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Supplemental information

Metabolic signature and response to glutamine deprivation are independent of p53 status in B cell malignancies

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Supplemental information titles and legends

Figure S1: p53 loss confers a survival advantage to DNA damage-induced cell death but does not dictate changes in metabolic activity or growth in malignant B-cells, Related to Figure 1. (A) PGA-1 cells with different *TP53* status were treated with various concentrations of fludarabine for 24 hours, viability was measured by flow cytometry using Mito-O/To-Pro-3 staining (N=3). Data are presented as mean \pm SEM and differences were analysed with two-way ANOVA with Tukey's multiple comparisons test (* $p < 0,05$). **TP53KO* #1 vs WT, #*TP53KO* #2 vs WT, \$*TP53KO* #3 vs WT. (B) LME-1 cell lines were subjected to 5 Gy IR, and 48 hours later viability was assessed by flow cytometry using Mito-O/To-Pro-3 staining (N=1). (C) Metabolomics analyses were performed after 48h of culture in all cell lines, as shown in Figure 1B. Pathway topology analysis was performed by grouping all samples according to p53 status (*TP53WT* N=6, *TP53KO* N=9) using MetaboAnalyst v5.0. X-axis represents the impact of the pathway on the differences between *TP53WT* and KO, Y-axis shows $-\log_{10}(p\text{-value})$. Node size is relative to pathway impact and colouring is based on p-value. (D) Relative abundance of PPP intermediates in each cell line is shown, each dot represents an independent sample. (E) *TP53WT* and *TP53KO* cells were cultured in complete RPMI medium for 48 h and Seahorse Mito Stress Test was performed. Basal OCR and basal ECAR were measured (PGA-1 N=6; OCI-Ly10 N=2, LME-1 N=2, Nalm-6 N=2). Each dot represents an independent sample. W: WT, M: Mock. (F) *TP53WT* and *TP53KO* cells were cultured in complete medium, growth rate was measured by counting cells with CasyCounter over time (Nalm-6, N=2 (presented as mean \pm SEM); PGA-1, N=1; OCILy10, N=2; LME-1, N=1).

Fig S2. *TP53* loss does not rewire glucose metabolism in PGA-1 cells, Related to Figure 2E. PGA-1 WT, MOCK, *TP53KO* #1 and #2 cells were cultured in complete media or glutamine deprivation (Gln-) for 24h and subsequently incubated in the presence of 5mM $^{13}\text{C}_6$ -Glucose. Mean enrichment indicates the ^{13}C -labelled fraction of a given metabolite relative to the total amount of that metabolite within a sample. WT cells grown in complete media had to be excluded from the analysis due to low quality of MS peaks. Additional data regarding nucleotide synthesis is shown in Figure 2E.

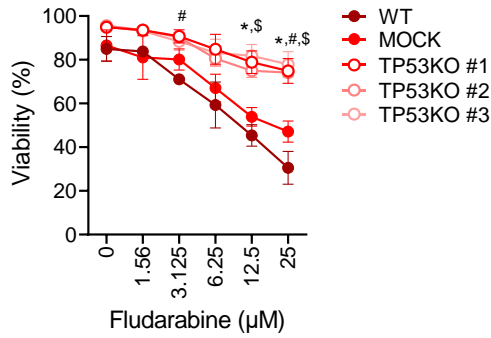
Fig S3. Glutamine deprivation induces the integrated stress response pathway in malignant B-cell lines, Related to Figure 3. Cells were cultured in control media or glutamine deprivation (Gln-) for A) 24 hours or B) 72 hours before RNA extraction. *CHOP* and *ATF4* mRNA levels were measured by RT-qPCR. (N=1).

Table S1. Raw data from pathway analysis, Related to Figure 1C. Pathway analysis was performed on cell-line specific metabolites (see heatmap, Fig. 1C) using MetaboAnalyst 5.0. A cut-off of $FDR < 0.061$ was applied, pathways above the threshold are listed.

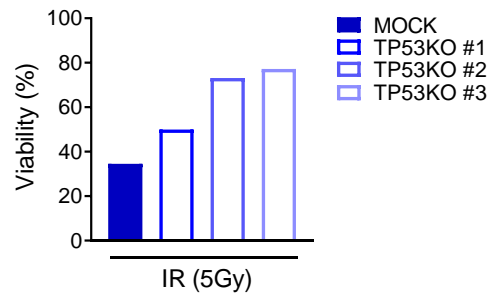
Table S2. CGH analysis of cell lines, Related to Figure 1. CGH array was performed on genomic DNA from all cell lines and also from PGA-1 and Nalm-6 TP53KO. Alterations were identified and listed.

Figure S1

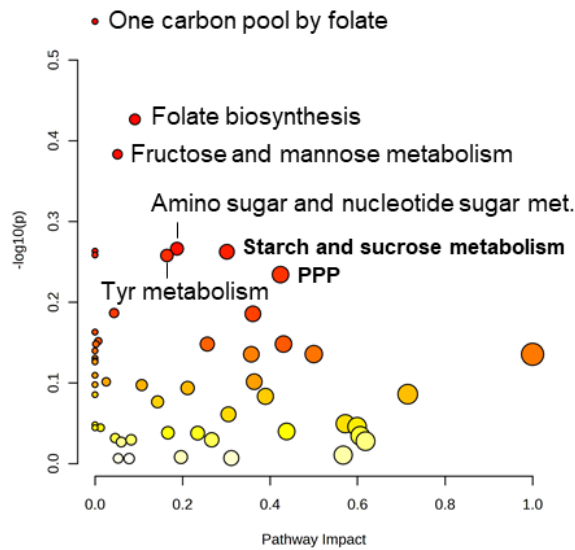
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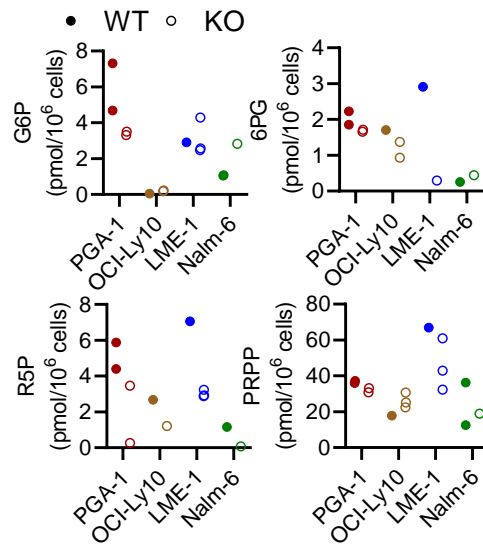
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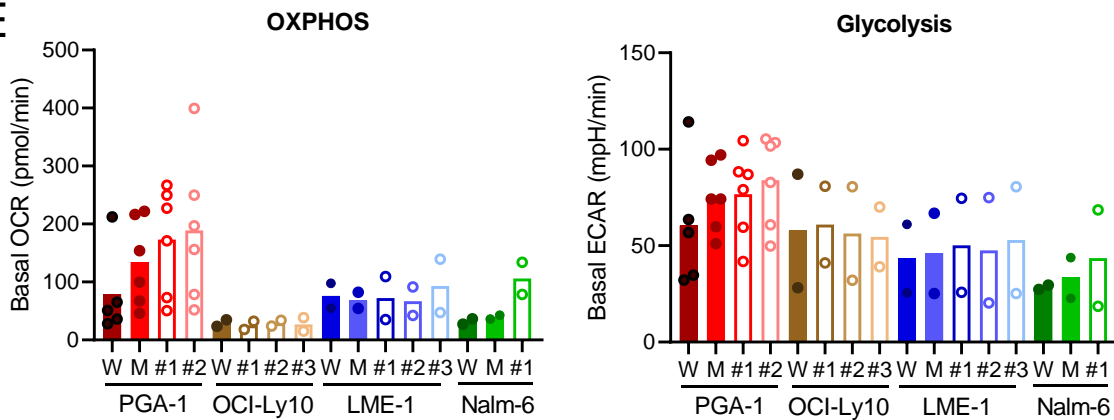
C



D



E



F

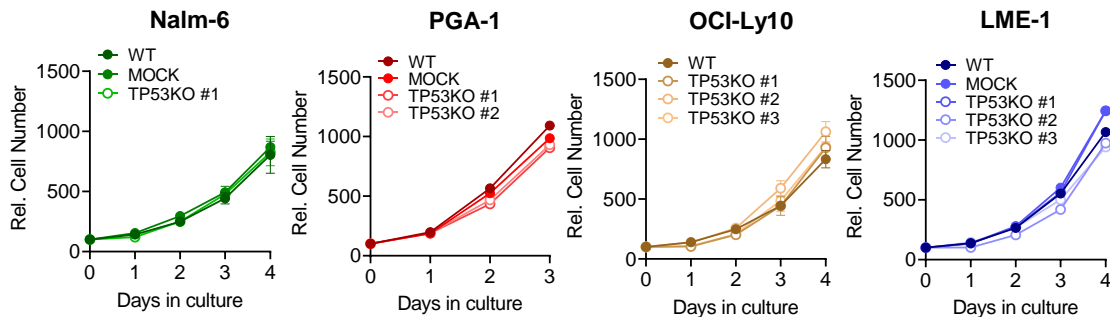


Figure S2

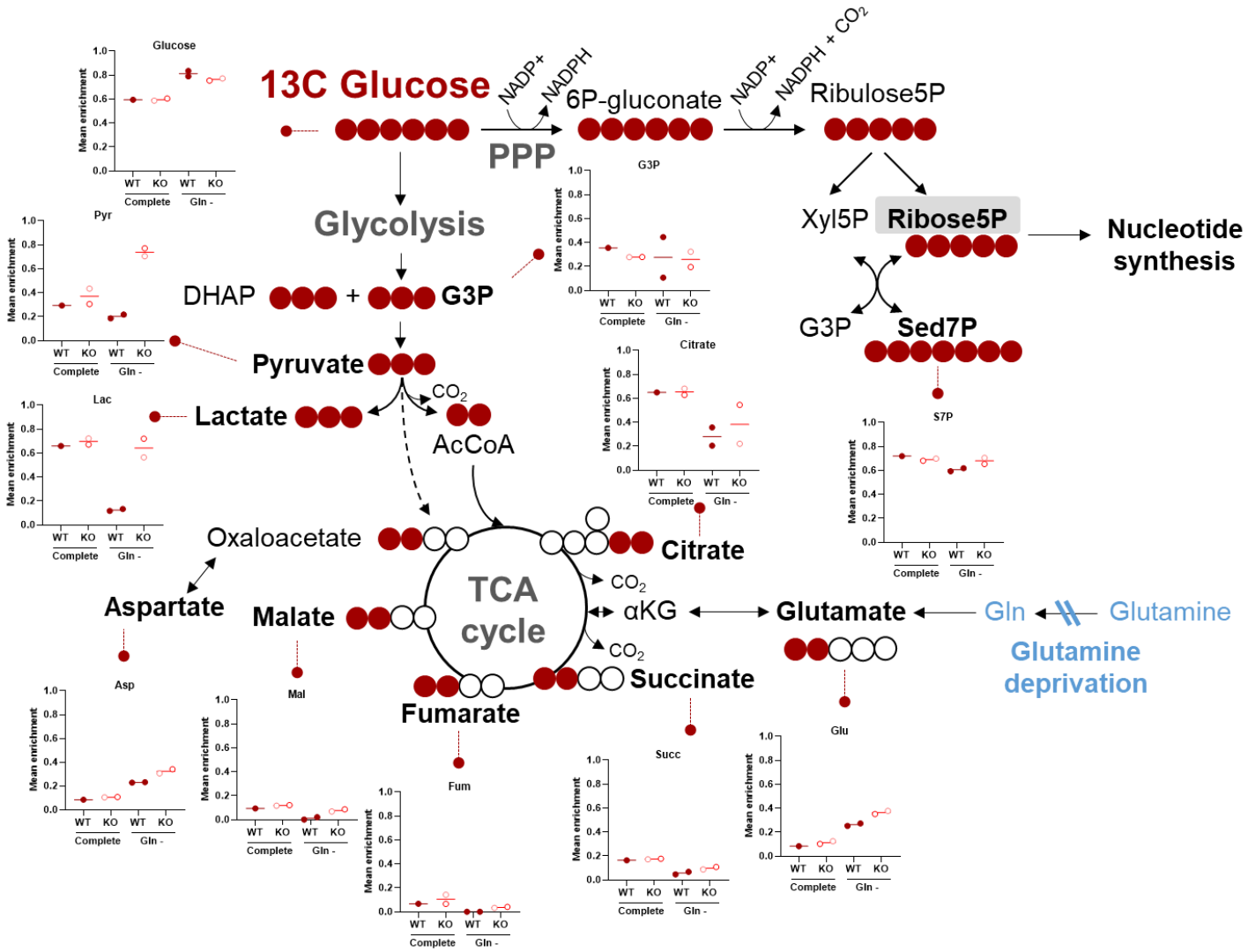
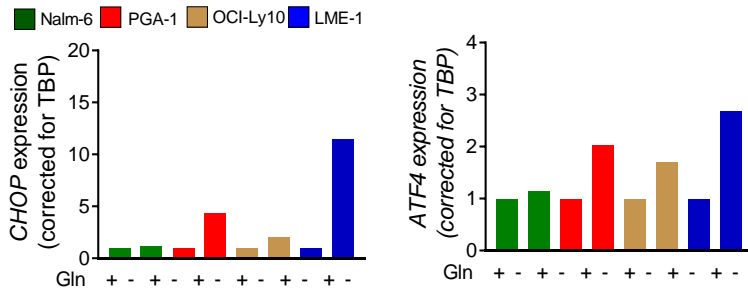


Figure S3

A



B

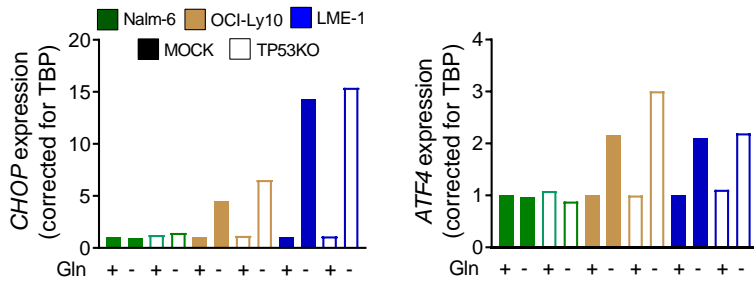


Table S1

Cell line	Pathway	FDR
PGA-1	Aminoacyl-tRNA biosynthesis	5.33E-07
	Purine metabolism	1.63E-06
	Arginine biosynthesis	0.000156
	Alanine, aspartate and glutamate metabolism	0.001013
	Citrate cycle (TCA cycle)	0.001013
	Glutathione metabolism	0.006463
	Arginine and proline metabolism	0.026901
	Valine, leucine and isoleucine biosynthesis	0.026901
OCI-Ly10	Glutathione metabolism	0.008541
	Butanoate metabolism	0.00969
	Arginine and proline metabolism	0.00969
	Synthesis and degradation of ketone bodies	0.014774
	beta-Alanine metabolism	0.014774
	Alanine, aspartate and glutamate metabolism	0.02911
LME-1	Purine metabolism	0.001349
NALM-6	Alanine, aspartate and glutamate metabolism	0.060941
	Glyoxylate and dicarboxylate metabolism	0.060941
	Glycine, serine and threonine metabolism	0.060941

Table S2

Cell line	CGH analysis	N° alterations
Nalm-6	arr[GRCh37] 5q31.3q33.1(141139631_150060364)x1[1.0],(9p)x2 hmz,9p21.3(20314006_22342373)x0[0.9]	3
PGA-1	arr[GRCh37] (2)x2 hmz,3q26.1q29(164216758_197851986)x3[1.0],(6)x1[0.35],(12)x3[0.45],13q21.1q3 3.2(58053767_105805914)x3[0.95],13q34(112592793_115107733)x1[0.95], (15)x2 hmz,16q11.2q24.3(46464488_90163275)x2 hmz	8
OCI-Ly10	arr[GRCh37] (X)x1[0.8],2p25.3p13.1(12770_74306073)x3[0.8],2q13q37.3(111743145_24277591 0)x2 hmz,3p26.3p24.3(61891_23610434)x4[0.7], 3p22.2p21.31(37107696_46803420)x3[0.7],3q24q25.33(147043014_160322956)x1 [0.85],5p15.3p15.31(113576_9312391)x3[0.7],6q15q25.1(91354194_150242165)x 1[0.8],7p21.3p21.1(9058438_19836065)x0[0.7],7p21.1q36.3(19839465_159119707)x3[0.7],8p23.3q12.1(168483_60290816)x2 hmz,8q12.1q24.12(60197960_122165704)x3[0.7], 8q24.12q24.22(122173681_136249330)x4[0.7],8q24.22q24.3(136250869_1462957 71)x3[0.7],9p24.3p13.1(203861_38787480)x3[0.7] hmz, 12p13.33q24.31(173786_120700268)x3[0.7],12q24.31(120707559_123661437)x1[0.7],12q24.31q24.33(123676131_133777902)x3[0.7],13q11q13.3(19436286_35959 599)x3[0.7],13q14.12q34(45647120_115107733)x3[0.7],18p11.32p11.21(2766336_ 15101911)x3[0.7],18q12.3q23(42088033_78014123)x3[0.7],19q13.2q13.43(425086 02_58956888)x4[0.7],20q13.2q13.33(53152594_62915555)x1[0.85],22q13.1q13.33 (37713571_51197838)x3[0.7]	21
LME-1	arr[GRCh37] (X)x1[0.8],1p13.2p11.2(112595773_120618050)x1[0.85],1q21.1q25.3(143932349_ 185080166)x4[0.85],1q25.3q44(185081909_249224684)x3[0.85], 3q11.1q29(93778974_197851986)x3[0.85],(4)x2 hmz,4q27q28.2(121090359_130798773)x1[0.85],4q32.3q35.2(165642090_190957 473)x1[0.85], 5p15.33q11.2(113576_56013926)x4[0.8],6p25.3q11.1(156974_62336066)x3[0.85],(7)x3[0.85],8p23.3p11.1(168483_43778914)x2 hmz, 8q11.1q24.3(46839735_146295771)x3[0.85],11p15.5p11.12(198509_51563636)x2 hmz,11p11.12q25(51486091_134938470)x3[0.85],(13)x2 hmz, 13q31.2q34(89737577_115107733)x3[0.85],15q21.1q26.3(48589631_102429112)x 3[0.85],17p13.3q11.2(525_29287256)x3[0.85], 17q21.31q25.3(44841599_81041938)x3[0.85],19p13.3p13.11(260911_18432929)x 3[0.7],(20,21)x3[0.85]	22
Nalm-6 <i>TP53</i> KO	arr[GRCh37] (1)x3[0.45],5q31.3q33.1(141262652_150060364)x1[0.9],(8)x1[0.45],(9p)x2 hmz,9p21.3(20314006_22342373)x0[0.9],(14)x1[0.4],(20)x3[0.25]	7
PGA-1 <i>TP53</i> KO#1	arr[GRCh37] (2)x2 hmz,3q26.1q29(164122391_197851986)x3[0.8],(6)x1[0.45],(9)x1[0.15],(12)x3[0.35], 13q21.1q33.2(58063254_105702427)x3[0.75], (15)x2 hmz,16q11.2q24.3(46464488_90163275)x2 hmz,(18)x1[0.35]	9