

Supplementary material

USP48 and A20 synergistically promote cell survival in *Helicobacter pylori*-infection

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Figure legends

Figure S1. USP48 stabilises nuclear RelA in *H. pylori* infection

a NCI-N87 cells were transfected with siRNA¹, or **b** siRNA⁵ against USP48 and infected with *H. pylori* for indicated times. Subcellular fractions were subjected to IB for analysis of the indicated proteins. Data information: Data shown are representative for at least two independent experiments. The band intensity was quantified using ImageJ software (NIH). GAPDH, C23 served as load controls and indicate for the purity of the subcellular fractions.

Figure S2. USP48 deubiquitinylates RelA

AGS cells were transfected with recombinant human USP48 (rhUSP48) and infected with *H. pylori* for the indicated times. MG132 was added 30 min after infection. IP with an anti-RelA antibody was performed at the indicated times in the presence of NEM and OPT, followed by IB analysis of the indicated proteins. Data information: Data shown are representative of at least two independent experiments.

Figure S3. USP48 prolongs A20 *de novo* synthesis

a NCI-N87 cells were transfected with siRNA against USP48 and infected with *H. pylori* for indicated times. **b** AGS cells were transfected with siRNA against USP48 and infected with *H. pylori* strain P12 for indicated times. Whole-cell lysates were subjected to IB for analysis of the indicated proteins. Data information: Data shown are representative for at least two independent experiments.

Figure S4. Caspase-8 cleavage contributes to *H. pylori* associated apoptotic cell death

a AGS cells were transfected with siRNA against caspase-8 and infected with *H. pylori* for indicated times. Whole-cell lysates were subjected to IB for analysis of the indicated proteins. **b** AGS cells were transfected with siRNA against caspase-8 and infected with *H. pylori* for 24 h. Cleaved caspase-3/7 expression was detected by the IncuCyte® S3 Live-Cell Analysis System. Scale bars = 100 μ m. Data shown depict the average of nine pictures from distinct regions. Error bars denote mean \pm SD. Data information: Data shown in (a) are representative for at least two independent experiments. Data shown in (b) are from two independent experiments. *P \leq 0.05 (Student's t-test).

Figure S5. USP48 suppresses apoptotic cell death

AGS cells were transfected with siRNA against USP48 (siRNA⁵) and infected with *H. pylori* for 24 h, followed by staining with annexin V/PI. Apoptotic cell death was analysed by flow cytometry. Data shown depict the average of two independent experiments. Error bars denote mean \pm SD. Data information: Data shown are representative for at least two independent experiments. *P \leq 0.05, **P \leq 0.01 (Student's t-test).

Figure S1

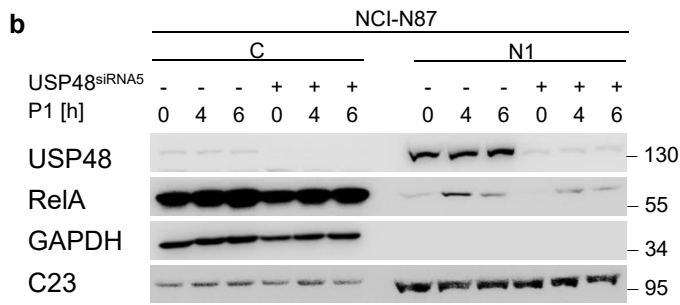
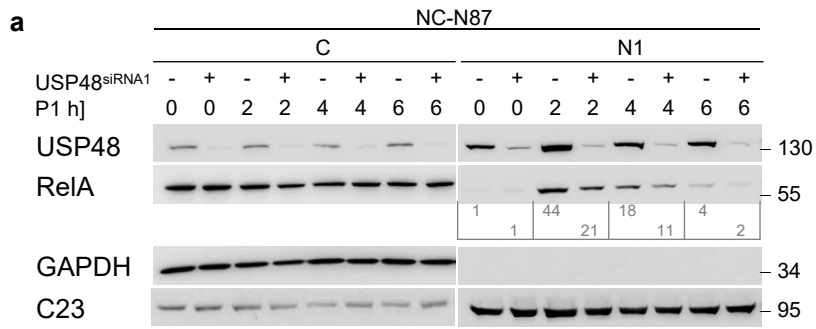


Figure S2

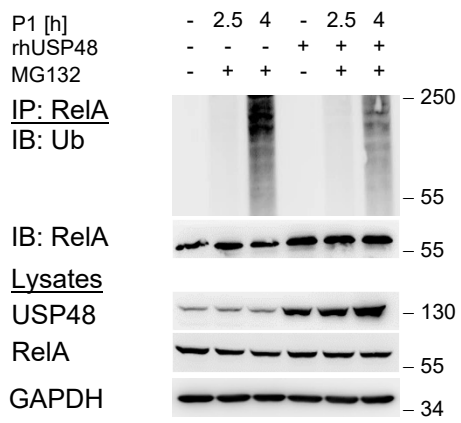


Figure S3

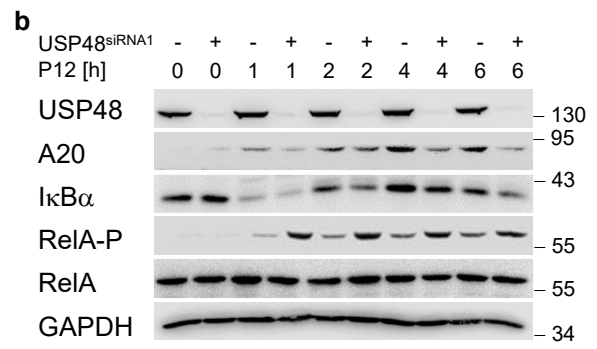
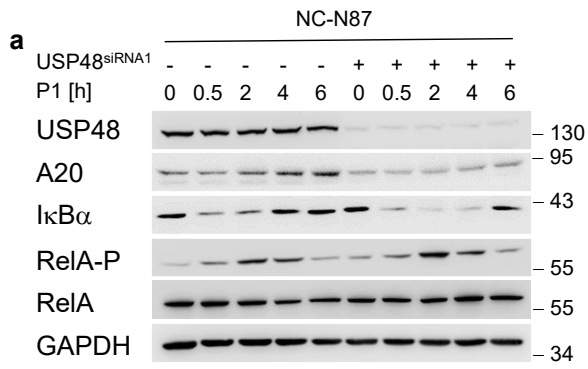


Figure S4

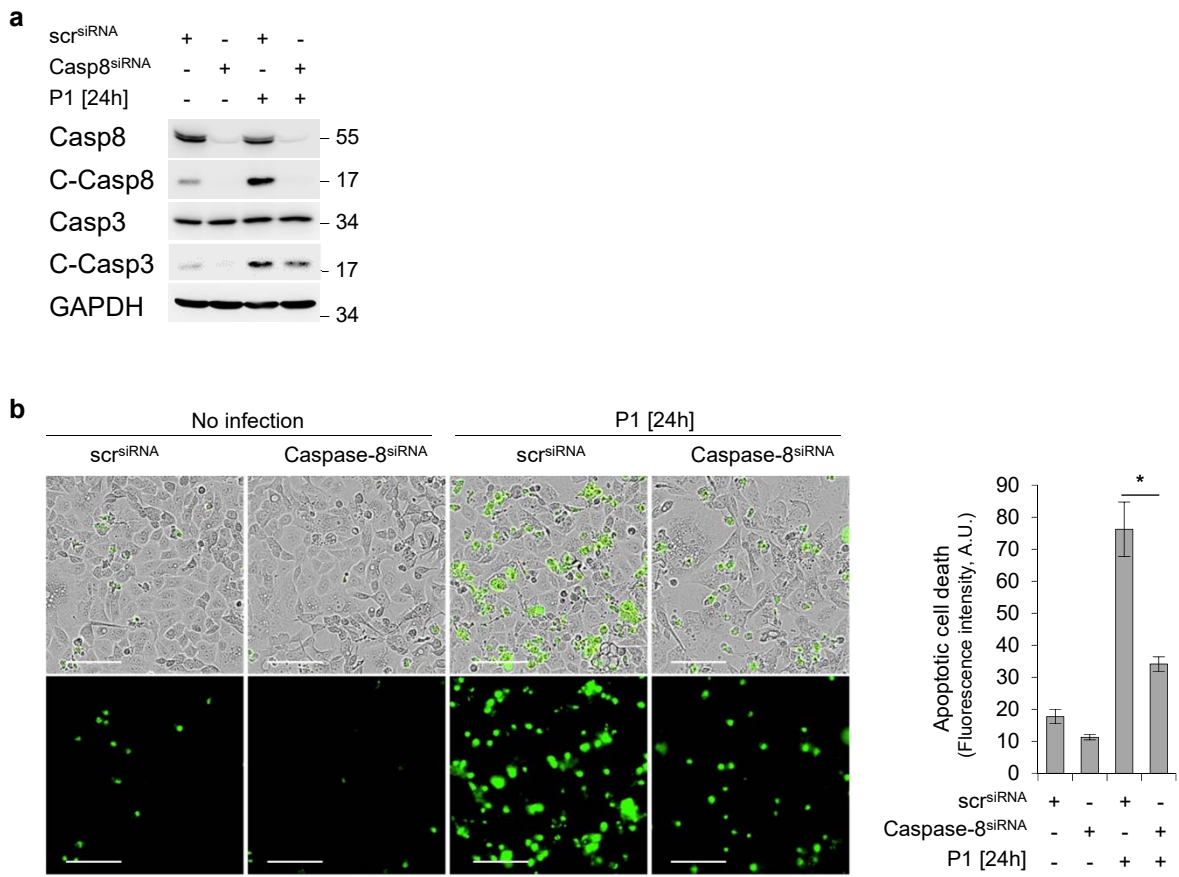
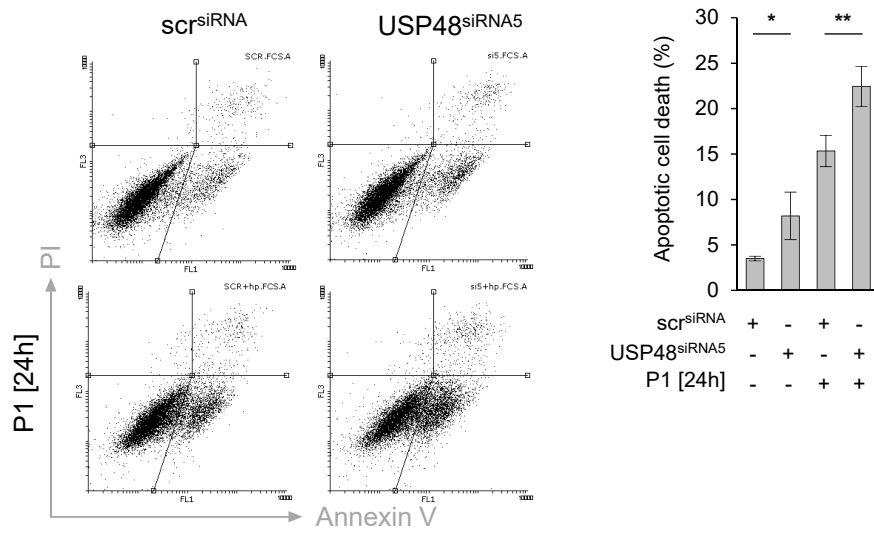
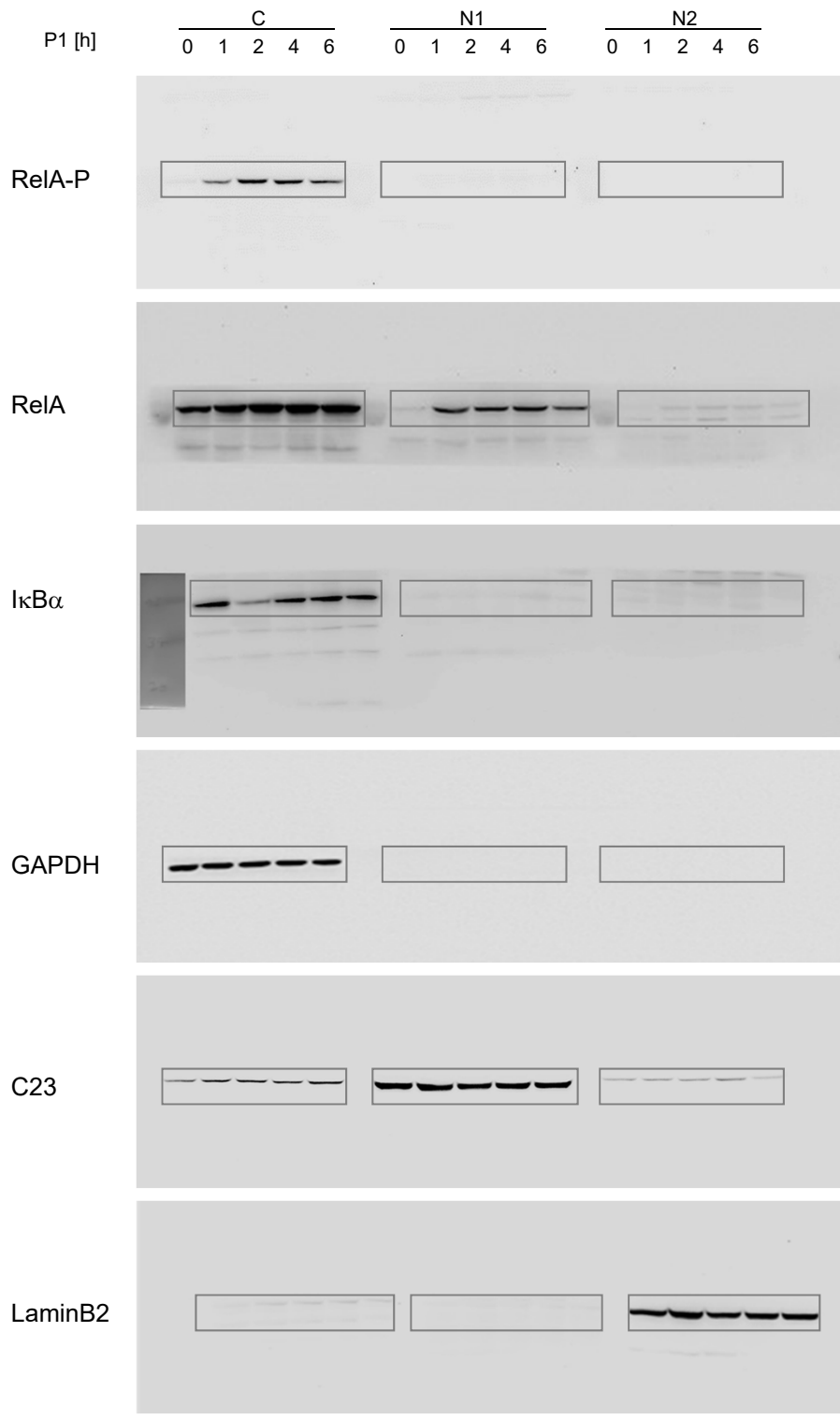


Figure S5



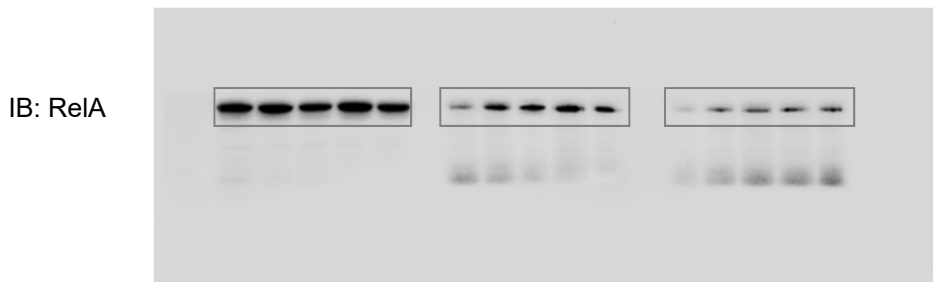
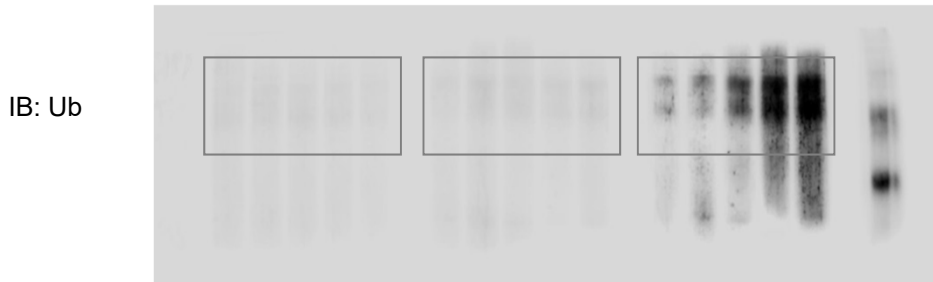
Uncropped blot for Fig 1a



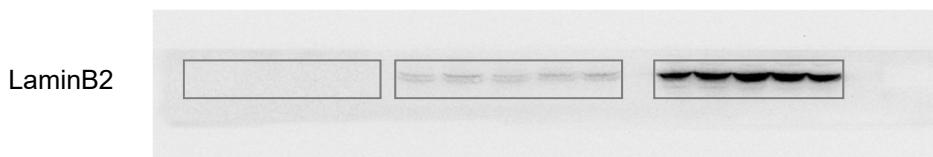
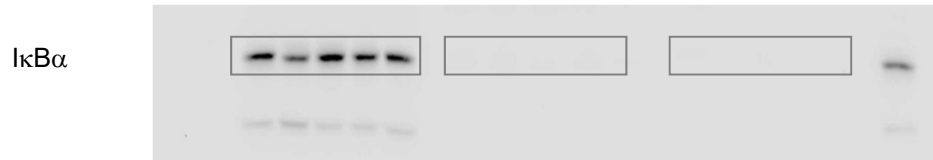
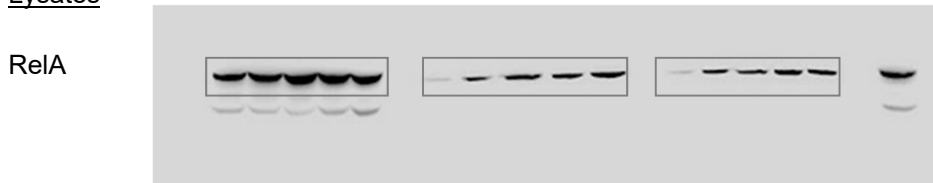
Uncropped blot for Fig 1d

	C					N1					N2					IgG
P1 [h]	0	1	2	4	6	0	1	2	4	6	0	1	2	4	6	C
MG132	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
LMB	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+

IP: RelA



Lysates

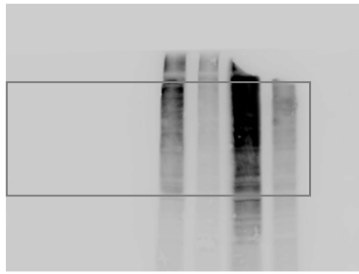


Uncropped blot for Fig 1e

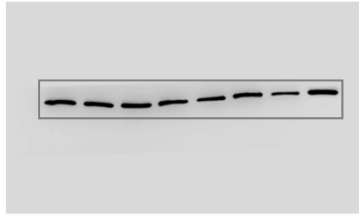
P1 [h]	0	0	2	2	4	4	6	6
Elongin B ^{siRNA}	-	+	-	+	-	+	-	+
MG132	-	-	+	+	+	+	+	+

IP: RelA

IB: Ub

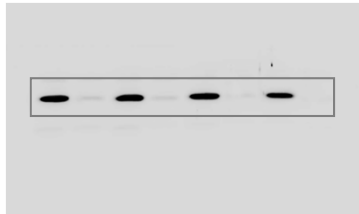


IB: RelA

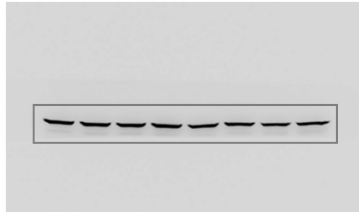


Lysates

Elongin B



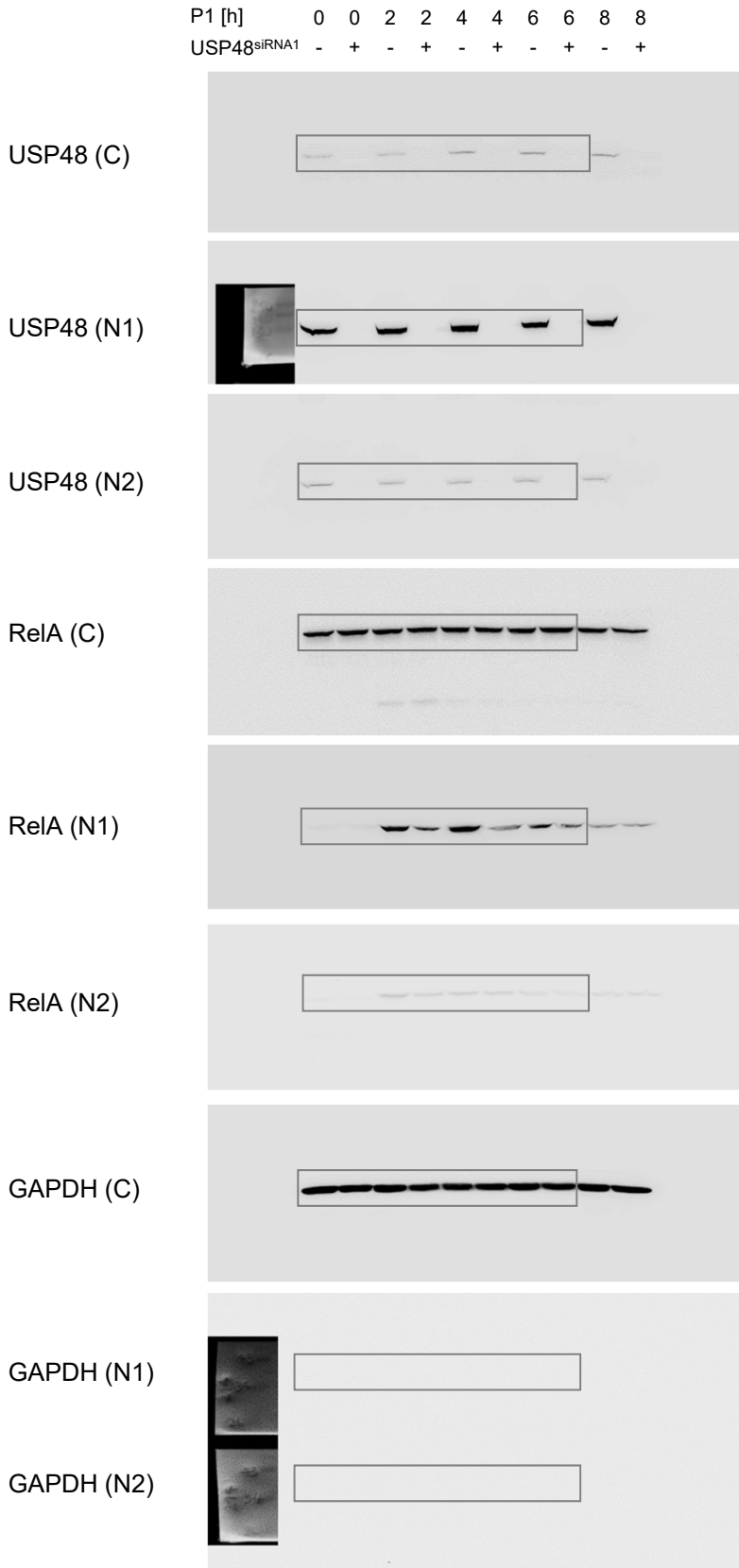
RelA



GAPDH



Uncropped blot for Fig 2a



Uncropped blot for Fig 2a

USP48^{siRNA1} 0 0 2 2 4 4 6 6 8 8
P1 [h] - + - + - + - + - +

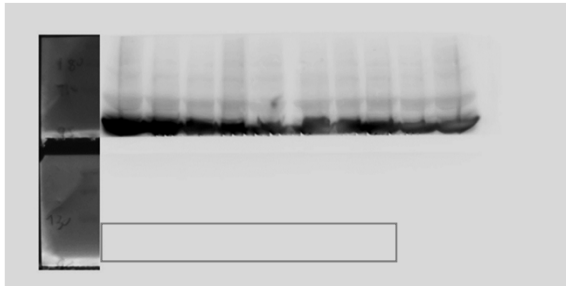
C23 (C)



C23 (N1)



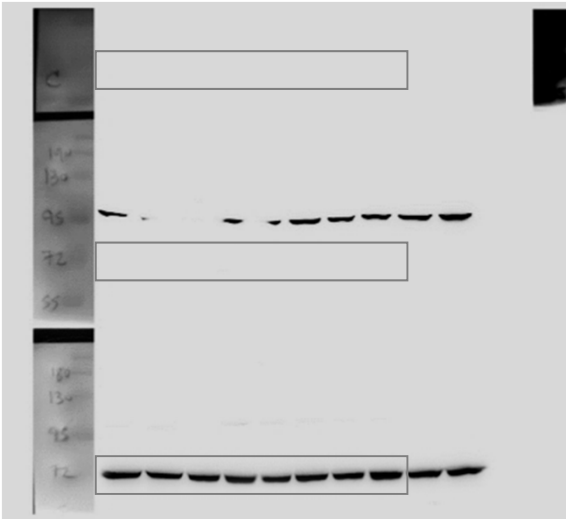
C23 (N1)



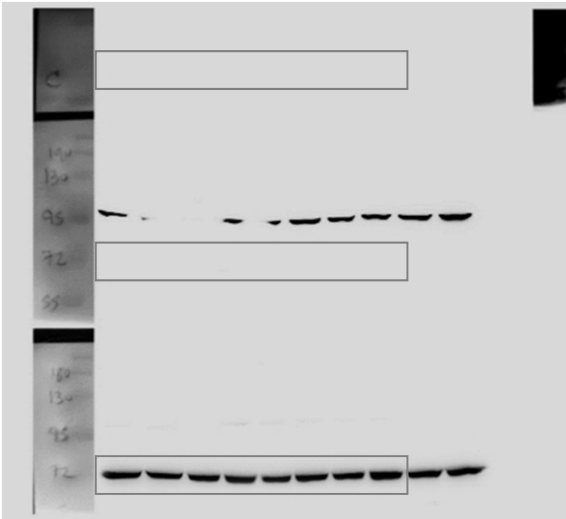
C23 (N2)



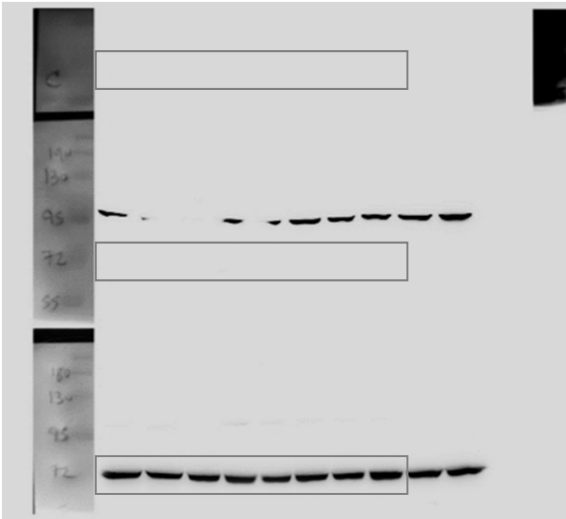
LaminB2 (C)



LaminB2 (N1)



LaminB2 (N2)



Uncropped blot for Fig 2b

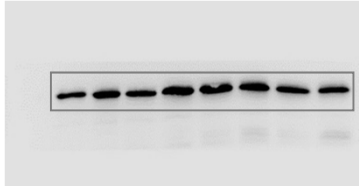
P1 [h]	0	0	2	2	4	4	6	6
USP48 ^{siRNA1}	-	+	-	+	-	+	-	+
MG132	-	-	+	+	+	+	+	+

IP: RelA

IB: Ub

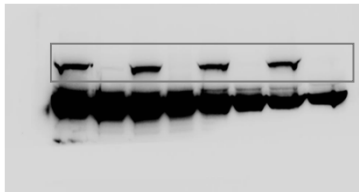


IB: RelA

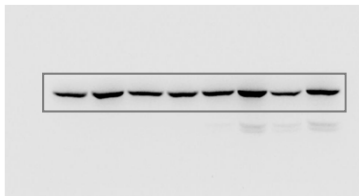


Lysates

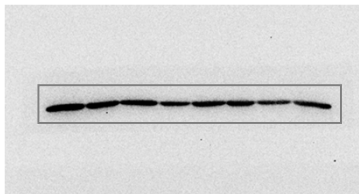
USP48



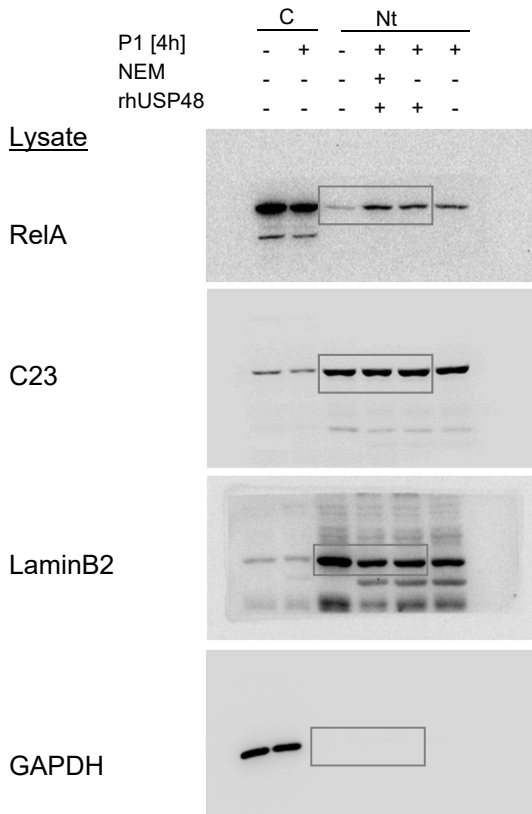
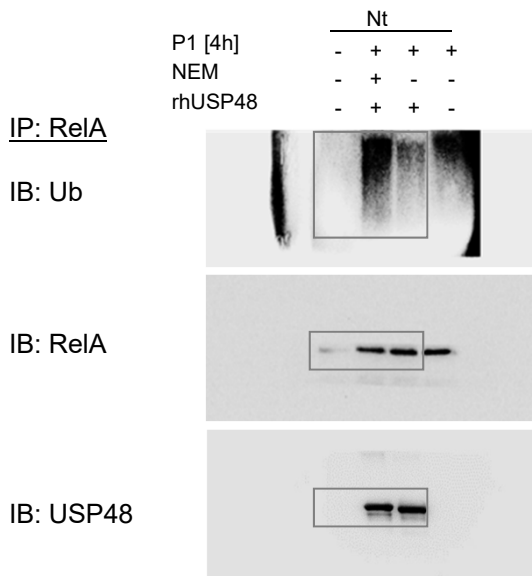
RelA



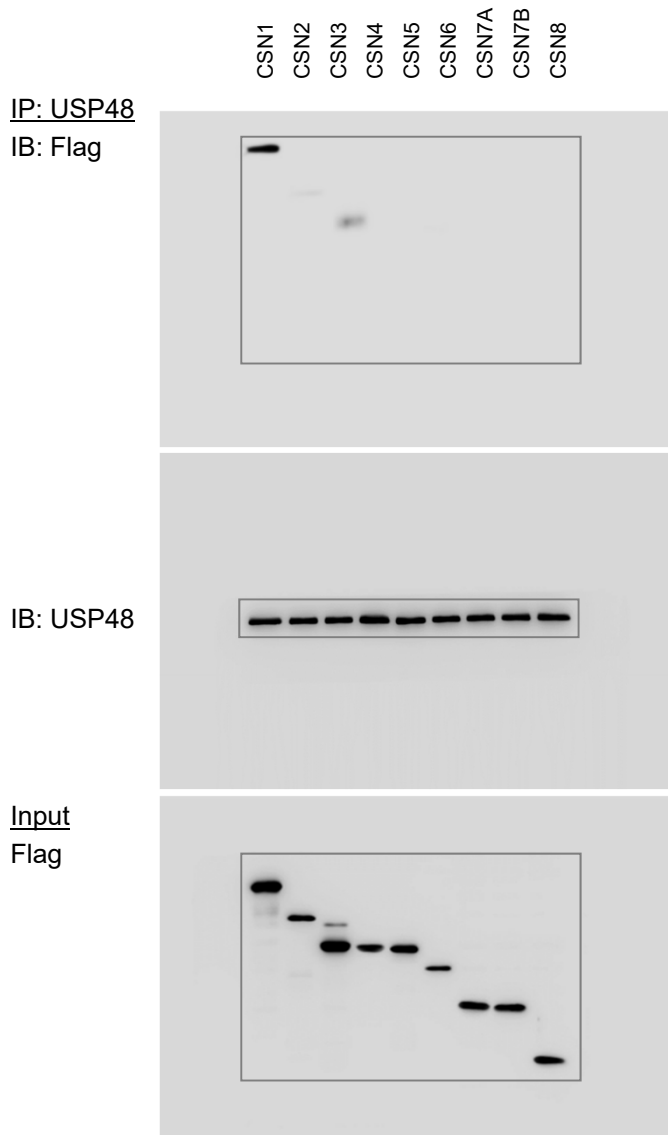
GAPDH



Uncropped blot for Fig 2c



Uncropped blot for Fig 2e

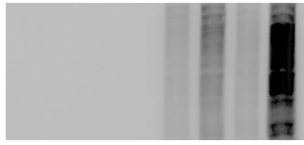


Uncropped blot for Fig 2f

P1 [h]	0	0	2	2	4	4	6	6
CSN2 ^{siRNA}	-	+	-	+	-	+	-	+
MG132	-	-	+	+	+	+	+	+

IP: RelA

IB: Ub



IB: RelA



Lysates

CSN2



CSN1



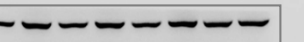
CSN5



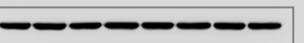
CSN6



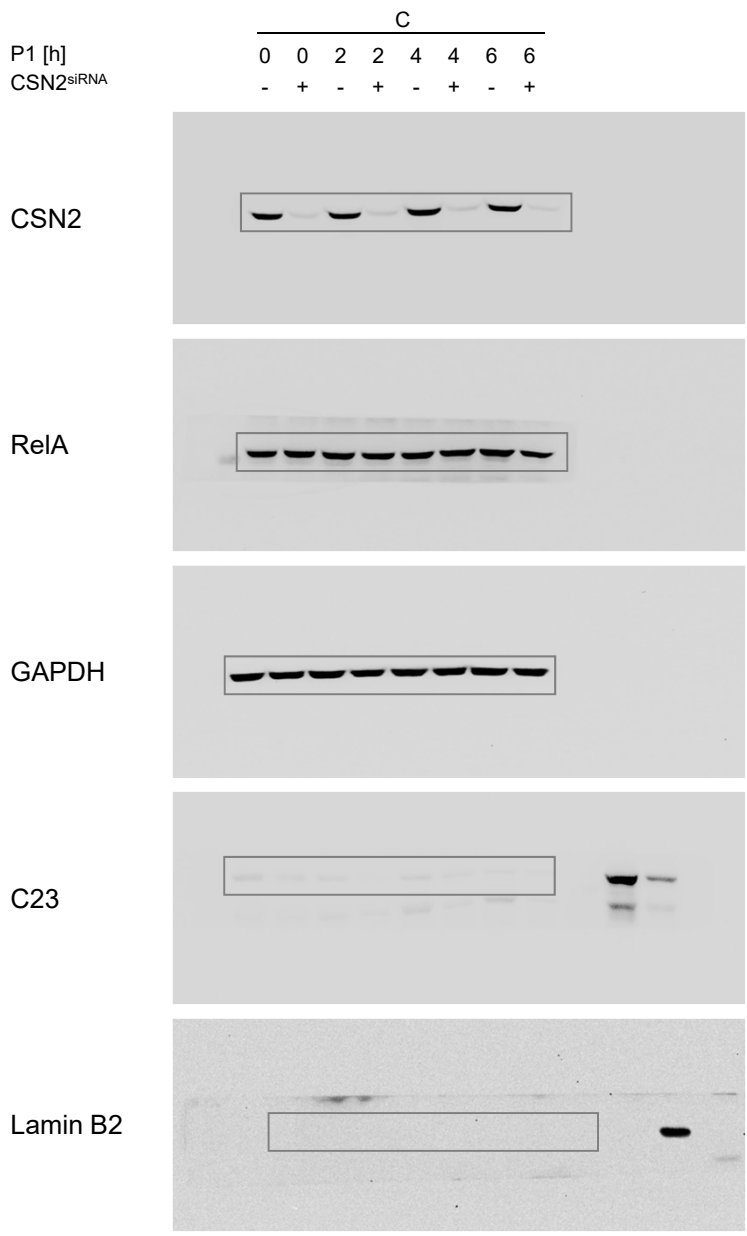
RelA



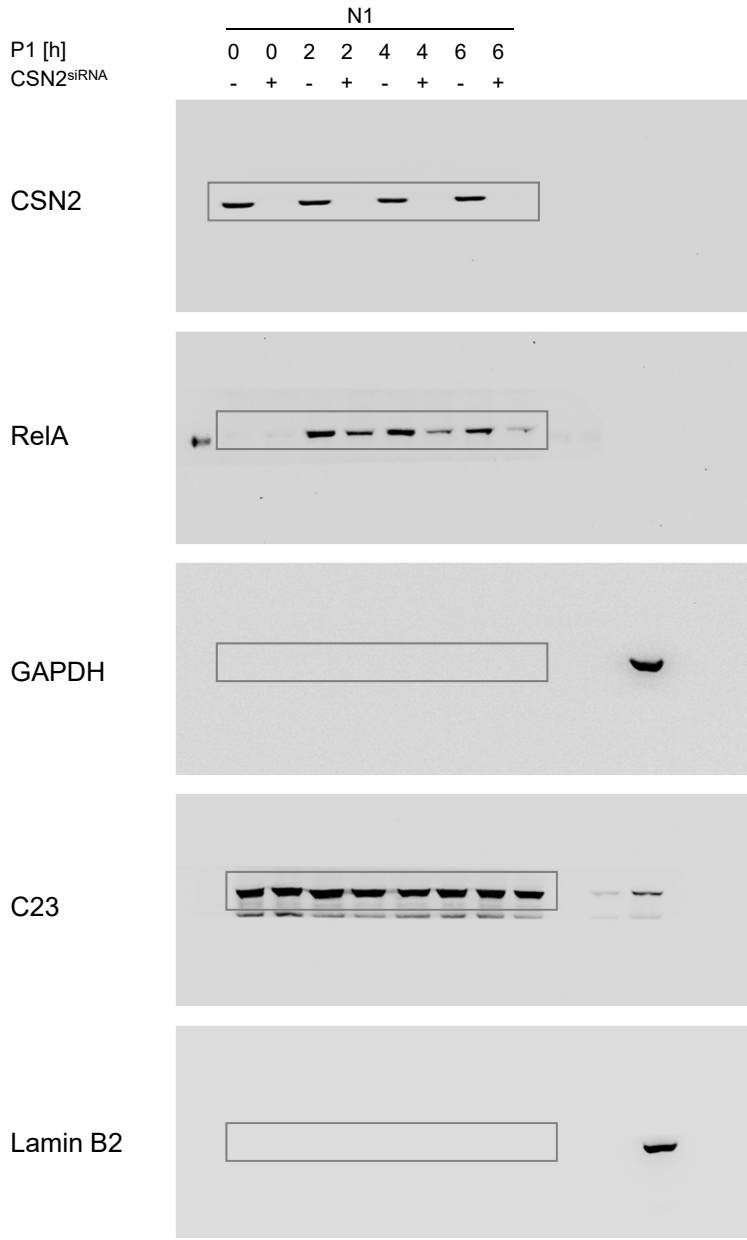
GAPDH



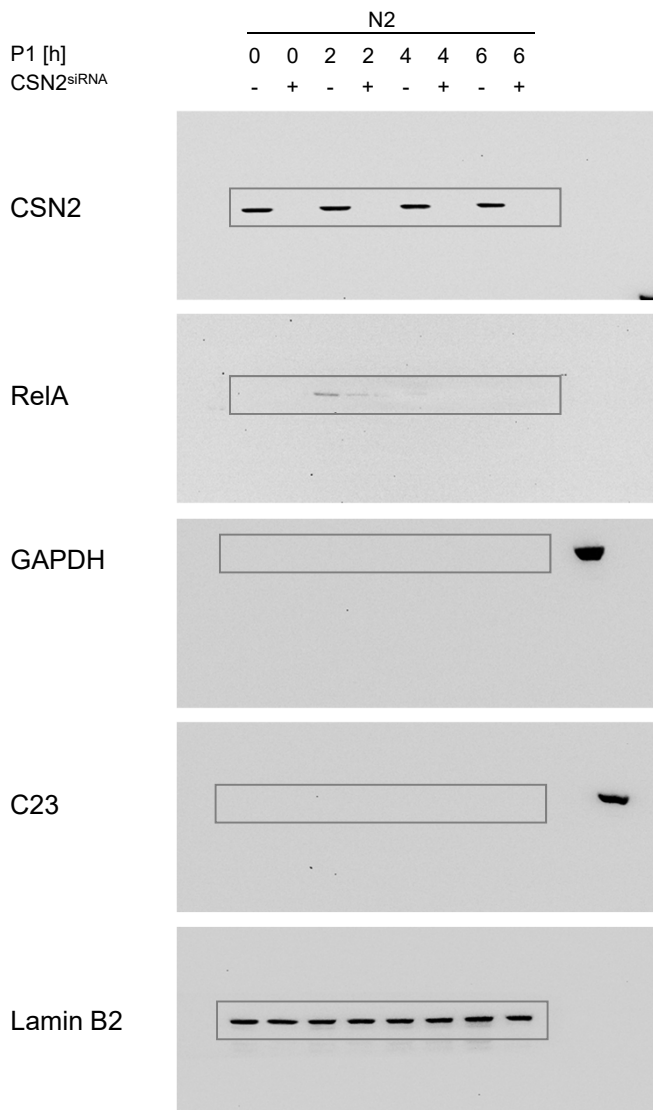
Uncropped blot for Fig 2g



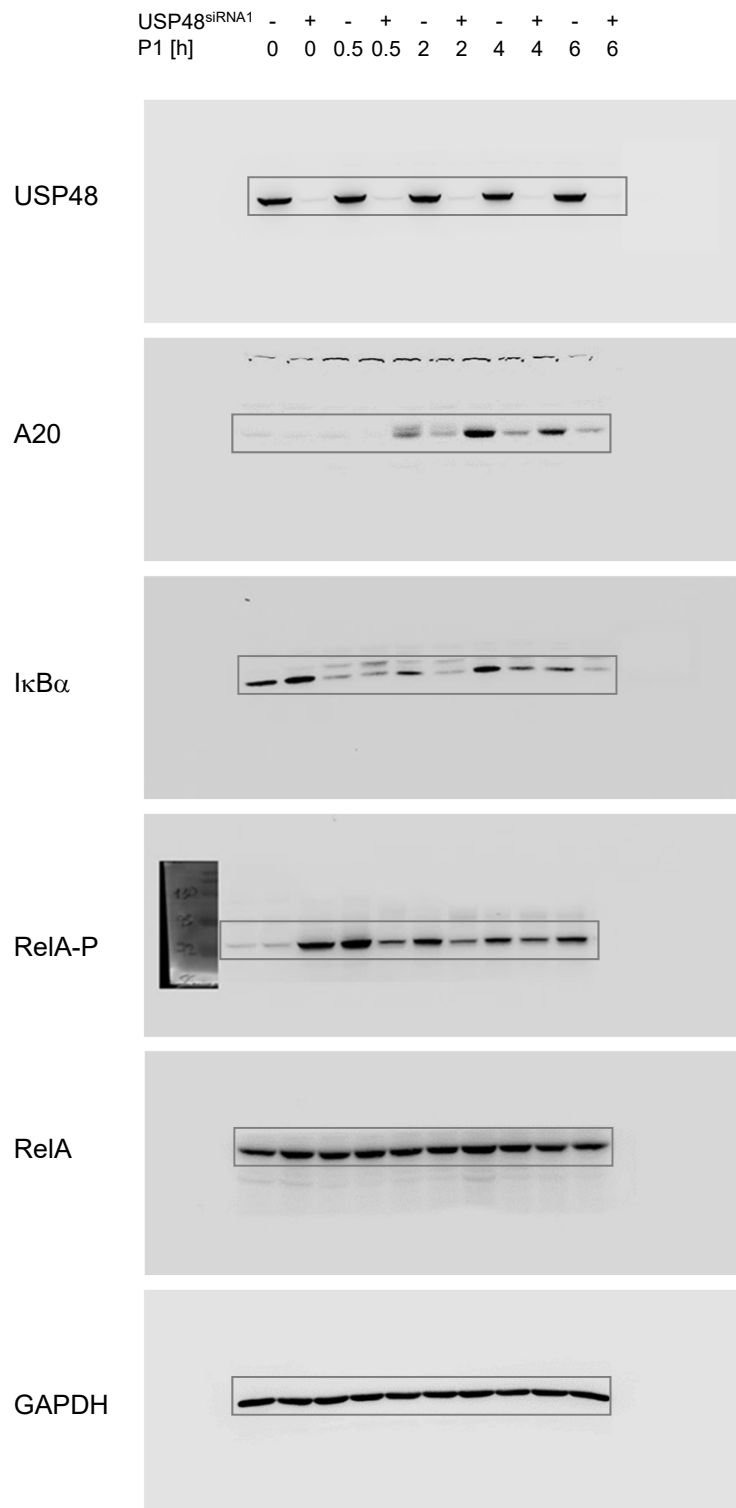
Uncropped blot for Fig 2g



Uncropped blot for Fig 2g



Uncropped blot for Fig 3b



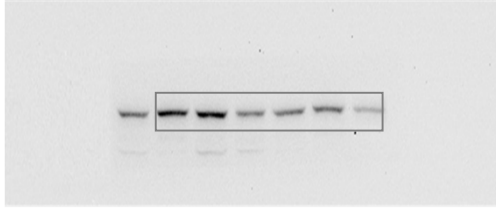
Uncropped blot for Fig 3c

scr ^{siRNA}	-	+	+	-	-	-	-
USP48 ^{siRNA1}	-	-	-	+	+	-	-
USP48 ^{siRNA5}	-	-	-	-	-	+	+
P1 [24h]	-	-	+	-	+	-	+

USP48



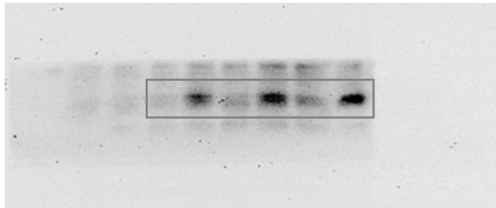
A20



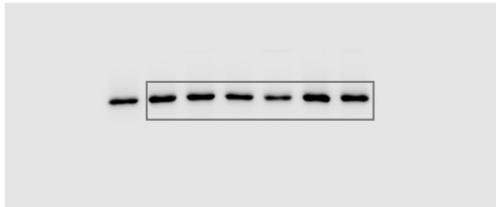
Casp8



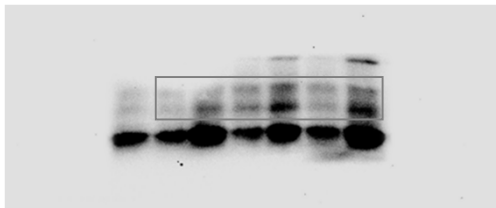
Cl. Casp8



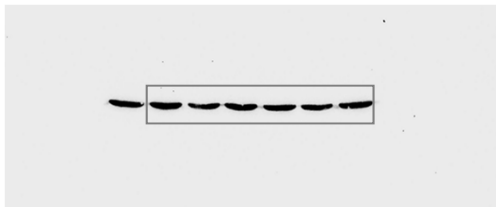
Casp3



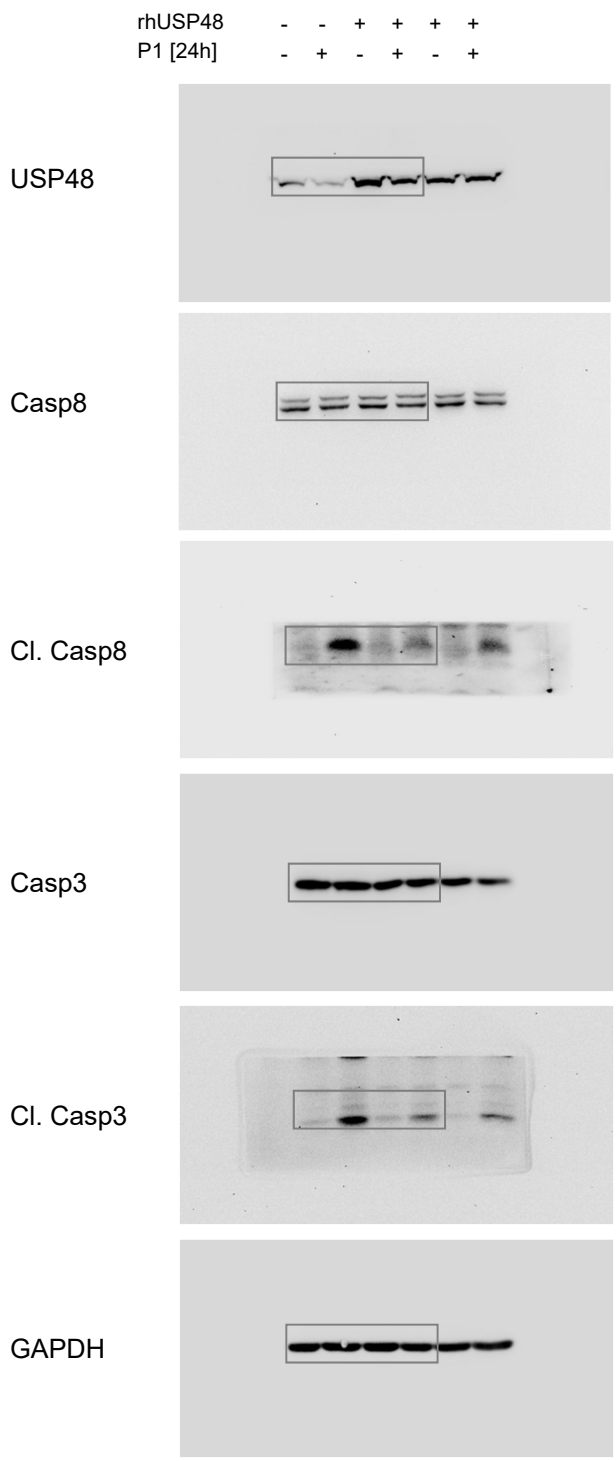
Cl. Casp3



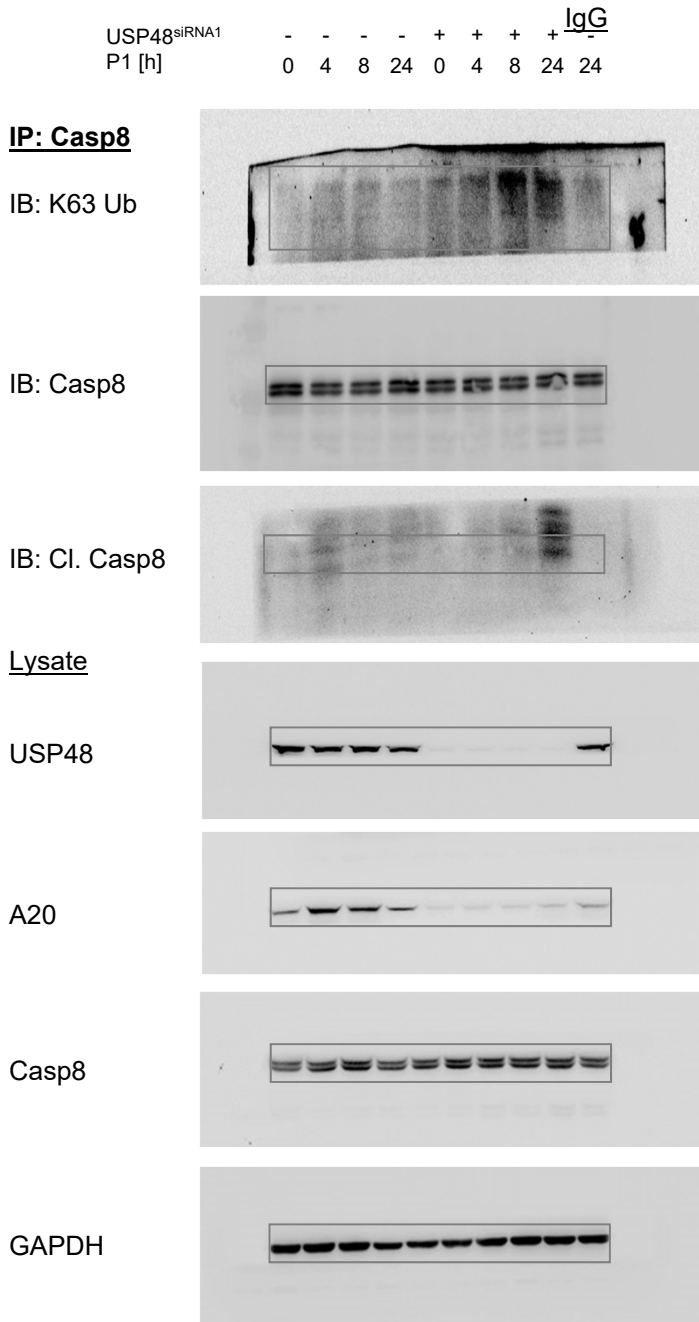
GAPDH



Uncropped blot for Fig 3d

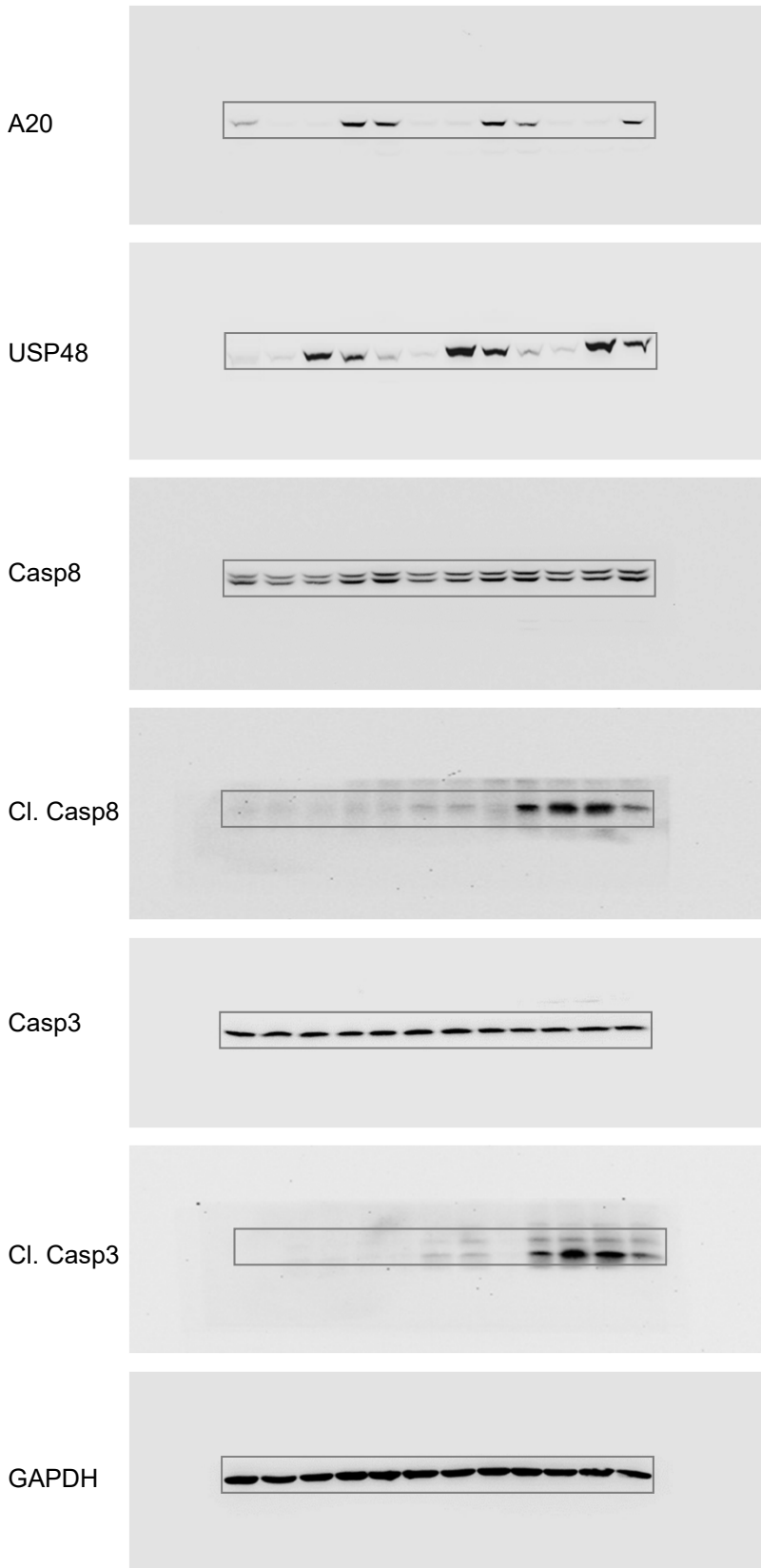


Uncropped blot for Fig 3e



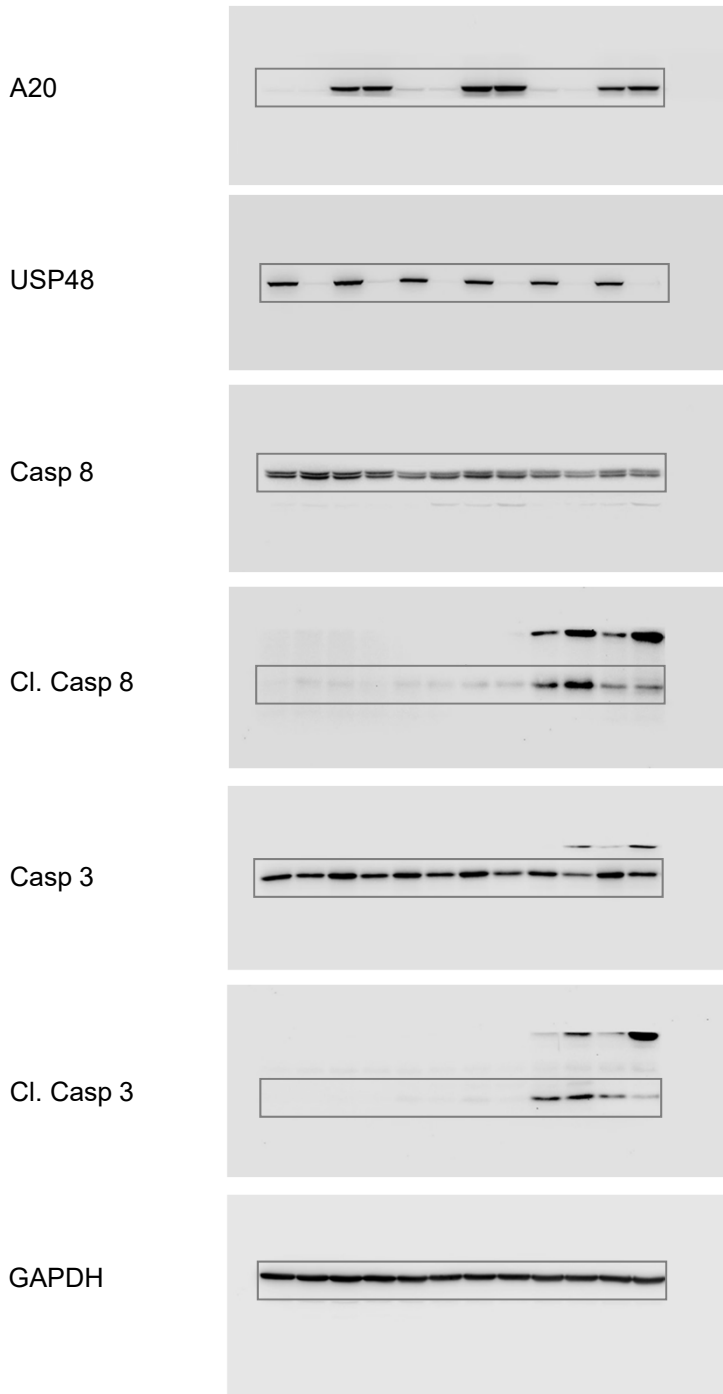
Uncropped blot for Fig 4a

A20 ^{siRNA}	-	+	+	-	-	+	+	-	-	+	+	-
rhUSP48	-	-	+	+	-	-	+	+	-	-	+	+
P1 [h]	-	-	-	-	8	8	8	8	16	16	16	16

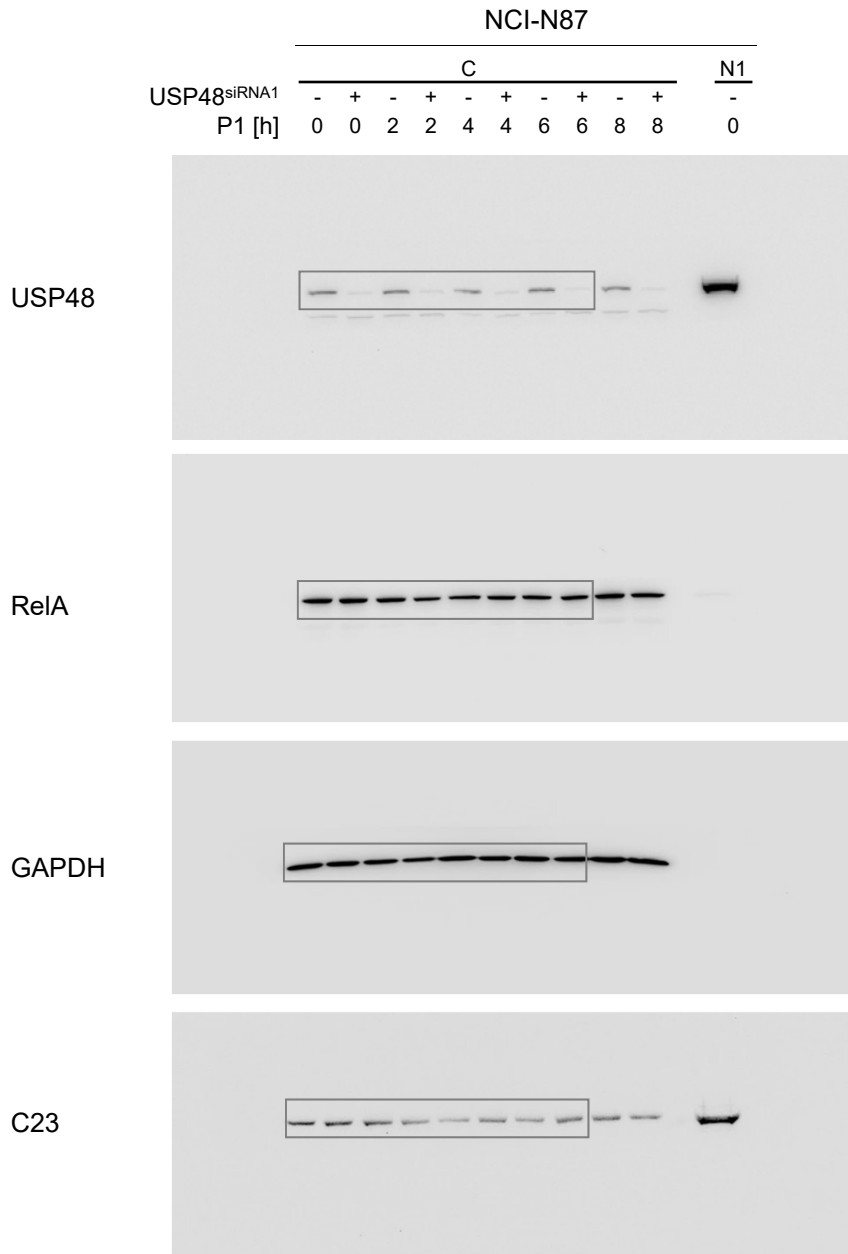


Uncropped blot for Fig 4c

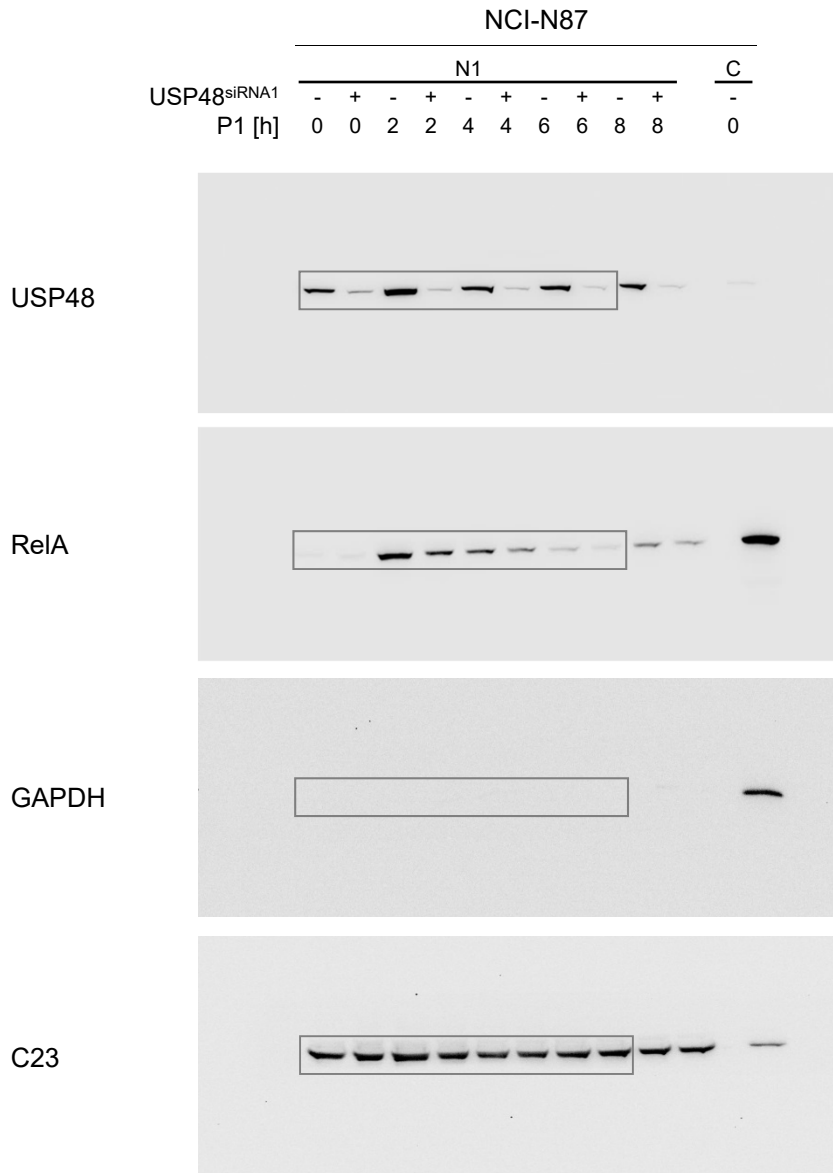
USP48 ^{siRNA}	-	+	-	+	-	+	-	+	-	+	-	+
rhA20	-	-	+	+	-	-	+	+	-	-	+	+
P1 [h]	-	-	-	-	8	8	8	8	16	16	16	16



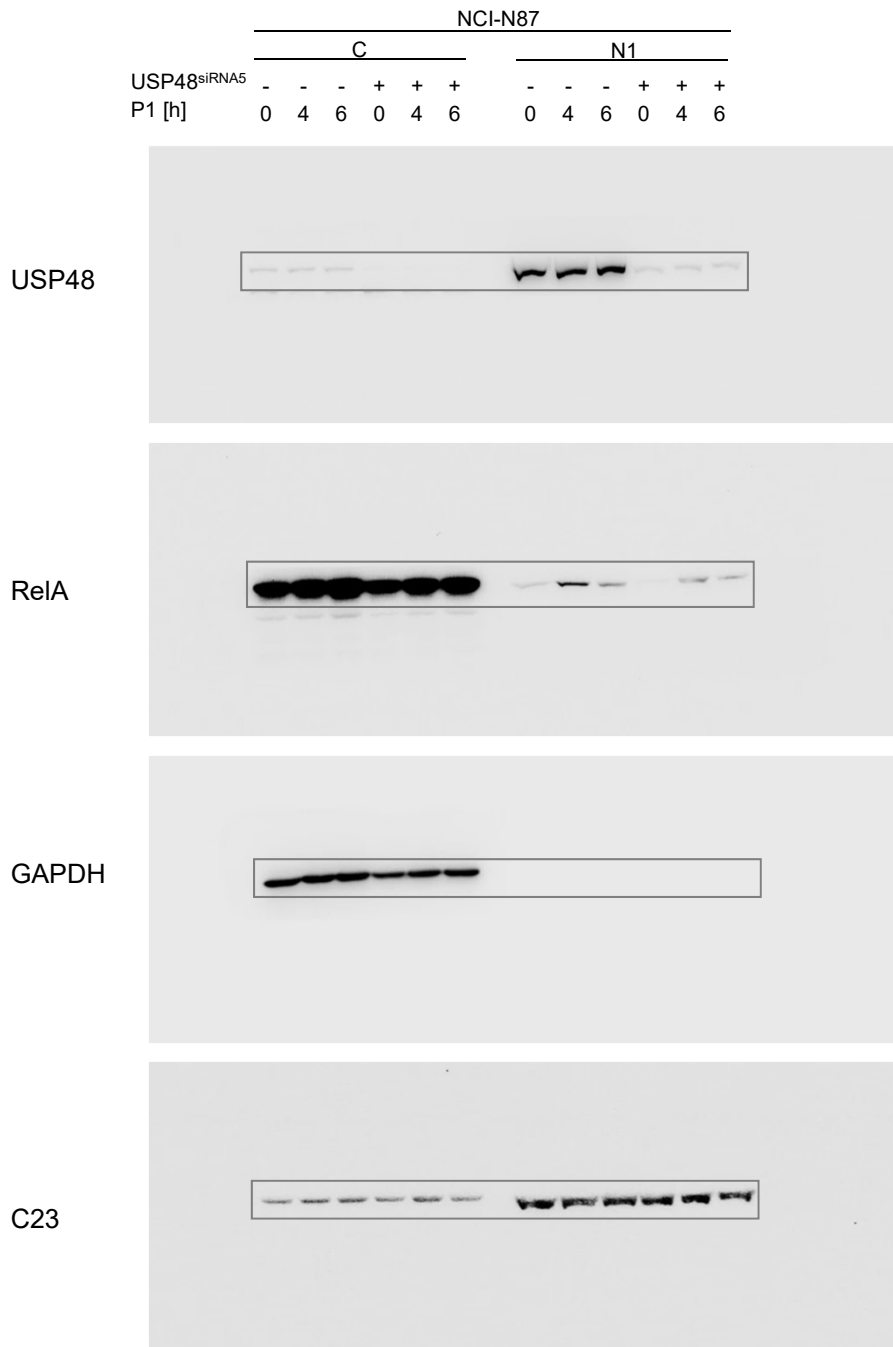
Uncropped blot for Fig S1a



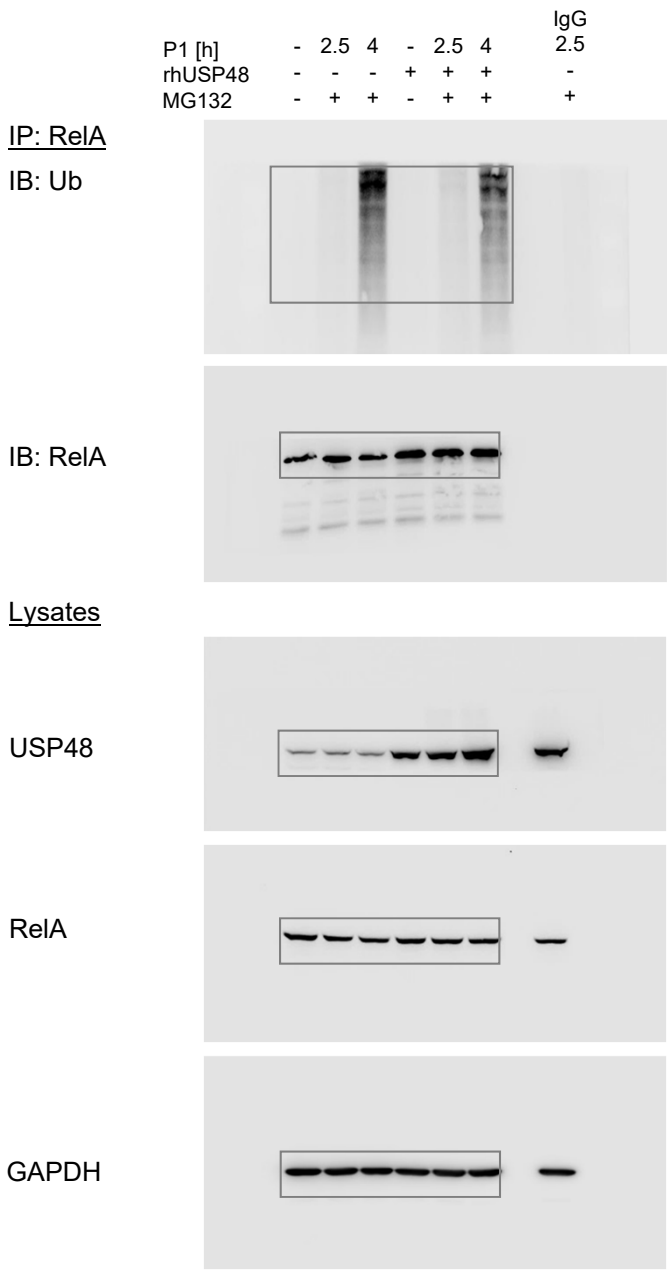
Uncropped blot for Fig S1a



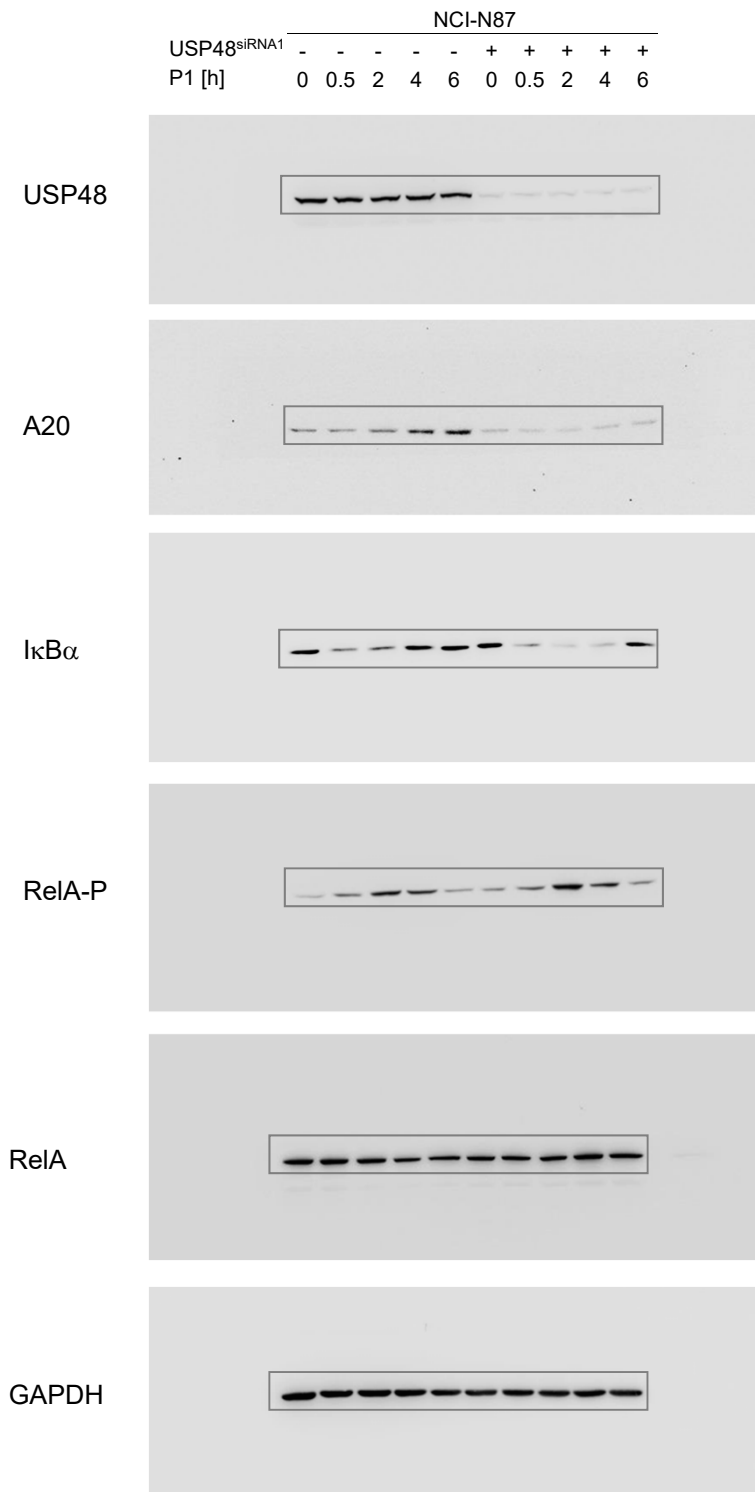
Uncropped blot for Fig S1b



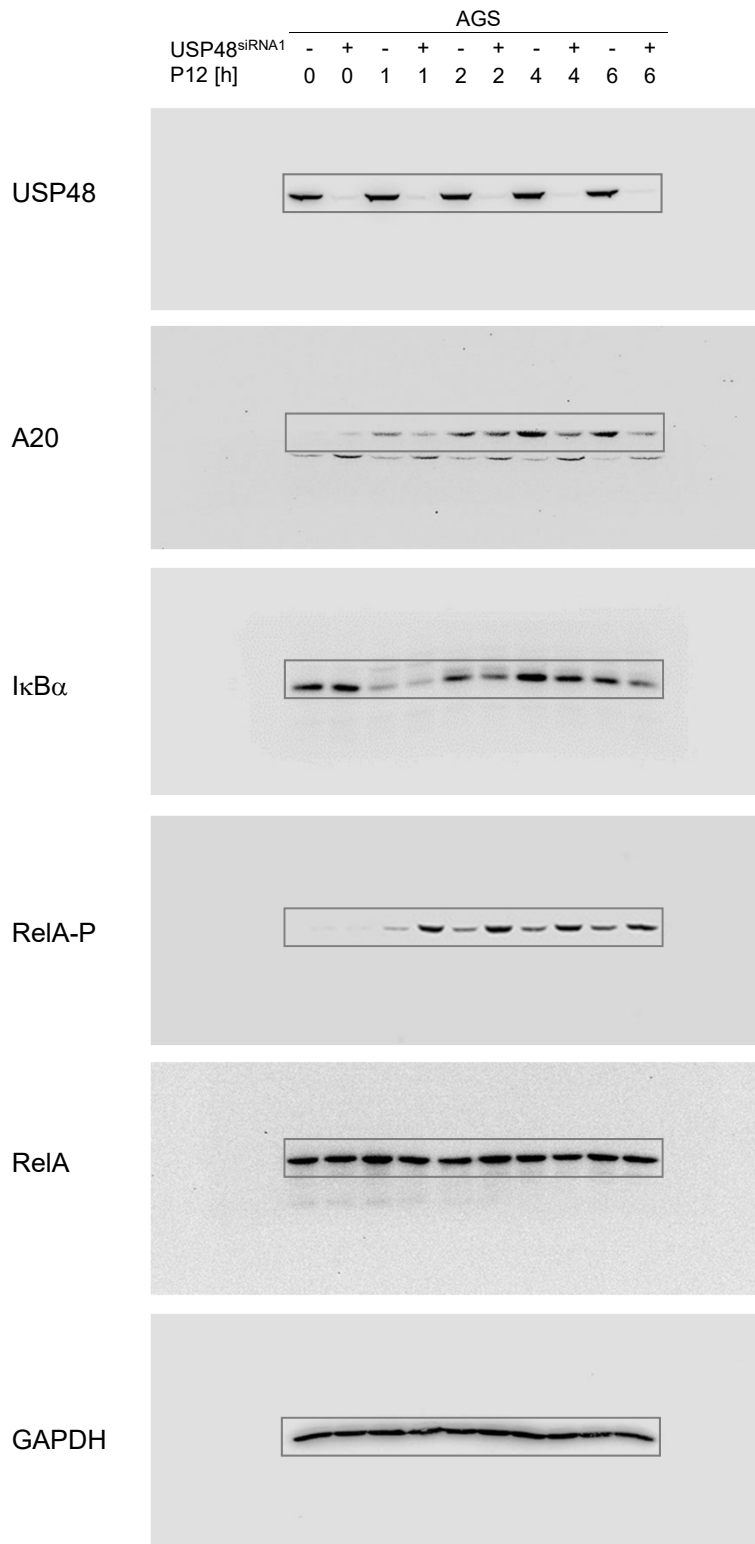
Uncropped blot for Fig S2



Uncropped blot for Fig S3a



Uncropped blot for Fig S3b



Uncropped blot for Fig S4a

scr ^{siRNA}	+	-	+	-	+	-	+	-
Casp8 ^{siRNA}	-	+	-	+	-	+	-	+
P1 [24h]	-	-	+	+	-	-	+	+

