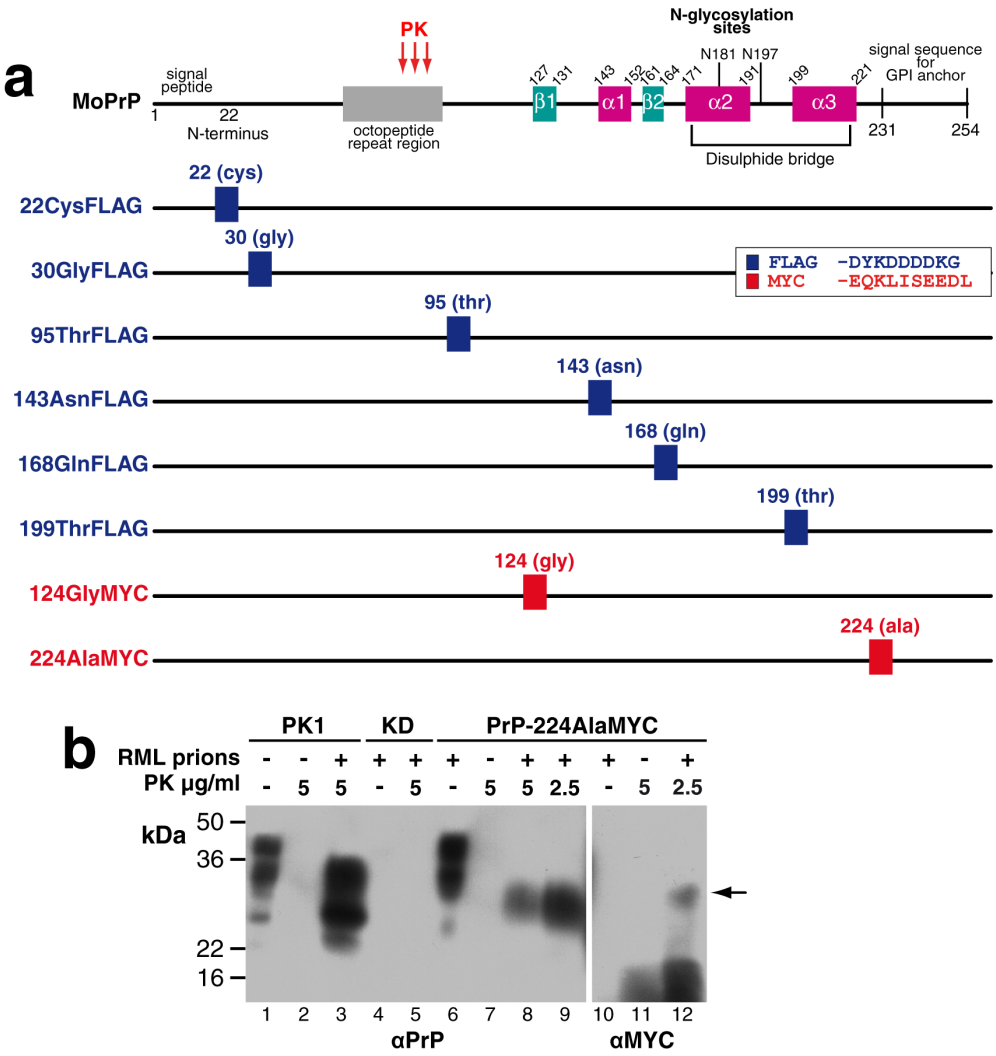
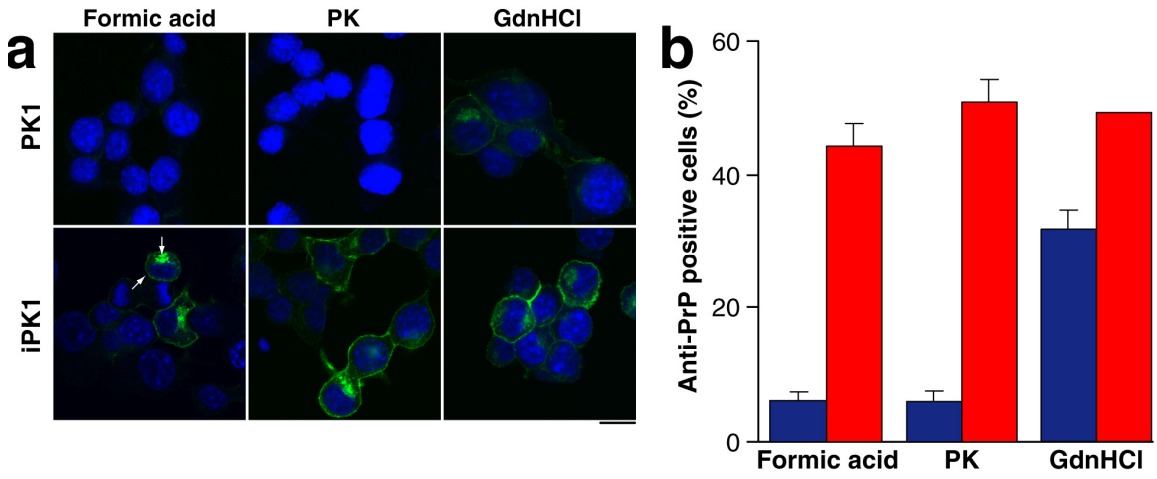
Supplementary Information



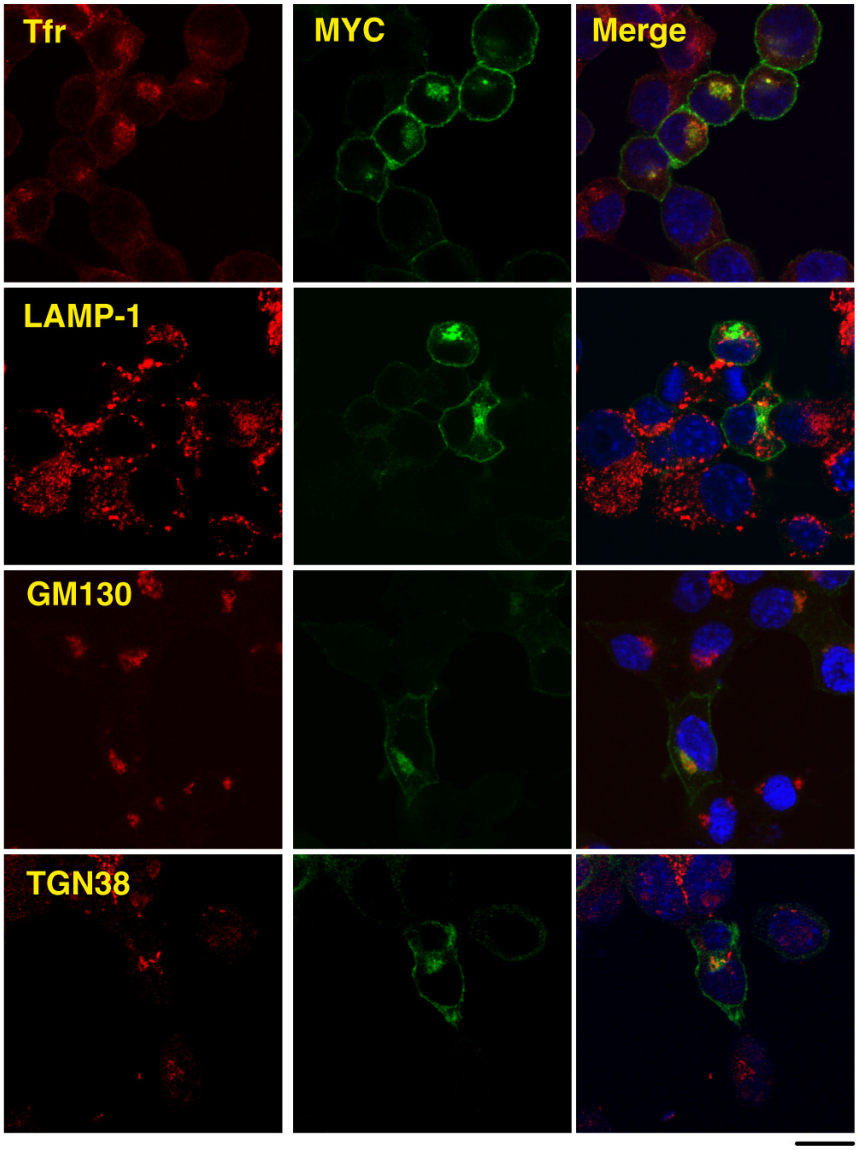
Supplementary Figure S1 Identification of PrP chimerae that support PrP^{Sc} propagation
a) Schematic representation of the domain structure of murine PrP. The insertion sites of the six FLAG tag and the two MYC tag constructs are detailed.
b) Western blots developed with anti-PrP and anti-MYC antibodies showing levels of PK resistant PrP^{Sc} in control and RML-prion infected PK1, PrP-KD and PrP-224AlaMYC cell extracts. The cell extracts were harvested after passage five and analysed undigested or subjected to PK digestion by the indicated concentrations of PK prior to SDS-PAGE. RML prion infected PK1 cells contain PK-resistant fragments characteristic of PrP^{Sc} (lane 3) whereas uninfected cells do not (lane 2). PrP-KD cells exposed to RML prions do not contain PrP^{Sc} demonstrating that no residual inocula derived PrP^{Sc} remains in the cells (lane 5). Consistent with the SCA data RML prion infected PrP-224AlaMYC cell extracts contain low levels of PrP^{Sc} such that the most abundant protease resistant fragment in RML prion infected cells was detected (lane 8). No PK resistant fragments were detected in uninfected cells treated in the same way (lane 7). Reducing the PK concentration increases the levels of MYC tagged-PrP^{Sc} (lane 9) and allows detection with anti-MYC antibodies (lane 12).



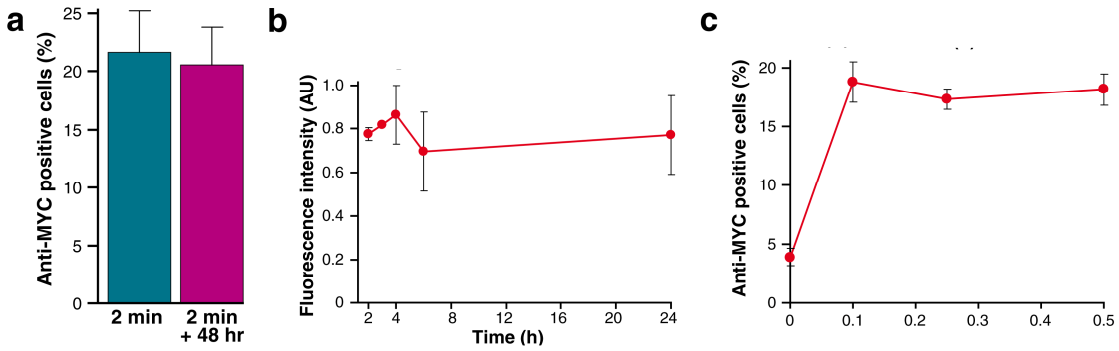
Supplementary Figure S2 Comparison of the methods to visualise PrP^{Sc} in RML prion infected cells.

a) Uninfected PK1 cells (top panels) or iPK1 cells (from chronically RML prion infected cultures, bottom panels) were fixed and treated with 98 % formic acid, proteinase K (PK) or guanidinium hydrochloride (GdnHCl) prior to staining with anti-PrP antibodies (green) and counter staining with DAPI (blue). Merged confocal images are shown; scale bar 20 µm. Uninfected cells show no staining after formic acid and PK treatment indicating that the PrP^{C} has been removed. Some residual staining was observed after GdnHCl treatment. RML prion infected cells contain formic acid and PK resistant PrP (PrP^{Sc}) with a characteristic plasma membrane/perinuclear compartment distribution (arrows).

b) Quantification of randomly chosen cell fields after the indicated treatments. Quantification of randomly chosen cell fields from uninfected PK1 cells (blue bars) or chronically RML prion infected ScPK1 cells (red bars). Approximately half of the RML prion infected PK1 cells contained PrP^{Sc}. The low level of background staining observed in the PK1 cells after formic acid and PK treatment is mostly attributable to clumped cells where access to the PrP^{C} is restricted. GdnHCl treatment was less effective at removing PrP^{C} staining. The mean ± SEM from three independent experiments are shown.



Supplementary Figure S3 Steady state subcellular distribution of MYC-tagged PrP^{Sc}
Chronically RML prion infected PrP-224AlaMYC cells were fixed and treated with 98 % formic acid prior to staining with anti-MYC antibodies (green) and marker antibodies for various cell compartments as indicated (red); the cells were counter stained with DAPI. Single channels and merged confocal images are shown as indicated; scale bar 20 µm. Partial colocalisation with markers for recycling endosomes (Tfr), lysosomes (LAMP1), Golgi stack (GM130), and trans- Golgi network (TGN38) was observed.

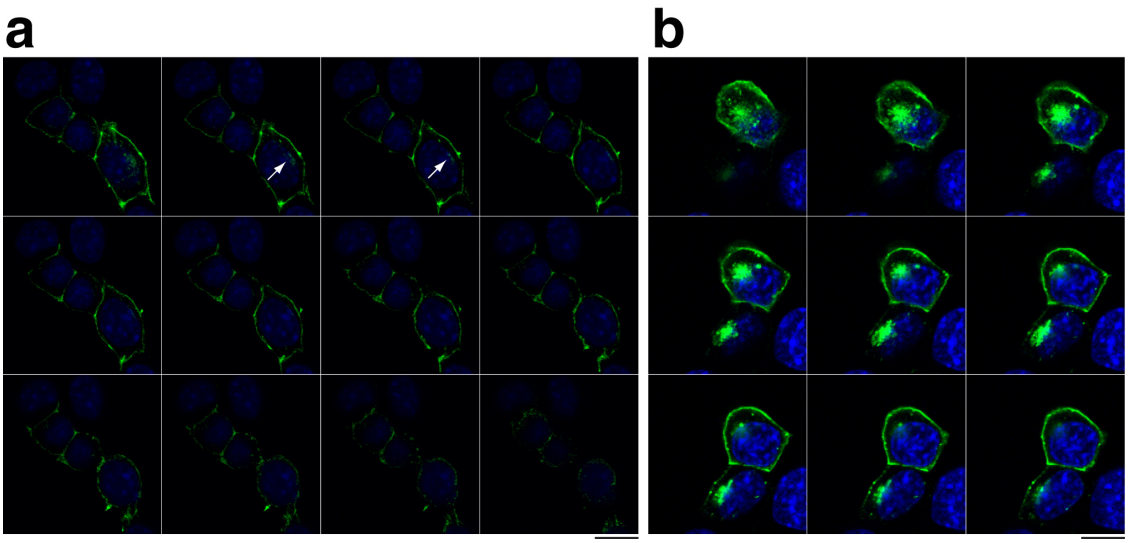


Supplementary Figure S4 Characterisation of PrP^{Sc} formation and propagation in PrP-224AlaMYC cells

a) Short RML prion exposure is sufficient to generate cells that continue to propagate PrP^{Sc} in the absence of RML prion seed. PrP-224AlaMYC cells were exposed to RML prions for 2 minutes and fixed immediately or washed and cultured in fresh media (without prions) for 48 h prior to fixation and formic acid extraction. The proportion of anti-MYC positive (RML prion infected) cells fixed at the indicated times was quantified. The mean ± SEM from three independent experiments are shown. The level of prion infected cells observed after 2 minutes RML prion exposure was maintained for 48 h in the absence of further prions, indicating the cells are irreversibly prion infected and stably propagate PrP^{Sc}.

b) PrP^{Sc} attains steady state levels rapidly following RML prion exposure. PrP-224AlaMYC cells were exposed to RML prions for the indicated times, fixed and formic acid extracted. The levels of anti-MYC label incorporated into individual cells (proportional to the PrP^{Sc} content) were measured using Volocity software. The mean ± SEM from three independent experiments are shown. PrP^{Sc} levels have attained steady state by 2h and do not change up to 24h. This suggests stable PrP^{Sc} metabolism has been established in 2 h or less.

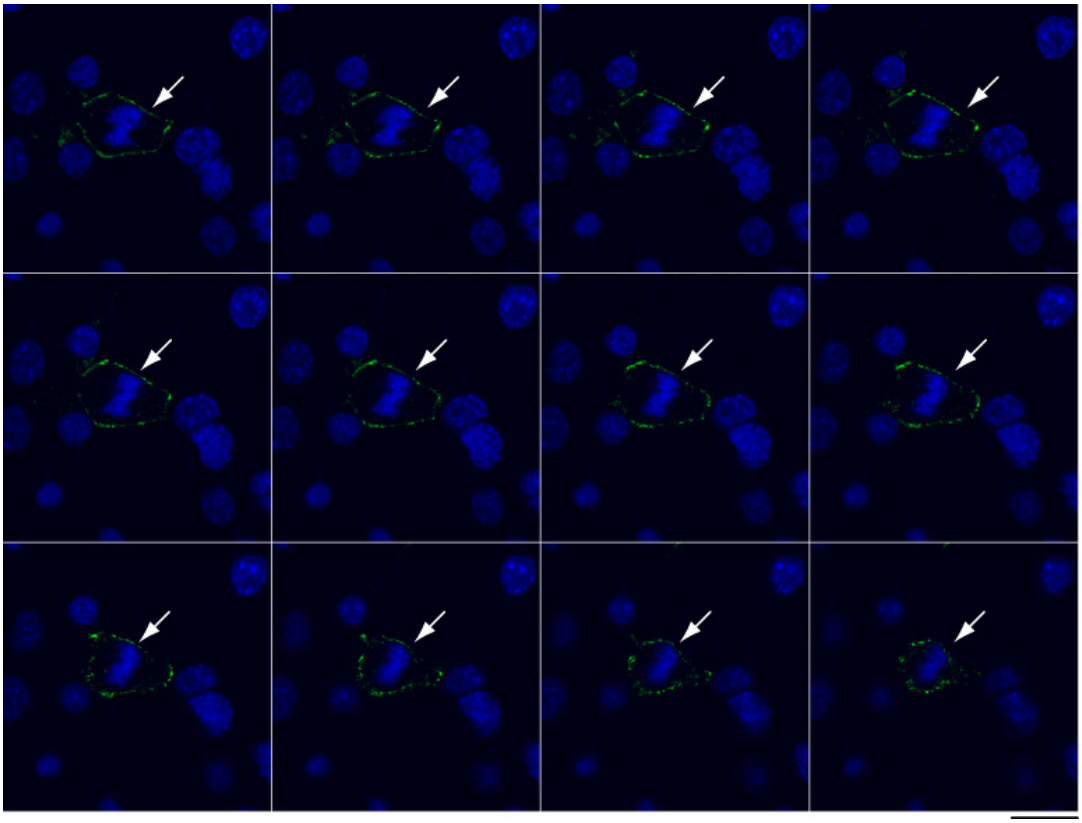
c) Incubations with increased RML inocula concentrations do not result in increased prion infection. PrP-224AlaMYC cells were exposed to various RML prion inocula concentrations for 180 minutes prior to fixation and formic acid extraction. The proportion of anti-MYC positive (RML prion infected) cells generated by each inocula concentration was quantified. The mean ± SEM from three independent experiments are shown. A maximum of 20% of our cells are infection competent and capable of MYC-tagged PrP^{Sc} production.



Supplementary Figure S5 Prion conversion first occurs at the cell surface within one minute of prion exposure

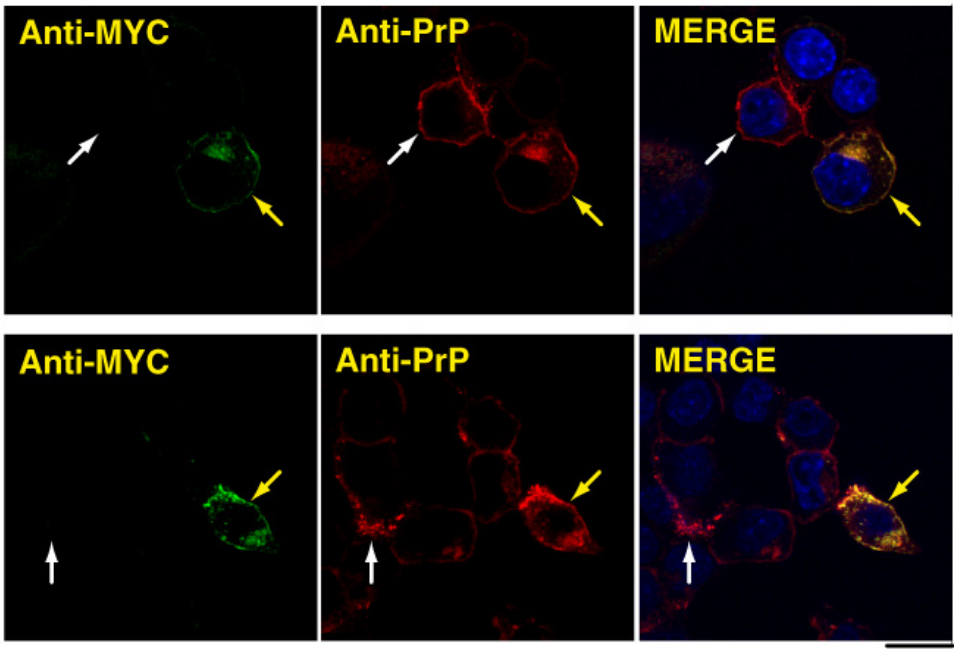
a) PrP-224AlaMYC cells were exposed to RML prions for one minute then fixed and formic acid extracted prior to staining with anti-MYC antibodies (green) and counter staining with DAPI (blue). Serial confocal slices are shown. The z stack series was used to generate the orthogonal projection shown in Fig. 4a (1 min); scale bar 10 µm. A cell fixed after one minute exposure to RML prions shows PrP^{Sc} immunostaining only at the cell surface. An adjacent cell in the field that has high levels of plasma membrane PrP^{Sc} also shows low intracellular levels of PrP^{Sc} (arrow).

b) Cells exposed to RML prions for 180 minutes were fixed and treated as in (a). Serial confocal sections show the typical distribution of PrP^{Sc} at steady state, with strong immunostaining at the plasma membrane and in the perinuclear region; scale bar 10 µm. The z stack series was used to generate the orthogonal projection shown in Fig. 4a (180 min).



Supplementary Figure S6 PrP^{Sc} forms at the cell surface.

Pre-cooled PrP-224AlaMYC cells were exposed to RML prions on ice for two minutes then fixed and formic acid extracted prior to staining with anti-MYC antibodies (green) and counter staining with DAPI (blue). Serial confocal sections are shown. The z stack series was used to generate the orthogonal projection shown in Fig 5b; scale bar 20 µm. Formic acid resistant PrP^{Sc} is formed on the cell surface of one of the cells in the field (arrow). No intracellular PrP^{Sc} was detected.



Supplementary Figure S7 Close association with inocula-derived PrP^{Sc} is not sufficient to generate *de novo* MYC-tagged PrP^{Sc} in the recipient cell

PrP-224AlaMYC cells were exposed to RML prions for 180 minutes then fixed and formic acid extracted. Merged confocal images of cells stained with anti-MYC (green) and anti-PrP antibodies (red) then counter stained with DAPI (blue) are shown; scale bar 20 µm. These images show that nearly all of the cells are closely associated with formic acid resistant PrP labelled with anti-PrP antibodies (red) but not anti-MYC antibodies; this is therefore PrP^{Sc} derived from the inocula. In the upper panel one of the cells shows particularly strong anti-PrP labelling at the plasma membrane (white arrow) while in the lower panel a cell with strong intracellular anti-PrP labelling is highlighted (white arrow). Despite this close association with high levels of inocula derived PrP^{Sc} these cells do not immunostain with anti-MYC antibodies (green) and therefore have not generated MYC-tagged PrP^{Sc}. An adjacent cell in each cell field (yellow arrow) is labelled with both anti-PrP antibodies and anti-MYC antibodies showing it has generated *de novo* MYC tagged PrP^{Sc}- these appear yellow in the merged image. This suggests that factors in addition to a simple physical association of cellular PrP^{C} with PrP^{Sc} affect prion conversion, an observation in agreement with previously published data.

Supplementary Table S1: Antibodies used in this study

	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-GFAP	GFAP	DAKO	N/A	1:1000	Rabbit IgG
Anti -ßactin	ßactin	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	1:400	mouse IgG1
Anti-TGN38	TGN38	AbD SeroTec	N/A	1:100	rabbit IgG
Anti-MYC (9B11)	MYC-tagged PrP (Prion protein)	Cell Signaling	N/A	1:1500	mouse IgG2a
Anti-CHC	Clathrin heavy chain	Cell Signaling	N/A	1:1000	Rabbit IgG

* used for immunofluorescence

** used for immunoblot and immunohistochemistry