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Supplemental Material to:

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Activation of the macroautophagic system in scrapie-infected experimental animals and human genetic prion diseases

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Figure S1: referring to Figures 3, 4, 10 and 11

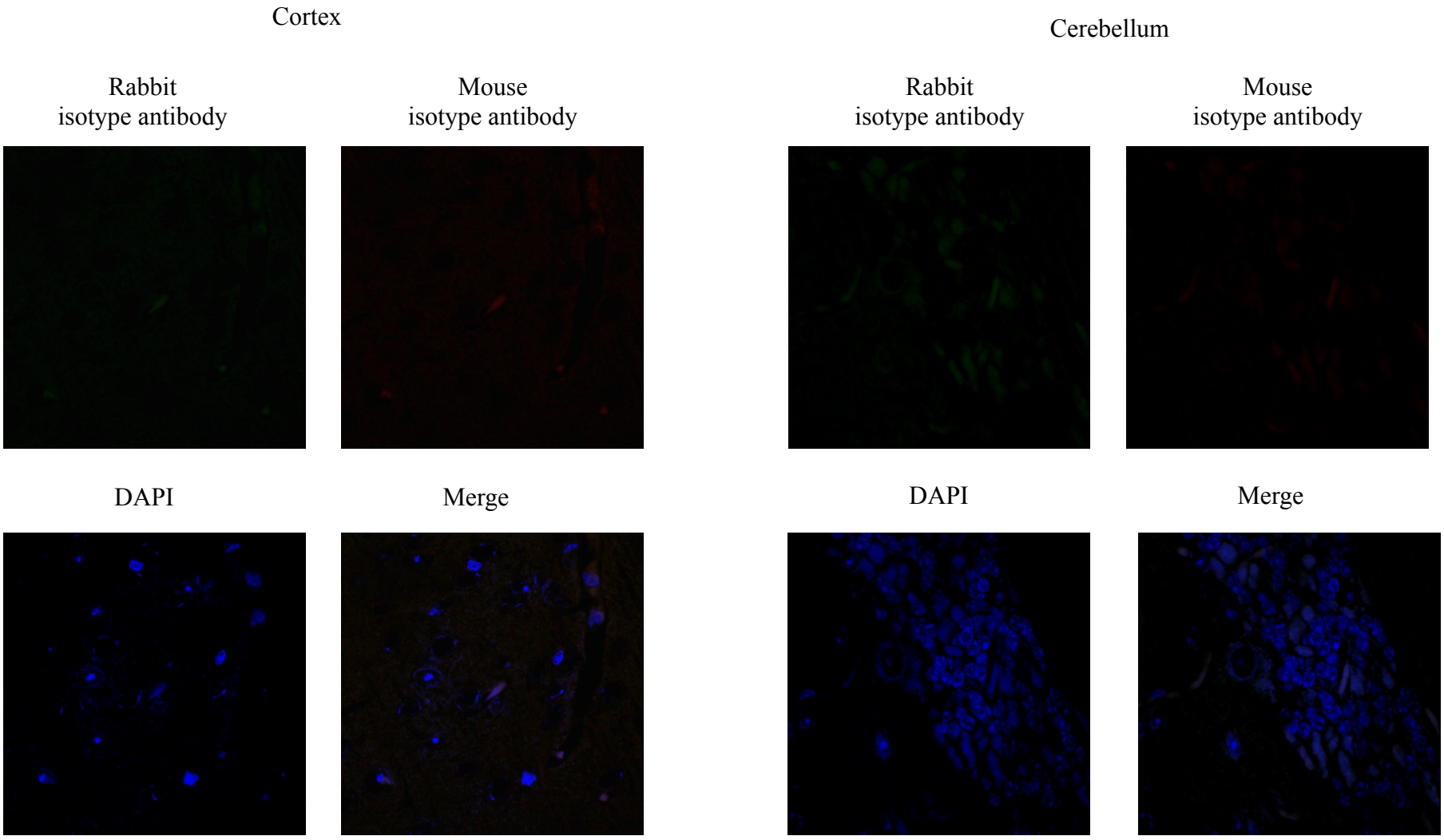
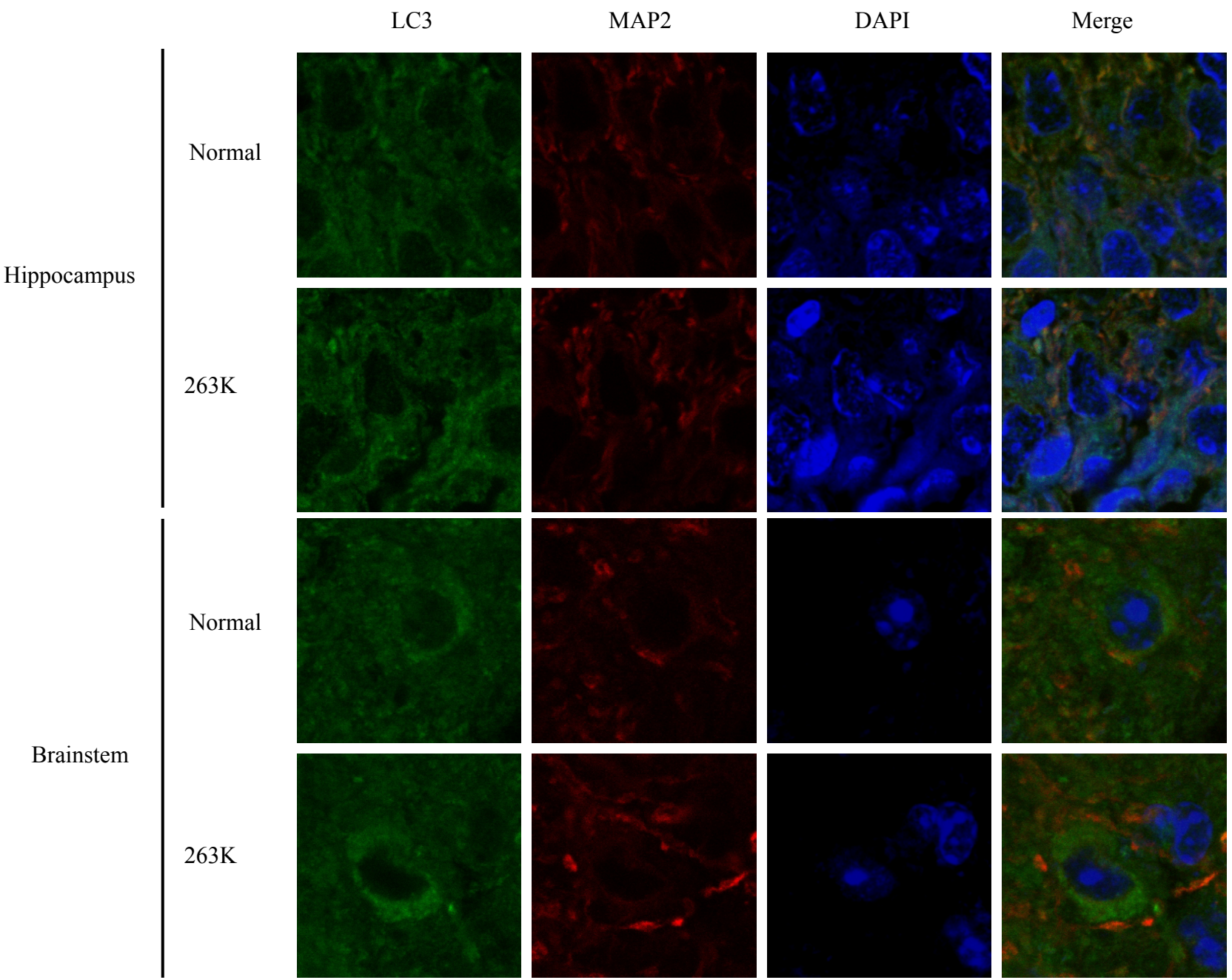
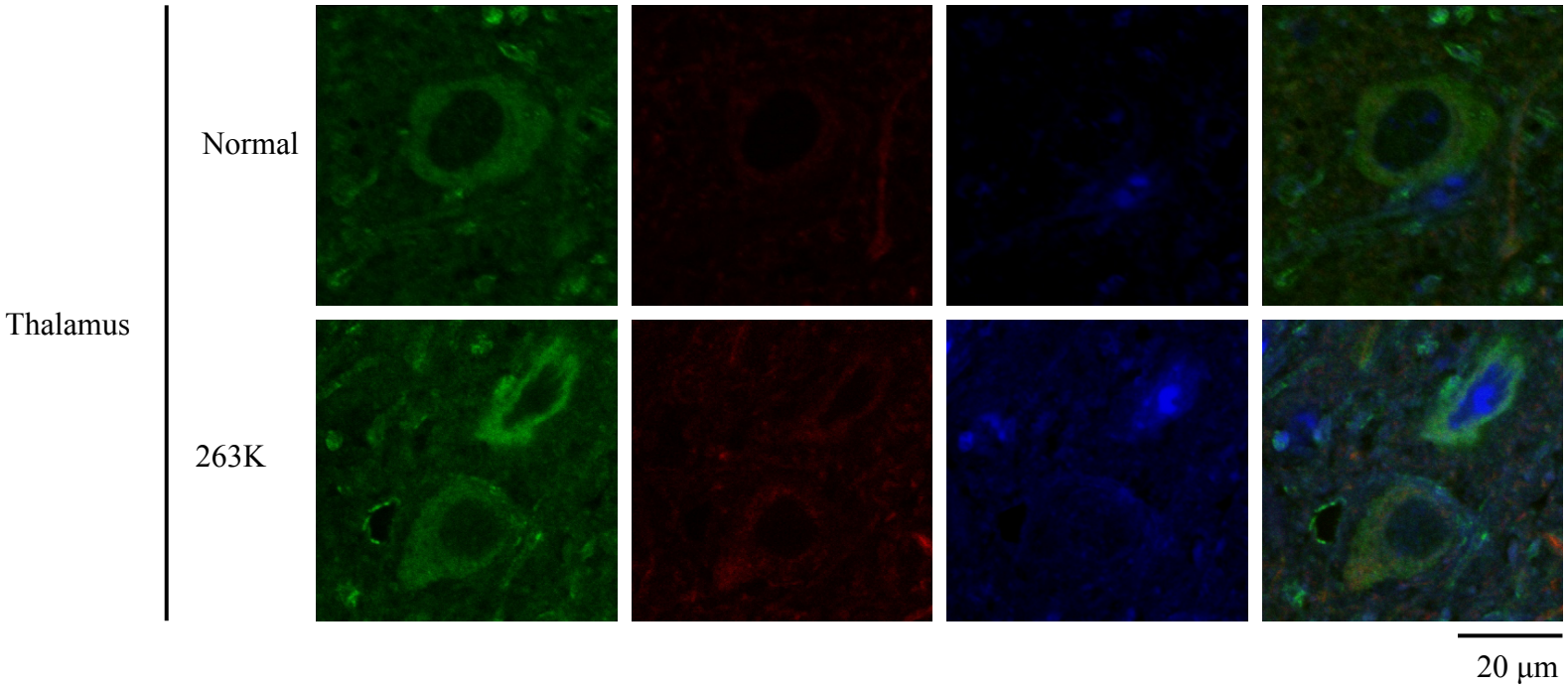


Figure S2: referring to Figure 3




Thalamus
Normal
263K
20 μm

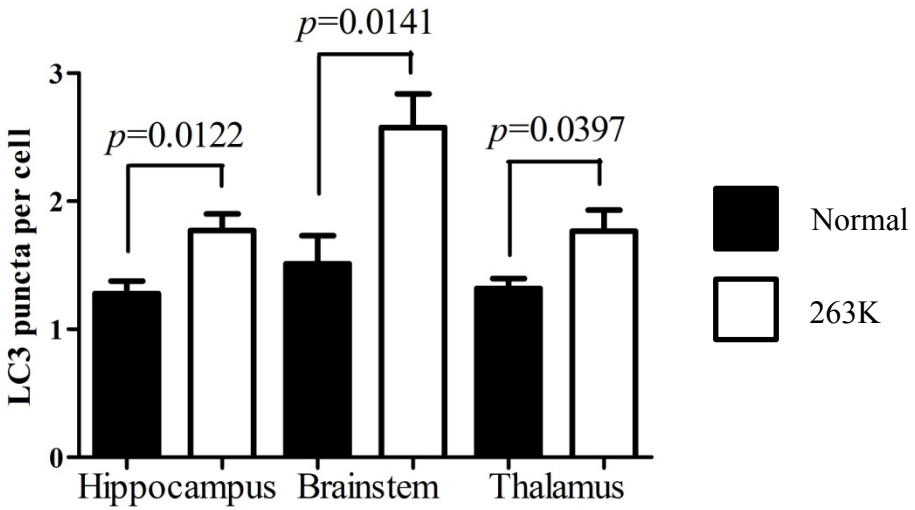
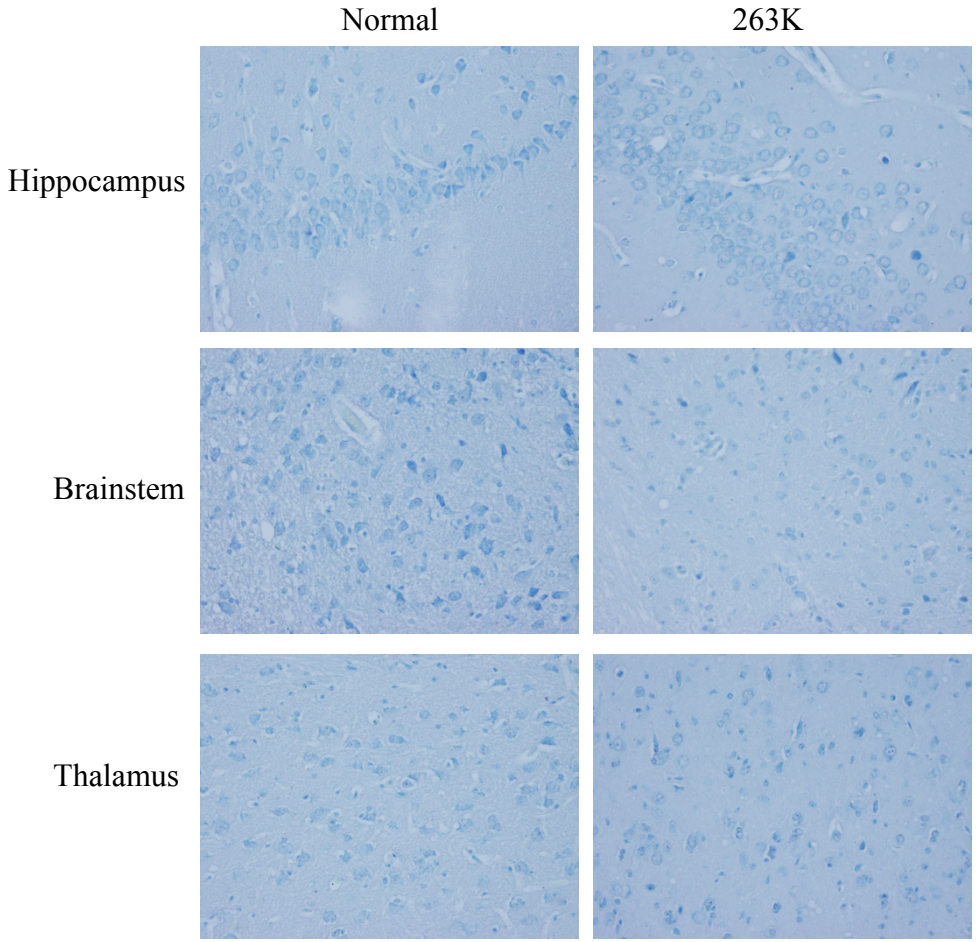

p=0.0141
p=0.0122
p=0.0397
LC3 puncta per cell
3
2
1
0
Hippocampus
Brainstem
Thalamus
Normal
263K

Figure S3: referring to Figure 4B



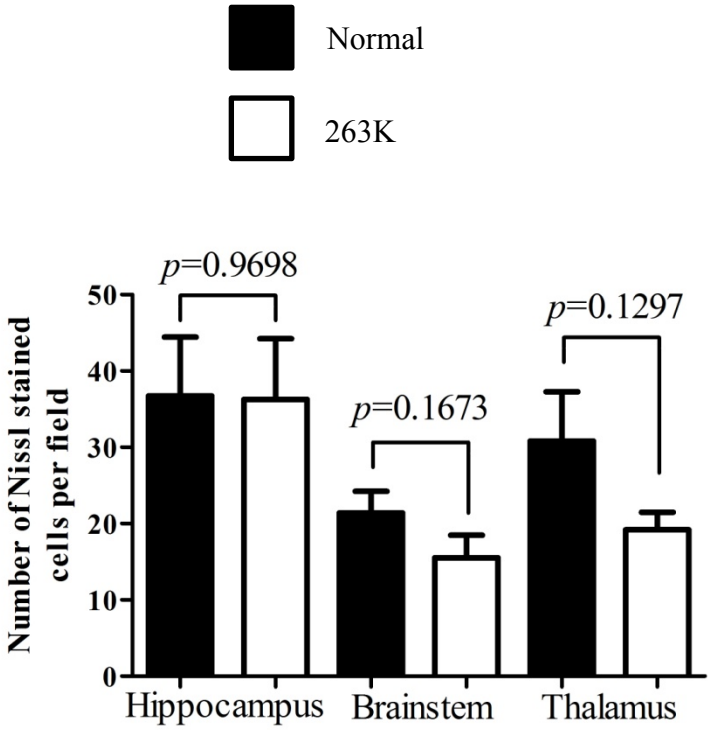


Figure S4: referring to Figure 4B

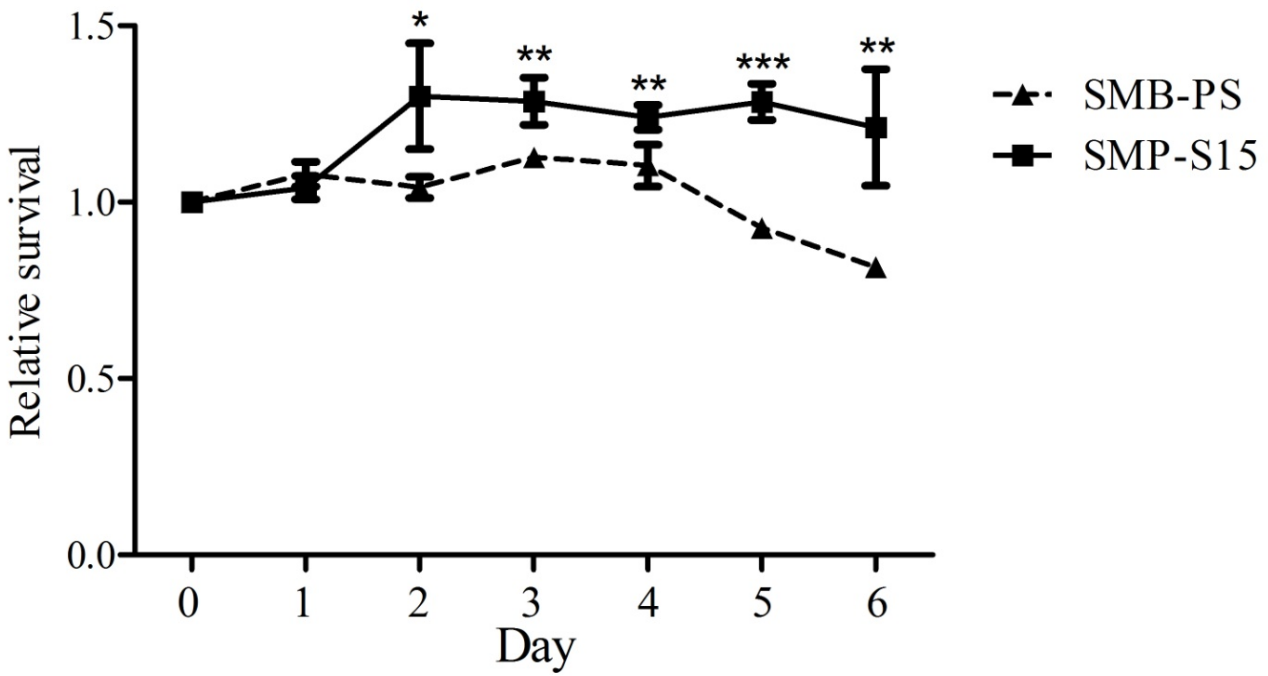


Figure S5: referring to Figures 7 and 8

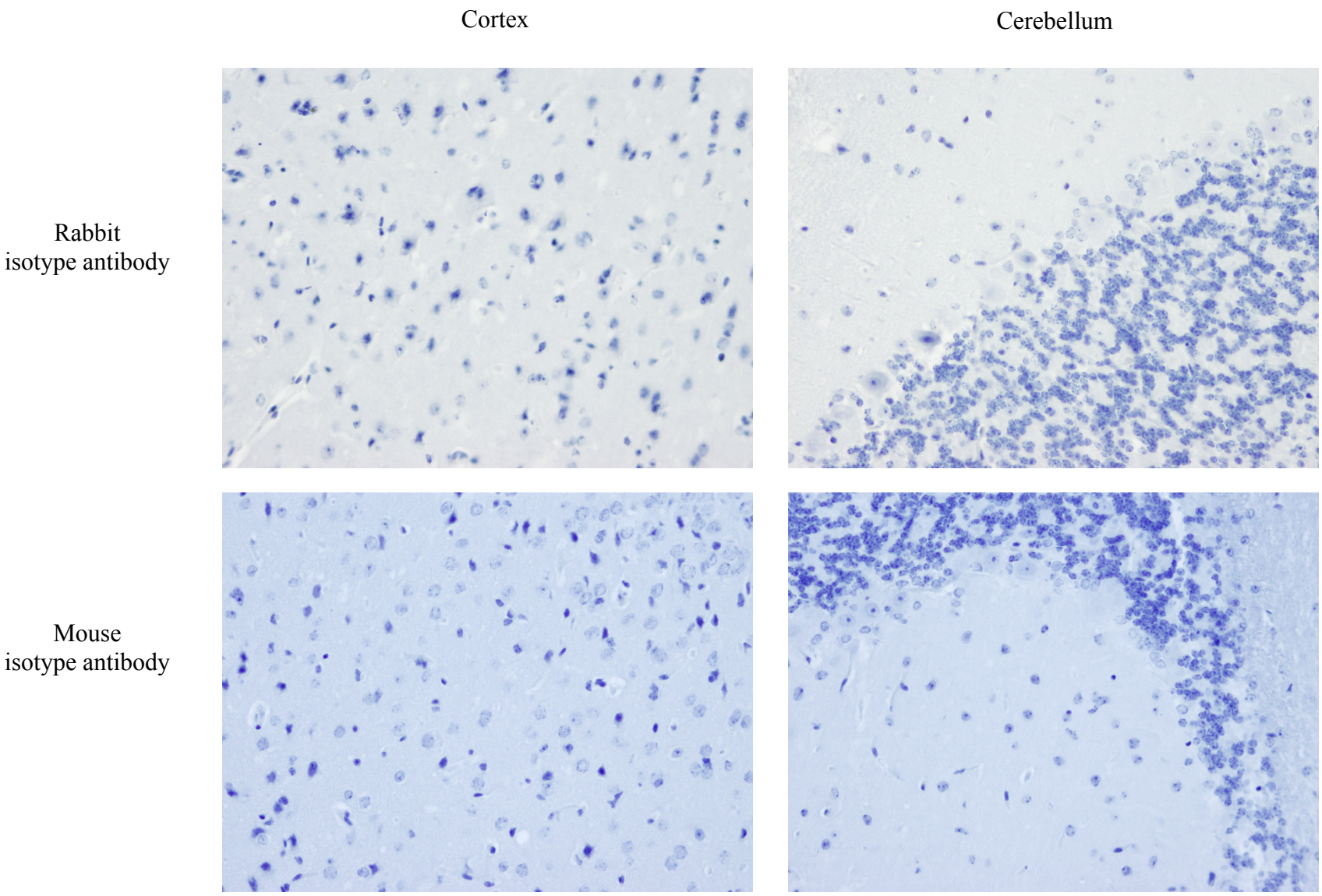
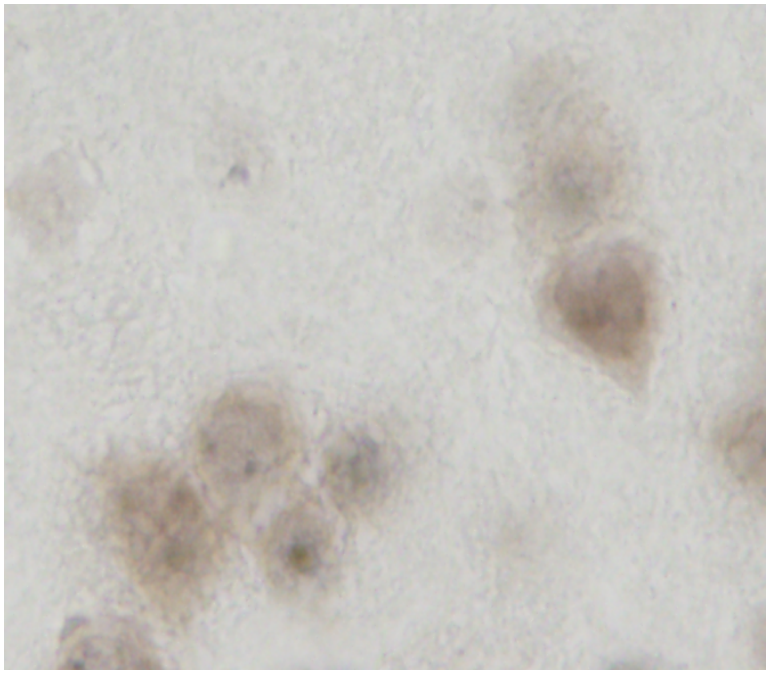


Figure S6: referring to Figure 7A

Normal



263K

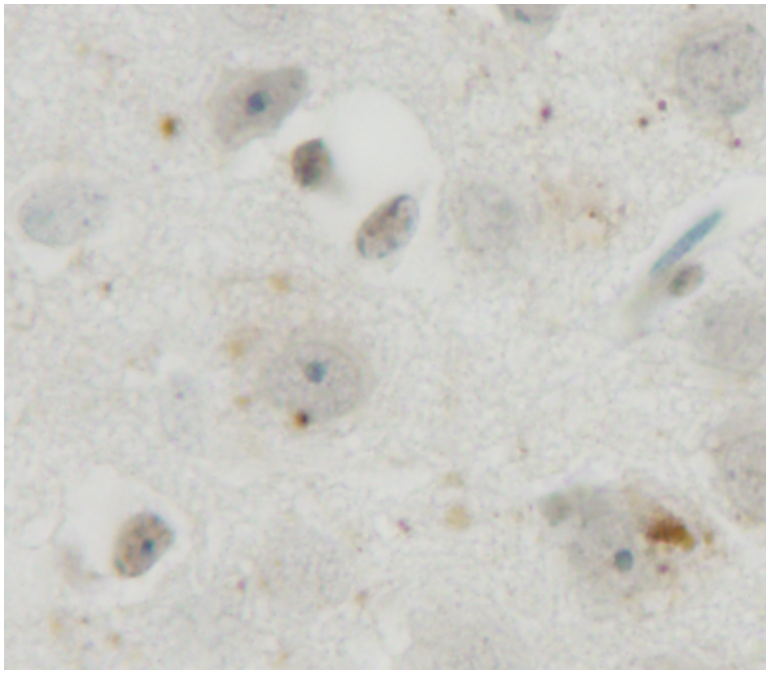


Figure S7: referring to Figure 5

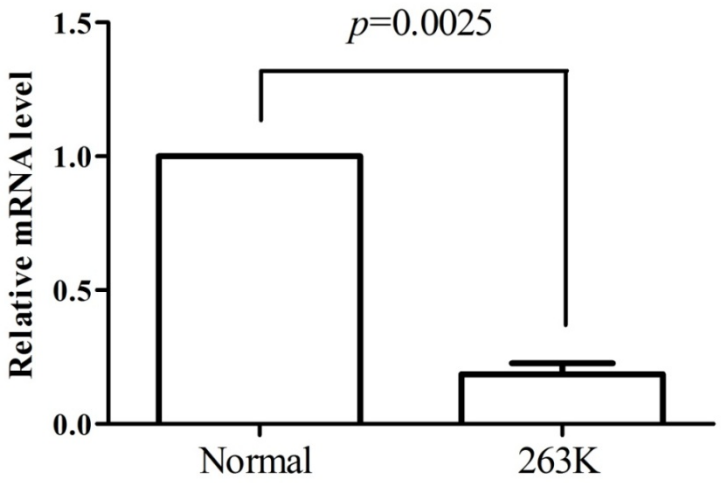
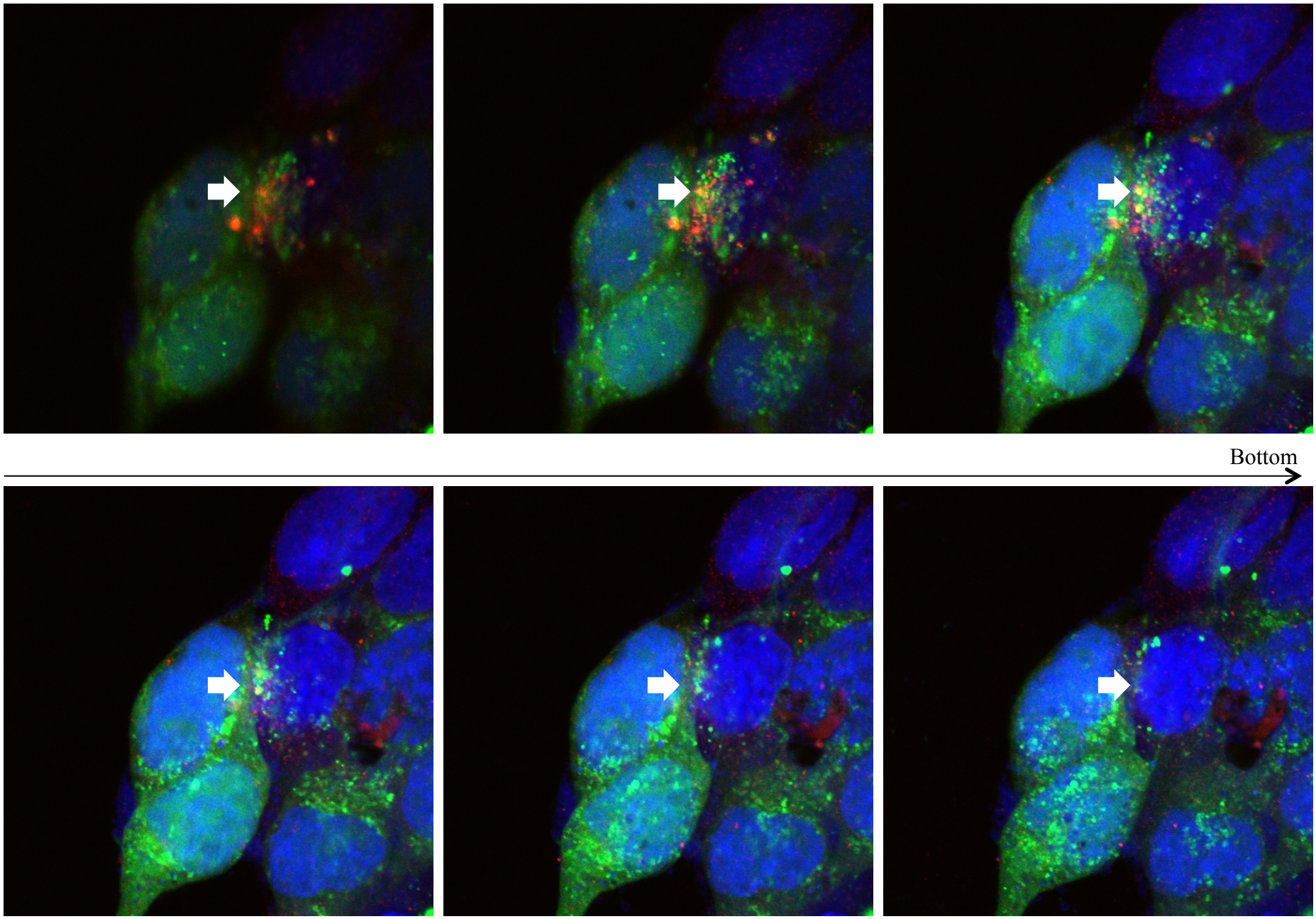


Figure S8: referring to Figure 11C and D

Top

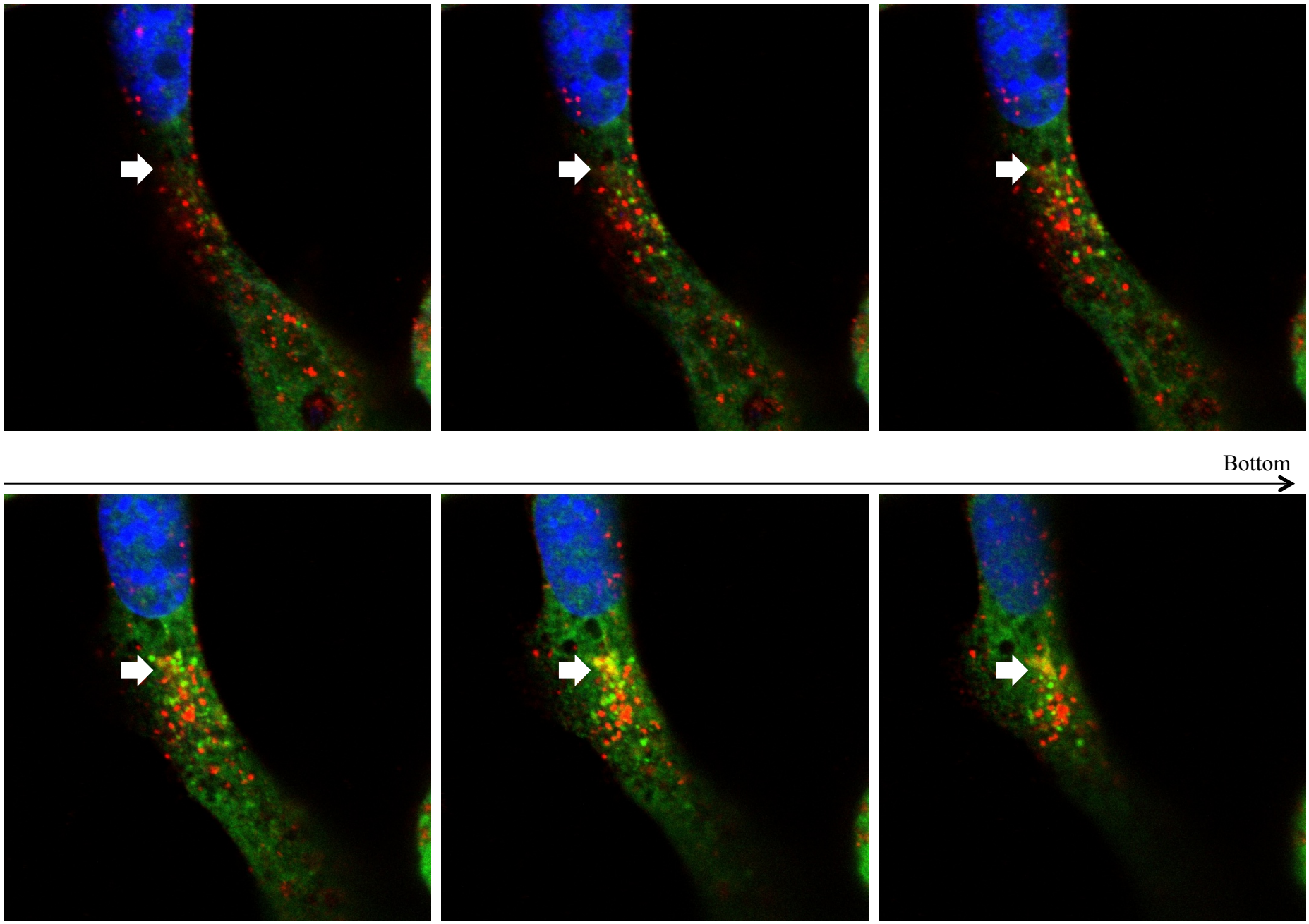
HEK293 expressing PrP-PG14



Bottom

SMB-S15

Top



Bottom

Figure S1. The negative controls of rabbit and mouse isotype antibodies for immunofluorescent staining in the tissue sections of cortex and the cerebellum regions of hamsters.

Figure S2. Autophagosomes localized in cytoplasm of neurons. Top: Double immunofluorescence staining for LC3 and MAP2 in the hippocampus, brainstem and thalamus regions of brain sections of scrapie agents 263K-infected and normal hamsters. Bar, 20 μm. Bottom: Quantity of LC3 puncta per cell. Graphical data denote mean ± SD.

Figure S3. Left: Nissl staining for neurodegeneration in the hippocampus, brainstem and thalamus regions (×40). Right: Quantity of neurons the hippocampus, brainstem, thalamus regions. Graphical data denote mean ± SD.

Figure S4. Relative survival of SMB-PS and SMB-S15 cells after treated by 3-MA. Each cell lines were exposed to 3-MA persistently from 0 to 6 days. The individual cells were maintained in the culture medium without 3-MA as the mock. Cells were harvested every 24 h and cell viability was evaluated with CCK-8 assays. Data were expressed as the ratio of OD value in 3-MA treated wells to that in untreated wells. Graphical data denote mean ± SD.

Figure S5. The negative controls of rabbit and mouse isotype antibodies for immunohistochemical staining in the tissue sections of cortex and the cerebellum regions of hamsters.

Figure S6. The inclusion bodies of polyubiquitinated protein in cortex (×100).

Figure S7. mRNA expression of MTOR in normal and scrapie agent 263K-infected hamsters. The data are normalized to that of the individual β-actin.

Figure S8. Serial optical section per 0.5μm of the merged graphs of the HEK293 co-transfected with pcDNA-PrP-PG14 and pEGFP-LC3 and the SMB-S15 cells transfected with pEGFP-LC3 by confocal microscopy after treatment with bafilomycin A_1. Cells were immunofluorescently stained for PrP (in HEK293) or PrP^{Sc} (in SMB-S15). Six scanning layers per merged graph from top left to bottom right represent the scans from top to bottom of the cells. The white arrows indicate the position of colocalization. Bar, 20 μm.

Table S1. Quantitative analysis of each gray numerical value in Fig. 9.

dpi	0	20	40	60	80
LC3- II	0±0.00	0±0.00	0±0.00	0±0.00	1±0.00*
BECN1	1±0.00	0.97±0.02	0.97±0.01	0.90±0.02*	0.49±0.02*
PIK3C3	0.63±0.02	0.71±0.08	0.66±0.04	0.67±0.02	1±0.00*
p-MTOR	1±0.00	1.00±0.01	1.03±0.01	0.33±0.06*	0.02±0.01*
MTOR	1±0.00	0.58±0.02*	0.57±0.02*	0.20±0.02*	0.02±0.00*
SQSTM1	1±0.00	0.96±0.01	0.85±0.04*	0.60±0.02*	0.57±0.02*
Polyubiquitinated protein	1±0.00	0.60±0.01*	0.19±0.01*	0.02±0.01*	0.02±0.01*
PrP^{Sc}	0±0.00	0.01±0.00	0.03±0.00*	0.38±0.00*	1±0.00*

*: statistically significant compared with control.