Supplemental Information

Mouse tissue harvest-induced hypoxia rapidly alters the in vivo metabolome, between-genotype metabolite level differences, and ^{13}C-tracing enrichments

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Supplemental object 1: Interactive and fully zoomable version of the urchin plot shown in Figure 1D. The urchin plot was produced using Plotly. Labels are revealed by placing your cursor over the vector termini.

Table S1: Liver delayed freezing metabolomic fold change data and associated p-values. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison test.

Table S2: Liver delayed freezing t50 urchin plot calculations.

Table S3: Heart time zero and 2-minutes delayed freezing metabolomic data and p-values. P-values were calculated by Student's t-test.

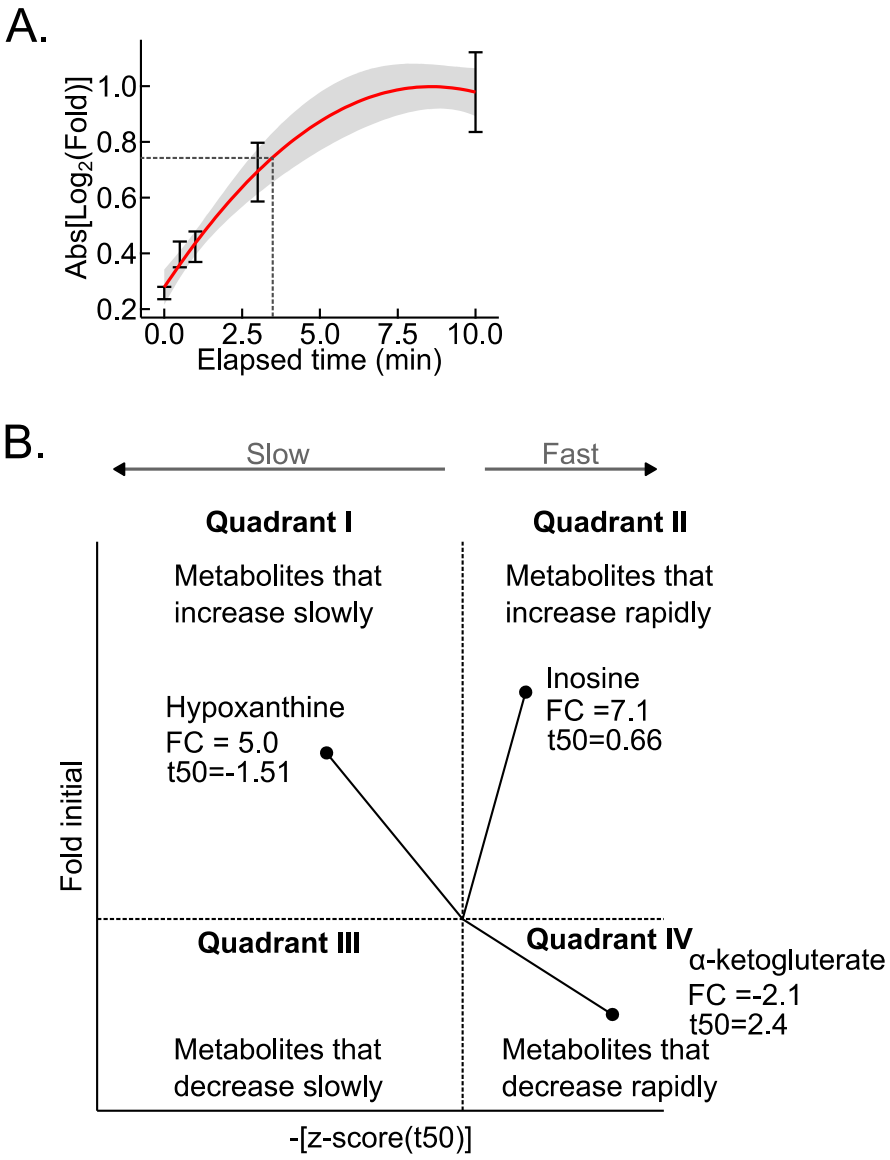
Table S4: Skeletal muscle time zero and 1-minute delayed freezing metabolomic data and p-values. P-values were calculated by Student's t-test.

Table S5: MPC-LivKO and WT delayed freezing time course metabolomic data and associated p-values. P-values between genotypes were calculated using the Student's t-test.

Table S6: MPC-LivKO/WT per metabolite ratios by delayed freezing time-point compared to MPC-LivKO/WT per metabolite ratios at time zero. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison test.

Table S7: ^{13}C isotopologue % enrichment and fold change data by delayed freezing time-point and associated p-values. P-values were calculated using a one-way ANOVA with the post-hoc Holm-Sidak multiple comparison test.

Figure S1

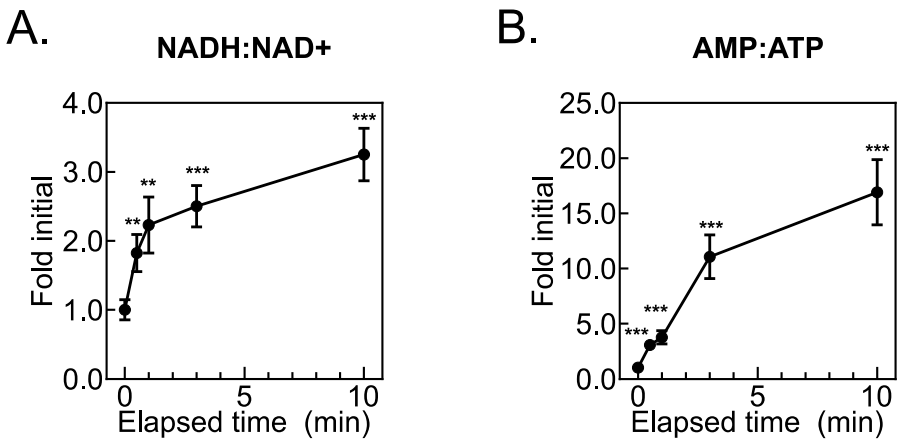


<u>Figure S1</u>: Supplement to Figure 1 - Delayed post-dissection freezing of the liver leads to broad metabolomic drift

(A) Line graph showing the total measured metabolome kinetic change by delayed freezing time-point. The 95% confidence interval is shaded gray. The dotted line is the approximate one-half maximum fold-change (t50) for the total measured metabolomic of 3.58 +/- 0.32 minutes (n = 5-6 biological replicates/metabolite).

(B) Example urchin plot showing line slopes as an interaction between the t50 kinetics and dynamic ranges of metabolite fold changes with delayed freezing. Metabolites were converted to line vectors displaying dynamic range (y-axis) and the negative z-score of the time required for one-half maximum fold change (-[z-score(t50)]; x-axis).

Figure S2

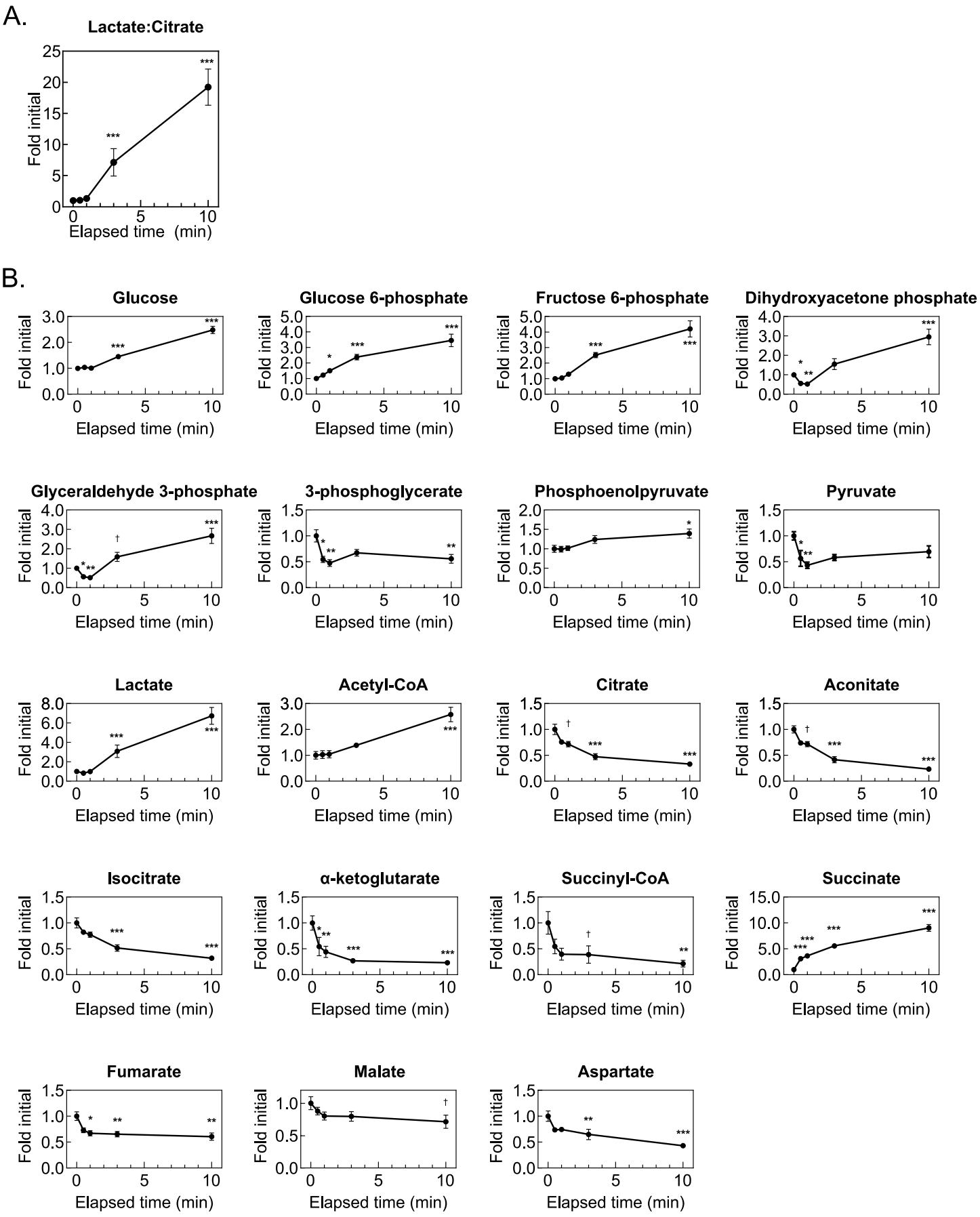


<u>Figure S2</u>: Supplement to Figure 2 - The post-dissection liver metabolome is rapidly remodeled by hypoxia

(A-B) Line graphs showing the NADH:NAD+ ratio (A) and AMP:ATP ratio (B) fold changes by delayed freezing time-point compared to time zero. Data are presented as the mean ± SEM. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison (n = 5-6 biological replicates).

**p < 0.01, ***p < 0.001.

Figure S3


A.
Lactate:Citrate
Fold initial
Elapsed time (min)
B.
Glucose
Glucose 6-phosphate
Fructose 6-phosphate
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
3-phosphoglycerate
Phosphoenolpyruvate
Pyruvate
Lactate
Acetyl-CoA
Citrate
Aconitate
Isocitrate
α-ketoglutarate
Succinyl-CoA
Succinate
Fumarate
Malate
Aspartate

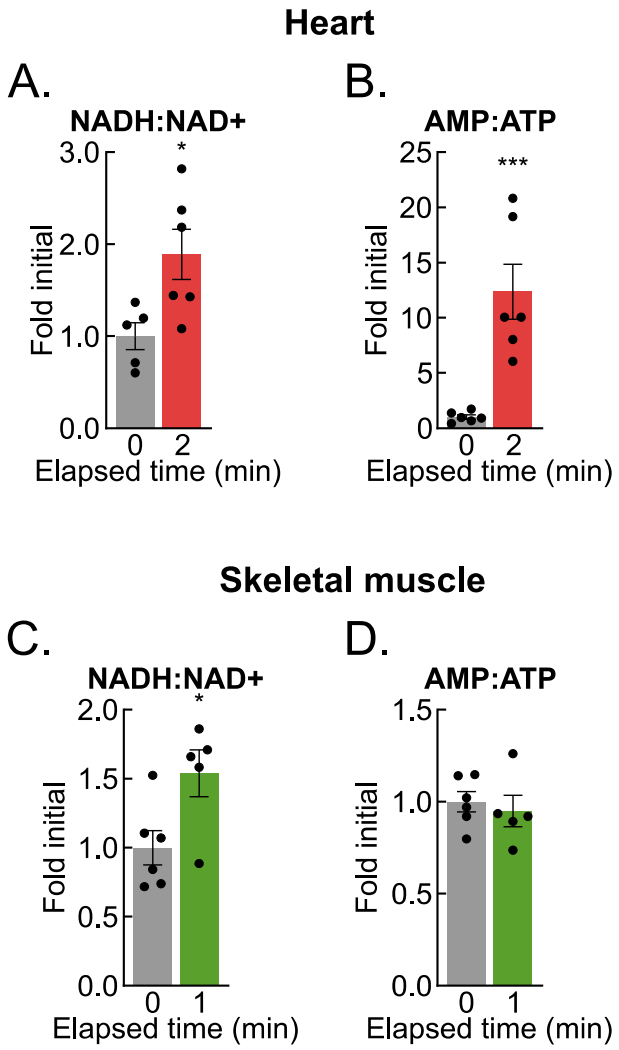
<u>Figure S3</u>: Supplement to Figure 3 - Post-dissection liver hypoxia leads to increased glycolytic and decreased TCA cycle activity

(A) Line graph showing the lactate:citrate ratio fold change by delayed freezing time-point compared to time zero. Data are presented as the mean ± SEM. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison (n = 5-6 biological replicates).

(B) Line graphs showing the Glycolytic and TCA cycle metabolite fold changes by delayed freezing time-point compared to time zero. Data are presented as the mean ± SEM. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison (n = 5-6 biological replicates).

$^{\dagger}p < 0.1$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Figure S4



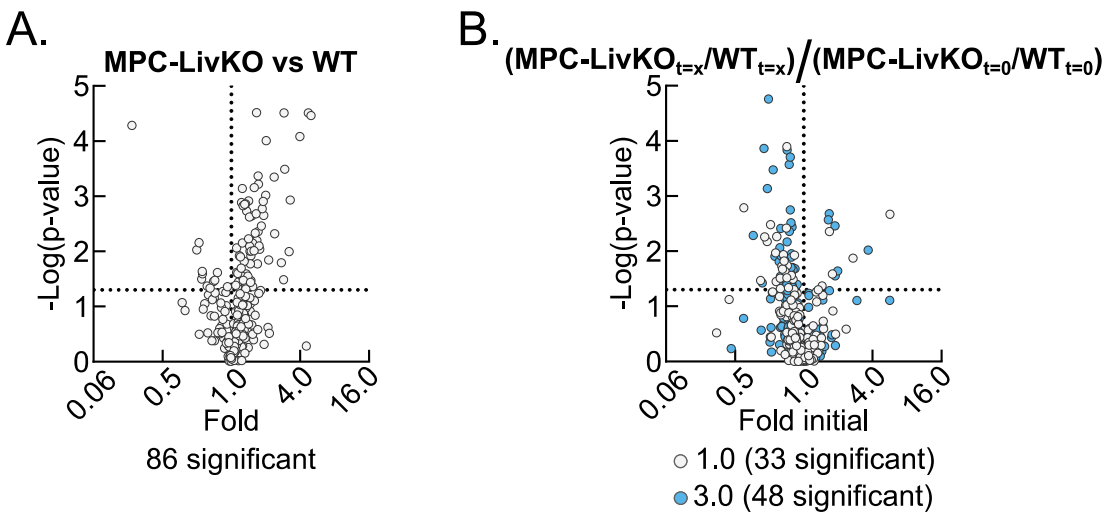
<u>Figure S4</u>: Supplement to Figure 5 - Post-dissection liver hypoxia leads to enormous increases in purine degradation metabolites

(A-B) Bar graphs showing the heart NADH:NAD+ ratio (A) and AMP:ATP ratio (B) fold changes at 2-minutes delayed freezing compared to time zero. Data are presented as the mean ± SEM. P-values were calculated using the Student's t-test (n = 5-6 biological replicates).

(C-D) Bar graph showing the skeletal muscle NADH:NAD+ ratio (C) and AMP:ATP ratio (D) fold changes at 1-minute delayed freezing compared to time zero. Data are presented as the mean ± SEM. P-values were calculated using the Student's t-test (n = 4-6 biological replicates).

*p < 0.05, ***p < 0.001.

Figure S5



<u>Figure S5</u>: Supplement to Figure 6 - Delayed liver freezing leads to both false negative and false positive between-genotype metabolite level differences

(A) Volcano plot showing the interaction between the MPC-LivKO compared to WT metabolite fold changes and associated p-values at time zero. The -log transformed p-values were calculated using the Student's t-test. The number of significant differences is listed in the panel (n = 4-5 biological replicates).

(B) Volcano plot showing the interaction between the MPC-LivKO/WT per metabolite ratios by delayed freezing time-point compared to the MPC-LivKO/WT per metabolite ratios at time zero. P-values were calculated by one-way ANOVA with the post-hoc Holm-Sidak multiple comparison test. The number of significant differences for each time point are listed in the panel (n = 4-5 biological replicates).