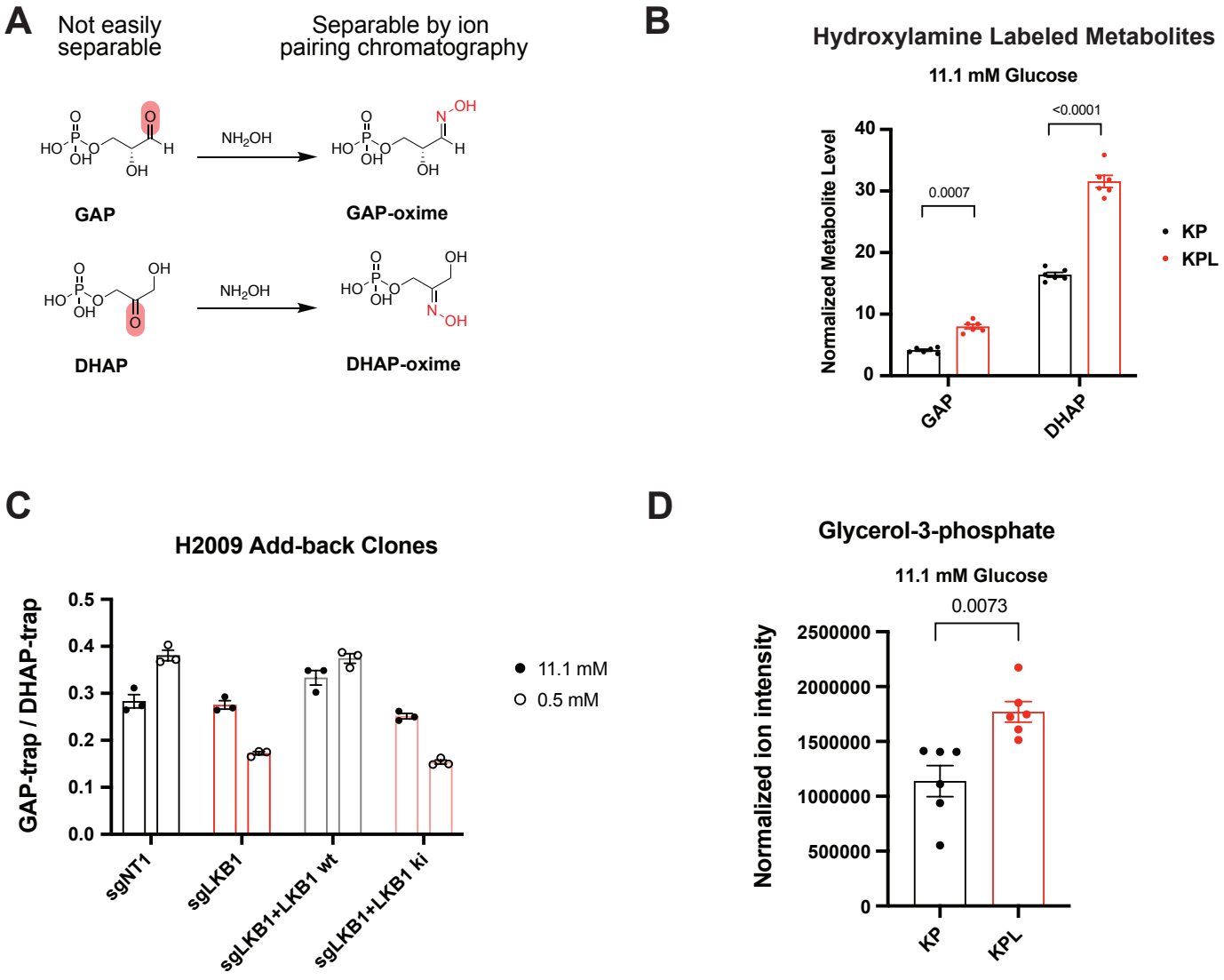

A
Not easily separable
Separable by ion pairing chromatography
NH2OH
GAP
GAP-oxime
DHAP
DHAP-oxime
B
Hydroxylamine Labeled Metabolites
11.1 mM Glucose
Normalized Metabolite Level
0.0007
<0.0001
KP
KPL
GAP
DHAP
C
H2009 Add-back Clones
GAP-trap / DHAP-trap
11.1 mM
0.5 mM
sgNT1
sgLKB1
sgLKB1+LKB1 wt
sgLKB1+LKB1 ki
D
Glycerol-3-phosphate
11.1 mM Glucose
0.0073
Normalized ion intensity
KP
KPL

Figure S3(i)

E

1,2-$^{13}C_2$ Glucose Tracing - 0.5 mM

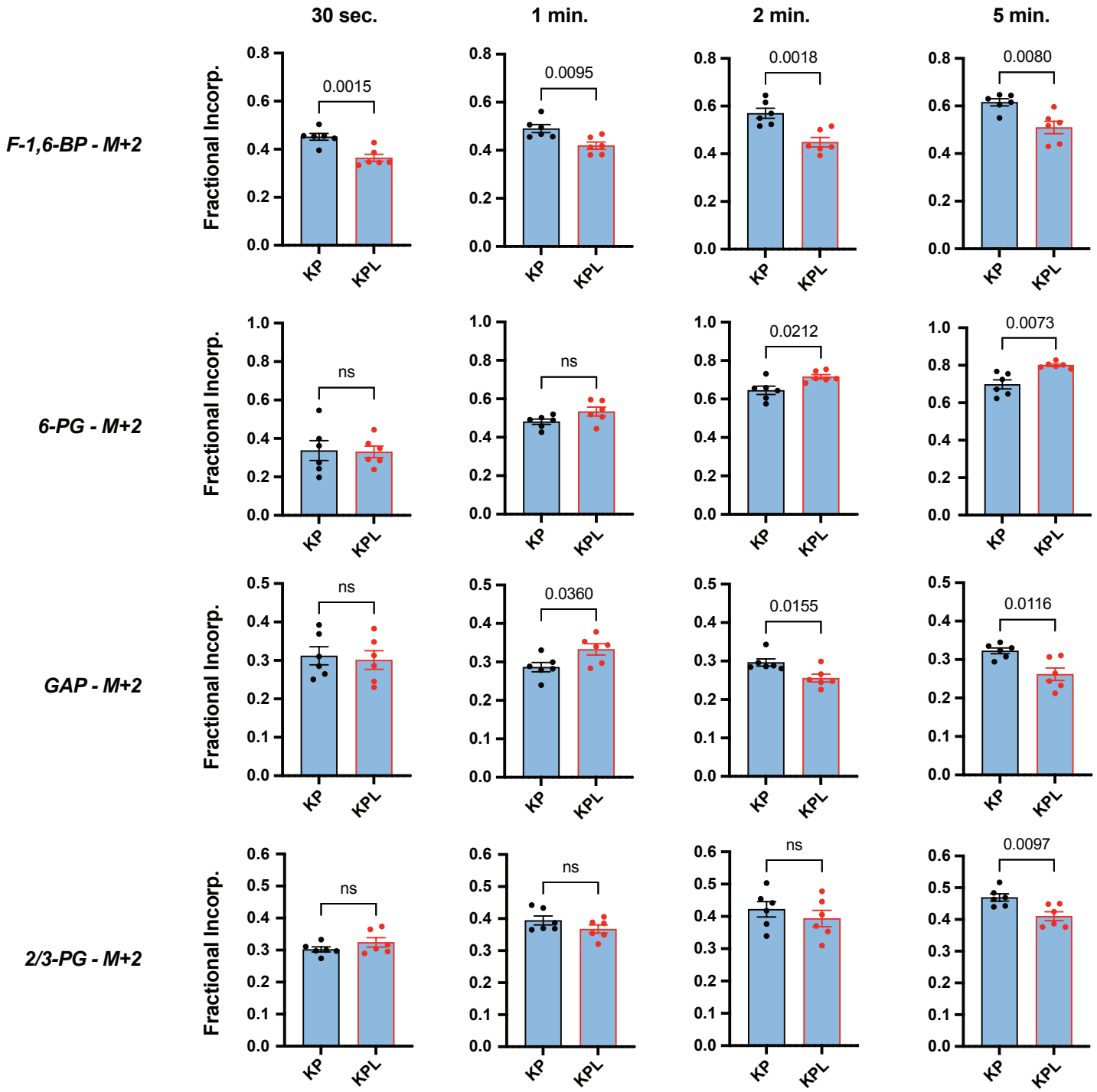
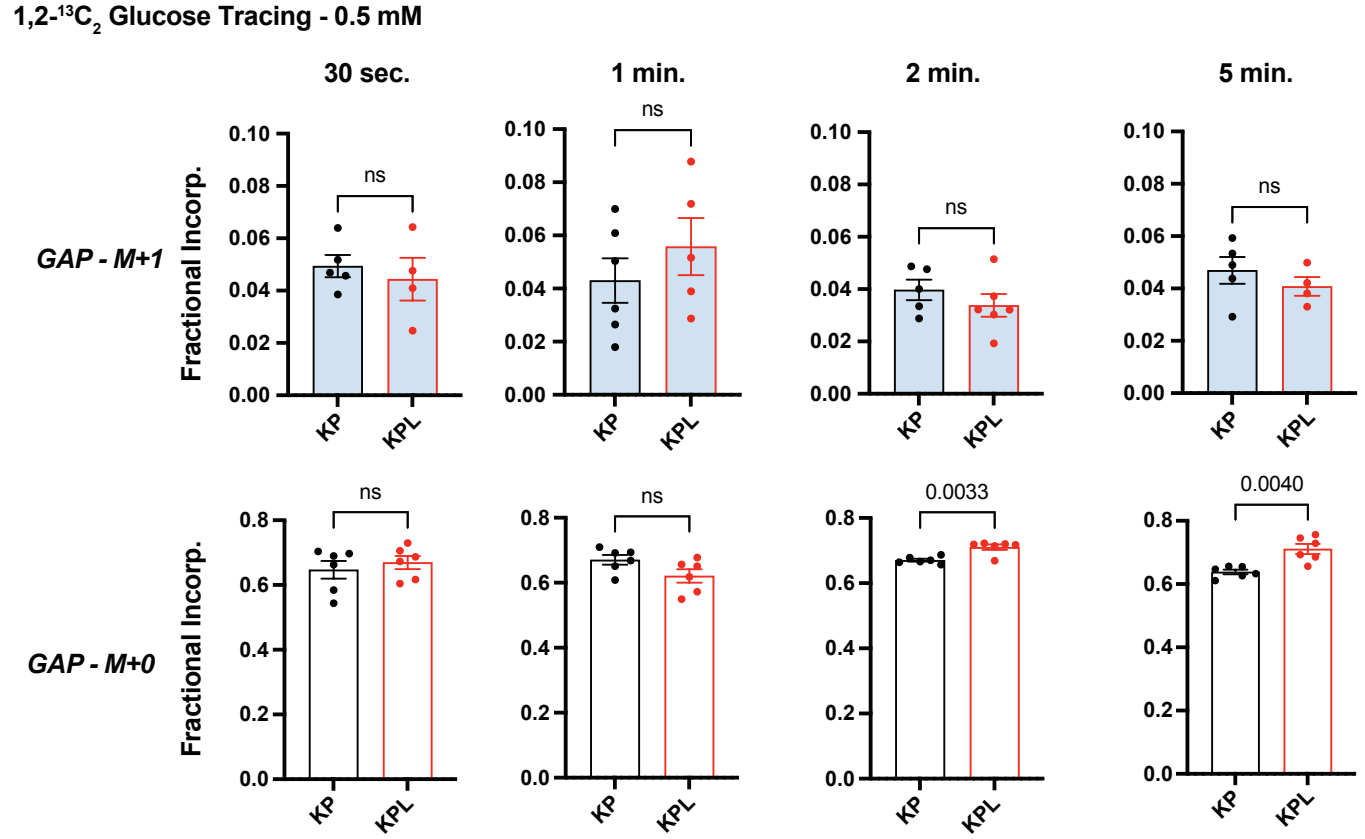


Figure S3(ii)

F



G

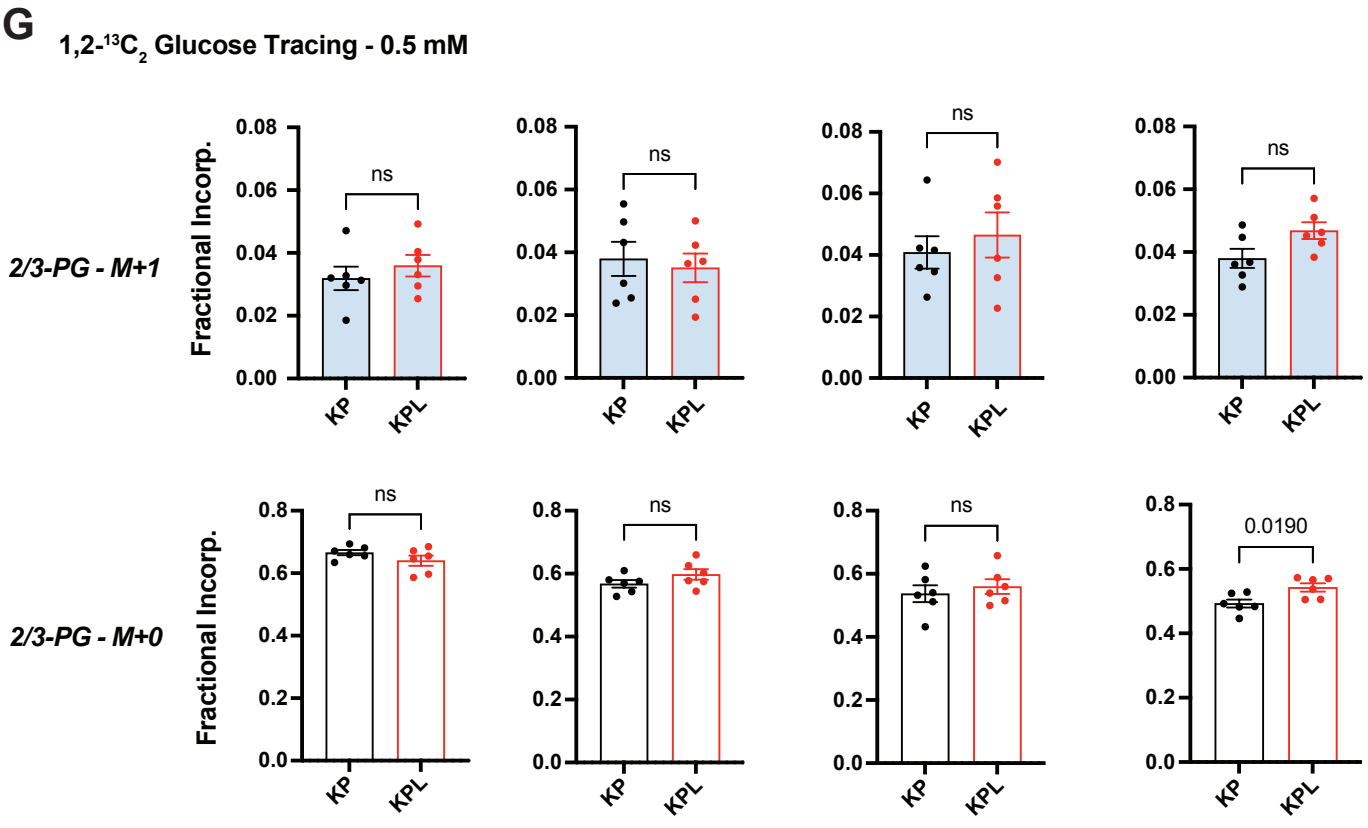


Figure S3(iii)

H

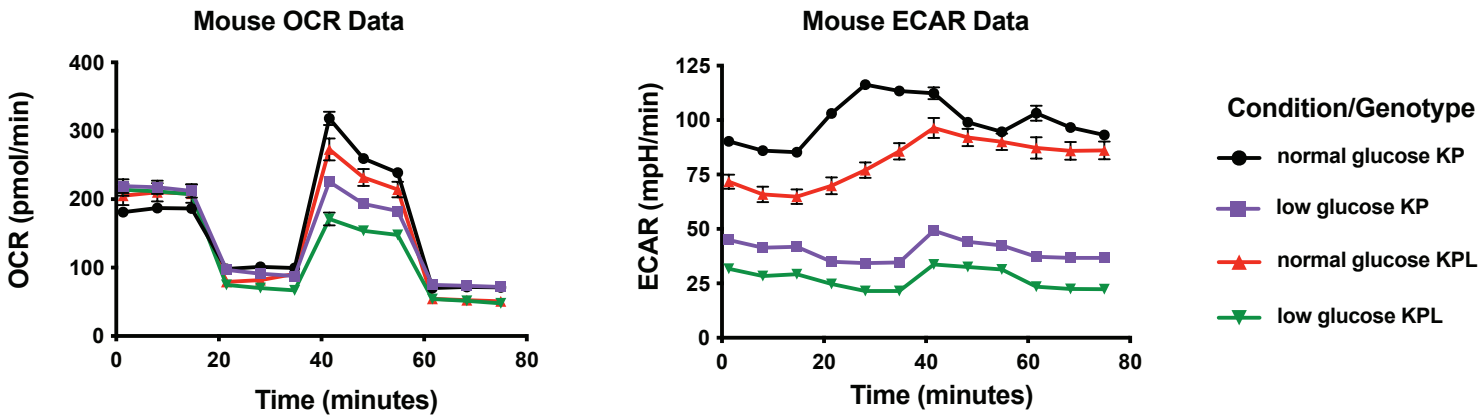


Figure S3(iv)

Figure S3. TPI1 phosphorylation regulates triose phosphate levels and metabolic flux.
(A) Schematic showing hydroxylamine chemical labeling and conversion of the triose phosphates; GAP and DHAP to their oxime derivatives. **(B)** *In-situ* chemical trapping metabolomics of hydroxylamine-labeled GAP and DHAP in H2009 clones (KP: sgNT1.1 and sgNT1.2; KPL: sgLKB1-3.1 and sgLKB1-3.7) treated in culture for 6 hours with 11.1 mM. Data presented are representative of three independent biological experiments each containing three technical replicates and reported as the mean (-/+s.e.m.). Cell number normalized across models 12 hours prior to assay and samples normalized to an exogenous standard, d_3-serine. Statistical significance determined by two-tailed paired t-test. **(C)** *In-situ* chemical trapping metabolomics of hydroxylamine-labeled GAP and DHAP in H2009 clones (KP: sgNT1.1 and sgNT1.2; KPL: sgLKB1-3.1 and sgLKB1-3.7) and additionally lines with transgenic expression of guide RNA resistant LKB1 wildtype (WT) (sgLKB1-3.1 + LKB1 WT and sgLKB1-3.7 + LKB1 WT) or LKB1 kinase inactive (KI) (sgLKB1-3.1 + LKB1 KI and sgLKB1-3.7 LKB1 KI) and treated in culture for 6 hours with 11.1 mM or 0.5 mM respectively. Data presented are representative of three independent biological experiments each containing two technical replicates and reported as the mean ratio (GAP-trap/DHAP-trap) (-/+s.e.m.). Cell number normalized across models 12 hours prior to assay and samples normalized to an exogenous standard, d_3-serine. **(D)** Normalized ion intensity of glycerol-3-phosphate from steady-state analysis of H2009 clones treated for 30 minutes with 11.1 mM glucose. Analysis conducted in H2009 isogenic clones (KP: sgNT1.1 and sgNT1.2; KPL: sgLKB1-3.1 and sgLKB1-3.7) in biological triplicate and reported as the mean (-/+s.e.m.). Statistical significance determined by two-tailed paired t-test. **(E)** Isotopic tracing results for M+2 isotopologues at 30 seconds, 1, 2 and 5 minute time points. Analysis conducted in H358 isogenic lines (KP: sgNT1.4 and sgNT1.6; KPL: sgLKB1-2.1 and sgLKB1-3.2) in biological triplicate (N=6 per genotype) and reported as the mean (-/+s.e.m.). Statistical significance determined by two-tailed paired t-test. **(F** and **G)** Isotopic tracing results for M+1 and M+0 isotopologues at 30 seconds, 1, 2 and 5 minute time points. Analysis conducted in H358 isogenic lines (KP: sgNT1.4 and sgNT1.6; KPL: sgLKB1-2.1 and sgLKB1-3.2) in biological triplicate (N=6 per genotype) and reported as the mean (-/+s.e.m.). Statistical significance determined by two-tailed paired t-test. **(H)** Mitochondrial Stress test results; Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) plotted over the course of the assay. Analysis conducted in mLUAD tumor cell-lines, N=6 per genotype and reported as the mean (-/+s.e.m.) and treated as indicated with normal (11.1 mM) or low (0.5 mM) glucose for 6 hr prior to analysis.