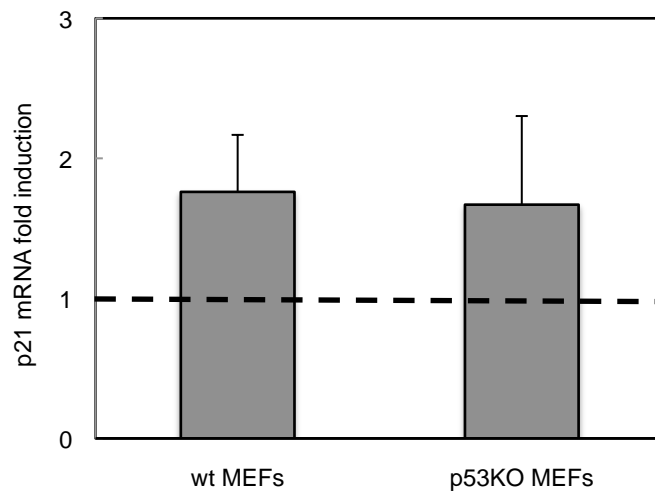


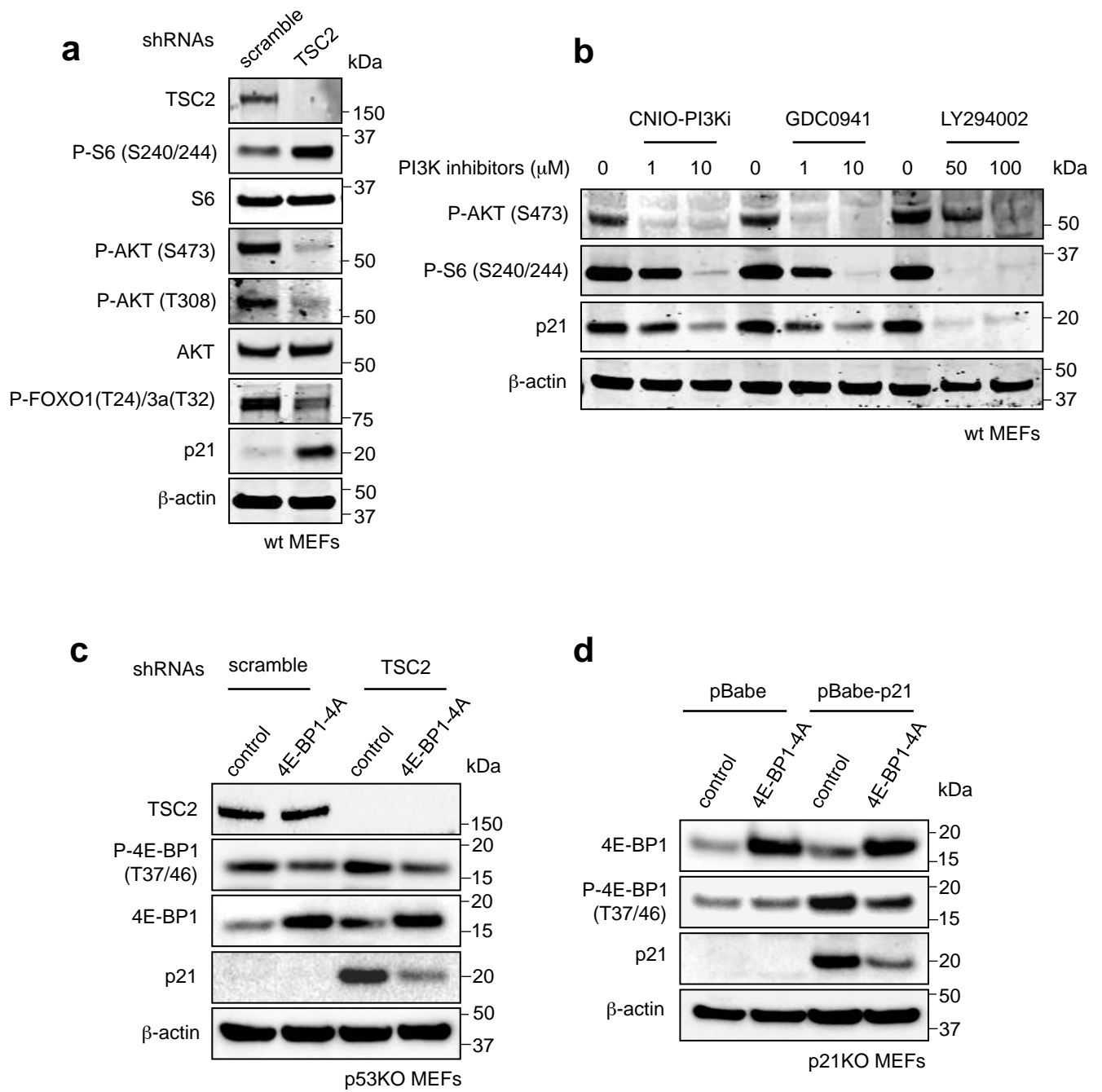
Supplementary Figure 1

- (a)** Western blot showing protein levels in wt MEFs infected with lentiviruses encoding either scramble or two different TSC2 shRNA sequences (TSC2-1 and TSC2-2).
- (b)** Western blot analysis of protein levels in wt MEFs following UVC irradiation ($1\text{J} / \text{cm}^2$) or TSC2-depletion by lentiviral infection. UVC-irradiated cells were lysed 30 min after irradiation.
- (c)** Western blot analysis of protein levels in wt MEFs treated with Doxorubicin ($1\ \mu\text{M} / 6$ hours) or depleted of TSC2 by lentiviral infection.
- (d)** Western blot depicting the impact of TSC2 depletion on p21 and p16 protein levels in both wt and p53KO MEFs. Two different TSC2 shRNA sequences (TSC2-1 and TSC2-2) were used to knock-down the endogenous protein.
- (e)** Representative images illustrating β -galactosidase activity in MEFs infected with either scramble or TSC2 shRNA-encoding lentiviruses. Scale bar $100\ \mu\text{m}$.

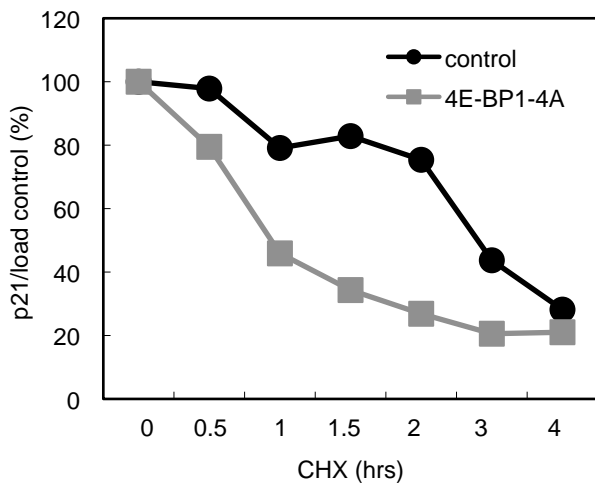
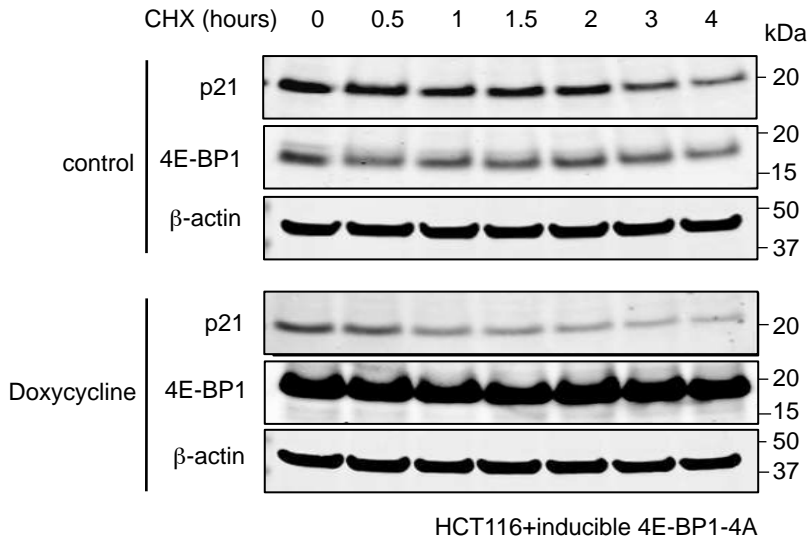
For each panel, all the Western blots correspond to samples from the same experiment; in some cases, samples were distributed in several electrophoretic gels run in parallel.

**Supplementary Figure 2**

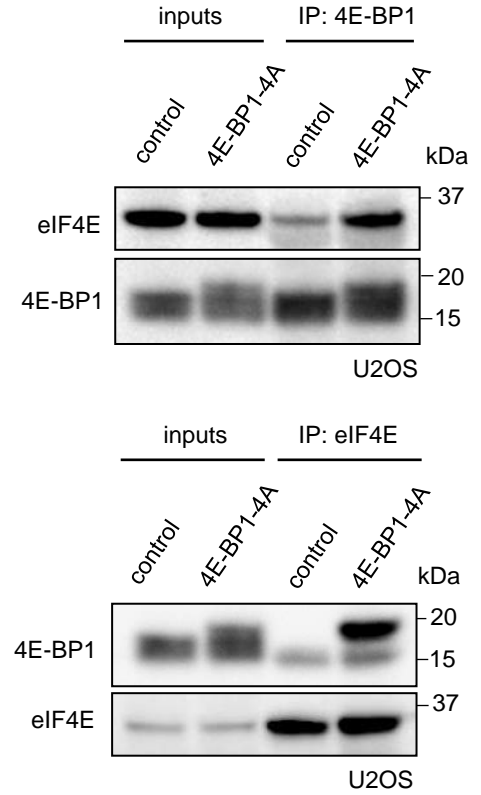
qRT-PCR measurement of *p21* mRNA levels in wt and p53KO MEFs depleted of TSC2 by infection with TSC2 shRNAs lentiviruses. Fold changes in *p21* mRNA are relative to scramble shRNA-infected cells. The graph represents the average of four independent infections \pm SD (n=4). No significance was found by statistical t-test analysis.



e



f

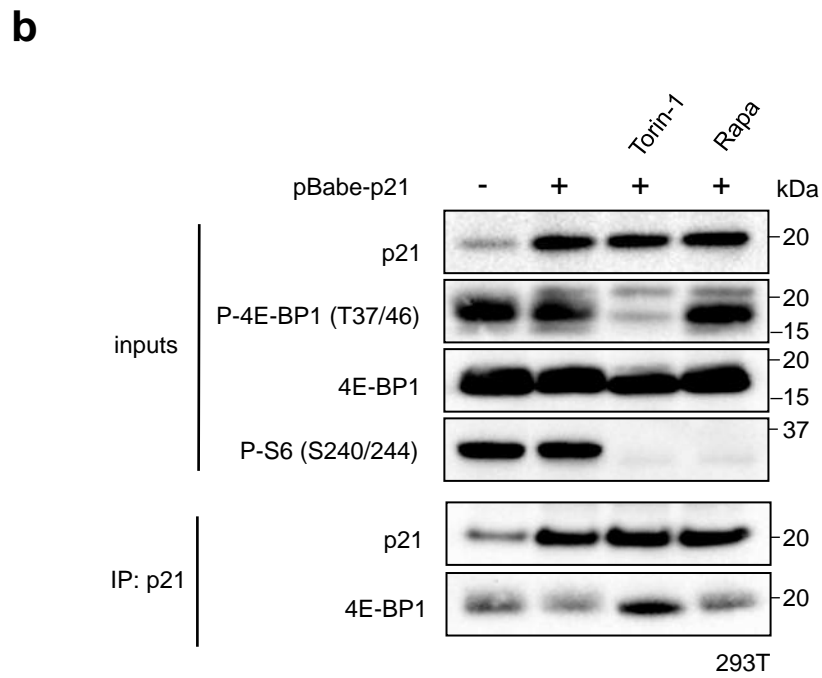
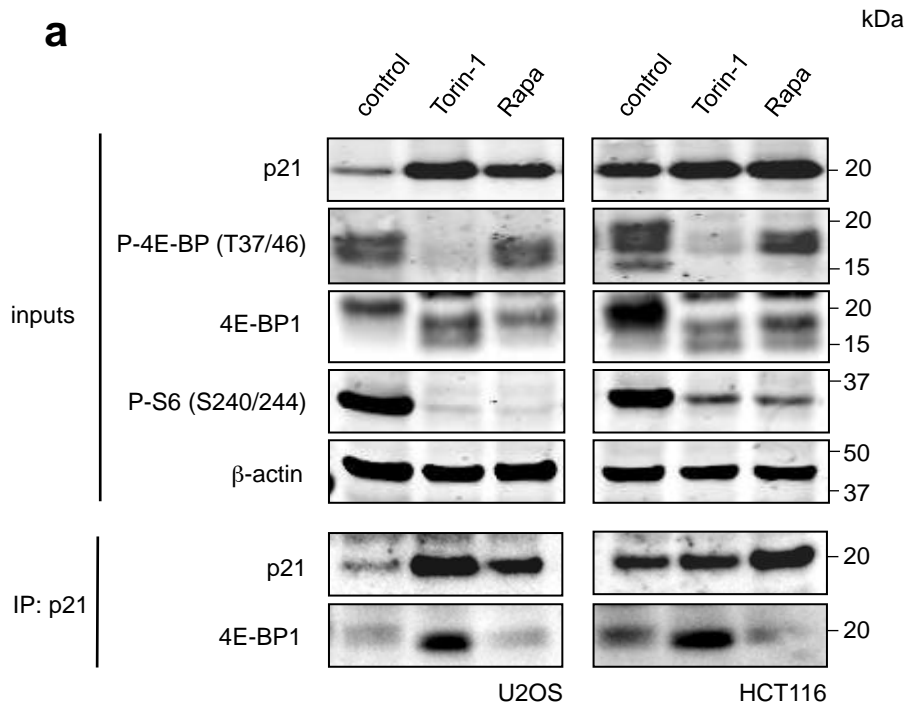


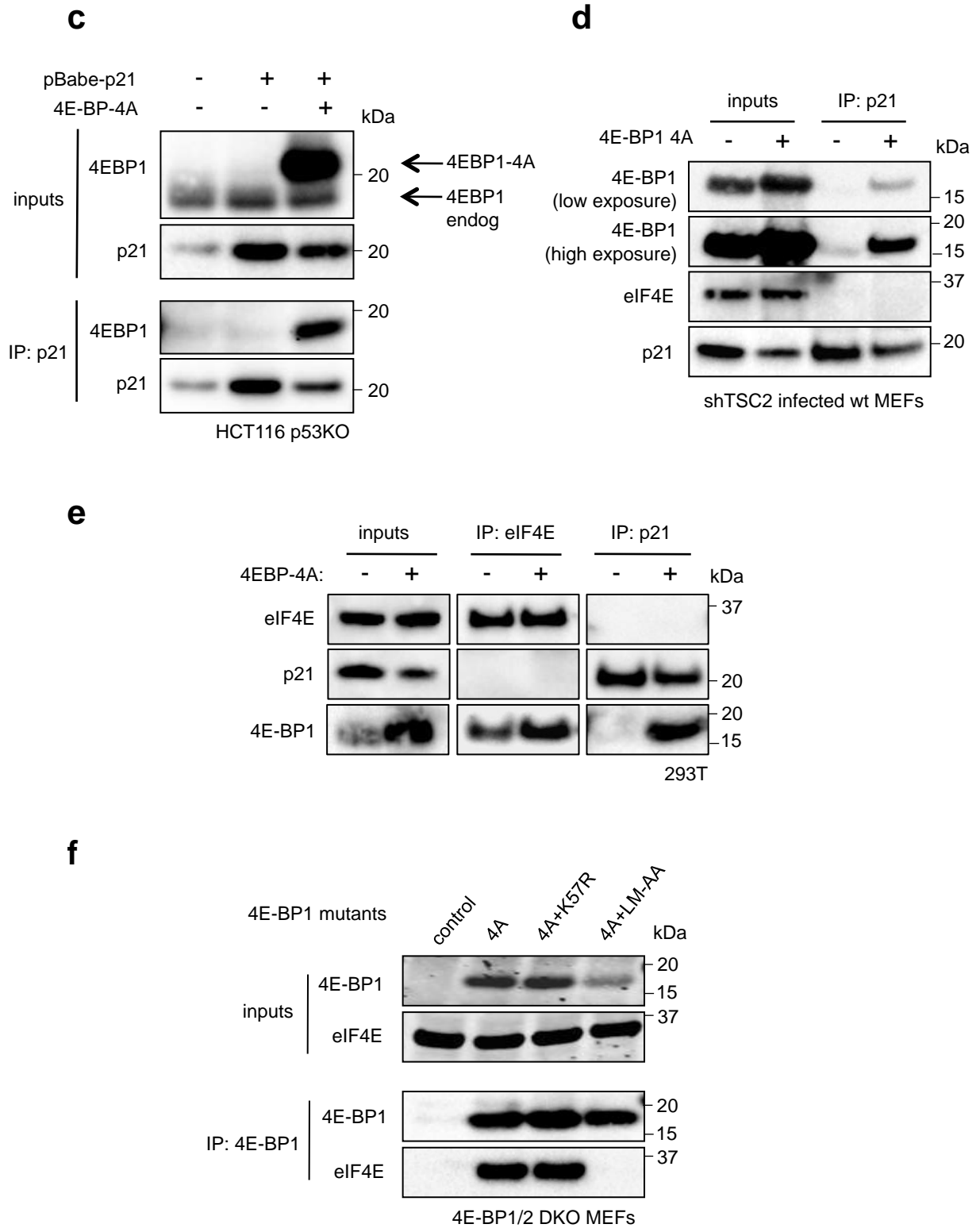
Supplementary Figure 3

- (a) Western blot showing the phosphorylation status of protein kinases belonging to the PI3K/AKT/mTORC1 pathway in MEFs infected with either control or TSC2 shRNA-encoding lentiviruses.
- (b) Western blot illustrating the effect of PI3K inhibitors on p21 protein levels in MEFs. Cells were treated for 24 hours with the indicated concentrations of the different drugs.
- (c) p53KO MEFs were simultaneously infected with either scramble or TSC2 shRNAs and either control or 4E-BP1-4A-encoding lentiviruses. After selection, protein levels were analyzed by western blot.
- (d) p21KO MEFs were infected with mouse p21, 4E-BP1-4A or their respectively control-encoding viruses. After selection protein levels were analyzed by western blot.
- (e) Control and doxycycline-treated (24 hours) 4E-BP-4A-inducible HCT116 cells were incubated with 10 μ M cyclohexamide (CHX) for the indicated times and, subsequently, p21 and 4E-BP1 levels were analyzed by Western blot (top). The densitometric quantification of p21 levels corrected by loading control is shown (bottom).
- (f) U2OS cells were transfected with control or 4E-BP1-4A-expressing plasmid. After 48 hours, cell lysates were immunoprecipitated with either anti-4E-BP1 (top) or anti-eIF4E antibodies (bottom). Inputs and co-immunoprecipitated proteins were analyzed by Western blot.

For each panel, all the Western blots correspond to samples from the same experiment; in some cases, samples were distributed in several electrophoretic gels run in parallel.

Supplementary Fig. 4 (part 1 out of 2)

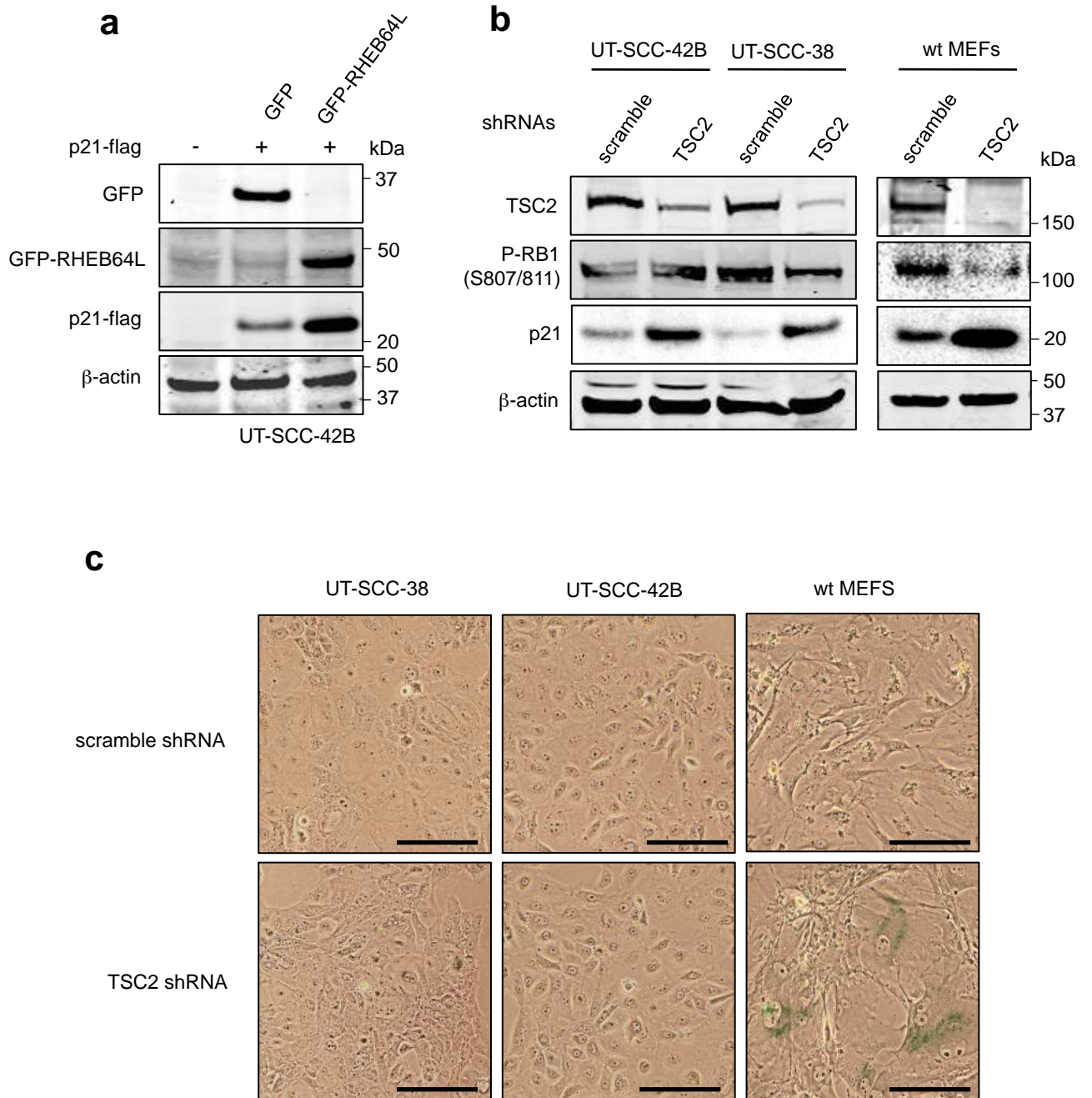




Supplementary Figure 4

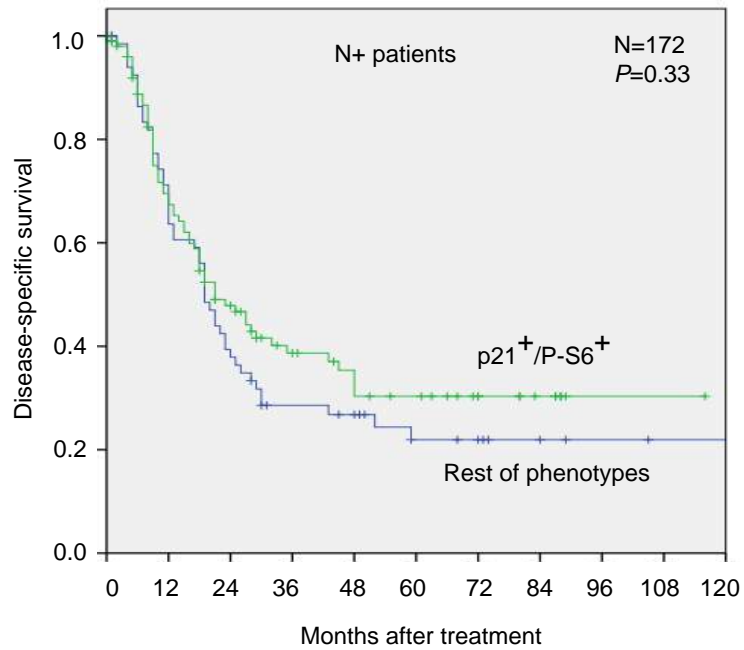
- (a)** U2OS and HCT116 cells were treated with Torin-1 (250 nM) or Rapamycin (Rapa; 100 nM) for 5 hours in the presence of the proteasomal inhibitor MG132. Cell lysates were immunoprecipitated with anti-p21 antibody and resolved by Western blot.
- (b)** 293T cells were transfected with either control or pBabe-p21 (human p21) plasmids and, 24 hours later, incubated with either torin-1 or rapamycin (Rapa) for 5 hours in the presence of the proteasomal inhibitor MG132. Following p21 immunoprecipitation, inputs and interacting proteins were analyzed by Western blot.
- (c)** p53KO HCT116 cells were co-transfected with human p21 and 4E-BP1-4A-expressing plasmids. Following 5 hours incubation with the proteasomal inhibitor MG132, p21 was immunoprecipitated and, subsequently, inputs and interacting proteins were analyzed by Western blot.
- (d)** MEFs were infected with TSC2 shRNAs together with either control or 4E-BP1-4A-encoding lentiviruses. Cells were incubated with the proteasomal inhibitor MG132 for 5 hours prior protein extraction.
- (e)** 293T cells were transfected with control or 4E-BP1-4A-encoding plasmids. Cell lysates were immunoprecipitated with either anti-eIF4E or anti-p21 antibodies and interacting proteins were resolved by Western blot.
- (f)** 4E-BP1/1 DKO MEFs were infected with the indicated 4E-BP1 mutant-encoding lentiviruses. Cell lysates were immunoprecipitated with anti-4E-BP1 antibodies and analyzed by Western blot.

For each panel, all the Western blots correspond to samples from the same experiment; in some cases, samples were distributed in several electrophoretic gels run in parallel.



Supplementary Figure 5

- (a) UT-SCC-42B cells were co-transfected with human p21-flag and either control, GFP or GFP-Rheb64L-encoding plasmids. Protein expression was analyzed by Western blot.
- (b) HNSCC cell lines (UT-SCC-38 and UT-SCC-42B) and wt MEFs were infected with either scramble or TSC2 shRNA-encoding lentiviruses. The phosphorylation levels of RB1 protein was analyzed by Western blot.
- (c) Representative images illustrating β -galactosidase activity in HNSCC cell lines infected with either scramble or TSC2 shRNA-encoding lentiviruses. Scale bar 100 μ m.
- (d) Graphic depiction of the mutations found in the genomic sequences of p53 in the HNSCC cell lines. UT-SCC-2 has point mutation in codon 275 which results in the change Cys>Phe. This is a known deleterious mutation (see IARC TP53 Database at <http://p53.iarc.fr>). UT-SCC-38 has a point mutation in the conserved splice donor site (intron position +5) of intron 4. Mutations in this sequence are known to result in a variety of alternatively spliced mRNAs encoding non-functional truncated proteins. UT-SCC-42B has a point mutation in codon 331, which results in a stop codon.
- (e) Western blot illustrating the effect of the Mdm2 inhibitor Nutlin3 on the levels of p21 and p53 proteins in different HNSCC cell lines. The human osteosarcoma cell line U2OS (wt p53) was included as a positive control.



Supplementary Figure 6

Kaplan-Meier disease-specific survival curves of patients with regional lymph node involvement at the time of diagnosis (N+) categorized by p21 and P-S6 (240/244) (n=172; $P=0.33$; log-rank test).

Supplementary Table 1. Cohort characteristics

Characteristics	Case no. (n=274)	%
Tumor localization		
Oropharynx	141	51
Larynx	65	24
Hypopharynx	68	25
Age		
>60	123	45
<60	151	55
Sex		
Male	262	96
Female	12	4
Tobacco smokers	264	96
Moderate	164	60
Heavy	100	37
Alcohol consumption	241	88
TNM stage		
I	18	7
II	14	5
III	42	15
IV	200	73
Differentiation		
W/D	100	37
M/D	110	40
P/D	64	23
pT classification		
T1	32	12
T2	48	17
T3	90	33
T4	104	38
LN metastasis		
Negative (N0)	56	20
Positive (N1-3)	172	63
HPV infection		
Negative	274	100
Positive	0	0

Supplementary Table 2. p53 and p21 expression in HNSCC

	p53 positive	p53 negative	<i>P</i> [†]
p21 positive	100	55	0.294
p21 negative	43	32	

[†]Fisher Exact test (two sided), *P*=0.312

Supplementary Table 3. P-S6 and p21 expression in HNSCC

	P-S6 positive	P-S6 negative	<i>P</i> [†]
p21 positive	143	11	<0.001
p21 negative	21	53	

[†]Fisher Exact test (two sided), *P*<0.001

Supplementary Table 4. Cox regression table

Parameter	HR (95% CI)	<i>P</i>
Localization (larynx)	0.86 (0.54-1.37)	0.52
T classification (T3-T4)	1.2 (0.79-1.81)	0.37
N classification (N+)	2.78 (1.7-4.56)	<0.001
Pathological grade (G3)	1.1 (0.87-1.4)	0.41
p21/P-S6 phenotype (+/+)	0.74 (0.52-1.05)	0.95

Supplementary Table 5. p21 and Ki67 expression in HNSCC

Ki67	0-25%	25-50%	51-75%	>75%	total
p21 positive	14	17	26	8	65
p21 negative	6	9	10	4	29
total	20	26	36	12	94

†Fisher Exact test (two sided), P=0.946

Supplementary Table 6. p21+/P-S6+ expression and tumour recurrence in HNSCC

	p21+/P-S6+	Rest of patients	P[†]
Recurrence	80	62	0.011
No-recurrence	63	23	

†Fisher Exact test (two sided), P=0.011

Supplementary Table 7. p21+/P-S6+ expression and distant metastasis

	p21+/P-S6+	Rest of patients	P[†]
Distant metastasis	46	43	<0.01
Absence of distant metastasis	97	42	

†Fisher Exact test (two sided), P=0.008

Supplementary Table 8. p21+/P-S6+ expression and tumor recurrence in N0 patients

	p21+/P-S6+	Rest of patients	<i>P</i> [†]
Recurrence	16	10	0.15
No-recurrence	24	6	

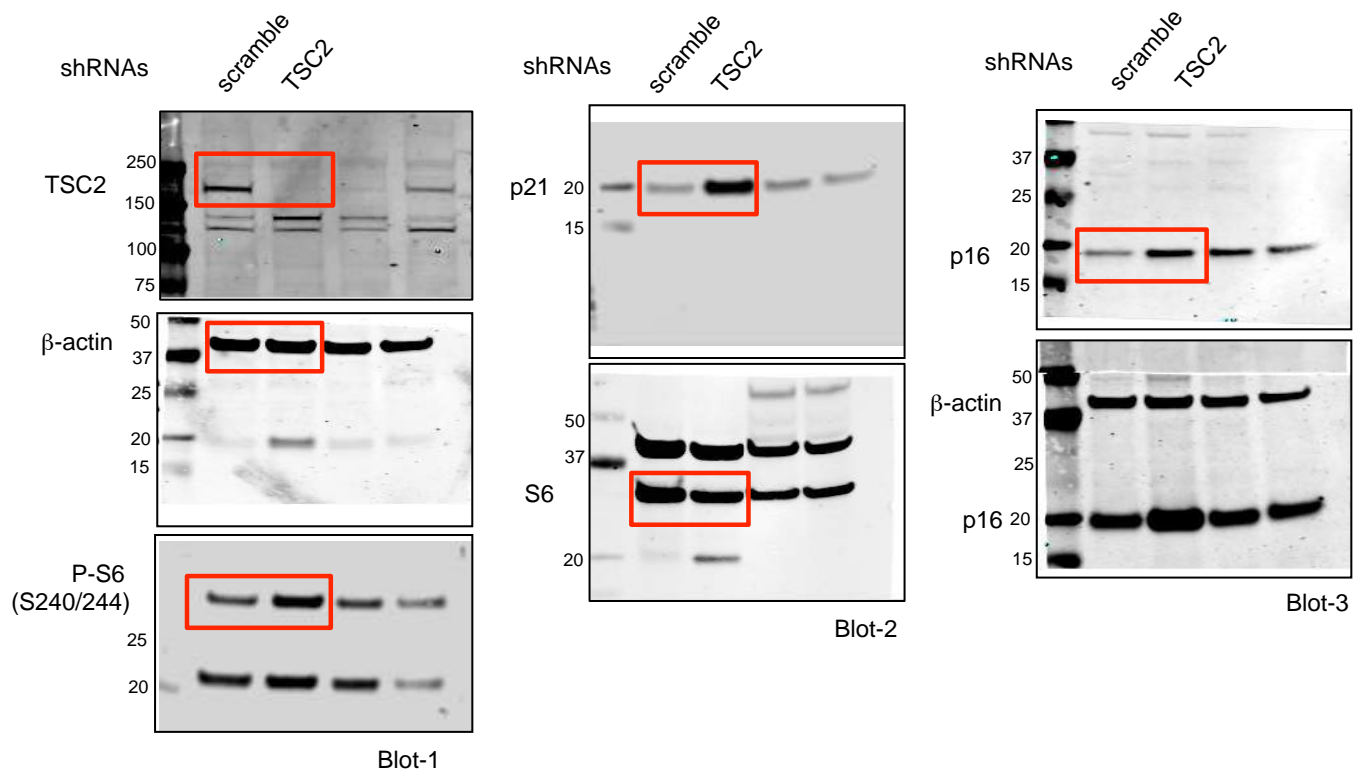
[†]Fisher Exact test (two sided), P=0.149

Supplementary Table 9. p21+/P-S6+ expression and distant metastasis in N0 patients

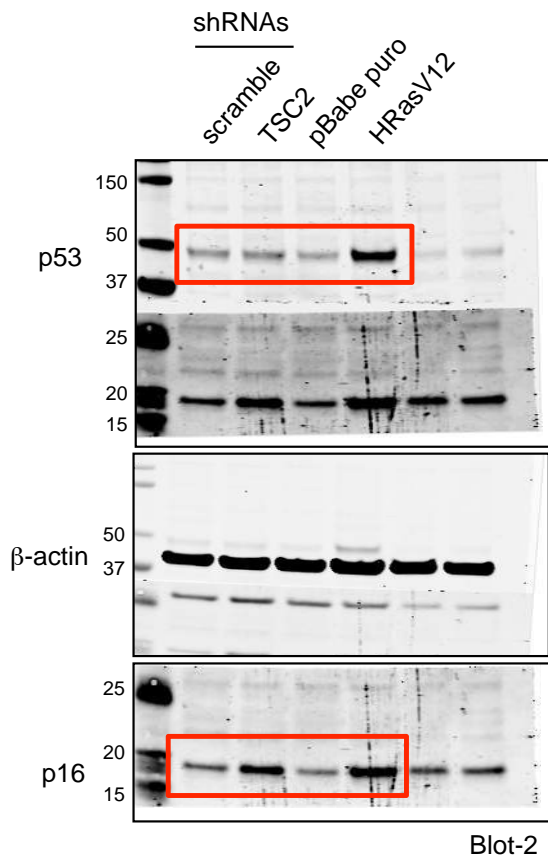
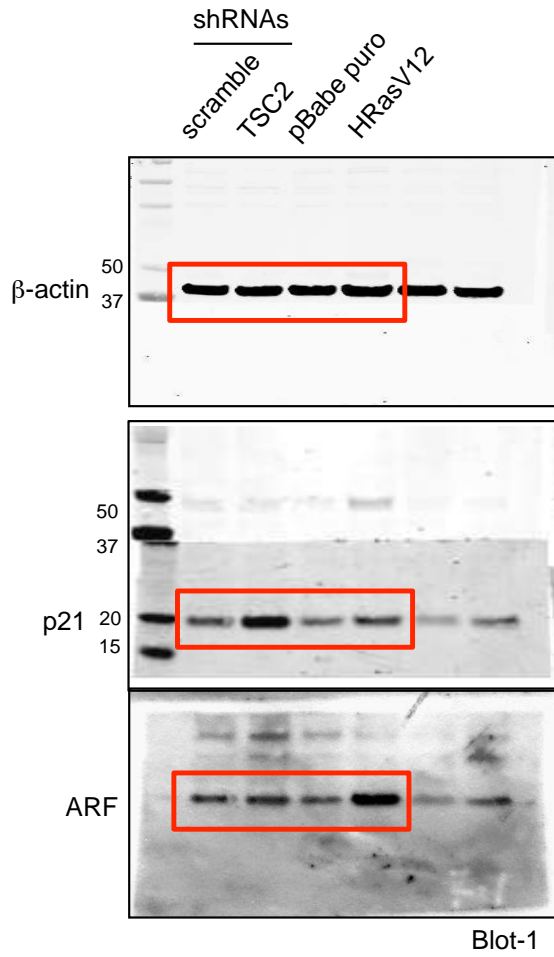
	p21+/P-S6+	Rest of patients	<i>P</i> [†]
Distant metastasis	8	6	0.19
Absence of distant metastasis	32	10	

[†]Fisher Exact test (two sided), P=0.189

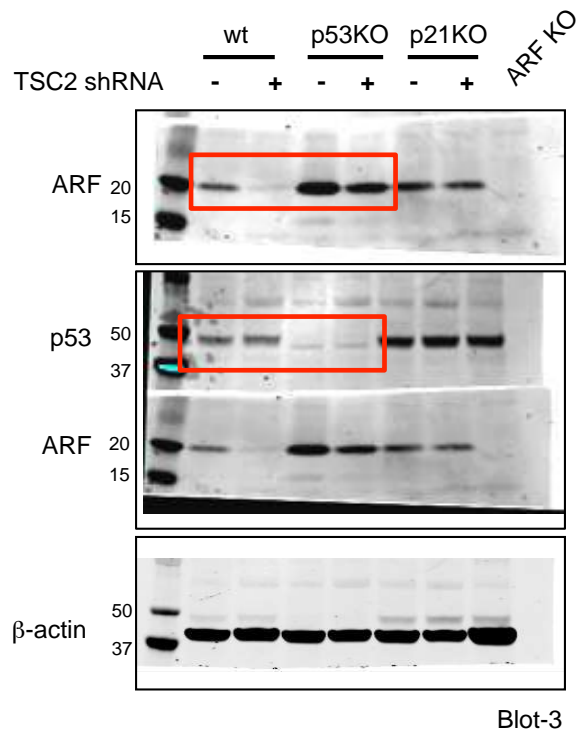
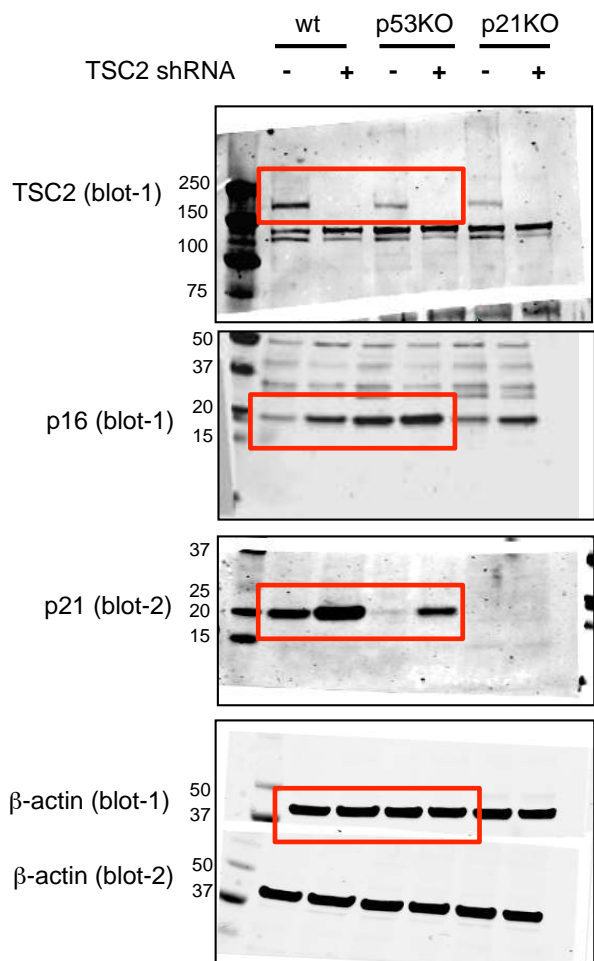
Supplementary Note 1



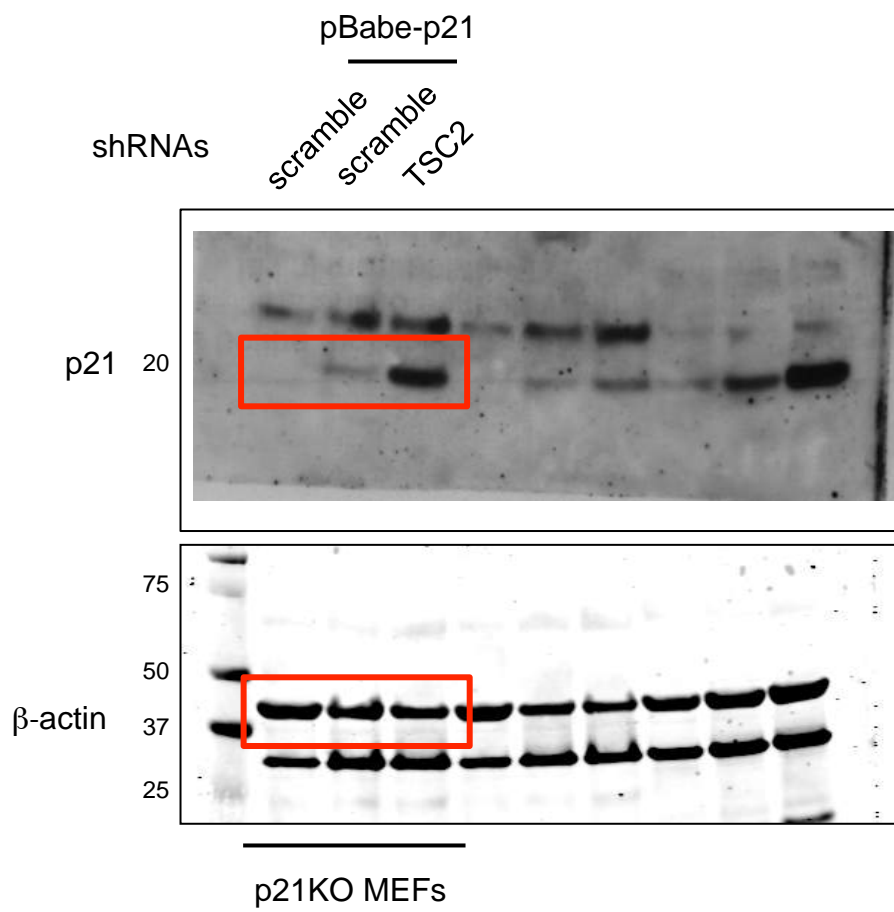
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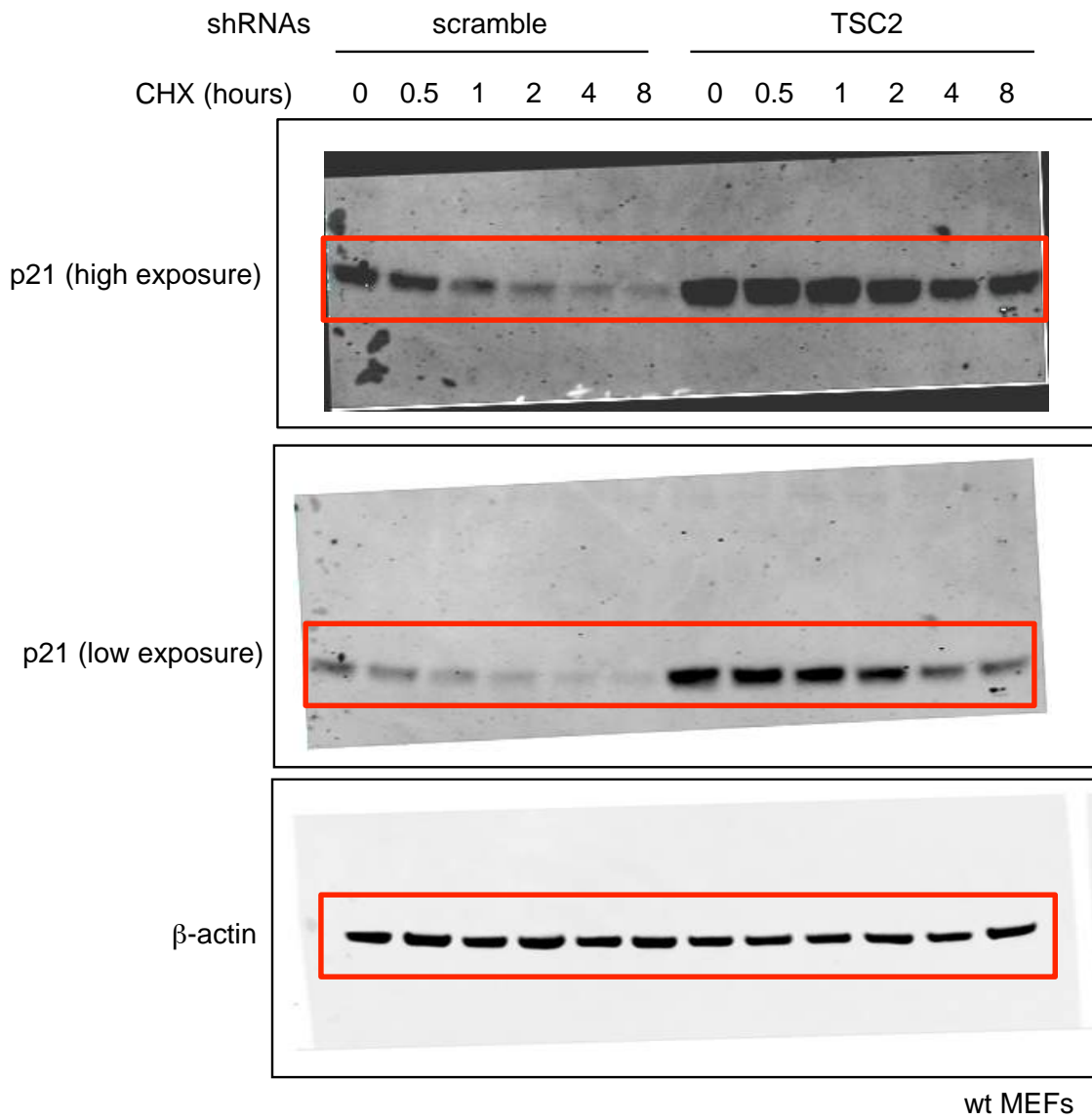
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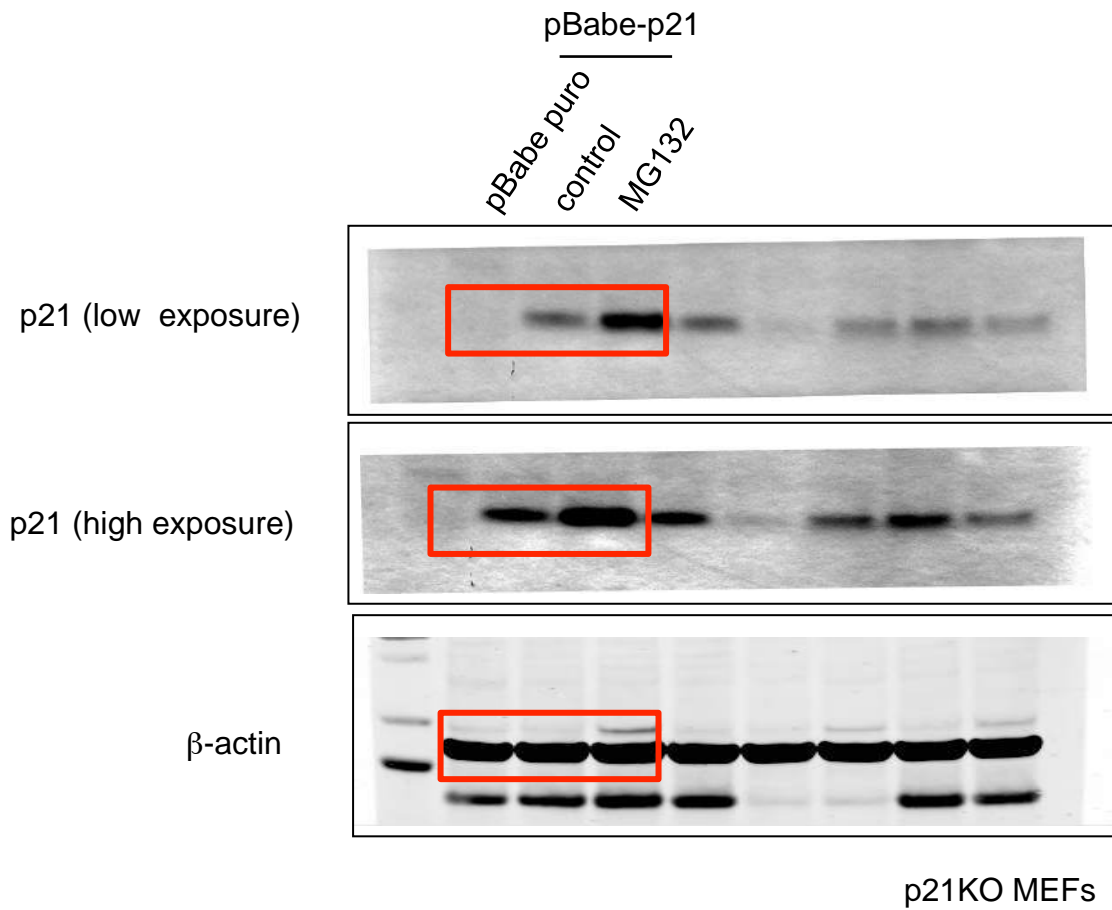
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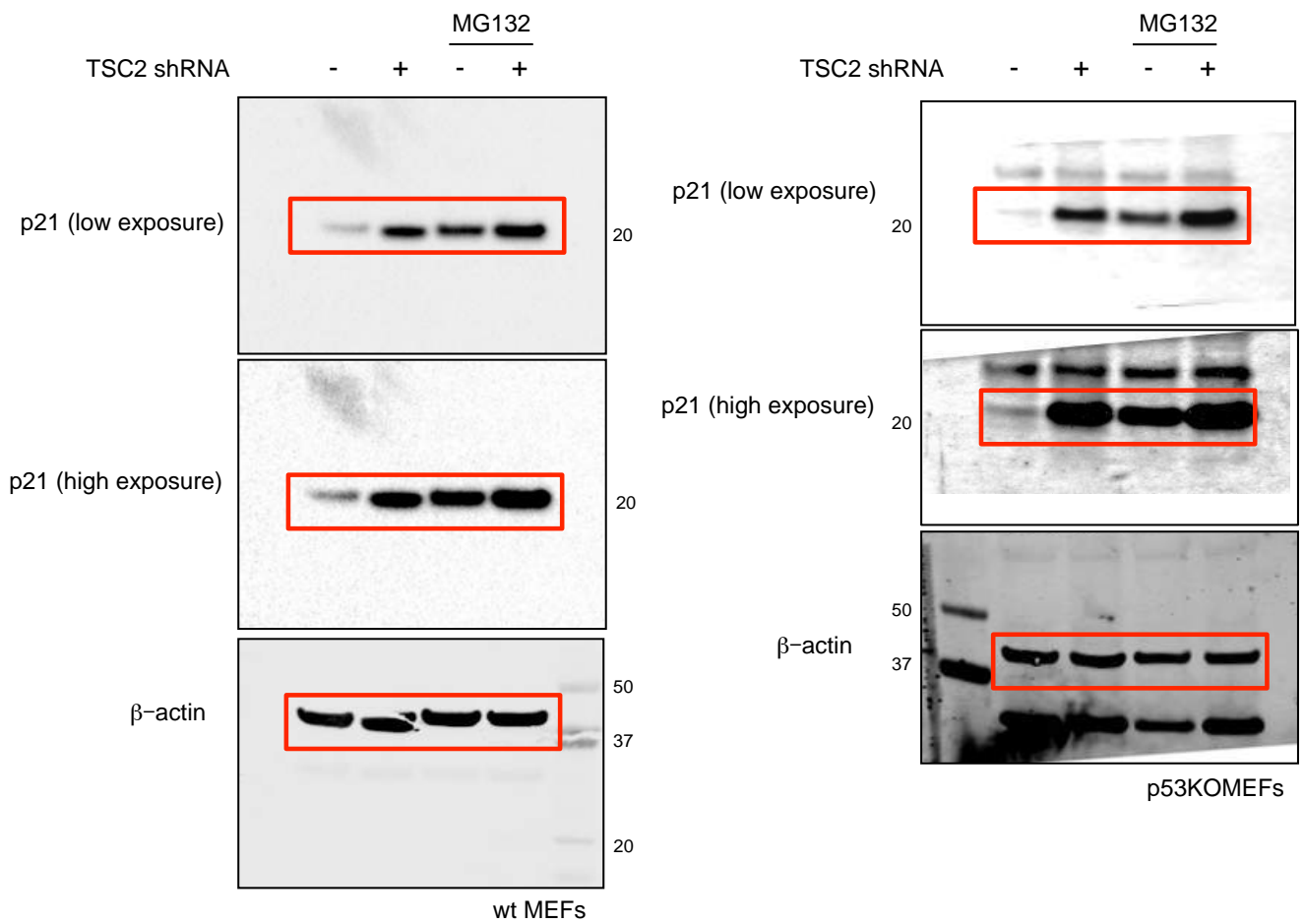
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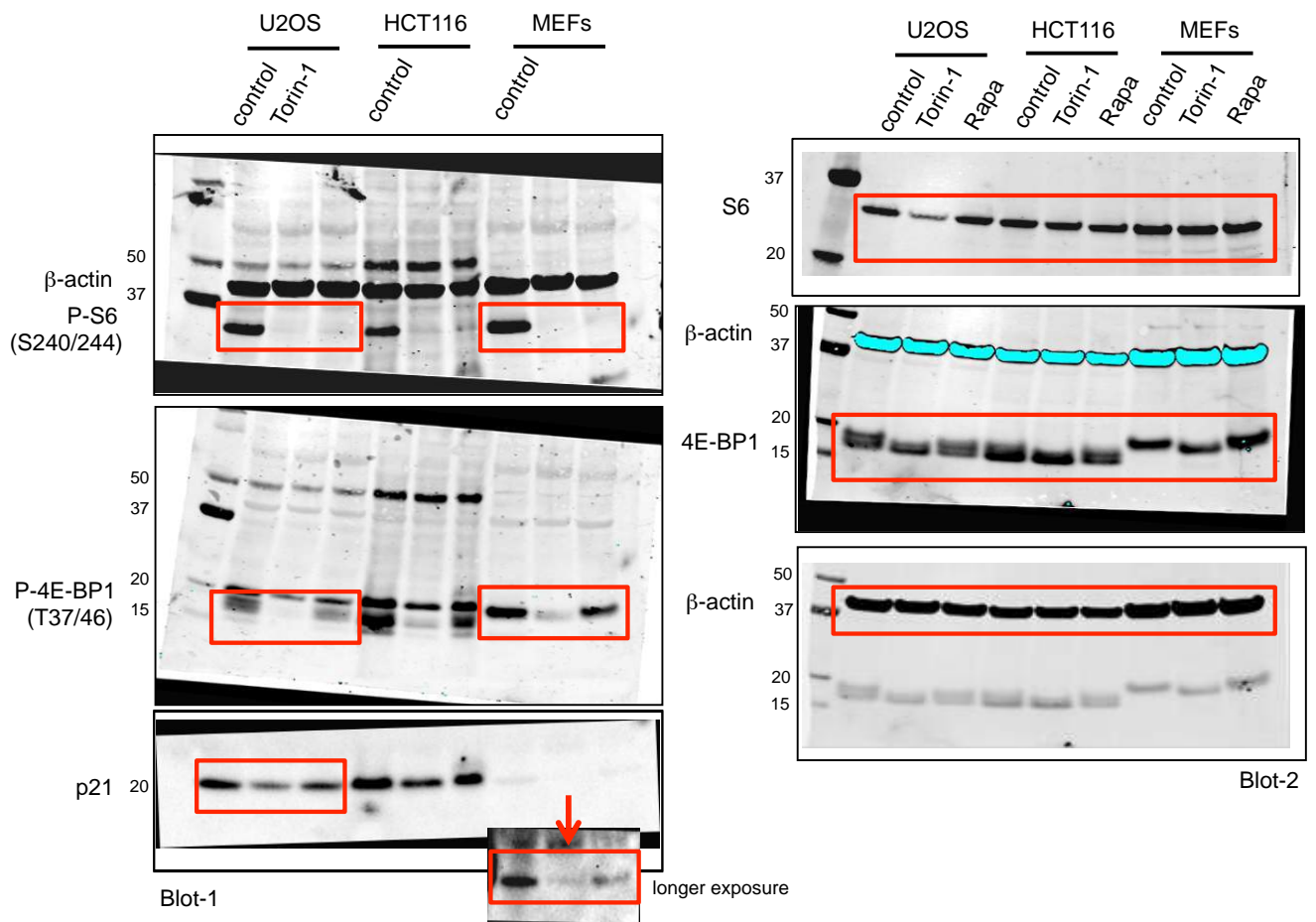
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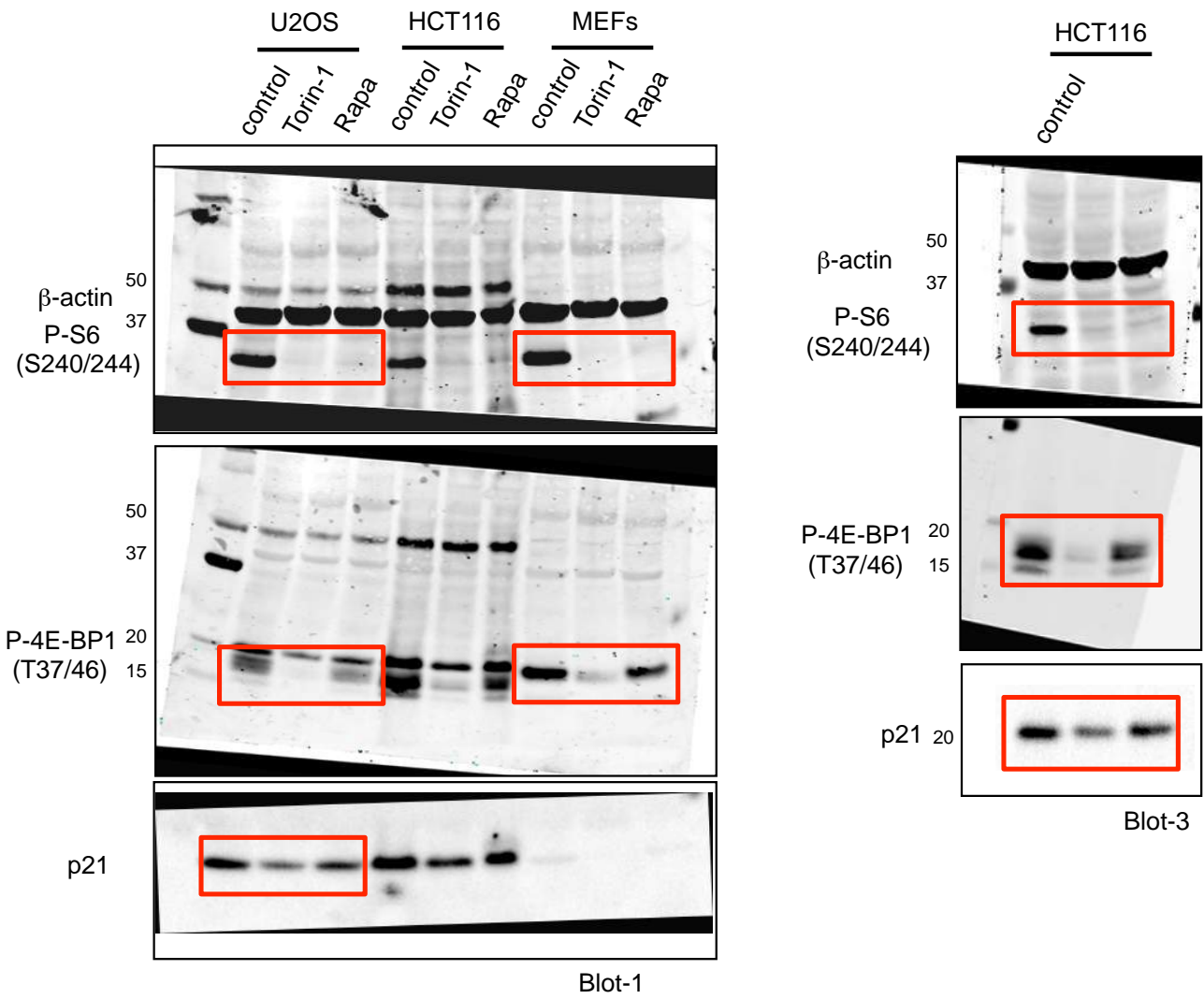
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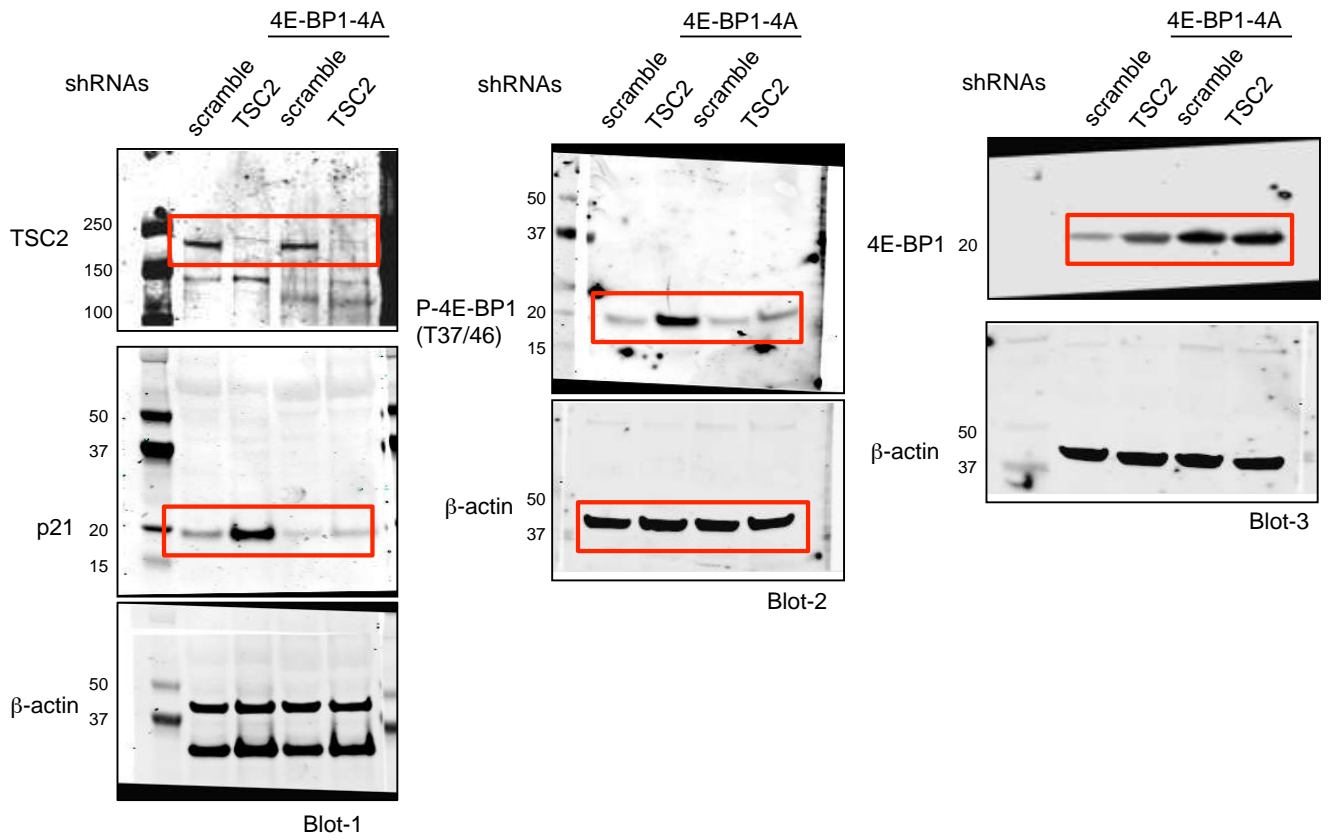
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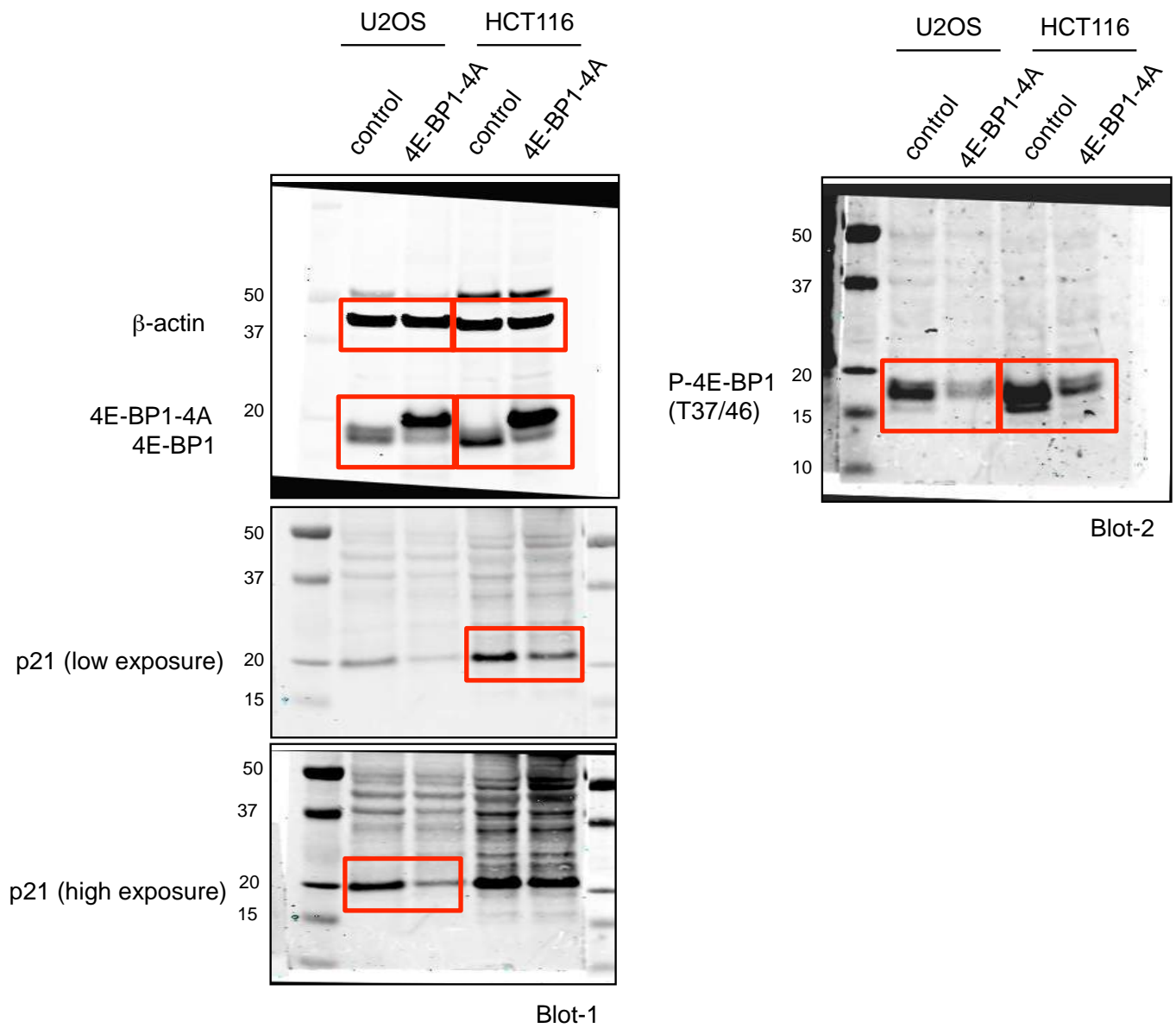
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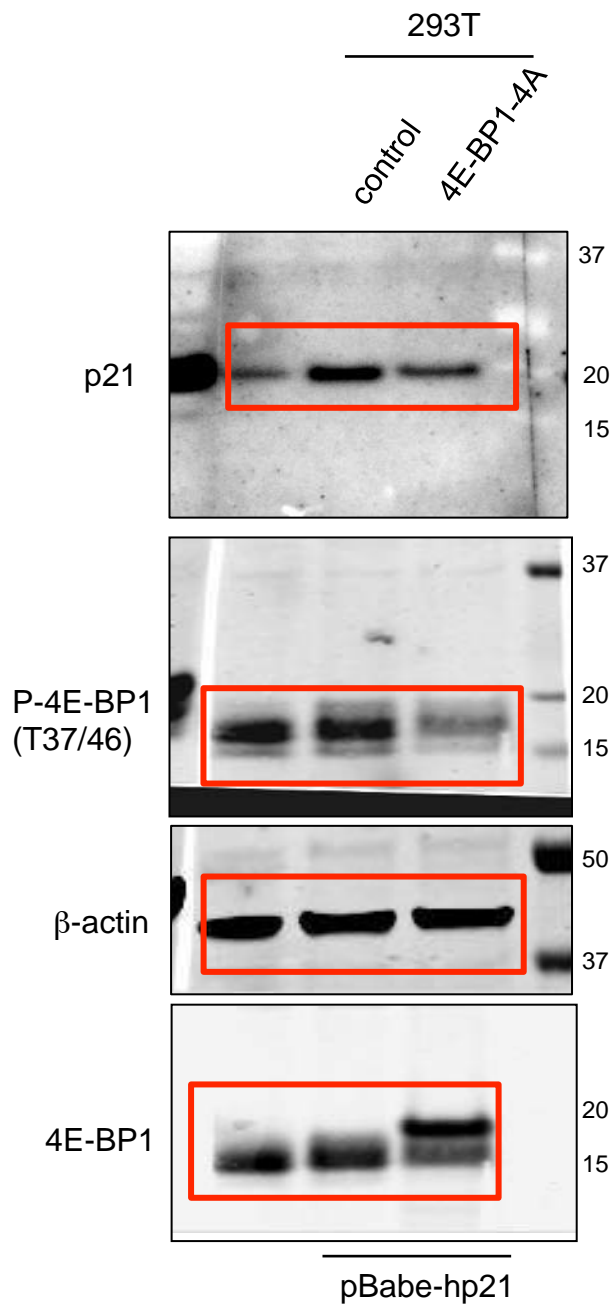
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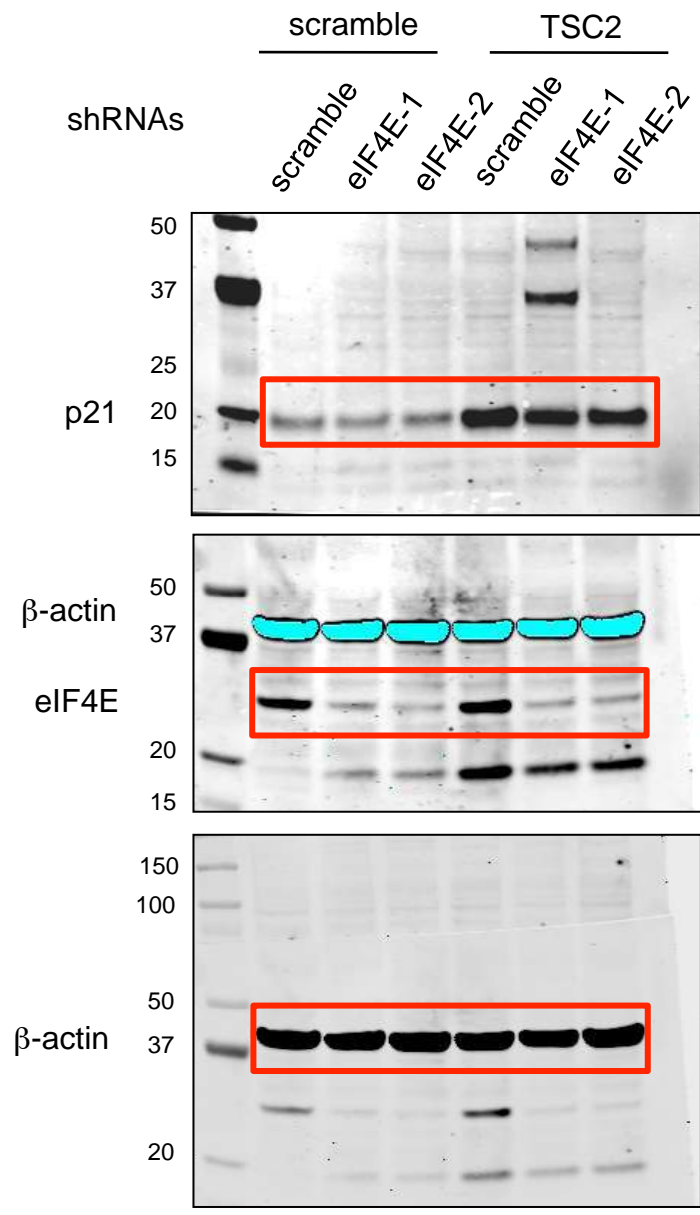
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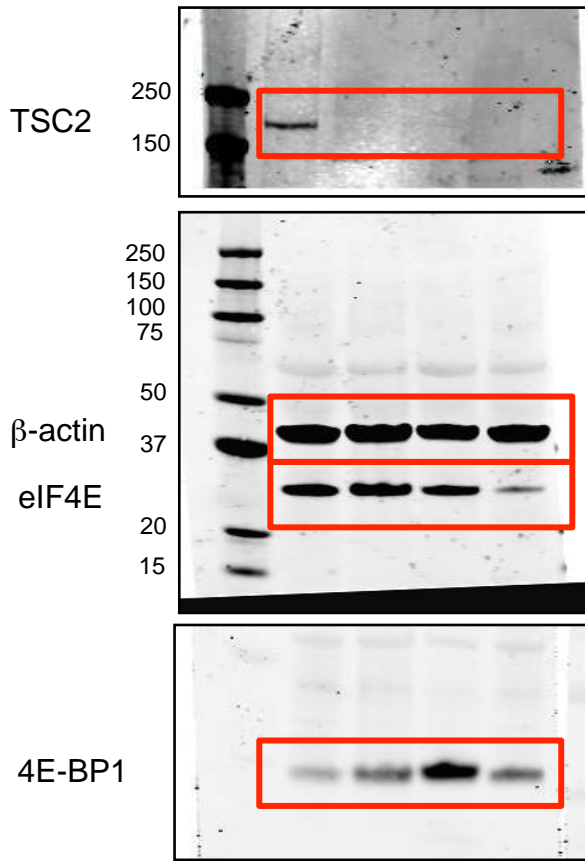


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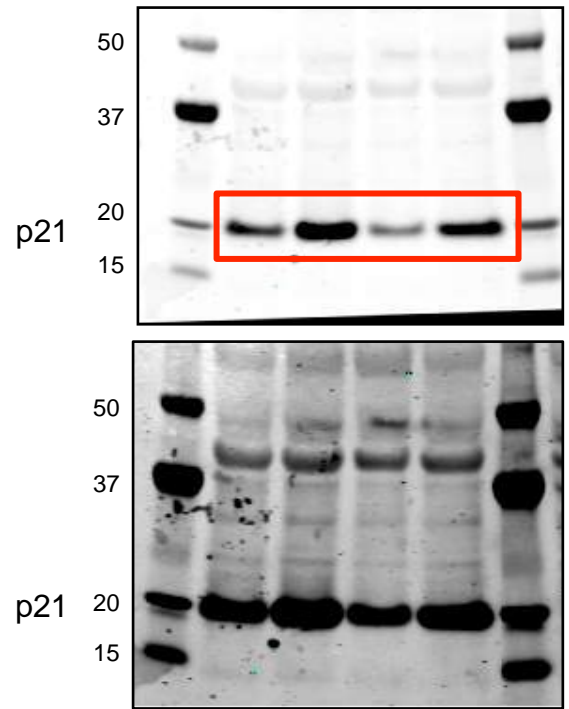
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4E-BP1 4A	-	-	+	-
eIF4E shRNA	-	-	-	+
TSC2 shRNA	-	+	+	+



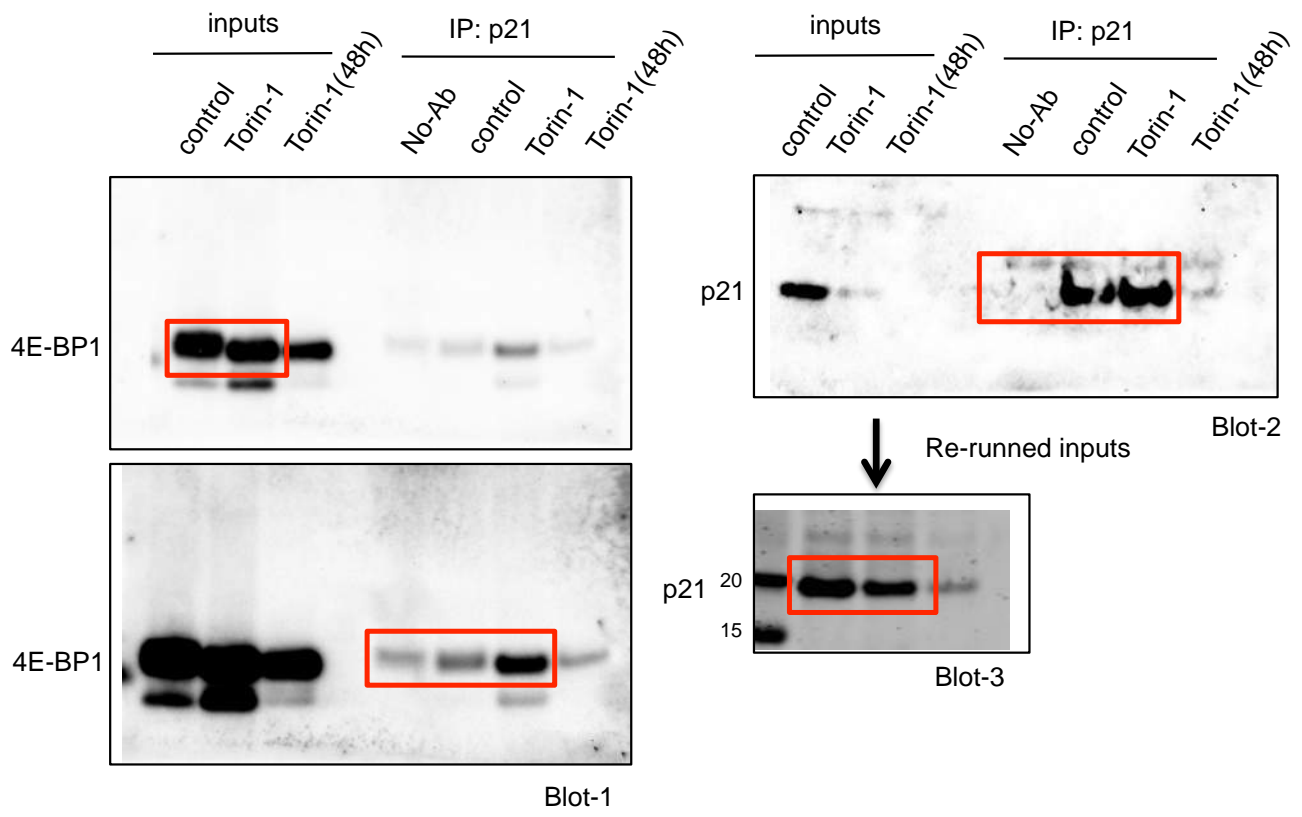
Blot-1

4E-BP1 4A	-	-	+	-
eIF4E shRNA	-	-	-	+
TSC2 shRNA	-	+	+	+

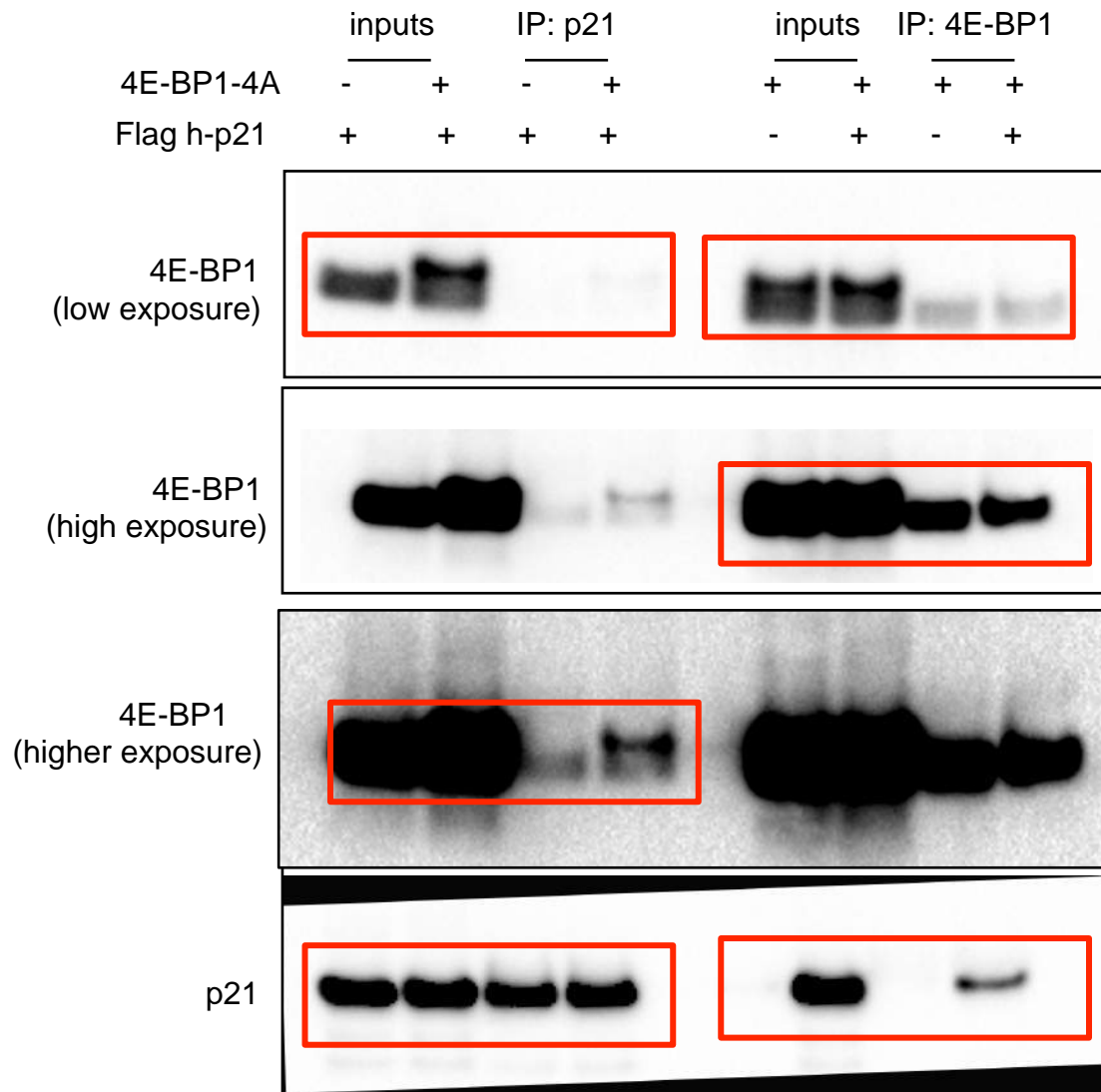


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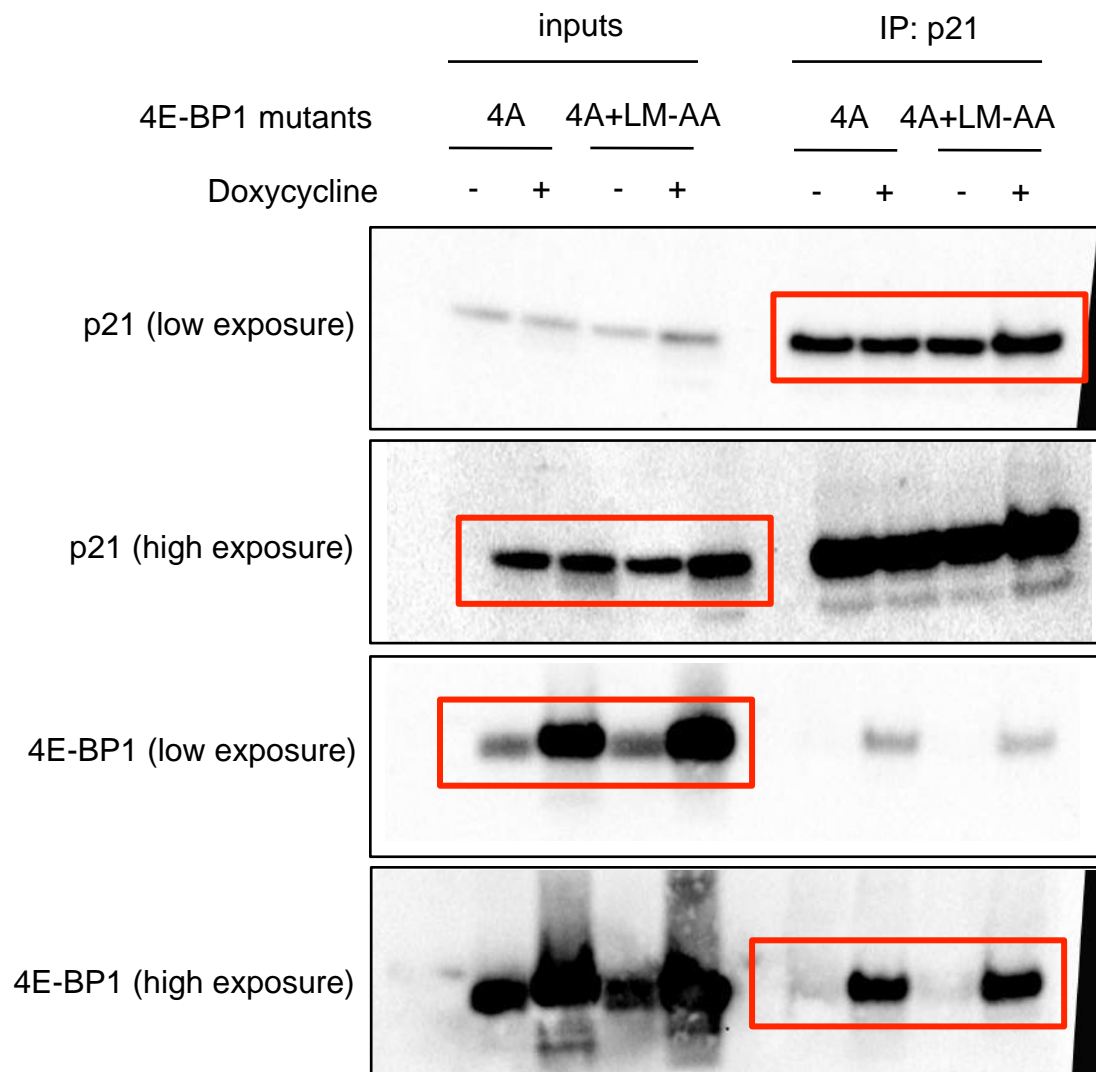
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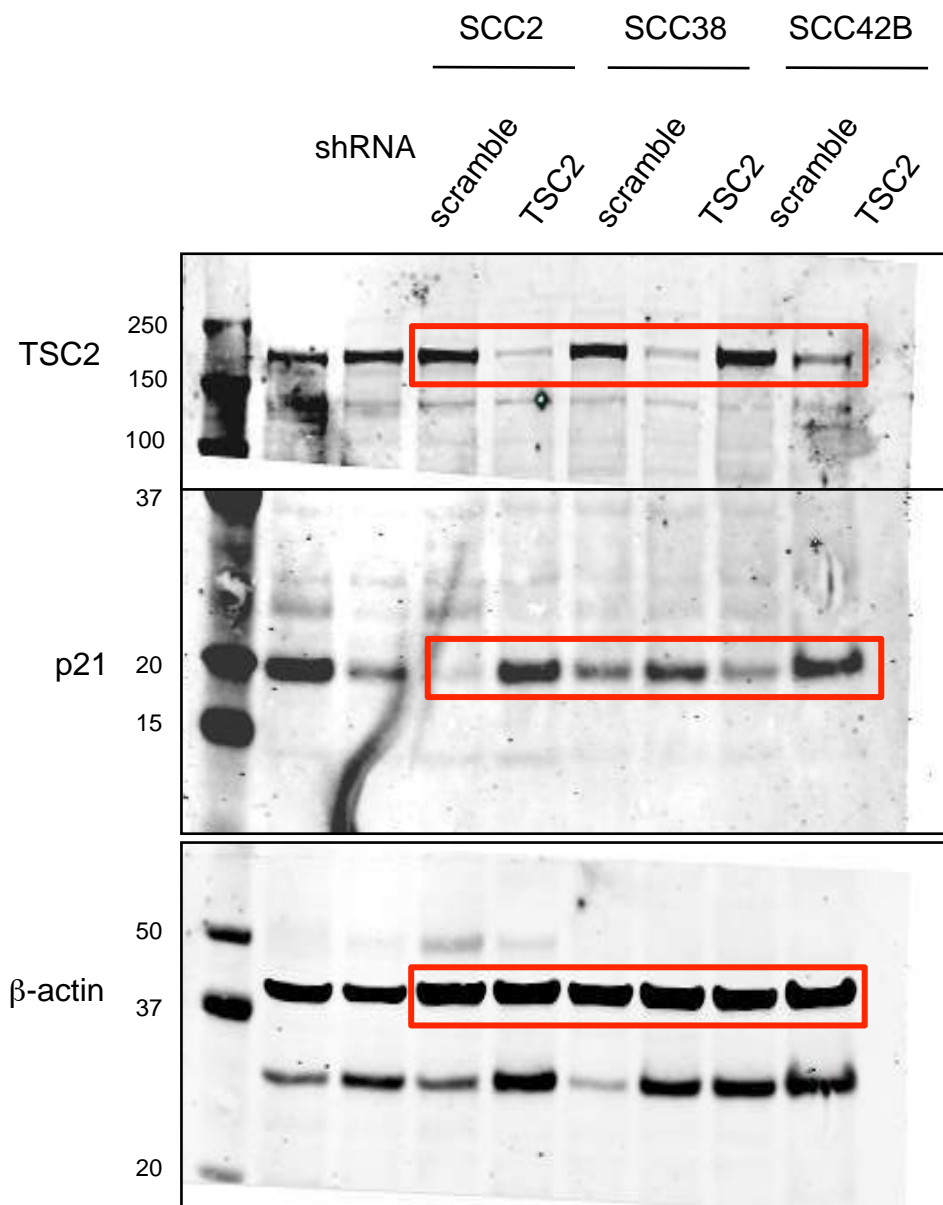
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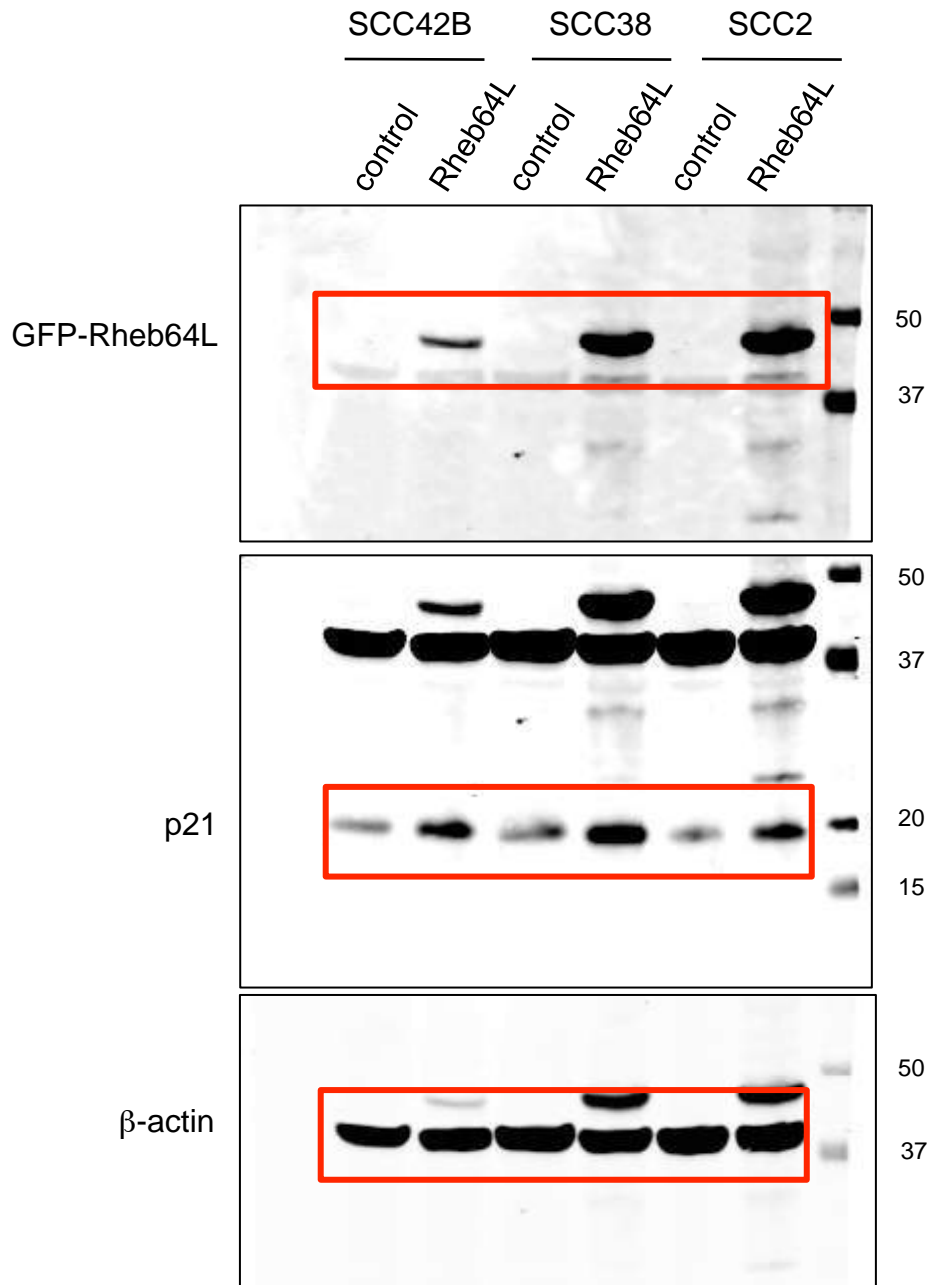
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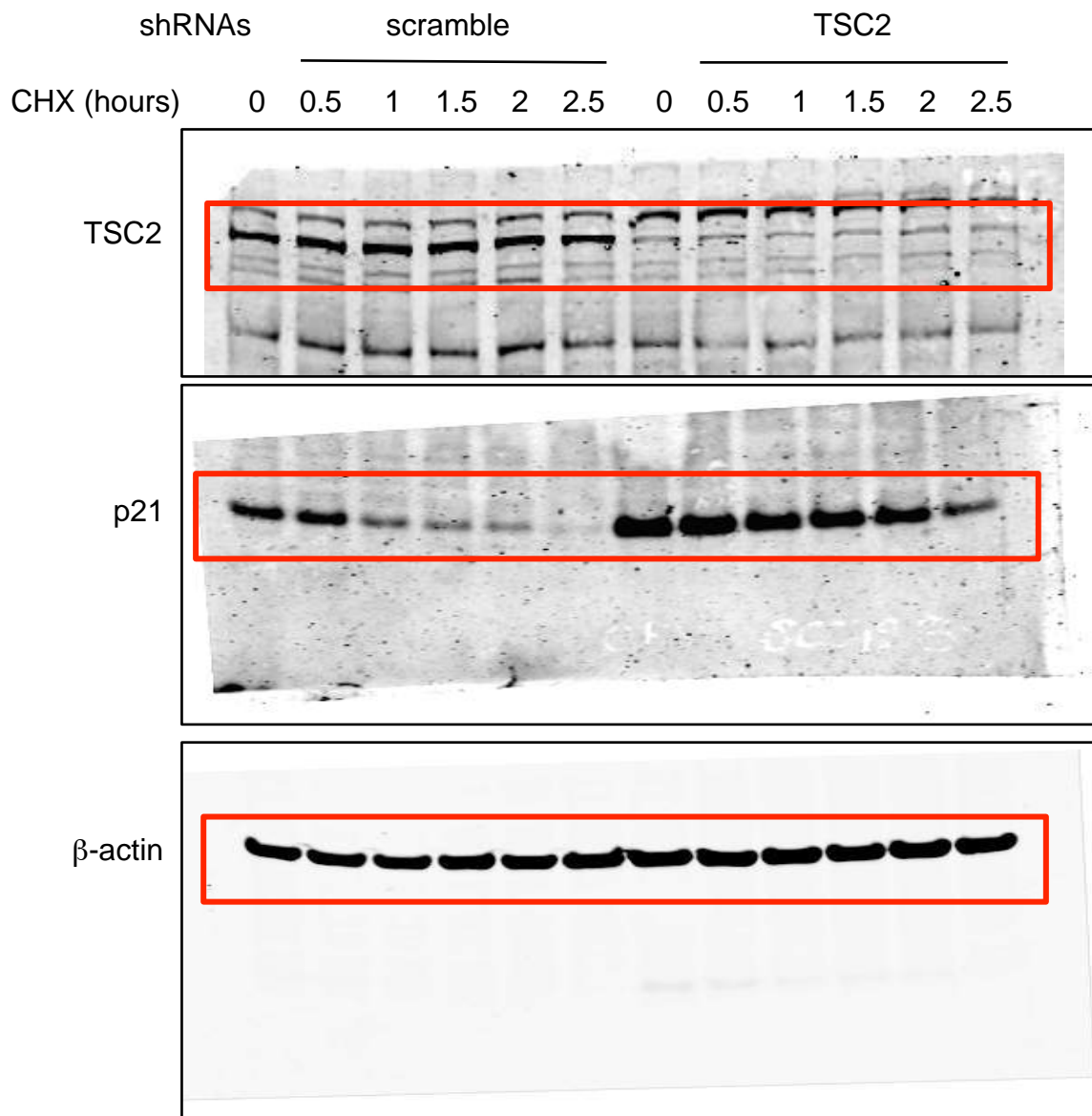
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Uncropped membranes for Figure 5c

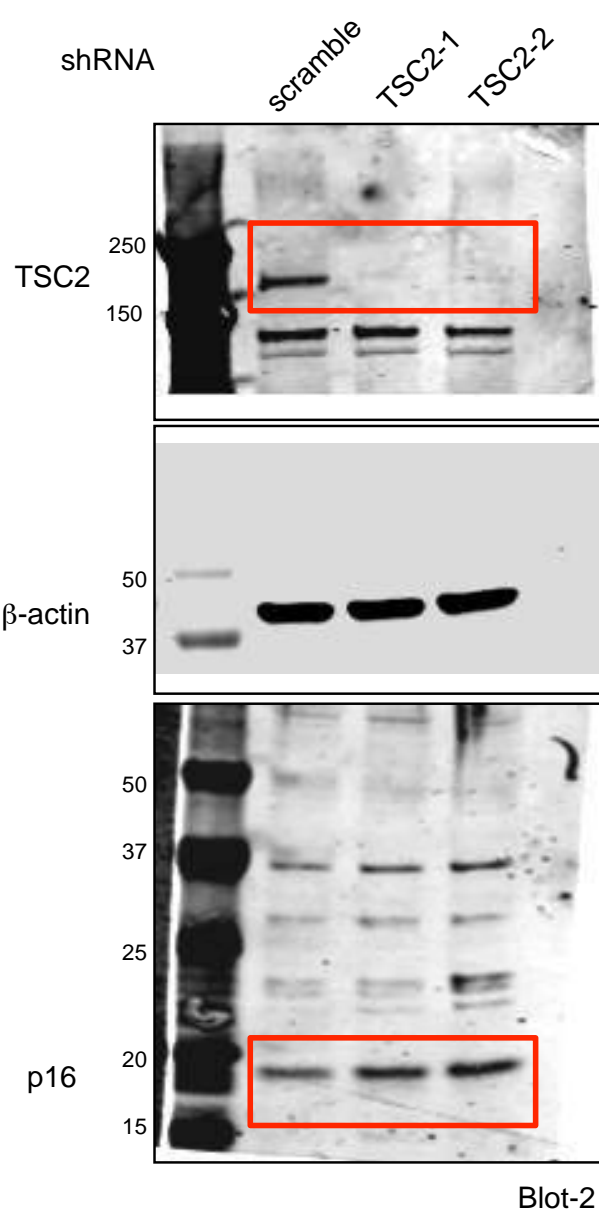
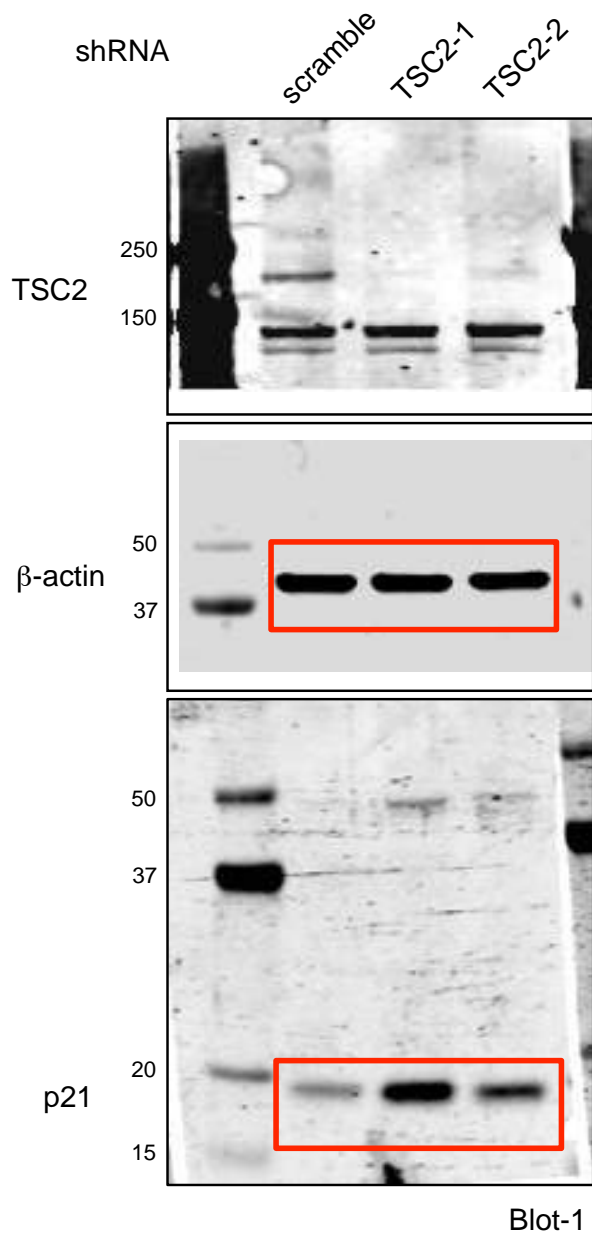


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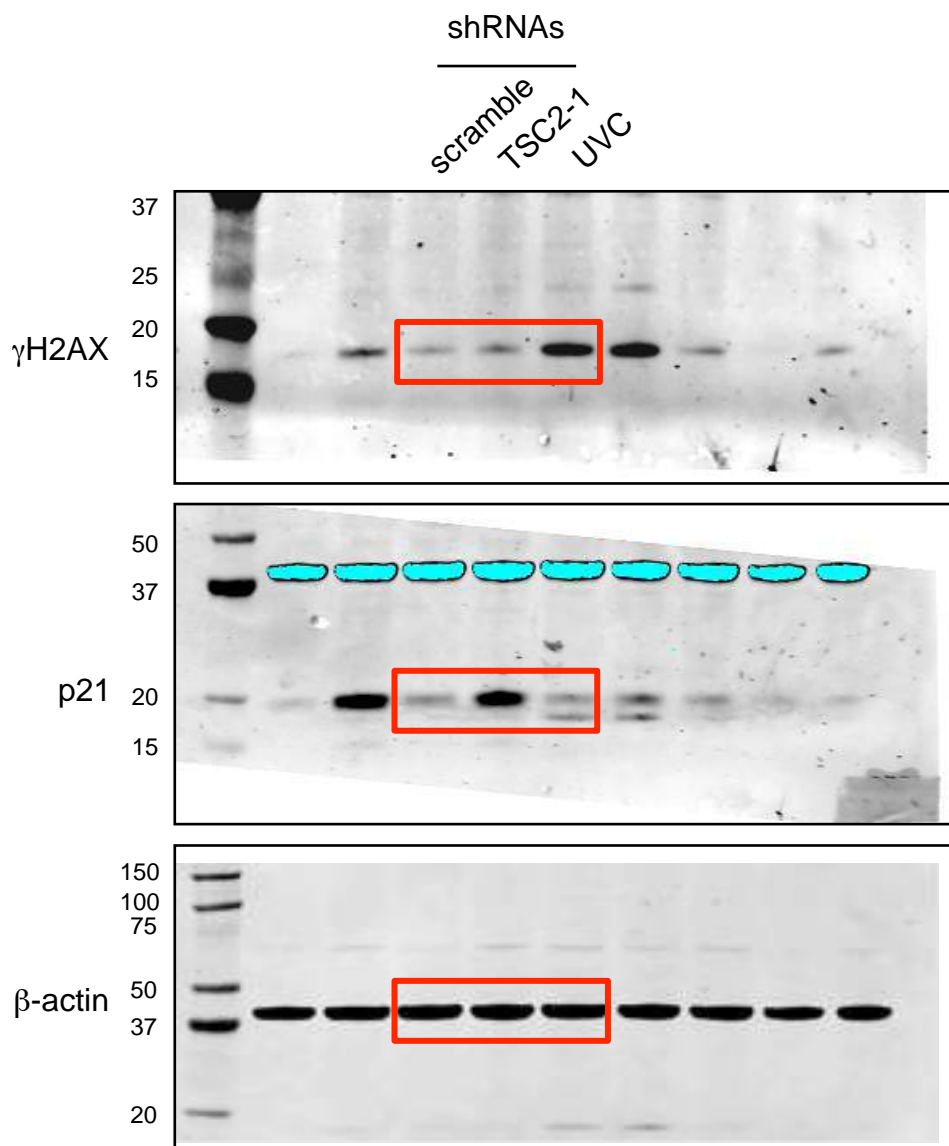


SCC42B

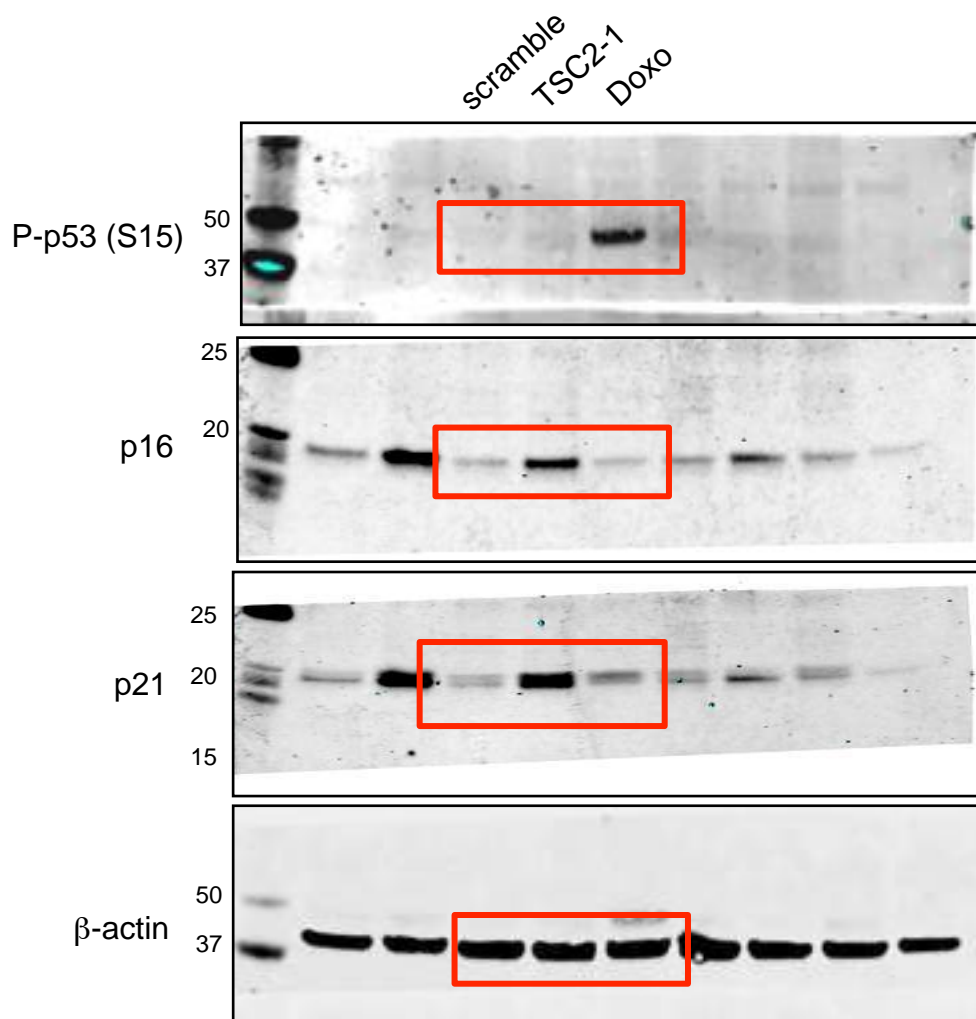
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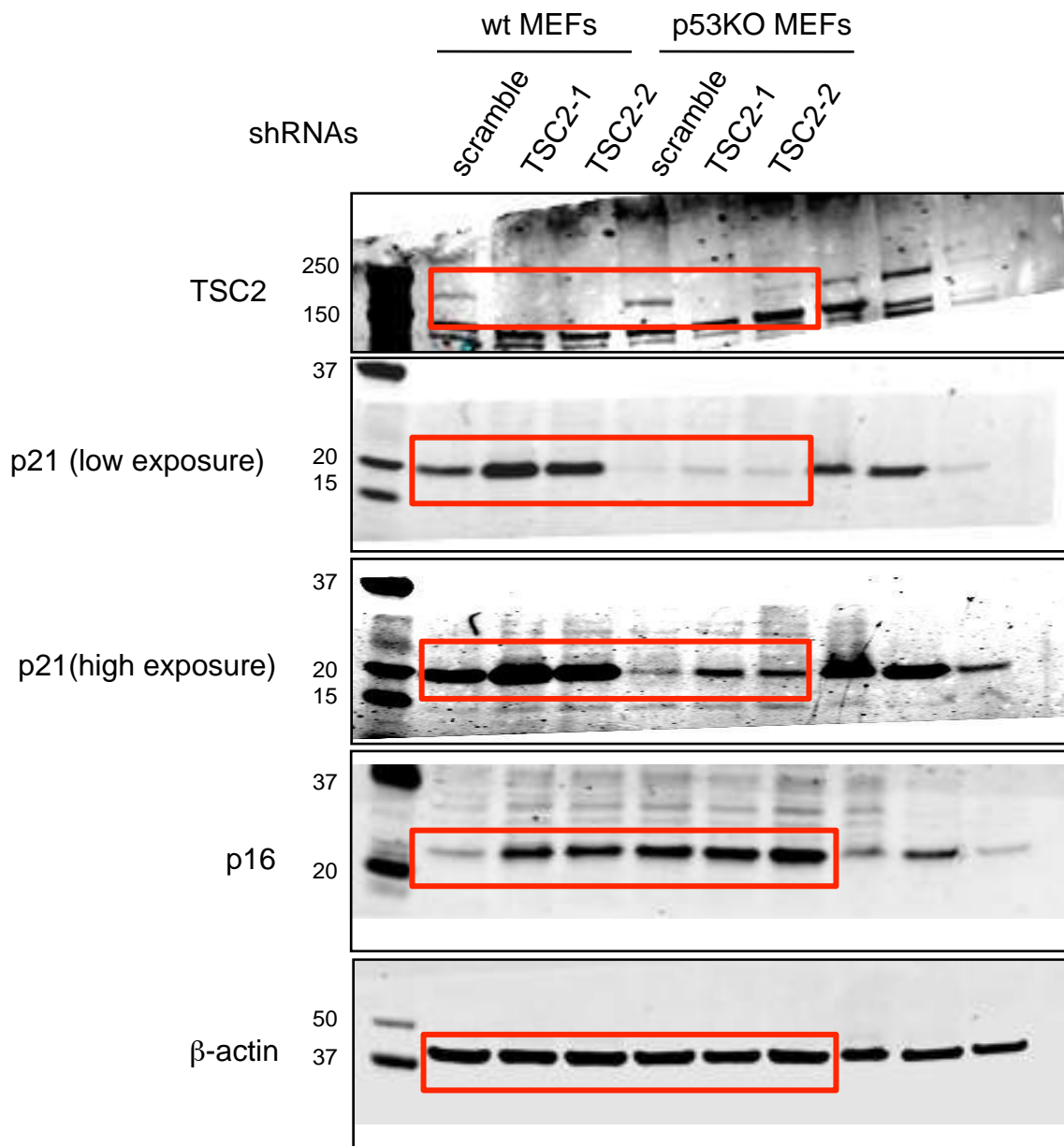
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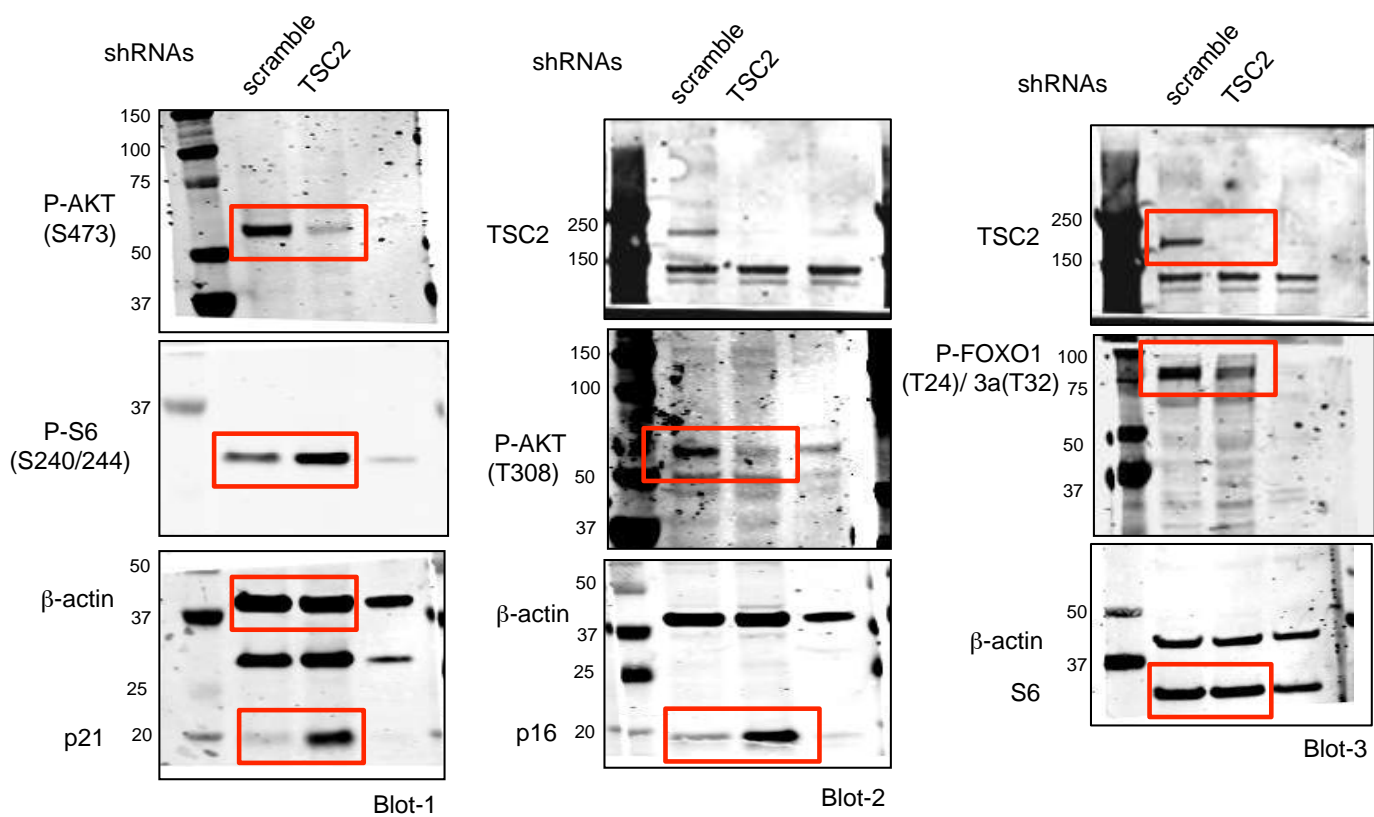
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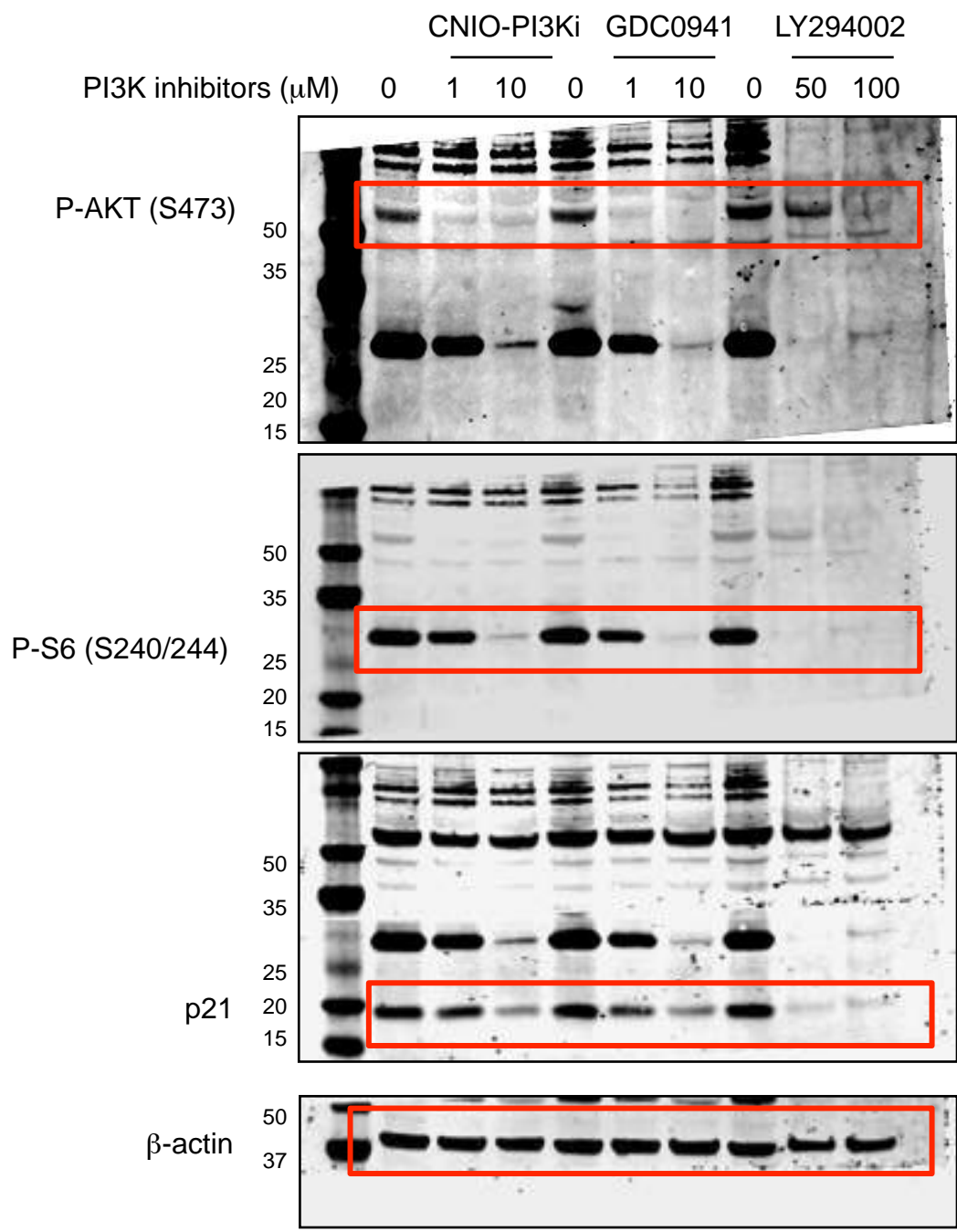
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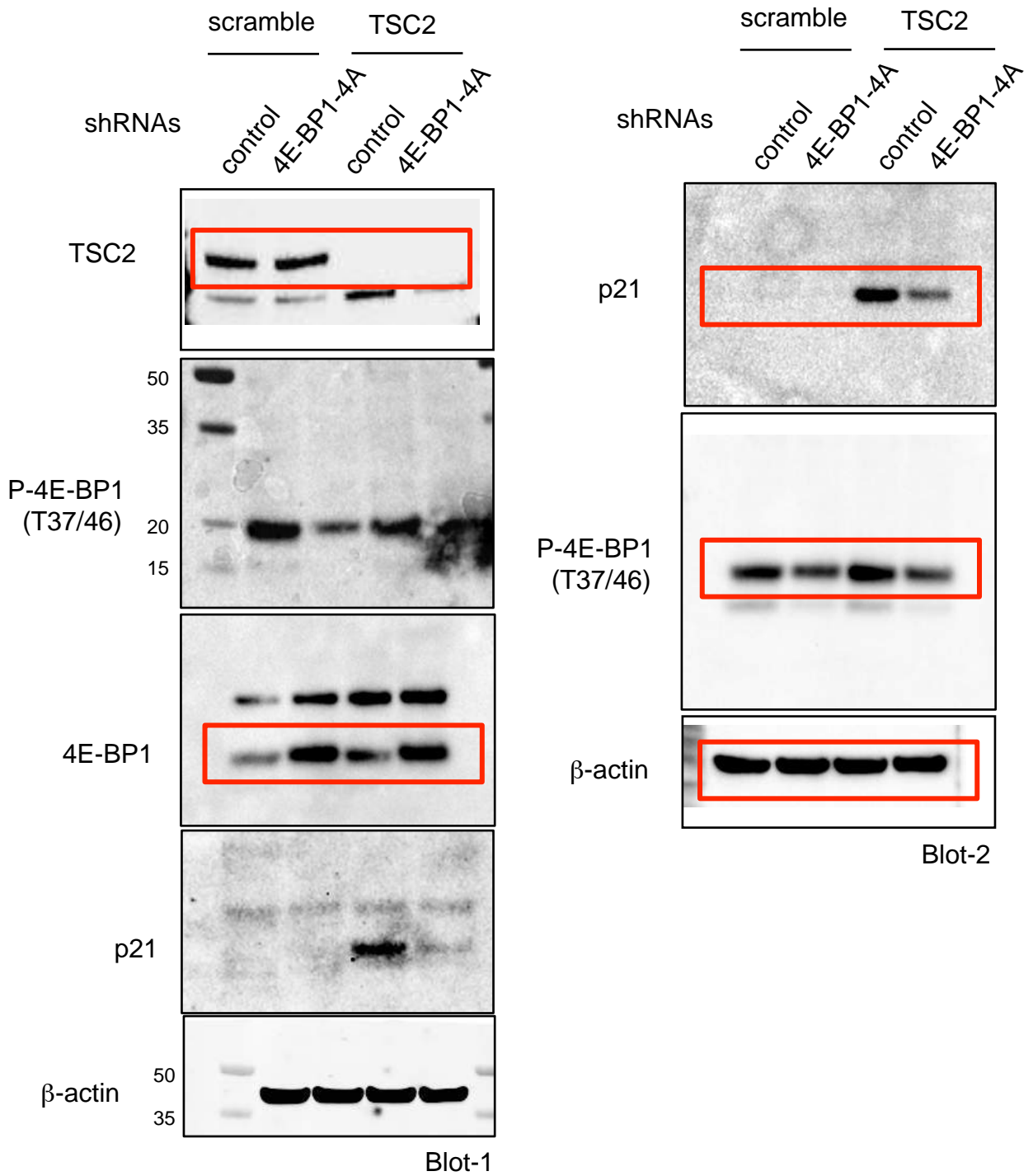
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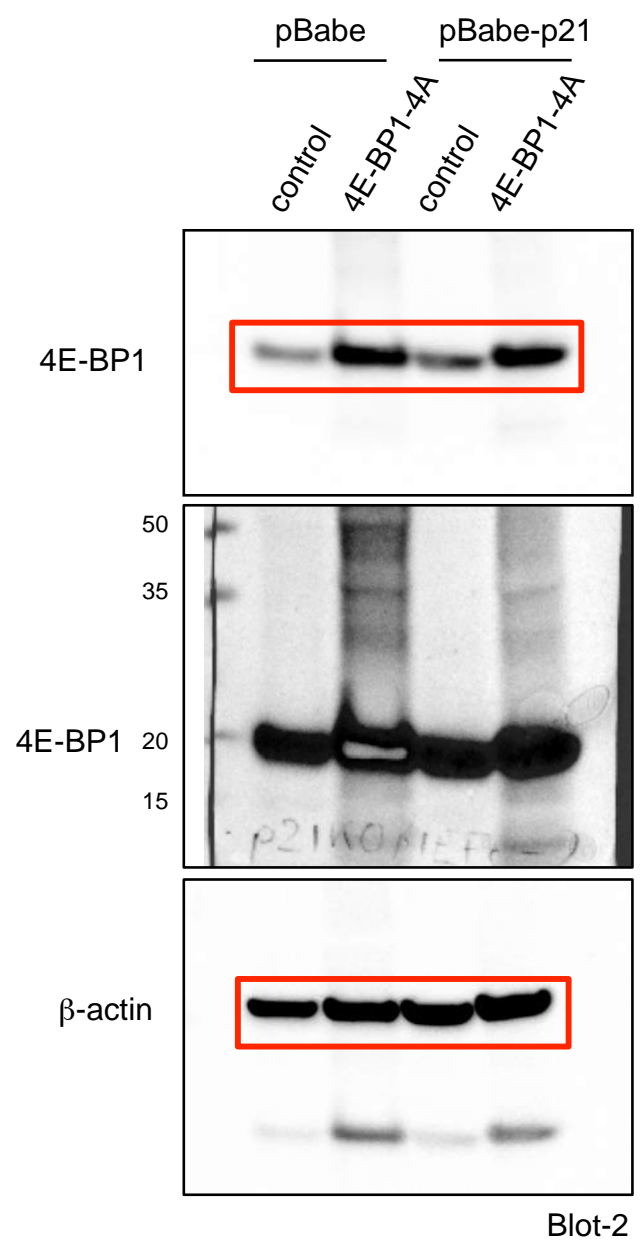
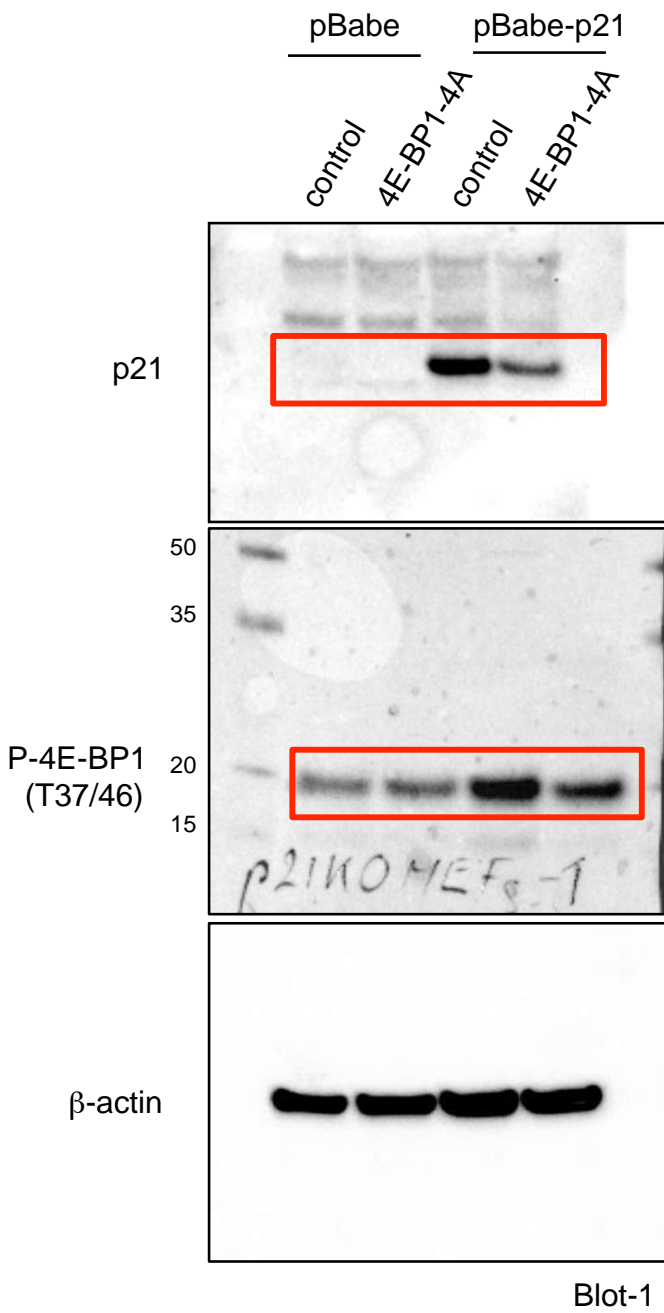
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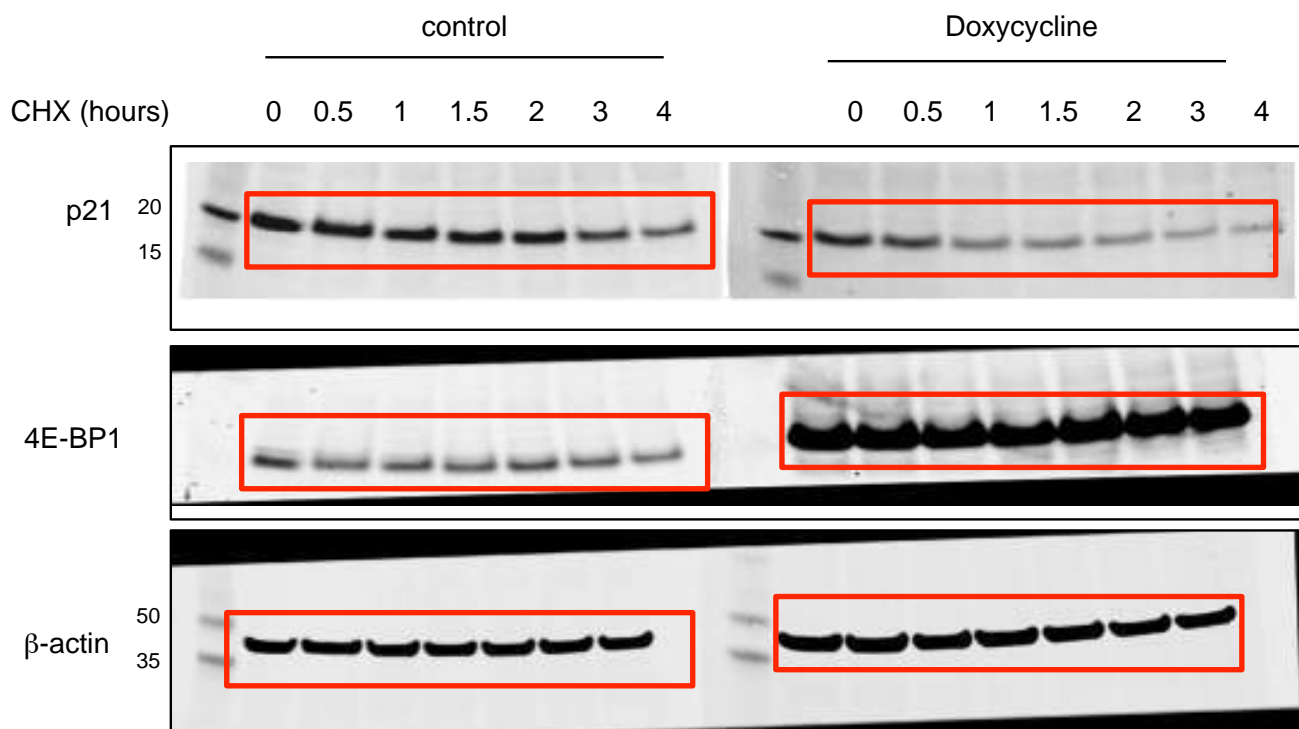
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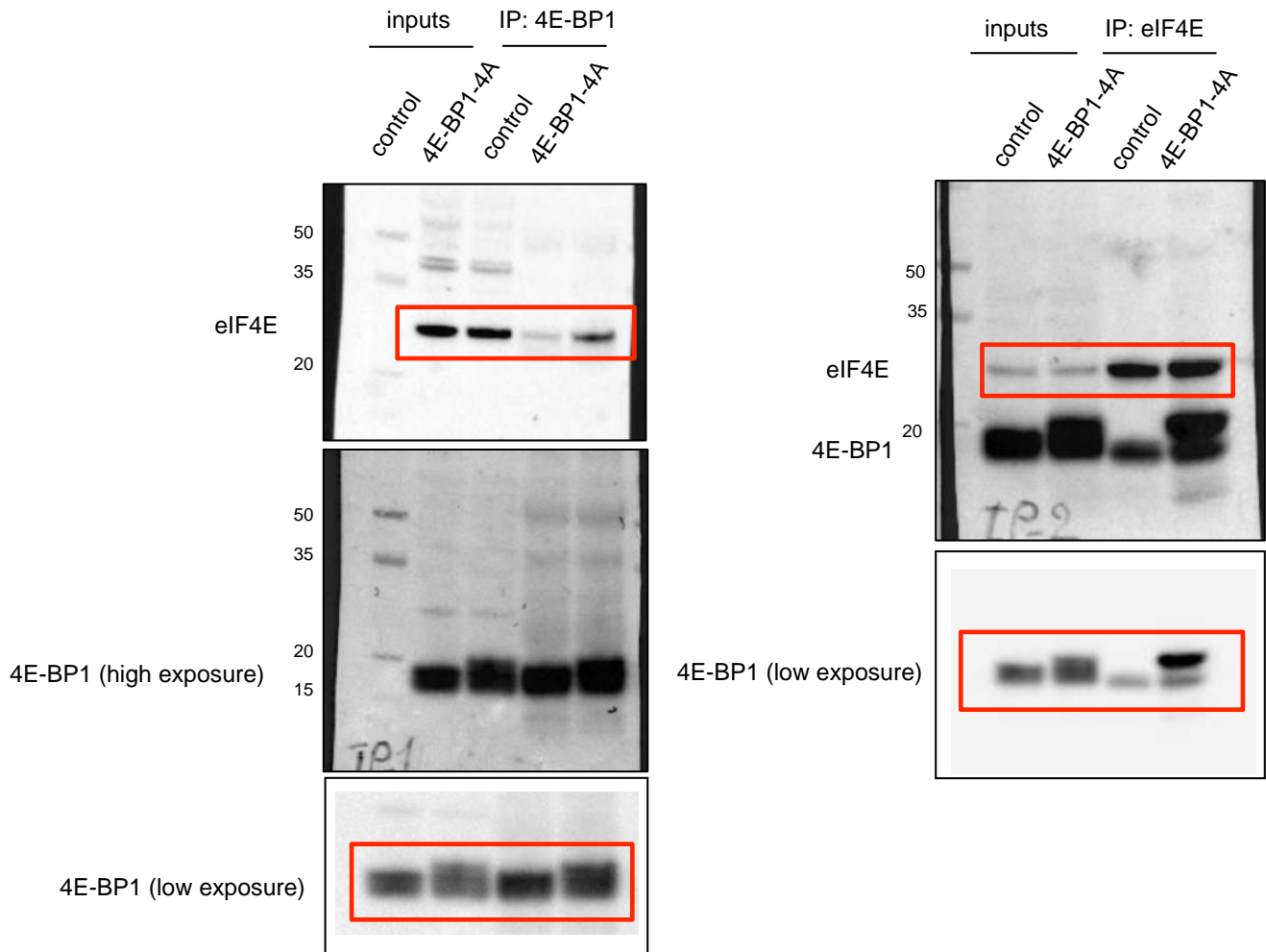
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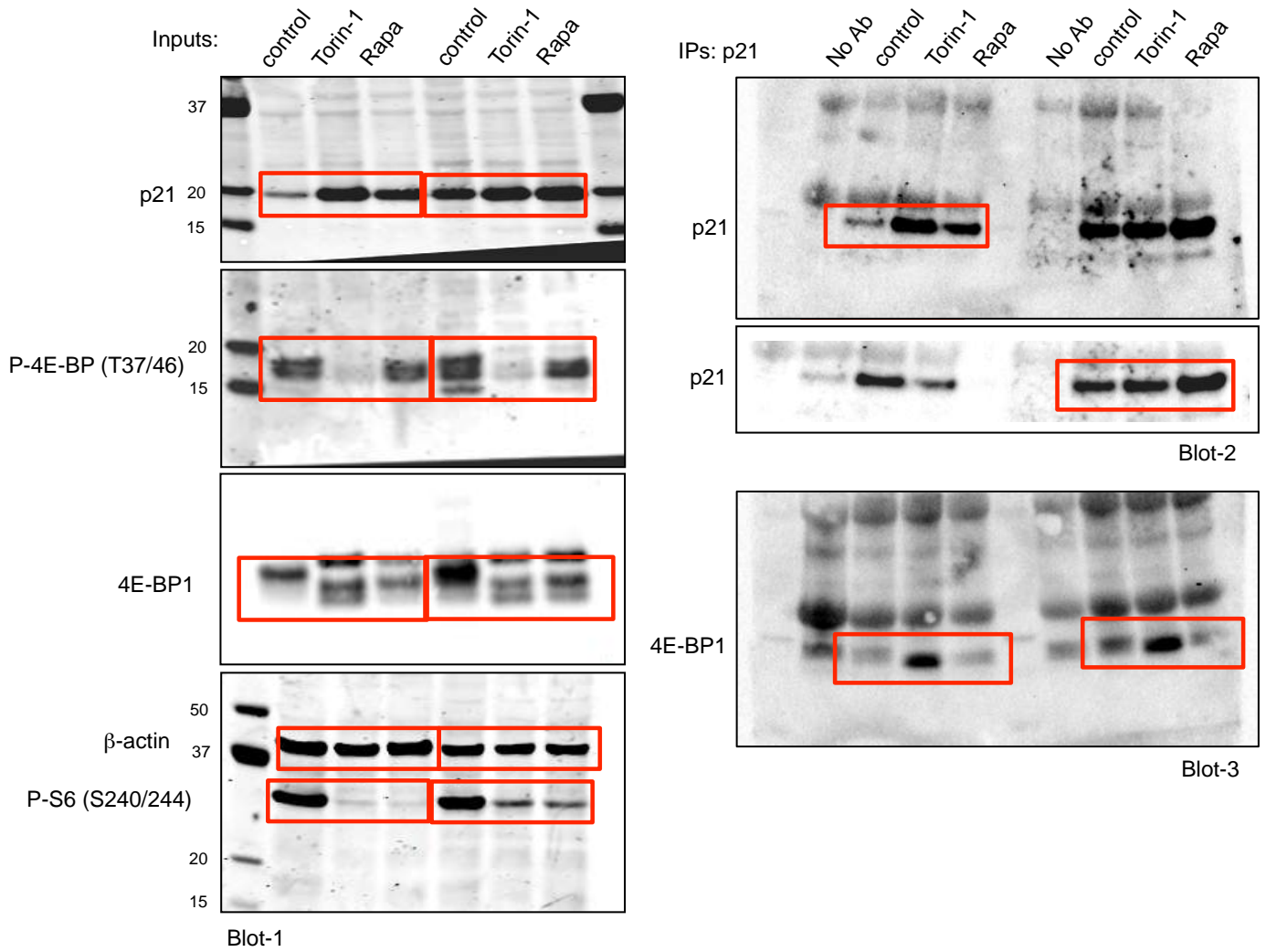
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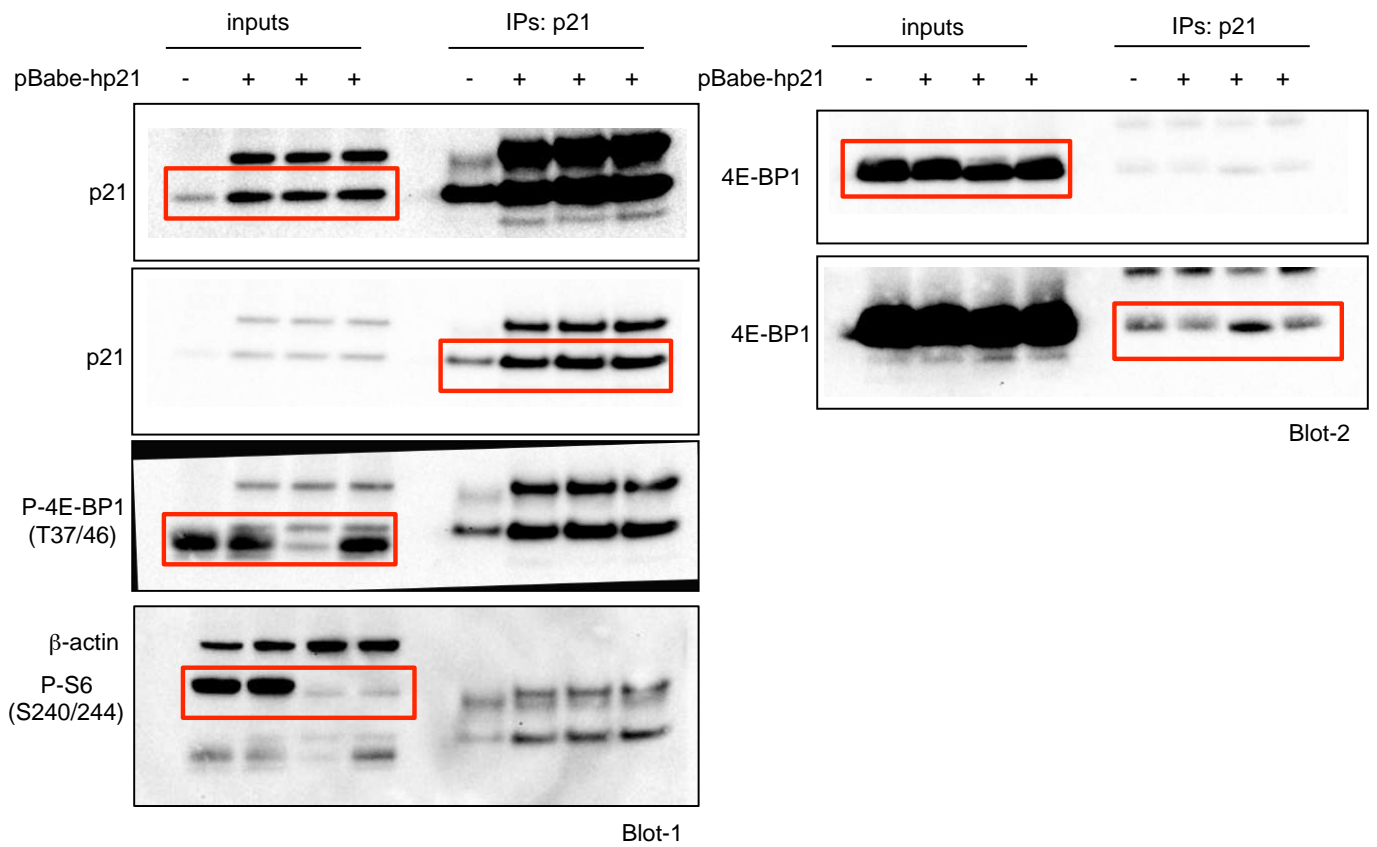
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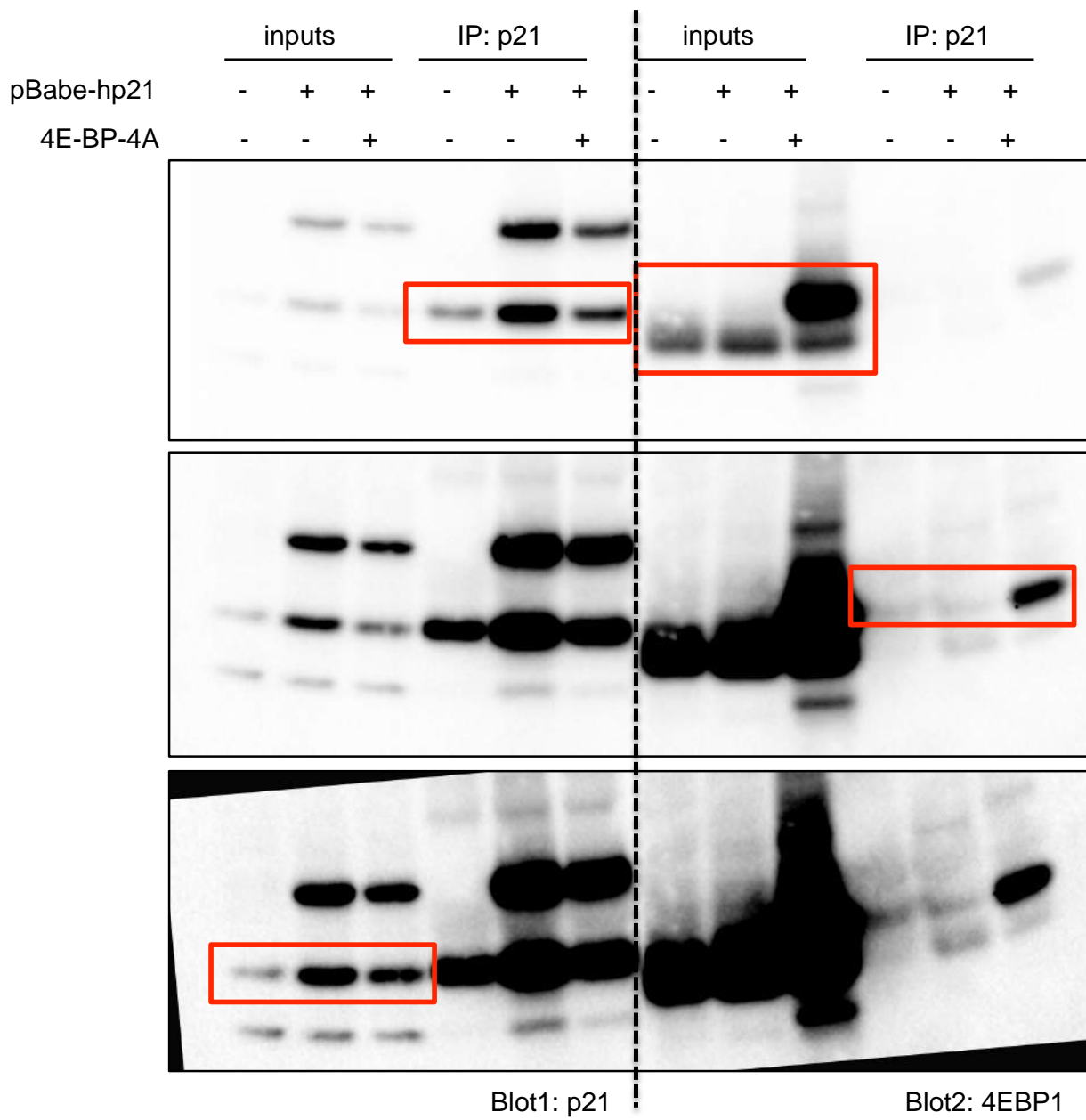
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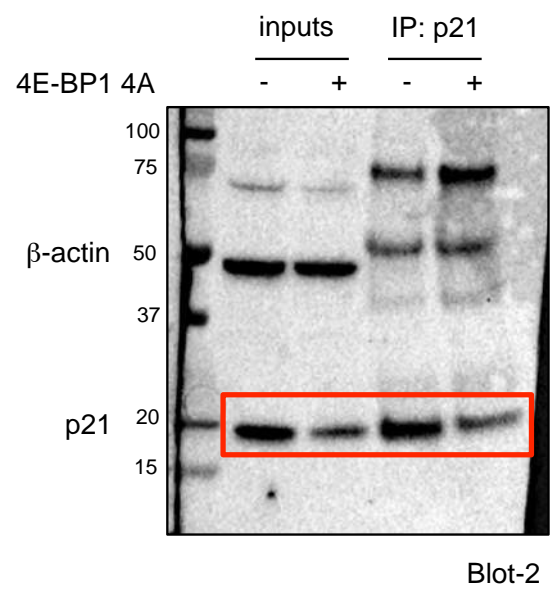
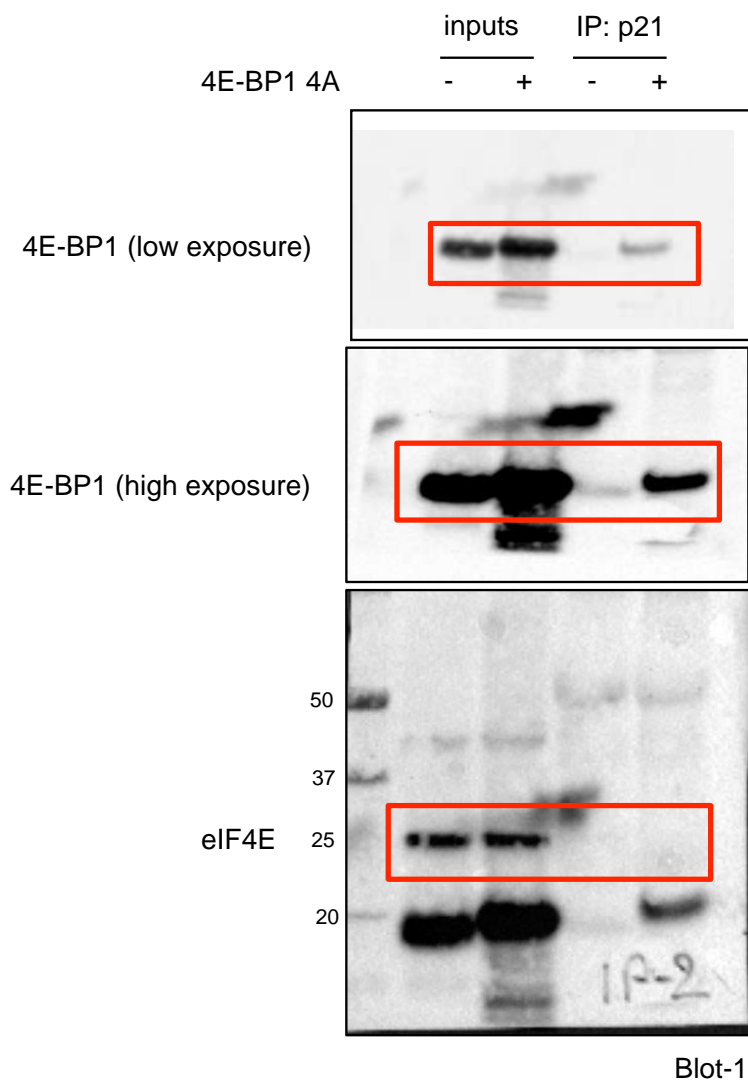
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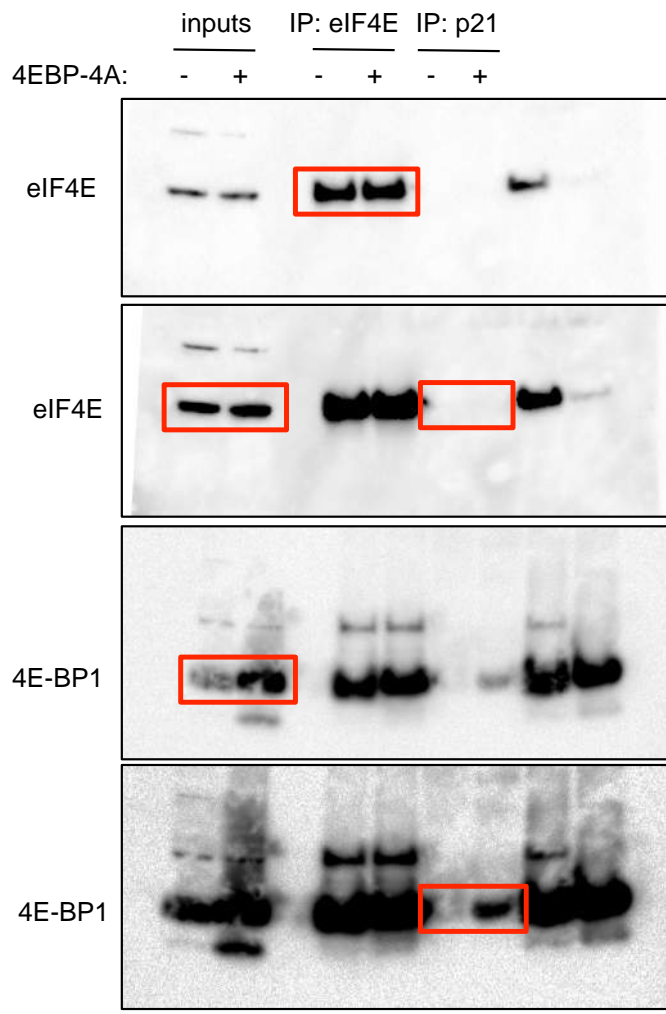
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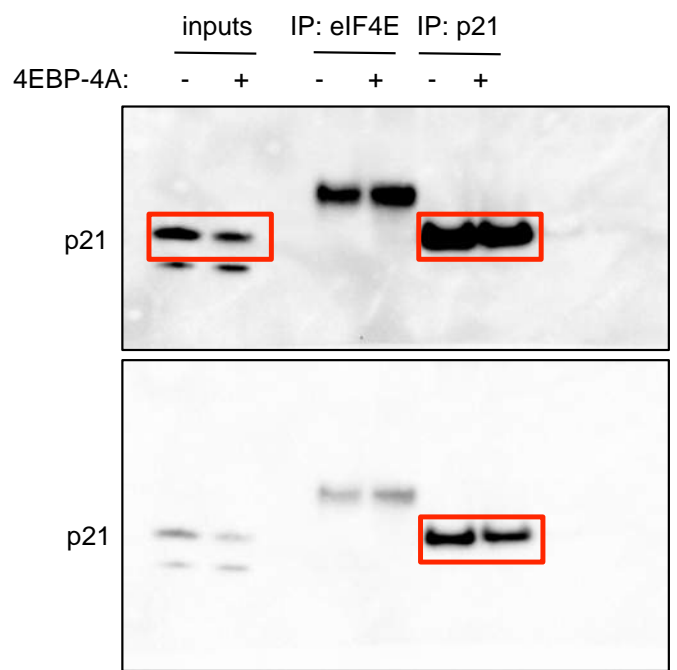
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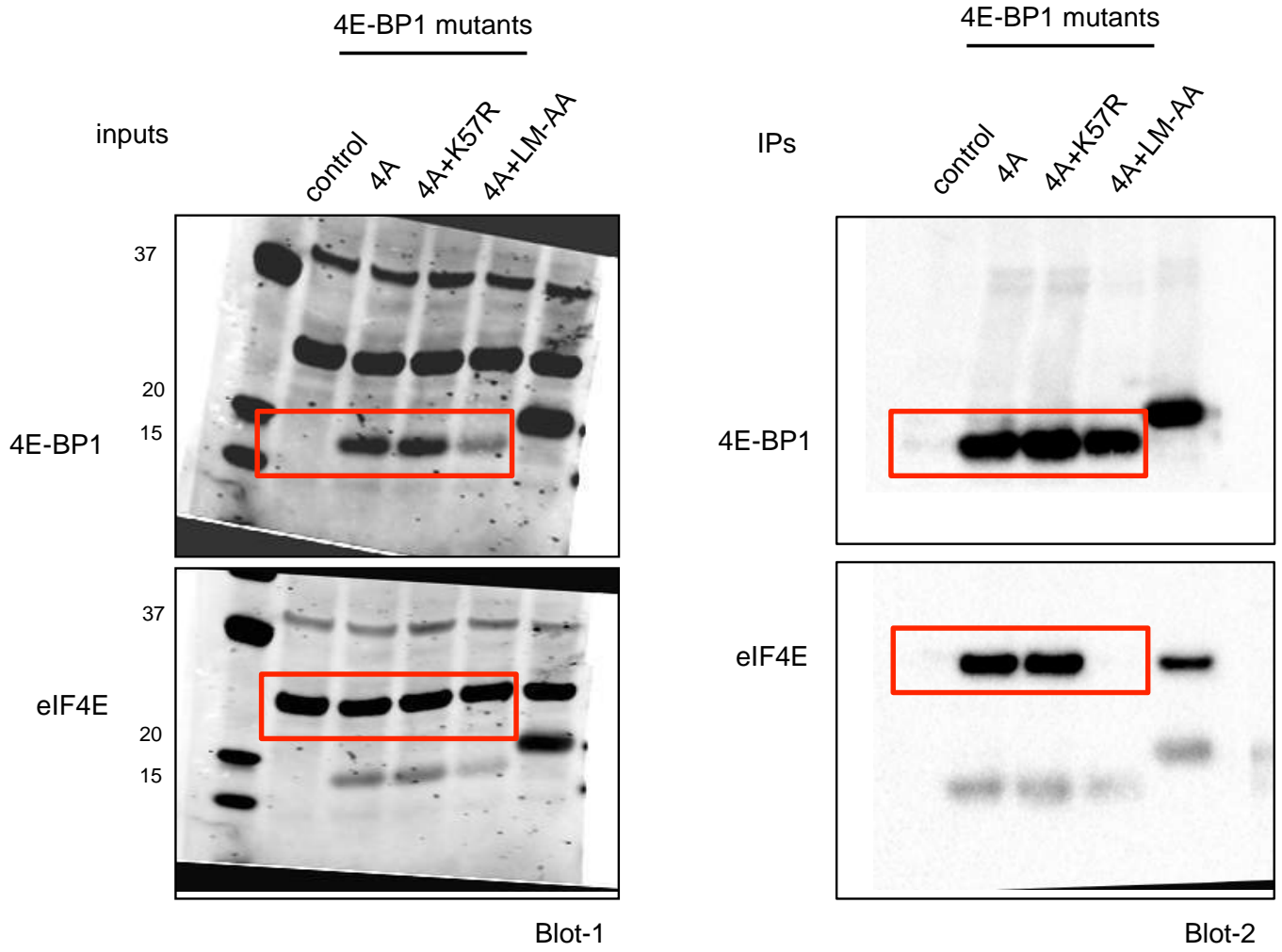


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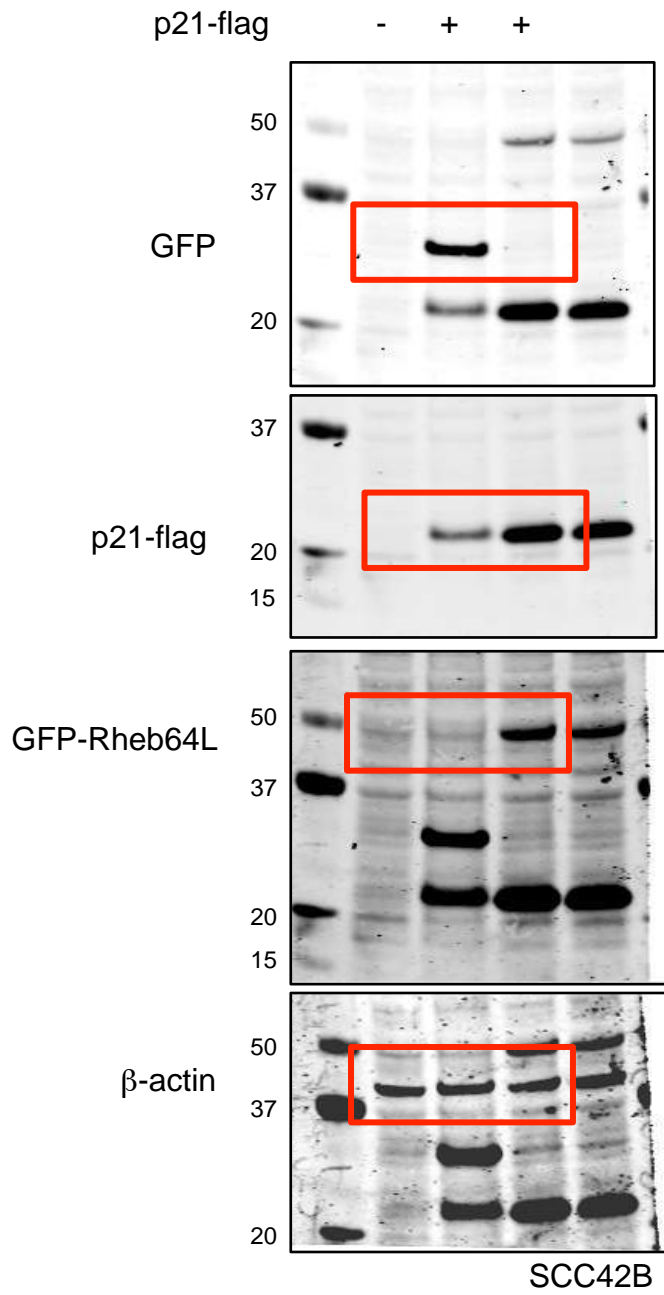


Blot-2

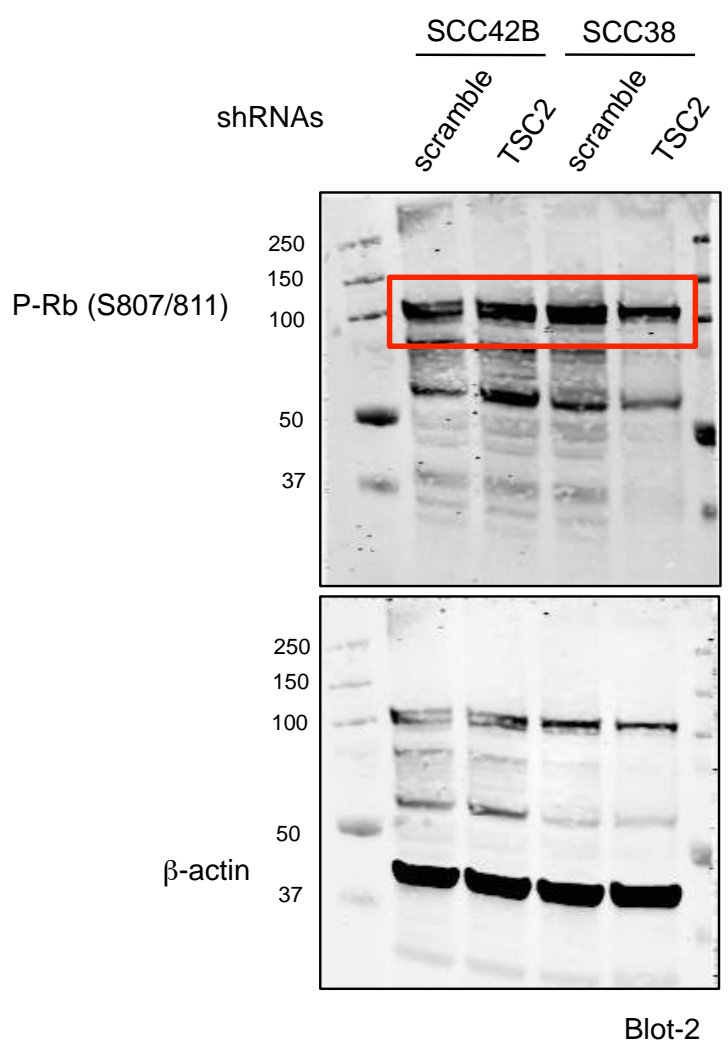
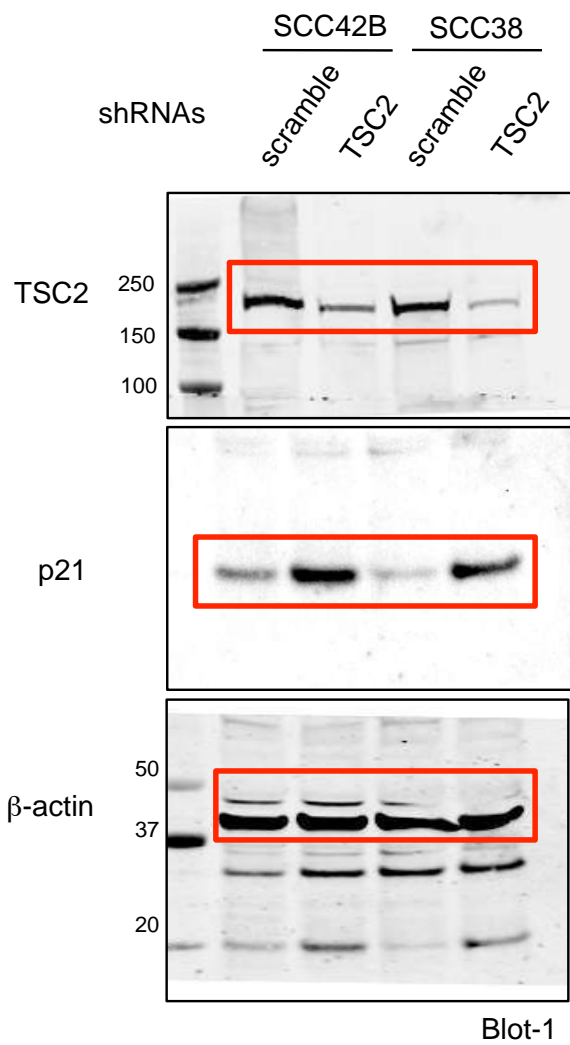
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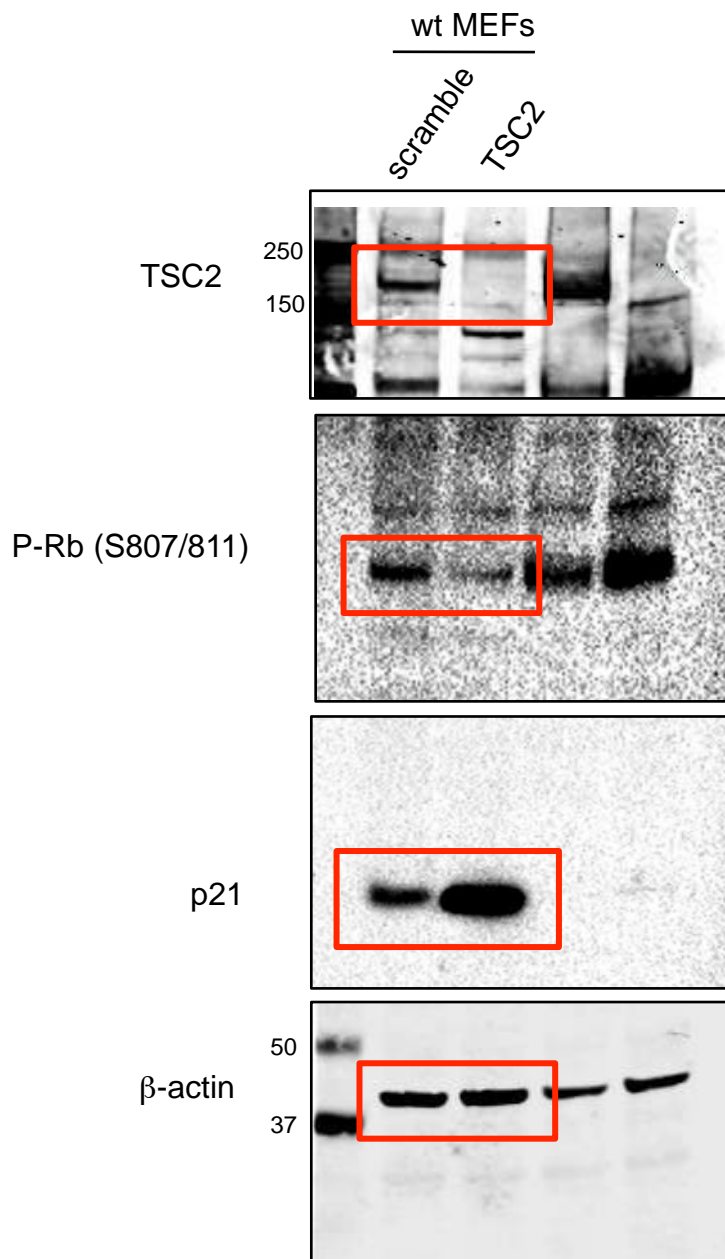
Uncropped membranes for Supplementary Fig. 4f



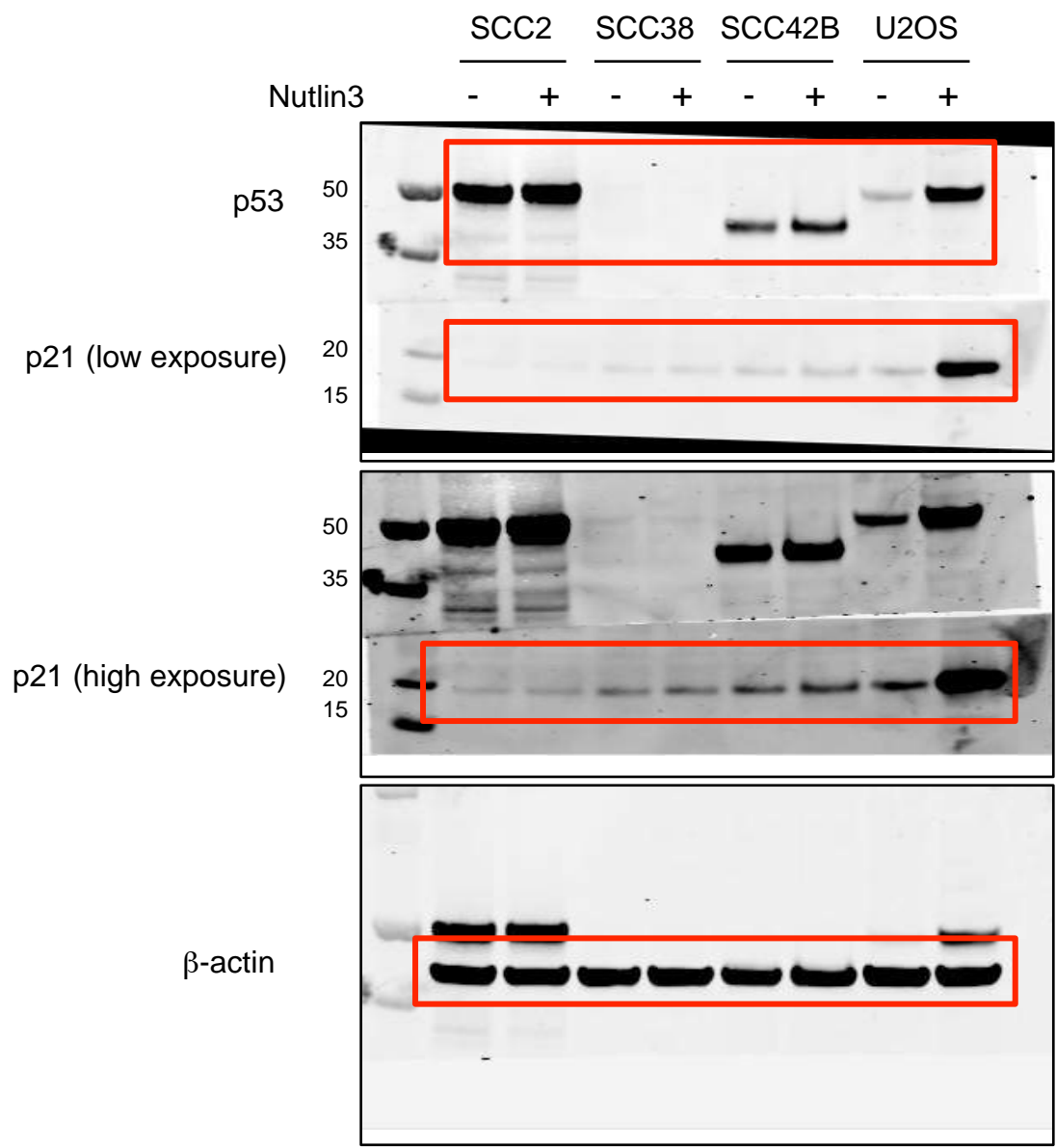
Uncropped membranes for Supplementary Fig. 5a



Uncropped membranes for Supplementary Fig. 5b (1)



Uncropped membranes for Supplementary Fig. 5b (2)



Uncropped membranes for Supplementary Fig. 5d