Fig. S1. PrP^{Sc}/PDI colocalisation following prion exposure. PrP-224AlaMYC cells were fixed at serial time points up to 16 min exposure to prions and processed to reveal PrP^{Sc} and PDI. The proportion of PrP^{Sc} that co-localises with PDI was quantified (mean ± s.e.m., n=4).

Fig. S2. Brefeldin A blocks PrP^{Sc} trafficking to the PNC. (**A**) PrP-224AlaMYC cells were pretreated with Brefeldin A (BFA, 10 µg/ml) for 15 min then exposed to prions for 16 min prior fixation and processing to reveal PrP^{Sc} (green) and GM130 (red). Nuclei were visualised with DAPI (blue). Merged confocal images are shown. Scale bar: 10 µm. After 16 min in control conditions PrP^{Sc} adopts a typical steady state distribution at the plasma membrane and concentrated near the nucleus in the PNC. BFA disperses the Golgi and prevents PrP^{Sc} accumulation in the perinuclear compartment. (**B**) Quantification of the proportion of cells showing typical steady state PrP^{Sc} distribution (the mean ± s.e.m., n=4 is shown). The data indicates PrP^{Sc} trafficking to the PNC is BFA sensitive. (**C**) Western blot analysis of lysates from ScPK1 cells treated with vehicle or BFA for 60 or 180 min. Blots were probed with anti-PrP or actin antibodies as indicated. PrP^{Sc} was revealed by limited proteinase K digests of lysates. BFA retards PrP maturation as shown by the appearance of faster migrating PrP species but full length PrP is still detectable after 60 and 180 min incubation. Note that PrP^{Sc} levels were maintained up to 60 min BFA treatment, only decreasing after prolonged exposure. This suggests the effects on PrP^{Sc} trafficking are not related to the reduction in levels, which only occur after longer BFA treatment.

Fig. S3. Effects of retrograde transport inhibitors on CTB trafficking. (**A**) PrP-224AlaMYC cells were pretreated with vehicle, BFA or Retro-2 for 15 min then CTB (10 µg/ml) was added. The cells were fixed after 30 min and processed to reveal CTB (red) and GM130 (a Golgi marker – green). Nuclei were visualised with DAPI (blue). Merged confocal images are shown. Scale bar: 10 µm. Under control conditions, CTB accumulates in the perinuclear compartment, closely colocalising with GM130. BFA disperses GM130 staining into distinct Golgi ministacks and inhibits CTB trafficking to the perinuclear compartment. Retro-2 has no gross effects on GM130 distribution but prevents CTB accumulation near the nucleus. (**B**) RNAi mediated knockdown of *Vps35*. Western blot analysis of lysates from PrP-224AlaMYC cells transfected with RNAi directed against VPS35 at the indicated concentrations (nM). The blots were probed with anti-VPS35 or actin antibodies as indicated. 100 nM RNAi oligo treatment resulted in approximately 70% knockdown of VPS35 expression.

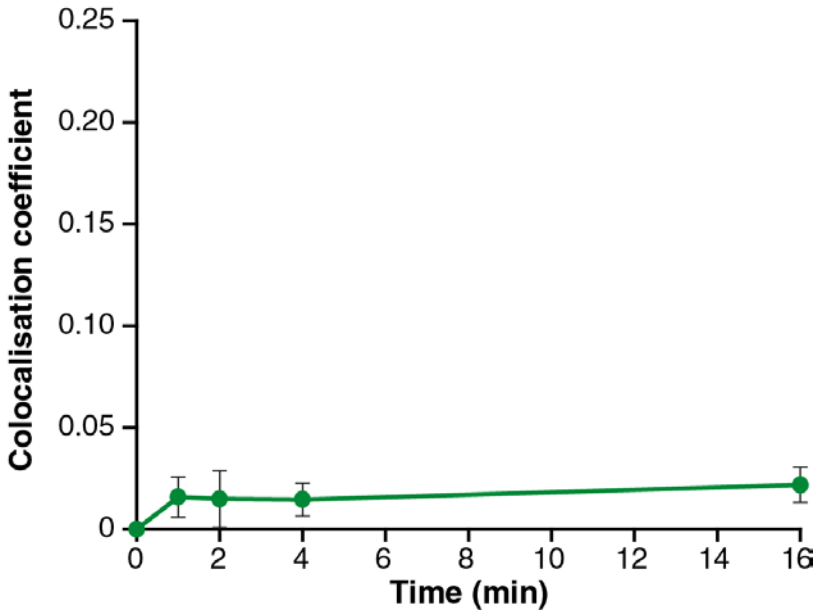
Fig. S4. Effects of lysosomal protease inhibitors on PrP^{Sc} levels and distribution. PrP-224AlaMYC cells were pretreated with vehicle, leupeptin, E64d or pepstatin A for 15 min then exposed to RML prions for 180 min. The cells were fixed and processed to reveal PrP^{Sc} and LAMP1 (red). Nuclei were visualised with DAPI (blue). Merged confocal images are shown. Scale bar: 10 µm. Under control conditions PrP^{Sc} is concentrated in the perinuclear compartment and shows limited colocalisation with LAMP1. E64d and leupeptin treatments have pronounced effects on PrP^{Sc}, increasing its levels in the cell periphery coincident with LAMP1 immunostaining. Pepstatin A treated cells looked similar to cells exposed to vehicle.

Fig. S5. Brefeldin A treatment inhibits PrP^{Sc} trafficking to the lysosome. (**A**) PrP-224AlaMYC cells were pre-treated with vehicle, BFA, leupeptin and E64d or BFA and leupeptin and E64d combined for 15 min then exposed to prions for 60 min prior fixation and processing to reveal PrP^{Sc} (green) and LAMP1 (red). Nuclei were visualised with DAPI (blue). Merged confocal images are shown. Scale bar: 10 µm. (**B**) PrP-224AlaMYC were treated as in panel A and the proportion of PrP^{Sc} that colocalises with LAMP1 was quantified (mean ± s.e.m., n=3, $^{**}P<0.01$, two tailed *t*-test). BFA has no effect on LAMP1 distribution but causes PrP^{Sc} to assume a dispersed distribution (BFA), preventing the build-up in the perinuclear compartment observed in control conditions (control). Despite this, a relatively low co-localisation with LAMP1 was detected. In contrast, leupeptin and E64d treatment causes a notable increase PrP^{Sc} co-localisation with LAMP1. Combined treatment with BFA and leupeptin and E64d resulted in a phenotype resembling single BFA treatment (i.e. PrP^{Sc} assumed a dispersed distribution with a relatively low co-localisation with LAMP1). PrP^{Sc} accumulation in the lysosome is BFA sensitive suggesting it is dependent on an intact Golgi.

Fig. S6. Proteasome activity in PrP-224AlaMYC cells treated with epoxomycin and MG262. (**A**) PrP-224AlaMYC cells treated with epoxomycin (1 µM) and MG262 (1 µM) for 2 h prior to the addition of MV152 (1 µM) for 1 h. Nuclei were visualised with DAPI (blue). Merged confocal images are shown. Scale bar: 10 µm. Little probe (red) was incorporated into the proteasome after epoxomycin and MG262 treatment. (**B**) Immunoblots of lysates from PrP-224AlaMYC cells treated with epoxomycin (1 µM) and MG262 (1 µM) 180 min. Blots were developed with the indicated antibodies. No changes in PrP^{C} or 20S levels were observed.

Fig. S7. PrP^{Sc} degradation in ScPK1 cells is dependent on lysosomal proteolysis, autophagy and proteasomal activity. (**A**) Immunoblots of lysates from ScPK1 cells treated with vehicle (control), leupeptin (200 µM) and E64d (2 µM), bafilomycin A (Baf A – 20 nM) or 3-methyladenine (3-MA – 10 mM) for 180 min. The blots were developed with the indicated antibodies. PrP^{Sc} was assayed after limited proteinase K digestion of cell lysates (+PK). Note the increased LC3II levels following Baf A treatment, indicating a block in autophagic flux. (**B**) Immunoblots of lysates from ScPK1 cells treated with vehicle (control), epoxomicin (Epox – 1 µM) or MG262 (1 µM) for 180 min and treated as in panel A. (**C**) Immunoblots similar to those in panels A and B were quantified to determine PrP^{Sc} levels (normalised to actin, mean ± s.e.m., n=4). Asterisks indicate significant difference from control levels ($P<0.05$, two tailed *t*-test). Unlike acutely infected PrP-224AlaMYC cells inhibitors of autophagy and the proteasome cause PrP^{Sc} accumulation.

Fig. S8. Surface PrP^{Sc} labelling and detection. Prion infected ScPK1 cells (+RML) and control PK1 cells not exposed to prions (−RML) were surface labelled with NHS-sulpho-biotin at 4°C for 30 min. Cell lysates were analysed directly, after limited proteinase K digestion (+PK), or after subsequent centrifugation to enrich for detergent-insoluble proteins (Det. P). Western blots were probed with anti-PrP antibodies or with NeutrAvidin-HRP to visualise biotinylated proteins. Proteinase K-resistant PrP (PrP^{Sc}) was recovered in the detergent-insoluble pellet from prion infected cells but not from control cells. The two right hand panels show the same sample made from ScPK1 lysates run in adjacent lanes on the same gel. The lanes were cut out and probed with anti-PrP antibodies or with NeutrAvidin-HRP as indicated (five times the amount of protein was loaded onto the lane probed with NeutrAvidn-HRP). Proteinase K-resistant, detergent-insoluble biotinylated proteins migrate with a similar molecular weight to PrP^{Sc}.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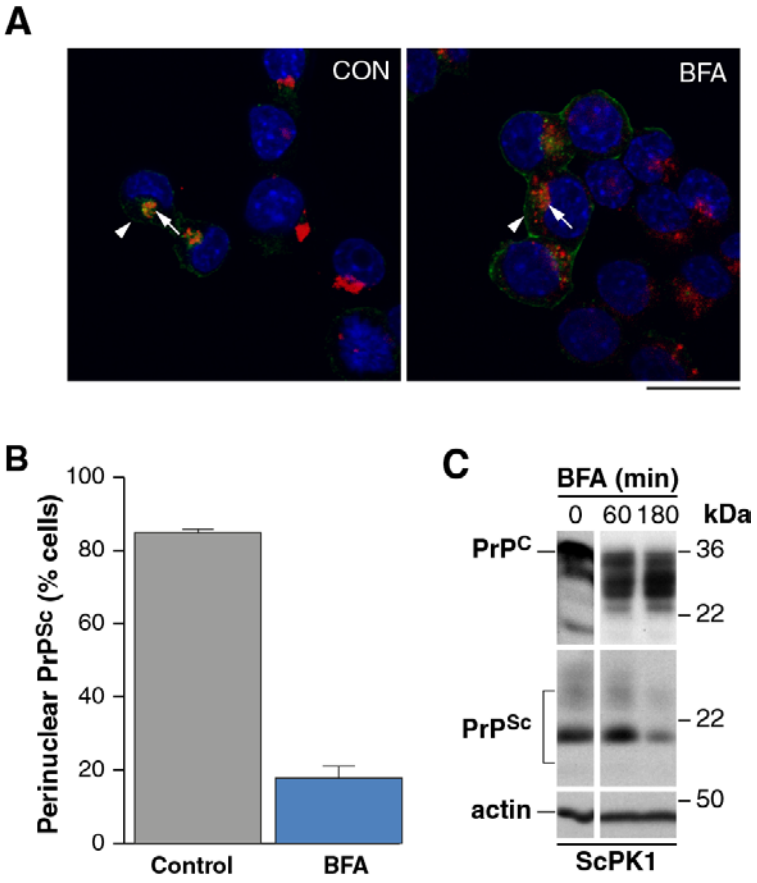
Time (min)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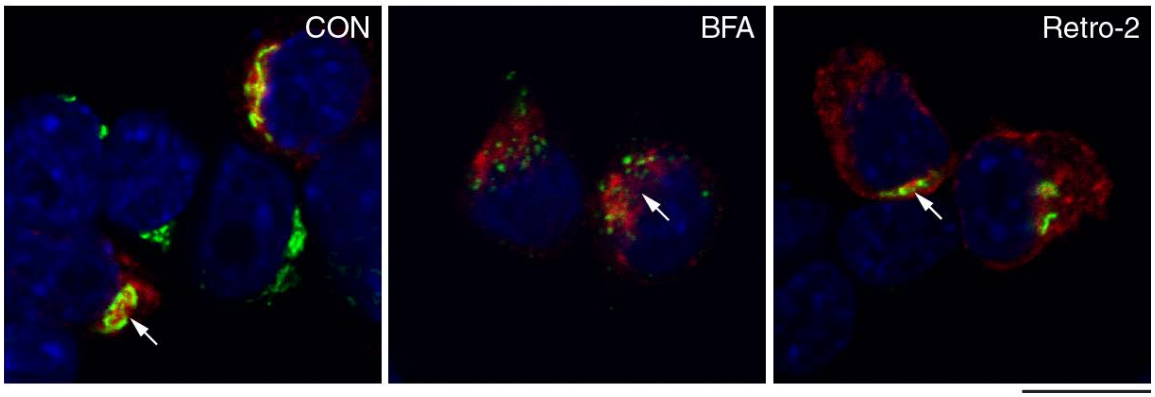

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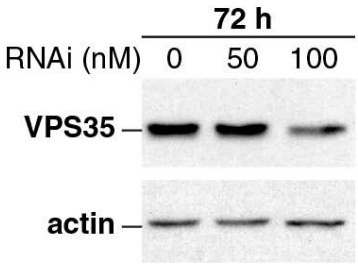

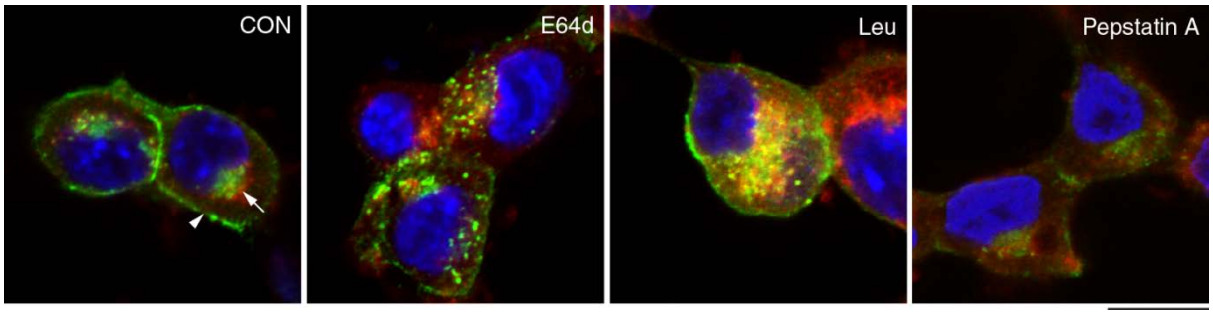
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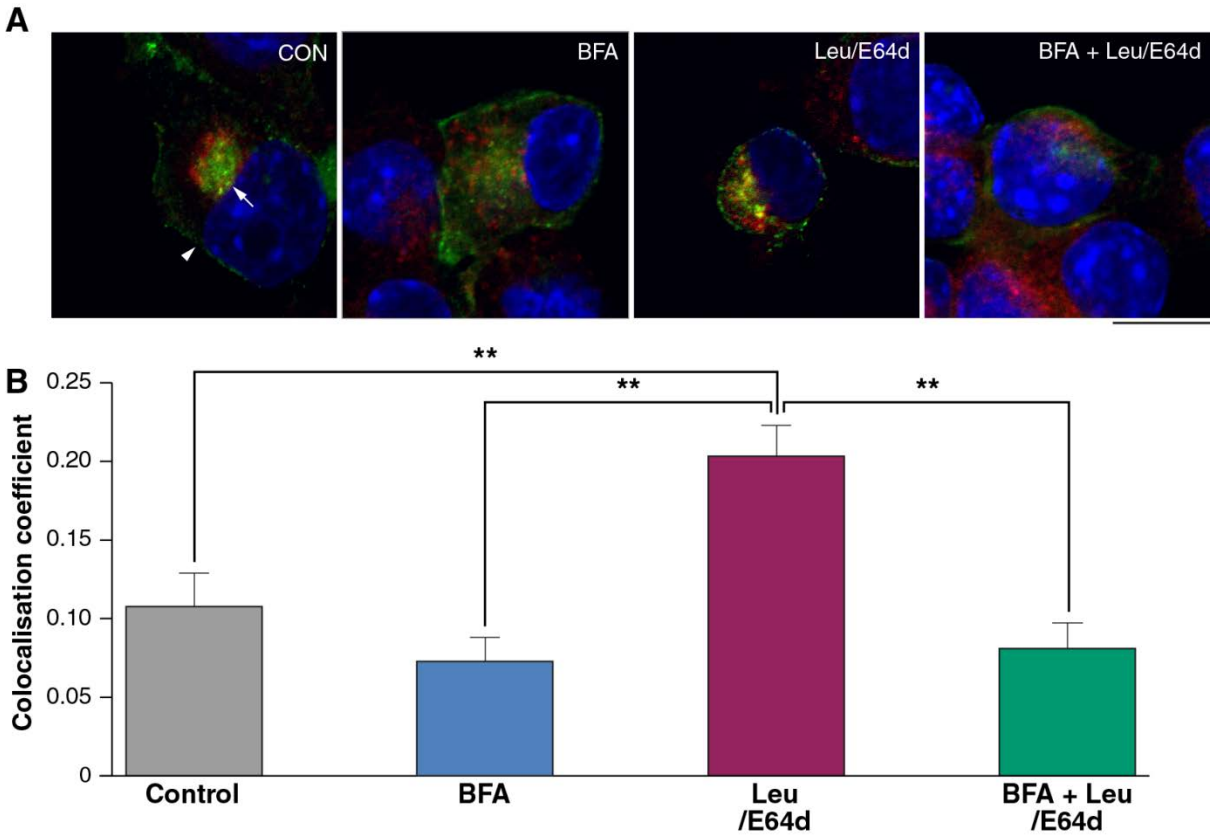
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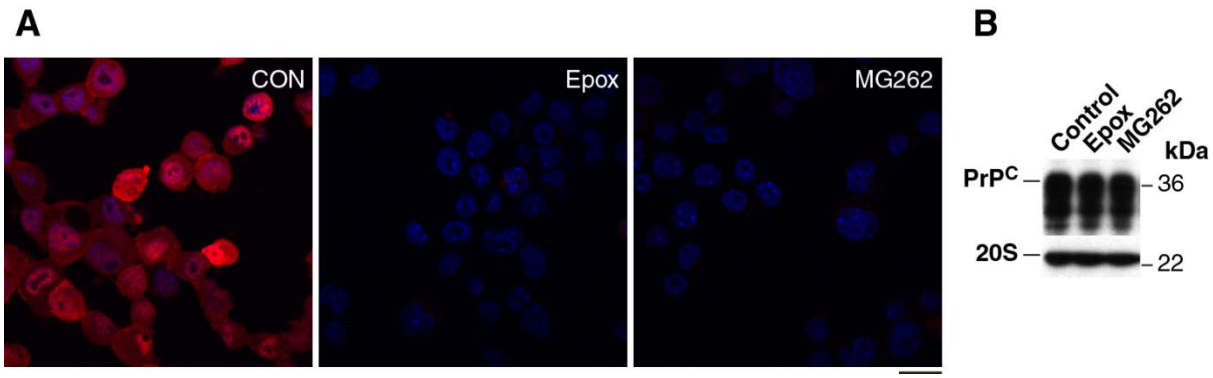
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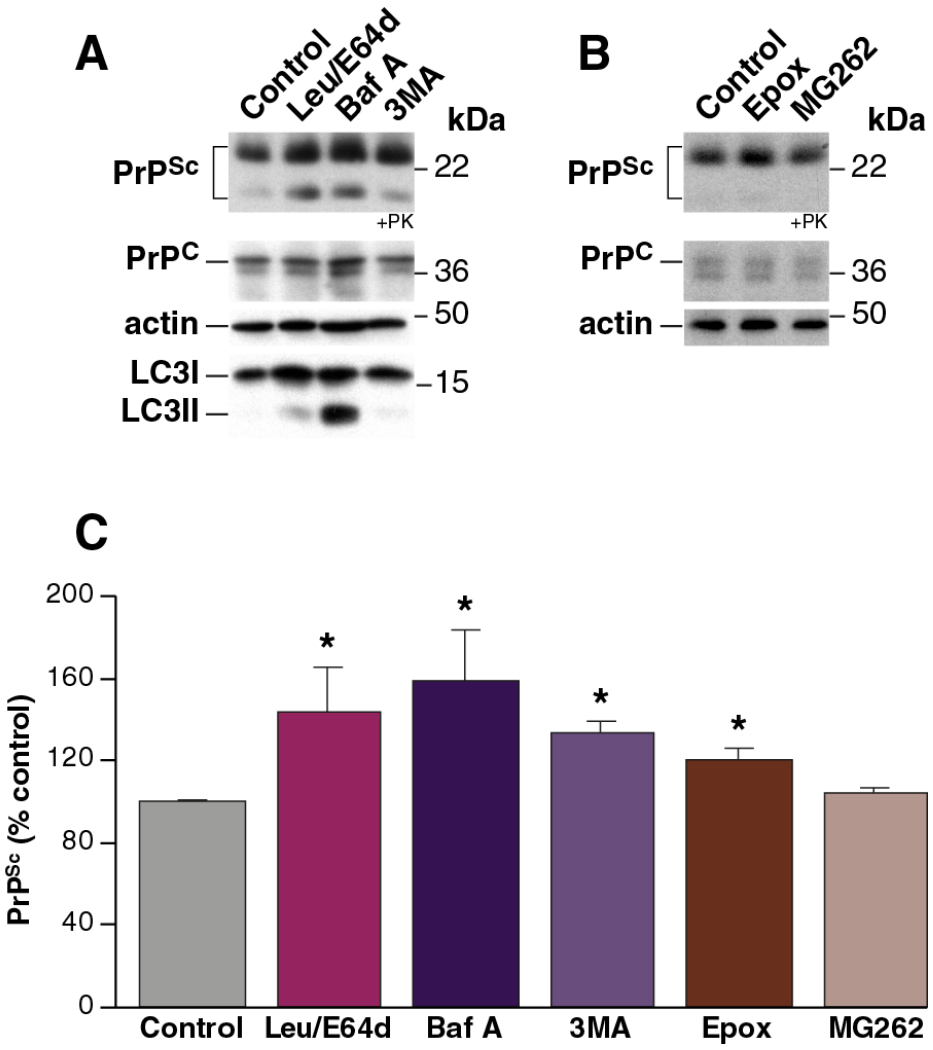
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15
B
Control
Epox
MG262
kDa
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22
+PK
PrP^C
36
actin
50
C
PrP^Sc (% control)
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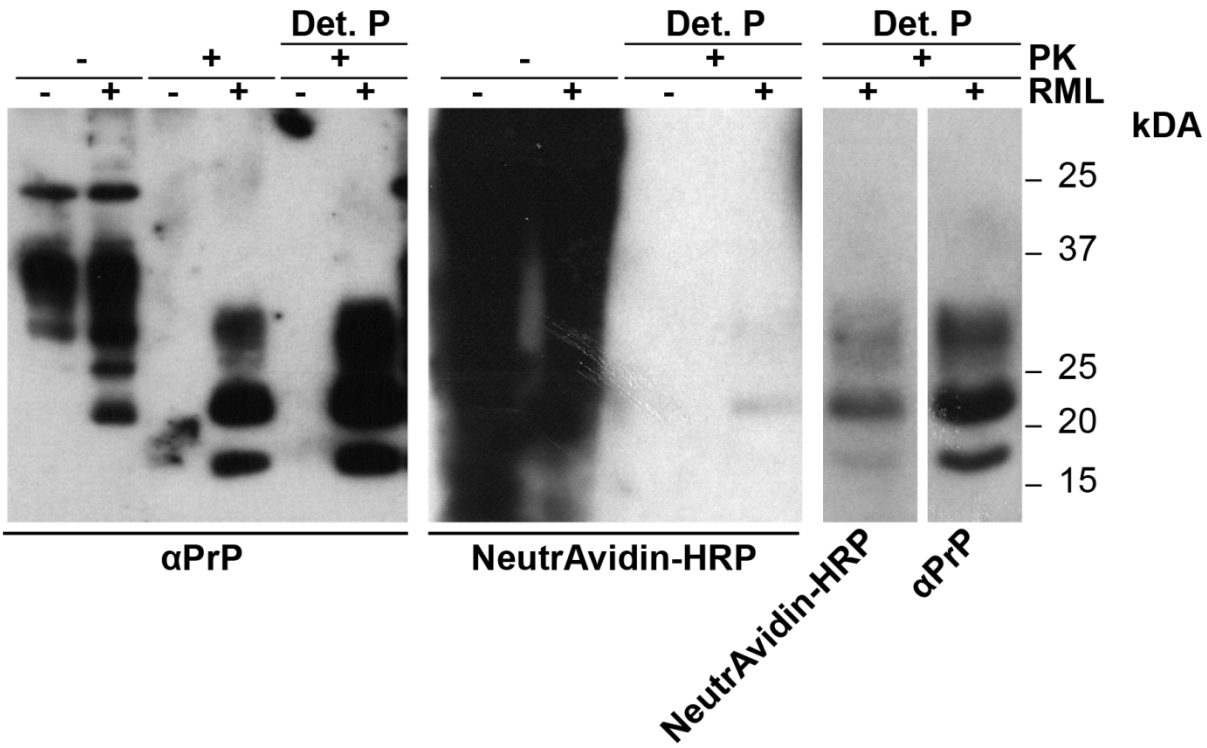
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NeutrAvidin-HRP
NeutrAvidin-HRP
αPrP

Table S1. Antibodies

	Detected protein	Source	Stock concentration (mg/ml)	Dilution	Isotype
Anti-PrP* (ICSM18)	PrP (Prion protein)	D-GEN	3	1:1000	mouse IgG1
Anti-PrP (ICSM35)**	PrP (Prion protein)	D-GEN	3	1:10,000	mouse IgG1
Anti-EEA1	Early endosome antigen-1	Cell Signaling		1:250	rabbit IgG
Anti-βactin	βactin	Sigma	N/A	1:10,000	mouse IgG1
Anti-LAMP-1	Lysosome-associated membrane protein 1	Santa Cruz	0.2	1:500	rat IgG2a
Anti-GM130	Golgi matrix protein of 130 KDa	BD Bioscience	0.25	1:500	mouse IgG1
Anti-TfR	Transferrin receptor	Invitrogen	0.5	1:1000	mouse IgG1
Anti-TGN46	TGN46	AbD SeroTec	N/A	1:200	rabbit IgG
Anti-MYC (9B11)	MYC-tagged PrP (Prion protein)	Cell Signaling		1:1500	mouse IgG2a
Anti-Rab 7	Rab7	Cell Signaling	N/A	1:250	rabbit IgG
Anti-Rab11	Rab11	Cell Signaling	N/A	1:250	rabbit IgG
Anti-VPS35	VPS35	AbCam	N/A	1:100* 1:500**	rabbit IgG
Anti-LC3B	LC3B	Cell Signaling	N/A	1:250* 1:1000**	rabbit IgG
Anti-ERK1/2	ERK1/2	Cell Signaling	N/A	1:1000	rabbit IgG

*Immunofluorescence use

**Immunoblot use