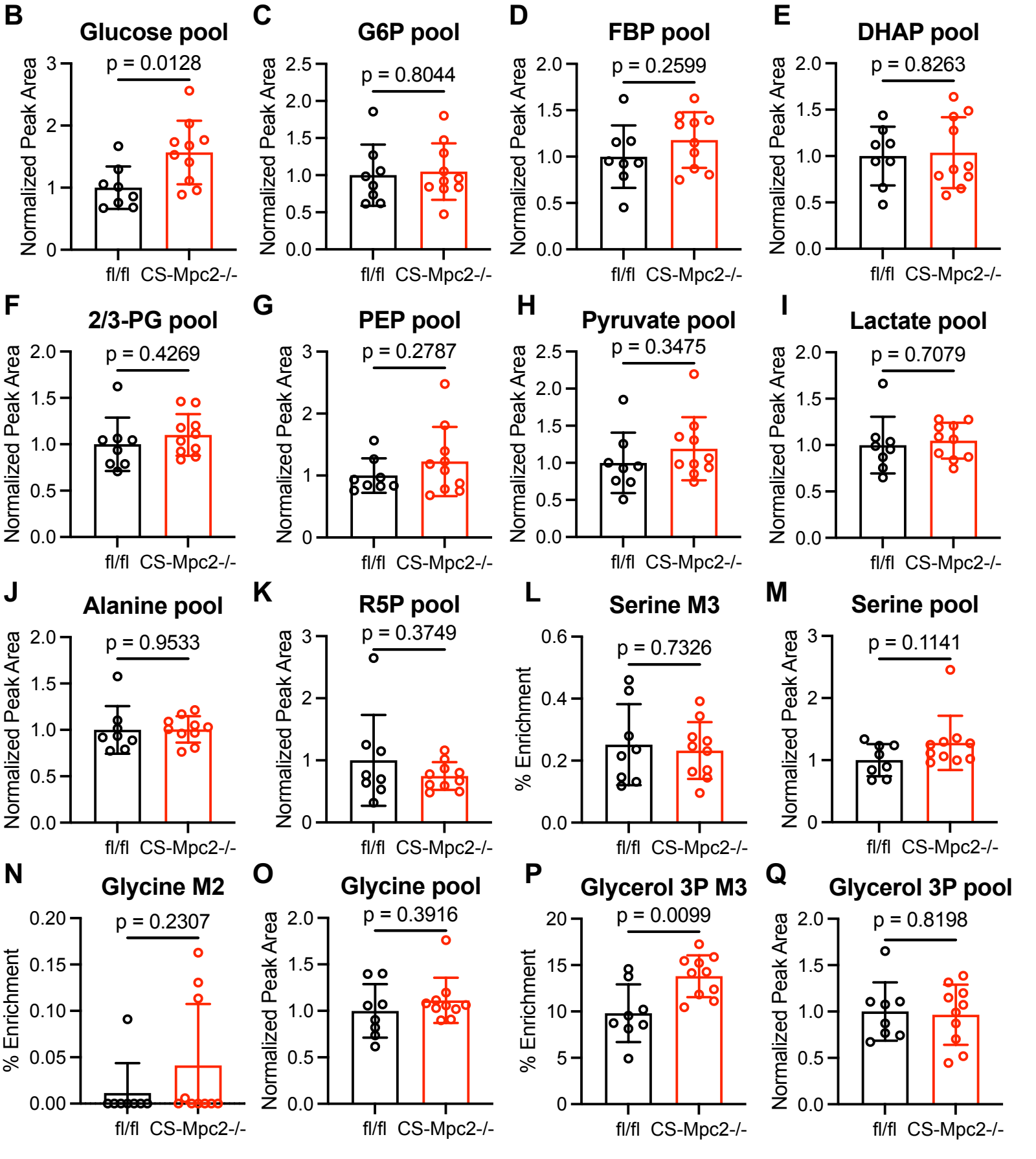




Supplemental Figure 4. No major changes in the pentose phosphate pathway in failing MPC-/- hearts. A, Schematic of ^{13}C enrichment of the pentose phosphate pathway from U-^{13}C-glucose. Metabolites in grey were not measured. **B** through **F**, ^{13}C % enrichment and normalized pool size of 6-phosphogluconate (PG) **(B)**, ribose-5-phosphate (R5P) **(C)**, sedoheptulose 7-phosphate (Sedo7P) **(D)**, serine **(E)**, and glycine **(F)**, in hearts from fl/fl and failing CS-Mpc2-/- mice injected with U-^{13}C-gluocse, n=11-12. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

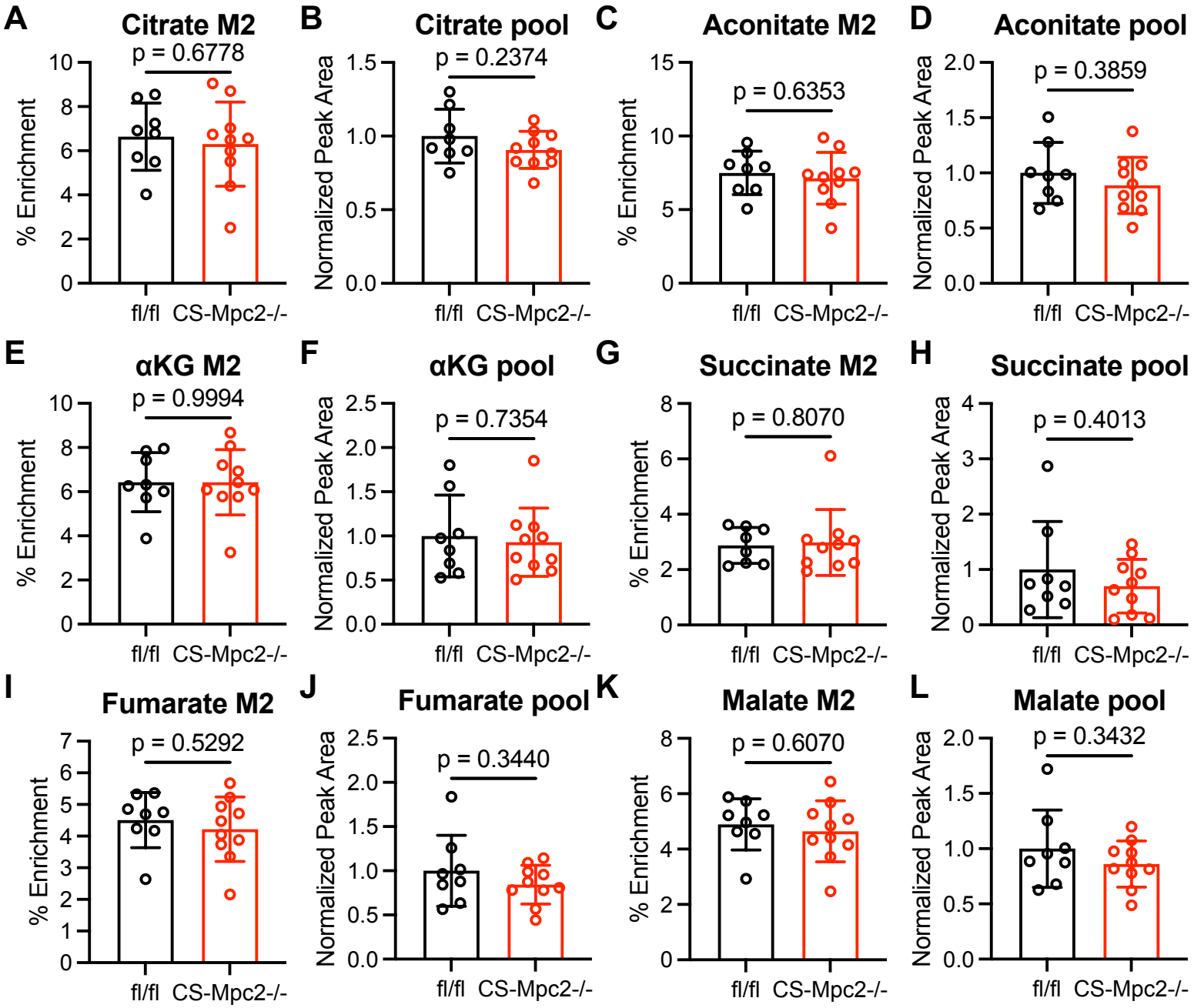
Supplemental Fig. 5 Glycolytic pool sizes in young, non-fatty MPC-/- hearts





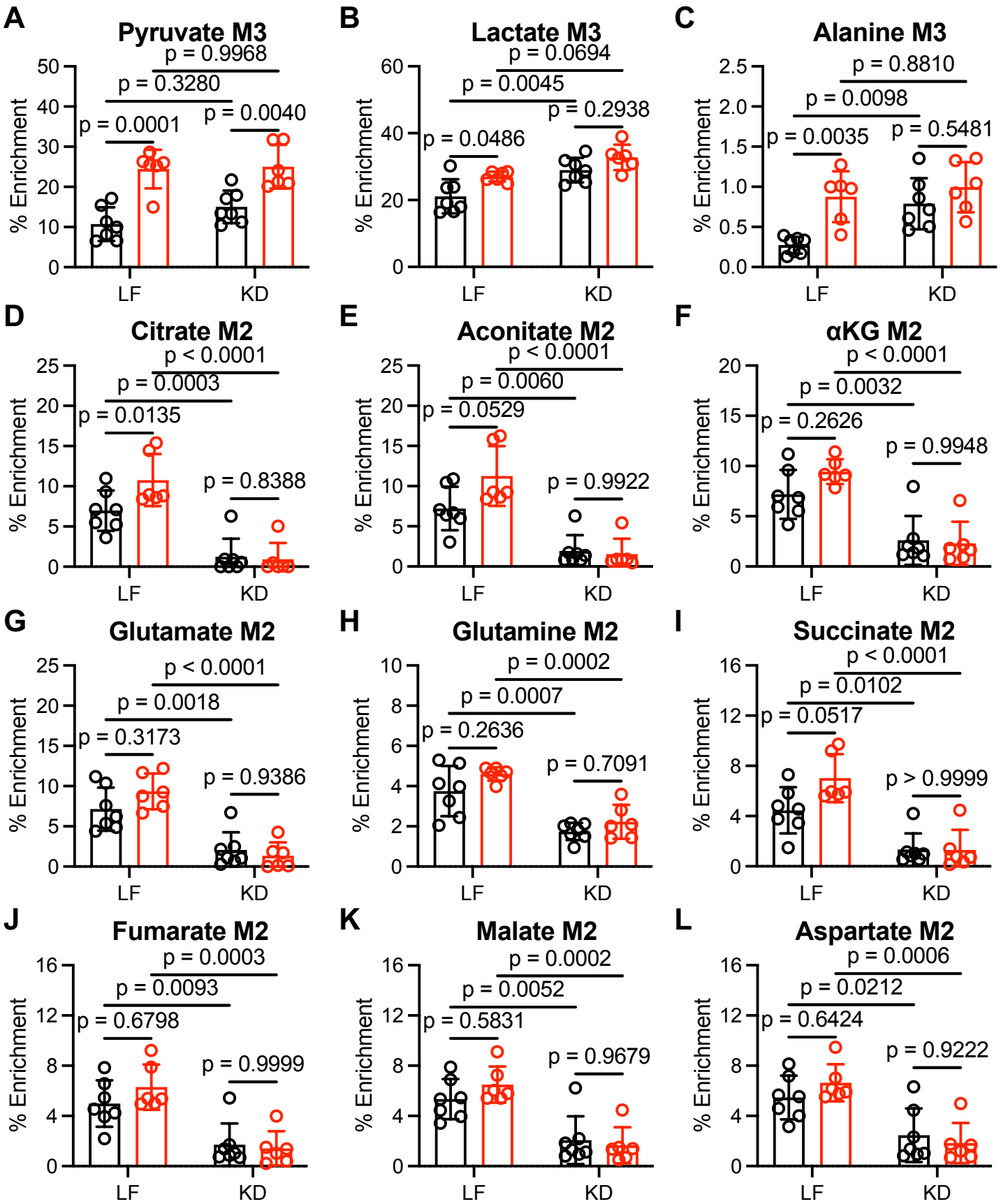
Supplemental Figure 5. Glycolytic pool sizes in young, non-failing MPC-/- hearts. A, Schematic of glycolysis and accessory glucose pathways. **B** through **Q**, Normalized pool sizes of glucose **(B)**, glucose-6-phosphate (G6P) (**C**), fructose bisphosphate (FBP) (**D**), dihydroxyacetone phosphate (DHAP) **(E)**, 2/3 phosphoglycerate (PG) **(F)**, phosphoenolpyruvate (PEP) **(G)**, pyruvate **(H)**, lactate **(I)**, alanine (J), ribose-5-phosphate (R5P) **(K)**, and the ^{13}C enrichment and pool size of serine **(L-M)**, glycine **(N-O)**, and glycerol 3-phosphate (glycerol 3P) **(P-Q)** in young non-failing CS-Mpc2-/- and fl/fl littermates injected with U-^{13}C-glucose, n=8-10. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

Supplemental Fig. 6. TCA cycle enrichment and pool sizes in young, non-failing CS-Mpc2-/- hearts



Supplemental Figure 6. TCA cycle enrichment and pool sizes in young, non-failing CS-Mpc2-/- hearts. A through **L**, ^{13}C % enrichment and normalized pool sizes of citrate (**A-B**), aconitate (**C-D**), α-ketoglutarate (αKG) (**E-F**), succinate (**G-H**), fumarate (**I-J**), and malate (**K-L**), in hearts of young, nonfailing CS-Mpc2-/- and fl/fl littermates injected with U-^{13}C-glucose, n=8-10. Data are presented as the mean±SD. Data were evaluated by unpaired, two-tailed Student's t-test with Welch correction.

Supplemental Fig. 7. Ketogenic diet decreases TCA cycle enrichment from glucose



Supplemental Figure 7. Ketogenic diet decreases TCA cycle enrichment from glucose. A through **L**, ^{13}C % enrichment of pyruvate (**A**), lactate (**B**), alanine (**C**), citrate (**D**), aconitate (**E**), α-ketoglutarate (αKG) (**F**), glutamate (**G**), glutamine (**H**), succinate (**I**), fumarate (**J**), malate (**K**), and aspartate (**L**) in hearts from fl/fl and CS-Mpc2-/- fed either low-fat (LF) or ketogenic diet (KD) and injected with U-^{13}C-glucose, n=6-7. Data are presented as the mean±SD. Data were evaluated by two-way analysis of variance (ANOVA) with Tukey post-hoc multiple-comparisons test.