

Supplemental Materials

Sequential Adaptive Changes in a c-Myc-Driven Model of Hepatocellular Carcinoma

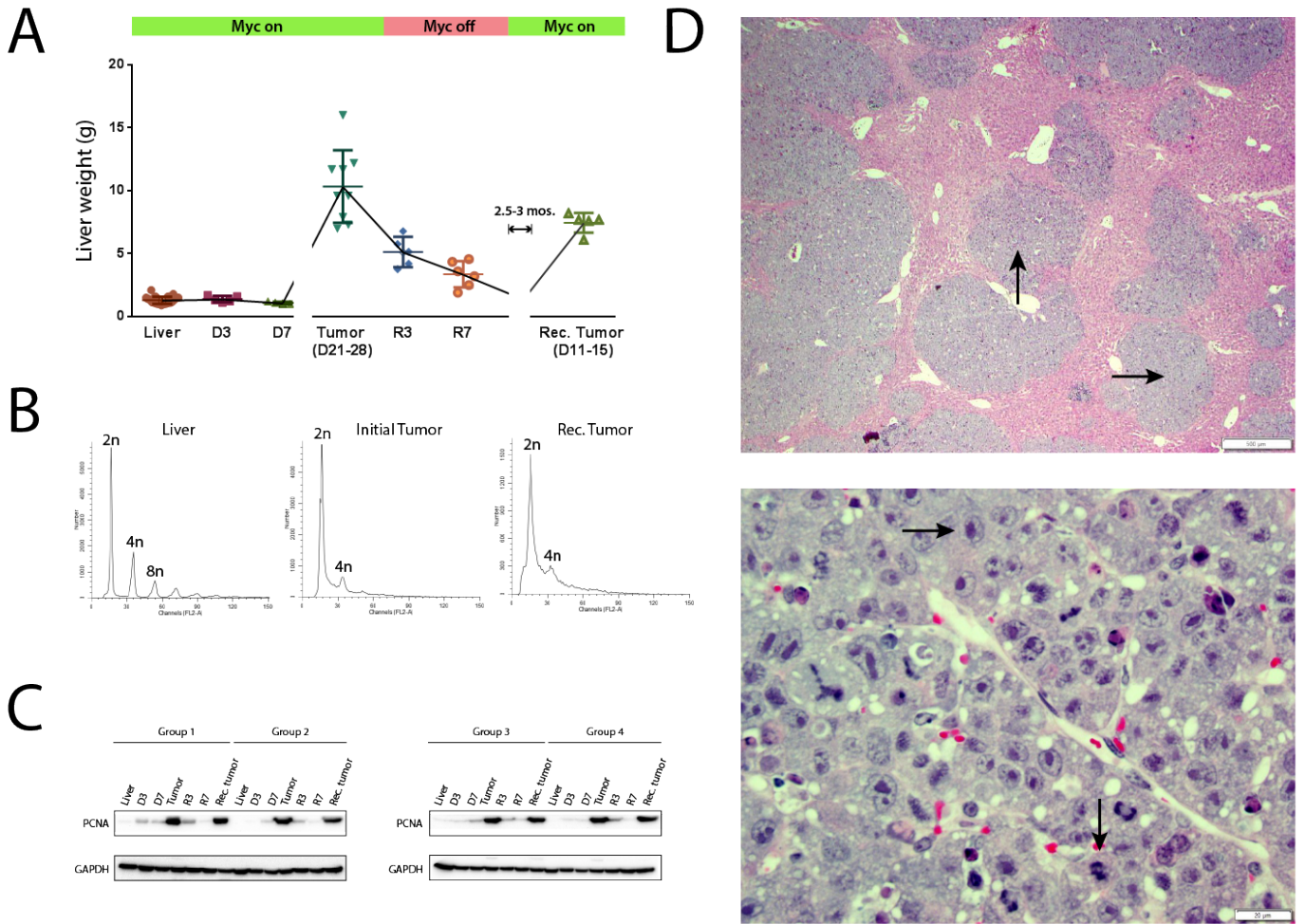
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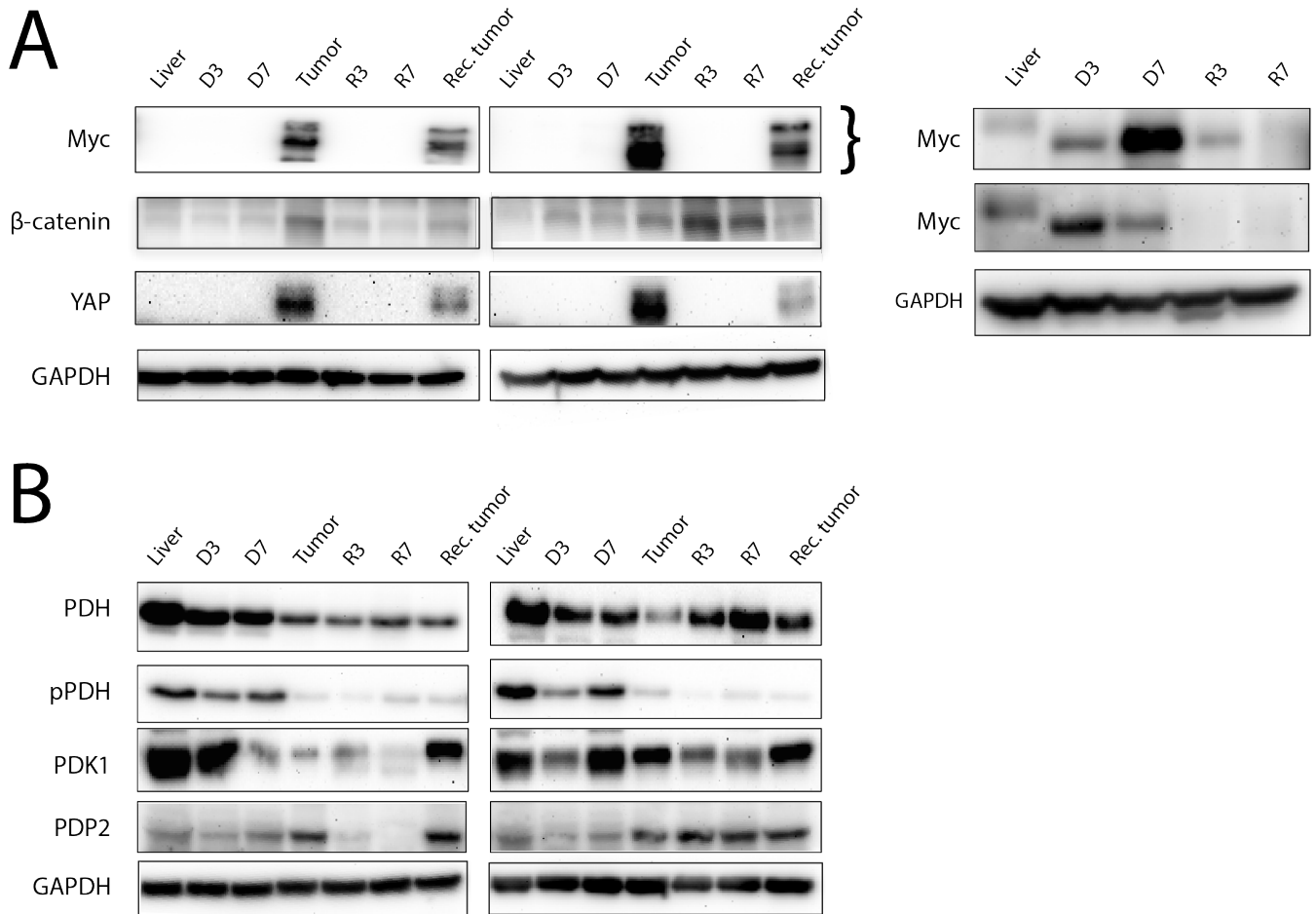
The Supplemental Materials for the manuscript “Sequential Adaptive Changes in a c-Myc-Driven Model of Hepatocellular Carcinoma” includes:

Supplemental Figures S1 – S7

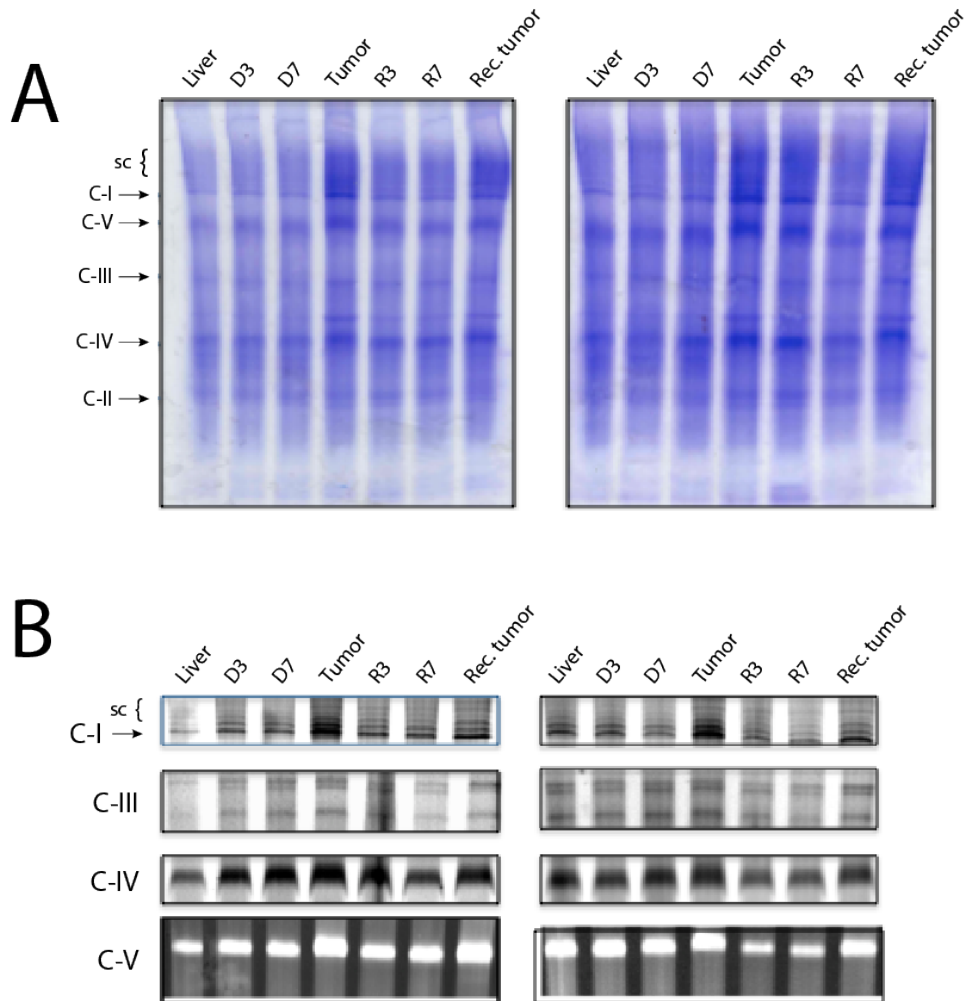
Supplemental Tables S1 – S6



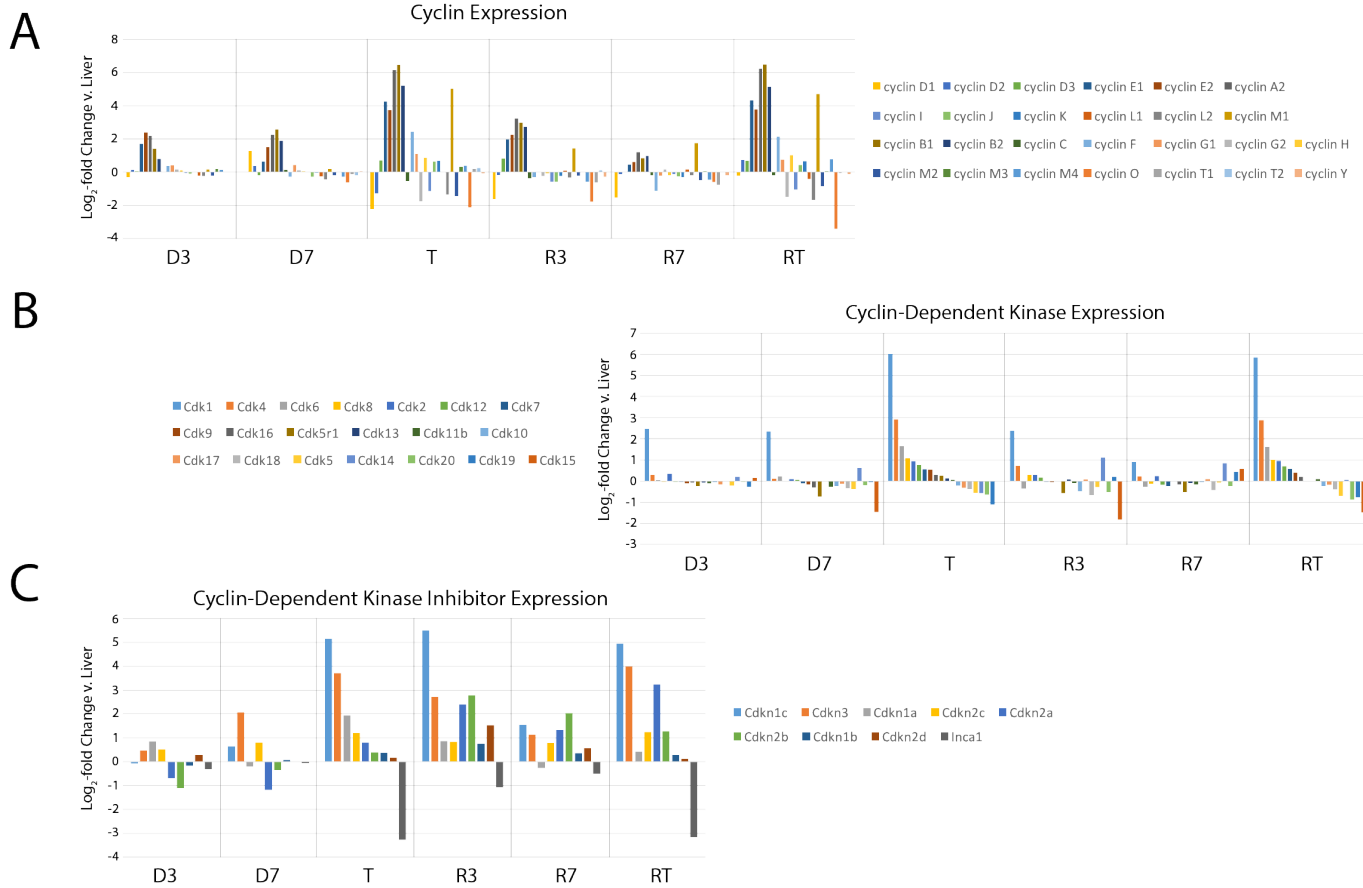
Supplemental Figure S1. Properties of initial and recurrent HCCs. *A*, Changes in liver weights in response to Myc de-regulation. “Liver” denotes organs used as control tissues at the beginning of the study and obtained from mice maintained continuously on doxycycline (Myc “off” state). D3 and D7 indicate the time (in days) at which livers were harvested following doxycycline removal and Myc induction (Myc “on” state). Initial tumors were harvested at 21-28 days with the remaining animals being returned to the “Myc off” state by resuming doxycycline. Regressing tumors were harvested at R3 and R7. Doxycycline was continued for 2-3 months in the remaining animals and then stopped again to generate recurrent tumors (Rec. Tumor). *B*, Typical examples of cell cycle analysis of livers and initial and recurrent tumors. Tumors demonstrated greater S phase fractions than control liver ($P = 0.002$), and recurrent tumors showed a greater fraction than initial tumors ($P = 0.001$). Groups were compared with ANOVA and Tukey’s multiple comparisons two-sided t-tests. $n = 4-6$ samples/group. *C*, PCNA expression in tissues from four groups of animals. Quantification of band density can be found in Supplemental Table S6. *D*, Upper panel: Low power histologic appearance of H&E-stained HCCs. Tumor nodules (arrows) were multifocal and generally in close proximity to blood vessels. The nodules were variably sized with pleomorphic tumor cells arranged in broad trabeculae, 6-8 cells thick in many areas and often displaying an acinar/cholangiocarcinoma-like arrangement around a lumen. Nodules typically possessed distinct margins with occasional off-shoots into the surrounding liver parenchyma. The cytoplasm was amphophilic with distinct borders and vesicular nuclei containing single or multiple prominent nucleoli. Black bar = 500 microns. Lower panel: Higher power view showing frequent mitoses (arrows). The degree of pleomorphism and the cytologic features were of a moderately differentiated hepatocellular carcinoma. Black bar = 20 microns.



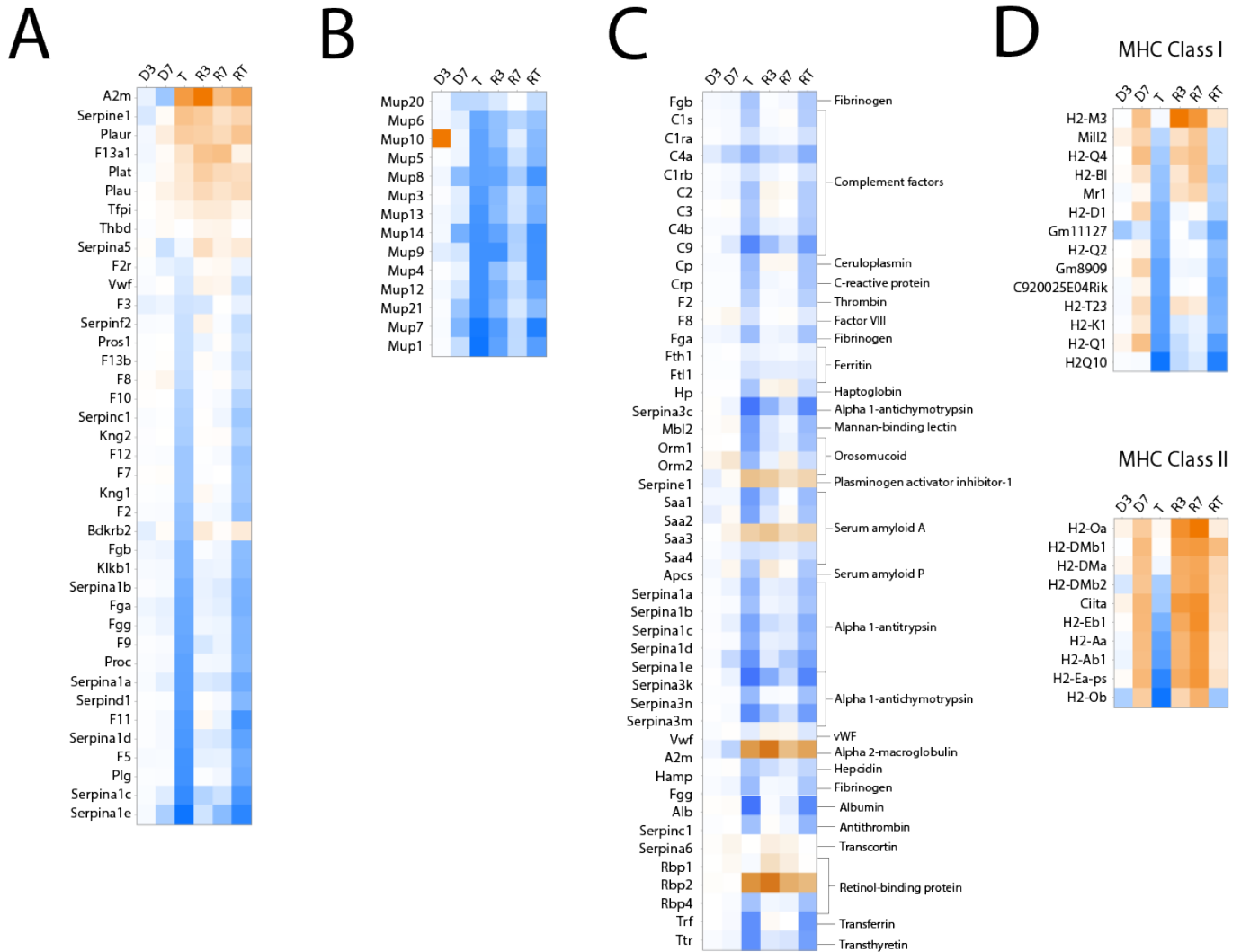
Supplemental Figure S2. Immuno-blot profiling of liver and tumor samples. *A*, Additional independent groups of samples similar to those depicted in Figure 1. Immuno-blots were performed for Myc, β -catenin and YAP. The immuno-blots for Myc on the far right are the same samples as those on the left but without tumors, to allow for a longer exposure and better visualization of Myc protein in other lanes. *B*, Additional sets of immuo-blots for PDH, pPDH, PDK1 and PDP2. Quantification of band density can be found in Supplemental Table S6.



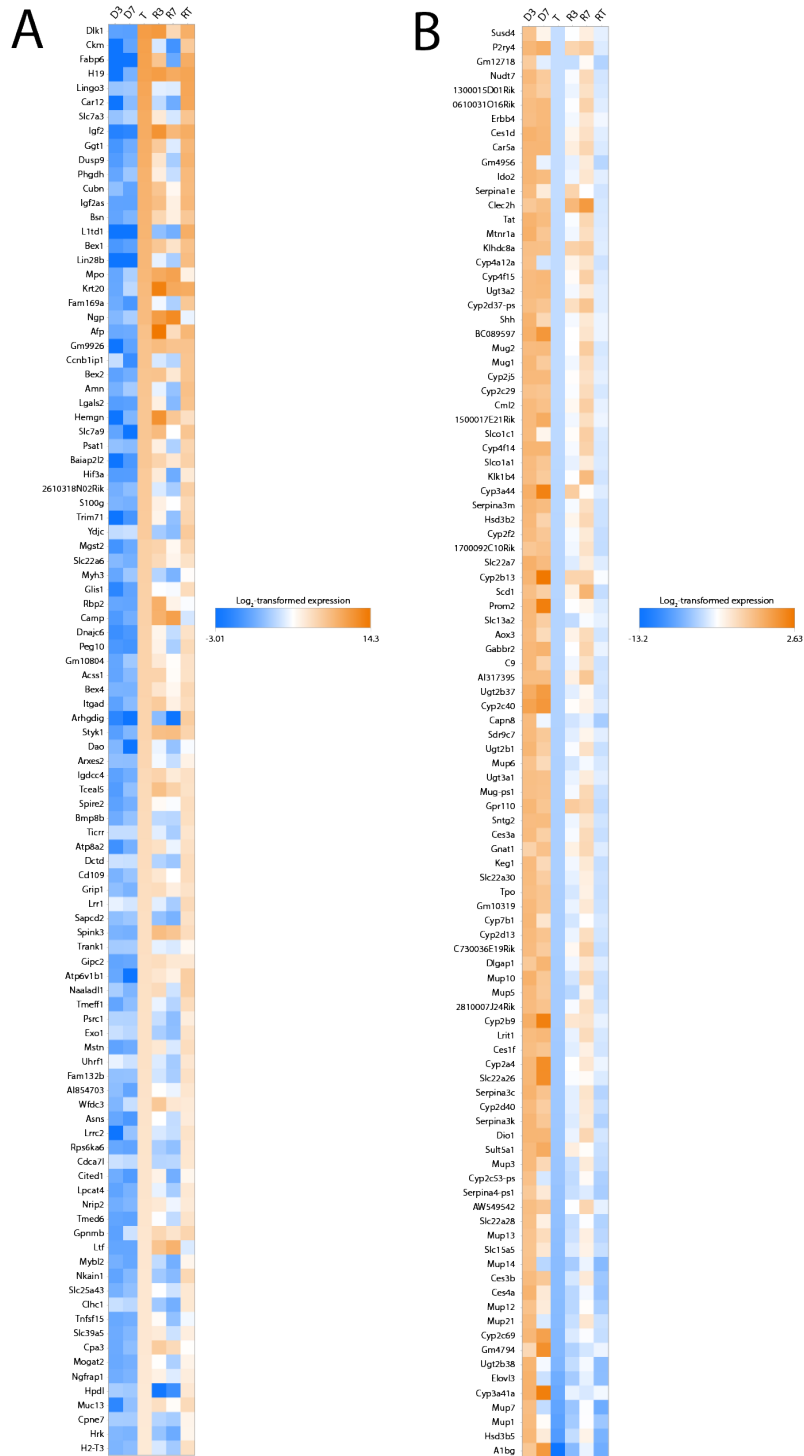
Supplemental Figure S3. Examples of BNGE and *in situ* assays of ETC Complexes I, III, IV and V. *A*, Typical BNGE results from two representative groups of mice. Each complex is denoted by an arrow. sc = supercomplexes comprised primarily of Complexes I, III and IV (1,2). *B*, *In situ* enzymatic assays for Complexes I, III, IV and V after normalizing to BNGE complex densities. Complex I = NADH ubiquinone oxidoreductase, Complex III = decylubiquinol cytochrome c oxidoreductase, Complex IV = cytochrome c oxidase, Complex V = ATPase (1).



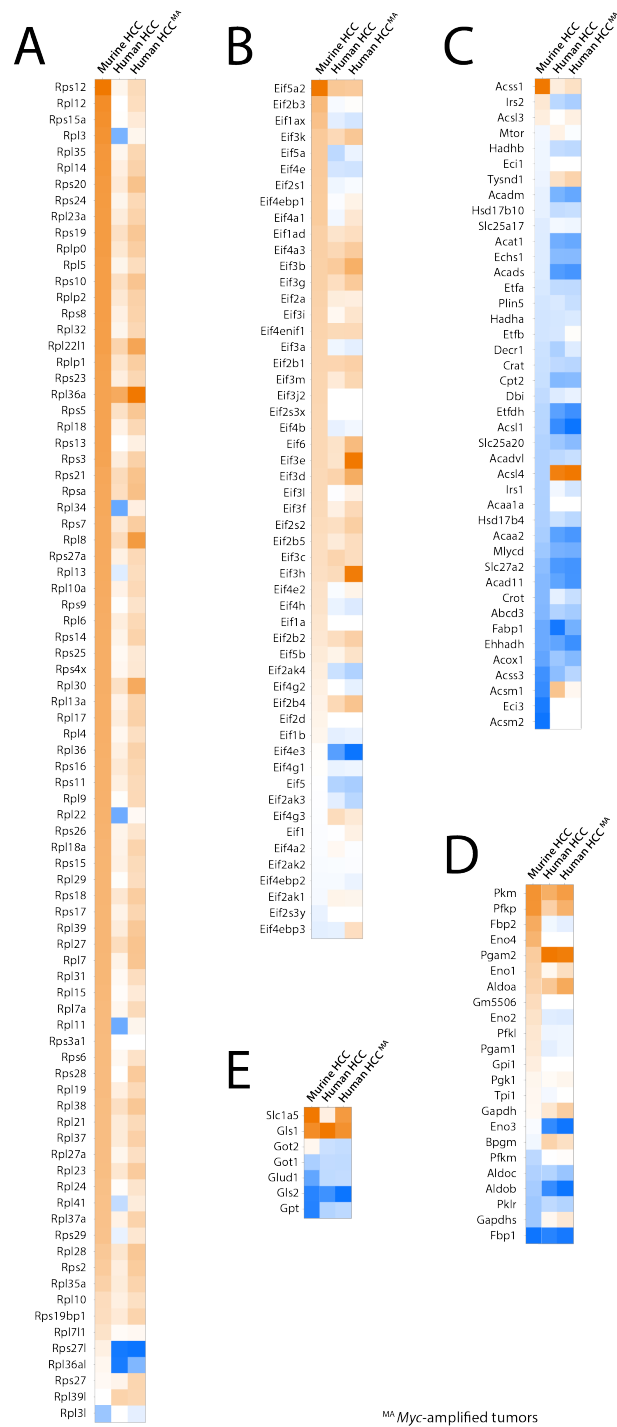
Supplemental Figure S4. Expression of transcripts encoding cell cycle regulators. A, Cyclins. B, Cyclin-dependent kinases. C, Cyclin-dependent kinase inhibitors.



Supplemental Figure S5. Loss of expression in tumors of select transcripts encoding proteins synthesized by mature hepatocytes. *A*, Transcripts encoding coagulation factors. Note the marked down-regulation of hepatocyte-specific transcripts encoding most members of the intrinsic, extrinsic and common clotting pathway including those for Factors II, V, VII, VIII, IX, X, XI and XIII. Transcripts for a variety of serpin proteases that regulate the clotting cascade (3) were also down-regulated, including antithrombin III (serpin 1C), protein C inhibitor (serpin A5) and heparin II co-factor (serpin D1). *B*, Heat map of major urinary protein (MUP) transcripts. Note that most of these were already dramatically down-regulated by D7, were further down-regulated by at least 100-fold in initial tumors, began to recover by R7 and were again markedly down-regulated in recurrent tumors. *C*, Heat map of acute phase reactant transcripts. *D*, Heat map of transcripts encoding major members of the major histocompatibility complex. All expression levels were obtained from RNA-Seq data and represent the mean of 5 samples/group.



Supplemental Figure S6. Expression of transcripts for the most highly de-regulated transcripts. *A*, An unbiased ranking of the 100 most highly up-regulated transcripts in initial tumors versus livers. Transcripts included are those for which a signal was obtained during RNA-seq and are displayed so as to show the most abundant tumor transcripts in the group at the top and the least abundant at the bottom. Fold differences for the group ranged from 3910-144. Collectively, these transcripts were, on average, up-regulated only 67% as highly in recurrent tumors as they were in initial tumors. *B*, A similar unbiased ranking of the 100 most down-regulated transcripts in initial tumors versus liver. Fold differences ranged from 95-145,000. As with the up-regulated transcripts, the average down-regulation for the group was 76% less pronounced in recurrent tumors. See Supplementary Tables 2 and 3 for full listings and relative expression levels for all transcripts.



Supplemental Figure S7. Expression of selected transcripts in human HCCs by *Myc*-amplification status. Heatmaps represent expression levels of transcripts from Figures 5 and 6 in 65 *Myc*-amplified HCCs, 298 non-*Myc*-amplified tumors, and 50 normal livers from The Cancer Genome Atlas (TCGA). For each group of transcripts, the degree of dysregulation (absolute value of fold-change relative to normal liver) was compared between *Myc*-amplified and non-*Myc* amplified tumors with Wilcoxon signed-rank tests. Ribosomal protein (A), eIF (B), fatty acid oxidation (C), and glycolytic (D) transcripts had a significantly greater degree of dysregulation in *Myc*-amplified tumors compared to non-*Myc*-amplified tumors ($P = <0.0001$, <0.0001 , 0.02, and 0.003, respectively). The degree of dysregulation for glutaminolytic transcripts (E) did not significantly differ between the tumor groups.

Antibody	Species	Vendor/ID	Dilution
Myc	Rabbit	Cell Signaling (13987)	1:1000
β -cat	Mouse	Fisher Scientific (BDB610154)	1:1000
Yap	Rabbit	Cell Signaling (4912)	1:1000
GAPDH	Mouse	Sigma-Aldrich (G8795)	1:20000
PDH	Mouse	Santa Cruz (SC-377092)	1:1000
pPDH	Rabbit	Calbiochem (ap1062)	1:2000
PDK1	Rabbit	Cell Signaling (3820)	1:1000
PDP2	Rabbit	BioVision (3944)	1:500
GLS-2	Rabbit	Abcam (ab113509)	1:1000
GluDH1	Rabbit	Cell Signaling (12793)	1:1000
CD45.2	Mouse	eBiosciences(17-0454)	1:200
CD11b (M1/70)	Rat	eBiosciences(45-0112)	1:200
Ly6G (RB6-8C5)	Rat	eBiosciences(13-5931)	1:200
CD68 (FA-11)	Rat	eBiosciences(25-0681)	1:200
MHC class II (AF6-120.1)	Mouse	eBiosciences(12-5320)	1:200
TCR- β (H57-597)	Hamster	eBiosciences(45-5961)	1:200
B220 (RA3-6B2)	Rat	BD Biosciences(553088)	1:200
CD4 (RM4-5)	Rat	BD Biosciences(553049)	1:200
CD8 (53-6.7)	Rat	eBiosciences(47-0081)	1:200
Ly6c (HK1.4)	Mouse	eBiosciences(53-5932)	1:200
Live/Dead dye	N/A	ThermoFisher(L34957)	1:1000
PCNA	Mouse	Santa Cruz (SC-56)	1:500

Supplemental Table S1. Antibodies used for the current studies.

GenBank ID	Gene	T v L log ₂ (Δ)	RT v L log ₂ (Δ)	GenBank ID	Gene	T v L log ₂ (Δ)	RT v L log ₂ (Δ)
13386	Dlk1	11.93	10.82	13142	Dao	8.30	5.40
12715	Ckm	11.71	9.06	76976	Arxes2	8.27	6.51
16204	Fabp6	11.65	10.90	56741	Igdcc4	8.10	7.63
14955	H19	11.58	11.45	331532	Tceal5	8.07	7.55
237403	Lingo3	11.35	11.34	234857	Spire2	8.03	7.85
76459	Car12	11.22	11.28	12164	Bmp8b	7.98	7.75
11989	Slc7a3	11.00	9.38	77011	Ticrr	7.95	7.36
16002	Igf2	10.96	10.94	50769	Atp8a2	7.92	7.64
14598	Ggt1	10.85	10.25	320685	Dctd	7.90	7.93
75590	Dusp9	10.82	10.55	235505	Cd109	7.84	7.89
236539	Phgdh	10.61	10.09	74053	Grip1	7.80	7.53
65969	Cubn	10.54	10.01	69706	Lrr1	7.79	8.07
111975	Igf2as	10.51	10.20	72080	Sapcd2	7.74	7.57
12217	Bsn	10.44	9.18	20730	Spink3	7.72	7.89
381591	L1td1	10.42	10.83	320429	Trank1	7.71	6.13
19716	Bex1	10.28	9.65	54120	Gipc2	7.65	7.13
380669	Lin28b	10.26	9.90	110935	Atp6v1b1	7.65	8.81
17523	Mpo	10.21	6.59	381204	Naaladl1	7.62	8.75
66809	Krt20	10.11	11.01	230157	Tmeff1	7.61	7.92
320557	Fam169a	10.01	9.21	56742	Psrl1	7.60	6.82
18054	Ngp	10.00	4.90	26909	Exo1	7.60	7.53
11576	Afp	9.65	10.28	17700	Mstn	7.59	7.87
100503565	Gm9926	9.52	9.50	18140	Uhrf1	7.57	7.26
239083	Ccnb1ip1	9.51	9.39	227358	Fam132b	7.52	7.63
12069	Bex2	9.40	9.41	243373	AI854703	7.50	7.30
93835	Amn	9.38	9.70	71856	Wfdc3	7.45	7.00
107753	Lgals2	9.32	9.52	27053	Asns	7.44	6.92
93966	Hemgn	9.32	7.70	74249	Lrrc2	7.42	7.47
30962	Slc7a9	9.31	9.38	67071	Rps6ka6	7.42	7.26
107272	Psat1	9.25	8.37	217946	Cdca71	7.40	7.24
207495	Baiap2l2	9.24	8.62	12705	Cited1	7.38	6.27
53417	Hif3a	9.09	7.04	99010	Lpcat4	7.38	7.14
70458	2610318N02Rik	9.08	8.73	60345	Nrip2	7.37	7.02
12309	S100g	9.05	8.18	66269	Tmed6	7.35	7.44
636931	Trim71	9.02	8.37	93695	GpnmB	7.30	8.37
69101	Ydjc	8.97	9.04	17002	Ltf	7.25	4.33
211666	Mgst2	8.70	8.37	17865	Mybl2	7.25	6.30
18399	Slc22a6	8.69	7.55	67149	Nkain1	7.21	8.19
17883	Myh3	8.67	5.83	194744	Slc25a43	7.21	7.16
230587	Glis1	8.63	7.94	69885	Clhc1	7.20	7.17
19660	Rbp2	8.61	5.47	326623	Tnfrsf15	7.20	5.20
12796	Camp	8.60	4.11	72002	Slc39a5	7.19	6.73
72685	Dnajc6	8.55	7.51	12873	Cpa3	7.18	5.76
170676	Peg10	8.52	8.06	233549	Mogat2	7.12	6.42
100038525	Gm10804	8.51	7.56	12070	Ngfrap1	7.11	6.82
68738	Acss1	8.48	7.56	242642	Hpd1	7.09	7.19
406217	Bex4	8.47	8.16	17063	Muc13	7.06	8.04
381924	Itgad	8.44	7.93	102278	Cpne7	7.05	6.37
14570	Arhgdig	8.44	8.90	12123	Hrk	7.04	6.34
243659	Styk1	8.44	8.45	15043	H2-T3	7.04	7.67

Supplemental Table S2. Top 100 most highly up-regulated transcripts in initial and recurrent tumors.

GenBank ID	Gene	T v L log ₂ (Δ)	RT v L log ₂ (Δ)	GenBank ID	Gene	T v L log ₂ (Δ)	RT v L log ₂ (Δ)
96935	Susd4	-6.57	-4.67	71773	Ugt2b1	-7.80	-6.31
57385	P2ry4	-6.58	-5.16	620807	Mup6	-7.88	-5.48
622167	Gm12718	-6.59	-7.60	105887	Ugt3a1	-7.89	-4.86
67528	Nudt7	-6.59	-5.74	17835	Mug-ps1	-7.90	-6.27
74161	1300015D01Rik	-6.59	-5.46	77596	Gpr110	-7.94	-6.81
68369	0610031O16Rik	-6.60	-4.97	268534	Sntg2	-7.95	-5.91
13869	Erbb4	-6.61	-3.92	382053	Ces3a	-7.95	-6.38
104158	Ces1d	-6.62	-4.90	14685	Gnat1	-8.01	-5.08
12352	Car5a	-6.62	-5.28	64697	Keg1	-8.02	-6.52
241041	Gm4956	-6.63	-7.32	319800	Slc22a30	-8.03	-6.14
209176	Ido2	-6.66	-4.84	22018	Tpo	-8.08	-6.40
20704	Serpina1e	-6.67	-5.83	381806	Gm10319	-8.13	-6.12
94071	Clec2h	-6.73	-5.50	13123	Cyp7b1	-8.15	-5.46
234724	Tat	-6.74	-5.38	68444	Cyp2d13	-8.18	-5.81
17773	Mtnr1a	-6.77	-5.15	402734	C730036E19Rik	-8.22	-6.50
213417	Klhdc8a	-6.79	-5.01	224997	Dlgap1	-8.33	-5.07
277753	Cyp4a12a	-6.79	-6.04	100039008	Mup10	-8.35	-6.27
106648	Cyp4f15	-6.79	-5.26	17844	Mup5	-8.35	-6.52
223337	Ugt3a2	-6.80	-4.93	76971	2810007J24Rik	-8.35	-5.81
627860	Cyp2d37-ps	-6.80	-4.97	13094	Cyp2b9	-8.36	-4.48
20423	Shh	-6.81	-4.27	239037	Lrit1	-8.47	-5.51
216454	BC089597	-6.82	-4.28	234564	Ces1f	-8.57	-5.78
17837	Mug2	-6.82	-5.51	13086	Cyp2a4	-8.62	-4.18
17836	Mug1	-6.82	-5.13	236149	Slc22a26	-8.67	-5.21
13109	Cyp2j5	-6.87	-4.65	16625	Serpina3c	-8.71	-7.45
13095	Cyp2c29	-6.88	-5.51	71754	Cyp2d40	-8.74	-6.41
93673	Cml2	-6.90	-4.54	20714	Serpina3k	-8.78	-7.66
668215	1500017E21Rik	-6.98	-4.17	13370	Dio1	-8.85	-5.72
58807	Slco1c1	-7.03	-5.30	57429	Sult5a1	-8.88	-6.40
64385	Cyp4f14	-7.04	-5.45	17842	Mup3	-9.01	-6.66
28248	Slco1a1	-7.13	-5.29	638988	Cyp2c53-ps	-9.06	-7.86
18048	Klk1b4	-7.18	-5.89	321018	Serpina4-ps1	-9.28	-8.16
337924	Cyp3a44	-7.23	-5.08	100993	AW549542	-9.37	-4.59
20717	Serpina3m	-7.23	-6.12	434674	Slc22a28	-9.53	-7.64
15493	Hsd3b2	-7.31	-6.08	100039089	Mup13	-9.69	-7.16
13107	Cyp2f2	-7.32	-6.53	277898	Slc15a5	-9.79	-6.42
73554	1700092C10Rik	-7.34	-6.62	100039116	Mup14	-9.93	-10.14
108114	Slc22a7	-7.34	-5.08	13909	Ces3b	-9.95	-8.83
13089	Cyp2b13	-7.35	-3.49	234677	Ces4a	-9.95	-9.17
20249	Scd1	-7.36	-6.57	100039054	Mup12	-10.06	-8.09
192212	Prom2	-7.45	-5.21	381531	Mup21	-10.16	-6.20
20500	Slc13a2	-7.48	-4.59	100043108	Cyp2c69	-10.32	-6.49
71724	Aox3	-7.49	-5.80	215895	Gm4794	-10.33	-4.22
242425	Gabbr2	-7.49	-4.39	100559	Ugt2b38	-10.60	-9.77
12279	C9	-7.56	-6.07	12686	Elov13	-11.44	-9.76
215929	A1317395	-7.65	-5.70	53973	Cyp3a41a	-11.83	-3.89
112417	Ugt2b37	-7.67	-6.04	100041658	Mup7	-12.28	-11.55
13099	Cyp2c40	-7.70	-5.13	17840	Mup1	-12.66	-8.84
170725	Capn8	-7.70	-8.30	15496	Hsd3b5	-13.22	-10.87
70061	Sdr9c7	-7.75	-4.84	117586	Albg	-17.15	-11.42

Supplemental Table S3. Top 100 most highly down-regulated transcripts in initial and recurrent tumors.

Gene	GenBank ID	Description	Initial Tumor Expression v Liver	Recurrent Tumor Expression v Liver
Igf1	16000	insulin-like growth factor 1	0.04	0.04
Igf2	16002	insulin-like growth factor 2	1994.25	1961.65
Igfbp1	16006	insulin-like growth factor binding protein 1	20.03	4.81
Igfbp2	16008	insulin-like growth factor binding protein 2	0.60	0.60
Igfbp3	16009	insulin-like growth factor binding protein 3	0.82	0.55
Igfbp4	16010	insulin-like growth factor binding protein 4	0.29	0.25
Igfbp5	16011	insulin-like growth factor binding protein 5	0.21	0.74
Igfbp6	16012	insulin-like growth factor binding protein 6	0.12	0.26
Igfbp7	29817	insulin-like growth factor binding protein 7	0.54	0.62
Igf2bp1	140486	insulin-like growth factor 2 mRNA binding protein 1	67.36	676.74
Igf2bp2	319765	insulin-like growth factor 2 mRNA binding protein 2	0.21	0.52
Igf2bp3	140488	insulin-like growth factor 2 mRNA binding protein 3	23.11	15.71
Igf1r	16001	insulin-like growth factor 1 receptor	0.69	2.12
Igf2r	16004	insulin-like growth factor 2 receptor	2.55	2.49
Igfals	16005	insulin-like growth factor binding protein, acid labile subunit	0.01	0.03
Insr	16337	insulin receptor	0.54	0.47
H19	14955	H19 fetal liver mRNA	3053.89	2794.88
Lin28b	380669	lin-28 homolog B (C. elegans)	1224.71	955.17

Supplemental Table S4. Relative expression of insulin-like growth factor-related transcripts in HCCs.

Target	Forward Primer	Reverse Primer	Annealing Temp
RAB27A	5'-TCGGATGGAGATTACGATTACCT-3'	5'-TTTTCCCTGAAATCAATGCCCA-3'	57 °C
ADAM19	5'-TCAGTGGCGGACTTCAGAAAG-3'	5'-GCAAAAAGGTGCTCGTTCTTC-3'	57 °C
H2DMB1	5'-ACCCACAGGACTTCACATAC-3'	5'-GGATACAGCACCCCAAATTCA-3'	57 °C
SFXN3	5'-CAGGAAGACTCCGACTGTGG-3'	5'-CCGCTGCGATTAGAGTAATTCAC-3'	57 °C
BOK	5'-AGGTAGTGTCCCTGTATTCCG-3'	5'-AAGGTCTTGCGTACAACTCC-3'	57 °C
NPNT	5'-GAAGCCTCGGCCCTGTAAG-3'	5'-AGCATGTATCCGTTGAGACAGTA-3'	57 °C
DHTKD1	5'-GCTGCTGCGGTTTATTGGTG-3'	5'-CAAGGCAAGTGTCTCTGCG-3'	60 °C

Supplemental Table S5. Primer sequences for qRT-PCR confirmation.

Target	Liver	D3	D7	Tumor	R3	R7	Rec. tumor
Figure 1							
Myc (short exposure)	0	0	0	1.0	0	0	0.83
β -catenin	1.0	2.9	2.5	3.3	3.9	2.4	2.3
YAP	0	0	0	1.0	0	0	0.56
GAPDH (A, short exposure)	1.0	1.1	0.92	1.1	1.1	1.1	0.98
Myc (long exposure)	0	1.0	0.57	-	0	0	-
GAPDH (A, long exposure)	1.0	1.2	1.0	-	1.5	1.9	-
PDH	1.0	0.67	0.79	0.66	0.53	0.76	0.27
pPDH	1.0	1.4	1.1	0.22	0.27	0.44	0.26
PDK1	1.0	0.97	0.86	0.52	0.12	0.02	0.54
PDP2	1.0	2.0	1.7	2.0	1.5	1.1	1.8
GAPDH (C)	1.0	0.80	1.0	0.97	1.0	0.97	0.96
Figure 5							
GLS1 (Set 1)	0	0	0.25	1.0	0.38	0.30	1.5
GLS1 (Set 2)	0	0.28	0.37	1.0	0.25	0.23	0.56
GLS1 (Set 3)	0	0.09	0.16	1.0	0	0	1.0
GLS1 (Set 4)	0.15	0.12	0.19	1.0	0.18	0.12	0.45
GLS2 (Set 1)	1.0	0.73	0.85	0.16	0.10	0.26	0.10
GLS2 (Set 2)	1.0	0.66	0.63	0.11	0.20	0.48	0.15
GLS2 (Set 3)	1.0	1.5	1.7	0.41	.69	0.64	0.27
GLS2 (Set 4)	1.0	0.99	1.0	0.34	0.23	0.67	0.46
GLUD1 (Set 1)	1.0	1.3	1.3	0.48	0.53	0.06	0.29
GLUD1 (Set 2)	1.0	1.1	1.4	0.28	0.68	0.46	0.52
GLUD1 (Set 3)	1.0	0.59	0.57	0.10	0.01	0.01	0.16
GLUD1 (Set 4)	1.0	0.69	0.48	0.07	0.29	0.55	0.24
GAPDH (Set 1)	1.0	1.0	0.94	1.5	1.1	1.2	0.89
GAPDH (Set 2)	1.0	0.92	0.93	1.1	1.0	0.94	0.76
GAPDH (Set 3)	1.0	1.0	1.1	1.1	0.88	0.82	0.76
GAPDH (Set 4)	1.0	1.3	1.3	0.97	1.0	1.0	1.4
Supplemental Figure 1							
PCNA (Set 1)	1.0	2.5	5.7	28.8	4.7	2.4	24.5
PCNA (Set 2)	1.0	5.7	7.6	138.7	32.3	6.6	121.6
PCNA (Set 3)	1.0	6.5	4.7	37.4	10.6	2.1	30.2
PCNA (Set 4)	1.0	1.2	7.2	101.8	11.2	4.3	77.4
GAPDH (Set 1)	1.0	0.98	0.76	0.94	0.73	0.65	0.50
GAPDH (Set 2)	1.0	0.85	0.76	0.94	0.90	0.91	1.2
GAPDH (Set 3)	1.0	0.70	0.63	0.59	0.54	0.60	0.55
GAPDH (Set 4)	1.0	0.93	0.79	1.0	1.0	1.1	1.3
Supplemental Figure 2							
Myc (short exposure, left)	0	0	0	1.0	0	0	.71
Myc (short exposure, right)	0	0	0	1.0	0	0	0.76
β -catenin (left)	1.0	0.73	0.84	4.7	1.5	1.0	2.5
β -catenin (right)	1.0	2.3	1.6	2.6	8.4	6.2	1.9
YAP (left)	0	0	0	1.0	0	0	0.54
YAP (right)	0	0	0	1.0	0	0	0.31
GAPDH (A, short exposure, left)	1.0	0.99	0.94	0.98	0.98	0.84	0.91
GAPDH (A, short exposure, right)	1.0	1.4	1.1	1.2	1.2	1.2	1.4
Myc (long exposure, top)	1.0	2.0	5.6	-	0.98	1.1	-
Myc (long exposure, bottom)	1.0	1.1	0.69	-	0.11	0.12	-
GAPDH (A, long exposure)	1.0	0.84	0.73	-	0.80	0.84	-
PDH (left)	1.0	0.81	0.64	0.34	0.24	0.38	0.26
PDH (right)	1.0	0.54	0.49	0.20	0.51	0.78	0.48
pPDH (left)	1.0	0.54	0.72	0.10	0.04	0.15	0.13
pPDH (right)	1.0	0.40	0.73	0.15	0.05	0.09	0.06
PDK1 (left)	1.0	0.78	0.17	0.08	0.17	0.09	0.46
PDK1 (right)	1.0	0.49	1.2	0.85	0.44	0.50	0.97
PDP2 (left)	1.0	0.27	0.61	1.1	0.27	0.11	1.3
PDP2 (right)	1.0	0.46	0.75	1.3	1.4	1.1	1.4
GAPDH (B, left)	1.0	1.1	1.0	0.96	1.0	1.0	0.96
GAPDH (B, right)	1.0	1.2	1.3	1.3	0.85	1.1	1.3

Supplemental Table S6. Western Blot Quantification with ImageJ. Western blot band densities were quantified with ImageJ and normalized to control liver. If no detectable control liver band was found, values were normalized to either tumor or D3 samples.

Supplemental References

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