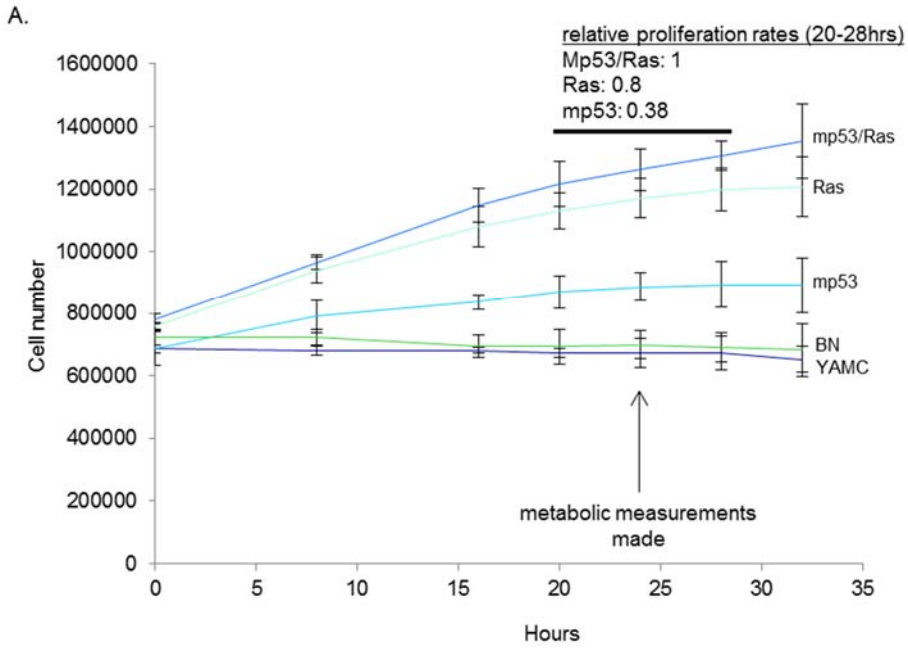
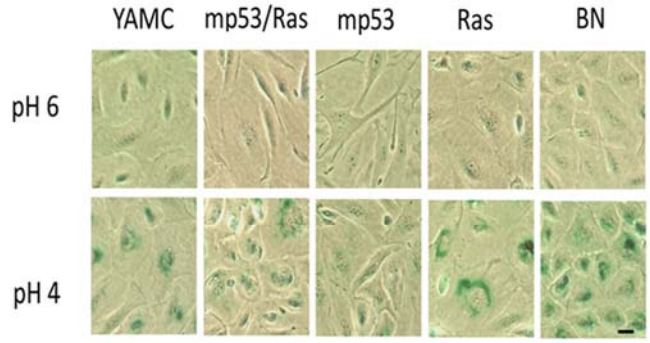


B.

Percentage of cells with β-galactosidase activity

	pH 6 (senescence associated)	pH 4 (acidic induced)
YAMC	<1%	91%
mp53/Ras	<1%	87%
mp53	<1%	88%
Ras	<1%	96%
BN	<1%	93%



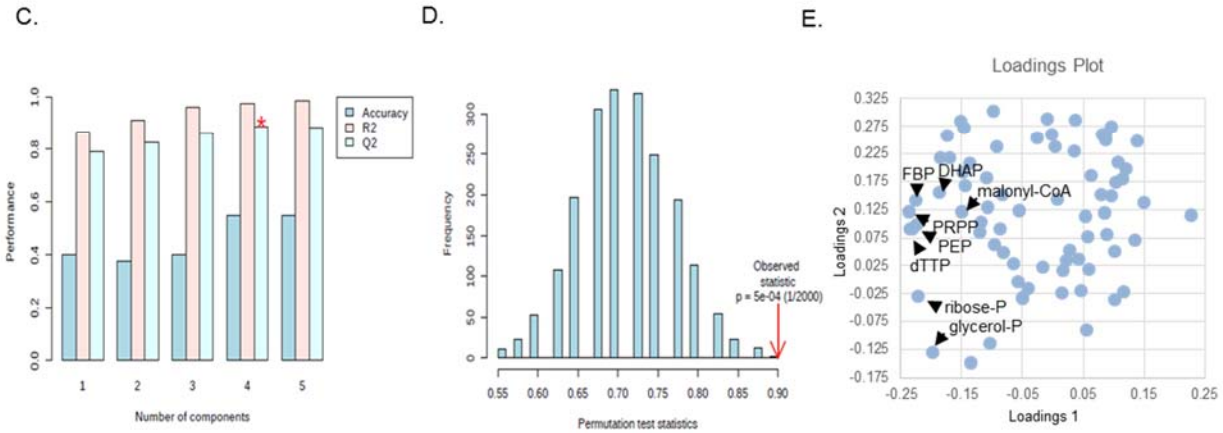


Figure S1, Related to Figure 1. The impact of oncogenic mutations on cellular proliferation and metabolism. **A.** Adherent cell proliferation rates of indicated YAMC cell derivatives prior to and following metabolite extraction and processing for LC-MS/MS. Proliferation rates indicated above graph are for time period +/- 4 hours from metabolite extraction. Data are means + S.D. **B.** Percentage of senescence associated and acidic β-gal activity depicted in representative images on right. Senescence associated (pH 6) and acidic (pH4) β-gal activity were measured at time of metabolite extraction and processing for LC-MS/MS. Values are represented as average of two independent experiments. Representative scale bar, 20 μm. **C.** PLS-DA model validation and classification with different number of components using the R caret package through Metaboanalyst. The red star indicates the optimum model. **D.** Permutation testing of the PLS-DA model shown in Fig 1F. Testing was performed using the separation distance based on the ratio of the between group sum of squares and the within group sum of squares (B/W ratio). The test statistic distribution frequency is plotted. **E.** Loadings plot for principal components 1 and 2 shown in Fig 1F, with indicated metabolites strongly correlated with the transformed state.

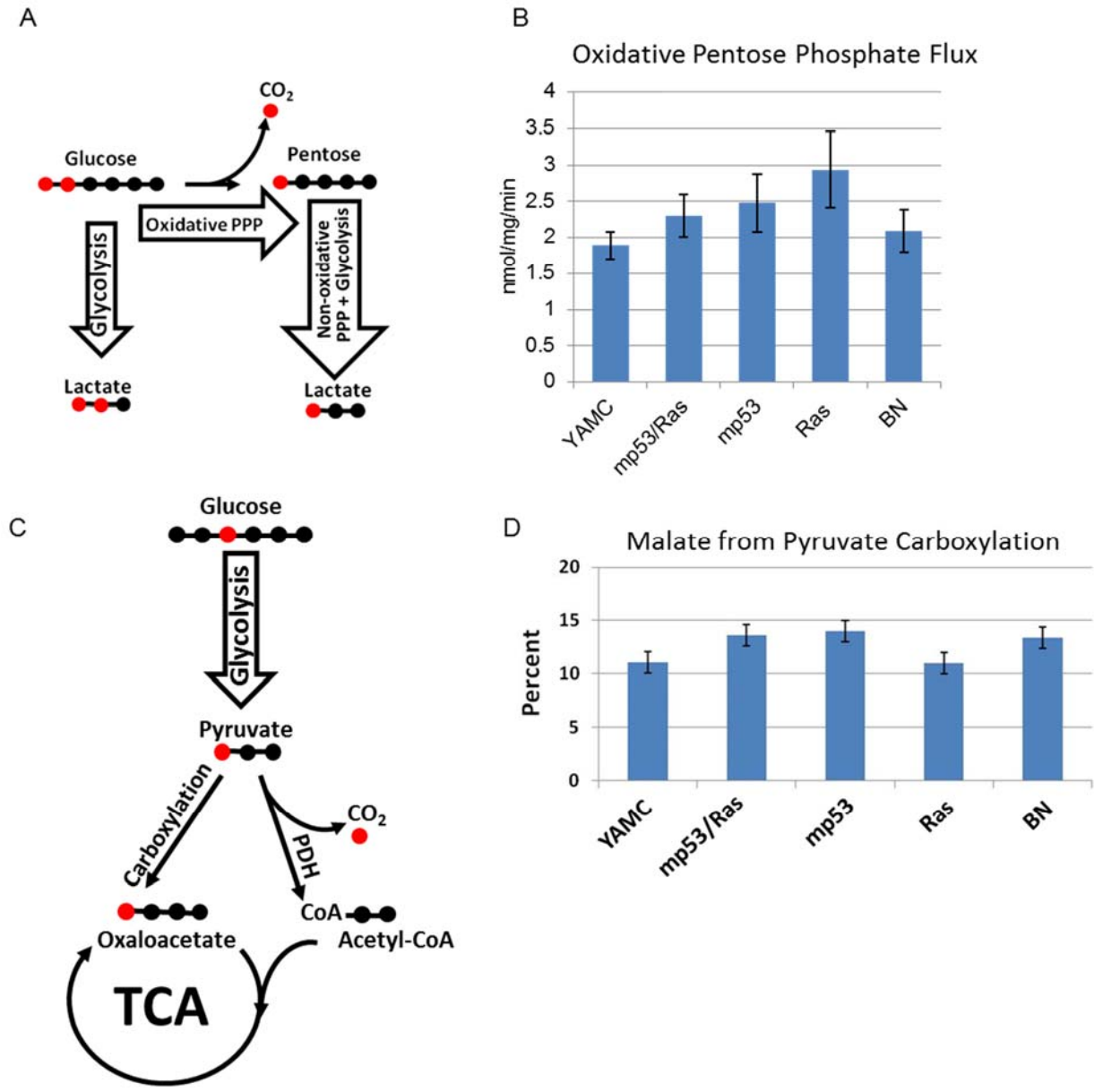


Figure S2, Related to Figures 1 and 4. The impact of oncogenic mutations on oxidative pentose phosphate pathway (PPP) and pyruvate carboxylation. A. Schematic of differential glucose carbon oxidation reactions and the downstream impact on lactate labeling. **B.** Parental murine colon crypt epithelial cells (Y), or cells retrovirally transduced with either vector controls (BN), DN-p53^{175H} (mp53), CA-Ras^{12V} (Ras), or both oncogenic alleles (mp53Ras) were pulsed with 1,2-^{13}C-glucose for 6h and then processed for LC-MS/MS. The ratio of labeled lactate containing one versus two ^{13}Carbon is indicative of the PPP:glycolytic flux ratio; nonoxidative PPP flux yields lactate containing one ^{13}C atom, whereas glycolytic flux yields two ^{13}C atoms. **C.** Schematic of TCA cycle labeling upon pyruvate carboxylation versus dehydrogenation (by PDH). **D.** The cells in (B) were pulsed with 3-^{13}C-glucose for 24h and then processed for LC-MS/MS. The percentage of 1-^{13}C-labeled malate represents the percentage of malate produced from pyruvate carboxylation. In contrast, pyruvate oxidation mediated by the pyruvate dehydrogenase complex releases this carbon as CO_2.

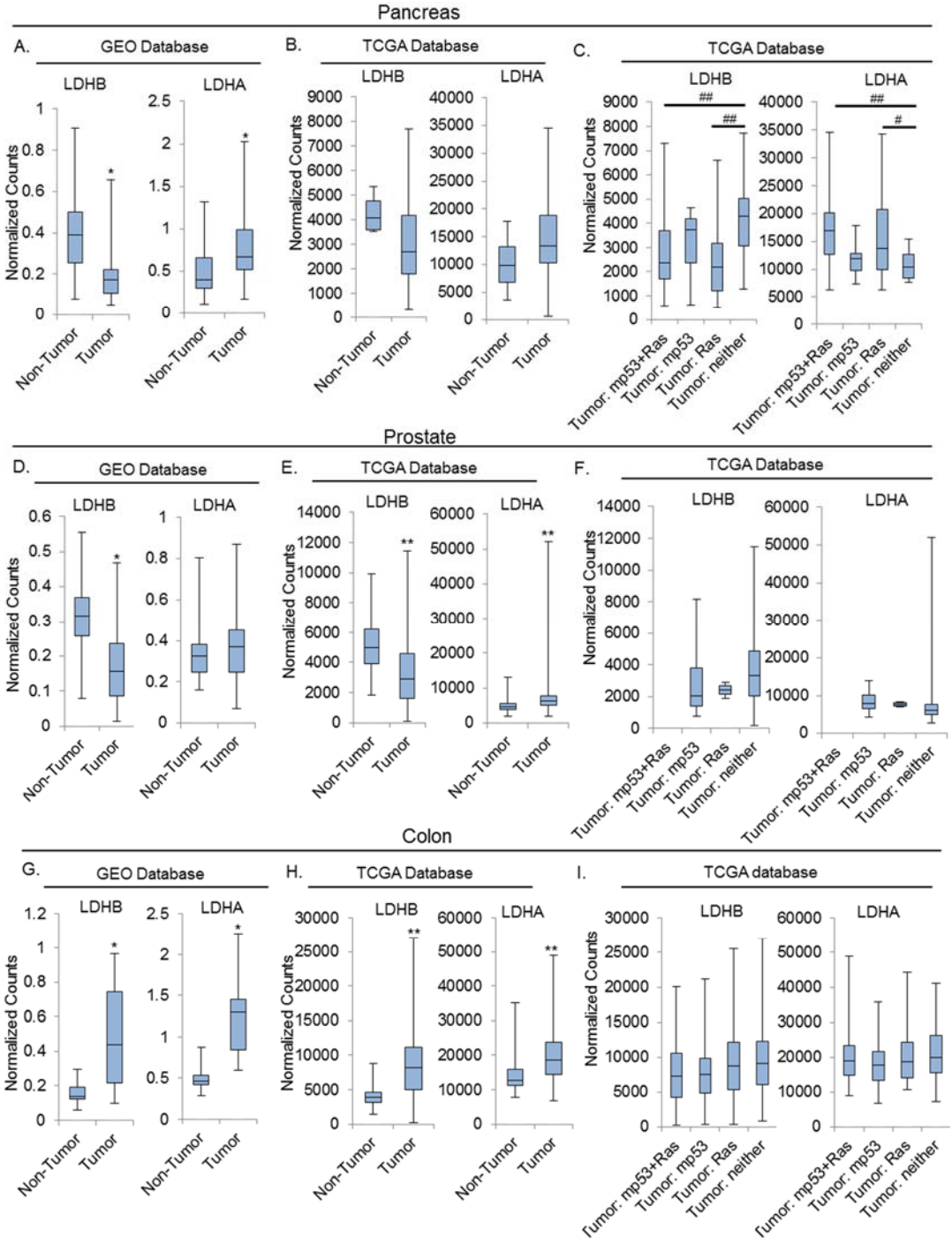


Figure S3, Related to Figure 2. Oncogenic LDH isoform switching in human cancers. A-H. LDHB or LDHA RNA expression in human pancreas, prostate, or colon tissue. (A, D, G) LDHB or LDHA RNA expression in tumor vs normal tissue based on cDNA microarray data from the Gene Expression Omnibus (GEO) database. (B, E, H) LDHB or LDHA RNA expression in tumor vs normal tissue based on expression data from the TCGA database. (C, F, I) LDHB or LDHA RNA expression in tumors with both p53 and Ras mutations (Tumor: mp53+Ras), tumors with p53 but not Ras mutation (Tumor: mp53), tumors with Ras but not p53 mutation (Tumor: Ras), and tumors with neither p53 nor Ras mutation (Tumor: neither). (A-H) *p<0.05 versus non-tumor and **p<.01 versus non-tumor, by student's t-test. #p<0.05 and ##p<.01, by ANOVA F-test and Tukey method. All y-axis values represent linear scale.

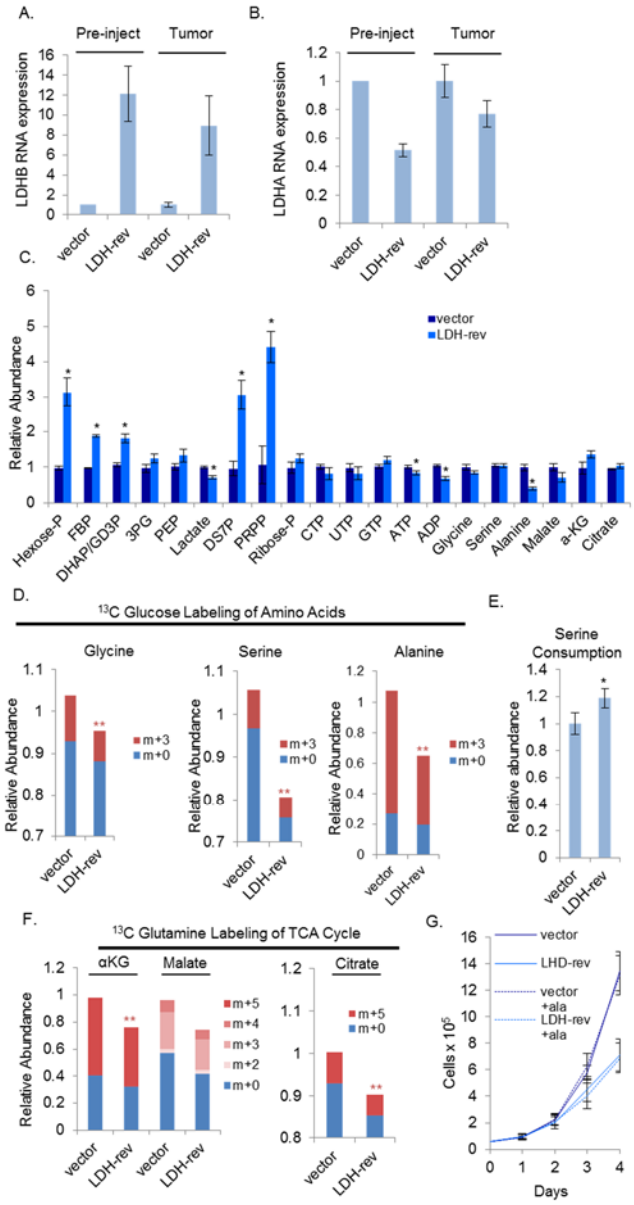


Figure S4, Related to Figure 2-4. Oncogenic LDH isoform switching is important for coupling of glycolysis to glutamine driven TCA anaplerosis. A-B. (A) LDHB or (B) LDHA RNA expression measured in indicated mp53/Ras derivative cell populations prior to implantation into mice (Pre-inject) and in tumors (Tumor) that arose from these cells following xeno-implantation. Expression was measured by real time quantitative PCR. Data are means + S.E. of the mean. **C.** Relative intracellular metabolite levels of indicated derivatives of mp53/Ras cells, determined by LC-MS/MS. Measurements made following 24 hr cell culture in 1% oxygen. Data are means + S.E. of the mean. *$p < 0.05$ versus vector control, by student's t-test. **D.** Cells were labeled with ^{13}C-glucose for 90 minutes at 1% oxygen and the relative abundance of ^{13}C- and ^{12}C-isoforms was determined by LC-MS/MS. Bars represent total relative abundance of a given metabolite and shading indicates isotopologue composition. Isotopologue species are indicated by m+n, with m+0 representing the unlabeled fraction. **$p<0.01$ for total ^{13}C-isotopologue abundance of indicated metabolite versus that metabolite's total ^{13}C-isotopologue abundance in vector control, by student's t-test. Measurements were made following 8-10 hr cell culture in 1% oxygen. **E.** Extracellular serine levels in culture media of indicated derivatives of mp53/Ras cells, determined by LC-MS/MS. Measurements were made following 8-10 hr cell culture in 1% oxygen and data are means + S.E. of the mean. *$p < 0.05$ versus vector control, by student's t-test. **F.** Cells were labeled with ^{13}C-glutamine for 90 minutes at 1% oxygen and the relative abundance of ^{13}C- and ^{12}C-isoforms was determined by LC-MS/MS. Bars represent total relative abundance of a given metabolite and shading indicates isotopologue composition. Isotopologue species are indicated by m+n, with m+0 representing the unlabeled fraction. **$p<0.01$ for total ^{13}C-isotopologue abundance of indicated metabolite versus that metabolite's total ^{13}C-isotopologue abundance in vector control, by student's t-test. Measurements were made following 8-10 hr cell culture in 1% oxygen. **G.** Adherent cell proliferation of indicated derivatives of mp53/Ras cells in 1% oxygen and in the presence of exogenously supplied alanine (+ala) when indicated. Data are means + S.D.

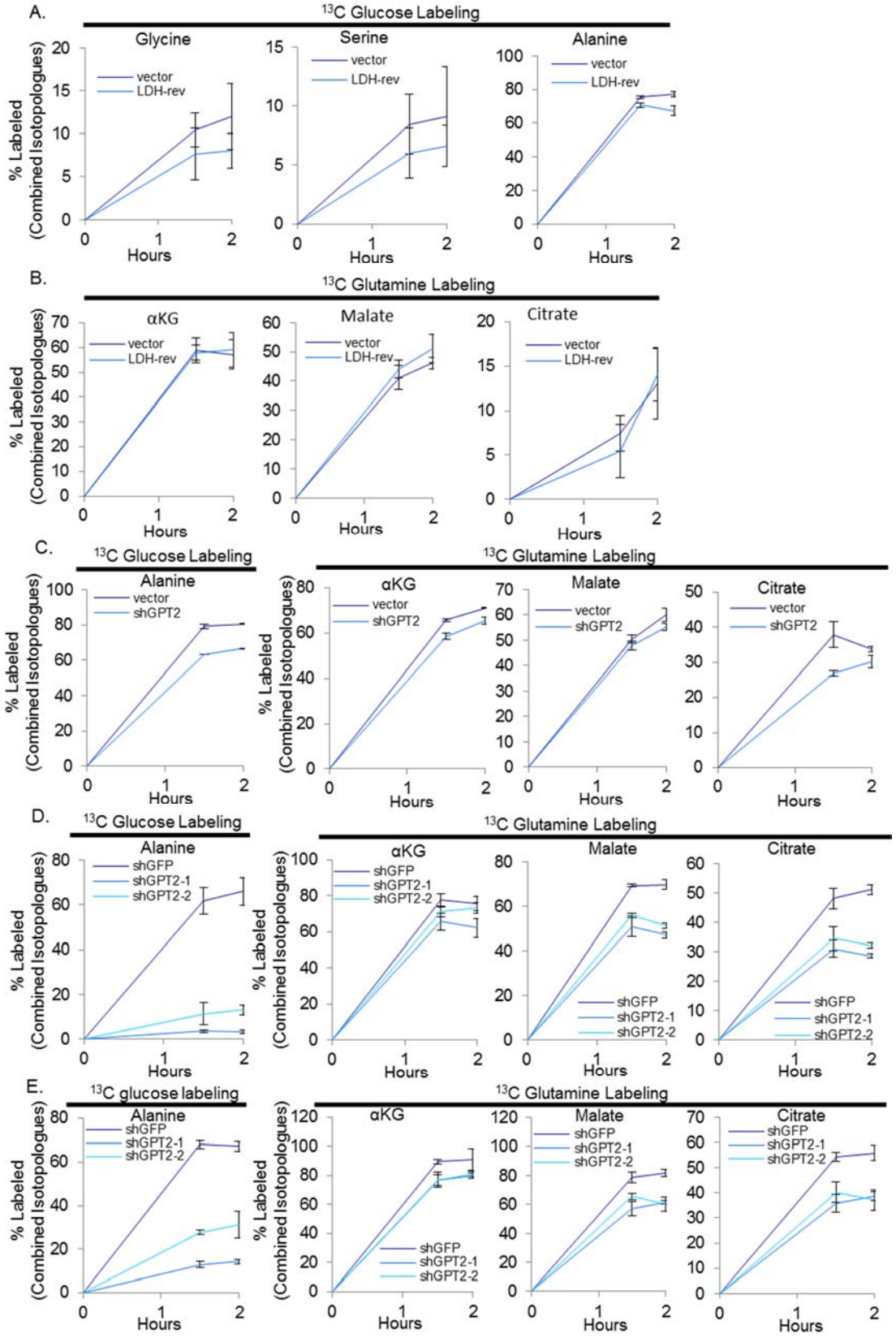


Figure S5, Related to Figures 3-6. Kinetic labeling of central carbon metabolic pools. A-E. Indicated derivatives of (A-C) mp53/Ras cells, (D) HCT116, or (E) SW480 cells were pulsed with ^{13}C-glucose or ^{13}C-glutamine for indicated time and the relative abundance of ^{12}C- and ^{13}C-isoforms was determined by LC-MS/MS. Graphs represent percentage of indicated metabolite pools that turnover from ^{12}C- to ^{13}C-isoforms over time, with ^{13}C representing the sum of all m+1 or greater species) Data are means + S.E. of the mean. (A-B) Labeling after 8-10 hr cell culture in 1% oxygen.

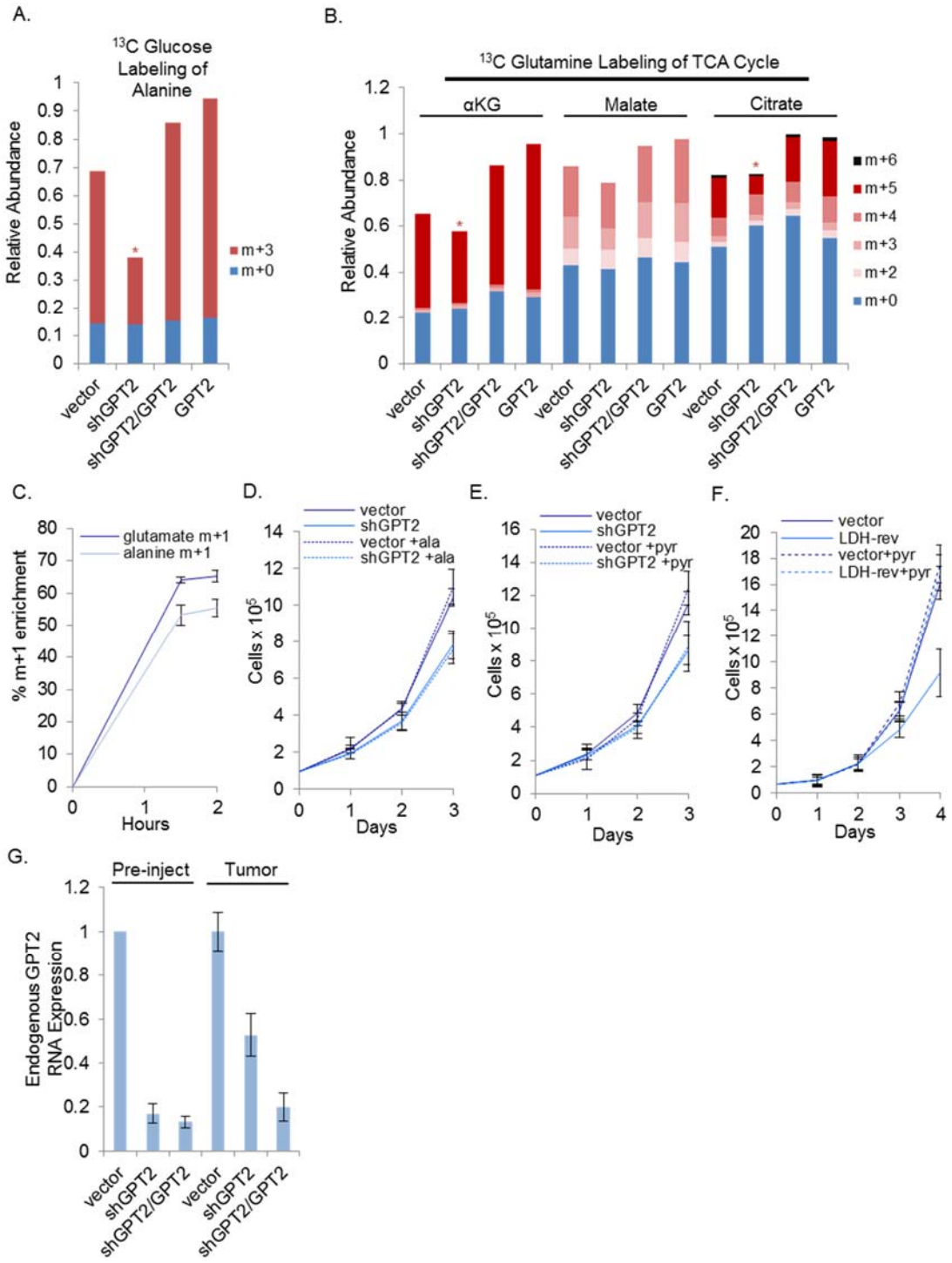


Figure S6, Related to Figure 5. GPT2 couples glycolysis to glutamine driven TCA anaplerosis in mp53/Ras cells. A-B. Cells were labeled with ^{13}C-glucose or ^{13}C-glutamine for 90 minutes and the relative abundance of ^{13}C- and ^{12}C-isoforms was determined by LC-MS/MS. Bars represent total relative abundance of a given metabolite and shading indicates isotopologue composition. Isotopologue species are indicated by m+n, with m+0 representing the unlabeled fraction. *p<0.01 for total ^{13}C-isotopologue abundance of indicated metabolite versus that metabolite's total ^{13}C-isotopologue abundance in all other cell lines, by student's t-test. **C.** Cells were labeled with α-^{15}N-glutamine and the relative abundance of ^{15}N- and ^{14}N-isoforms was determined by LC-MS/MS. Graph represents percentage of indicated metabolite pools that turnover from m+0 to m+1 over time. **D-F.** Adherent cell proliferation of indicated derivatives of mp53/Ras cells in the presence of exogenously supplied alanine (+ala) or pyruvate (+pyr) when indicated. Data are means + S.D. (F) proliferation assayed in 1% oxygen. **G.** Endogenous GPT2 RNA expression measured in indicated mp53/Ras derivative cell populations prior to implantation into mice (Pre-inject) and in tumors (Tumor) that arose from these cells following xeno-implantation. Expression was measured by real time quantitative PCR. Data are means + S.E. of the mean.

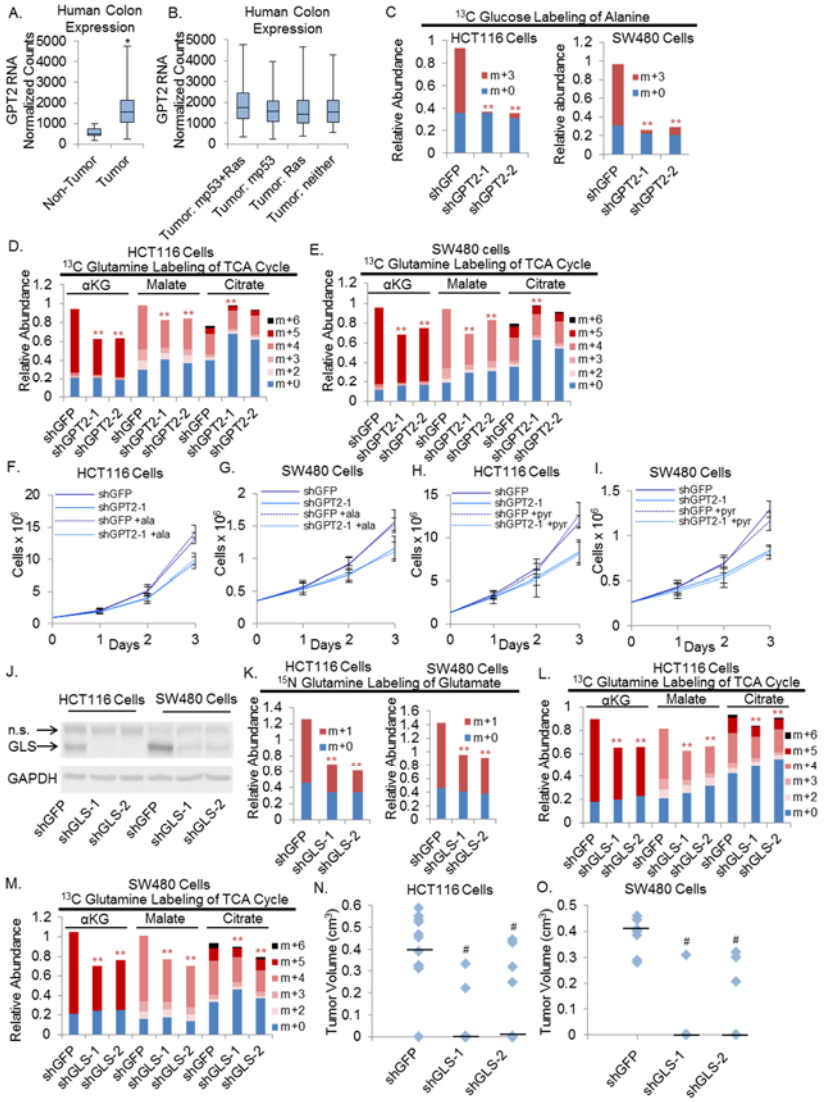


Figure S7, Related to Figure 6. GPT2 dependency of human colon cancer cells. A-B. GPT2 RNA expression in human colon tissue. (A) GPT2 RNA expression in tumor vs normal tissue based on expression data from the TCGA database. (B) GPT2 RNA expression in colon tumors with both p53 and Ras mutations (Tumor: mp53+Ras), tumors with p53 but not Ras mutation (Tumor: mp53), tumors with Ras but not p53 mutation (Tumor: Ras), and tumors with neither p53 nor Ras mutation (Tumor: neither). (A-B) *p<0.001 versus non-tumor, by student's t-test. All y-axis values represent linear scale. **C-E.** The indicated derivatives of HCT116 or SW480 human colon cancer cells were labeled with ^{13}C-glucose or ^{13}C-glutamine for 90 minutes and the relative abundance of ^{13}C- and ^{12}C-isoforms was determined by LC-MS/MS. Bars represent total relative abundance of a given metabolite and shading indicates isotopologue composition. Isotopologue species are indicated by m+n, with m+0 representing the unlabeled fraction. **p<0.01 for total ^{13}C-isotopologue abundance of indicated metabolite versus that metabolite's total ^{13}C-isotopologue abundance in shGFP control, by student's t-test. **F-I.** Adherent cell proliferation of indicated derivatives of HCT116 or SW480 human colon cancer cells in the presence of exogenously supplied alanine (+ala) or pyruvate (+pyr) when indicated. Data are means + S.D. **J.** Glutaminase (GLS) protein expression by western blot in indicated derivatives of HCT116 or SW480 cells: shGFP control (shGFP), GLS knockdown-1 (shGLS-1), or GLS knockdown-2 (shGPT2-2). GAPDH serves as loading control. **K-M.** The indicated derivatives of HCT116 or SW480 cells were labeled with α-^{15}N-glutamine or ^{13}C-glutamine for 90 minutes and the relative abundance of ^{15}N- and ^{14}N-isoforms and ^{13}C- and ^{12}C-isoforms was determined by LC-MS/MS. Bars represent total relative abundance of a given metabolite and shading indicates isotopologue composition. Isotopologue species are indicated by m+n, with m+0 representing the unlabeled fraction. **p<0.01 for total ^{13}C-isotopologue abundance of indicated metabolite versus that metabolite's total ^{13}C-isotopologue abundance in shGFP control, by student's t-test. **N-O.** Tumor volumes four weeks after 8-12 subcutaneous implantations of the indicated cell lines in the flanks of nude mice. Median tumor volume is indicated by horizontal line. *P < 0.01 versus shGFP, by student's t-test.

Table S1, Related to Figure 1: ANOVA analysis of metabolic parameters

Metabolic Process	Mean p-value	Significant at 0.05 by post hoc Fisher's LSD
Lactate Production	2.79E-6	mp53/Ras - YAMC; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - BN; mp53 - Ras; mp53 - BN
Glucose Consumption	5.11E-5	mp53/Ras - YAMC; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - BN; mp53 - YAMC; mp53 - BN; Ras - BN
Glutamine Consumption	7.58E-7	mp53/Ras - YAMC; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - BN; mp53 - Ras; Ras - YAMC; Ras - BN
Fatty Acid Biosynthesis	3.13E-10	mp53/Ras - YAMC; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - BN; mp53 - Ras; mp53 - YAMC; mp53 - BN; Ras - BN

Table S2, Related to Figure 1: Relative metabolite pool sizes

Metabolite	YAMC			mp53/Ras			mp53			Ras			BN		
1,3-diphosphateglycerate	3094	±	1144	6460	±	1406	4963	±	1202	2890	±	744	3447	±	1527
2-dehydro-D-gluconate	4001	±	702	6733	±	631	5876	±	4124	3759	±	662	3876	±	*
3-hydroxy-3-methylglutaryl-CoA	3846	±	*	6644	±	3356	4969	±	2565	2849	±	*	4194	±	1041
6-phospho-D-gluconate	4018	±	357	8366	±	1634	4746	±	1694	6404	±	2749	3525	±	1011
aconitate	3441	±	1385	2450	±	525	4903	±	1218	4136	±	1206	6490	±	767
adenosine 5'-phosphosulfate	5287	±	1515	3801	±	842	4960	±	1245	5095	±	1345	6223	±	997
ADP	5748	±	1524	4884	±	879	5588	±	907	5316	±	1202	7832	±	656
ADP-D-glucose	6166	±	2141	3928	±	2156	2429	±	915	3766	±	1428	4576	±	2079
allantoate	3625	±	974	6348	±	1212	5765	±	1069	3758	±	401	3338	±	761
AMP	9243	±	1023	4820	±	890	5553	±	789	7521	±	644	10000	±	681
anthranilate	5049	±	1311	5872	±	4128	2655	±	1056	6103	±	1764	5973	±	590
CDP	2784	±	493	6655	±	1635	4490	±	656	3308	±	676	3795	±	701
coenzyme A	2499	±	790	5291	±	2028	3489	±	452	5814	±	1645	3045	±	1114
CTP	3474	±	1175	7862	±	965	6216	±	867	4645	±	996	4567	±	606
dATP	4136	±	2249	2726	±	602	3203	±	1570	2706	±	1700	5204	±	1745
dCTP	3375	±	1017	7176	±	1190	5738	±	1189	3324	±	995	2926	±	479
deoxyribose-phosphate	5625	±	1898	7178	±	1677	6340	±	1019	7490	±	1773	5052	±	937
D-gluconate	3978	±	891	4716	±	758	4321	±	727	6180	±	1094	9067	±	489
D-glucono-lactone-6-phosphate	3256	±	1280	4021	±	802	4792	±	1115	4593	±	1889	4657	±	1934
dihydroxy-acetone phosphate	2137	±	651	8101	±	1899	6701	±	1426	2404	±	1344	1782	±	304
D-sedoheptulose-7-phosphate	5307	±	914	4215	±	670	4842	±	788	6809	±	741	6335	±	1025
dTTP	1770	±	428	7378	±	1043	5619	±	1043	2335	±	508	2070	±	410
fructose-6-phosphate	5401	±	1559	6010	±	1582	4670	±	787	4273	±	701	3276	±	229
fumarate	3836	±	1999	3557	±	1109	1815	±	460	3806	±	2089	4227	±	2001
GDP	5151	±	1288	4219	±	783	5761	±	1007	5200	±	1014	7490	±	832

glutathione[1]	4259	±	1053	3344	±	601	4360	±	722	2571	±	618	7961	±	955
glycerate	6745	±	1101	4256	±	1051	3476	±	1087	5717	±	995	4669	±	767
hexose-phosphate	6623	±	859	6543	±	638	5541	±	610	7071	±	954	4554	±	718
IDP	5234	±	1538	4358	±	742	5425	±	1215	4861	±	1178	7401	±	798
citrate/isocitrate	3423	±	1221	2329	±	543	3669	±	972	4043	±	1880	7331	±	1645
lactate	5429	±	969	8832	±	880	5761	±	931	5718	±	658	1649	±	441
myo-inositol	5519	±	*	5172	±	1451	7055	±	502	7676	±	167	6871	±	3129
N-acetyl-glucosamine-1-phosphate	7524	±	737	7513	±	971	7714	±	783	7609	±	1441	8379	±	442
NAD+	6321	±	1059	5651	±	1419	4443	±	646	6040	±	1047	4022	±	892
NADH	4511	±	1282	5961	±	1634	5615	±	1238	4148	±	1242	3694	±	581
NADP+	3818	±	1223	6057	±	1103	4887	±	780	6040	±	1221	6906	±	796
NADPH	2249	±	924	5570	±	1864	4097	±	907	6358	±	1496	3370	±	995
orotate	3560	±	793	2616	±	642	3332	±	1406	8123	±	1877	2966	±	599
orotidine-5'-phosphate	1512	±	1162	329	±	134	1275	±	1061	784	±	282	5919	±	4081
oxaloacetate	5645	±	4355	3588	±	707	3800	±	1200	2658	±	1368	8845	±	370
pantothenate	7961	±	1506	6971	±	589	5730	±	621	6155	±	950	8403	±	833
phenylpyruvate	5815	±	4185	3809	±	*	3139	±	551	2634	±	1247	2247	±	911
p-hydroxybenzoate	3057	±	1055	1911	±	672	2160	±	363	1379	±	178	7349	±	2651
propionyl-CoA	6431	±	1396	6441	±	2138	8598	±	1402	3940	±	211	4127	±	*
pyruvate	4038	±	969	5840	±	1181	2867	±	478	6096	±	1071	4110	±	595
quinolinate	4780	±	1711	3186	±	1017	1324	±	532	2627	±	1277	3852	±	1357
ribose-phosphate	2826	±	797	8611	±	772	6861	±	995	3122	±	431	2135	±	540
glycerol-3-phosphate	6809	±	715	7680	±	656	8676	±	404	3460	±	636	2365	±	451
succinate	5611	±	1306	4671	±	896	3729	±	900	4164	±	1208	5542	±	892
taurine	1872	±	392	442	±	127	471	±	*	534	±	126	9008	±	522
UDP	4805	±	1013	4468	±	1271	3486	±	845	5496	±	1230	3901	±	989
UDP-D-glucose	4837	±	992	7574	±	923	6750	±	883	4789	±	937	5721	±	407

UDP-D-glucuronate	5330	±	1464	6589	±	912	6480	±	948	5566	±	1091	5468	±	490
UDP-N-acetyl-glucosamine	6380	±	1146	7127	±	948	7519	±	994	5181	±	909	6550	±	604
xanthine	3490	±	2175	1141	±	157	1199	±	110	1387	±	267	4682	±	2076

Averages and SEM from four independent experiments performed in duplicate (n=8).
* indicates that metabolite was not found in enough replicates to yield an SEM
[a]Measurement of oxidized glutathione is representative of total glutathione pool due to oxidation during sample preperation

Table S3, Related to Figure 1: Absolute Metabolite Pool Sizes (pmol/mg protein)

Metabolite	YAMC			mp53/Ras			mp53			Ras			BN		
3-Phosphoglycerate	678	±	104	1200	±	194	802	±	131	633	±	144	734	±	133
5-phosphoribosyl 1-pyrophosphate	151	±	58	856	±	125	351	±	59	129	±	49	168	±	27
acetyl-CoA	216	±	59	388	±	84	421	±	160	150	±	36	523	±	150
α-ketoglutarate	29725	±	10848	38189	±	9995	22510	±	3511	19948	±	4854	26147	±	4802
ATP	81543	±	23836	98726	±	18503	102482	±	16591	94429	±	20555	119458	±	9398
citrate/isocitrate	11769	±	4309	6081	±	1482	10779	±	2795	8378	±	2968	15196	±	2865
CTP	4035	±	1364	9130	±	1121	7218	±	1007	5394	±	1157	5304	±	704
glyceraldehyde-3-phosphate/ DHAP	181	±	55	686	±	161	568	±	121	204	±	114	151	±	26
fructose bisphosphate	3458	±	756	6267	±	744	4554	±	852	2354	±	615	1693	±	97
GTP	12020	±	3203	11221	±	1991	10883	±	1696	10320	±	2632	12148	±	2422
malate	11821	±	3478	14748	±	1770	12445	±	2636	12282	±	3128	21515	±	2023
malonyl-CoA	41	±	12	100	±	23	70	±	25	39	±	8	39	±	11
phosphoenolpyruvate	292	±	27	1383	±	228	761	±	134	430	±	61	433	±	100
UTP	11777	±	3887	24475	±	3476	18895	±	3603	12683	±	4163	15262	±	2425

Averages and SEM from ≥ 3 independent experiments performed in duplicate.

Table S4, Related to Figure 1: Important features identified by One-way ANOVA and post-hoc analysis

	Compound	p.value	-LOG10(p)	FDR	Fisher's LSD
1	taurine	4.01E-22	21.397	2.97E-20	BN - mp53; BN - mp53/Ras; BN - Ras; BN - YAMC; YAMC - mp53; YAMC - mp53/Ras; YAMC - Ras
2	sn-glycerol-3-phosphate	1.62E-10	9.7914	5.98E-09	mp53 - BN; mp53/Ras - BN; YAMC - BN; mp53 - Ras; mp53 - YAMC; mp53/Ras - Ras; YAMC - Ras
3	5-phosphoribosyl 1-pyrophosphate	1.17E-08	7.9326	2.88E-07	mp53/Ras - BN; mp53/Ras - mp53; mp53 - Ras; mp53 - YAMC; mp53/Ras - Ras; mp53/Ras - YAMC
4	phosphoenolpyruvate	4.55E-07	6.3423	8.41E-06	mp53/Ras - BN; mp53/Ras - mp53; mp53 - YAMC; mp53/Ras - Ras; mp53/Ras - YAMC
5	D-gluconate	8.94E-07	6.0488	1.32E-05	BN - mp53; BN - mp53/Ras; BN - Ras; BN - YAMC; Ras - mp53; Ras - YAMC
6	ribose-phosphate	3.23E-06	5.4907	3.98E-05	mp53 - BN; mp53/Ras - BN; mp53 - Ras; mp53 - YAMC; mp53/Ras - Ras; mp53/Ras - YAMC
7	dTTP	4.03E-06	5.3951	4.26E-05	mp53 - BN; mp53/Ras - BN; mp53 - Ras; mp53 - YAMC; mp53/Ras - Ras; mp53/Ras - YAMC
8	lactate	3.99E-05	4.3993	0.000369	mp53 - BN; mp53/Ras - BN; Ras - BN; YAMC - BN; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - YAMC
9	glutathione disulfide	6.62E-05	4.1789	0.000545	BN - mp53; BN - mp53/Ras; BN - Ras; BN - YAMC
10	fructose-1-6-bisphosphate	0.000146	3.8344	0.001084	mp53 - BN; mp53/Ras - BN; mp53 - Ras; mp53/Ras - Ras; mp53/Ras - YAMC
11	D-glyceraldehdye-3-phosphate	0.002539	2.5954	0.017077	mp53/Ras - BN; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - YAMC
12	3-phosphoglycerate	0.007416	2.1298	0.045734	mp53/Ras - BN; mp53/Ras - mp53; mp53/Ras - Ras; mp53/Ras - YAMC
13	dCTP	0.011465	1.9406	0.06526	mp53/Ras - BN; mp53/Ras - Ras; mp53/Ras - YAMC
14	CTP	0.014036	1.8528	0.07419	mp53/Ras - BN; mp53 - YAMC; mp53/Ras - Ras; mp53/Ras - YAMC
15	pyruvate	0.032798	1.4842	0.15813	mp53/Ras - mp53; Ras - mp53
16	malonyl-CoA	0.03419	1.4661	0.15813	mp53/Ras - BN; mp53/Ras - Ras; mp53/Ras - YAMC
17	UDP-D-glucose	0.043639	1.3601	0.18996	mp53/Ras - Ras; mp53/Ras - YAMC

Table S5, Related to Figures 3-5: isotopologue labeling in mp53/Ras cells

	^{13}C-glucose labeling						^{13}C-glutamine labeling					
	glycine		*serine*		*alanine*		*aKG*		*malate*		*citrate*	
	1.5hrs	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**
Vector (LDH-rev)	m+0: 90% m+3: 10%	m+0: 88% m+3: 12%	m+0: 91% m+3: 9%	m+0:91% m+3: 9%	m+0: 25% m+3: 75%	m+0: 23% m+3: 77%	m+0: 41% m+5: 59%	m+0: 43% m+5: 57%	m+0: 59% m+2: 3% m+3: 28% m+4: 10%	m+0: 54% m+2: 5% m+3: 31% m+4: 10%	m+0: 93% m+5: 7%	m+0: 87% m+5: 13%
LDH-rev	m+0: 92% m+3: 8%	m+0: 92% m+3: 8%	m+0: 94% m+3: 6%	m+0:93% m+3: 7%	m+0: 29% m+3: 71%	m+0: 33% m+3: 67%	m+0: 42% m+5: 58%	m+0: 41% m+5: 59%	m+0: 56% m+2: 4% m+3: 31% m+4: 9%	m+0: 50% m+2: 7% m+3: 35% m+4: 8%	m+0: 95% m+5: 5%	m+0: 86% m+5: 14%
Vector (shGPT2)	---	---	---	---	m+0: 21% m+3: 79%	m+0: 19% m+3: 81%	m+0: 34% m+3: 2% m+4: 2% m+5: 62%	m+0: 29% m+3: 3% m+4: 4% m+5: 64%	m+0: 50% m+2: 8% m+3: 16% m+4: 26%	m+0: 39% m+2: 10% m+3: 18% m+4: 33%	m+0: 62% m+2: 3% m+3: 3% m+4: 10% m+5: 21% m+6: 1%	m+0: 66% m+2: 2% m+3: 4% m+4: 13% m+5: 13% m+6: 2%
shGPT2	---	---	---	---	m+0: 37% m+3: 63%	m+0: 33% m+3: 67%	m+0: 41% m+3: 3% m+4: 2% m+5: 54%	m+0: 35% m+3: 3% m+4: 3% m+5: 59%	m+0: 52% m+2: 11% m+3: 12% m+4: 25%	m+0: 45% m+2: 10% m+3: 15% m+4: 30%	m+0: 73% m+2: 3% m+3: 3% m+4: 10% m+5: 10% m+6: 1%	m+0: 70% m+2: 2% m+3: 4% m+4: 11% m+5: 12% m+6: 1%

Indicated derivatives of mp53/Ras cells were pulsed with ^{13}C-glucose or ^{13}C-glutamine for indicated time and the relative abundance of ^{12}C- and ^{13}C-isoforms was determined by LC-MS/MS. Values represent percentage of indicated metabolite pools for all detectable isotopologues (m+n), with m+0 representing the unlabeled fraction.

Table S6, Related to Figure 6: isotopologue labeling in HCT116 cells

	^{13}C-glucose labeling		^{15}N-glutamine labeling		^{13}C-glutamine labeling					
	alanine		*glutamate*		*aKG*		*malate*		*citrate*	
	1.5hrs	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**
shGFP	m+0: 38% m+3: 62%	m+0: 34% m+3: 66%	m+0: 38% m+1: 62%	m+0: 36% m+1: 64%	m+0: 23% m+3: 3% m+4: 3% m+5: 72%	m+0: 24% m+3: 3% m+4: 2% m+5: 71%	m+0: 31% m+2: 10% m+3: 12% m+4: 47%	m+0: 30% m+2: 14% m+3: 10% m+4: 46%	m+0: 52% m+2: 4% m+3: 4% m+4: 29% m+5: 8% m+6: 3%	m+0: 49% m+2: 5% m+3: 4% m+4: 32% m+5: 7% m+6: 3%
shGPT2-1	m+0: 96% m+3: 4%	m+0: 97% m+3: 3%	---	---	m+0: 36% m+3: 2% m+4: 2% m+5: 60%	m+0: 38% m+3: 2% m+4: 2% m+5: 58%	m+0: 48% m+2: 9% m+3: 7% m+4: 36%	m+0: 53% m+2: 7% m+3: 6% m+4: 34%	m+0: 69% m+2: 3% m+3: 3% m+4: 19% m+5: 5% m+6: 1%	m+0: 72% m+2: 2% m+3: 2% m+4: 19% m+5: 4% m+6: 1%
shGPT2-2	m+0: 88% m+3: 11%	m+0: 87% m+3: 13%	---	---	m+0: 31% m+3: 3% m+4: 2% m+5: 65%	m+0: 27% m+3: 4% m+4: 4% m+5: 65%	m+0: 43% m+2: 10% m+3: 7% m+4: 40%	m+0: 49% m+2: 10% m+3: 8% m+4: 33%	m+0: 66% m+2: 3% m+3: 3% m+4: 21% m+5: 5% m+6: 1%	m+0: 68% m+2: 1% m+3: 5% m+4: 22% m+5: 4% m+6: 0%
shGLS-1	---	---	m+0: 49% m+1: 51%	m+0: 51% m+1: 49%	m+0: 3% m+3: 0% m+4: 0% m+5: 69%	m+0: 30% m+3: 0% m+4: 0% m+5: 70%	m+0: 40% m+2: 11% m+3: 8% m+4: 41%	m+0: 38% m+2: 13% m+3: 7% m+4: 42%	m+0: 59% m+2: 4% m+3: 4% m+4: 22% m+5: 10% m+6: 1%	m+0: 63% m+2: 1% m+3: 2% m+4: 18% m+5: 14% m+6: 1%
shGLS-2	---	---	m+0: 56% m+1: 44%	m+0: 52% m+1: 48%	m+0: 34% m+3: 0% m+4: 0% m+5: 66%	m+0: 37% m+3: 0% m+4: 0% m+5: 63%	m+0: 48% m+2: 10% m+3: 6% m+4: 36%	m+0: 43% m+2: 14% m+3: 8% m+4: 35%	m+0: 61% m+2: 4% m+3: 3% m+4: 22% m+5: 10% m+6: 1%	m+0: 66% m+2: 2% m+3: 3% m+4: 20% m+5: 7% m+6: 2%

Indicated derivatives of HCT116 cells were pulsed with ^{13}C-glucose , ^{13}C-glutamine, or ^{15}N-glutamine for indicated time and the relative abundance of ^{12}C- and ^{13}C-isoforms was determined by LC-MS/MS. Values represent percentage of indicated metabolite pools for all detectable isotopologues (m+n), with m+0 representing the unlabeled fraction.

Table S7, Related to Figure 6: Isotopologue Labeling in SW480 Cells

	^{13}C-glucose labeling		^{15}N-glutamine labeling		^{13}C-glutamine labeling					
	alanine		*glutamate*		*aKG*		*malate*		*citrate*	
	1.5hrs	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**	**1.5hrs**	**2hrs**
shGFP	m+0: 32% m+3: 68%	m+0: 33% m+3: 67%	m+0: 32% m+1: 68%	m+0: 29% m+1: 71%	m+0: 10% m+3: 2% m+4: 3% m+5: 85%	m+0: 9% m+3: 2% m+4: 3% m+5: 86%	m+0: 20% m+2: 6% m+3: 12% m+4: 62%	m+0: 18% m+2: 6% m+3: 12% m+4: 64%	m+0: 45% m+2: 3% m+3: 4% m+4: 31% m+5: 13% m+6: 4%	m+0: 44% m+2: 3% m+3: 4% m+4: 29% m+5: 17% m+6: 3%
shGPT2-1	m+0: 87% m+3: 13%	m+0: 86% m+3: 14%	---	---	m+0: 22% m+3: 2% m+4: 2% m+5: 74%	m+0: 20% m+3: 3% m+4: 3% m+5: 74%	m+0: 42% m+2: 5% m+3: 8% m+4: 45%	m+0: 39% m+2: 6% m+3: 7% m+4: 48%	m+0: 63% m+2: 2% m+3: 3% m+4: 22% m+5: 9% m+6: 1%	m+0: 61% m+2: 2% m+3: 3% m+4: 25% m+5: 8% m+6: 1%
shGPT2-2	m+0: 72% m+3: 28%	m+0: 69% m+3: 31%	---	---	m+0: 23% m+3: 2% m+4: 2% m+5: 73%	m+0: 19% m+3: 1% m+4: 4% m+5: 76%	m+0: 35% m+2: 6% m+3: 8% m+4: 51%	m+0: 40% m+2: 9% m+3: 6% m+4: 45%	m+0: 59% m+2: 2% m+3: 4% m+4: 25% m+5: 9% m+6: 1%	m+0: 63% m+2: 2% m+3: 1% m+4: 26% m+5: 7% m+6: 1%
shGLS-1	---	---	m+0: 55% m+1: 45%	m+0: 57% m+1: 43%	m+0: 35% m+3: 0% m+4: 0% m+5: 65%	m+0: 31% m+3: 0% m+4: 0% m+5: 69%	m+0: 23% m+2: 11% m+3: 9% m+4: 57%	m+0: 26% m+2: 10% m+3: 9% m+4: 55%	m+0: 51% m+2: 4% m+3: 5% m+4: 29% m+5: 10% m+6: 1%	m+0: 55% m+2: 4% m+3: 3% m+4: 31% m+5: 7% m+6: 0%
shGLS-2	---	---	m+0: 57% m+1: 43%	m+0: 54% m+1: 46%	m+0: 33% m+3: 0% m+4: 0% m+5: 67%	m+0: 34% m+3: 0% m+4: 0% m+5: 66%	m+0: 19% m+2: 10% m+3: 10% m+4: 61%	m+0: 24% m+2: 7% m+3: 8% m+4: 61%	m+0: 47% m+2: 4% m+3: 6% m+4: 28% m+5: 14% m+6: 3%	m+0: 46% m+2: 2% m+3: 5% m+4: 29% m+5: 16% m+6: 2%

Indicated derivatives of SW480 cells were pulsed with ^{13}C-glucose , ^{13}C-glutamine, or ^{15}N-glutamine for indicated time and the relative abundance of ^{12}C- and ^{13}C-isoforms was determined by LC-MS/MS. Values represent percentage of indicated metabolite pools for all detectable isotopologues (m+n), with m+0 representing the unlabeled fraction.

Supplemental Experimental Procedures

Plasmids

Murine lactate dehydrogenase-B (LDHB) cDNA was purchased from Open Biosystems and cloned into the retroviral vector pBabePuro3 (Morgenstern and Land, 1990). shRNA's targeting murine lactate dehydrogenase-A (LDHA) and murine glutamic pyruvate transaminase 2 (GPT2) were cloned into the retrovirus vectors pSUPERretro.hygro and .puro (OligoEngine) respectively. shRNAs targeting human GPT2 and human glutaminase (GLS) were purchased from Open Biosystems and expressed with pLKO.1 vector system. shRNA resistant GPT2 cDNA was generated by restriction digest removal of shRNA target sequence within 3' UTR of a full length murine cDNA. Both full length cDNAs were purchased from Open Biosystems and cloned into the lentiviral vector pLenti6/UbC/V5-GW/*lacZ*.For shRNA target sequences and mutagenesis primer sequences see below.

Measurement of senescence associated-β-gal and acidic-β-gal activity

Senescence associated and acidic β-gal activity was performed as described previously (Debacq-Chainiaux et al., 2009). Briefly, cells were washed twice with PBS and fixed in PBS containing 2% formaldehyde (vol/vol) and 0.2% glutaraldehyde for 5 min. at room temperature. At the end of 5 min. fixation solution was removed and cells were washed twice with PBS. Staining solution containing 40 mM citric acid/Na phosphate buffer, 5 mM K4[Fe(CN)6] 3H2O, 5 mM K3[Fe(CN)6], 150 mM sodium chloride, 2 mM magnesium chloride and 1 mg ml − 1 X-gal in distilled water, was calibrated to pH 6 to detect senescence associated β-gal activity and to pH 4 for acidic β-gal activity as a positive control. Staining solution was added to wells and cells were incubated at 37°C for 16 hrs. After the incubation, cells were washed twice with PBS and imaged using bright field microscopy. Percentages were calculated by counting at least 100 cells from each condition.

LC-MS/MS Methodology

Cells were maintained in culture as indicated above. Cellular media of murine cells was changed to serum free medium with HEPES (10mM) 24 hours and again one hour prior to metabolite extraction or metabolic tracer addition, while human colon cancer cell media was only changed one hour prior. The same conditions were used for measurements in hypoxia, except cells were moved to 1% oxygen for the indicated time prior to extraction. For all labeling experiments, labeling medium was prepared from serum free media with HEPES (10mM), lacking either glucose or glutamine and supplemented with either 12C or U-13C labeled glucose (2 g/L) or 12C or U-13C, or α-15N labeled glutamine (300 mg/L) (Cambridge Isotope Laboratories). Isotope labeling experiments were performed at a time at which label accumulation had plateaued of the metabolic pools of interest had plateaued, i.e. approaching pseudo-steady state, as verified by empirical analysis of their labeling kinetics. Glucose labeling was based upon total 13C labeling (i.e. the sum of all isotopologues) of a given metabolite pool, as a percentage of total pool size over time (see Fig S5 and Table S5-7). Glutamine-derived citrate labeling in the murine-derived colon cells was an exception, as its labeling did not always plateau (Fig S5B); however, this did not impact our conclusions. Labeled (13C) or control medium (12C) was aspirated at indicated times and metabolic activity was quenched with immediate addition of 4 ml of -78˚C methanol: water (80:20). Plates were stored on dry ice for at least 10 minutes following quenching. Cells were scraped, and the resulting suspension vortexed vigorously, then centrifuged at 4˚C for 5 minutes at 2000 x g. Supernatants were collected and remaining cell pellets were re-extracted twice more with 80% methanol. All extractions were pooled for each sample. Extracts were then either dried under nitrogen gas and resuspended in 200 µl of 50:50 methanol:water or, for measurement of redox sensitive NAD and NADH levels, were directly analyzed following methanol extraction. Amino acids were derivatized prior to analysis. Specifically, 100 µl of the above methanol extracts was derivatized with 1 µl benzyl chloroformate and 5 µl trimethylamine. All samples were then centrifuged at 4˚C for 5 minutes at full speed to pellet insoluble material and analyzed using reverse phase chromatography with an ion-paring reagent in a Shimadzu HPLC coupled to a Thermo Quantum triple quadrupole mass spectrometer running in negative mode with selected-reaction-monitoring (SRM) specific scans. Specific chromatography conditions and mass spectrometry parameters were as described in(DeVito et al., 2014; Munger et al., 2008).

LC-MS/MS data were analyzed using the publically available mzrock machine learning toolkit (http://code.google.com/p/mzrock/), which automates SRM/HPLC feature detection, grouping, signal to noise classification, and comparison to known metabolite retention times (Melamud et al., 2010). Extracted ion chromatograms of metabolite-specific SRM peak heights were normalized by their cellular protein content as measured by Bradford assay. For relative quantification, protein-normalized peak heights were further normalized by the maximum value for a specific metabolite measured across the samples run on a given day. This normalization serves to reduce the impact of inter-day mass spectrometry variability while preserving relative differences between samples. For absolute quantifications, the metabolite pool abundances of the mp53/Ras cells were quantified based on comparison to standard curves. Extracts from the mp53/Ras cells were subsequently used as a standard, run in the same sample set, to estimate the absolute metabolite abundances of the YAMC cells and their derivatives.

Measurement of fatty acid biosynthesis.

Fatty acid biosynthesis was evaluated largely as indicated previously (Harwood et al., 2003). Briefly, fatty acid biosynthesis was assayed by measuring the incorporation of [1-14C]-acetic acid (NEN Radiochemicals) into cellular lipids. Following 48 hours without selective drugs, cells were plated in 6-well plates under standard culture conditions and serum starved for 24 hours under indicated oxygen conditions. To begin the assay, 1.25 ml of RPMI containing 1 μCi of 56.2 mCi $mmol^{-1}$ [1-14C]-acetic acid was added to each well, and cells were incubated at 39°C for 2 h. After incubation, cells were washed with PBS and dissolved in 1 ml of 0.2M KOH. Samples were then saponified by the addition of 0.6 ml of 50% KOH and 4 ml of ethanol, followed by a 2-h incubation at 75°C and then an overnight incubation at room temperature. After saponification, the samples were extracted two times with 9 ml of hexane. The hexane-containing fractions were discarded, and the remaining fractions containing fatty acids were acidified to pH 2 by the addition of 1 ml 12 M HCl. After acidification, the samples were extracted one time with 8 ml hexane. The organic fractions were dried under N2, resuspended in 100 μl of 1:1 chloroform-hexane, dissolved in 2 ml of Ecoscint O liquid scintillation fluid, and assessed for radioactivity using a Beckman LS6500 liquid scintillation counter. Results were normalized for protein content measured by Bradford assay. Significant differences were identified through one-way ANOVA analysis with subsequent Fisher's LSD post-hoc testing (see Table S1).

Western Blot

Prior to lysis, cells were grown without selective drugs for a minimum of 48 hr at 39°C, in standard culture media. Cells were lysed for 30 minutes in RIPA buffer (1% NP-40, 0.1% SDS, 0.5% deoxycholic acid, 5 mM EDTA, 50 mM Tris-HCL, pH 7.4, 150 mM NaCL, protease inhibitor cocktail tablet) at 4°C, followed by 10 minutes of centrifugation at 13,000xg to clear insoluble material. Bradford protein assay (Bio-Rad) was used to measure lysate protein concentrations and 50ug was separated by SDS-PAGE. Protein bands were visualized using horse-radish peroxidase-coupled secondary antibodies (Pierce) and ECL+ kit (Amersham). The following primary antibodies were used: goat polyclonal anti-LDHA (Santa Cruz), mouse monoclonal anti-LDHB (Abcam), rabbit monoclonal anti-GLS (Abcam), rabbit monoclonal anti-GAPDH (Cell Signaling), and mouse monoclonal anti-γ tubulin (Sigma).

Real-time quantitative PCR

Cells were grown without selective drugs for a minimum of 48 hr under standard culture conditions, prior to harvesting for RNA isolation. Total RNA was isolated from cells using RNeasy Mini Kit (Quiagen). cDNA was produced for qPCR analysis by denaturing 5ug RNA at 90°C for 5 minutes, then mixing with 400 μM dNTP mixture, 10 mM DTT, 1x SuperScript II reverse transcriptase buffer, 0.3 ng random hexamer primer, 1μl SuperScript II reverse transcriptase, and 1 μl RNaseOUT RNase inhibitor for a total reaction volume of 50μl (all components from Invitrogen). RT reactions were carried out for 2 hours at 42°C, followed by heat inactivation of RT enzyme by 10 minute incubation at 70°C. SYBR Green-based quantitative PCR was performed by mixing 1ul cDNA/RT reaction with 0.2 μM forward and reverse primer mix of gene-specific qPCR primers (IDT) and 1x Bio-Rad iQ SYBR Green master mix. Reactions were run on an iCycler (Bio-Rad) using the following conditions: 3 minutes at 95°C to activate polymerase, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 45 seconds to amplify products, then 40 cycles of 94°C with 1°C step-down for 30 seconds to generate melt curves. Gene expression was calculated using the ΔΔCt method, as previously described (McMurray et al., 2008), and normalized to RhoA. See below for primer sequences.

shRNA target sequences:

The following sequences were targeted for shRNA-mediated knockdown of gene expression:

Murine LDHA shRNA - CATGGCAGCCTCTTCCTTAAAAC
Murine GPT2 shRNA - TATCTGGATTATATGCCTAGC
Human GPT2 shRNA 1 - TTCAGGATCGTAGAAATGCCG
Human GPT2 shRNA 2 - ATCTGGAGAGTACACGTTGTC
Human GLS shRNA 1 - TATTTCGAACTGCTTCAGGGC
Human GLS shRNA 2 - ATATACCAACCATGTCTGTGC

RT-PCR Primers

The following gene specific qPCR primers were used:
Murine LDHB Forward - GGGAGCTTGTTCCTCCAGAC
Murine LDHB Reverse - TGGGTGGAAACCACGATGAT

Murine LDHA Forward - GGCATGGCTTGGCCATCAGTATC
Murine LDHA Reverse - GGAGATCCATCATCTCGCCCTTGA

Murine GPT2 Forward - CTTTGCCTGGGAAGAGAAG

Murine GPT2 Reverse - TGGAGGTGGAGTGGAAG

Murine endogenous GPT2 Forward - GGAAGTAGCCGCATCCATAA
Murine endogenous GPT2 Reverse - AATCTCAGAACTCTGGAGCATATC

Human GPT2 Forward - CATGGACATTGTCGTGAACC
Human GPT2 Reverse - TTACCCAGGACCGACTCCTT

Murine RhoA Forward - AGCTTGTGGTAAGACATGCTTG
Murine RhoA Reverse - GTGTCCCATAAAGCCAACTCTAC

Human RhoA Forward - TGGAAAGACATGCTTGCTCAT
Human RhoA Reverse - GCCTCAGGCGATCATAATCTTC

Gene expression in human cancer

GEO: publically available cDNA microarray datasets were used to assess the expression of LDHA, LDHB, and GPT2 in human cancers of colon (Saaf et al., 2007), pancreas (Lowe et al., 2007) and prostate (Lapointe et al., 2004). The ratio of background normalized mean fluorescence intensities of Cy-5 labeled sample and Cy-3 labeled reference mRNA at each microarray element was used as a standardized measure of the abundance for the corresponding mRNAs. Statistical significance in differential gene expression between non-tumor and tumor tissue was determined by using Student's t-test

TCGA: Level 3 Illumina RNAseq normalized counts were collected for colon, pancreatic and prostate tumors and their corresponding non-tumor tissues. Changes in expression of genes were identified as significantly different by using Benferroni adjusted p-values between tumor and normal samples using t-tests. Relationship between p53/Ras mutational status and expression of genes were studied by partitioning the RNASeq data for the tumors using sample matched somatic mutation information for the presence or absence of Ras and/or p53 mutations. Variance in gene expression between groups were analyzed by using ANOVA F-test in conjunction with Tukey method to determine samples that have means that differ from each other significantly.

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