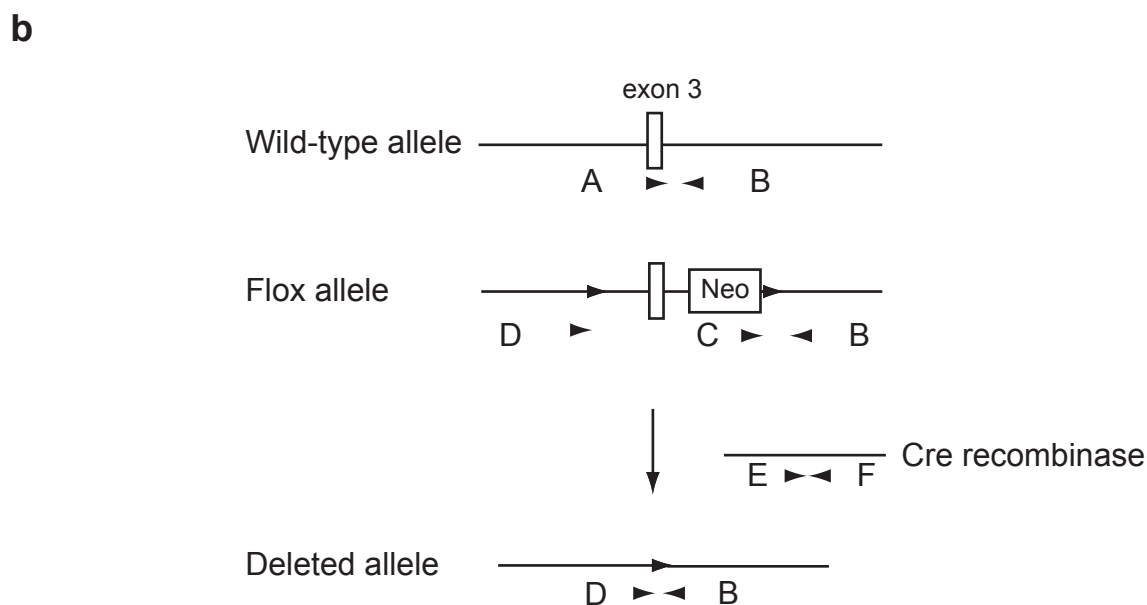
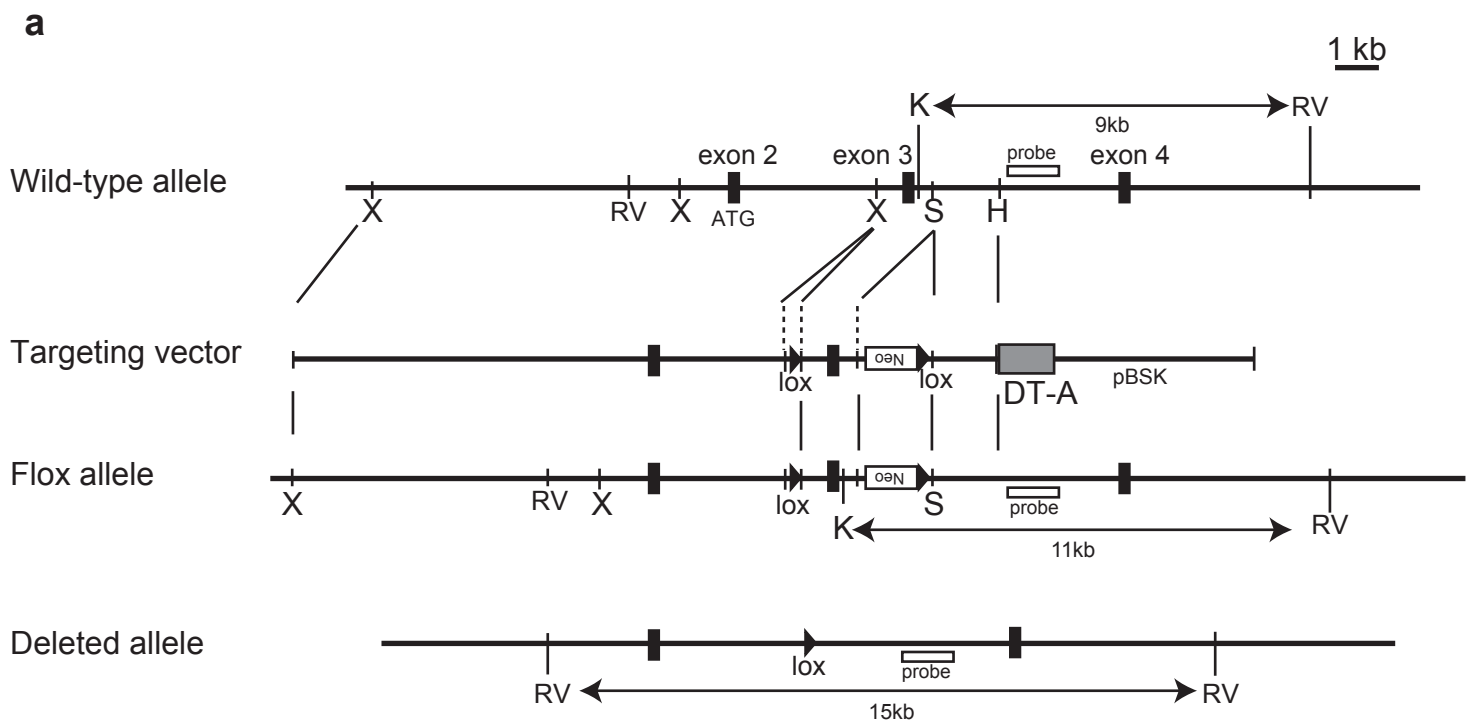


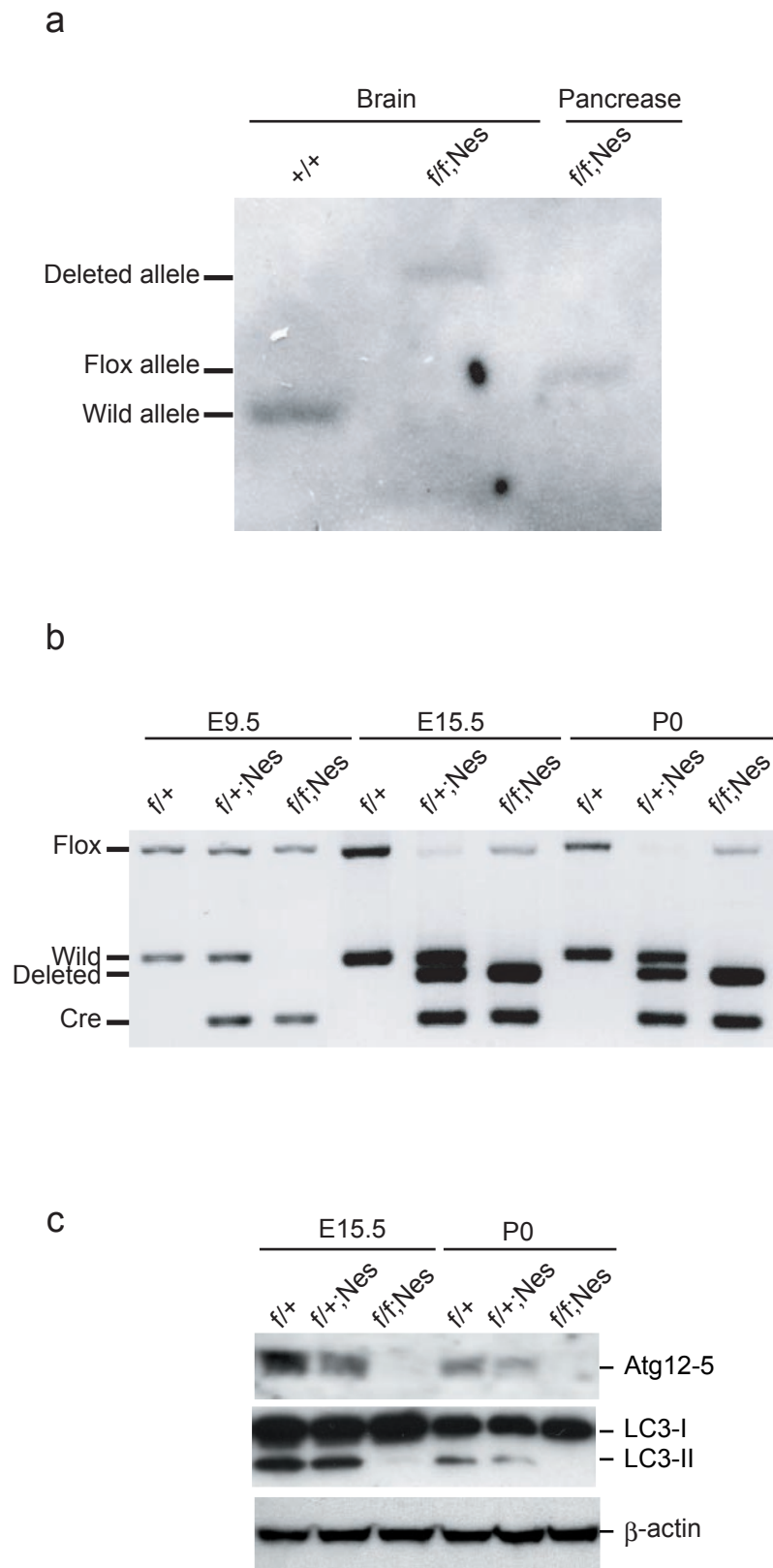
**Supplementary Figure S1** Ubiquitin-positive IBs accumulate in *Atg5*<sup>-/-</sup> tissues. *Atg5*<sup>-/-</sup> neonatal tissues were fixed and decalcified. Whole-mount paraffin sections were prepared and stained with an anti-ubiquitin antibody (1B3). Representative regions of liver, anterior horn of the spinal cord, pons, dorsal root ganglia, anterior lobe of pituitary gland, and cerebral cortex are shown. Scale bar, 10  $\mu$ m.



**Supplementary Figure S2** Targeting and genotyping strategies for the *Atg5<sup>flx/flx</sup>* mice.

(a) The restriction map of the wild-type *Atg5* allele, the targeting construct, *flx* allele, and the deleted allele after Cre-mediated recombination. Black boxes indicate exons 2-4. Probes used for Southern blot analysis are indicated by open boxes. The *EcoRV* and *KpnI*-digested fragments detected by probes are depicted. Restriction enzymes are as follows: X; *XbaI*, RV; *EcoRV*, H; *HindIII*, S; *SpeI*, K; *KpnI*.

(b) The positions of PCR primers in the wild-type *Atg5* allele, the targeting construct, the *flx* allele, and the deleted allele. Primers A and B were used for amplification of the wild-type *Atg5* allele; primers C and B for the *flx* allele; primers D and B for the deleted allele; and primers E and F for detection of the Cre recombinase gene.

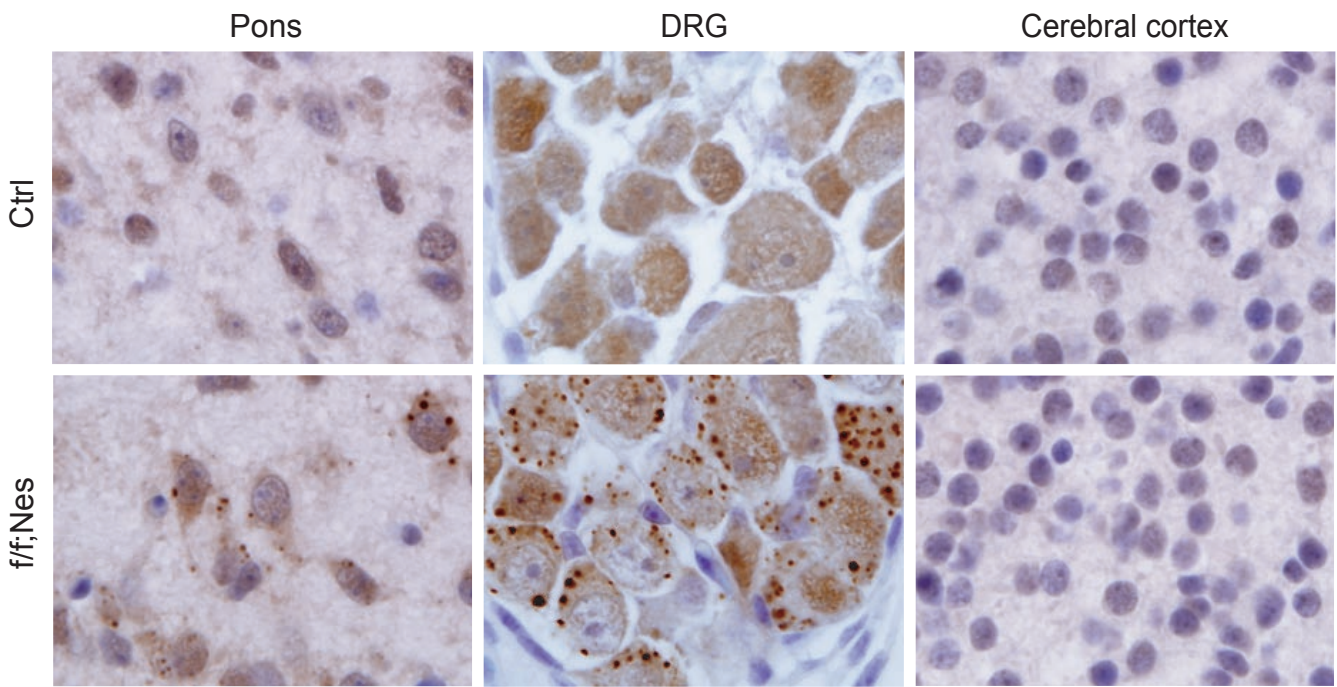


**Supplementary Figure S3** *Atg5* gene deletion in *Atg5<sup>flox/flox</sup>;Nestin-Cre* mice.

(a, b) Genomic DNA extracted from *Atg5<sup>+/+</sup>* and *Atg5<sup>flox/flox</sup>;Nestin-Cre* mice at eight weeks of age (a), and embryos (b) were analyzed by Southern blot analysis (a) and PCR (b).

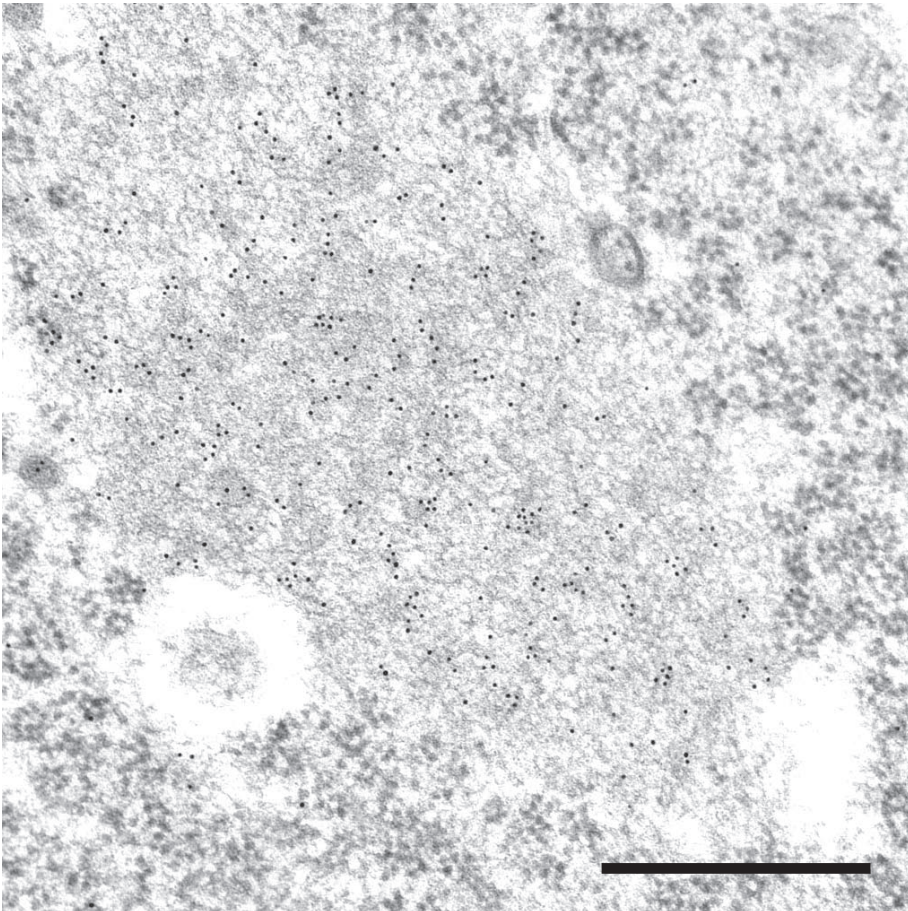
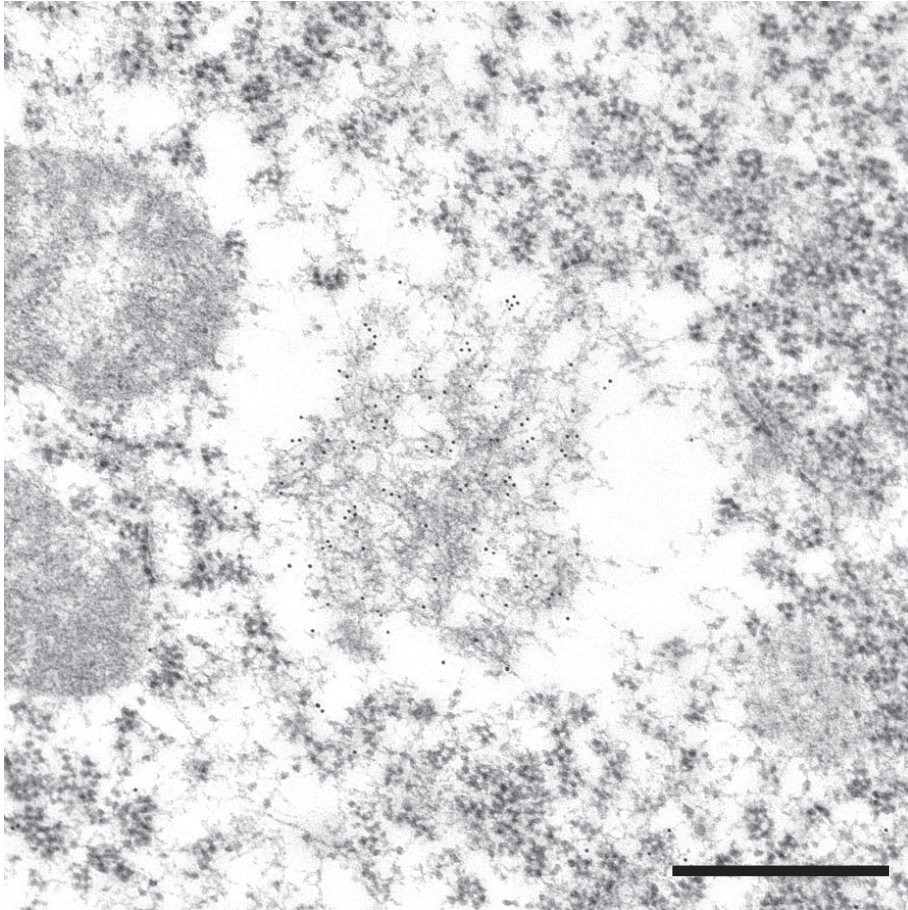
The probe and primers used were indicated in Supplementary Figure S2.

(c) Immunoblot analysis of Atg5 and LC3 performed as described in Figure 1.

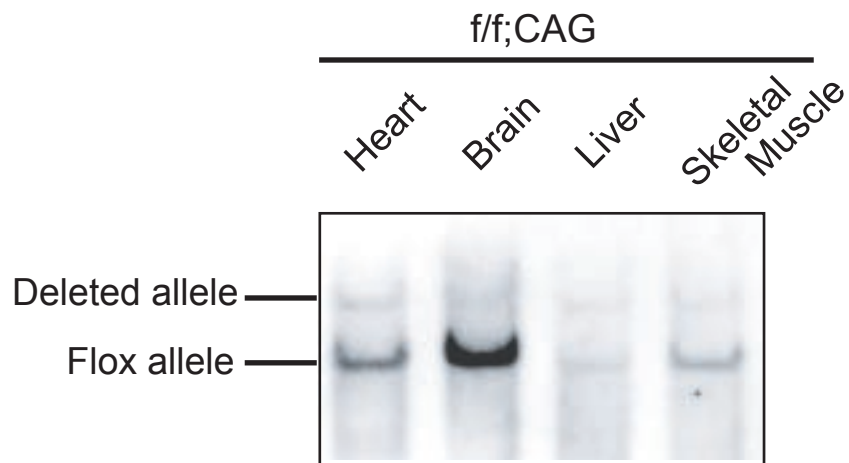


**Supplementary Figure S4** Ubiquitin-positive IBs accumulated in *Atg5<sup>flox/flox</sup>;Nestin-Cre* neonates. Tissue sections of pons, DRG, and cerebral cortex were prepared from day 0 neonates of *Atg5<sup>flox/flox</sup>;Nestin-Cre* and control (ctrl; *Atg5<sup>flox/+</sup>;Nestin-Cre*) mice as described in Supplementary Figure S1 and stained with anti-ubiquitin antibody (1B3). IBs accumulated in the pons and DRG, but not in the cerebral cortex. Scale bar, 10  $\mu$ m.

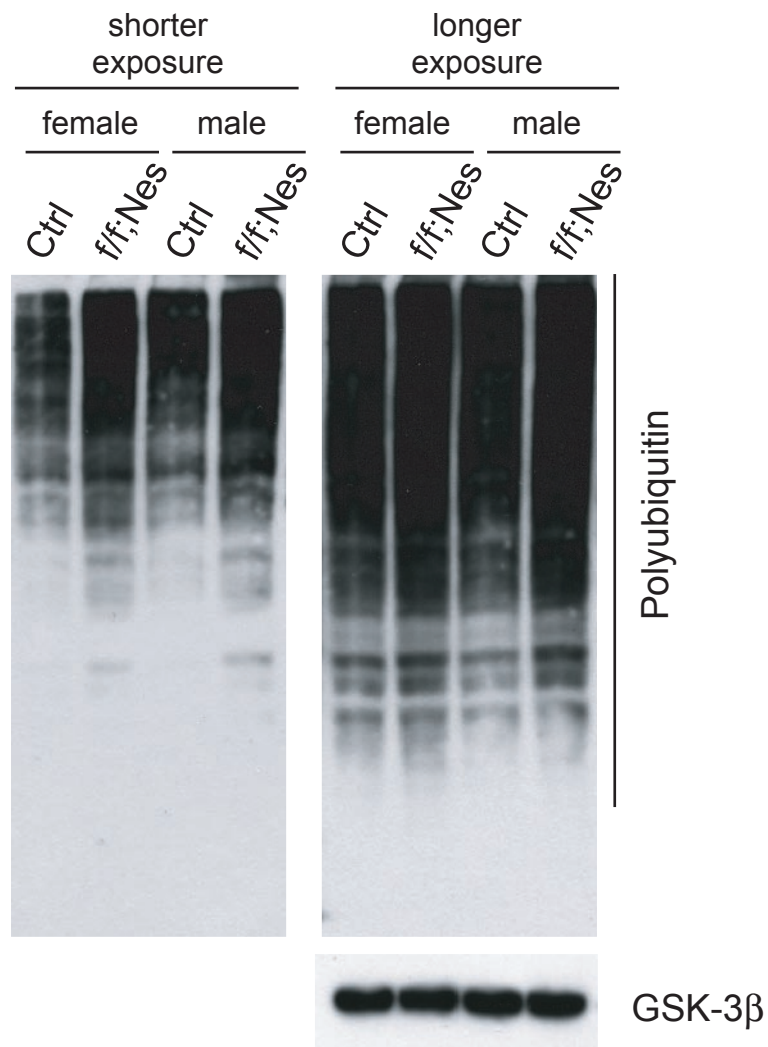




**Supplementary Figure S5** Larger images of the electron microscopic data shown in Fig. 3c.

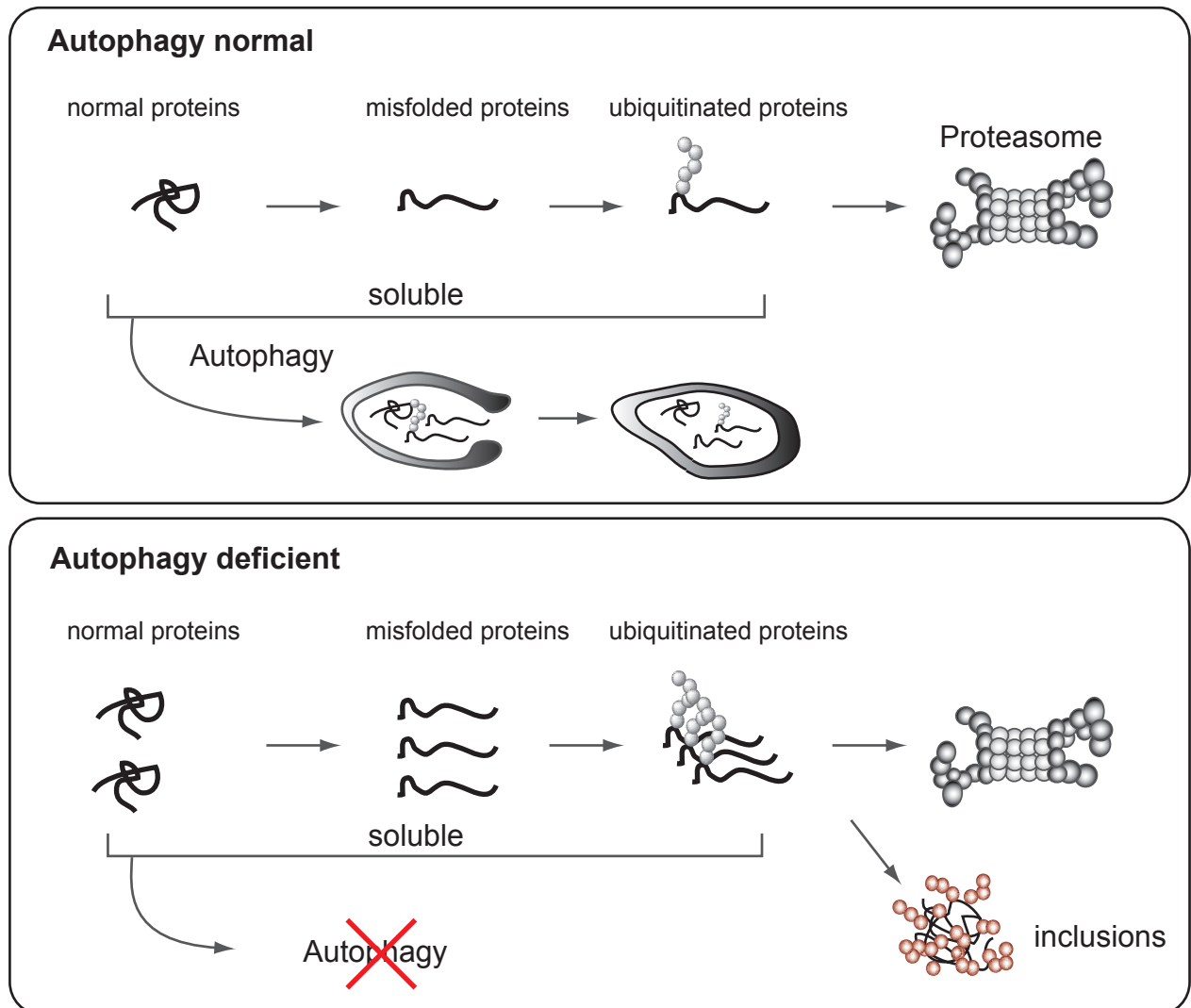


**Supplementary Figure S6** Southern blot analysis of genomic DNA extracted from various tissues of *Atg5<sup>flox/flox</sup>;CAG-Cre* mice.



**Supplementary Figure S7** Immunoblot analysis of brain lysates.

Whole-brain lysates from control (ctrl; *Atg5<sup>flox/+</sup>;Nestin-Cre*) and *Atg5<sup>flox/flox</sup>;Nestin-Cre* mice at six weeks of age were subjected to immunoblot analysis with antibodies to polyubiquitin (FK2) and GSK-3β (loading control). The right panel of the polyubiquitin blot is a longer exposure of the left panel. Accumulation of ubiquitinated proteins was very similar in male and female mice.



**Supplementary Figure S8** Model for the role of autophagy in protein quality control. Under normal conditions, autophagy constitutively degrades diffuse cytosolic proteins in basically a non-selective manner, whereas ubiquitinated proteins are efficiently degraded by the proteasome. If autophagy is blocked, general protein turnover is impaired, resulting in the accumulation of misfolded and ubiquitinated proteins, which ultimately aggregate within the cytoplasm.