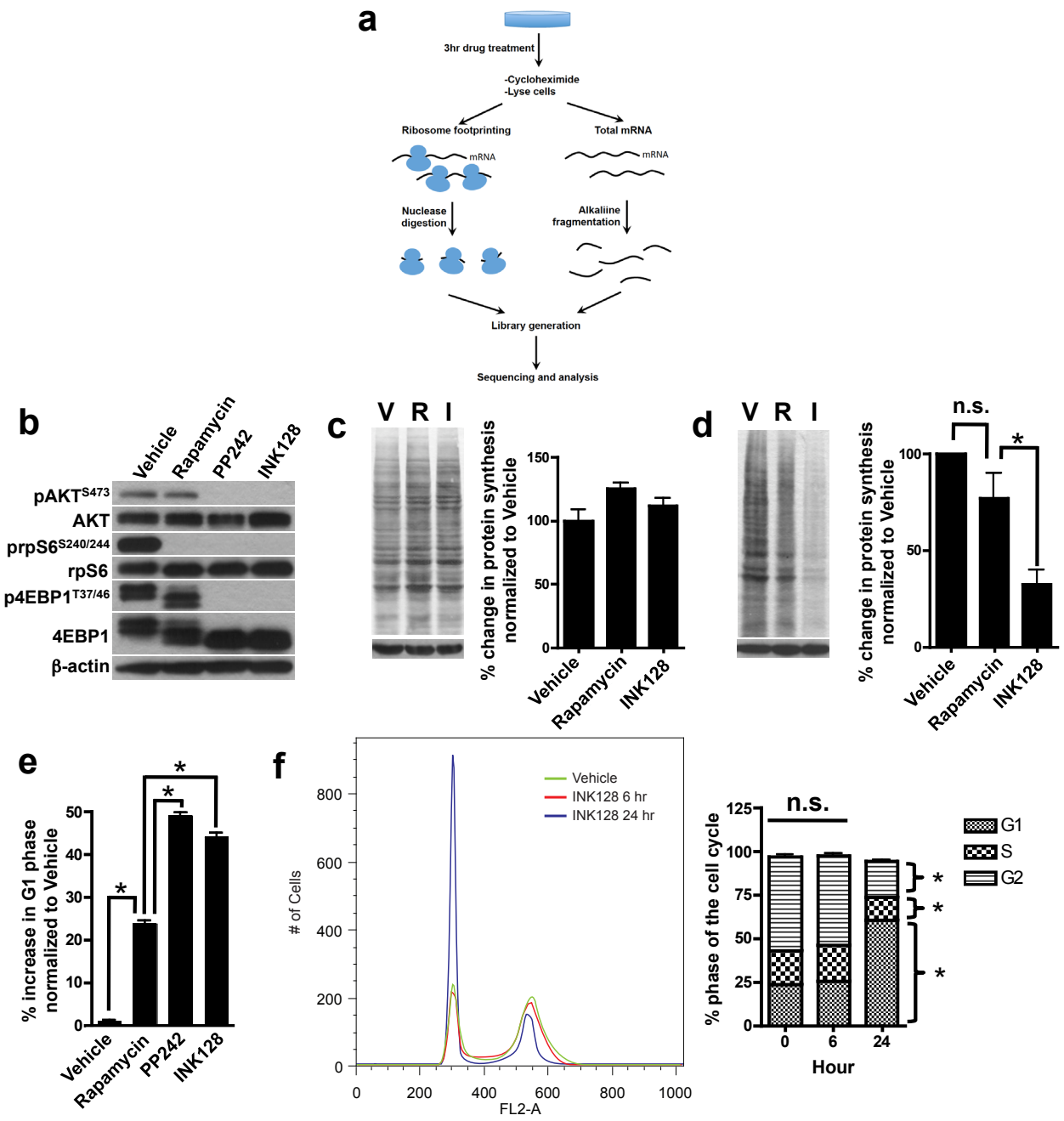
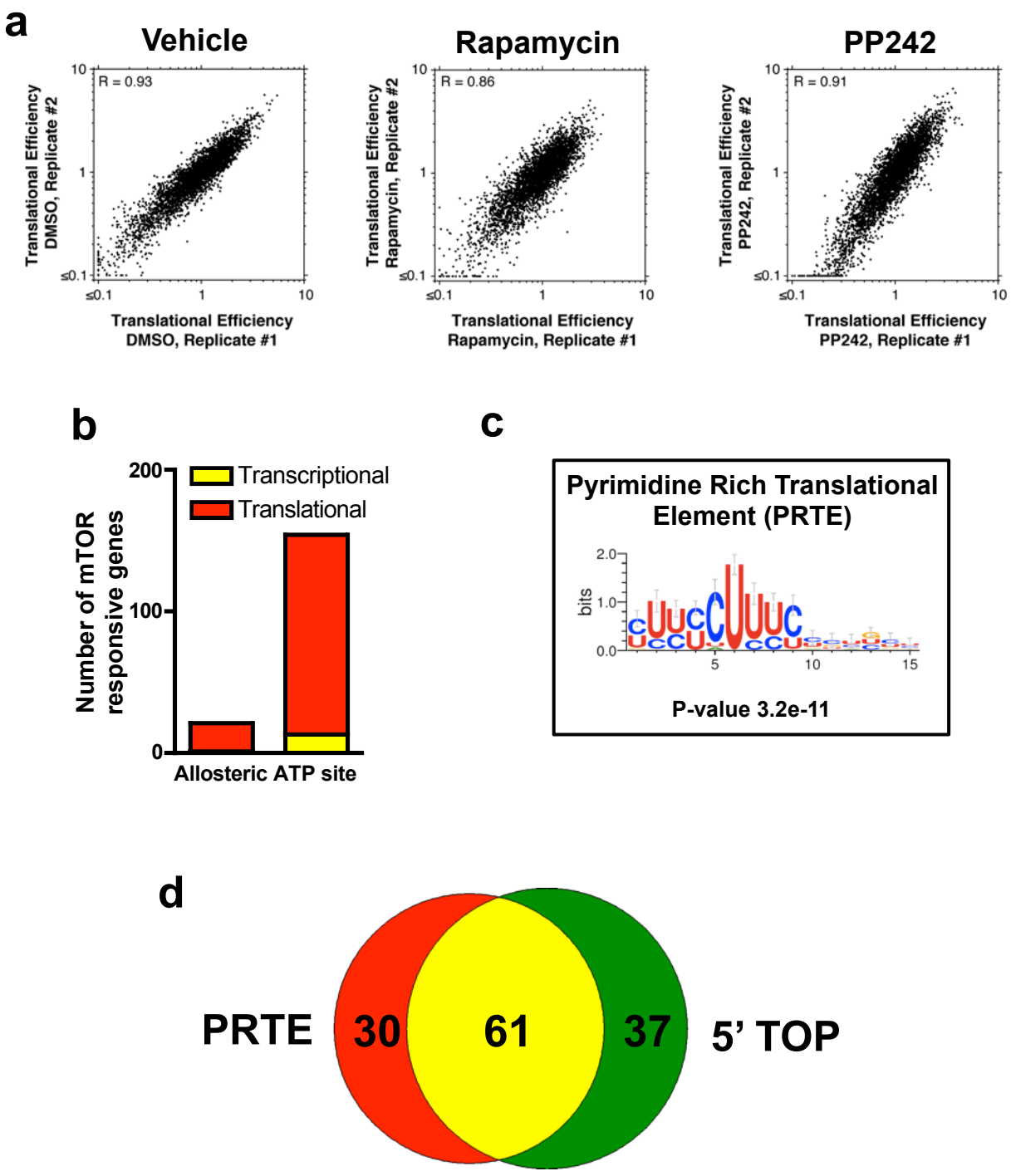
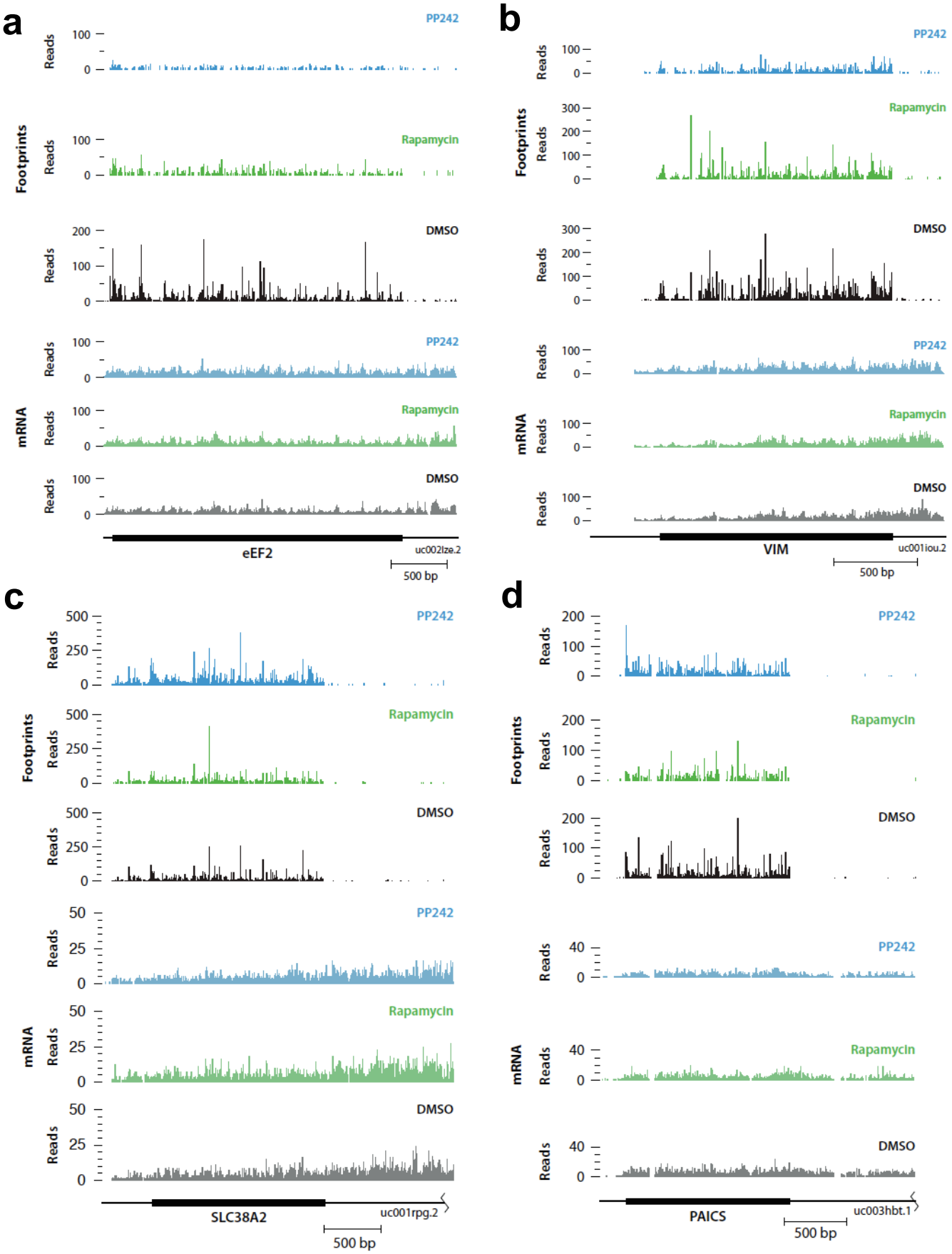
doi:10.1038/nature10912



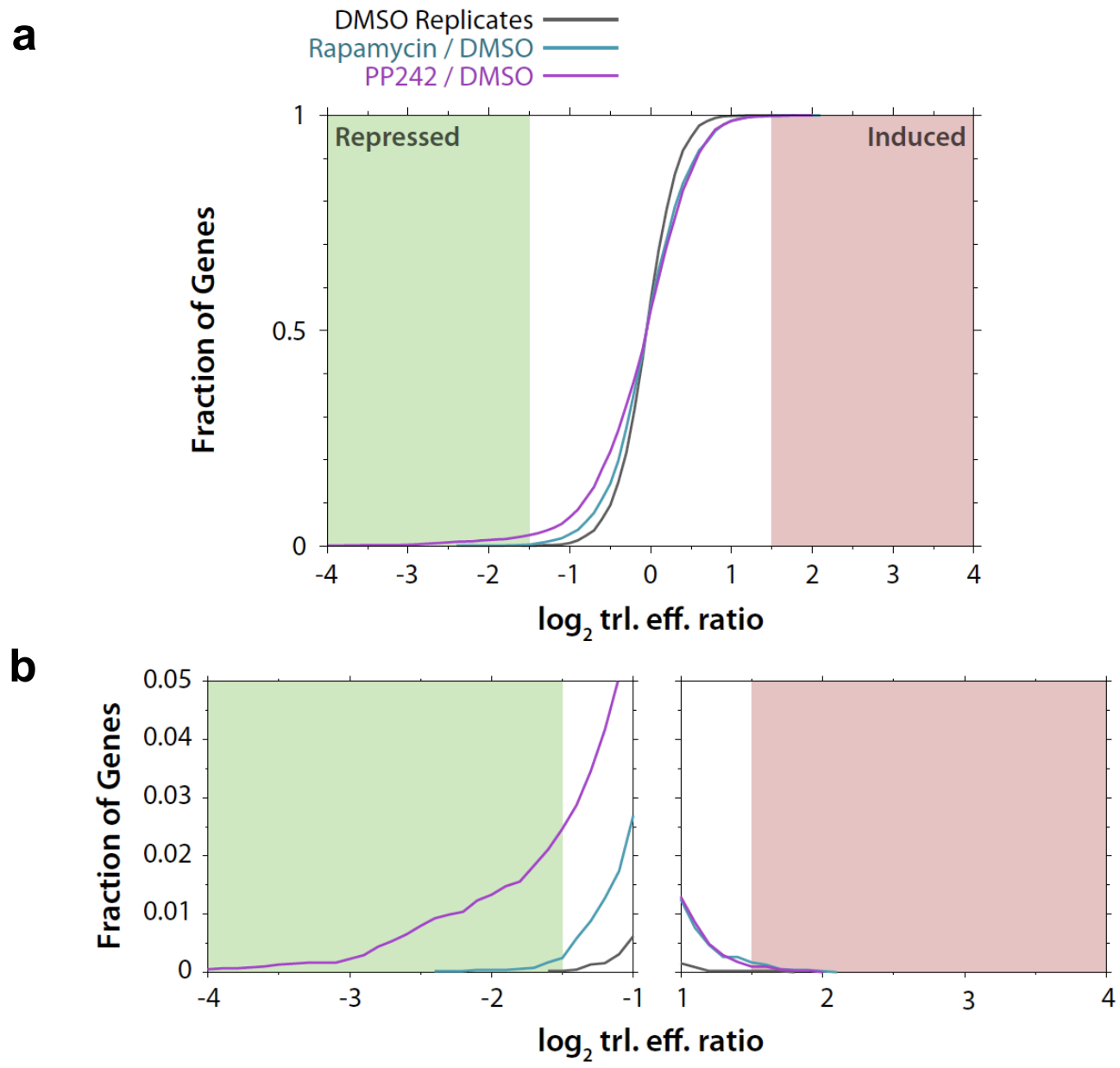
Supplementary Figure 1. Validation of mTOR inhibitors in PC3 prostate cancer cell line. (a) Schematic of ribosome profiling of human prostate cancer cells. (b) Representative western blot analysis from 3 independent experiments of PC3 prostate cancer cells treated with rapamycin (50 nM), PP242 (2.5 μM), or INK128 (200 nM) for 3 hours. (c) Representative [^{35}S]-methionine incorporation in PC3 cells after 6-hour treatment with rapamycin (50 nM) or INK128 (200 nM)(left panel). Quantification of [^{35}S]-methionine incorporation (right panel, n = 4, mean ± SEM). (d) Representative [^{35}S]-methionine incorporation in PC3 cells after 14-hour treatment with rapamycin (50 nM) or INK128 (200 nM)(left panel). Quantification of [^{35}S]-methionine incorporation (right panel, n = 4, mean ± SEM, * P<0.05 ANOVA). (e) Cell cycle analysis of PC3 cells after treatment with rapamycin (50 nM), PP242 (2.5 μM), or INK128 (200 nM) for 48 hours (mean ± SEM, n = 3, * P<0.001 ANOVA). (f) Cell cycle analysis of PC3 cells after 0-, 6-, or 24-hour treatment with INK128 (200 nM) (mean ± SEM, n = 3, * P<0.001 ANOVA). n.s. – not statistically significant. V = vehicle, R = rapamycin, I = INK128.



Supplementary Figure 2. Inter-experimental correlation of ribosome profiling per treatment condition and tally of mTOR responsive genes. The Pyrimidine Rich Translational Element (PRTE) within the 5' UTRs of mTOR sensitive mRNAs. (a) Correlation plots from 2 independent ribosome profiling experiments after a 3-hour treatment with rapamycin (50 nM) or PP242 (2.5 μM). (b) Number of translationally and transcriptionally regulated mRNA targets of mTOR after 3-hour drug treatments. (c) Pyrimidine Rich Translational Element (PRTE) present within the 5' UTRs of 63% of mTOR responsive translationally regulated mRNAs. (d) Venn diagram of the number of mTOR sensitive genes that possess a PRTE (red), 5' TOP (green), or both (yellow).



Supplementary Figure 3. Read count profiles for *eEF2*, vimentin, *SLC38A2*, and *PAICS*. (a) Ribosome footprint and RNA-Seq profiles for *eEF2*. Read count profiles are shown for each nucleotide position in the uc002lze.2 transcript, with the *eEF2* coding sequence marked. Ribosome footprints were assigned to specific A site nucleotide positions based on their length. (b) Ribosome footprint and RNA-Seq profiles for vimentin. (c) Ribosome footprint and RNA-Seq profiles for *SLC38A2*. (d) Ribosome footprint and RNA-Seq profiles for *PAICS*.



Supplementary Figure 4. False Discovery Rate computation. (a) The cumulative distribution of $\log_2$ fold-change values is shown for three comparisons, considering only genes passing the minimum read count criterion in that comparison. The DMSO replicate represents a comparison of full biological replicates of the control DMSO-only treatment condition. The rapamycin and PP242 conditions show the ratio of drug-treated to DMSO-treated samples within a single experiment. The fold-change threshold chosen based on PP242 translational repression, described below, is shown. (b) The extremes of the $\log_2$ fold-change cumulative distributions, showing the complementary cumulative distribution function for positive extreme values on the right. We used the cumulative distribution of fold-change values between the DMSO replicates as an estimate to the error distribution for measurements in drug treatment comparisons. That is, the fraction of genes above a given absolute value fold-change level in the comparison of biological replicates should reflect the fraction of genes above that level by chance in any measurement. At a cutoff of $\log_2$ fold-change of +/- 1.5, we detect 2.5% (95% CI, 2.1% - 2.9% by Agresti-Coull) of genes in the PP242 / DMSO comparison and only 0.044% (95% CI, 0.001% - 0.172%) of genes in the DMSO replicate comparison. The estimated false discovery rate is therefore q = 0.018 in the PP242 / DMSO comparison at this fold-change threshold.

	Gene	Description	Rapamycin		PP242	
			mRNA	TrlEff	mRNA	TrlEff
1	EEF2	eukaryotic translation elongation factor 2	0.56	-1.45	0.90	-4.40
2	RPS12	ribosomal protein S12	0.12	-1.39	0.05	-4.17
3	RPL12	ribosomal protein L12	-0.01	-0.74	0.17	-3.99
4	RPS2	ribosomal protein S2	0.11	-0.78	0.23	-3.94
5	RPL13A	ribosomal protein L13a	0.20	-1.38	0.35	-3.75
6	RPL18A	ribosomal protein L18a	0.22	-1.03	0.31	-3.61
7	EEF1A1	eukaryotic translation elongation factor 1 alpha 1	0.46	-1.57	0.29	-3.53
8	RPL28	ribosomal protein L28 isoform 1	0.64	-1.59	0.55	-3.48
9	RPS28	ribosomal protein S28	0.20	-1.25	0.55	-3.36
10	RPS27	ribosomal protein S27	0.07	-1.55	0.06	-3.35
11	RPL34	ribosomal protein L34	0.11	-1.27	-0.04	-3.05
12	RPL27A	ribosomal protein L27a	0.12	-1.05	0.09	-3.04
13	RPL10	ribosomal protein L10	0.13	-0.79	0.36	-2.99
14	EEF1D	eukaryotic translation elongation factor 1 delta	0.30	-1.02	0.52	-2.98
15	GLTSCR2	glioma tumor suppressor candidate region gene 2	0.51	-1.03	1.02	-2.94
16	RPL3	ribosomal protein L3 isoform a	0.28	-1.29	0.28	-2.89
17	PABPC1	poly(A) binding protein, cytoplasmic 1	0.52	-1.47	0.14	-2.88
18	RPL37A	ribosomal protein L37a	0.12	-0.94	0.09	-2.87
19	RPS5	ribosomal protein S5	0.23	-1.06	0.30	-2.86
20	RPL21	ribosomal protein L21	0.14	-1.31	-0.11	-2.83
21	RPS15A	ribosomal protein S15a	0.03	-0.98	0.04	-2.81
22	GNB2L1	guanine nucleotide binding protein (G protein)	0.32	-1.02	0.38	-2.80
23	RPL11	ribosomal protein L11	0.13	-1.11	0.23	-2.78
24	RPS20	ribosomal protein S20 isoform 1	0.14	-1.79	-0.10	-2.78
25	RPL7A	ribosomal protein L7a	0.31	-1.05	0.22	-2.76
26	RPS19	ribosomal protein S19	0.28	-0.76	0.34	-2.76
27	RPS21	ribosomal protein S21	-0.06	-0.51	0.15	-2.76
28	RPLP0	ribosomal protein P0	0.23	-0.57	0.17	-2.73
29	RPS9	ribosomal protein S9	0.15	-0.47	0.46	-2.73
30	RPS3	ribosomal protein S3	0.35	-0.93	0.33	-2.72
31	CRTAP	cartilage associated protein	0.29	-1.33	0.28	-2.70
32	FAM128B	hypothetical protein LOC80097	0.32	-0.20	0.68	-2.68
33	RPL32	ribosomal protein L32	0.32	-1.20	0.12	-2.67
34	EEF1G	eukaryotic translation elongation factor 1, gamma	0.31	-1.17	0.10	-2.65
35	RPL22L1	ribosomal protein L22-like 1	0.09	-0.77	0.36	-2.63
36	YB1	Y-box binding protein 1	0.11	-0.72	0.30	-2.62
37	EIF4B	eukaryotic translation initiation factor 4B	0.71	-1.36	0.58	-2.61
38	RPLP2	ribosomal protein P2	0.46	-1.44	0.31	-2.61
39	RPS16	ribosomal protein S16	0.28	-0.78	0.31	-2.59
40	VIM	vimentin	0.54	-0.50	0.73	-2.58
41	GAMT	guanidinoacetate N-methyltransferase isoform b	0.10	0.38	0.18	-2.53
42	HSPA8	heat shock 70kDa protein 8 isoform 1	-0.18	-0.51	-0.66	-2.53
43	RPL39	ribosomal protein L39	0.30	-2.11	-0.42	-2.53
44	AHCY	adenosylhomocysteinase isoform 1	0.30	-0.38	0.48	-2.51
45	EEF1A2	eukaryotic translation elongation factor 1 alpha 2	0.26	-0.24	0.64	-2.50
46	RPL10A	ribosomal protein L10a	0.23	-1.00	0.18	-2.49
47	PABPC4	poly A binding protein, cytoplasmic 4 isoform 1	0.40	-0.81	0.51	-2.48
48	RPS4X	ribosomal protein S4, X-linked X isoform	0.20	-1.22	0.06	-2.48
49	RPL31	ribosomal protein L31 isoform 2	-0.10	-0.49	0.05	-2.47
50	RPLP1	ribosomal protein P1 isoform 1	0.52	-0.96	0.30	-2.46
51	RPS11	ribosomal protein S11	0.20	-0.74	0.33	-2.46
52	RPL26	ribosomal protein L26	0.17	-1.39	-0.02	-2.43
53	RPL14	ribosomal protein L14	0.30	-1.08	0.04	-2.42
54	RPL37	ribosomal protein L37	0.11	-0.64	0.14	-2.39
55	RPL7	ribosomal protein L7	0.12	-1.13	-0.08	-2.37
56	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	0.23	-0.93	0.29	-2.36
57	RPS8	ribosomal protein S8	0.15	-0.41	0.16	-2.36
58	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	0.31	-0.49	0.36	-2.34
59	RPL8	ribosomal protein L8	0.29	-0.59	0.49	-2.31
60	RPL29	ribosomal protein L29	0.23	-0.67	0.27	-2.30
61	RPS3A	ribosomal protein S3a	0.29	-1.21	-0.17	-2.29
62	RPL18	ribosomal protein L18	0.29	-0.71	0.33	-2.26
63	RPL36	ribosomal protein L36	0.21	-0.46	0.44	-2.25
64	AGRN	agrin precursor	0.11	0.44	0.69	-2.17
65	TPT1	tumor protein, translationally-controlled 1	0.19	-1.22	-0.12	-2.15
66	RPL36A	ribosomal protein L36a	0.13	-1.18	-0.01	-2.13
67	SLC25A5	adenine nucleotide translocator 2	0.41	-0.46	0.24	-2.13
68	TKT	transketolase isoform 1	0.18	-0.40	0.50	-2.13
69	LMF2	lipase maturation factor 2	0.40	0.27	0.95	-2.12
70	RPL13	ribosomal protein L13	0.28	-0.57	0.35	-2.12

Supplementary Figure 5. Representative list of significant translationally regulated PP242 responsive genes. mRNA expression and translational efficiency changes after 3-hour treatment with rapamycin (50 nM) or PP242 (2.5 μM)(FDR <0.05) in PC3 cells (log_2 fold-change)(Red highlights denote genes with the Pyrimidine Rich Translational Elements (PRTE)).

	Gene	Description	Rapamycin		PP242	
			mRNA	TrlEff	mRNA	TrlEff
71	CTSH	cathepsin H isoform b	-0.09	0.17	0.17	-2.10
72	FAM83H	FAM83H	0.09	0.26	0.66	-2.09
73	RPS29	ribosomal protein S29 isoform 2	-0.07	-0.67	0.11	-2.09
74	RPL23	ribosomal protein L23	0.07	-1.01	0.05	-2.08
75	RPS25	ribosomal protein S25	0.04	-0.71	-0.13	-2.08
76	TUBB3	tubulin, beta, 4	0.23	0.52	0.72	-2.07
77	RPS10	ribosomal protein S10	0.21	-0.82	0.24	-2.06
78	FASN	fatty acid synthase	-0.37	0.39	0.40	-2.03
79	RPL17	ribosomal protein L17	-0.06	-0.82	0.01	-2.03
80	EIF2S3	eukaryotic translation initiation factor 2, S3	0.41	-1.27	0.09	-2.02
81	RPL30	ribosomal protein L30	0.06	-0.97	-0.07	-1.97
82	ACTG1	actin, gamma 1 propeptide	0.13	-0.33	0.41	-1.96
83	COL6A2	alpha 2 type VI collagen isoform 2C2	0.00	0.43	0.86	-1.95
84	UBA52	ubiquitin and ribosomal protein L40 precursor	0.20	-0.41	0.25	-1.95
85	ALKBH7	spermatogenesis associated 11 precursor	0.16	0.39	0.82	-1.94
86	RPL5	ribosomal protein L5	0.20	-1.35	0.04	-1.94
87	PGLS	6-phosphogluconolactonase	0.23	-0.24	0.64	-1.92
88	CSDA	cold shock domain protein A	0.29	-0.70	0.38	-1.91
89	RPL6	ribosomal protein L6	0.04	-0.74	0.02	-1.90
90	RPS24	ribosomal protein S24 isoform d	0.08	-1.19	-0.11	-1.88
91	RPL22	ribosomal protein L22	0.05	-0.86	-0.02	-1.87
92	AP2A1	adaptor-related protein complex 2, alpha 1	0.25	-0.22	0.56	-1.86
93	NAP1L1	nucleosome assembly protein 1-like 1	0.26	-1.00	0.10	-1.85
94	RPS14	ribosomal protein S14	0.09	-0.71	0.14	-1.84
95	ETHE1	ETHE1 protein	0.64	-0.37	0.81	-1.79
96	CCNI	cyclin I	0.50	-0.69	0.32	-1.78
97	MTA1	metastasis associated 1	0.22	-0.54	0.33	-1.75
98	EIF3H	eukaryotic translation initiation factor 3, H	0.21	-0.97	0.00	-1.74
99	RPL9	ribosomal protein L9	0.09	-1.01	-0.23	-1.74
100	RPS23	ribosomal protein S23	0.18	-1.36	-0.08	-1.74
101	RPS6	ribosomal protein S6	0.13	-1.09	-0.10	-1.74
102	RPS7	ribosomal protein S7	0.05	-0.89	-0.13	-1.74
103	RPL19	ribosomal protein L19	0.21	-0.71	0.18	-1.73
104	RPL4	ribosomal protein L4	0.29	-0.97	0.04	-1.73
105	RPS13	ribosomal protein S13	0.07	-0.83	-0.22	-1.73
106	C21orf66	GC-rich sequence DNA-binding factor candidate	-0.48	0.05	-0.42	-1.72
107	LCMT1	leucine carboxyl methyltransferase 1 isoform a	0.22	-0.01	-0.09	-1.72
108	EIF3L	eukaryotic translation initiation factor 3, L	0.36	-0.93	0.22	-1.70
109	IPO7	importin 7	0.05	-0.68	0.02	-1.70
110	PC	pyruvate carboxylase	0.19	-0.61	0.53	-1.69
111	RPS27A	ubiquitin and ribosomal protein S27a	0.13	-0.81	-0.15	-1.69
112	SECTM1	secreted and transmembrane 1 precursor	0.06	0.50	0.67	-1.69
113	RPL41	ribosomal protein L41	0.01	-1.48	-0.13	-1.68
114	TSC2	tuberous sclerosis 2 isoform 1	-0.06	0.49	0.32	-1.66
115	COL18A1	alpha 1 type XVIII collagen isoform 3	-0.10	0.24	0.52	-1.65
116	CHP	calcium binding protein P22	0.72	-1.19	0.45	-1.64
117	PACS1	phosphofurin acidic cluster sorting protein 1	0.06	0.08	0.50	-1.64
118	BRF1	transcription initiation factor IIIB	0.08	0.42	0.44	-1.63
119	PTGES2	prostaglandin E synthase 2	-0.05	-0.08	0.36	-1.63
120	C2orf79	hypothetical protein LOC391356	0.16	-0.41	0.02	-1.62
121	PGM1	phosphoglucomutase 1	0.69	-0.67	0.46	-1.62
122	SLC19A1	solute carrier family 19 member 1	-0.18	-0.33	0.39	-1.62
123	CD44	CD44 antigen isoform 1	0.60	-0.77	0.59	-1.59
124	RPL24	ribosomal protein L24	0.12	-0.53	-0.09	-1.59
125	NCLN	nicalin	0.14	0.20	0.47	-1.58
126	RPL15	ribosomal protein L15	0.11	-0.71	0.20	-1.58
127	CLPTM1	cleft lip and palate associated transmembrane	0.04	-0.12	0.50	-1.56
128	ECSIT	evolutionarily conserved signaling intermediate	0.07	0.17	0.45	-1.56
129	EEF1B2	eukaryotic translation elongation factor 1 beta 2	0.14	-0.98	-0.10	-1.56
130	PFDN5	prefoldin subunit 5 isoform alpha	0.21	-0.44	0.04	-1.55
131	PNKP	polynucleotide kinase 3' phosphatase	-0.01	0.96	0.68	-1.55
132	SEPT8	septin 8 isoform a	0.12	0.59	0.10	-1.55
133	CIRBP	cold inducible RNA binding protein	0.30	0.09	0.86	-1.54
134	ABCB7	ATP-binding cassette, sub-family B, member 7	0.33	-0.39	0.44	-1.53
135	ARD1A	alpha-N-acetyltransferase 1A	0.15	-0.14	0.12	-1.53
136	EVPL	envoplakin	0.03	0.36	0.72	-1.53
137	LAMA5	laminin alpha 5	-0.41	1.00	0.56	-1.53
138	MYH14	myosin, heavy chain 14 isoform 3	0.28	0.07	0.83	-1.53
139	RABGGTB	RAB geranylgeranyltransferase, beta subunit	-0.40	-0.88	0.07	-1.53
140	RPL27	ribosomal protein L27	0.09	-0.49	-0.19	-1.53
141	RPS15	ribosomal protein S15	0.15	0.10	0.44	-1.53
142	IMPDH2	inosine monophosphate dehydrogenase 2	0.20	-0.61	0.20	-1.52
143	SIGMAR1	sigma non-opioid intracellular receptor 1	0.50	-0.41	0.72	-1.52
144	ATP5G2	ATP synthase, H+ transporting, mitochondrial F0	0.50	-0.68	0.33	-1.51
145	C3orf38	hypothetical protein LOC285237	-0.31	0.95	-0.99	1.53

	Gene	Description	Rapamycin		PP242	
			mRNA	TrlEff	mRNA	TrlEff
1	EEF2	eukaryotic translation elongation factor 2	0.39	-1.12	0.76	-3.60
2	EEF1A1	eukaryotic translation elongation factor 1 alpha 1	0.43	-1.58	0.36	-3.21
3	RPL13A	ribosomal protein L13a	0.15	-1.25	0.30	-3.10
4	RPS12	ribosomal protein S12	0.11	-1.22	0.04	-3.00
5	RPL12	ribosomal protein L12	0.07	-0.94	0.12	-2.95
6	RPS27	ribosomal protein S27	0.10	-1.54	0.07	-2.71
7	RPS28	ribosomal protein S28	0.01	-0.80	0.28	-2.67
8	RPL18A	ribosomal protein L18a	0.17	-0.82	0.23	-2.63
9	RPL34	ribosomal protein L34	0.11	-1.12	0.04	-2.63
10	RPL28	ribosomal protein L28 isoform 1	0.24	-1.09	0.22	-2.54
11	RPL27A	ribosomal protein L27a	0.06	-0.96	0.07	-2.53
12	CRTAP	cartilage associated protein	0.29	-1.17	0.33	-2.50
13	RPL10	ribosomal protein L10	0.09	-0.79	0.25	-2.46
14	RPS20	ribosomal protein S20 isoform 1	0.18	-1.35	-0.01	-2.46
15	RPL21	ribosomal protein L21	0.14	-1.25	-0.04	-2.45
16	RPL3	ribosomal protein L3 isoform a	0.18	-1.08	0.22	-2.44
17	RPL39	ribosomal protein L39	0.17	-1.65	-0.15	-2.41
18	RPL37A	ribosomal protein L37a	0.08	-1.02	0.01	-2.38
19	VIM	vimentin	0.36	-0.40	0.67	-2.38
20	EEF1D	eukaryotic translation elongation factor 1 delta	0.18	-0.84	0.35	-2.37
21	GNB2L1	guanine nucleotide binding protein (G protein)	0.19	-0.77	0.27	-2.35
22	RPS19	ribosomal protein S19	0.15	-0.74	0.23	-2.34
23	RPL32	ribosomal protein L32	0.22	-0.97	0.11	-2.33
24	RPS15A	ribosomal protein S15a	0.07	-0.96	0.07	-2.31
25	RPL11	ribosomal protein L11	0.09	-1.08	0.14	-2.31
26	RPL7A	ribosomal protein L7a	0.17	-0.74	0.15	-2.30
27	YB1	Y-box binding protein 1	0.11	-0.59	0.24	-2.30
28	RPS9	ribosomal protein S9	0.10	-0.60	0.34	-2.27
29	EIF4B	eukaryotic translation initiation factor 4B	0.55	-1.21	0.61	-2.27
30	EEF1G	eukaryotic translation elongation factor 1, gamma	0.21	-1.15	0.15	-2.26
31	RPS2	ribosomal protein S2	0.07	-0.56	0.20	-2.25
32	RPS5	ribosomal protein S5	0.14	-0.77	0.23	-2.25
33	HSPA8	heat shock 70kDa protein 8 isoform 1	-0.21	-0.46	-0.40	-2.25
34	RPS3A	ribosomal protein S3a	0.22	-1.15	-0.06	-2.17
35	RPS3	ribosomal protein S3	0.22	-0.92	0.24	-2.16
36	RPL10A	ribosomal protein L10a	0.16	-0.94	0.14	-2.16
37	RPS25	ribosomal protein S25	0.04	-0.89	-0.04	-2.13
38	GLTSCR2	glioma tumor suppressor candidate region gene 2	0.31	-0.68	0.70	-2.12
39	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	0.18	-0.86	0.27	-2.12
40	RPLP2	ribosomal protein P2	0.26	-1.18	0.14	-2.10
41	RPL31	ribosomal protein L31 isoform 2	-0.02	-0.62	0.05	-2.10
42	PABPC1	poly(A) binding protein, cytoplasmic 1	0.35	-1.44	0.16	-2.09
43	RPS21	ribosomal protein S21	-0.01	-0.60	0.09	-2.09
44	RPS4X	ribosomal protein S4, X-linked X isoform	0.18	-1.15	0.12	-2.06
45	RPLP1	ribosomal protein P1 isoform 1	0.28	-1.09	0.12	-2.06
46	RPL7	ribosomal protein L7	0.15	-1.06	0.01	-2.02
47	RPL26	ribosomal protein L26	0.15	-1.11	0.02	-2.00
48	PABPC4	poly A binding protein, cytoplasmic 4 isoform 1	0.24	-0.80	0.40	-1.98
49	RPL36A	ribosomal protein L36a	0.13	-1.11	-0.01	-1.98
50	EEF1A2	eukaryotic translation elongation factor 1 alpha 2	0.03	-0.03	0.40	-1.94
51	TPT1	tumor protein, translationally-controlled 1	0.24	-1.22	0.01	-1.94
52	AHCY	adenosylhomocysteinase isoform 1	0.20	-0.23	0.38	-1.93
53	RPL22L1	ribosomal protein L22-like 1	0.15	-0.68	0.39	-1.90
54	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	0.17	-0.27	0.28	-1.90
55	RPL30	ribosomal protein L30	0.11	-0.99	0.01	-1.89
56	RPS11	ribosomal protein S11	0.11	-0.59	0.20	-1.88
57	RPL29	ribosomal protein L29	0.10	-0.50	0.20	-1.88
58	RPL14	ribosomal protein L14	0.07	-0.68	-0.02	-1.85
59	RPL36	ribosomal protein L36	0.09	-0.43	0.28	-1.85
60	EIF2S3	eukaryotic translation initiation factor 2, S3	0.33	-1.04	0.15	-1.85
61	RPL23	ribosomal protein L23	0.09	-0.92	0.07	-1.82
62	RPS16	ribosomal protein S16	0.13	-0.38	0.19	-1.81
63	SLC25A5	adenine nucleotide translocator 2	0.21	-0.30	0.15	-1.80
64	RPL17	ribosomal protein L17	0.05	-0.93	0.07	-1.80
65	RPL37	ribosomal protein L37	0.11	-0.68	0.10	-1.79
66	RPL8	ribosomal protein L8	0.12	-0.40	0.29	-1.79
67	NAP1L1	nucleosome assembly protein 1-like 1	0.24	-0.97	0.15	-1.79
68	RPS10	ribosomal protein S10	0.16	-0.69	0.19	-1.78
69	IPO7	importin 7	0.20	-0.83	0.26	-1.75
70	RPS8	ribosomal protein S8	0.09	-0.44	0.14	-1.74
71	RPL5	ribosomal protein L5	0.17	-1.11	0.06	-1.73
72	RPS24	ribosomal protein S24 isoform d	0.11	-1.16	-0.01	-1.73

Supplementary Figure 6. Mean list of translationally regulated PP242 responsive genes. mRNA expression and translational efficiency changes after 3-hour treatment with rapamycin (50 nM) or PP242 (2.5 μM) in PC3 cells (log_2 fold-change) (data represents the average of 2 independent experiments).

	Gene	Description	Rapamycin		PP242	
			mRNA	TrlEff	mRNA	TrlEff
73	EEF1B2	eukaryotic translation elongation factor 1 beta 2	0.12	-1.10	-0.06	-1.70
74	RPL6	ribosomal protein L6	0.09	-0.68	0.06	-1.68
75	RPS23	ribosomal protein S23	0.15	-1.19	-0.03	-1.68
76	RPL18	ribosomal protein L18	0.08	-0.42	0.18	-1.65
77	RPS29	ribosomal protein S29 isoform 2	-0.01	-0.69	0.11	-1.65
78	RPS6	ribosomal protein S6	0.14	-1.06	-0.02	-1.65
79	RPL22	ribosomal protein L22	0.08	-0.89	0.00	-1.64
80	UBA52	ubiquitin and ribosomal protein L40	0.12	-0.22	0.18	-1.62
81	RPLP0	ribosomal protein P0	0.15	-0.42	0.12	-1.61
82	RPS27A	ubiquitin and ribosomal protein S27a	0.16	-0.89	-0.04	-1.61
83	RPL9	ribosomal protein L9	0.16	-1.00	-0.08	-1.59
84	TKT	transketolase isoform 1	0.02	-0.11	0.33	-1.58
85	RPL13	ribosomal protein L13	0.14	-0.38	0.26	-1.56
86	EIF3H	eukaryotic translation initiation factor 3,	0.16	-0.79	0.09	-1.54
87	RPS13	ribosomal protein S13	0.07	-0.82	-0.08	-1.54
88	RPS7	ribosomal protein S7	0.11	-0.76	-0.04	-1.51
89	RPS14	ribosomal protein S14	0.10	-0.60	0.16	-1.50
90	RPL4	ribosomal protein L4	0.22	-0.85	0.10	-1.50
91	FAM128B	hypothetical protein LOC80097	0.06	0.27	0.43	-1.47
92	EIF3L	eukaryotic translation initiation factor 3L	0.28	-0.85	0.21	-1.47
93	RABGGTB	RAB geranylgeranyltransferase, beta subunit	-0.20	-0.84	0.20	-1.46
94	FASN	fatty acid synthase	-0.37	0.47	0.30	-1.42
95	RPL24	ribosomal protein L24	0.11	-0.63	0.00	-1.41
96	ACTG1	actin, gamma 1 propeptide	0.02	-0.07	0.28	-1.40
97	PFDN5	prefoldin subunit 5 isoform alpha	0.11	-0.51	0.04	-1.38
98	LMF2	lipase maturation factor 2	0.22	0.39	0.62	-1.36
99	RPL19	ribosomal protein L19	0.14	-0.66	0.11	-1.35
100	PGM1	phosphoglucomutase 1	0.40	-0.55	0.23	-1.35
101	CCNI	cyclin I	0.29	-0.45	0.24	-1.33
102	IMPDH2	inosine monophosphate dehydrogenase 2	0.11	-0.39	0.21	-1.33
103	AP2A1	adaptor-related protein complex 2, alpha 1	0.09	-0.04	0.42	-1.32
104	AGRN	agrin precursor	0.01	0.51	0.50	-1.29
105	COL6A2	alpha 2 type VI collagen isoform 2C2	-0.08	0.43	0.57	-1.29
106	CD44	CD44 antigen isoform 1	0.34	-0.46	0.43	-1.29
107	RPL41	ribosomal protein L41	0.04	-1.15	-0.01	-1.28
108	ALKBH7	spermatogenesis associated 11 precursor	0.06	0.28	0.51	-1.27
109	RPL27	ribosomal protein L27	0.05	-0.33	-0.13	-1.23
110	RPL15	ribosomal protein L15	0.11	-0.51	0.19	-1.20
111	RPS15	ribosomal protein S15	-0.01	0.03	0.21	-1.19
112	CLPTM1	cleft lip and palate associated transmembrane	0.07	0.26	0.41	-1.13
113	FAM83H	FAM83H	-0.17	0.71	0.33	-1.11
114	PGLS	6-phosphogluconolactonase	0.03	0.20	0.21	-1.11
115	MTA1	metastasis associated 1	0.00	-0.05	0.21	-1.09
116	TSC2	tuberous sclerosis 2 isoform 1	-0.15	0.34	0.21	-1.09
117	PACS1	phosphofurin acidic cluster sorting protein 1	0.07	0.04	0.45	-1.09
118	CIRBP	cold inducible RNA binding protein	0.14	0.10	0.54	-1.08
119	SLC19A1	solute carrier family 19 member 1	-0.36	0.23	0.10	-1.07
120	ECSIT	evolutionarily conserved signaling intermediate	-0.04	0.41	0.26	-1.06
121	ARD1A	alpha-N-acetyltransferase 1A	-0.04	0.01	0.03	-1.05
122	C21orf66	GC-rich sequence DNA-binding factor candidate	-0.30	-0.09	-0.31	-1.03
123	ATP5G2	ATP synthase, H+ transporting, mitochondrial F0	0.29	-0.28	0.17	-1.01
124	LAMA5	laminin alpha 5	-0.32	0.87	0.40	-0.94
125	PNKP	polynucleotide kinase 3' phosphatase	-0.24	0.74	0.33	-0.79
126	EVPL	envoplakin	-0.08	0.30	0.38	-0.79
127	NCLN	nicalin	-0.05	0.67	0.29	-0.76
128	PTGES2	prostaglandin E synthase 2	-0.19	0.52	0.17	-0.65
129	GAMT	guanidinoacetate N-methyltransferase isoform b	n/a	n/a	n/a	n/a
130	CTSH	cathepsin H isoform b	n/a	n/a	n/a	n/a
131	TUBB3	tubulin, beta, 4	n/a	n/a	n/a	n/a
132	CSDA	cold shock domain protein A	n/a	n/a	n/a	n/a
133	ETHE1	ETHE1 protein	n/a	n/a	n/a	n/a
134	LCMT1	leucine carboxyl methyltransferase 1 isoform a	n/a	n/a	n/a	n/a
135	PC	pyruvate carboxylase	n/a	n/a	n/a	n/a
136	SECTM1	secreted and transmembrane 1	n/a	n/a	n/a	n/a
137	COL18A1	alpha 1 type XVIII collagen isoform 3	n/a	n/a	n/a	n/a
138	CHP	calcium binding protein P22	n/a	n/a	n/a	n/a
139	BRF1	transcription initiation factor IIIB	n/a	n/a	n/a	n/a
140	C2orf79	hypothetical protein LOC391356	n/a	n/a	n/a	n/a
141	SEPT8	septin 8 isoform a	n/a	n/a	n/a	n/a
142	ABCB7	ATP-binding cassette, sub-family B, member 7	n/a	n/a	n/a	n/a
143	MYH14	myosin, heavy chain 14 isoform 3	n/a	n/a	n/a	n/a
144	SIGMAR1	sigma non-opioid intracellular receptor 1	n/a	n/a	n/a	n/a
145	C3orf38	hypothetical protein LOC285237	n/a	n/a	n/a	n/a

	Gene	RefSeq ID	Chromosome	Strand (+/-)	5' TOP Position	PRTE Position
1	AP2A1	NM_014203	19	+	50270268	50270306
2	CCNI	NM_006835	4	-	77997142	77997076
3	CD44	NM_000610	11	+	35160717	35160813
4	CHP	NM_007236	15	+	41523519	41523536
5	CRTAP	NM_006371	3	+	33155506/33155554	33155540
6	eEF1A2	NM_001958	20	-	62130436	62129175
7	eEF1B2	NM_021121	2	+	207024619	207024665
8	eEF1G	NM_001404	11	-	62341490/62341335	62341383
9	eEF2	NM_001961	19	-	3985461	3985423
10	eIF4B	NM_001417	12	+	53400240	53400250
11	GAPDH	NM_002046	12	+	6643684	6643717
12	GNB2L1	NM_006098	5	-	180670906	180670818
13	HNRNPA1	NM_031157	12	+	54674529	54674571
14	HSPA8	NM_006597	11	-	122932844	122932806
15	IPO7	NM_006391	11	+	9406199	9406255
16	LCMT1	NM_016309	16	+	25123101	25123114
17	NAP1L1	NM_004537	12	-	76478465	76478429
18	PABPC1	NM_002568	8	-	101734315	101734151
19	PACS1	NM_018026	11	+	65837839	65837922
20	PGM1	NM_002633	1	+	64059078	64059107
21	RABGGTB	NM_004582	1	+	76251941	76251928
22	RPL10	NM_006013	X	+	153626718	153626846
23	RPL12	NM_000976	9	-	130213677	130213648
24	RPL13	NM_000977/NM_033251	16	+	89627090	89627102/89627202
25	RPL14	NM_001034996	3	+	40498830	40498906
26	RPL15	NM_002948	3	+	23958639	23958711
27	RPL17	NM_000985	18	-	47018849	47017964
28	RPL22	NM_000983	1	-	6259654	6259645
29	RPL22L1	NM_001099645	3	-	170587984	170587976
30	RPL23	NM_000978	17	-	37009989	37010013
31	RPL29	NM_000992	3	-	52029911	52029904
32	RPL31	NM_001098577	2	+	101618755	101618739
33	RPL32	NM_001007074	3	-	12883040	12883002
34	RPL34	NM_000995/NM_033625	4	+	109541733	109541743/109541769
35	RPL36	NM_033643/NM_015414	19	+	5690307	5690319/5690493
36	RPL36A	NM_021029	X	+	100645999	100645981
37	RPL37	NM_000997	5	-	40835324	40835314
38	RPL37A	NM_000998	2	+	217363567	217363526
39	RPL39	NM_001000	X	-	118925591	118925564
40	RPL4	NM_000968	15	-	66797185	66797143
41	RPL41	NM_001035267	12	+	56510417	56510539
42	RPL5	NM_000969	1	+	93297597	93297656
43	RPL6	NM_000970	12	-	112847409	112847256
44	RPL8	NM_000973/NM_033301	8	-	146017775	146017709
45	RPLP0	NM_053275	12	-	120638910	120638652
46	RPLP2	NM_001004	11	+	809968	810006
47	RPS10	NM_001014	6	-	34393846	34393715
48	RPS11	NM_001015	19	+	49999690	49999677
49	RPS14	NM_001025070	5	-	149829300/149829186	149829107
50	RPS15A	NM_001030009	16	-	18801656	18801604
51	RPS2	NM_002952	16	-	2014827	2014653

Supplementary Figure 7. 5'TOP and PRTE genomic positions in 61 mTOR sensitive genes that possess both motifs based on DBTSS release 8.0 (slashes demarcate 5'TOPs or PRTEs in putative mRNA isoforms).

52	RPS20	NM_001146227	8	-	56987065	56986992
53	RPS27A	NM_001177413	2	+	55459824	55459920
54	RPS3A	NM_001006	4	+	152020780	152020789
55	RPS5	NM_001009	19	+	58898636	58898691
56	RPS6	NM_001010	9	-	19380234	19380207
57	RPS9	NM_001013	19	+	54704726	54704775
58	SECTM1	NM_003004	17	-	80291646	80291674/80291639
59	TPT1	NM_003295	13	-	45915318	45915222
60	UBA52	NM_003333	19	+	18682670	18683218
61	VIM	NM_003380	10	+	17271277	17271358

	Gene	Description	Rapamycin		PP242	
			mRNA	TrlEff	mRNA	TrlEff
1	MAPK6	mitogen-activated protein kinase 6	0.13	-2.43	0.10	-0.29
2	RPL39 *	ribosomal protein L39	0.30	-2.11	-0.42	-2.53
3	RPS20 *	ribosomal protein S20 isoform 1	0.14	-1.79	-0.10	-2.78
4	PRKD3	protein kinase D3	-0.22	-1.72	-0.46	0.68
5	UBTD2	dendritic cell-derived ubiquitin-like protein	0.19	-1.64	0.25	0.27
6	RPL28 *	ribosomal protein L28 isoform 1	0.64	-1.59	0.55	-3.48
7	RBPJ	recombining binding protein suppressor of	1.09	-1.58	0.17	-0.03
8	EEF1A1 *	eukaryotic translation elongation factor 1 alpha	0.46	-1.57	0.29	-3.53
9	UCHL5	ubiquitin carboxyl-terminal hydrolase L5	-0.08	-1.56	-0.51	0.40
10	RPS27 *	ribosomal protein S27	0.07	-1.55	0.06	-3.35
11	SDCCAG10	serologically defined colon cancer antigen 10	-0.19	-1.50	-0.37	0.23
12	MAPKAPK2	mitogen-activated protein kinase-activated	-0.21	1.50	-0.22	0.92
13	NFATC2IP	nuclear factor of activated T-cells, 2IP	-0.16	1.54	0.08	0.35
14	GTPBP3	GTP binding protein 3 (mitochondrial) isoform V	-0.73	1.56	0.15	-0.83
15	C17orf28	hypothetical protein LOC283987	-0.44	1.66	0.21	-0.20
16	VHL	von Hippel-Lindau tumor suppressor isoform 1	-0.23	1.67	0.43	0.52
17	DDX51	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51	-0.24	1.68	0.17	-0.51
18	DGCR2	integral membrane protein DGCR2	-0.66	1.69	0.05	0.02
19	CCNA1	cyclin A1 isoform a	-0.51	1.81	-0.33	0.66
20	NR2F1	nuclear receptor subfamily 2, group F, member 1	0.05	1.94	0.87	-0.09
21	ACD	adrenocortical dysplasia homolog isoform 1	-0.96	2.06	0.20	-1.02

Supplementary Figure 8. List of rapamycin sensitive translationally regulated genes after 3-hour treatment with rapamycin (50 nM) or PP242 (2.5 μM) in PC3 cells (* = targets which overlap with PP242, $\log_2$ fold-change).

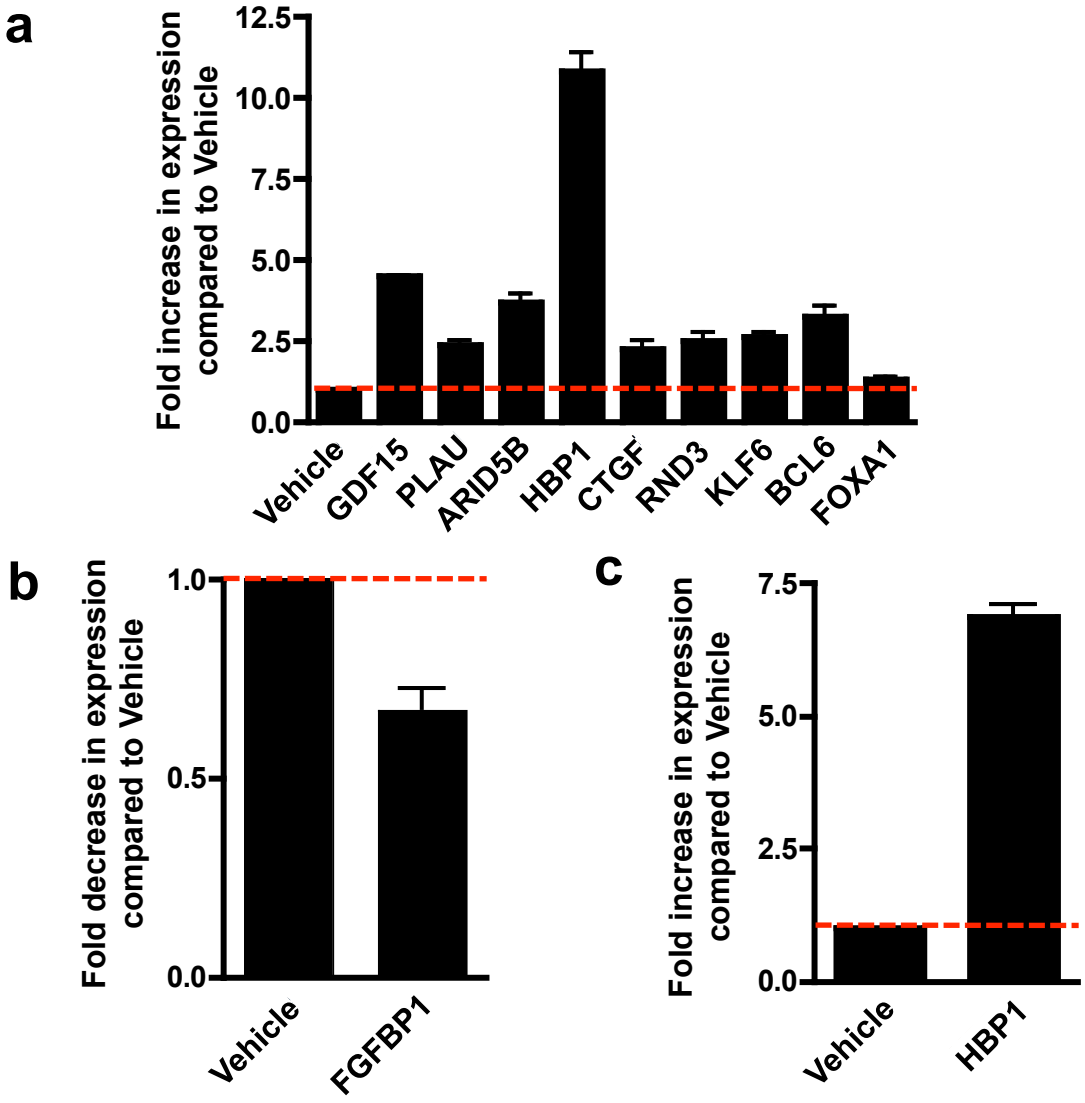
a

	Gene	Description	mRNA
1	FGFBP1	fibroblast growth factor binding protein 1	-1.75
2	BRIX1	ribosome biogenesis protein BRX1 homolog	-1.51
3	FOXA1	forkhead box A1	1.45
4	CYR61	cysteine-rich, angiogenic inducer, 61 precursor	1.47
5	MT2A	metallothionein 2A	1.47
6	SOX4	SRY (sex determining region Y)-box 4	1.51
7	BCL6	B-cell lymphoma 6 protein isoform 1	1.59
8	KLF6	Kruppel-like factor 6 isoform A	1.75
9	RND3	ras homolog gene family, member E precursor	1.78
10	CTGF	connective tissue growth factor precursor	1.80
11	HBP1 *	HMG-box transcription factor 1	1.88
12	ARID5B	AT rich interactive domain 5B (MRF1-like)	1.93
13	PLAU	plasminogen activator, urokinase isoform 1	2.04
14	GDF15	growth differentiation factor 15	3.02

b

	Gene	Description	mRNA
1	HBP1	HMG-box transcription factor 1	1.75

Supplementary Table 9. PP242 and rapamycin transcriptional targets. (a) List of PP242 sensitive transcriptionally regulated genes upon 3-hour treatment with PP242 (2.5 μM) in PC3 cells (* = target which overlaps with rapamycin)($\log_2$ fold-change). (b) Rapamycin sensitive transcriptionally regulated genes upon 3-hour treatment with rapamycin (50 nM) in PC3 cells ($\log_2$ fold-change).

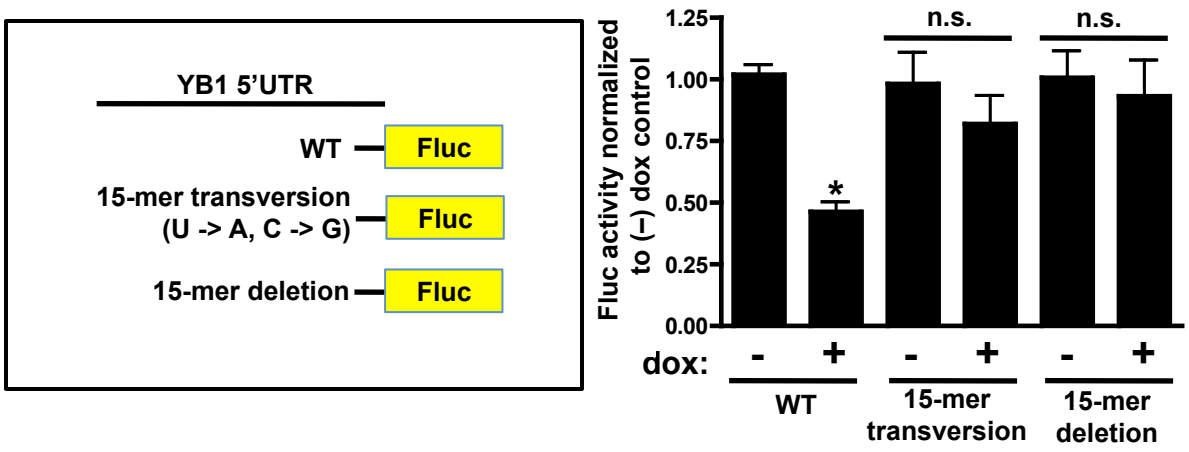


Supplementary Figure 10. Transcriptionally regulated mTOR targets. (a and b) qPCR validation of up-regulated or down-regulated transcripts identified by RNA-Seq upon 3-hour PP242 treatment (2.5 μM) in PC3 cells (mean ± SEM, n = 3). (c) qPCR validation of up-regulated transcript identified by RNA-Seq upon 3-hour rapamycin treatment (50 nM) in PC3 cells (mean ± SEM, n = 3).

a

	Gene	Description
Known pro-invasion genes	YB1	Y-box binding protein 1
	VIM	vimentin
	MTA1	metastasis associated 1
	CD44	CD44
Putative pro-invasion genes	ACTG1	actin G1
	TUBB3	tubulin, beta, 4
	COL6A2	alpha 2 type VI collagen isoform 2C2
	COL18A1	alpha 1 type XVIII collagen isoform 3 precursor
	SEPT8	septin 8 isoform a
	LAMA5	laminin alpha 5
	MYH14	myosin, heavy chain 14 isoform 3

b



Supplementary Figure 11. mTOR sensitive translationally regulated gene invasion signature. Mutation of the Pyrimidine Rich Translational Element abrogates sensitivity to eIF4E. (a) 4 known pro-invasion genes and 7 putative pro-invasion genes discovered through ribosome profiling. (b) Schematic of *YB1* 5'UTR cloning (WT, transversion mutant, and deletion mutant of the PRTE (position +20-34, uc001chs.2)) into pGL3-Promoter (Left panel). Firefly luciferase activity in PC3-4EBP1^{M} cells after a 24-hour pre-treatment with 1µg/ml doxycycline followed by transfection of respective 5'UTR constructs (mean ± SEM, n = 7, * P<0.0001, t-test)(Right panel). n.s. – not statistically significant.

a

Profile	INK128
Molecular weight (Da):	309
mTOR biochemical IC_{50} (nM):	1.0
Class I PI3K biochemical IC_{50} (nM): PI3Kα PI3Kβ PI3Kδ PI3Kγ	 219 5293 230 221
Cellular potency (PC3 cell, EC_{50} nM): - Inhibition of proliferation - Inhibition of pathway (pS6/pAkt)	 17 <10
Plasma protein binding (%): (m/r/d/mk/h)	48 / 65 / 47 / 62 / 71
Pharmacokinetics (mouse; 1.0 mg/kg; p.o.): Cmax T_{max} (hr)/$T_{1/2}$ (hr): Bioavailability:	500nM 0.25 - 0.5/1.5 90%
In vivo potency (tumor growth inhibition): (ED_{90} mg/kg; po, qd)	1.0

b

Enzyme	K_i (nM)
mTOR	1.4
PI3Kα	152
PI3Kβ	4700
PI3Kγ	165

	K_d (μM)	k_{on} (1/s *1/μM)	k_{off} (1/s)
mTOR	0.0003	18.4	0.0053

c

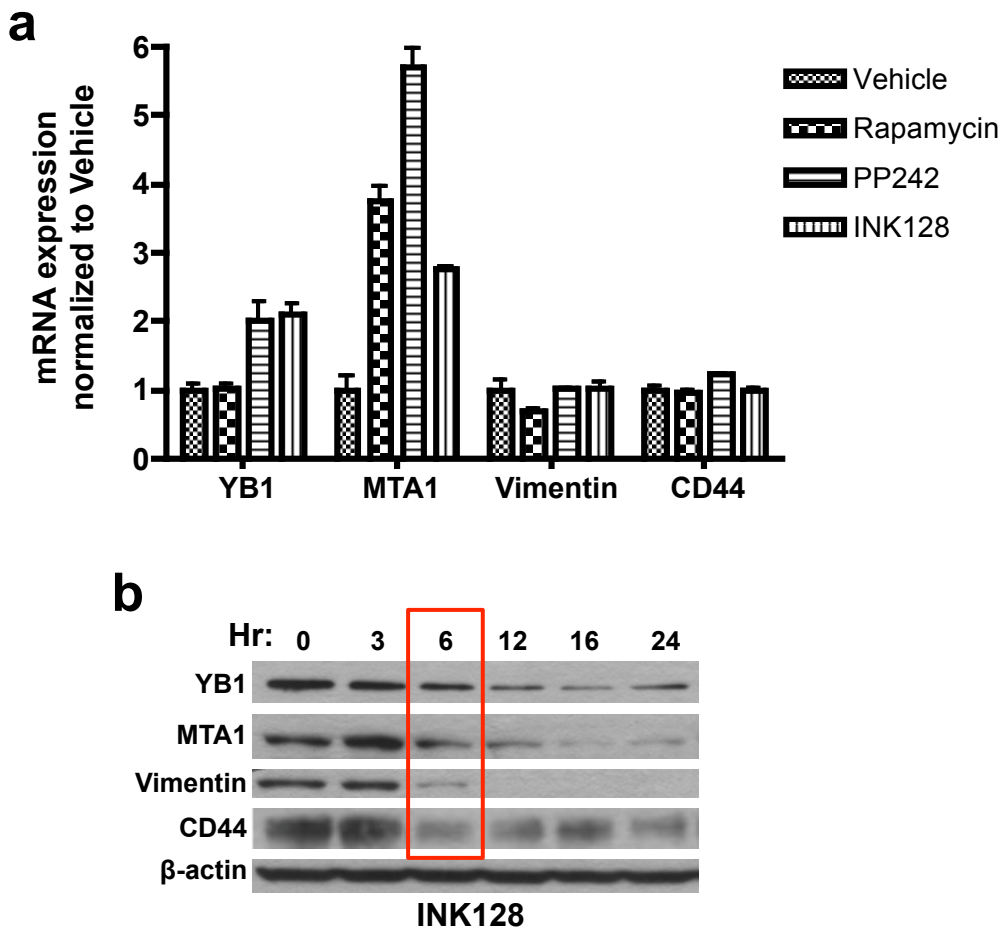
Lipid-PIKK selectivity	(% inhibition at 1000nM, 100nM):
PI3K Class II	
PI3KC2α	(85, 44)
PI3KC2β	(21, 9)
PI3K Class III	
VPS34	(0, 0)
PI4K	
PI4Kα	(0, 0)
PI4Kβ	(0, 0)
PIKK	
DNA-PK (IC_{50} nM)*	10

* INK128 inhibits DNA-PK activity in the cell only at high concentration (1 mM) while it inhibits mTOR activity in the cell at less than 10 nM

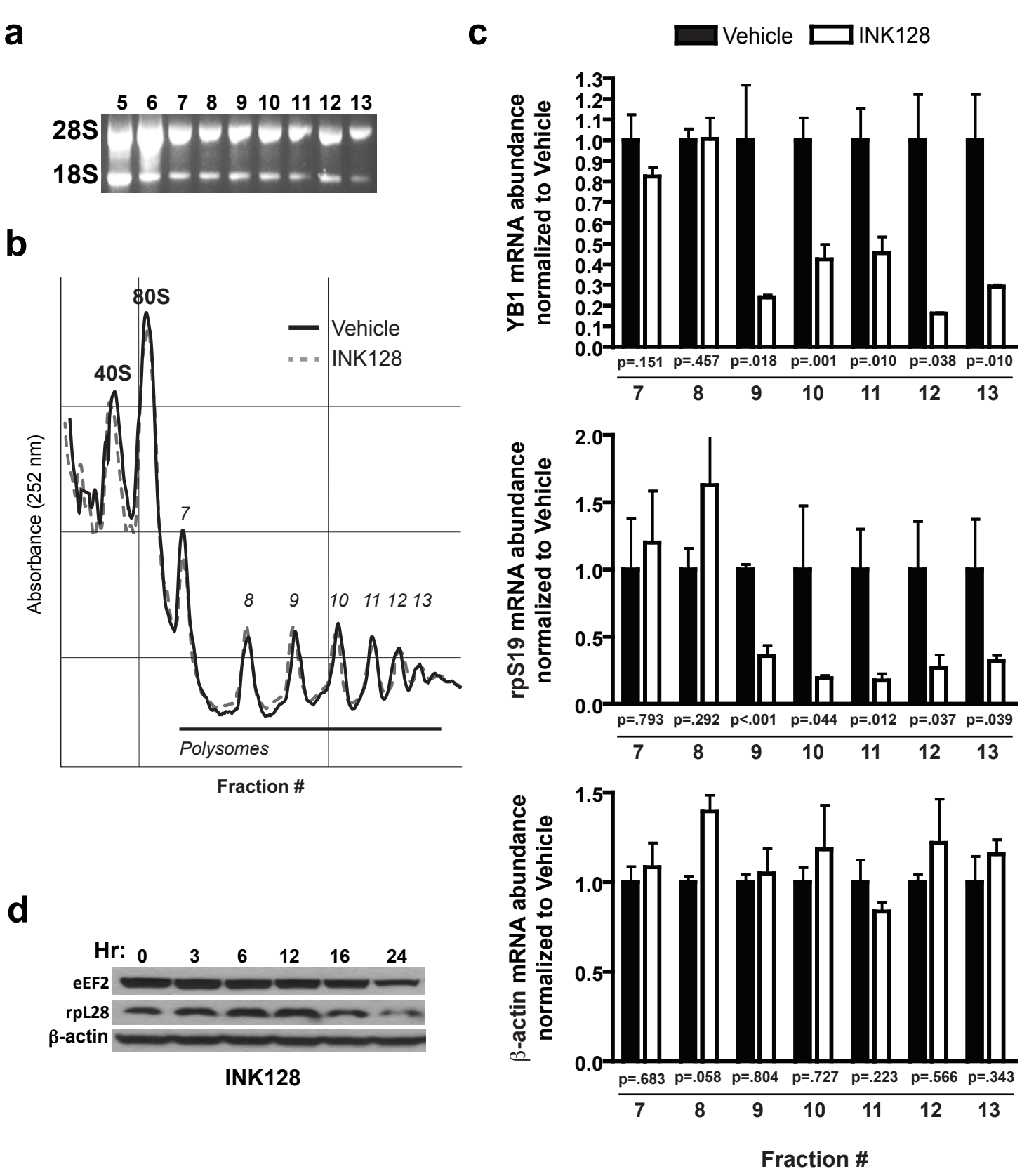
Supplementary Figure 12. Preclinical properties of INK128 (a) INK128 displayed a maximal oral tumor growth inhibition of ED_{90} at 1.0 mg/kg in xenograft solid tumor mouse models due to its biochemical, cellular potency and favorable pharmacokinetic properties. In detail, the percentage of protein binding of INK128 was determined in mouse, rat, dog, monkey, and human plasma at CEREP. Mouse pharmacokinetic parameters were assessed after acute dosing with INK128 at 0.3, 1.0, and 3.0 mg/kg by oral gavage resulting in plasma C_{max} values of 200, 600, and 1940 nM and AUC_{last} values of 500, 1300, and 2560 hr•ng/mL, respectively. INK128 displayed consistent and predictable oral pharmacokinetic parameters such as dose-linear plasma exposures across mouse, but also rat and cynomolgus monkey, with rapid absorption (T_{max} ranged from 0.25 hr to 5.0 hrs) and high oral bioavailability (%F ranging from 70% to 98%) supporting once-daily administration. In mouse tumor models, pharmacokinetic analysis displayed a dose-dependent increase in INK128 levels in plasma and tumor. Tumor concentrations appeared to be slightly higher than plasma concentrations. INK128 was well tolerated at efficacious doses in *in vivo* pharmacology studies. **(b)** INK128 was screened in biochemical assays with mTOR, PI3Kα, PI3Kβ and PI3Kγ to determine the K_i for each target. The average K_i values were determined using the Cheng-Pursoff equation. K_d, K_{on}, and K_{off} values were determined using the Proteros Reporter Displacement Assay. **(c)** Lipid kinase selectivity of INK128. The values indicate percent inhibition at 1 mM and 100 nM of INK128.

Kinase Tested	% Inhibition
ABL1	48
AKT1 (PKB alpha)	0
ALK	18
AURKA (Aurora A)	13
ABL1 E255K	45
ABL1 G250E	27
ABL1 T315I	9
ABL1 Y253F	62
ABL2 (Arg)	39
ACVR1B (ALK4)	25
ADRBK1 (GRK2)	3
ADRBK2 (GRK3)	-4
AKT2 (PKB beta)	5
AKT3 (PKB gamma)	19
AMPK A1/B1/G1	2
AMPK A2/B1/G1	16
AURKB (Aurora B)	8
AURKC (Aurora C)	4
AXL	6
BLK	50
BMX	13
BRAF	9
BRAF V599E	8
BRSK1 (SAD1)	2
BTK	13
CAMK1 (CaMK1)	-14
CAMK1D (CaMKI delta)	8
CAMK2A (CaMKII alpha)	2
CAMK2B (CaMKII beta)	7
CAMK2D (CaMKII delta)	15
CAMK4 (CaMKIV)	0
CDC42 BPA (MRCKA)	2
CDC42 BPB (MRCKB)	2
CDK1/cyclin B	-2
CDK2/cyclin A	-7
CDK5/p25	4
CDK5/p35	1
CDK7/cyclin H/MNAT1	-37
CHEK1 (CHK1)	2
CHEK2 (CHK2)	15
CLK1	6
CLK2	5
CLK3	3
CSF1R (FMS)	76
CSK	23
CSNK1A1 (CK1 alpha 1)	13
CSNK1D (CK1 delta)	73
CSNK1E (CK1 epsilon)	87
CSNK1G1 (CK1 gamma 1)	2
CSNK1G2 (CK1 gamma 2)	4
CSNK1G3 (CK1 gamma 3)	12
CSNK2A1 (CK2 alpha 1)	1
CSNK2A2 (CK2 alpha 2)	4
DAPK1	13
DAPK3 (ZIPK)	-1
DCAMKL2 (DCK2)	2
DYRK1A	-1
DYRK1B	3
DYRK3	31
DYRK4	-4
EEF2K	6
EGFR (ErbB1)	10
EGFR (ErbB1) L858R	11
EGFR (ErbB1) L861Q	13
EGFR (ErbB1) T790M	5
EGFR (ErbB1) T790M L858R	9
EPHA1	37
EPHA2	21
EPHA3	4
EPHA4	11
EPHA5	16
EPHA8	29
EPHB1	16
EPHB2	15
EPHB3	10
EPHB4	9
ERBB2 (HER2)	3
ERBB4 (HER4)	5
FER	-4
FES (FPS)	5
FGFR1	27
FGFR2	36
FGFR3	17
FGFR3 K650E	37
FGFR4	32
FGR	76
FLT1 (VEGFR1)	5
FLT3	81
FLT3 D835Y	25
FLT4 (VEGFR3)	20
FRAP1 (mTOR)	101
FRK (PTK5)	36
FYN	55
GRK4	-3
GRK5	4
GRK6	-5
GRK7	-2
GSK3A (GSK3 alpha)	5
GSK3B (GSK3 beta)	-2
HCK	41
HIPK1 (Myak)	1
HIPK4	7
IGF1R	3
IKBKB (IKK beta)	1
IKBKE (IKK epsilon)	1
INSR	4
INSRR (IRR)	14
IRAK4	5
ITK	0
JAK1	4
JAK2	-2
JAK2 JH1 JH2	-11
JAK2 JH1 JH2 V617F	4
JAK3	3
KDR (VEGFR2)	30
KIT	13
KIT T670I	4
LCK	66
LRRK2	16
LRRK2 G2019S	7
LTK (TYK1)	9
LYN A	63
LYN B	65
MAPK1 (ERK2)	-1
MAP2K1 (MEK1)	18
MAP2K2 (MEK2)	28
MAP2K6 (MKK6)	-3
MAP3K8 (COT)	2
MAP3K9 (MLK1)	16
MAP4K2 (GCK)	6
MAP4K4 (HGK)	15
MAP4K5 (KHS1)	6
MAPK10 (JNK3)	-5
MAPK11 (p38 beta)	13
MAPK12 (p38 gamma)	7
MAPK13 (p38 delta)	1
MAPK3 (ERK1)	6
MAPK8 (JNK1)	12
MAPK9 (JNK2)	4
MAPKAPK2	4
MAPKAPK3	2
MAPKAPK5 (PRAK)	1
MARK1 (MARK)	9
MARK2	13
MARK3	6
MARK4	13
MATK (HYL)	3
MET (cMet)	7
MELK	32
MERTK (cMER)	16
MET M1250T	3
MINK1	4
MST1R (RON)	1
MST4	-5
MUSK	15
MYLK2 (skMLCK)	15
NEK1	7
NEK2	1
NEK4	2
NEK6	-2
NEK7	13
NEK9	-4
NTRK1 (TRKA)	33
NTRK2 (TRKB)	29
NTRK3 (TRKC)	66
PAK2 (PAK65)	9
PAK3	-17
PAK4	4
PAK6	5
PAK7 (KIAA1264)	-4
PASK	0
PDGFRA (PDGFR alpha)	50
PDGFRA D842V	50
PDGFRA T674I	8
PDGFRA V561D	81
PDGFRB (PDGFR beta)	24
PDK1	14
PHKG1	5
PHKG2	2
PIM1	-2
PIM2	0
PKN1 (PRK1)	0
PLK1	-1
PLK2	3
PLK3	-3
PRKACA (PKA)	39
PRKCA (PKC alpha)	22
PRKCB1 (PKC beta I)	8
PRKCB2 (PKC beta II)	3
PRKCD (PKC delta)	2
PRKCE (PKC epsilon)	9
PRKCG (PKC gamma)	23
PRKCH (PKC eta)	6
PRKCI (PKC iota)	4
PRKCN (PKD3)	17
PRKCQ (PKC theta)	4
PRKCZ (PKC zeta)	1
PRKD1 (PKC mu)	21
PRKD2 (PKD2)	11
PRKG1	4
PRKG2 (PKG2)	4
PRKX	0
PTK2 (FAK)	12
PTK2B (FAK2)	5
PTK6 (Brk)	53
RAF1 (cRAF) Y340D Y341D	34
RET	51
RET V804L	8
RET Y791F	71
ROCK1	0
ROCK2	1
ROS1	13
RPS6KA1 (RSK1)	1
RPS6KA2 (RSK3)	0
RPS6KA3 (RSK2)	4
RPS6KA4 (MSK2)	11
RPS6KA5 (MSK1)	4
RPS6KA6 (RSK4)	0
RPS6KB1 (p70S6K)	-23
SGK (SGK1)	4
SGK2	2
SGKL (SGK3)	2
SNF1LK2	20
SRC	12
SRC N1	23
SRMS (Srm)	30
SRPK1	-1
SRPK2	-3
STK22B (TSSK2)	1
STK22D (TSSK1)	1
STK23 (MSSK1)	5
STK24 (MST3)	-3
STK25 (YSK1)	1
STK3 (MST2)	1
STK4 (MST1)	3
SYK	-1
TAOK2 (TAO1)	6
TBK1	-4
TEK (Tie2)	-5
TYK2	2
TYRO3 (RSE)	18
YES1	43
ZAP70	2

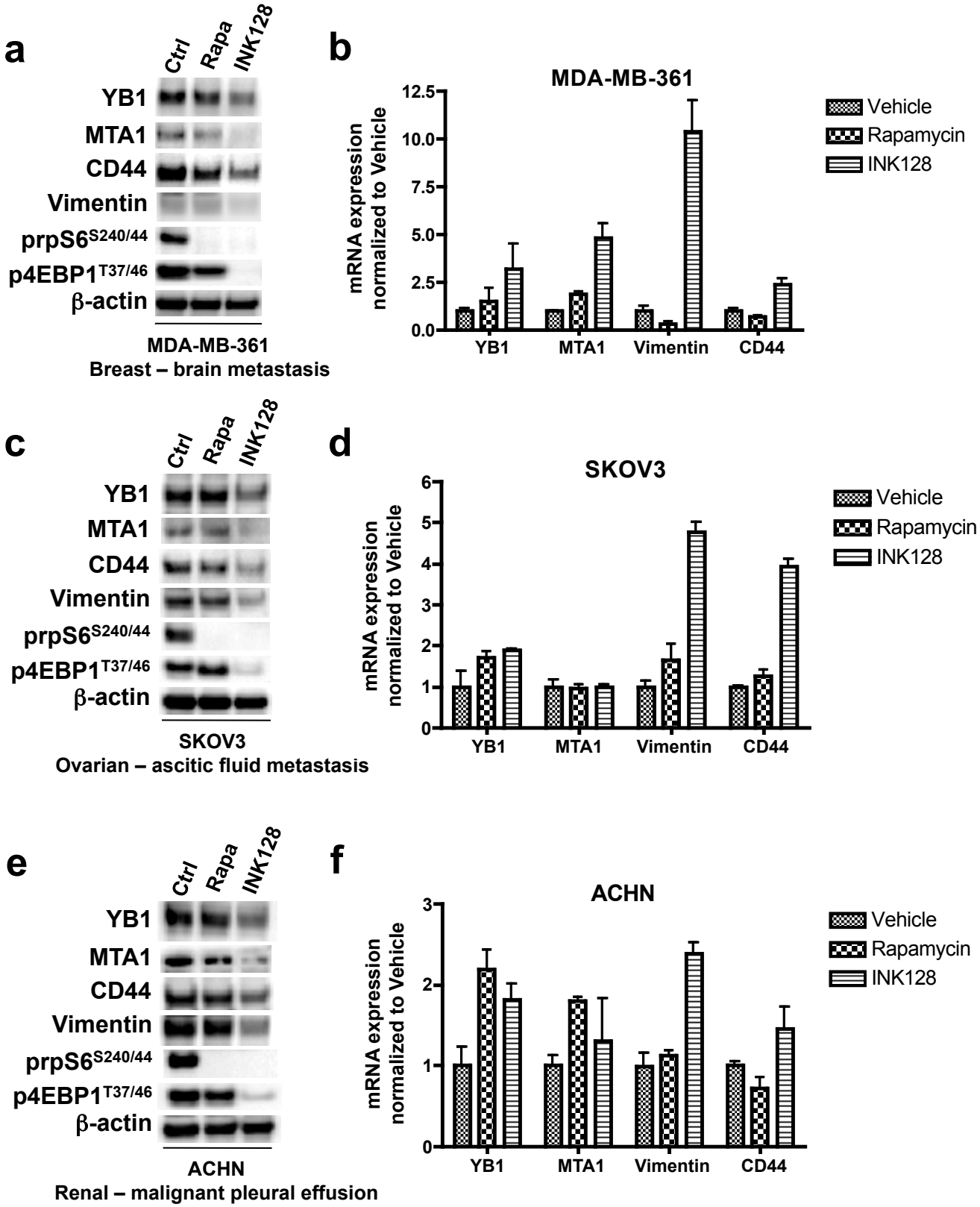
Supplementary Figure 13. INK128 screened against 243 kinases. INK128 was screened against a panel of 243 kinases using the Invitrogen SelectScreen™ kinase profiling service. The values indicate percent inhibition at 1 μM of INK128.



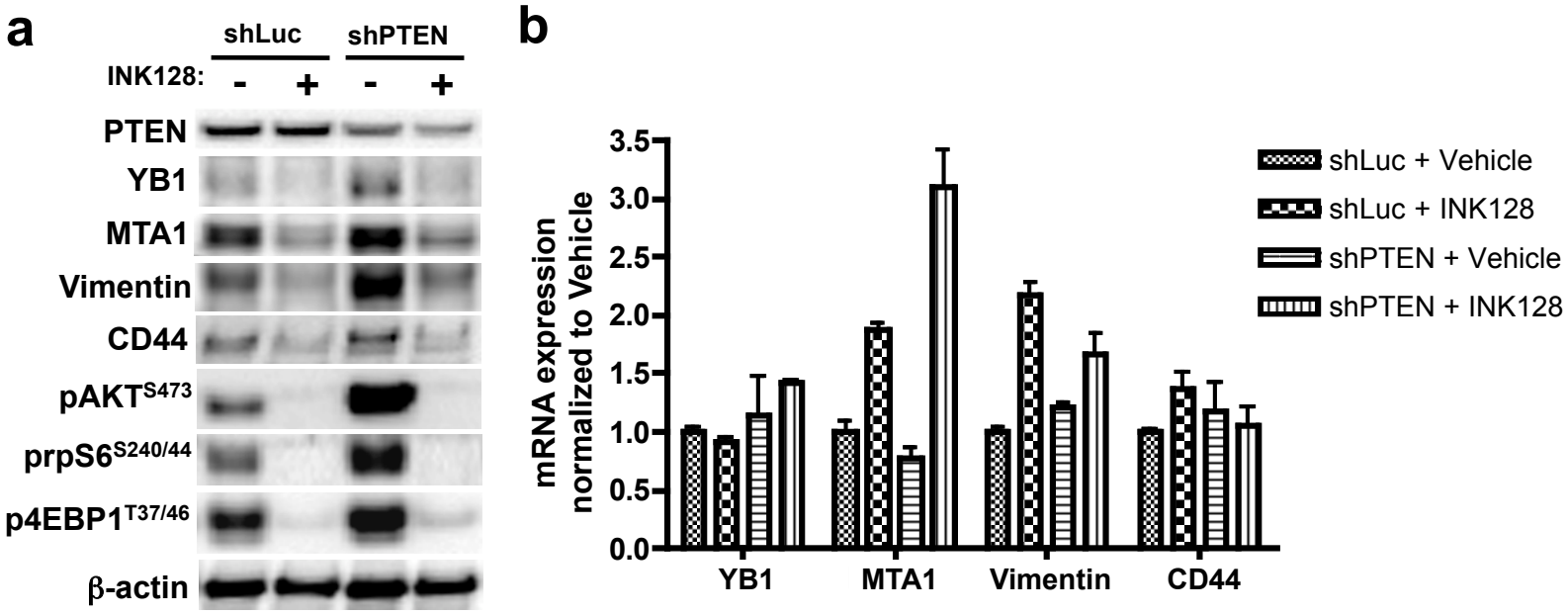
Supplementary Figure 14. ATP site inhibition of mTOR does not reduce transcript levels of the 4 invasion genes. INK128 time course. (a) mRNA expression of *YB1*, *MTA1*, vimentin, and *CD44*, relative to β-actin upon treatment with rapamycin (50 nM), PP242 (2.5 μM), or INK128 (200 nM) for 48 hours in PC3 cells (mean ± SEM, n = 3). (b) Representative western blot of 3 independent experiments showing a time course of invasion gene expression before and after treatment with INK128 (200 nM) in PC3 cells.



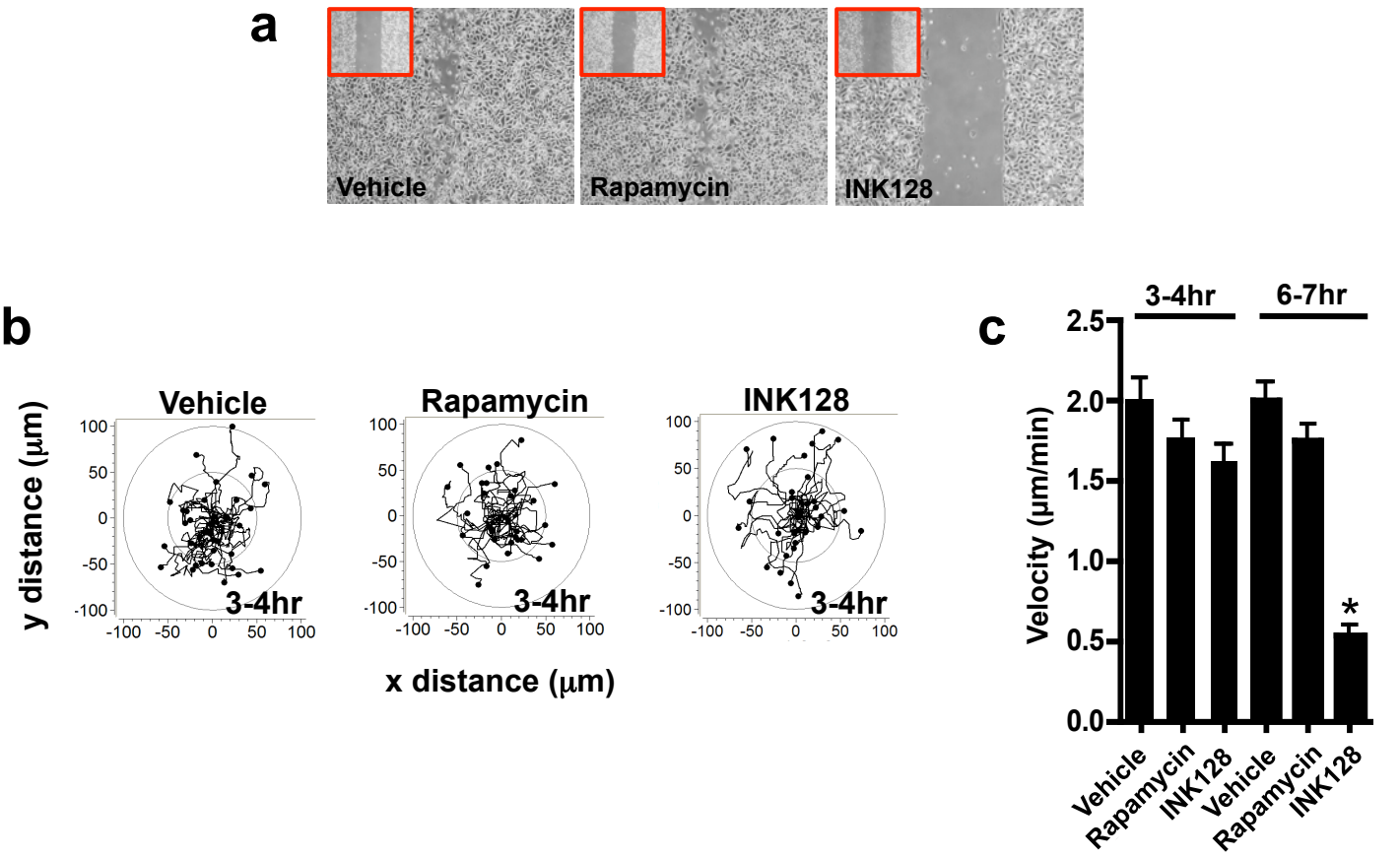
Supplementary Figure 15. Polysome analysis after 3-hour INK128 treatment. (a) Ethidium bromide staining of rRNA species in individual fractions. Fractions 7-13 were determined to be polysome-associated fractions. (b) Overlay of polysome profiles from PC3 cells treated with vehicle (solid line) or INK128 (100 nM)(dotted line). (c) qPCR analysis of *YB1* and *rpS19* mRNAs that show differential association in polysome fractions after INK128 (100 nM) treatment (mean ± SEM, n = 6). The bottom graph shows that there is no change in β-actin mRNA association in polysome fractions between treatments. P-values (t-test) for each polysome fraction are shown. (d) Representative western blot of 3 independent experiments showing a time course of eEF2 and rpL28 expression before and after treatment with INK128 (200 nM) in PC3 cells.



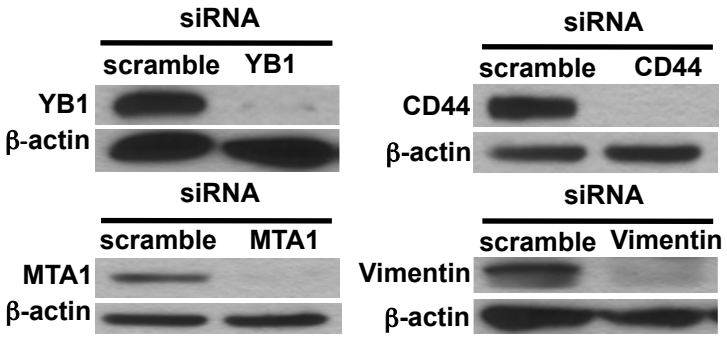
Supplementary Figure 16. 4-gene invasion signature is responsive to INK128 but not rapamycin in metastatic cell lines. (a-b) Representative western blot (a) and qPCR analysis (b) of MDA-MB-361 cells after 48-hour treatment with INK128 (200 nM). (c-d) Representative western blot (c) and qPCR analysis (d) of SKOV3 cells after 48-hour treatment with INK128 (200 nM). (e-f) Representative western blot (e) and qPCR analysis (f) of ACHN cells after 48-hour treatment with INK128 (200 nM). Westerns = representative western blot of 2 independent experiments. QPCR – n = 3. All data represent mean ± SEM.



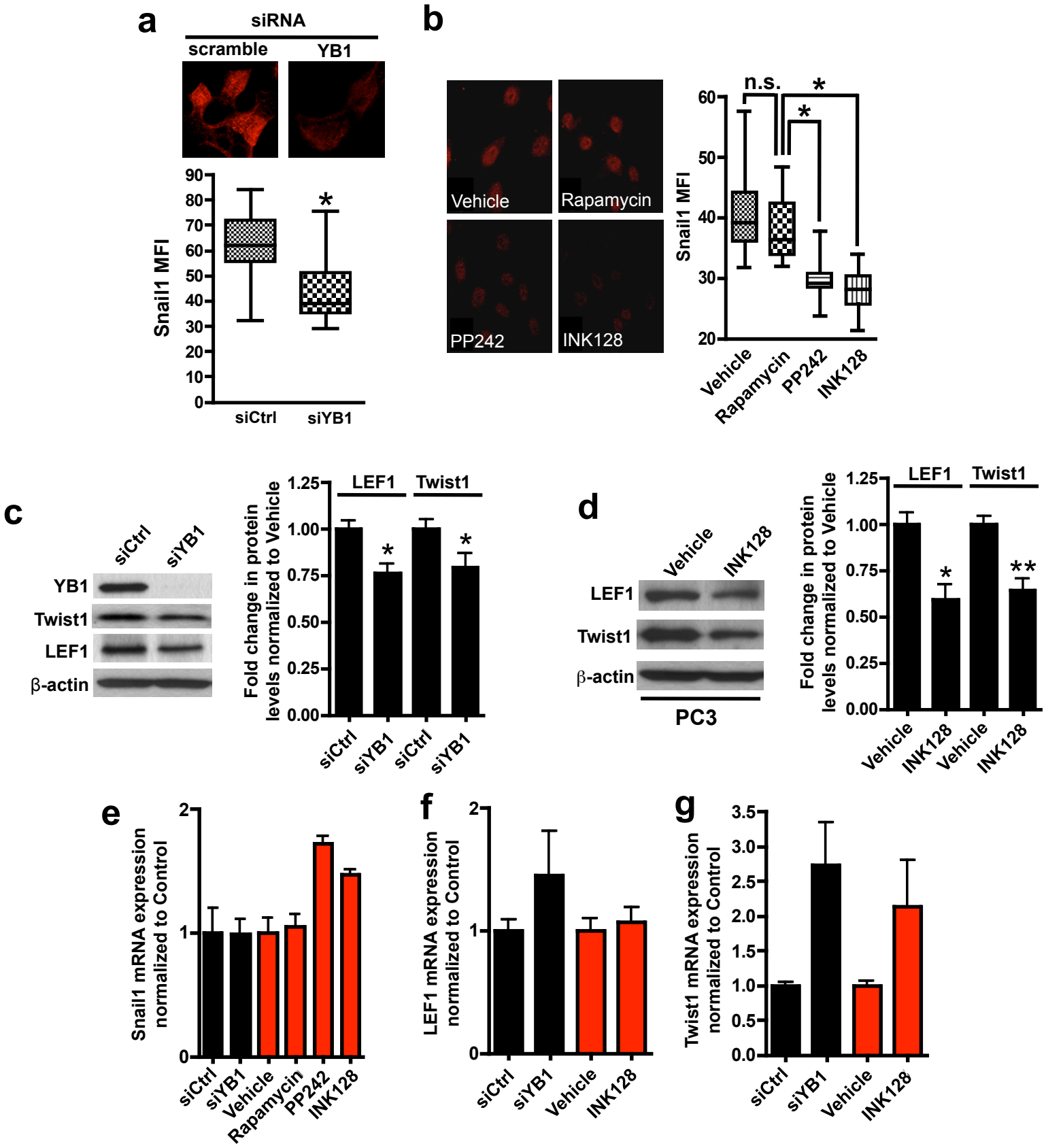
Supplementary Figure 17. *PTEN* gene silencing in the A498 *PTEN* positive renal carcinoma cell line induces the post-transcriptional expression of the 4-gene invasion signature. (a-b) Representative western blot (a) and qPCR analysis (b) of A498 cells after stable silencing of *PTEN* and 24 hour INK128 treatment (200 nM). Western = representative western blot of 2 independent experiments. QPCR - n = 3. All data represent mean ± SEM.



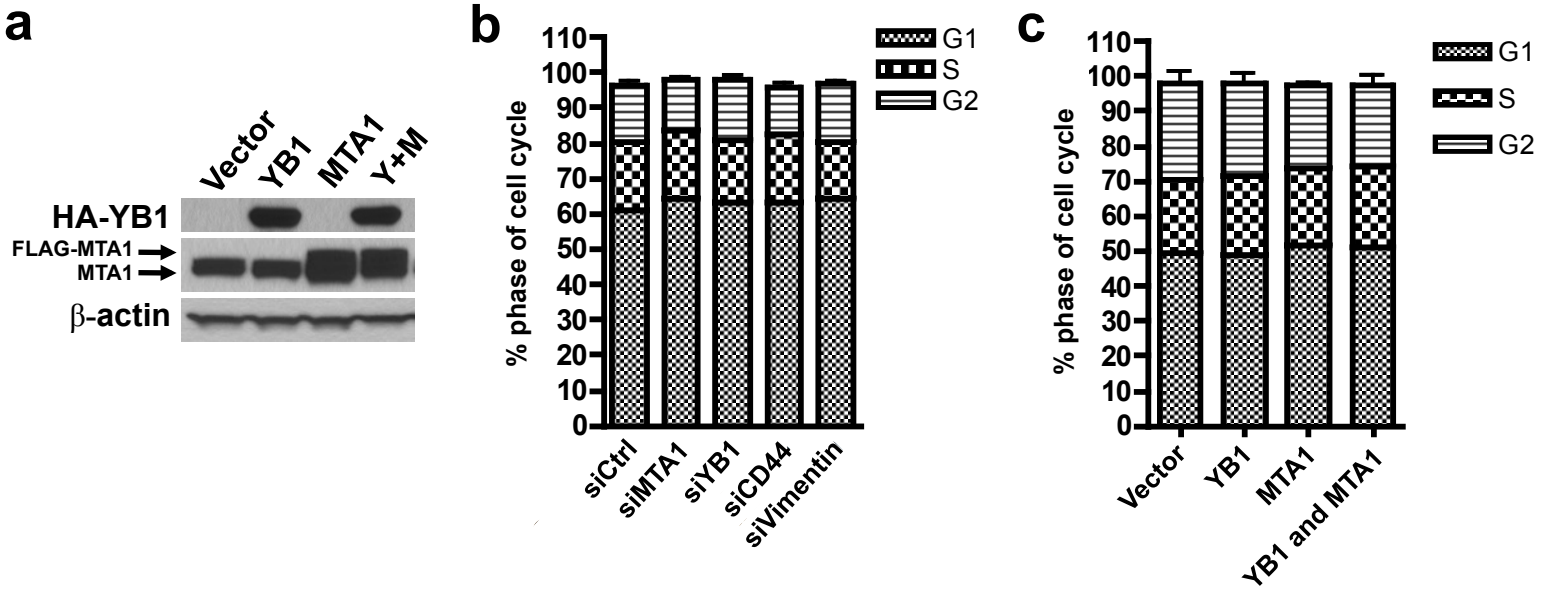
Supplementary Figure 18. INK128 inhibits cell migration in PC3 prostate cancer cells as early as 6 hours after drug treatment. (a) Representative wound healing assay of 3 independent experiments in PC3 cells treated with rapamycin (50 nM) or INK128 (200 nM) for 40 hours. Inset (red box) represents wound at 0 hours. (b) Migration patterns of individual GFP-labeled PC3 cells during hours 3-4 after treatment with rapamycin or INK128 (34 cells per condition). (c) Average velocity of GFP-labeled PC3 cells during hours 3-4 or 6-7 after treatment with rapamycin 50 nM or INK128 200 nM (mean ± SEM, n = 34 cells per condition, * P<0.001, ANOVA).



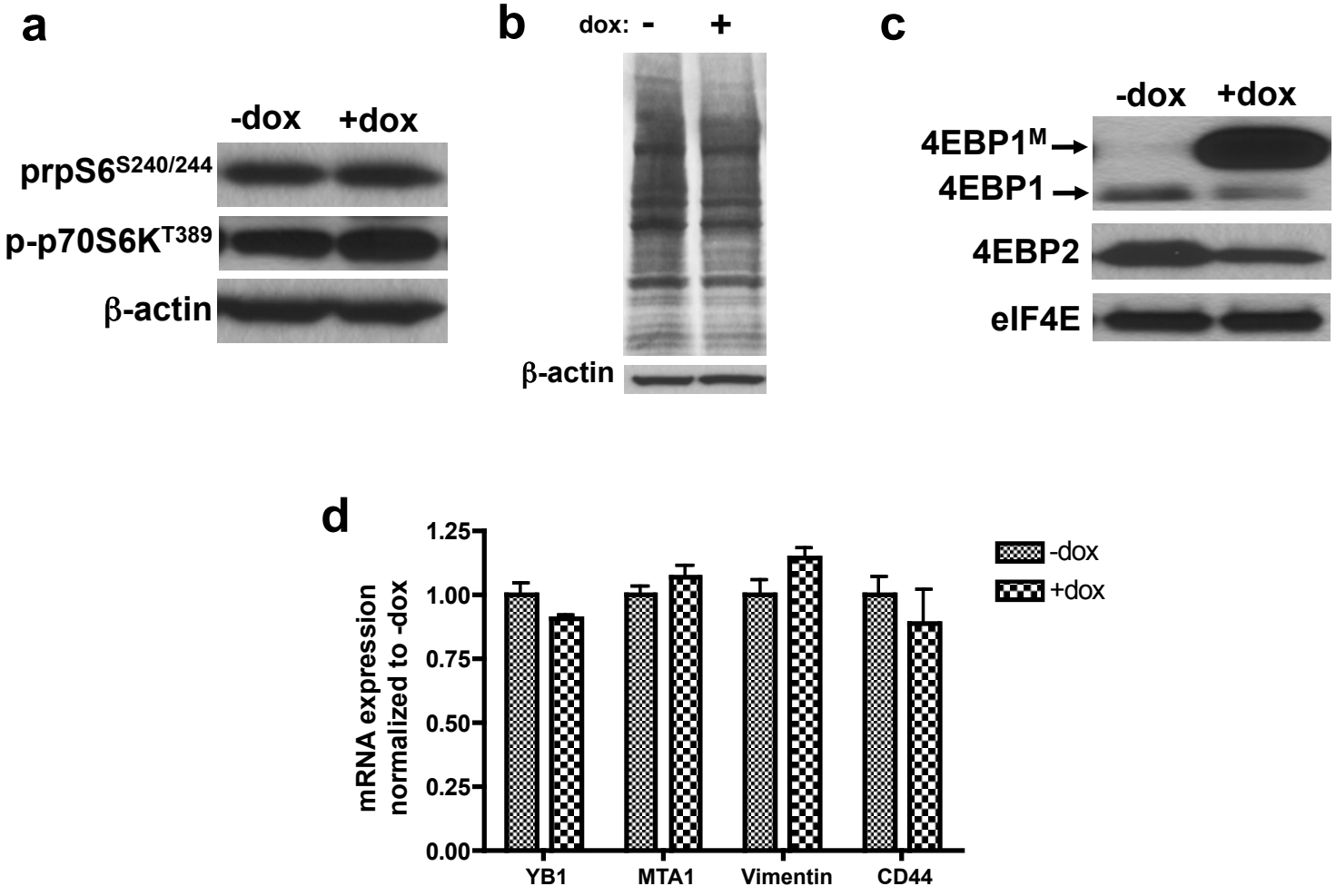
Supplementary Figure 19. Knockdown of the 4 invasion genes in PC3 prostate cancer cells. YB1, CD44, MTA1, and Vimentin protein levels after 48 hours of gene silencing in PC3 cells.



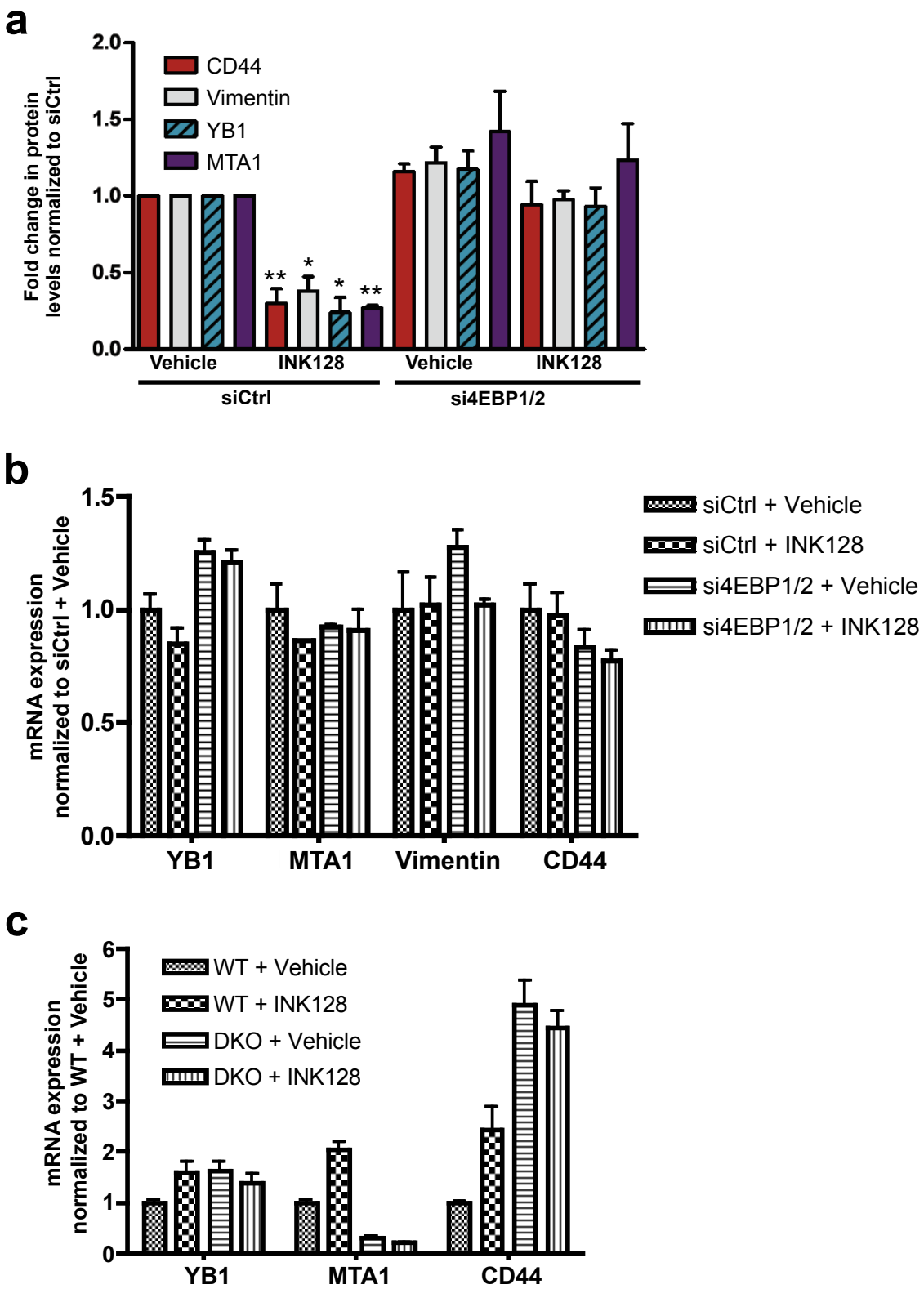
Supplementary Figure 20. *YB1* knockdown and ATP site inhibition of mTOR decreases the protein levels but not mRNA levels of YB1 target genes. (a) Snail1 immunofluorescence in PC3 cells after 48 hours of *YB1* gene silencing. Representative Snail1 immunofluorescence (top panels), box plot of Snail1 mean fluorescence intensity per cell (MFI)(n = 26 siCtrl cells, n = 15 siYB1 cells, * P = 0.001, t-test)(bottom panel). (b) Snail1 immunofluorescence in PC3 cells after treatment with rapamycin (50 nM), PP242 (2.5 μM), or INK128 (200 nM). Representative Snail1 immunofluorescence (left panel), box plot of Snail1 mean fluorescence intensity per cell (MFI)(n = 16 vehicle treated cells, n = 26 rapamycin treated cells, n = 28 PP242 treated cells, n = 27 INK128 treated cells, * P < 0.05, ANOVA)(right panel). (c) Representative western blot (left panel) and quantification of protein levels (right panel) for LEF1 and Twist1 after *YB1* gene silencing (mean ± SEM, n = 6, * P<0.05, t-test). (d) Representative western blot (left panel) and quantification of protein levels (right panel) for LEF1 and Twist1 after INK128 treatment (mean ± SEM, n = 6, * P<0.005, t-test). (e-g) *Snail1* (e), *LEF1* (f), or *Twist1* (g) mRNA expression normalized to β-actin after *YB1* gene knockdown or treatment with rapamycin (50nM), PP242 (2.5 μM) or INK128 (200 nM) in PC3 cells (mean ± SEM, n = 3).



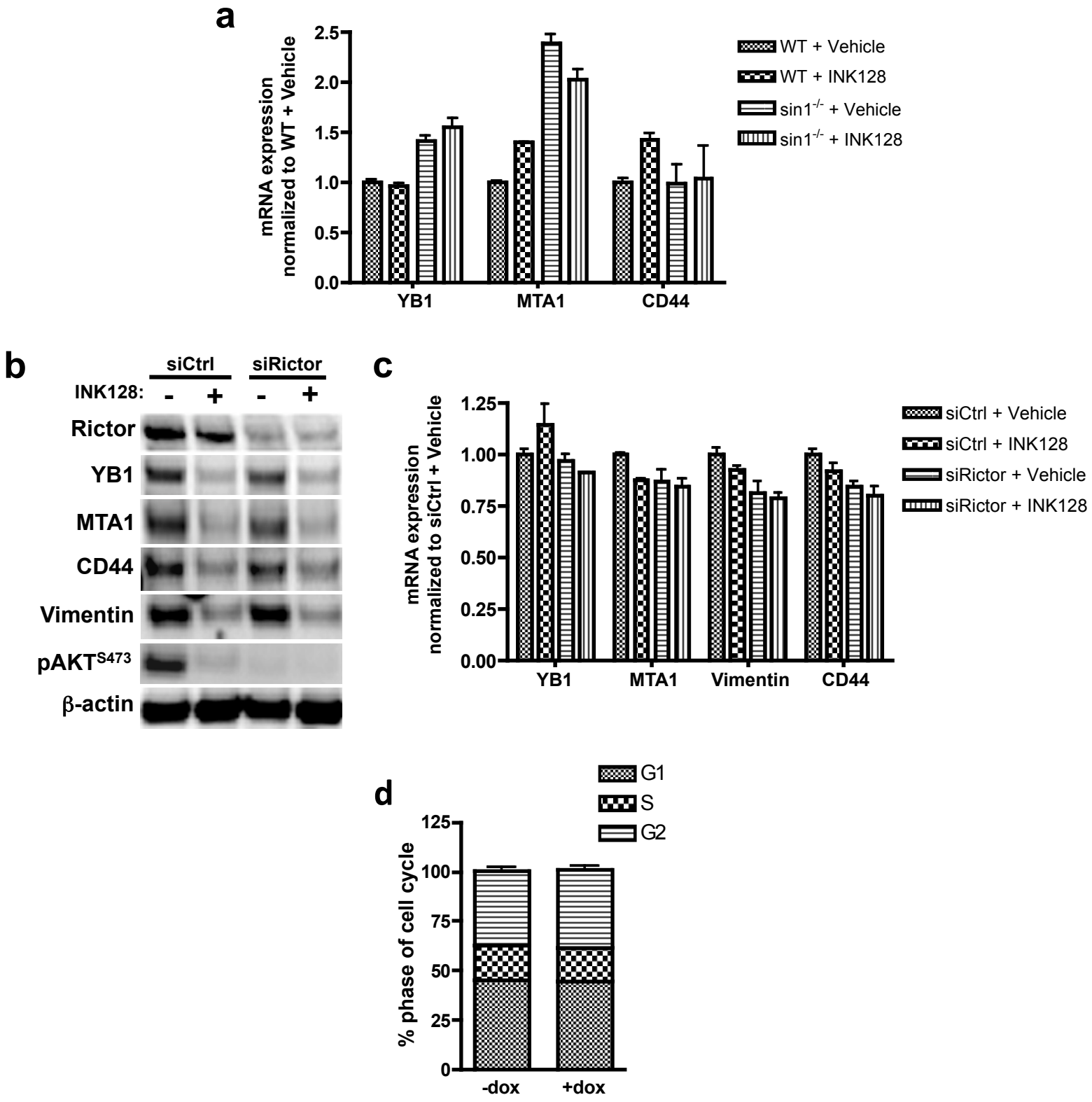
Supplementary Figure 21. Effects of invasion gene knockdown or over expression in PC3 and BPH-1 cells, respectively on the cell cycle. (a) HA-YB1 and Flag-MTA1 protein levels after 48 hours of over expression in non-transformed BPH-1 prostate epithelial cells (Y = YB1, M = MTA1). (b) Cell cycle analysis in PC3 cells after knockdown of respective genes (mean ± SEM, n = 3). (c) Cell cycle analysis upon over expression of *YB1* and/or *MTA1* in BPH-1 cells. (mean ± SEM, n = 3).



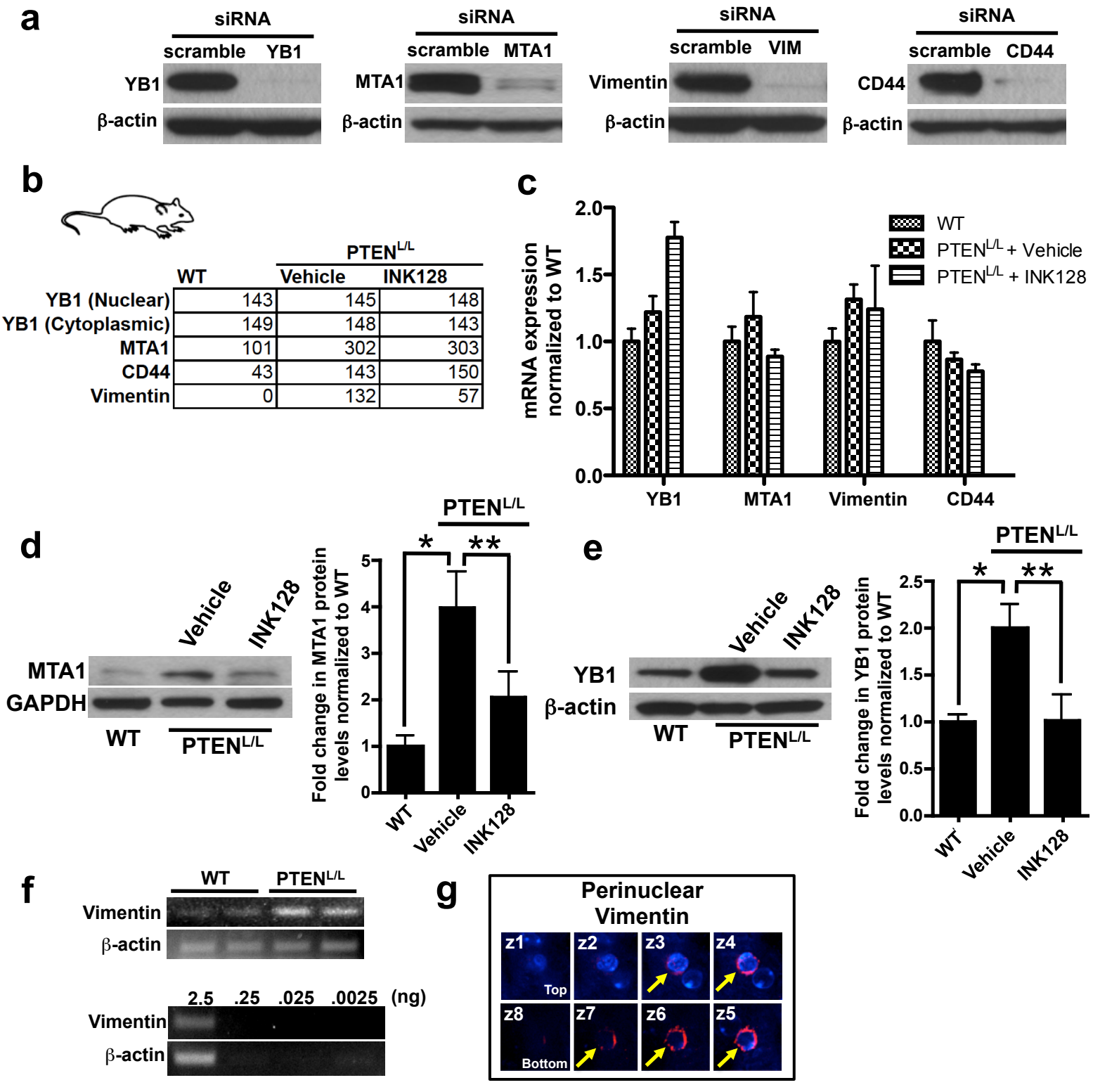
Supplementary Figure 22. The 4EBP1^{M} does not augment mTORC1 function or global protein synthesis in PC3 cells. (a) Representative western blot from 3 independent experiments of phospho-p70S6K^{T389} and phospho-rpS6$^{S240/244}$ after a 48-hour treatment with and without 1μg/ml doxycycline in PC3-4EBP1^{M} cells. (b) Representative [^{35}S]-methionine incorporation from 2 independent experiments in PC3-4EBP1^{M} cells (48 hours, doxycycline 1μg/mL)(mean + SEM). (c) Representative cap-binding assay from 2 independent experiments after 48-hour treatment with 1μg/ml doxycycline in PC3-4EBP1^{M} cells. (d) mRNA expression of *YB1*, *MTA1*, Vimentin, and *CD44* relative to β-actin after 48-hour treatment with 1μg/ml doxycycline in PC3-4EBP1^{M} cells (mean ± SEM, n = 3).



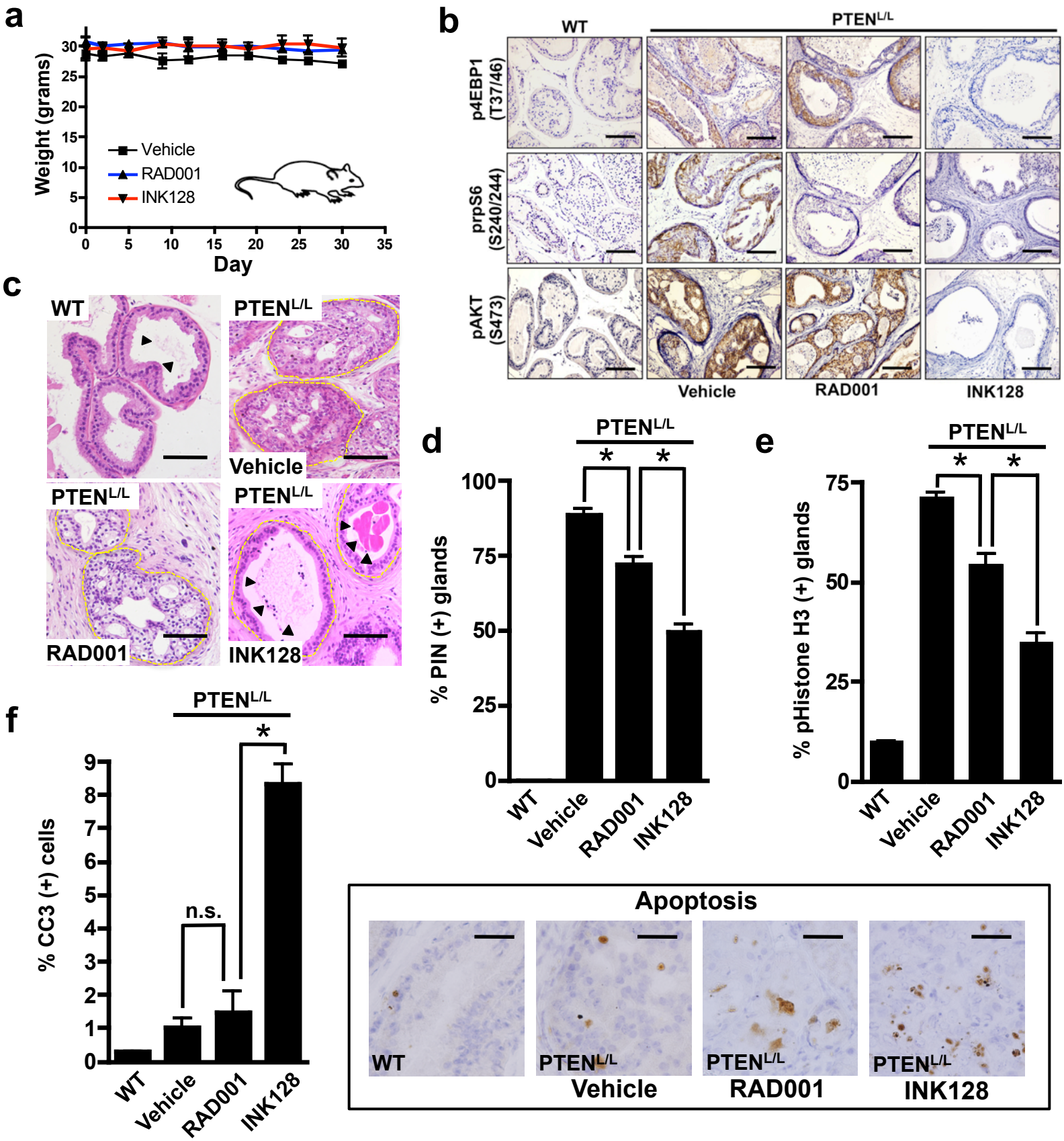
Supplementary Figure 23. The 4EBP/eIF4E axis imparts sensitivity to mTOR ATP site inhibition. (a) Quantification of western blots from 3 independent experiments of PC3 cells after 48 hours of *4EBP1*/*4EBP2* knockdown followed by 24-hour INK128 treatment. (n = 3, * p<0.05, ** p<0.01, ANOVA). (b) mRNA expression of *YB1*, *MTA1*, vimentin, and *CD44* relative to β-actin after 48 hours of gene silencing of *4EBP1* and *4EBP2* followed by a 24-hour INK128 treatment (200 nM)(mean ± SEM, n = 3). (c) mRNA expression of *YB1*, *MTA1*, and *CD44* in WT and *4EBP1*/*4EBP2* DKO MEFs treated with 200 nM INK128 for 24 hours (mean ± SEM, n = 3).



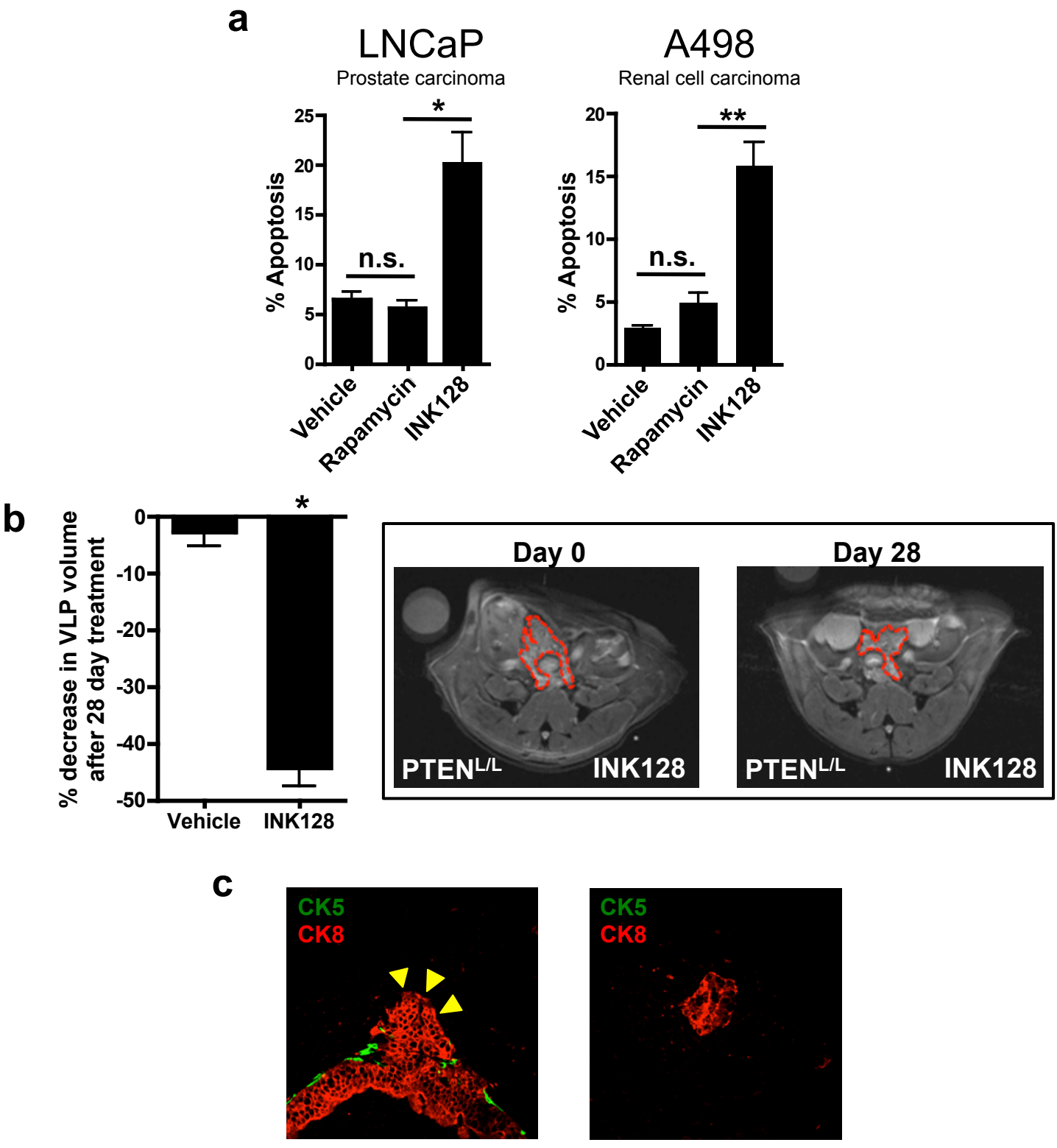
Supplementary Figure 24. mTORC2 does not control the expression of the 4-gene invasion signature. (a) mRNA expression of *YB1*, *MTA1*, and *CD44* relative to β-actin after a 24-hour treatment with INK128 (200 nM) in *mSin1*$^{-/-}$ MEFs (mean ± SEM, n = 3). (b) Representative western blot analysis from 2 independent experiments of PC3 prostate cancer cells after 48 hours of *rictor* gene silencing followed by a 24-hour treatment with INK128 (200 nM). (c) mRNA expression of *YB1*, *MTA1*, vimentin, and *CD44* relative to β-actin in PC3 prostate cancer cells after 48 hours of *rictor* gene silencing followed by a 24-hour treatment with INK128 (200 nM) in PC3. (mean ± SEM, n = 3). (d) Cell cycle analysis of PC3-4EBP1^{M} cells after treatment with 1μg/ml doxycycline for 48 hours (mean ± SEM, n = 3).



Supplementary Figure 25. Complete mTOR inhibition decreases the expression of the 4-gene invasion signature at the level of translational control *in vivo* in *PTEN*$^{L/L}$ mice. (a) Validation of antibodies used for immunofluorescence after 48-hour gene silencing of respective genes in PC3 cells. (b) Number of individual CK5+ and/or CK8+ cells measured in 3 separate mice for mean fluorescence intensity of respective protein targets in WT and *PTEN*$^{L/L}$ mouse prostates. (c) mRNA expression of *YB1*, *MTA1*, vimentin, and *CD44* relative to β-actin in WT and *PTEN*$^{L/L}$ mice after 28 days of treatment with INK128 (1 mg/kg daily) (mean ± SEM, n = 3 mice/arm). (d) Representative western blot of MTA1 from whole prostate tissue in WT and *PTEN*$^{L/L}$ mice after 28 days of treatment with INK128 (1 mg/kg daily) (left panel) and quantitation relative to β-actin protein levels (right panel) (mean ± SEM, n = 3 mice/arm, * P=0.02, ** P=0.04, t-test) (e) Representative western blot of YB1 from whole prostate tissue in WT and *PTEN*$^{L/L}$ mice after 28 days of treatment with INK128 (1 mg/kg daily) (left panel) and quantitation relative to β-actin protein levels (right panel) (mean ± SEM, n = 4 mice/arm, * P=0.002, ** P=0.04, t-test) (f) Semi-quantitative RT-PCR of vimentin and β-actin for WT and *PTEN*$^{L/L}$ FACS sorted murine prostate luminal epithelial cells (top panel). RT-PCR of a serial dilution of WT prostate luminal epithelial cell (bottom panel) (g) Z-series of perinuclear vimentin in a *PTEN*$^{L/L}$ CK8 + prostate epithelial cell (red – vimentin, blue – DAPI, 0.4 μm per section, yellow arrows point to perinuclear vimentin).



Supplementary Figure 26. Preclinical efficacy of complete mTOR blockade *in vivo* (a) Mouse weights measured every 3 days over the course of the preclinical trial (mean ± SEM, n = 3/arm). (b) Representative phospho-specific immunohistochemistry of downstream mTOR targets in the ventral prostate (VP) of 9-month-old WT or *PTEN*$^{L/L}$ mice after 28 days of treatment with INK128 (1 mg/kg daily) or RAD001 (10 mg/kg daily)(n = 6 mice/treatment arm). Scale bar = 100 μm. (c) Representative histology of 9-month-old WT or *PTEN*$^{L/L}$ mice VP after 28 days of treatment with vehicle, RAD001 (10 mg/kg daily), or INK128 (1 mg/kg daily). Yellow dotted lines encircle prostate glands. Black triangles refer to prostatic secretions. Scale bar = 50 μm. (d) Quantification of PIN+ glands in treated mice (mean ± SEM, n = 6 mice/arm, * P<0.001, ANOVA). (e) Proliferation measured by phospho-histone H3 positive glands in the prostates of 9-month-old WT or *PTEN*$^{L/L}$ mice treated with RAD001 (10 mg/kg daily) or INK128 (1 mg/kg daily)(mean ± SEM, n = 3 mice/arm, * P<0.01, ANOVA). (f) Apoptosis measured by cleaved caspase 3 (CC3) positive cells in the prostates of 9-month-old WT or *PTEN*$^{L/L}$ mice treated with RAD001 (10 mg/kg daily) or INK128 (1 mg/kg daily) (mean ± SEM, n = 3 mice/arm, * P<0.01, ANOVA)(Left panel). Representative CC3 images (right panel). Scale bar = 25 μm.



Supplementary Figure 27. INK128 induces apoptosis in specific cancer cell lines and decreases primary prostate cancer volume *in vivo*. (a) Apoptosis in LNCaP (n = 3) and A498 (n = 2) cancer cells after treatment with rapamycin (50 nM), or INK128 (200 nM) for 48 hours (mean ± SEM, * P<0.001, ** P<0.05, ANOVA, n.s. = not statistically significant). (b) Percentage decrease in ventral and lateral prostate volume in 9-month-old *PTEN*$^{L/L}$ after a 28-day treatment with vehicle or INK128 (1mg/kg daily) measured by MRI (left panel)(mean ± SEM, n = 4 mice/arm, * P = 0.0008, t-test). Representative MRI images of the *PTEN*$^{L/L}$ ventral and lateral prostate on day 0 and day 28 of treatment with INK128 (right panel)(red dotted lines encircle the ventral and lateral prostate). (c) Additional images of prostate cancer invasion in the *PTEN*$^{L/L}$ prostate (14-month-old mouse).