Supplemental Data

TDP-43 proteinopathy occurs independently of autophagic substrate accumulation and underlies nuclear defects in Niemann-Pick C disease

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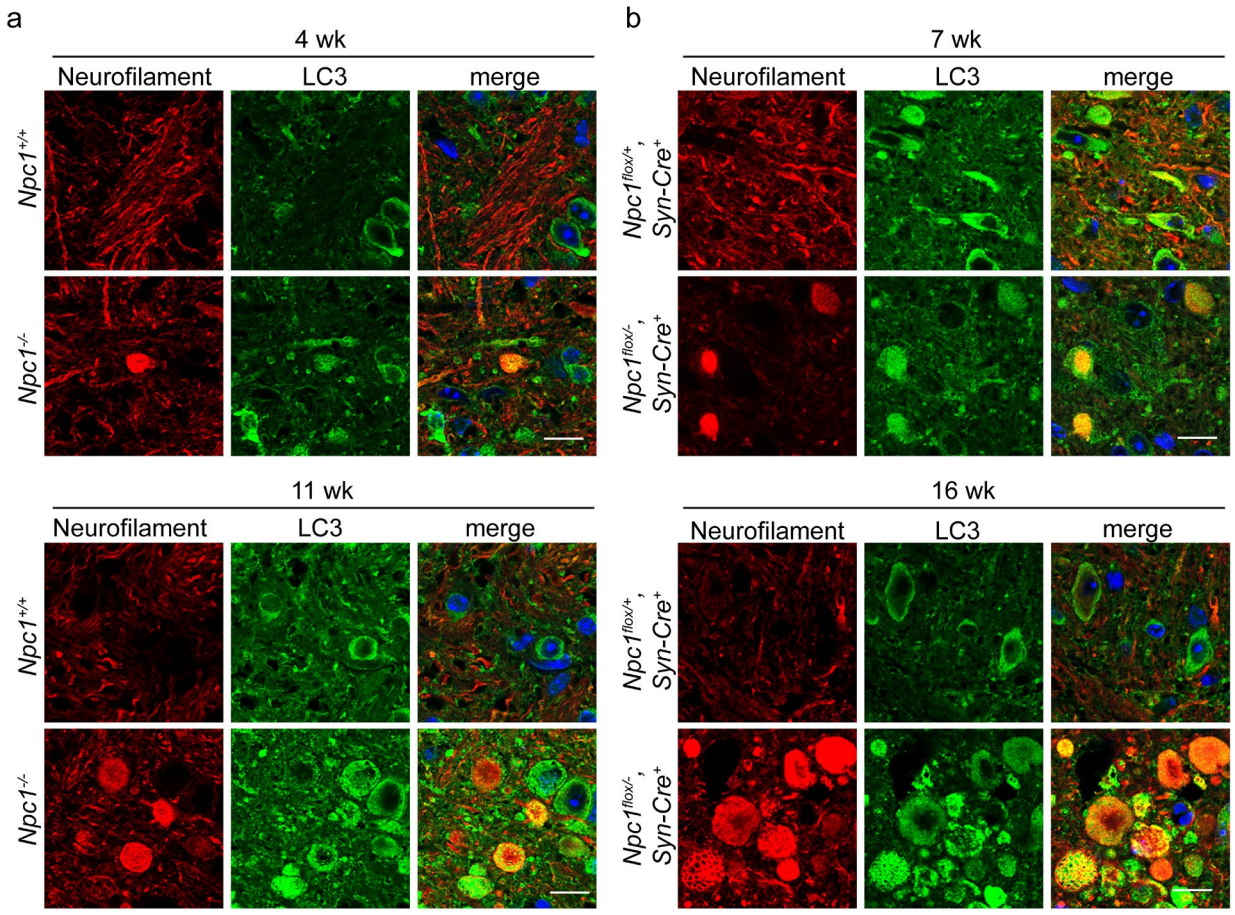
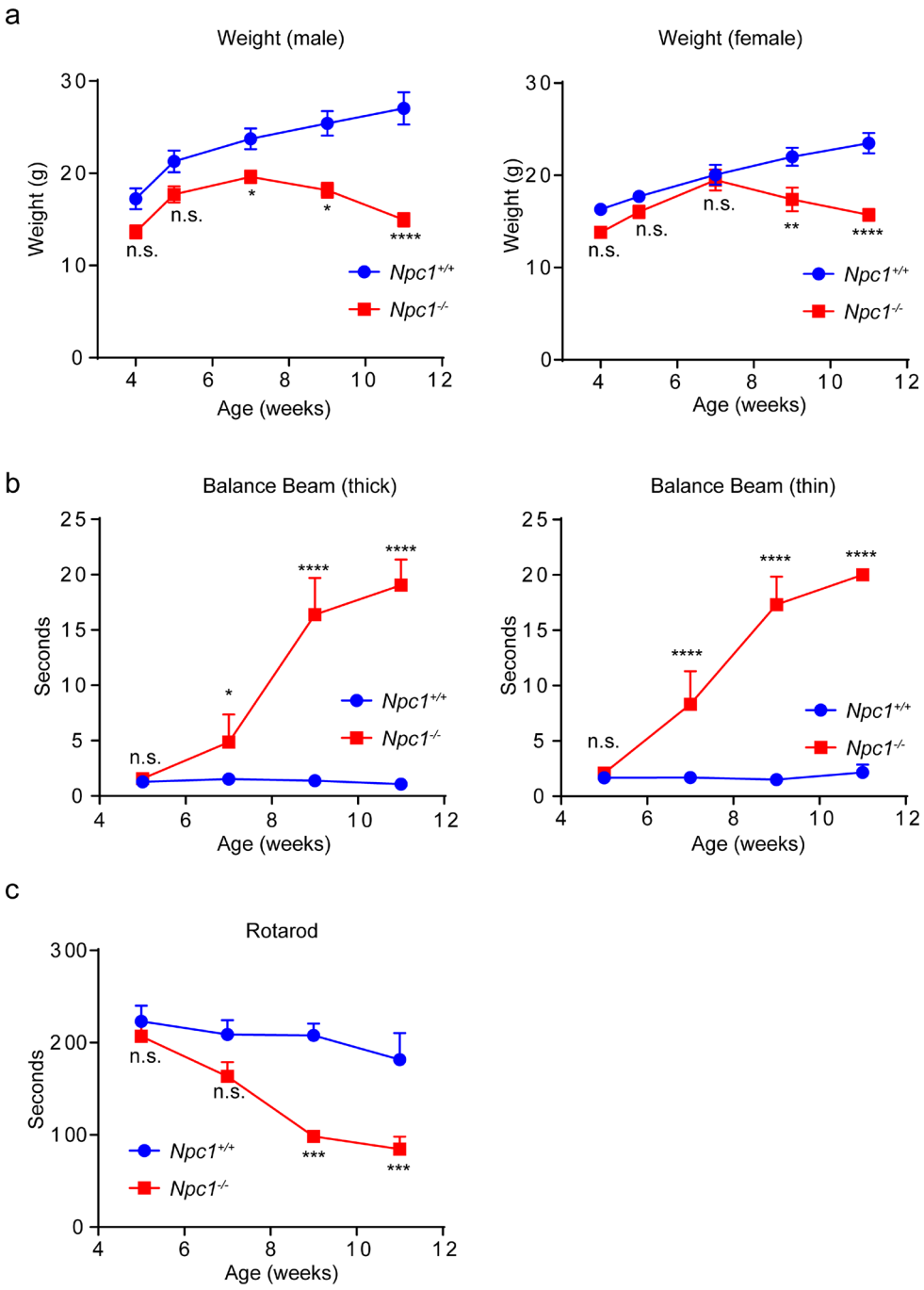


Supplementary Figure 1. Age-dependent accumulation of axonal spheroids

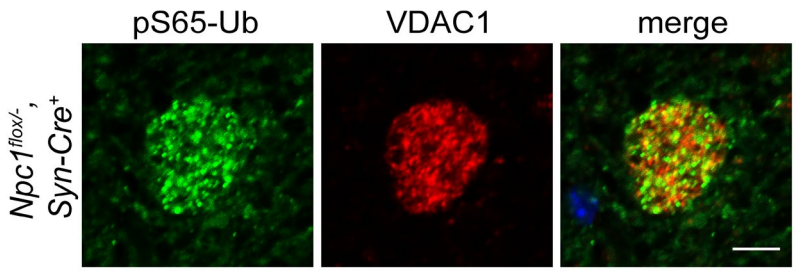
(a-b) Brain sections were stained with neurofilament and LC3 to identify axonal spheroids in the brainstem. Sections were imaged by confocal microscopy. Scale bar: 10 µm.



Supplementary Figure 2. Age-dependent progression of weight loss and motor deficits of *Npc1*$^{-/-}$ mice

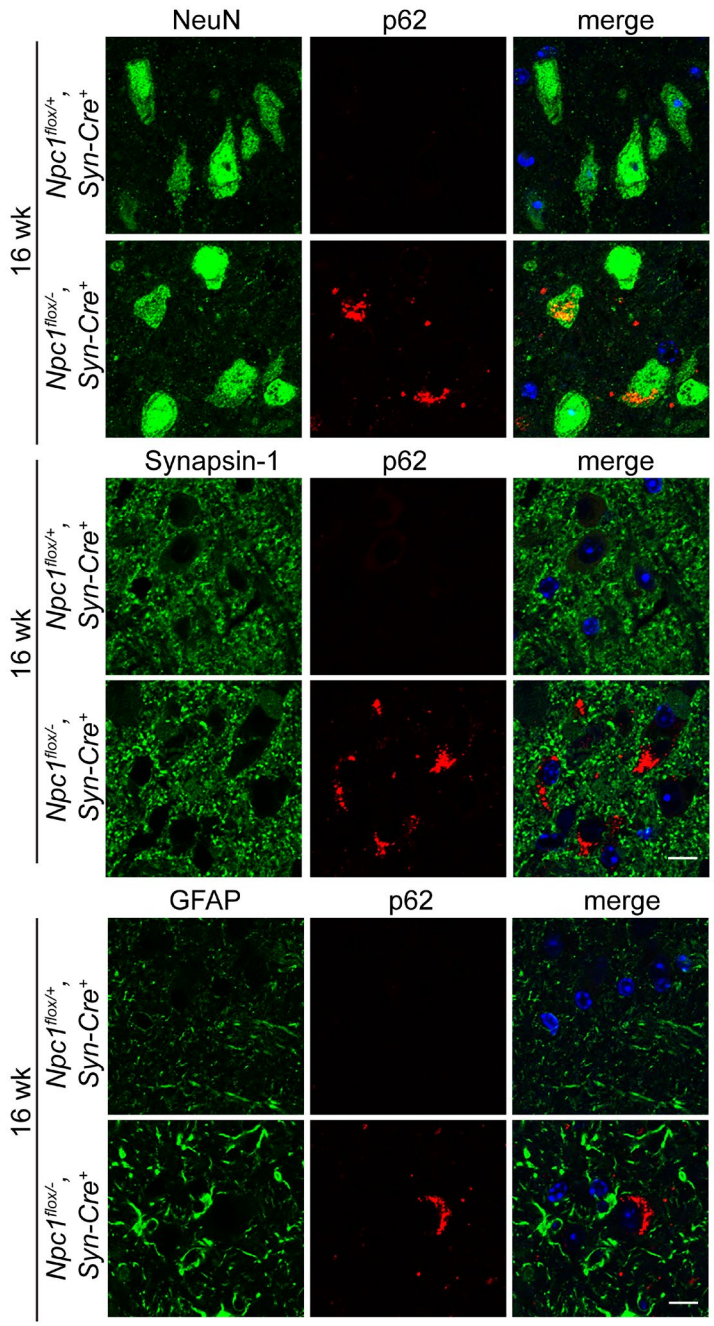
(a) Body weight of male and female *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice. N=4-5 mice per sex and genotype. **(b)** Age-dependent performance on balance beam using thick or thin beams. N=4-6

mice per genotype. **(c)** Age-dependent performance on accelerating rotarod. N=4-6 mice per genotype. Data are shown as mean ± s.e.m. n.s., not significant, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 by **(a-c)** two-way ANOVA with Sidak's multiple comparisons test.



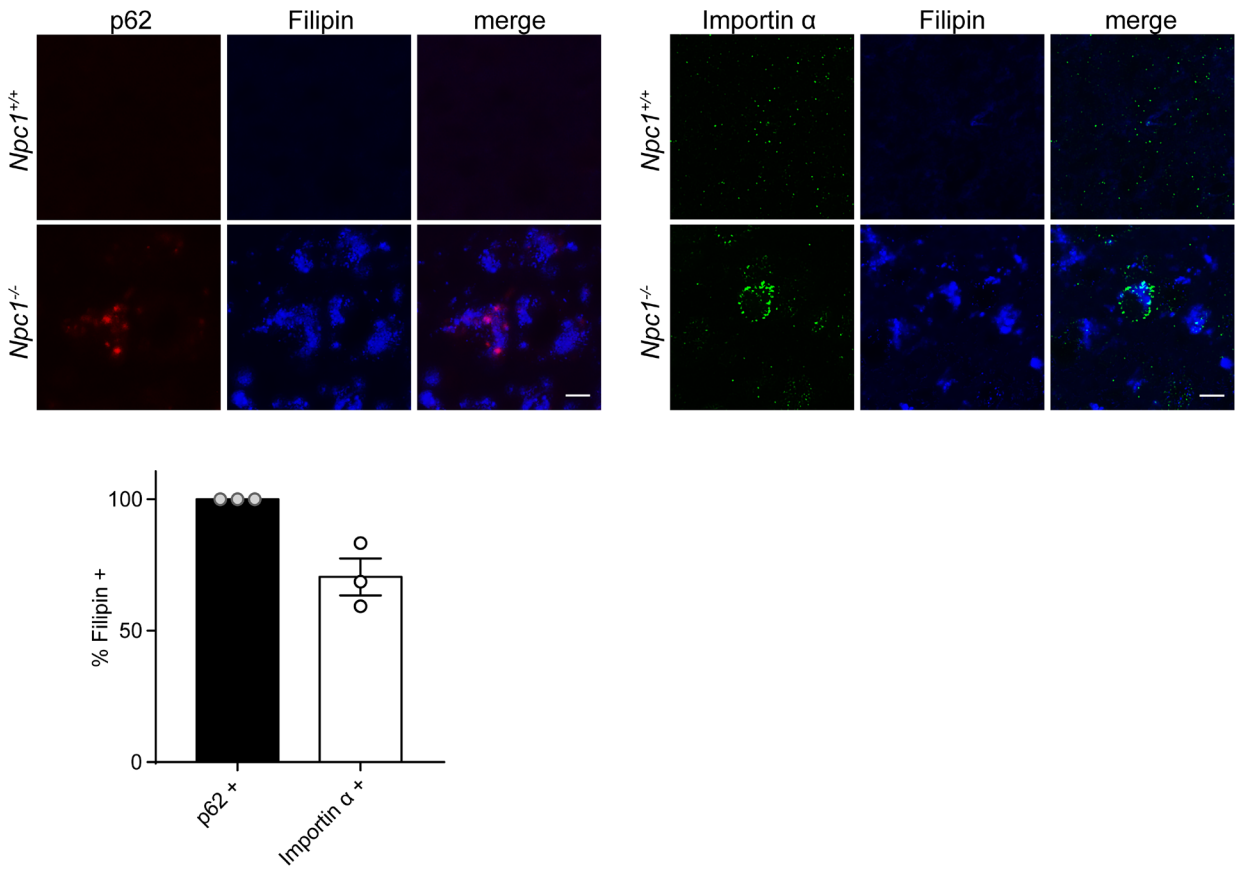
Supplementary Figure 3. pS65-Ub co-localizes with mitochondrial marker VDAC1

Brainstem from 16-week *Npc1*$^{flox/-}$*, Syn-Cre*$^{+}$ mice were stained with pS65-Ub and VDAC1 and imaged by confocal microscopy. Mander's coefficient = 0.84. Scale bar: 5 µm.



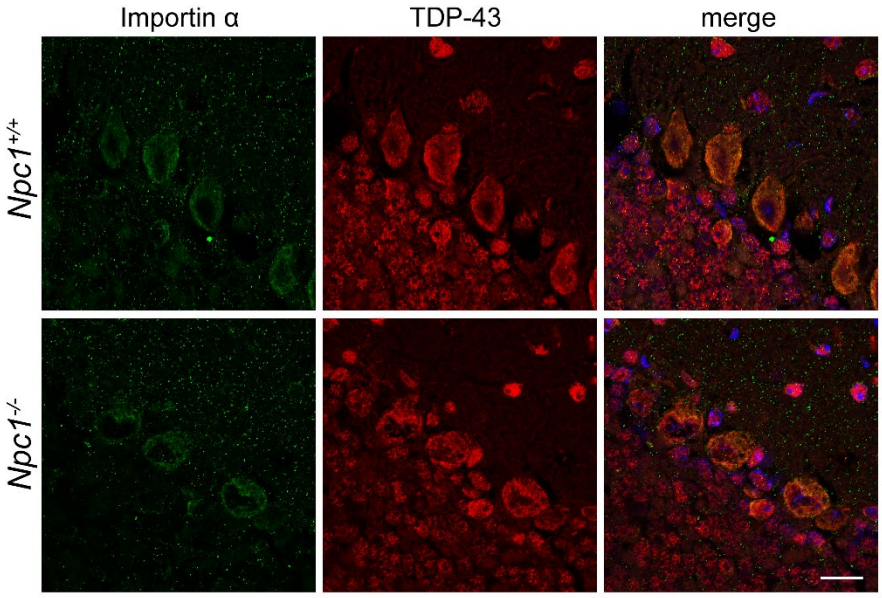
Supplementary Figure 4. p62 accumulates in neuron cell bodies and not in presynaptic terminals or astrocytes

Brain sections from 16-week *Npc1*$^{flox/+}$, *Syn-Cre*$^{+}$ and *Npc1*$^{flox/-}$, *Syn-Cre*$^{+}$ mice were stained with the indicated markers and imaged by confocal microscopy. Scale bar: 10 µm.



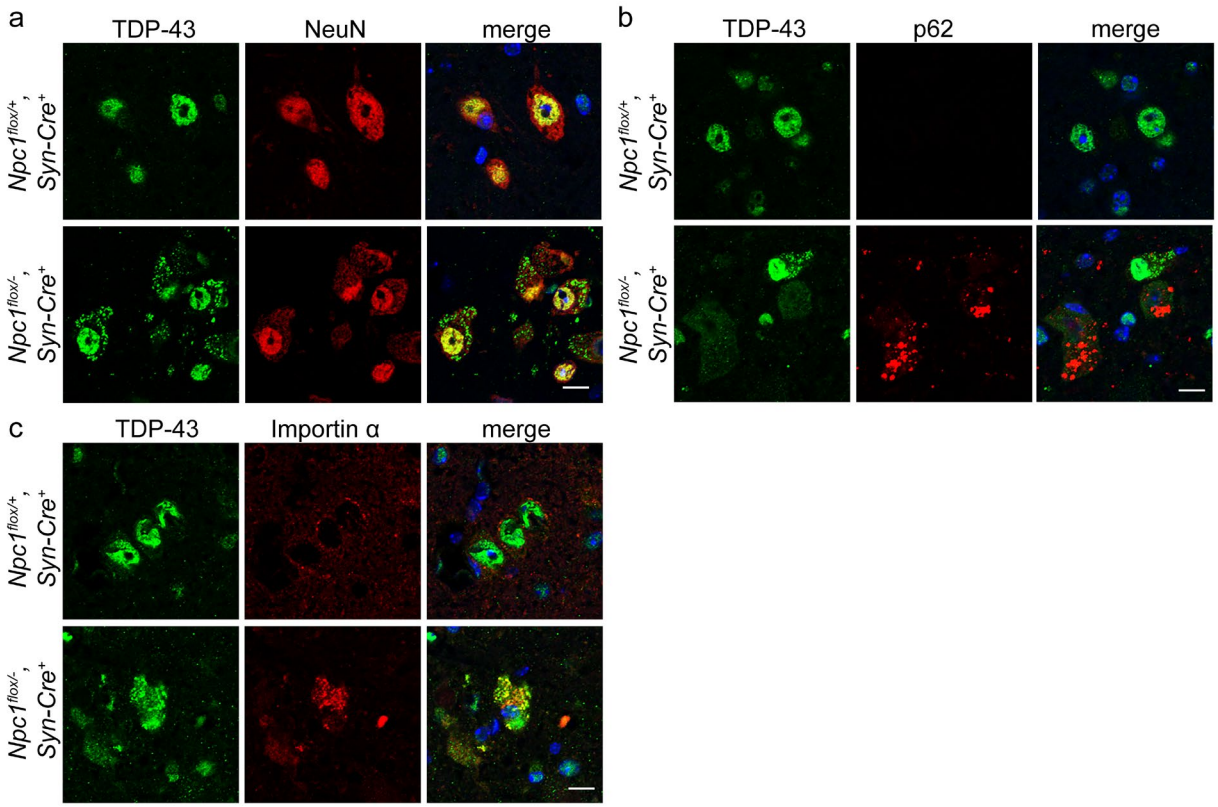
Supplementary Figure 5. Cholesterol accumulates in both p62+ and importin α+ neurons

Brain sections from 11-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice were stained with the indicated markers and imaged by fluorescent microscopy. Scale bar: 10 µm. The percentage of p62+ or importin α+ cells that stain for filipin is shown below. Fifteen to twenty importin α+ or p62+ cells were quantified per mouse, N=3 mice. Data are shown as mean ± s.e.m.



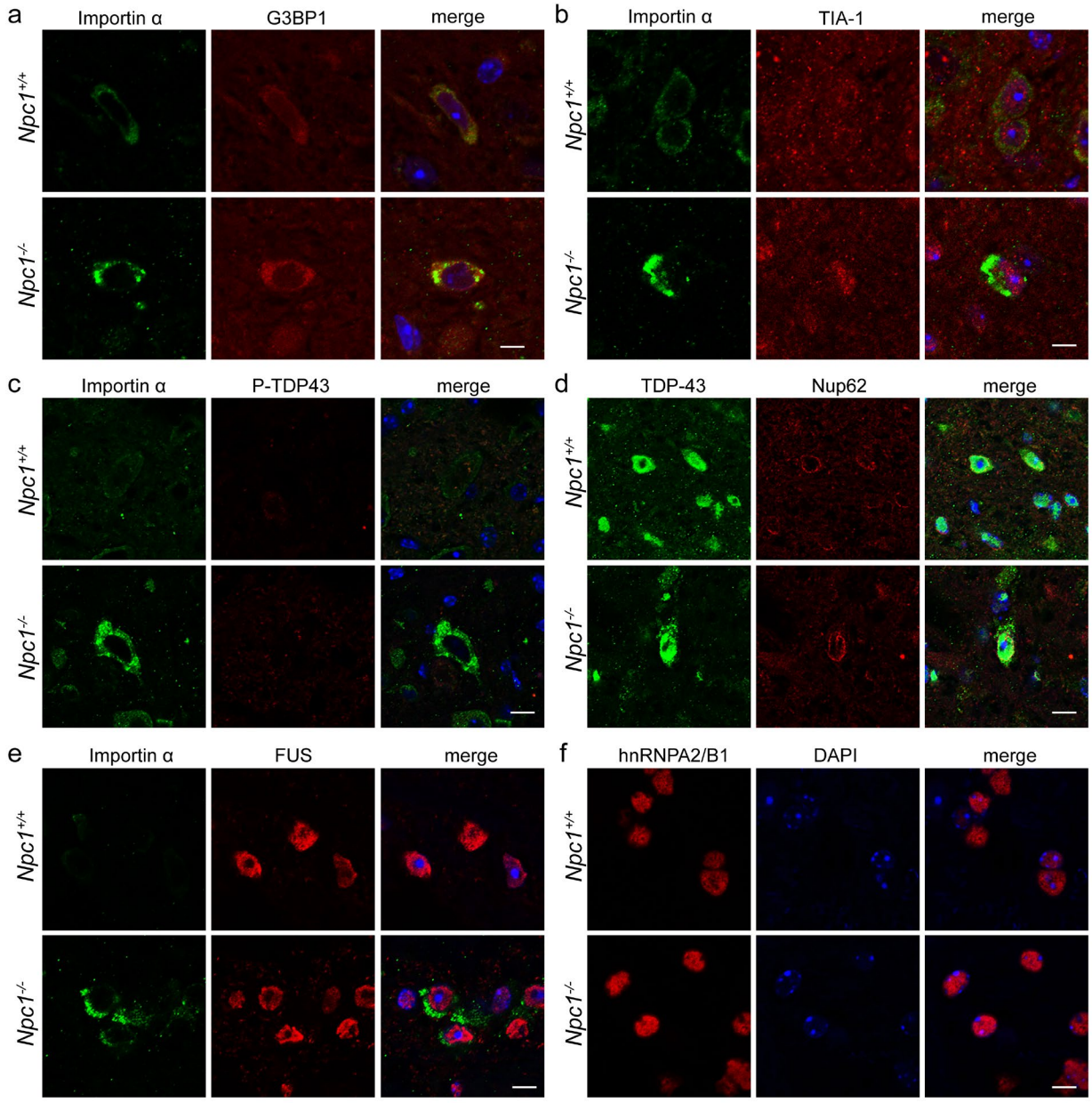
Supplementary Figure 6. TDP-43 and importin α mislocalization is not observed in the cerebellum

Brain sections of 11-week mice were stained with importin α and TDP-43 and imaged by confocal microscopy. Scale bar: 25 µm.



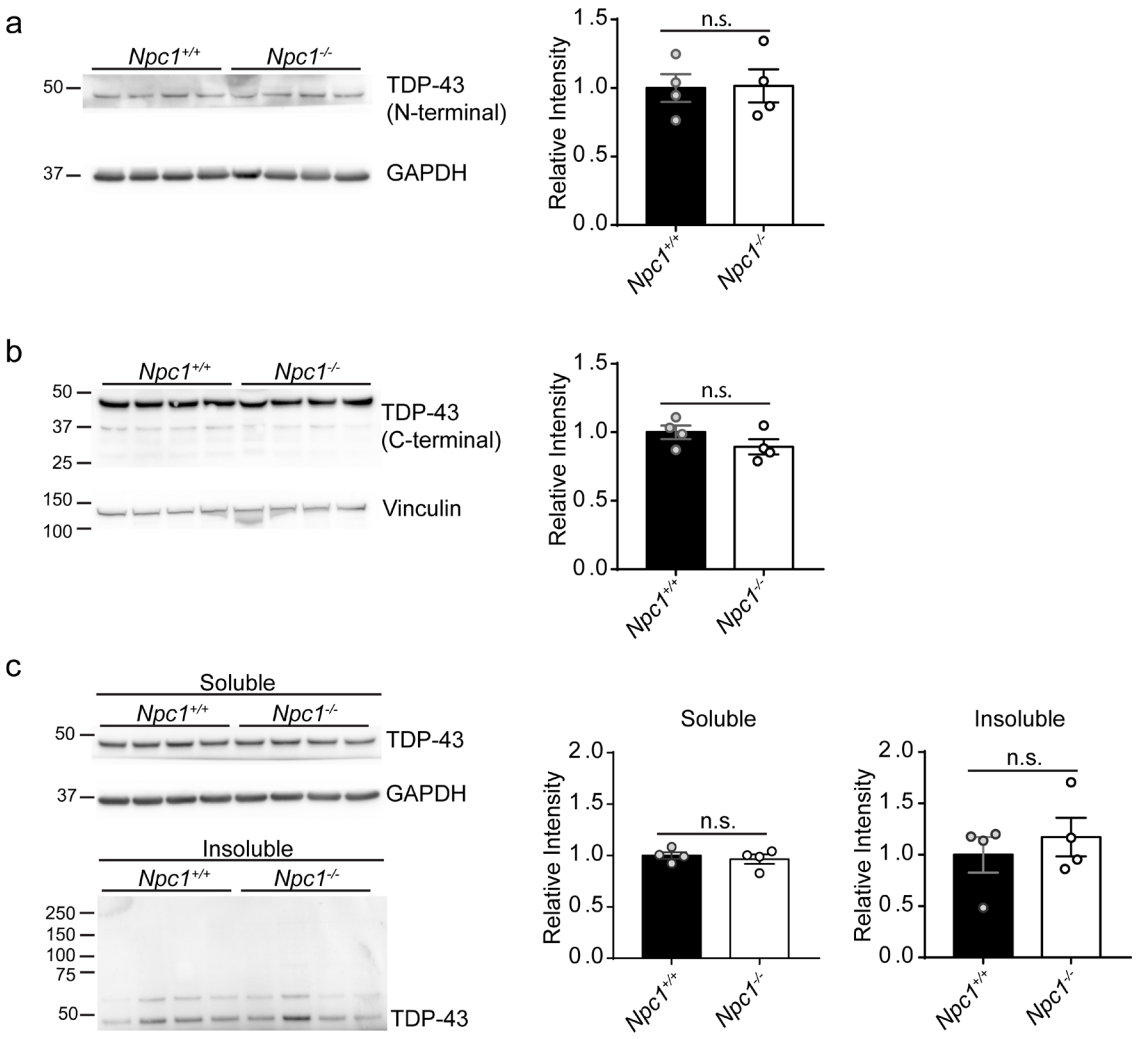
Supplementary Figure 7. TDP-43 mislocalization in neurons occurs cell autonomously

(a-c) Brainstem from 16-week *Npc1*$^{flox/+}$, *Syn-Cre*$^{+}$ and *Npc1*$^{flox/-}$, *Syn-Cre*$^{+}$ mice was stained with the indicated markers and imaged by confocal microscopy. Scale bar: 10 µm.



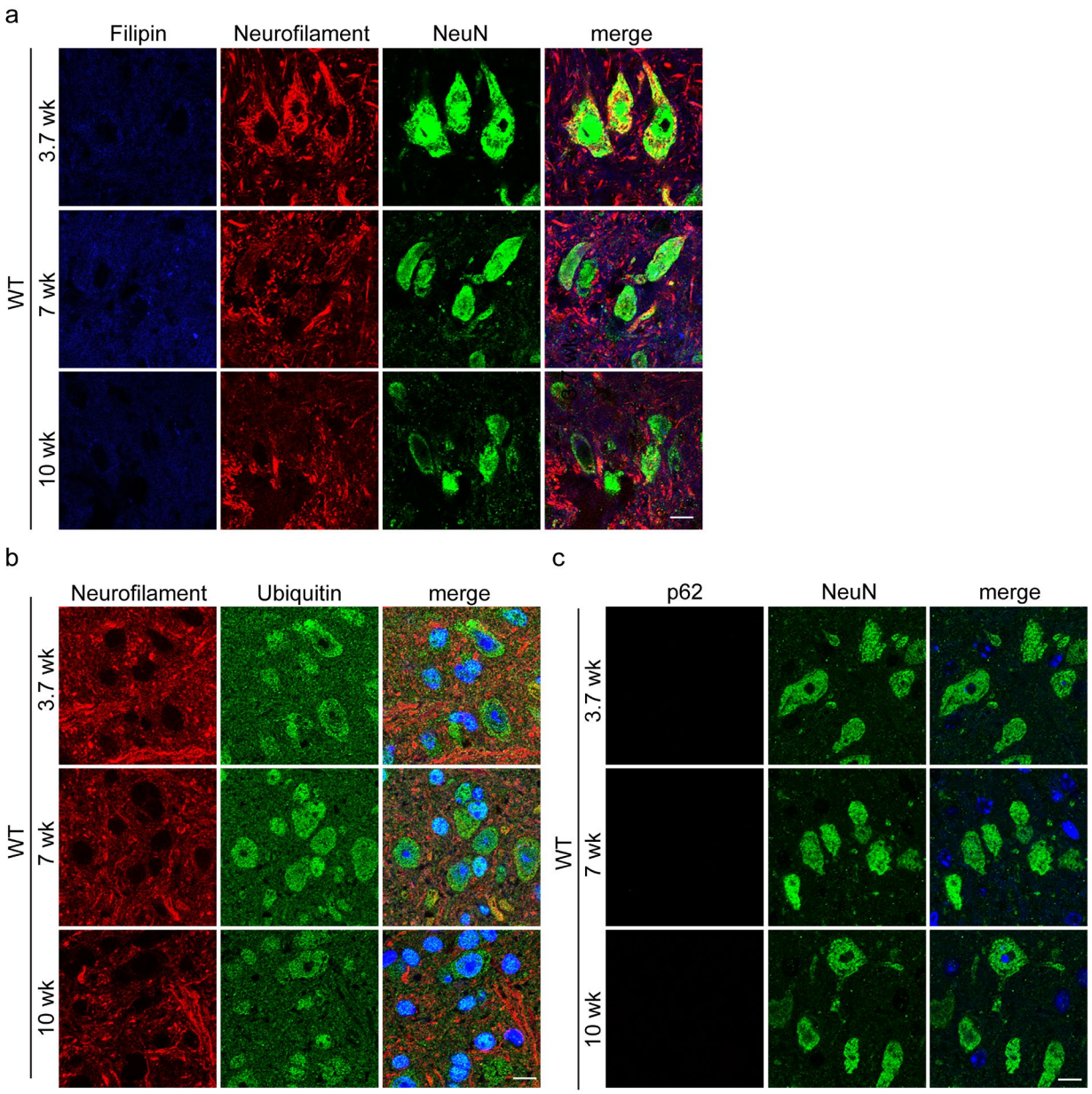
Supplementary Figure 8. *Npc1*$^{-/-}$ mice do not accumulate stress granules, P-TDP43, mislocalized Nup62, or mislocalized FUS and hnRNPA2/B1

(a-f) Brainstem from 11-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice was stained with the indicated markers and imaged by confocal microscopy. Scale bar: 10 μm.



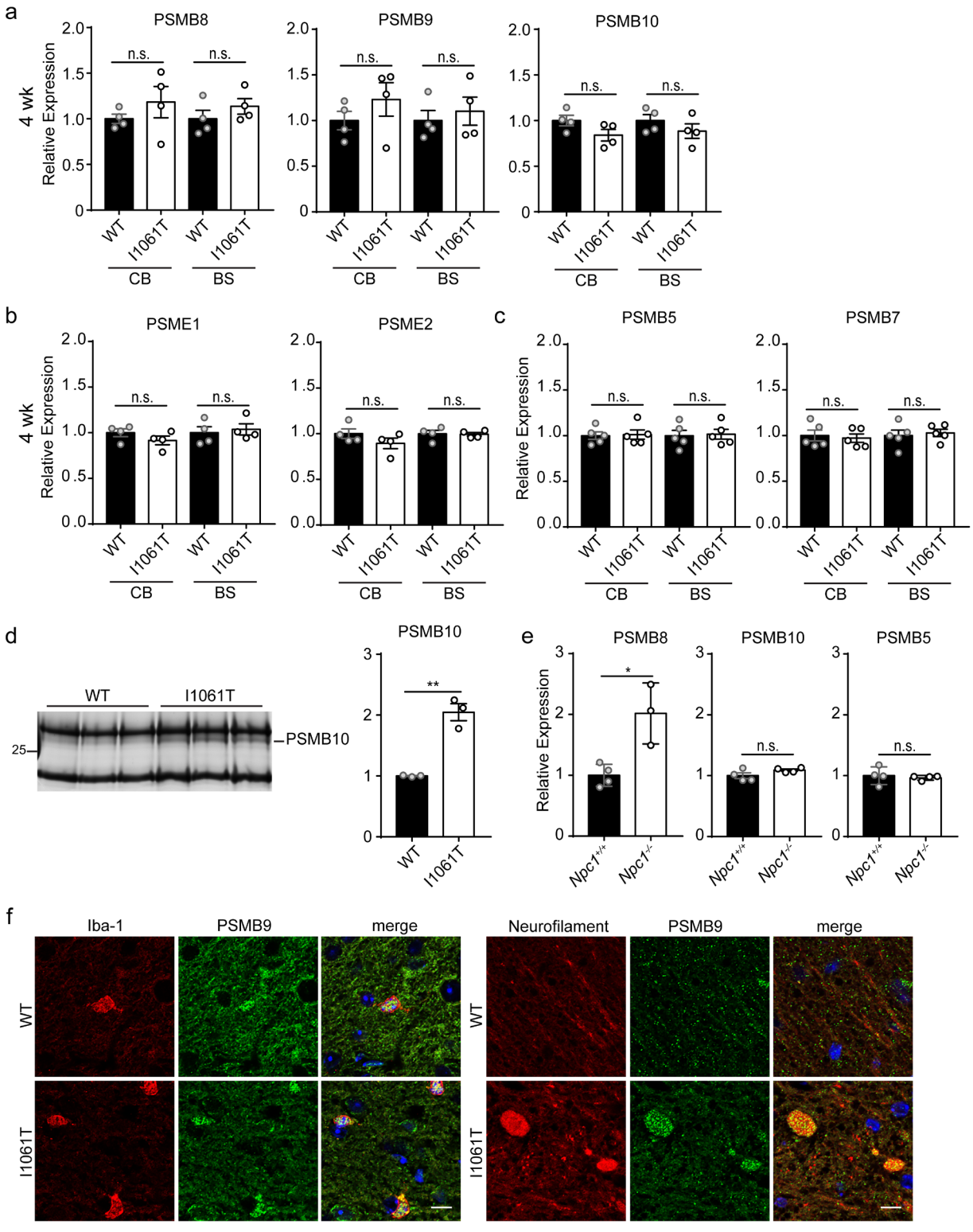
Supplementary Figure 9. TDP-43 protein levels are unchanged and C-terminal fragments do not accumulate in *Npc1*$^{-/-}$ mice

(a-b) The relative abundance of total TDP-43 was determined in the brainstem of 11-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice using an N-terminal **(a)** or C-terminal **(b)** TDP-43 antibody. Quantified at right. N=4 mice per genotype. **(c)** Western blot analysis of **s**oluble and insoluble TDP-43 in the brainstem of 11-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$. N=4 mice per genotype. Data are shown as mean ± s.e.m. n.s., not significant by **(a-c)** Student's t-test **(a)** t=0.0987 **(b)** t=1.424 **(c)** t=0.6154 (soluble); t= 0.6732 (insoluble)



Supplementary Figure 10. WT mice do not accumulate cholesterol or autophagic substrates

(a-c) Brainstem from 3.7, 7 and 10-week WT mice was stained with the indicated markers and imaged by confocal microscopy. Scale bar: 10µm.



Supplementary Figure 11. Age-dependent induction of the immunoproteasome in *Npc1-I1061T* and *Npc1*$^{-/-}$ mice

(a-c) Relative expression of immunoproteasome 20S core **(a)**, alternative lid **(b)** and constitutive proteasome **(c)** subunits was determined by qPCR in the cerebellum (CB) and brainstem (BS) of 4-week **(a-b)** and 12-week **(c)** WT and *Npc1-I1061T* mice. N=4-5 mice per genotype. **(d)** Lysates from 8-week brainstem were incubated with a BODIPY-labeled activity-based probe, then resolved by SDS-PAGE. Quantification at right, N=3 mice per genotype. **(e)** Relative expression of immunoproteasome and constitutive proteasome subunits was determined by qPCR in the BS of 4-week *Npc1*$^{-/-}$ mice. N=3-4 mice per genotype. **(f)** Brainstem from 11-week WT and *Npc1-I1061T* mice was stained with the indicated markers and imaged by confocal microscopy. Scale bar: 10 μm. Data are shown as mean ± s.e.m. n.s., not significant, **P ≤ 0.005 by **(a-e)** Student's t-test **(a)** t=1.023 (CB PSMB8); t=1.088 (BS PSMB8); t=1.11 (CB PSMB9); t=0.5413 (BS PSMB9); t=1.873 (CB PSMB10); t=1.126 (BS PSMB10) **(b)** t=1.307 (CB PSME1); t=0.4092 (BS PSME1); t=1.353 (CB PSME2); t=0.1487 (BS PSME2) **(c)** t=0.1711 (CB PSMB5); t=0.1898 (BS PSMB5); t=0.3676 (CB PSMB7); t=0.4048 (BS PSMB7) **(d)** t=7.489 **(e)** t=3.833 (PSMB8); t=1.787 (PSMB10); t=0.4553 (PSMB5)