Figure S1

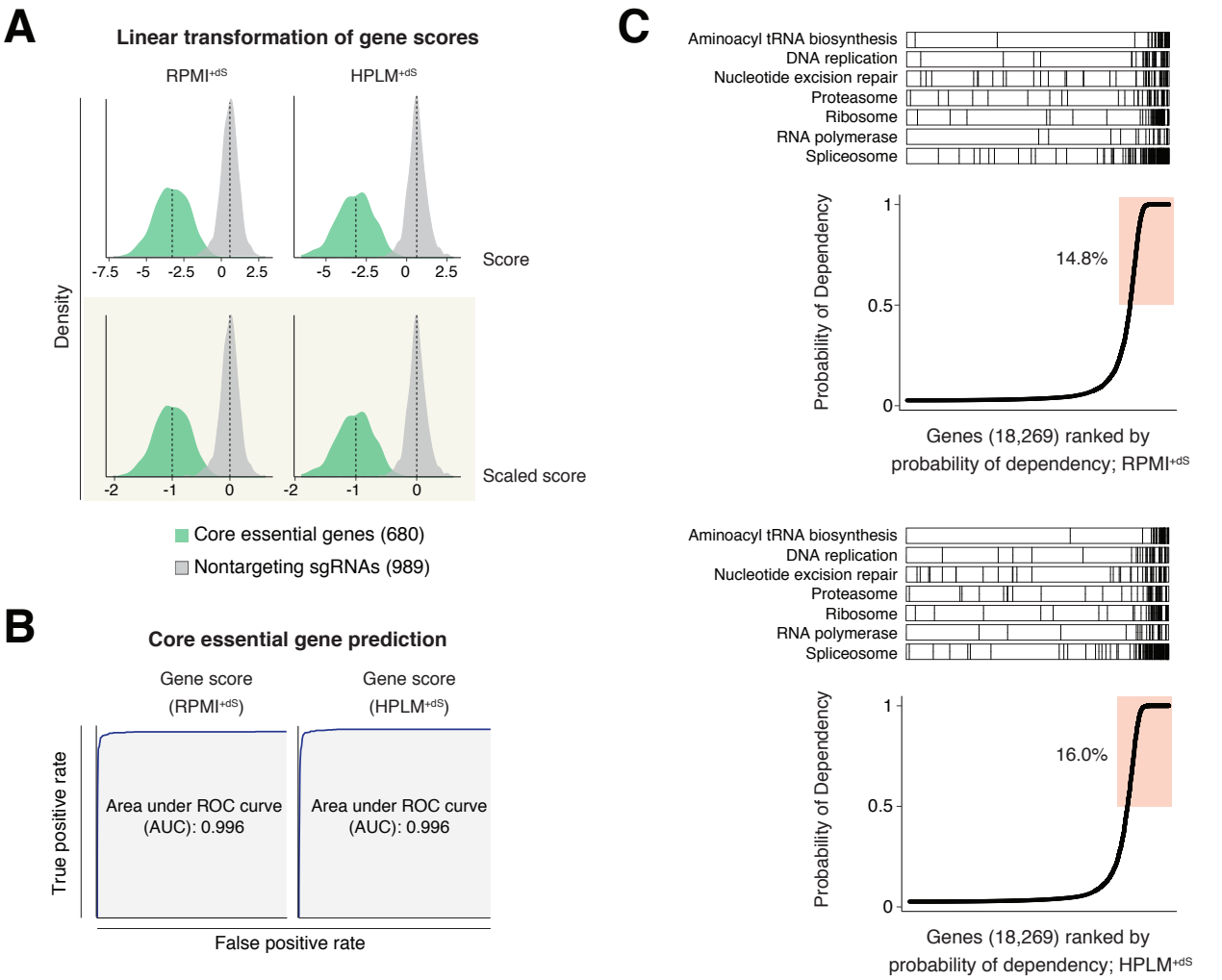

A
Linear transformation of gene scores
RPMI+dS
HPLM+dS
Density
Score
Scaled score
Core essential genes (680)
Nontargeting sgRNAs (989)
B
Core essential gene prediction
Gene score (RPMI+dS)
Gene score (HPLM+dS)
True positive rate
Area under ROC curve (AUC): 0.996
Area under ROC curve (AUC): 0.996
False positive rate
C
Aminoacyl tRNA biosynthesis
DNA replication
Nucleotide excision repair
Proteasome
Ribosome
RNA polymerase
Spliceosome
Probability of Dependency
14.8%
Genes (18,269) ranked by probability of dependency; RPMI+dS
16.0%
Genes (18,269) ranked by probability of dependency; HPLM+dS

Figure S1. Gene score scaling and genome-wide screen performance, Related to Figure 1

(A) Raw (top) and scaled (bottom) score distributions for 680 CEGs (gene score) and 989 non-targeting sgRNAs (depletion score) from genome-wide K562 screens.
(B) Receiver operator characteristic (ROC) curves for the prediction of CEGs using datasets from genome-wide K562 screens.
(C) Plots of targeted genes ranked by probability of dependency from genome-wide K562 screens in RPMI^{+dS} (top) and HPLM^{+dS} (bottom). Shaded region indicates probability > 0.5. Barcode plots depict the distribution of genes involved in several fundamental cellular processes.

Figure S2

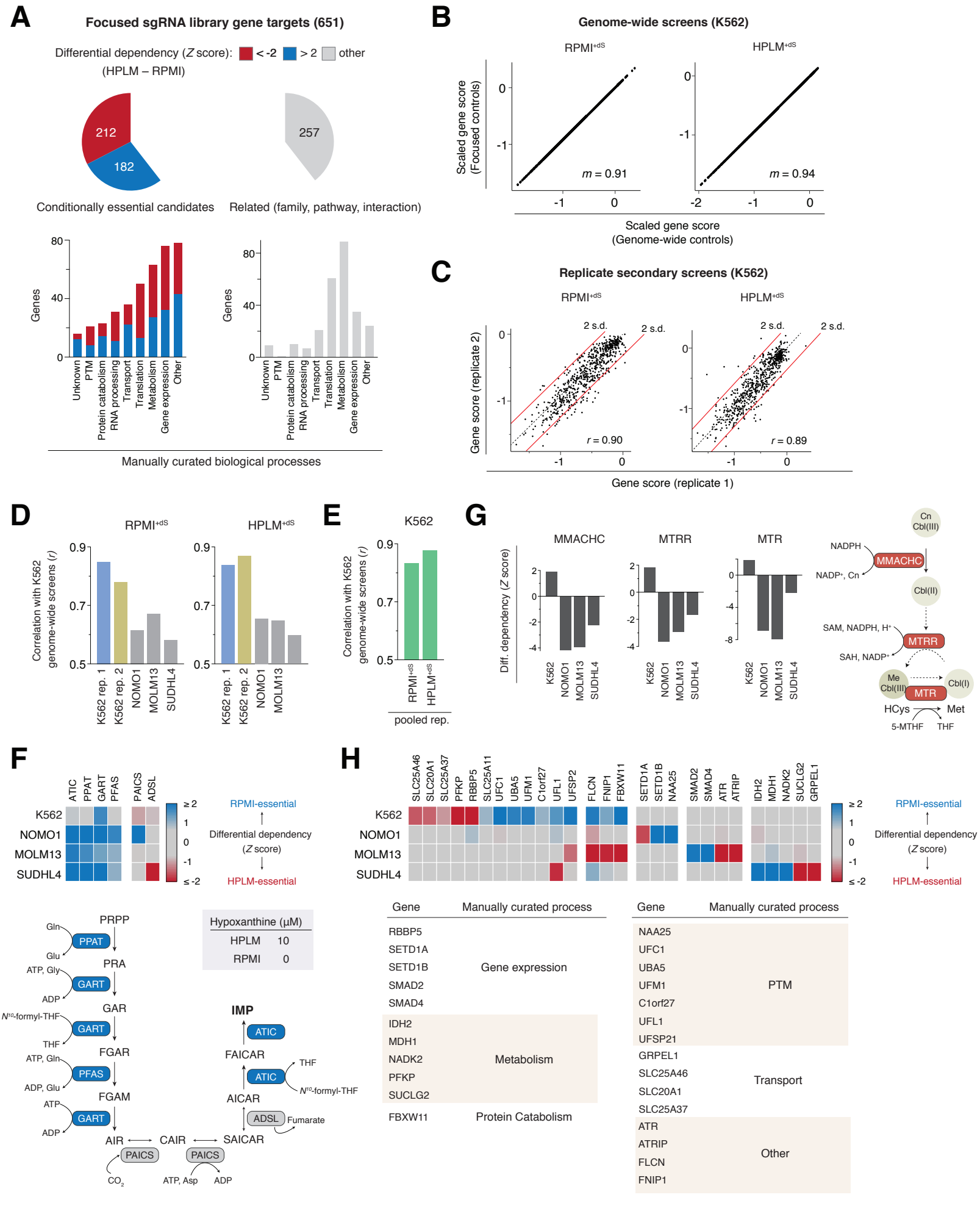

A
Focused sgRNA library gene targets (651)
Differential dependency (Z score): < -2 > 2 other
(HPLM – RPMI)
212
182
257
Conditionally essential candidates
Related (family, pathway, interaction)
Genes
Unknown
PTM
Protein catabolism
RNA processing
Transport
Translation
Metabolism
Gene expression
Other
Manually curated biological processes
B
Genome-wide screens (K562)
RPMI+dS
HPLM+dS
Scaled gene score (Focused controls)
m = 0.91
m = 0.94
Scaled gene score (Genome-wide controls)
C
Replicate secondary screens (K562)
2 s.d.
r = 0.90
r = 0.89
Gene score (replicate 2)
Gene score (replicate 1)
D
Correlation with K562 genome-wide screens (r)
K562 rep. 1
K562 rep. 2
NOMO1
MOLM13
SUDHL4
E
K562
RPMI+dS
HPLM+dS
pooled rep.
G
MMACHC
MTRR
MTR
Diff. dependency (Z score)
Cn Cbl(III)
NADPH
NADP+, Cn
Cbl(II)
SAM, NADPH, H+
SAH, NADP+
Me Cbl(III)
Cbl(I)
HCys
Met
5-MTHF
THF
F
ATIC
PPAT
GART
PFAS
PAICS
ADSL
RPMI-essential
Differential dependency (Z score)
HPLM-essential
PRPP
PPAT
PRA
GART
GAR
GART
FGAR
PFAS
FGAM
GART
AIR
CAIR
SAICAR
PAICS
PAICS
CO2
ATP, Asp
ADP
IMP
ATIC
FAICAR
ATIC
THF
AICAR
ADSL
Fumarate
Hypoxanthine (μM)
HPLM 10
RPMI 0
H
SLC25A46
SLC20A1
SLC25A37
PFKP
RBBP5
SLC25A11
UFC1
UBA5
UFM1
C1orf27
UFL1
UFSP2
FLCN
FNIP1
FBXW11
SETD1A
SETD1B
NAA25
SMAD2
SMAD4
ATR
ATRIP
IDH2
MDH1
NADK2
SUCLG2
GRPEL1
Gene
Manually curated process
RBBP5
SETD1A
SETD1B
SMAD2
SMAD4
Gene expression
IDH2
MDH1
NADK2
PFKP
SUCLG2
Metabolism
FBXW11
Protein Catabolism
NAA25
UFC1
UBA5
UFM1
C1orf27
UFL1
UFSP21
PTM
GRPEL1
SLC25A46
SLC20A1
SLC25A37
Transport
ATR
ATRIP
FLCN
FNIP1
Other

Figure S2. Focused sgRNA library design and correlation analyses; additional examples of genes with cell-specific conditional CRISPR phenotypes, Related to Figure 2

(A) Fraction of genes targeted by the focused sgRNA library with indicated conditional phenotypes from genome-wide K562 screens (top). Composition of focused sgRNA library gene targets by manually curated cellular processes (bottom).

(B) Comparison of gene scores from genome-wide K562 screens scaled by using CEG and non-targeting sgRNA control sets from either the genome-wide or focused sgRNA library. *m*, slope.

(C) Comparison of gene scores from replicate secondary K562 screens. *r*, Pearson's correlation coefficient.

(D) Gene score correlations between secondary screens in four cell lines and genome-wide K562 screens.

(E) Gene score correlations between pooled secondary K562 screen datasets and genome-wide K562 screens.

(F) Heatmap of conditional phenotypes for the indicated genes (top). Schematic for the de novo purine biosynthesis pathway (bottom). Enzymes encoded by the four genes that share a common RPMI-essential phenotype in three cell lines are shaded blue and catalyze irreversible pathway steps based on annotations in the KEGG Pathway database (Kanehisa et al., 2017). Data for secondary K562 screens are from pooled replicates in panels F-H.

(G) Conditional phenotypes for *MMACHC* (left), *MTRR* (middle), and *MTR* (right). Schematic for the processing of CnCbl (III) to Cbl (II) and downstream conversion of HCys to Met coupled with the reductive generation of Cbl(I) to MeCbl (III) (furthest right). Cbl(I), cob(I)alamin; Cbl(II), cob(II)alamin; CnCbl(III), cyanocob(III)alamin; MeCbl(III), methylcob(III)alamin; 5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate.

(H) Heatmap of conditional phenotypes for the indicated genes (top). Manually curated processes for each gene (bottom).

Figure S3

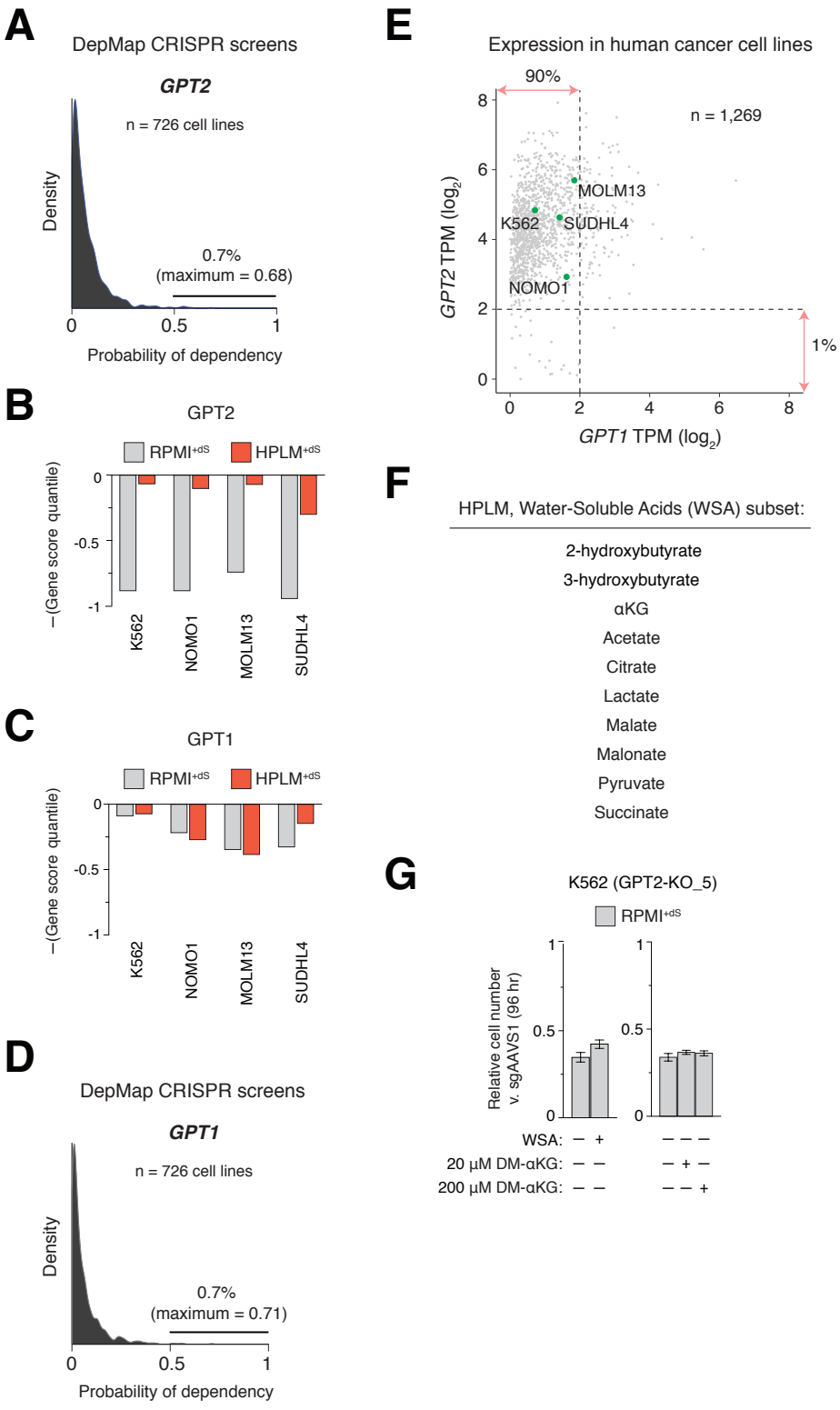

A
DepMap CRISPR screens
GPT2
n = 726 cell lines
Density
0.7%
(maximum = 0.68)
Probability of dependency
B
GPT2
RPMI+dS
HPLM+dS
−(Gene score quantile)
K562
NOMO1
MOLM13
SUDHL4
C
GPT1
D
DepMap CRISPR screens
GPT1
n = 726 cell lines
0.7%
(maximum = 0.71)
E
Expression in human cancer cell lines
90%
n = 1,269
MOLM13
K562
SUDHL4
NOMO1
1%
GPT2 TPM (log2)
GPT1 TPM (log2)
F
HPLM, Water-Soluble Acids (WSA) subset:
2-hydroxybutyrate
3-hydroxybutyrate
αKG
Acetate
Citrate
Lactate
Malate
Malonate
Pyruvate
Succinate
G
K562 (GPT2-KO_5)
RPMI+dS
Relative cell number
v. sgAAVS1 (96 hr)
WSA:
20 μM DM-αKG:
200 μM DM-αKG:

Figure S3. Additional data related to identification of a gene-nutrient interaction between *GPT2* and alanine, Related to Figure 3

(A) Density plot for probability of dependency values annotated for *GPT2* across CRISPR screens from DepMap. Probability > 0.5 is the reference threshold for essentiality.

(B-C) Gene score quantiles for *GPT2* (B) and *GPT1* (C) from secondary screens. Data for secondary K562 screens are from pooled replicates.

(D) Density plot for probability of dependency values annotated for *GPT1* across CRISPR screens from DepMap. Probability > 0.5 is the reference threshold for essentiality.

(E) Comparison between *GPT1* and *GPT2* RNA levels in human cancer lines from RNA-seq data in DepMap. Labeled points indicate cell lines in this study. TPM, transcripts per million.

(F) Metabolites that comprise the water-soluble acids (WSAs) subset of HPLM components.

(G) Relative growth of *GPT2*-knockout versus control cells (mean ± SD, $n = 3$). DM, dimethyl.

Figure S4

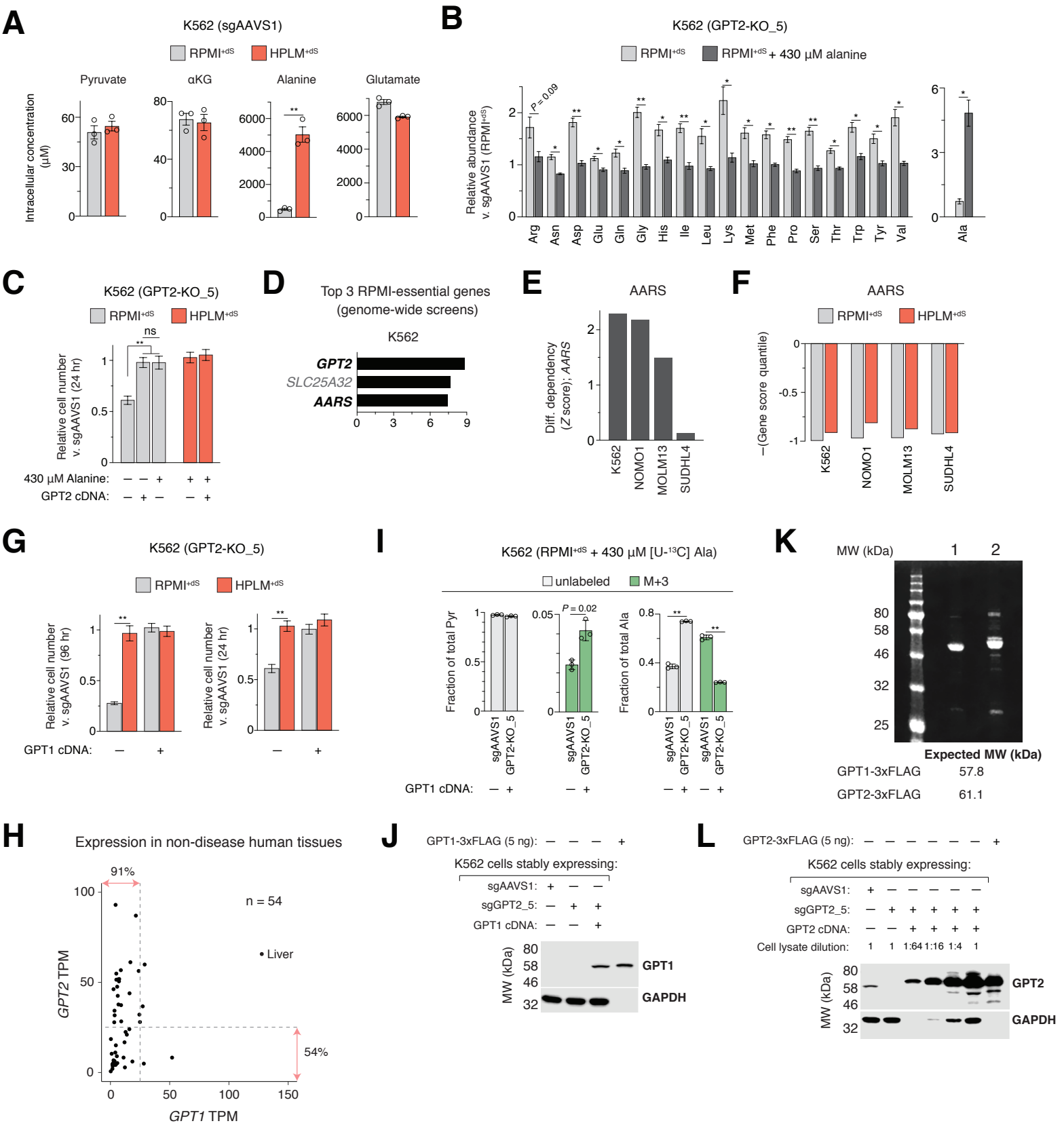

A
K562 (sgAAVS1)
RPMI+dS
HPLM+dS
Pyruvate
αKG
Alanine
Glutamate
Intracellular concentration (μM)
B
K562 (GPT2-KO_5)
RPMI+dS
RPMI+dS + 430 μM alanine
Relative abundance v. sgAAVS1 (RPMI+dS)
P = 0.09
Arg Asn Asp Glu Gln Gly His Ile Leu Lys Met Phe Pro Ser Thr Trp Tyr Val Ala
C
K562 (GPT2-KO_5)
RPMI+dS
HPLM+dS
ns
Relative cell number v. sgAAVS1 (24 hr)
430 μM Alanine:
GPT2 cDNA:
D
Top 3 RPMI-essential genes (genome-wide screens)
K562
GPT2
SLC25A32
AARS
E
AARS
Diff. dependency (Z score); AARS
K562 NOMO1 MOLM13 SUDHL4
F
AARS
RPMI+dS
HPLM+dS
−(Gene score quantile)
K562 NOMO1 MOLM13 SUDHL4
G
K562 (GPT2-KO_5)
RPMI+dS
HPLM+dS
Relative cell number v. sgAAVS1 (96 hr)
Relative cell number v. sgAAVS1 (24 hr)
GPT1 cDNA:
I
K562 (RPMI+dS + 430 μM [U-13C] Ala)
unlabeled
M+3
Fraction of total Pyr
Fraction of total Ala
P = 0.02
sgAAVS1 GPT2-KO_5
GPT1 cDNA:
K
MW (kDa)
1 2
80 58 46 32 25
Expected MW (kDa)
GPT1-3xFLAG 57.8
GPT2-3xFLAG 61.1
H
Expression in non-disease human tissues
91%
n = 54
• Liver
54%
GPT2 TPM
GPT1 TPM
J
GPT1-3xFLAG (5 ng):
K562 cells stably expressing:
sgAAVS1:
sgGPT2_5:
GPT1 cDNA:
MW (kDa)
80 58 46 32
GPT1
GAPDH
L
GPT2-3xFLAG (5 ng):
K562 cells stably expressing:
sgAAVS1:
sgGPT2_5:
GPT2 cDNA:
Cell lysate dilution: 1 1 1:64 1:16 1:4 1
MW (kDa)
80 58 46 32
GPT2
GAPDH

Figure S4. Additional data related to protein synthesis underlies the *GPT2*-alanine interaction and human GPTs show markedly different K_M values for pyruvate, Related to Figure 4

(A) Concentrations of GPT reaction components in control (sgAAVS1) K562 cells following 24 hr culture (mean ± SEM, $n = 3$, $^{**}P < 0.01$).

(B) Relative amino acid levels in *GPT2*-knockout cells following culture in either RPMI^{+dS} (light gray) or RPMI^{+dS} containing 430 μM alanine (dark gray) versus control cells in RPMI^{+dS} (mean ± SEM, $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.005$).

(C) Relative growth of *GPT2*-knockout versus control cells (mean ± SD, $n = 3$, $^{**}P < 0.005$). ns, not significant.

(D) Top three scoring RPMI-essential hits from genome-wide K562 screens.

(E) Conditional phenotypes for *AARS*. Data for secondary K562 screens are from pooled replicates in panels E-F.

(F) Gene score quantiles for *AARS* from secondary screens.

(G) Relative growth of *GPT2*-knockout versus control cells (mean ± SD, $n = 3$, $^{**}P < 0.005$).

(H) Comparison of *GPT1* versus *GPT2* RNA levels in non-disease human tissues from RNA-seq data in GTEx. Point corresponding to 'liver' is indicated. Dotted lines indicate TPM (transcripts per million) = 25.

(I) Fractional labeling of pyruvate (left) and alanine (right) following culture of cells in RPMI^{+dS} containing [U-^{13}C]-alanine (mean ± SD, $n = 3$, $^{**}P < 0.005$). M+3, incorporation of three ^{13}C.

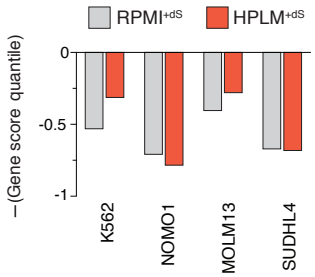
(J) Immunoblot for expression of GPT1 in cells and from purified GPT1-3xFLAG. M.W. standards are annotated. GAPDH served as a loading control.

(K) Pseudocolor Coomassie-stained gel imaged using a LICOR Odyssey FC. 1: GPT1-3xFLAG, 2: GPT2-3xFLAG (see STAR Methods for note on observed M.W. versus those in immunoblots of the same purified proteins).

(L) Immunoblot for expression of GPT2 in cells and from purified GPT2-3xFLAG. M.W. standards are annotated. Cells transduced with cDNA were loaded in serial dilution as indicated. GAPDH served as a loading control.

Figure S5

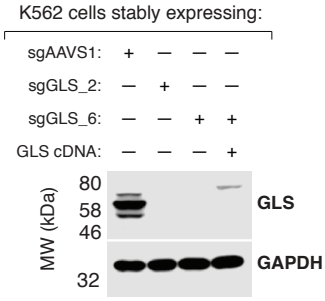
A



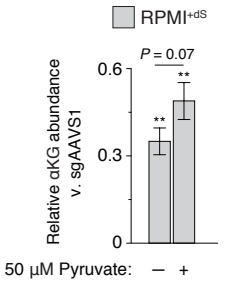
B

Concentration (μM)	RPMI+dS	HPLM+dS
Cystine	170 ± 10	97 ± 5.5
Cysteine	0	40

C



D



E

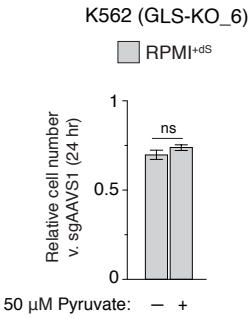


Figure S5. Additional data related to identification of a gene-nutrient interaction between *GLS* and pyruvate, Related to Figure 5

(A) Gene score quantiles for *GLS* from secondary screens. Data for secondary K562 screens are from pooled replicates

(B) Measured concentrations of cystine in $RPMI^{+dS}$ and $HPLM^{+dS}$ (mean ± SD, $n = 3$). Cysteine could not be detected by the metabolite profiling method but is also a defined component in HPLM.

(C) Immunoblot for expression of GLS. M.W. standards are annotated. GAPDH served as a loading control.

(D) Relative αKG levels in *GLS*-knockout cells following 24 hr culture in either $RPMI^{+dS}$ or $RPMI^{+dS}$ containing 50 μM pyruvate versus control cells in $RPMI^{+dS}$ (mean ± SEM, $n = 3$, $^{**}P < 0.005$).

(E) Relative growth of *GLS*-knockout versus control cells in the indicated conditions (mean ± SD, $n = 3$). ns, not significant.

Figure S6

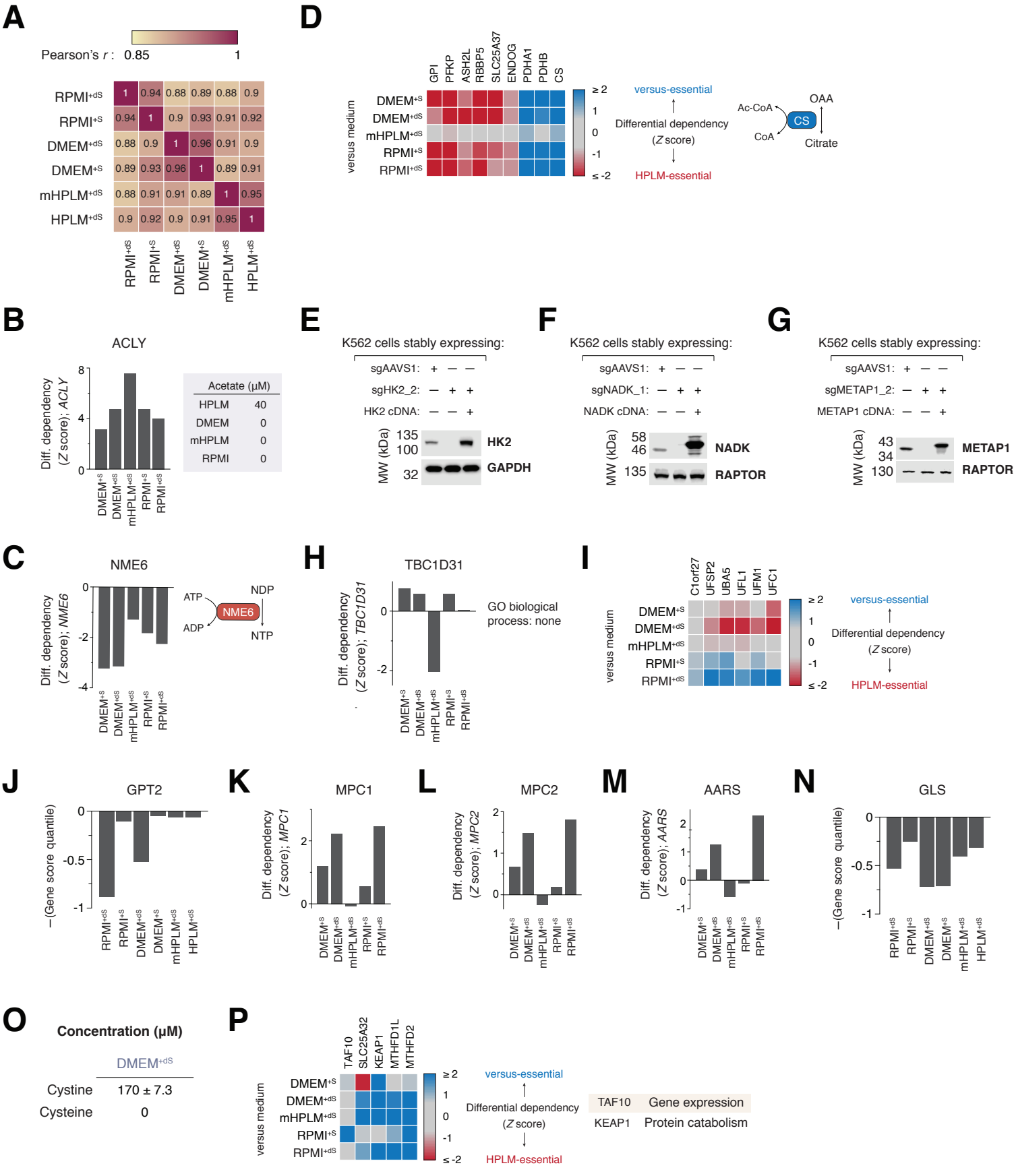

A
Pearson's r : 0.85 1
RPMI+dS 1 0.94 0.88 0.89 0.88 0.9
RPMI+S 0.94 1 0.9 0.93 0.91 0.92
DMEM+dS 0.88 0.9 1 0.96 0.91 0.9
DMEM+S 0.89 0.93 0.96 1 0.89 0.91
mHPLM+dS 0.88 0.91 0.91 0.89 1 0.95
HPLM+dS 0.9 0.92 0.9 0.91 0.95 1
D
GPI PFKP ASH2L RBBP5 SLC25A37 ENDOG PDHA1 PDHB CS
versus medium
DMEM+S
DMEM+dS
mHPLM+dS
RPMI+S
RPMI+dS
≥ 2 1 0 -1 ≤ -2
versus-essential
Differential dependency (Z score)
HPLM-essential
Ac-CoA CoA CS OAA Citrate
B
ACLY
Diff. dependency (Z score); ACLY
Acetate (μM)
HPLM 40
DMEM 0
mHPLM 0
RPMI 0
E
K562 cells stably expressing:
sgAAVS1: + — —
sgHK2_2: — + +
HK2 cDNA: — — +
MW (kDa) 135 100 HK2
32 GAPDH
F
K562 cells stably expressing:
sgAAVS1: + — —
sgNADK_1: — + +
NADK cDNA: — — +
MW (kDa) 58 46 NADK
135 RAPTOR
G
K562 cells stably expressing:
sgAAVS1: + — —
sgMETAP1_2: — + +
METAP1 cDNA: — — +
MW (kDa) 43 34 METAP1
130 RAPTOR
C
NME6
Diff. dependency (Z score); NME6
ATP ADP NME6 NDP NTP
H
TBC1D31
Diff. dependency (Z score); TBC1D31
GO biological process: none
I
C1orf27 UFSP2 UBA5 UFL1 UFM1 UFC1
versus medium
DMEM+S
DMEM+dS
mHPLM+dS
RPMI+S
RPMI+dS
≥ 2 1 0 -1 ≤ -2
versus-essential
Differential dependency (Z score)
HPLM-essential
J
GPT2
−(Gene score quantile)
K
MPC1
Diff. dependency (Z score); MPC1
L
MPC2
Diff. dependency (Z score); MPC2
M
AARS
Diff. dependency (Z score); AARS
N
GLS
−(Gene score quantile)
O
Concentration (μM)
DMEM+dS
Cystine 170 ± 7.3
Cysteine 0
P
TAF10 SLC25A32 KEAP1 MTHFD1L MTHFD2
versus medium
DMEM+S
DMEM+dS
mHPLM+dS
RPMI+S
RPMI+dS
≥ 2 1 0 -1 ≤ -2
versus-essential
Differential dependency (Z score)
HPLM-essential
TAF10 Gene expression
KEAP1 Protein catabolism

Figure S6. Additional examples of genes with conditional phenotypes and additional data related to phenotypes for loss of *GPT2*, *MPC1/2*, *AARS*, and *GLS*, Related to Figure 6

(A) Gene score correlations between secondary K562 screens in six conditions. Data for screens in RPMI^{+dS} and HPLM^{+dS} are from pooled replicates in panels A-D; H-N; and P.

(B-C) HPLM-relative phenotypes for *ACLY* (B) and *NME6* (C). Defined acetate levels in each basal medium (B, right). Reaction catalyzed by NME6 (C, right).

(D) Heatmap of HPLM-relative phenotypes for the indicated genes (left). Reaction catalyzed by CS (right). Remaining genes are highlighted in Figure 2.

(E-G) Immunoblots for expression of HK2 (E), NADK (F), and METAP1 (G). M.W. standards are annotated. GAPDH (E) and RAPTOR (F and G) served as loading controls.

(H) HPLM-relative phenotypes for *TBC1D31*.

(I) Heatmap of HPLM-relative phenotypes for genes that encode components of the UFMylation machinery.

(J) Gene score quantiles for *GPT2* from secondary screens.

(K-M) HPLM-relative phenotypes for *MPC1* (B), *MPC2* (C), and *AARS* (D).

(N) Gene score quantiles for *GLS* from secondary screens.

(O) Measured concentration of cystine DMEM^{+dS} (mean ± SD, n = 3). Cysteine is not a defined component in DMEM.

(P) Heatmap of HPLM-relative phenotypes for the indicated genes (left). Manually curated processes for TAF10 and KEAP1 (right). Remaining genes are highlighted in Figure 2.