Figure S1

Supplementary Figures

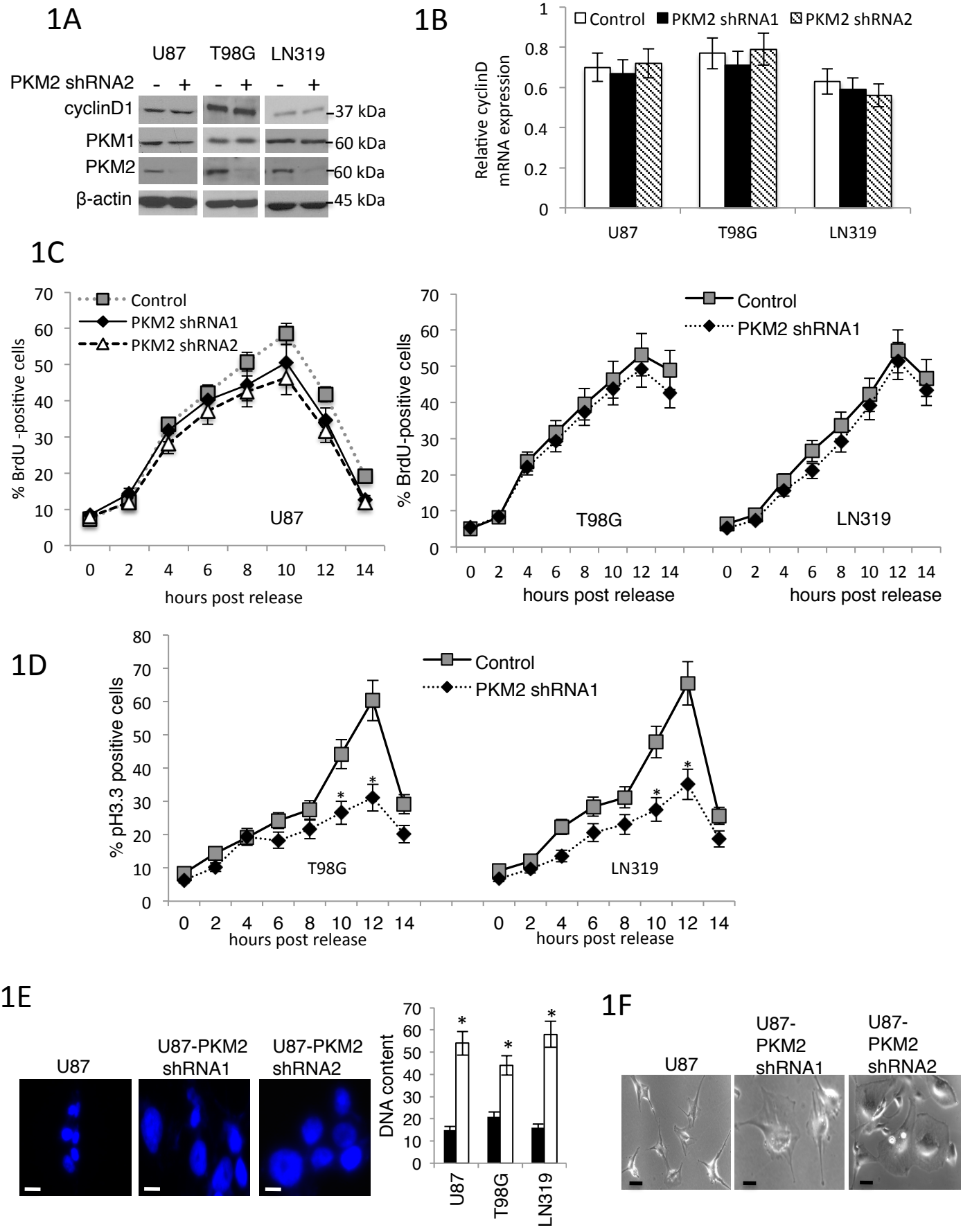
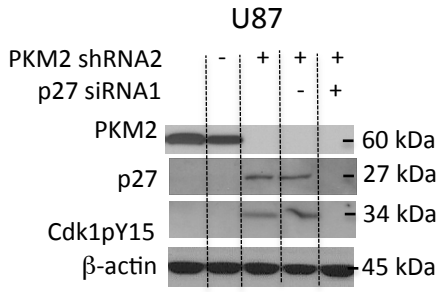


Figure S1

1G



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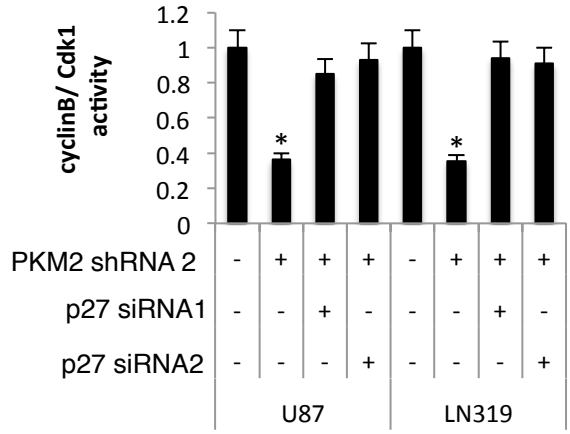

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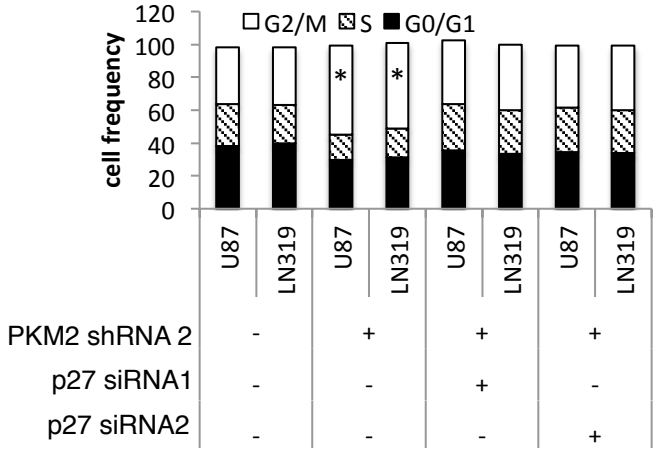

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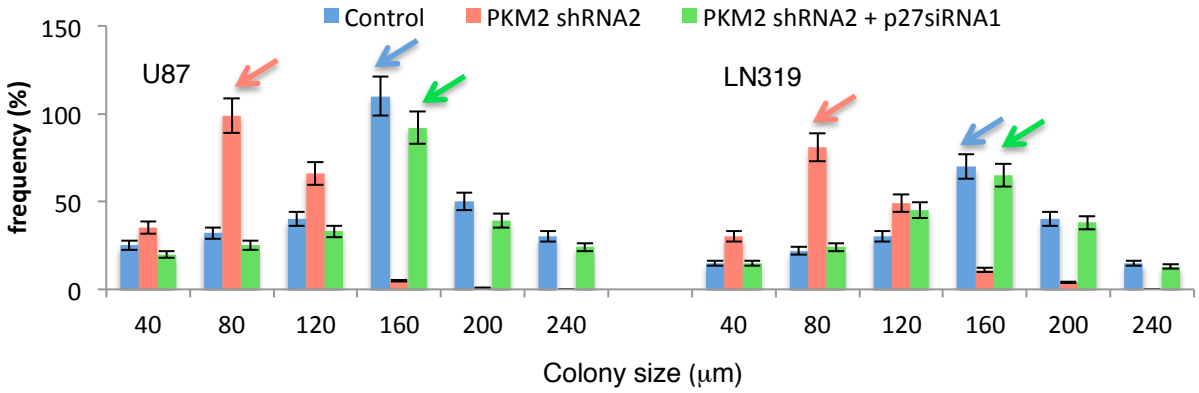

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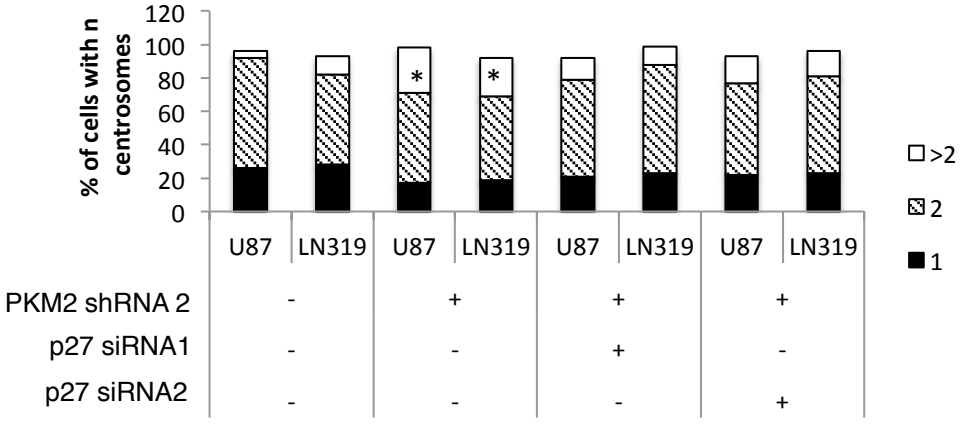


Figure S2

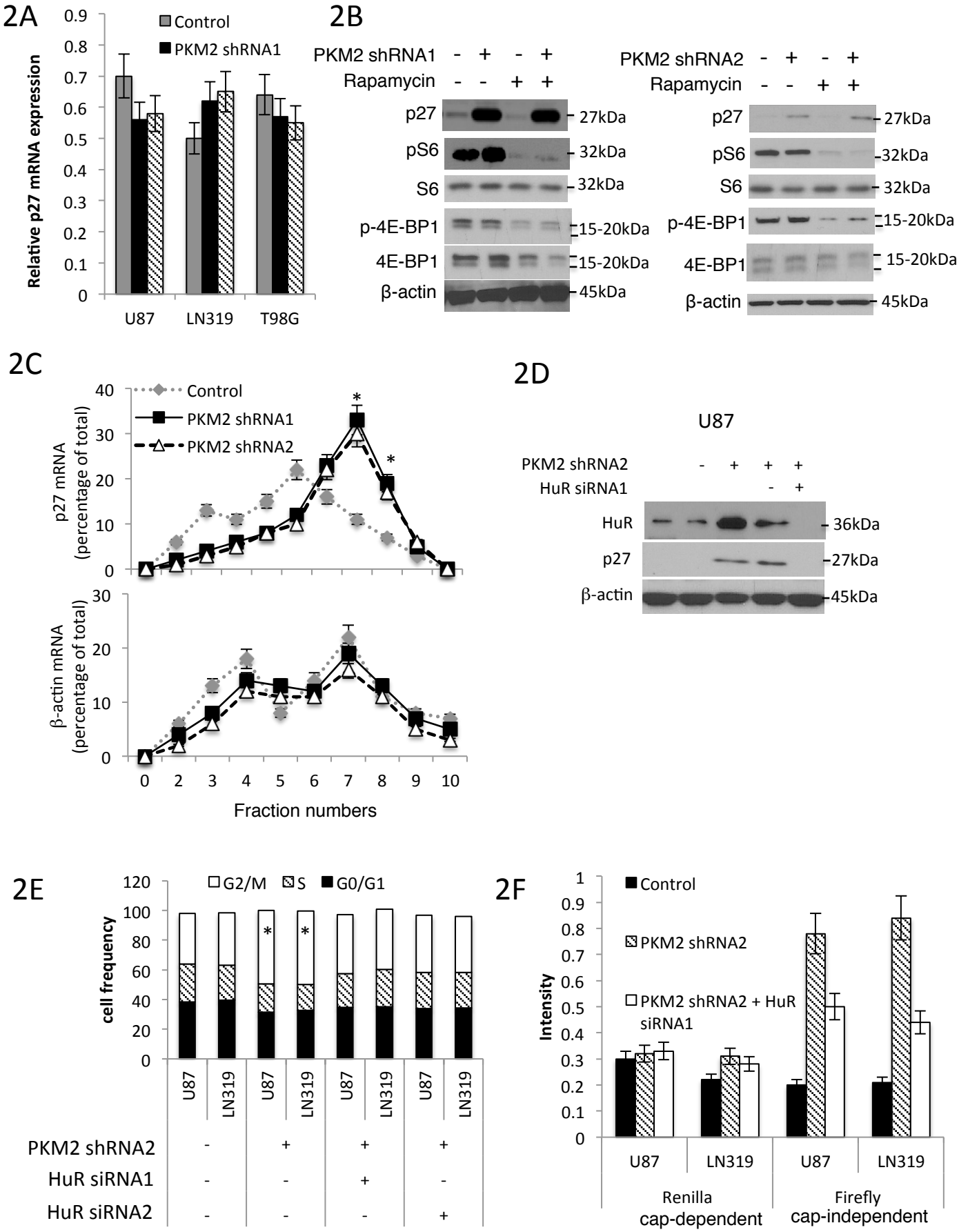
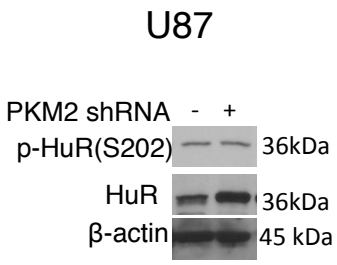

2A
Relative p27 mRNA expression
Control
PKM2 shRNA1
U87
LN319
T98G
2B
PKM2 shRNA1
Rapamycin
PKM2 shRNA2
p27
pS6
S6
p-4E-BP1
4E-BP1
β-actin
27kDa
32kDa
15-20kDa
45kDa
2C
p27 mRNA (percentage of total)
β-actin mRNA (percentage of total)
Fraction numbers
2D
U87
HuR siRNA1
HuR
36kDa
2E
cell frequency
G2/M
S
G0/G1
PKM2 shRNA2
HuR siRNA1
HuR siRNA2
2F
Intensity
PKM2 shRNA2 + HuR siRNA1
Renilla cap-dependent
Firefly cap-independent

Figure S3

3A



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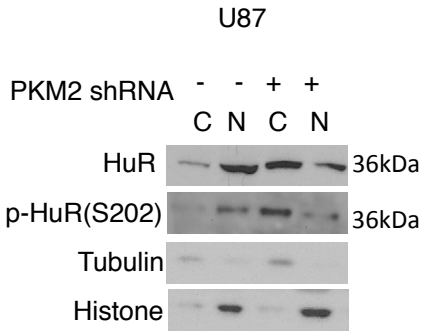

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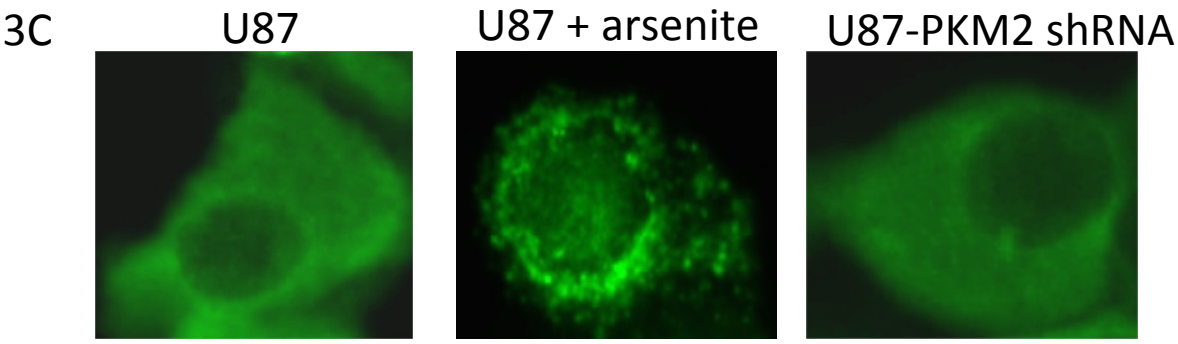


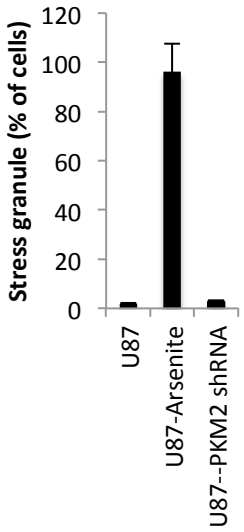
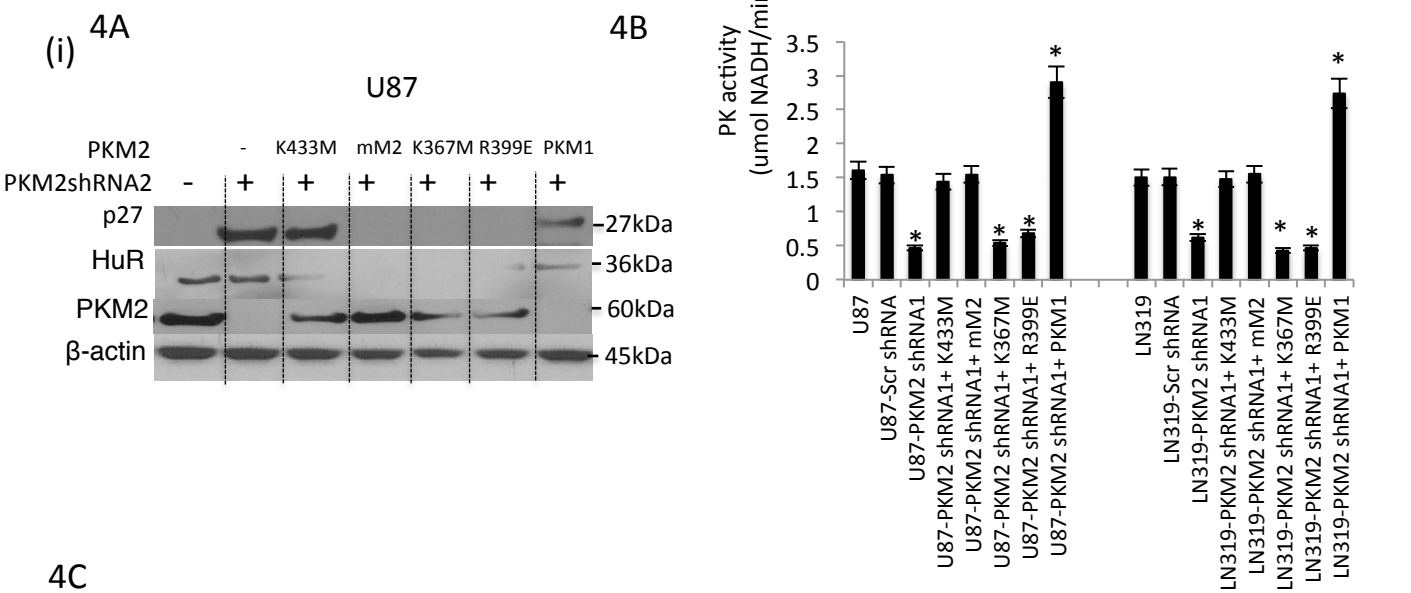
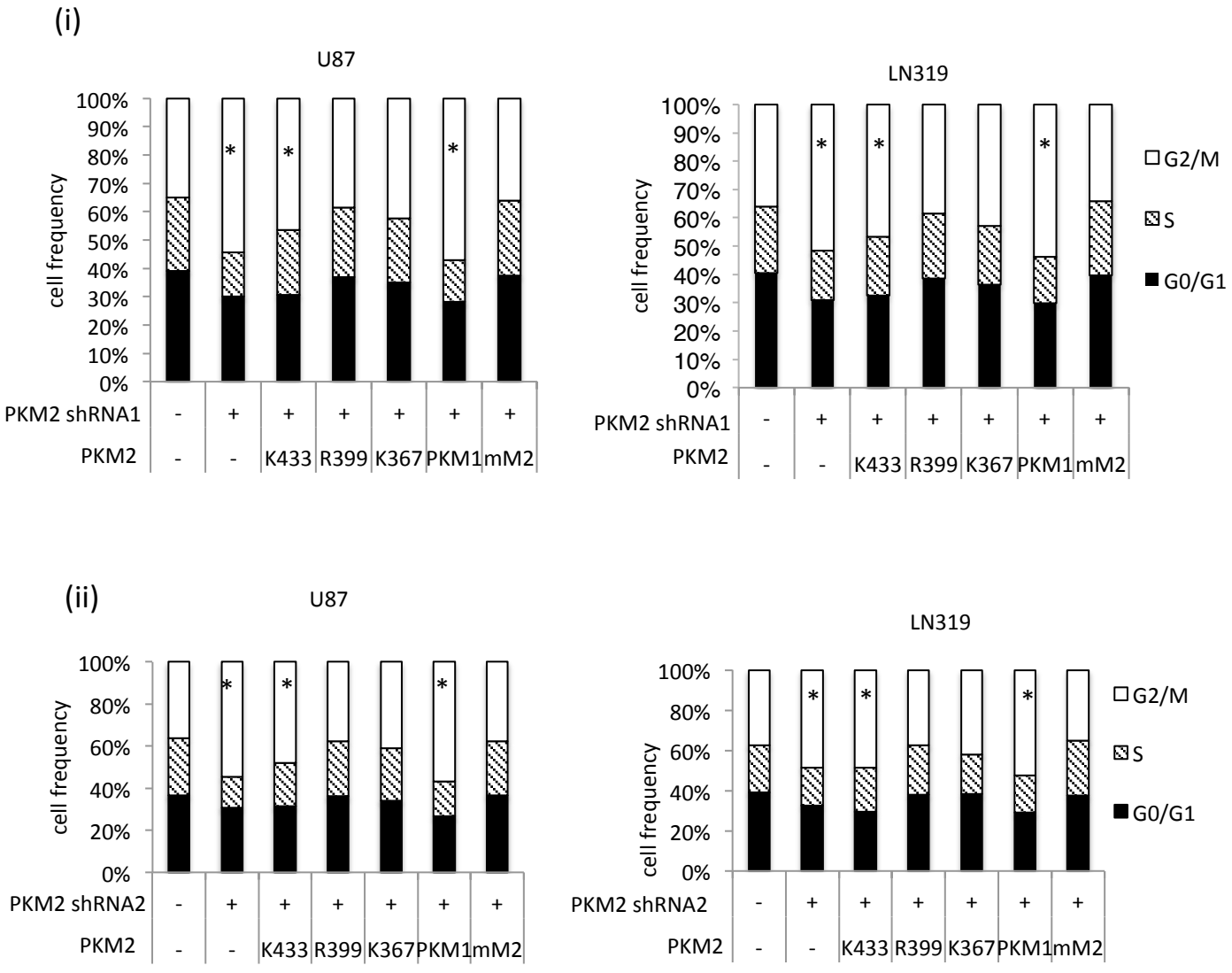


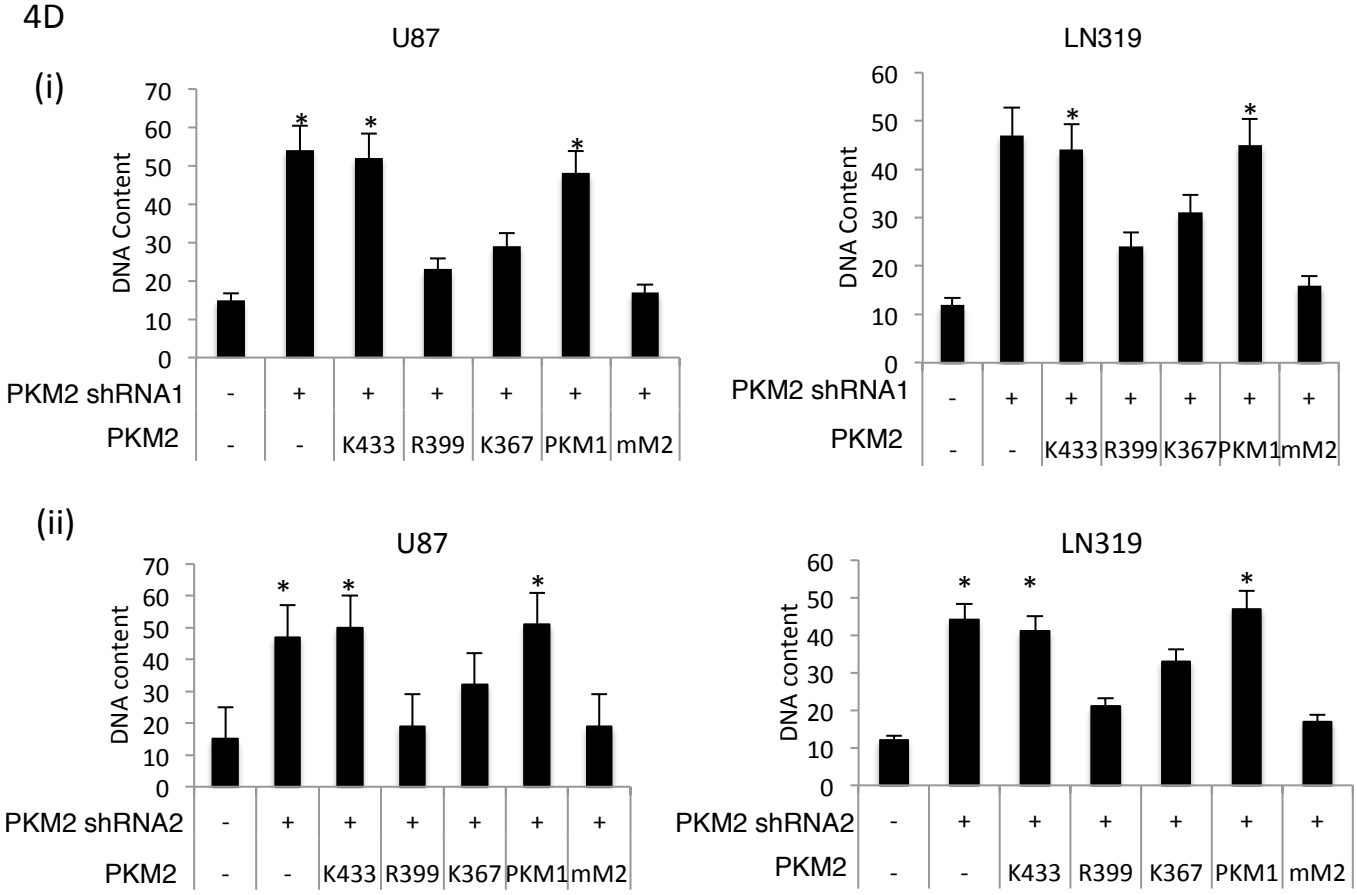
Figure S4


4A
(i)
U87
PKM2
PKM2shRNA2
-
K433M
mM2
K367M
R399E
PKM1
p27
HuR
PKM2
β-actin
27kDa
36kDa
60kDa
45kDa
4B
PK activity (umol NADH/min)
U87
U87-Scr shRNA
U87-PKM2 shRNA1
U87-PKM2 shRNA1+ K433M
U87-PKM2 shRNA1+ mM2
U87-PKM2 shRNA1+ K367M
U87-PKM2 shRNA1+ R399E
U87-PKM2 shRNA1+ PKM1
LN319
LN319-Scr shRNA
LN319-PKM2 shRNA1
LN319-PKM2 shRNA1+ K433M
LN319-PKM2 shRNA1+ mM2
LN319-PKM2 shRNA1+ K367M
LN319-PKM2 shRNA1+ R399E
LN319-PKM2 shRNA1+ PKM1

4C


(i)
U87
LN319
cell frequency
G2/M
S
G0/G1
PKM2 shRNA1
PKM2
K433
R399
K367
PKM1
mM2
(ii)
U87
LN319
PKM2 shRNA2

4D


(i)
U87
DNA Content
70
60
50
40
30
20
10
0
PKM2 shRNA1 - + + + + + +
PKM2 - - K433 R399 K367 PKM1 mM2
LN319
DNA Content
60
50
40
30
20
10
0
PKM2 shRNA1 - + + + + + +
PKM2 - - K433 R399 K367 PKM1 mM2
(ii)
U87
DNA content
70
60
50
40
30
20
10
0
PKM2 shRNA2 - + + + + + +
PKM2 - - K433 R399 K367 PKM1 mM2
LN319
DNA content
60
50
40
30
20
10
0
PKM2 shRNA2 - + + + + + +
PKM2 - - K433 R399 K367 PKM1 mM2

4E

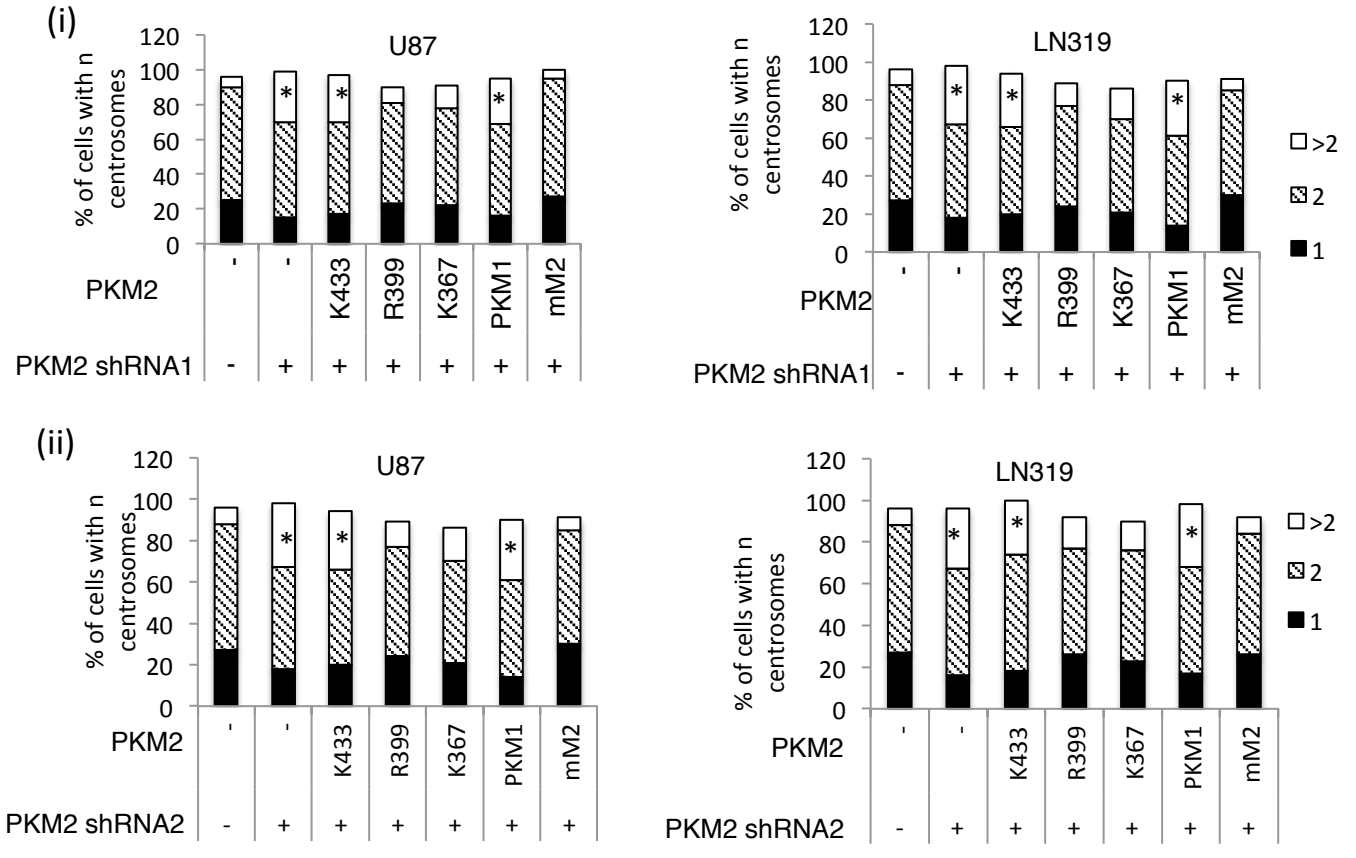
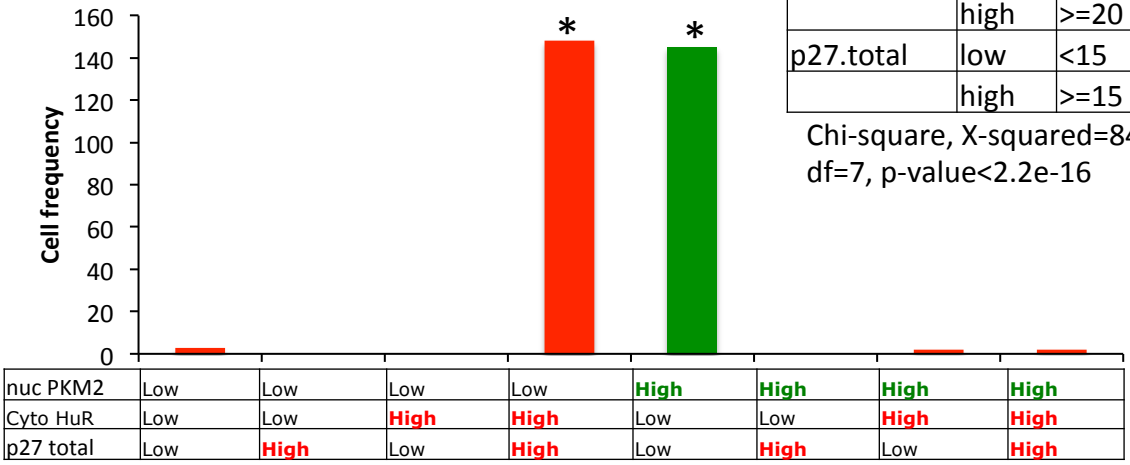

(i)
U87
% of cells with n centrosomes
120
100
80
60
40
20
0
PKM2 - - K433 R399 K367 PKM1 mM2
PKM2 shRNA1 - + + + + + +
LN319
% of cells with n centrosomes
120
100
80
60
40
20
0
PKM2 - - K433 R399 K367 PKM1 mM2
PKM2 shRNA1 - + + + + + +
>2
2
1
(ii)
U87
% of cells with n centrosomes
120
100
80
60
40
20
0
PKM2 - - K433 R399 K367 PKM1 mM2
PKM2 shRNA2 - + + + + + +
LN319
% of cells with n centrosomes
120
100
80
60
40
20
0
PKM2 - - K433 R399 K367 PKM1 mM2
PKM2 shRNA2 - + + + + + +
>2
2
1

Figure S5



nuc PKM2	Low	Low	Low	Low	**High**	**High**	**High**	**High**
Cyto HuR	Low	Low	**High**	**High**	Low	Low	**High**	**High**
p27 total	Low	**High**	Low	**High**	Low	**High**	Low	**High**

With cut points defined by kmeans

Nuc PKM2	low	< 15
	high	>=15
Cyto HuR	low	<20
	high	>=20
p27.total	low	<15
	high	>=15

Chi-square, X-squared=84, df=7, p-value<2.2e-16

Table-S1

GBM-1 (High nuclear PKM2)

	PKM2		**Hur**		**p27 total**
Cell #	Nuclear	Cyto	Nuclear	cyto	
1	18	78	85	9	10
2	22	71	82	8	9
3	22	70	81	6	7
4	24	81	82	5	7
5	17	83	81	7	6
6	19	70	80	8	5
7	21	72	79	7	8
8	20	75	76	8	10
9	20	76	94	7	10
10	18	76	76	10	11
11	18	79	84	7	8
12	17	78	83	5	4
13	22	71	88	8	11
14	16	79	87	6	8
15	21	76	89	8	4
16	20	74	94	7	7
17	15	80	87	6	5
18	22	82	88	8	10
19	21	71	86	9	12
20	24	73	84	6	10
21	21	74	83	8	7
22	19	74	89	7	6
23	16	81	91	8	8
24	8	78	89	6	5
25	17	73	87	9	11
26	21	71	82	7	5
27	22	79	81	8	11
28	22	69	78	9	14
29	24	71	76	8	11
30	16	70	81	7	9
31	22	72	82	6	4
32	23	74	89	8	7
33	21	73	78	4	6
34	22	78	79	5	8
35	20	71	84	3	10
36	24	74	82	6	11
37	19	79	80	4	5
38	18	69	89	5	7
39	21	73	76	8	11
40	20	74	74	6	9
41	18	71	82	8	9
42	16	77	81	3	4
43	24	73	79	7	6
44	23	78	80	8	7
45	22	79	72	9	8
46	21	69	91	6	4
47	19	72	83	5	6
48	17	74	86	4	8
49	17	71	84	5	10
50	22	72	85	6	7
Avg	19.84	74.56	83.18	6.76	7.92

GBM-2 (High nuclear PKM2)

	PKM2		**Hur**		**p27**
Cell #	Nuclear	Cyto	Nuclear	Cyto	Total
1	18	66	89	9	5
2	26	69	88	11	11
3	22	76	86	13	12
4	21	79	79	5	11
5	21	82	81	7	11
6	23	79	88	11	10
7	24	76	83	10	5
8	25	69	80	9	4
9	18	78	76	8	6
10	21	75	91	7	6
11	22	76	93	9	5
12	24	82	80	11	7
13	19	84	85	10	11
14	17	76	80	8	10
18	3	88	84	7	12
16	19	86	89	4	10
17	18	82	87	11	8
18	16	81	78	9	9
19	17	82	94	8	6
17	18	79	95	9	7
21	21	74	90	7	7
22	23	73	79	87	8
23	20	72	81	67	9
24	23	76	75	9	4
25	21	81	88	16	5
26	20	76	83	13	9
27	22	83	89	11	5
28	21	76	79	9	7
29	19	77	81	4	10
30	18	81	80	11	11
31	21	77	86	12	12
32	20	79	87	9	10
33	21	81	79	8	9
34	23	76	91	8	4
35	21	74	94	7	7
36	20	73	90	9	5
37	18	71	91	6	9
38	17	76	78	9	4
39	16	78	85	10	9
40	19	81	84	8	10
41	20	75	76	7	9
42	19	80	92	5	8
43	18	69	96	11	7
44	20	76	73	10	9
45	22	74	76	9	4
46	23	73	81	8	7
47	19	81	85	7	5
48	18	78	85	10	9
49	15	77	87	9	7
50	14	75	84	4	5
Avg	19.68	77.16	84.62	11.52	7.8

GBM-3 (High nuclear PKM2)

	PKM2		**Hur**		**p27**
cell #	Nuclear	Cyto	Nuclear	Cyto	Total
1	28	68	95	0	4
2	34	65	94	1	3
3	29	70	89	1	4
4	30	72	96	0	5
5	27	75	96	0	6
6	25	74	96	0	11
7	26	81	97	0	3
8	32	65	93	0	4
9	33	64	98	1	7
10	28	67	99	2	8
11	29	65	97	2	2
12	25	64	100	0	0
13	26	72	95	2	0
14	32	73	94	1	0
15	33	63	98	0	2
16	36	62	94	2	0
17	21	74	95	2	4
18	29	66	98	2	0
19	25	65	96	2	7
20	28	64	94	1	7
21	27	67	96	1	6
22	31	65	96	1	4
23	33	66	97	1	3
24	27	70	100	0	3
25	29	65	100	0	3
26	31	66	100	0	3
27	33	64	100	0	7
28	34	61	100	0	9
29	24	71	100	0	0
30	27	69	100	0	2
31	29	60	95	1	1
32	31	65	96	1	1
33	27	64	94	1	2
34	32	67	97	2	6
35	27	70	94	2	3
36	28	69	95	3	4
37	29	65	98	0	8
38	32	66	99	1	4
39	33	68	98	0	2
40	30	65	96	1	1
41	27	71	100	0	3
42	29	68	98	0	2
43	32	64	99	1	0
44	29	63	98	0	2
45	28	68	97	1	3
46	30	65	100	0	0
47	29	67	97	1	6
48	27	71	98	0	4
49	31	64	97	2	1
50	29	66	99	1	3
Avg	29.22	67.18	96.96	0.8	3.46

GBM-4 (Low nuclear PKM2)

	PKM2		**Hur**		**p27**
Cell #	Nuclear	Cyto	Nuclear	Cyto	Total
1	10	85	39	55	29
2	2	97	42	58	23
3	11	84	31	57	24
4	10	86	39	59	32
5	8	88	41	50	31
6	6	91	32	58	29
7	4	92	33	57	28
8	4	89	35	58	31
9	1	95	39	51	28
10	11	84	37	55	24
11	12	82	39	57	32
12	6	89	32	50	33
13	3	91	41	53	25
14	12	82	43	52	27
15	10	84	39	54	24
16	9	87	37	55	32
17	3	93	36	57	31
18	8	89	40	59	22
19	10	84	42	54	24
20	11	85	39	49	26
21	9	87	33	53	28
22	8	89	31	59	22
23	11	84	33	51	26
24	14	82	36	58	21
25	10	89	38	52	30
26	6	91	37	56	28
27	4	93	35	54	29
28	9	87	38	51	26
29	7	92	39	52	24
30	12	85	32	57	26
31	10	89	41	55	28
32	11	90	32	51	22
33	3	94	38	55	25
34	11	86	36	52	24
35	14	83	39	51	28
36	15	80	33	50	29
37	3	94	32	59	31
38	5	91	43	58	28
39	10	87	38	51	31
40	14	83	42	50	31
41	8	89	43	59	27
42	9	82	39	52	24
43	6	91	44	54	32
44	11	85	36	51	28
45	11	87	39	58	25
46	12	86	43	57	32
47	6	91	35	56	33
48	9	89	38	59	30
49	13	83	40	50	24
50	15	82	39	59	23
Avg	8.74	87.56	37.56	54.56	27.4

GBM-5 (Low nuclear PKM2)

	PKM2		**Hur**		**p27**
Cell #	Nuclear	Cyto	Nuclear	Cyto	Total
1	1	99	31	68	35
2	0	98	30	61	26
3	1	98	27	65	31
4	0	96	29	60	33
5	0	97	31	59	31
6	0	98	33	64	30
7	0	96	35	58	27
8	0	96	37	68	29
9	1	95	31	69	35
10	1	95	29	64	31
11	1	93	27	63	36
12	1	96	29	65	32
13	0	97	33	67	31
14	0	99	31	58	33
15	0	100	29	54	30
16	0	100	29	69	29
17	0	99	30	71	27
18	1	97	32	73	32
19	2	94	34	57	34
20	1	99	32	59	37
21	0	100	33	69	33
22	1	96	31	62	31
23	0	97	36	60	29
24	1	95	37	64	34
25	0	96	33	58	36
26	0	98	39	69	33
27	0	96	29	64	31
28	0	94	27	66	32
29	0	100	26	65	34
30	1	98	33	68	37
31	1	97	32	61	29
32	1	96	34	65	28
33	2	94	35	62	24
34	1	95	32	66	29
35	1	97	31	69	33
36	2	93	30	58	35
37	0	98	32	71	38
38	1	94	29	73	31
39	0	100	29	64	30
40	1	94	27	60	32
41	0	93	33	62	29
42	1	94	35	57	31
43	0	96	37	66	34
44	2	94	29	64	35
45	1	97	34	62	27
46	2	92	32	59	29
47	0	97	29	65	28
48	1	94	25	68	30
49	2	93	31	61	31
50	2	94	33	59	29
Avg	0.68	96.28	31.44	63.78	31.42

GBM-6 (Low nuclear PKM2)

	PKM2		**Hur**		**p27**
cell #	Nuclear	Cyto	Nuclear	Cyto	Total
1	6	93	32	51	45
2	9	88	30	50	38
3	5	92	27	65	33
4	6	91	37	56	41
5	5	91	33	40	39
6	4	93	38	59	39
7	9	86	26	54	45
8	11	84	28	52	43
9	8	90	35	48	37
10	8	88	34	54	40
11	9	85	32	44	37
12	11	86	30	45	41
13	12	84	28	51	39
14	6	93	32	50	43
15	5	94	27	52	38
16	8	88	34	55	44
17	11	86	29	47	37
18	11	86	38	49	41
19	6	91	34	51	40
20	8	89	29	60	38
21	10	87	32	45	33
22	7	89	31	58	48
23	7	91	35	44	42
24	6	92	36	60	37
25	5	93	32	51	39
26	11	88	33	56	41
27	9	87	36	59	40
28	6	92	37	57	42
29	9	88	39	46	38
30	9	87	32	64	33
31	11	87	36	50	48
32	13	85	37	57	39
33	10	88	36	51	49
34	9	86	33	56	38
35	8	90	34	59	36
36	5	93	28	56	49
37	8	87	27	54	37
38	7	91	22	45	44
39	9	86	25	56	42
40	11	85	28	55	39
41	10	87	31	41	42
42	9	88	26	51	51
43	8	87	27	45	48
44	5	94	34	58	44
45	6	92	28	48	34
46	11	88	29	51	36
47	9	87	31	48	38
48	7	88	27	57	42
49	13	85	33	54	43
50	6	91	30	59	40
Avg	8.24	88.74	31.56	52.48	40.6

Supplemental Figure Legends:

Figure S1 (related to main Figure 1).

(A) U87, T98G and LN319 glioma cells were lenivirally infected with a scrambled shRNA (PKM2 shRNA, control) or a second shRNA construct targeting human PKM2 (PKM2 shRNA2+). Following drug selection, polyclonal populations were examined by Western blot for cyclin D1, PKM1, PKM2 and β-actin expression

(B) Unsynchronized cells infected with a scrambled shRNA (control) or one of two shRNAs construct targeting human PKM2 were analyzed by quantitative PCR for levels of cyclin D1 mRNA using cyclin D1-specific primers. Values were normalized to β-actin, n=3.

(C,D) U87, T98G and LN319 cells from Figure 1A were serum starved for 48 hrs, after which serum was added and the cells were pulse-labeled with BrdU for the final 15 min prior to harvesting at the times indicated and assessed for the percentage of BrdU+ (C) or mitotic pH3.3+ (D) cells by immunohistochemistry and FACS. *, $p<.05$, n=3.

(E, F) Cells from panel A and Figure S1A were fixed and incubated with DAPI, after which the cells were examined for DNA content (E, solid bars, control; open bars, PKM2 knock-down), and cell size (F). *, $p<.05$, n=3. Bar in 1E, F=5 μm.

(G, H) Levels of PKM2, p27, phosphoY15-Cdk1, and β-actin (G), and cyclin B/Cdk1 activity (normalized to control)(H), in U87, T98, and LN319 cells from Figure S1A, or cells additionally containing scramble siRNA (p27 siRNA-) or one of two siRNAs targeting p27 (p27 siRNA1 & p27 siRNA2). *, $p<.05$, n=3.

(I) FACS-based cell cycle distribution in U87, T98G, and LN319 cells from panels S1G and H. *, $p<.05$, n=3.

(J, K) Colony size (J) and centrosome number (K) in U87, T98G, and LN319 cells from panel S1G. Arrows in panel J indicate the mode for each group. *, $p<.05$, n=3.

Figure S2 (related to main Figure 2)

(A) U87, T98G, and LN319 cells from Figure 1A and S1A were analyzed by quantitative PCR for levels of p27 mRNA using p27-specific primers. Values were normalized to β-actin, n=3.

(B) Levels of p27, pS6,S6, phospho 4E-BP1 and 4E-BP1 in U87 cells lentivirally infected with scr shRNA (PKM2 shRNA-) or one of two different shRNA targeting PKM2 (PKM2 shRNA1 or 2) and/or incubated with rapamycin (100 nM, 18 hr).

(C) Control or PKM2 knockdown U87 cells from Figure 1A and S1A were lysed and subjected to sucrose density gradient centrifugation, with subsequent RNA from fractions containing unassembled ribosomal subunits (fractions 2 to 5) or assembled polyribosomes (fractions 6 to 10) analyzed for p27 and β-actin mRNA content by quantitative PCR. *, $p<.05$, n= 3.

(D-F) Levels of HuR, p27, and β-actin (D), cell cycle distribution (E), and cap-dependent and cap-independent translation of p27 mRNA (F) in cells from Figure S1A also expressing non-targeted (HuR siRNA-) or one of two HuR-targeted (HuR siRNA1 or HuR siRNA2) siRNA. *, $p<.05$, n=3.

Figure S3 (related to main Figure 3)

(A, B) Levels of HuR, pHuR S 202, and β-actin (A) or histone H3 and tubulin (B) in total cell lysates (A) or the cytoplasmic (C) and nuclear (N) fractions of U87 cells expressing a scramble or PKM2-targeted shRNA as assessed by Western blot.

(C) Control, sodium arsenite-treated (250 μM, 45 min), or PKM2 shRNA-expressing U87 cells were fixed and subjected to immunofluorescence analysis using an antibody specific for the stress marker TIA-1. The graph represents the percentage of cells analyzed (200 per group) containing at least one foci of TIA-1 staining (stress granule).

Figure S4 (related to main Figure 4)

(A) Western blot analysis of levels of p27, HuR, PKM2, and βactin in total cell lysates from control or PKM2 shRNA 2-expressing U87 cells expressing WT (mM2) or R399E, K367M, or K433E forms of PKM2, or PKM1.

(B) PK activity in U87 and LN319 cell from panel 4F. *, $p<.05$, n=3.

(C-E) FACS-based cell cycle distribution (C), DNA content (D), and centrosome number (E) in U87 (i panels) and LN319 (ii panels) cells expressing a scrambled shRNA (PKM2 shRNA-) or one of two shRNA targeting human PKM2 (PKM1 or 2 shRNA1), and also containing empty vector (PKM2-) or WT (mM2) or R399E, K367M, or K433E forms of PKM2, or PKM1. *, $p<.05$, n=3.

Figure S5 (related to main Figure 5)

(A) 300 individual cells derived from of three primary human GBM each determined by PKM2 immunohistochemistry to have low or high expression of nuclear PKM2 were

analyzed for the nuclear/cytoplasmic percentage distribution of HuR and PKM2 immunofluorescence, and total p27 immunofluorescence. Unsupervised k-means clustering (with k=2) was then used to define the high/low cut-points for nuclear PKM2, cytoplasmic HuR, and p27 expression (listed), and the cells were placed into bins representing all 8 possible combinations of high/low expression, after which a Chi-square goodness of fit test was applied.

Table S1 (related to main Figure 5), Values for the nuclear/cytoplasmic percentage distribution of PKM2 and HuR fluorescence, and for p27 total fluorescence (relative to that noted in an average of 5 immune infiltrate cells in the same field), in 50 cells derived from each of 3 primary GBM expressing high or low levels of nuclear PKM2, respectively.