SUPPLEMENTAL FIGURE LEGENDS

Figure S1. NM-HA does not form visible aggregates in N2a cells as detected by anti-Sup35p-N rat monoclonal antibody 7H5. N2a cells were transfected with a NM-HA construct and studied 48 h post transfection. NM-HA was detected using rat monoclonal antibody 7H5. ***A)*** N2a cells expressing NM-HA were fixed and stained for NM-HA (red). Nuclei were visualized using Hoechst staining (blue). No visible foci were observed. Untransfected cells were not stained by the antibody. Scale bar: 10 µm. ***B)*** Sedimentation assay of NM-HA. Lysate fractions were subjected to SDS gel electrophoresis. The fractions of insoluble (pellet = P) and soluble recombinant proteins (supernatant = S) are shown. As a control, untransfected cells were lysed and lysates fractioned into soluble and insoluble fractions. NM-HA remains exclusively in the supernatant. ***C)*** Proteinase K digestion of NM-HA. Cell lysates were subjected to PK treatment using the indicated amounts of PK. NM-HA was completely digested upon treatment with 5 µg/ml PK for 30 min.

Figure S2. PrP_{cyto}, N-PrP and NM-PrP aggregates are not located in intracellular organelles or vesicles. N2a cells expressing the constructs were fixed and stained for PrP_{cyto}, N-PrP and NM-PrP with mAb 3F4 48 h post transfection. Nuclei were visualized using Hoechst staining. Merged images demonstrate missing co-localization of aggregates with intracellular compartments. ***A)*** Co-staining for ER marker Calnexin. ***B)*** Transfected N2a cells were co-stained for Rab6. ***C)*** N2a cells were transfected with constructs and Rab4-GFP, a marker for early endosomes. ***D)*** N2a cells were co-transfected with the constructs and Rab11-GFP that visualizes late and recycling endosomes. Results were independent of the cell line. Scale bars: 10 µm.

Figure S3. Analysis of recombinantly produced PrP-M, NM and NM-PrP by SDS-PAGE and subsequent protein staining with Coomassie Blue. Samples of the respective proteins (2 µg of protein per lane) were subjected to SDS-PAGE after 0, 5, 21 and 28 days of incubation. LMW = Low Molecular Weight protein standard (BIORAD). Numbers of the lanes refer to days of incubation.

Fig. S1

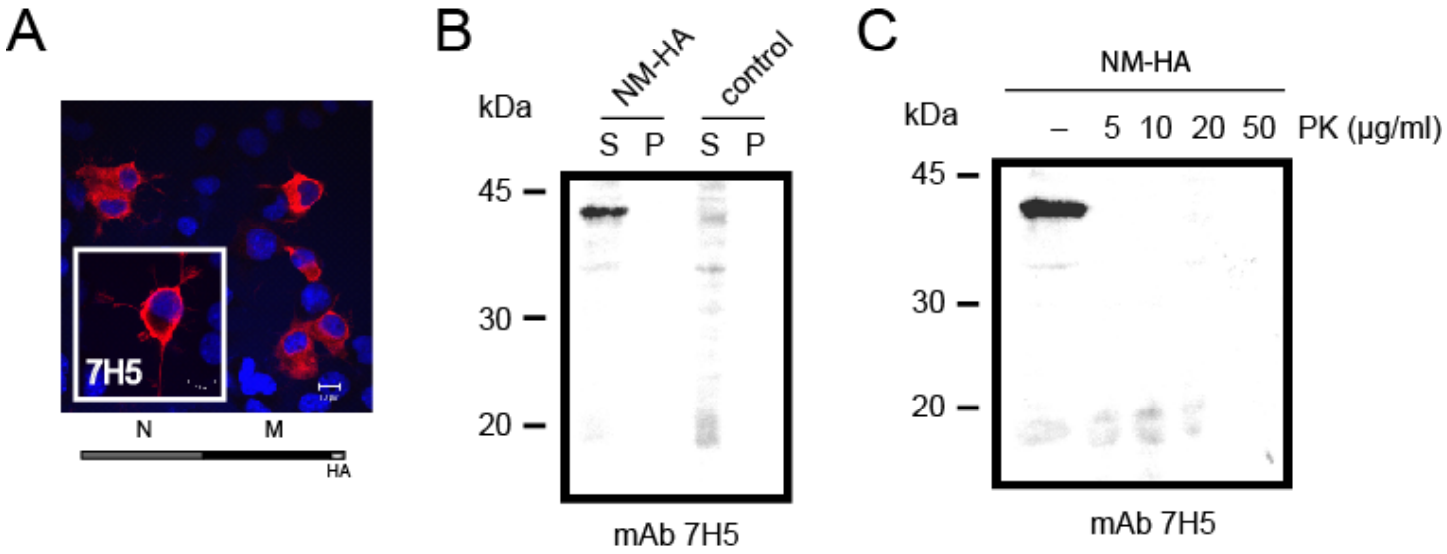

A
7H5
N
M
HA
B
kDa
NM-HA
control
S P S P
45
30
20
mAb 7H5
C
NM-HA
kDa
– 5 10 20 50 PK (μg/ml)
45
30
20
mAb 7H5

Fig. S2

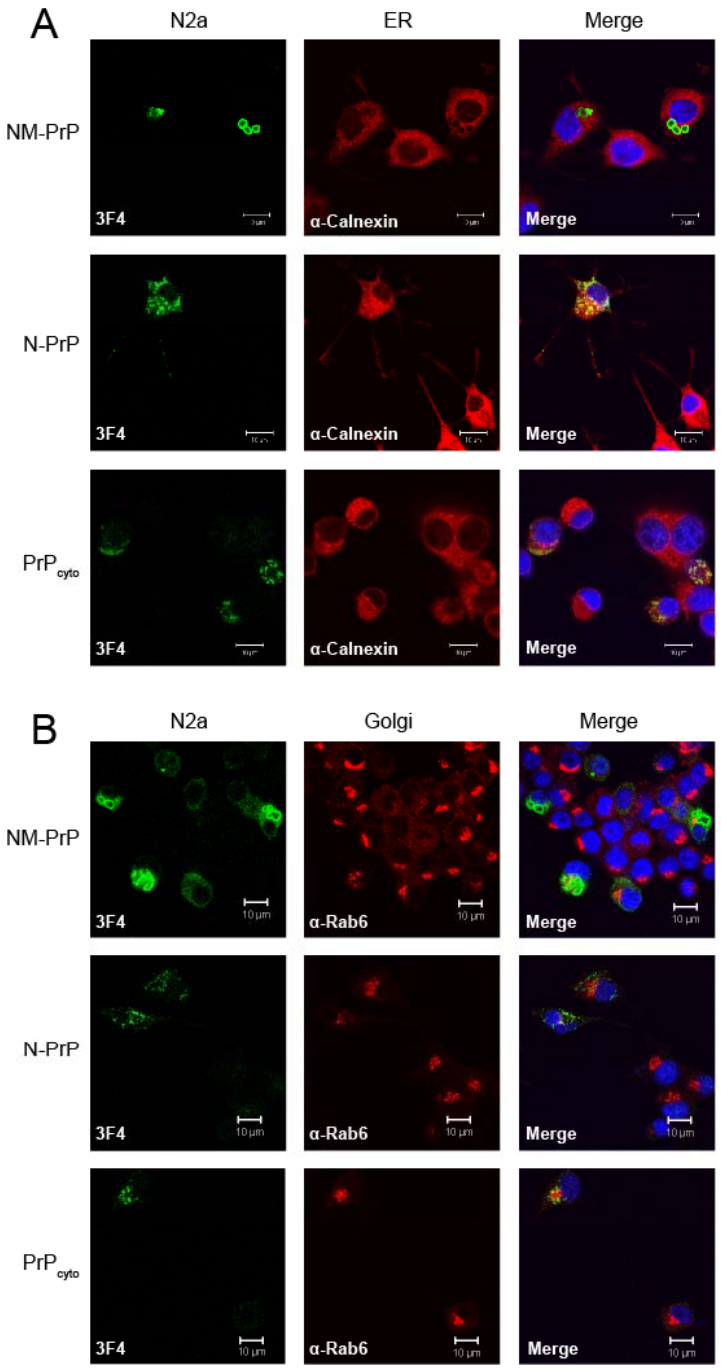

A
N2a
ER
Merge
NM-PrP
N-PrP
PrP cyto
3F4
α-Calnexin
Merge
B
N2a
Golgi
Merge
NM-PrP
N-PrP
PrP cyto
3F4
α-Rab6
Merge
10 μm

Fig. S2

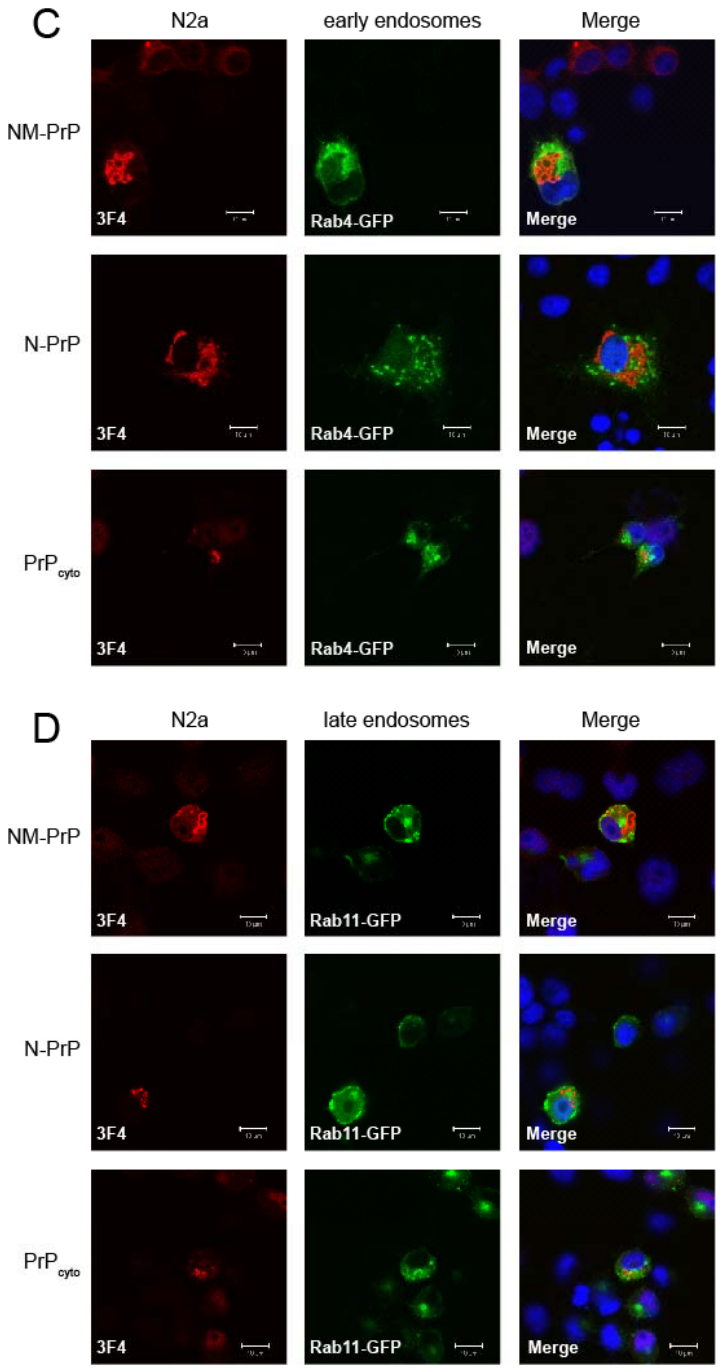
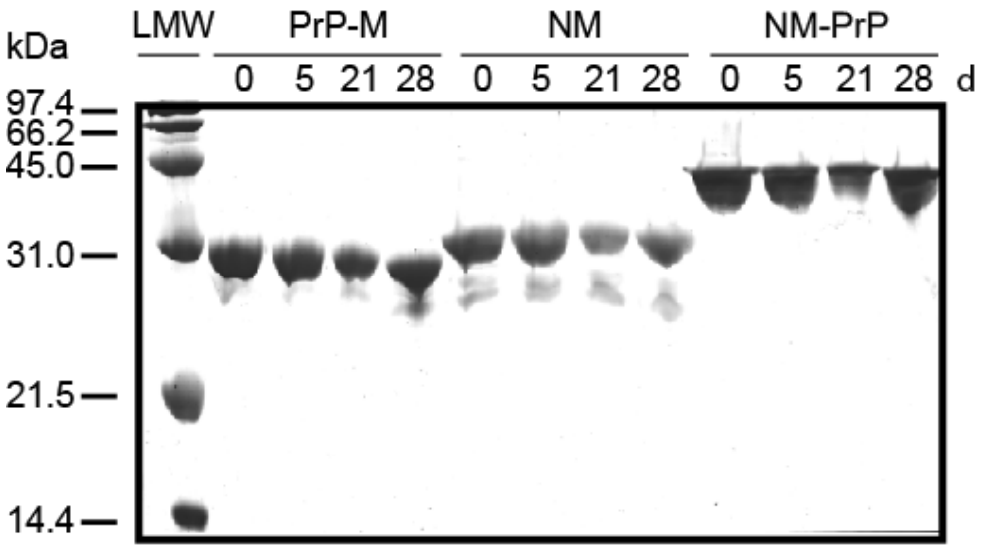

C
N2a
early endosomes
Merge
NM-PrP
N-PrP
PrP cyto
3F4
Rab4-GFP
Merge
D
N2a
late endosomes
Merge
NM-PrP
N-PrP
PrP cyto
3F4
Rab11-GFP
Merge

Fig. S3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LMW
PrP-M
NM
NM-PrP
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97.4
66.2
45.0
31.0
21.5
14.4