Supporting Information for

Original article

Cathepsin D overexpression in the nervous system rescues lethality and Aβ42 accumulation of cathepsin D systemic knockout *in vivo*

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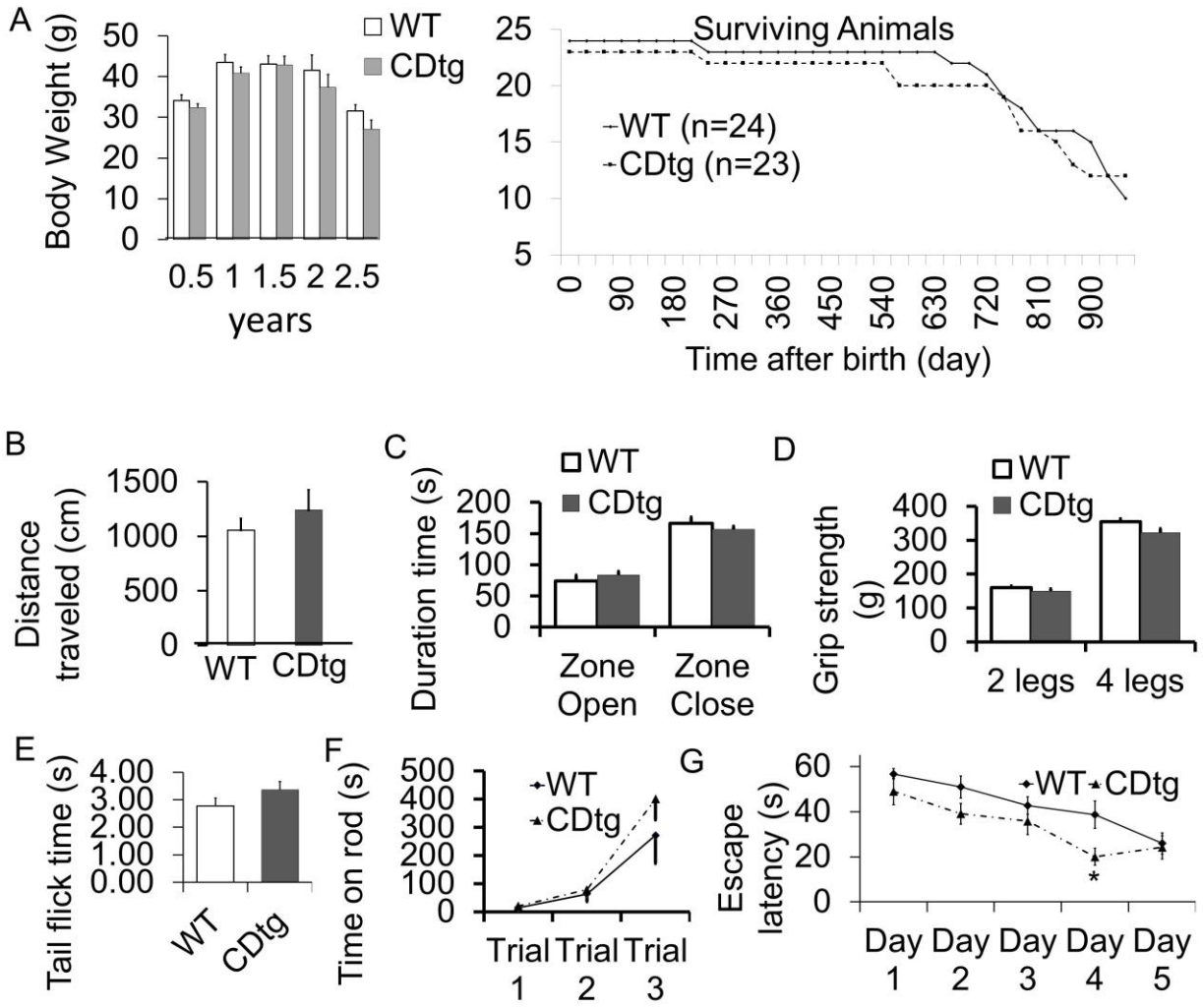


Figure S1 Normal body weight, lifespan, and behaviors of CDtg mice. A) The body weight of CDtg mice are indistinguishable from wildtype mice (*n*=8–24 male). Both wildtype and CDtg mice start to die of natural causes at 22 months of age and median lifespan of 30 months of age (male). At one year of age (*n*=6 each genotype): **B)** Performance in open field was examined and the total distance traveled within examination windows was indistinguishable for the CDtg mice compared to the wildtype mice; **C)** Performance in zero maze was examined and CDtg mice spent similar time in open and close zone as wildtype mice; **D)** Grip strength was similar between Cdtg and wildtype mice; **E)** Tail flick time was similar between Cdtg and wildtype mice; **F)** CDtg mice exhibit modest increase of time on rod compared to wildtype mice on the accelerating rotarod (*P*=0.046); and **G)** Performance in Morris water maze were also similar between CDtg and control mice, except on Day 4. Data=mean±SEM. Behavioral data were analyzed using two-way repeated

measures analysis of variance (RM ANOVA). Animal genotype is a grouping factor while the repeated observation within animal is the repeated measure. No differences in Water maze and rotarod performance were found at 1.5 years of age (*n*=8–9 male each genotype) and in open field and zero maze tests at 2 years of age (*n*=6–7 male each genotype) (data not shown).

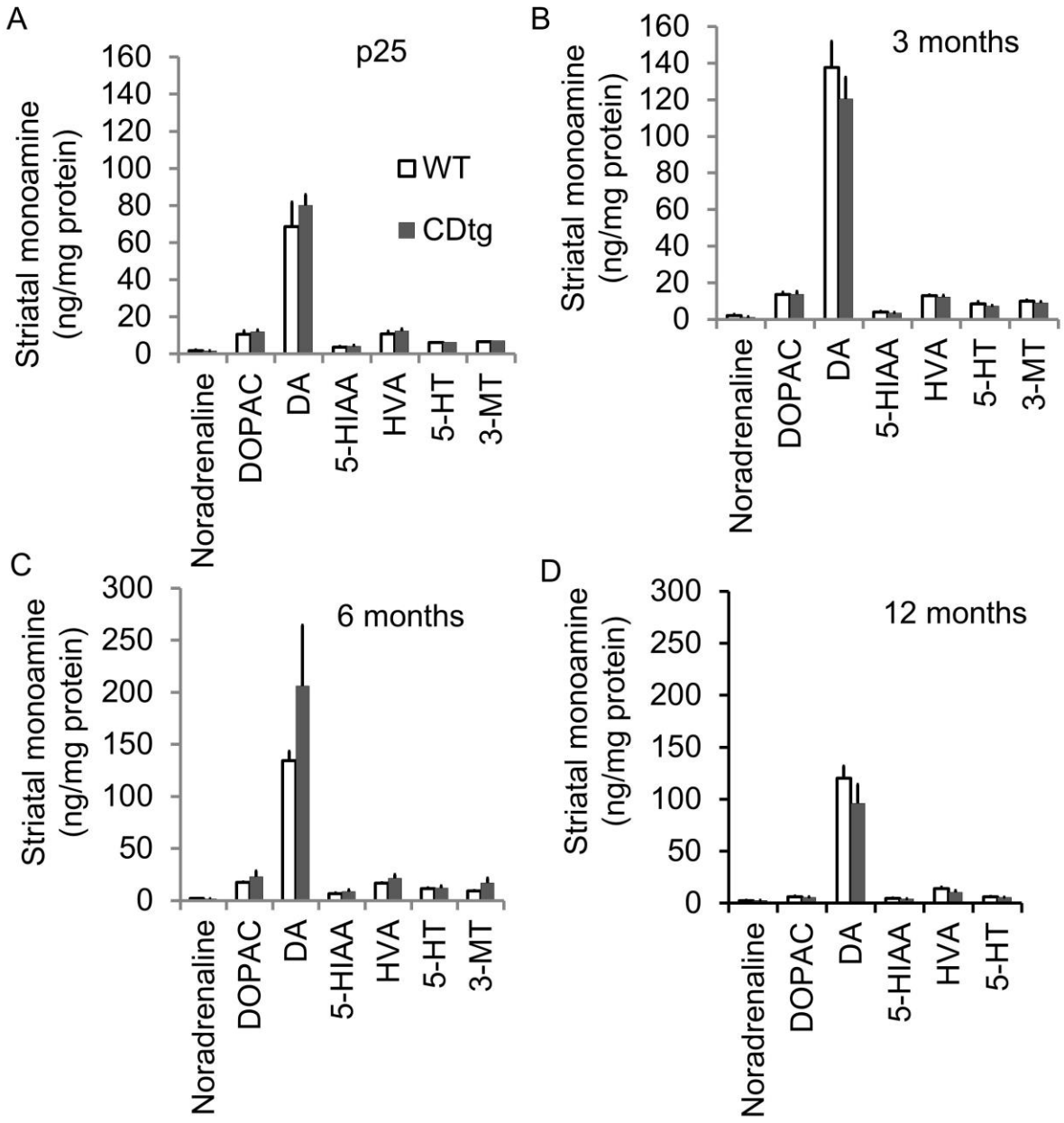


Figure S2 Normal striatal monoamine levels in CDtg mice. Normal striatal monoamine levels in CDtg mice as assessed by HPLC analyses at **A)** postnatal 25 (P25) (*n*=7), **B)** 3 mo of age (*n*=3), **C)** 6 mo of age (*n*=7), and **D)** 1 year of age (*n*=3). Data = mean ± SEM.

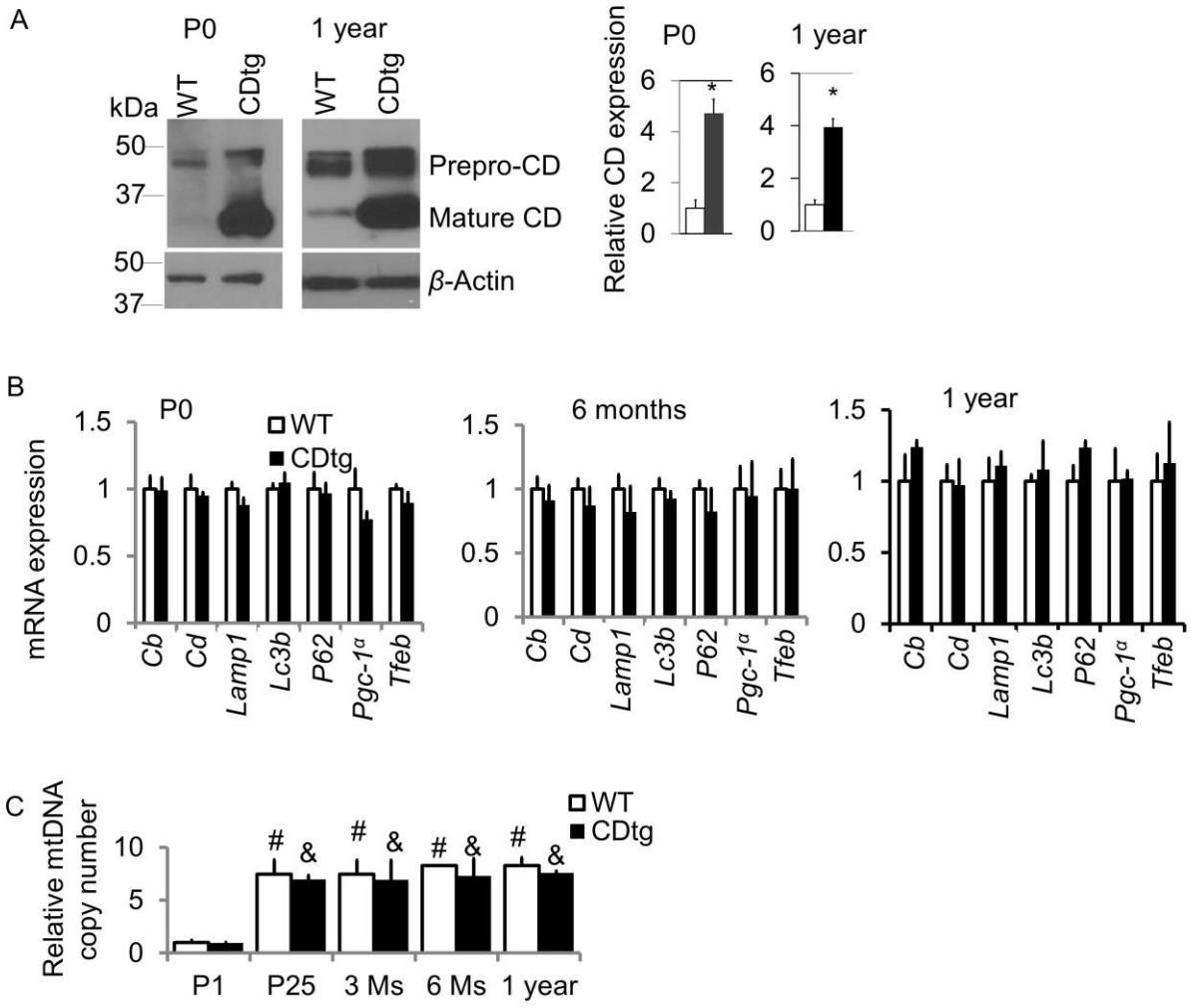


Figure S3 Normal levels of autophagy related mRNAs and mitochondrial copy numbers in CDtg mice. A) Western blot analysis of cortical lysates from WT and CDtg mice (P0 and 1 year old) for Ctsd. *β*-Actin was used as a protein loading control. Quantification of the band intensity was done by image J software 1.48e NIH, USA. **B)** Real-time RT-PCR analyses of indicated mRNA, with *β*-actin as a control. Similar mRNA expression of *Cb*, endogenous mouse *Ctsd*, *Lamp1*, *Map1-lc3*, *Sqstm1/P62*, *Ppagc1a* and *Tfeb* in CDtg mice compared to WT mice (*n*=3 each genotype at P0, 6 months and 1 year of age. **C)** mtDNA copy number was measured by real-time PCR normalized to nuclear DNA. Similar mtDNA copy number at P1, P25, 3 months, 6 months,

and 1 year of age in CDtg mice compared to WT mice. Data=mean±SEM (n = 4) normalized to WT. Comparison between two groups was performed using unpaired Student t-test. *P < 0.05 compared to WT, $^{\#}P$<0.05 compared to WT P1, $^{\&}P$<0.05 compared to CDtg P1.

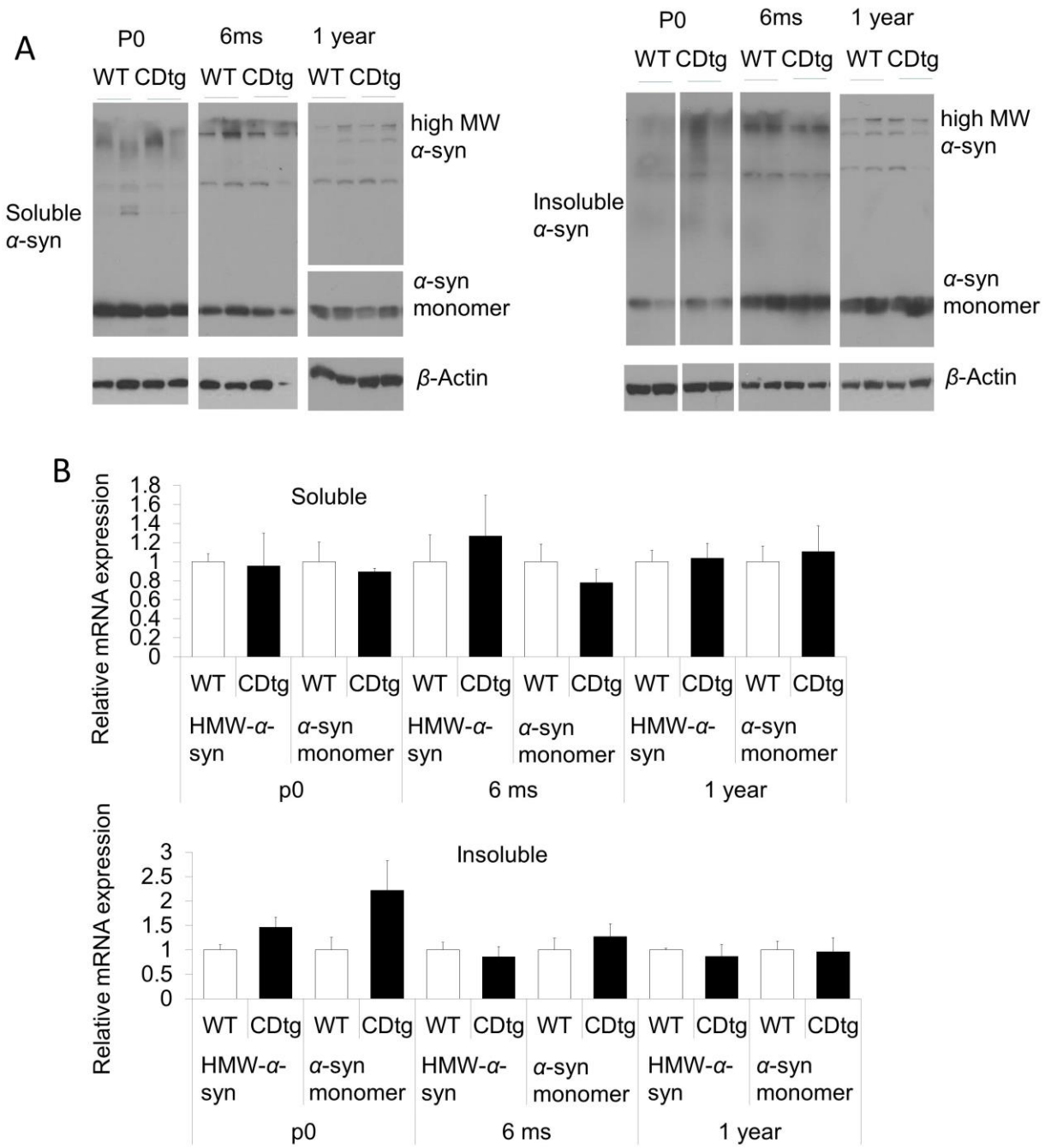


Figure S4 Similar endogenous α-synuclein levels in CDtg and wildtype (WT) mouse cortex. **A)** Western blot analyses of 1% Triton-X100 soluble and insoluble (pellet dissolved in sample

buffer containing 2% SDS) α-synuclein with protein extracts from cortex of CDtg and WT mouse brains. **B)** Quantification of immunoreactive bands for both monomer and higher molecular weight species, n=3 mice each genotype. β-actin was used as a protein loading control. Quantification of the band intensity was done by image J software 1.48e NIH, USA. Data=mean±SEM normalized to WT. Comparison between two groups was performed using unpaired Student t-test.

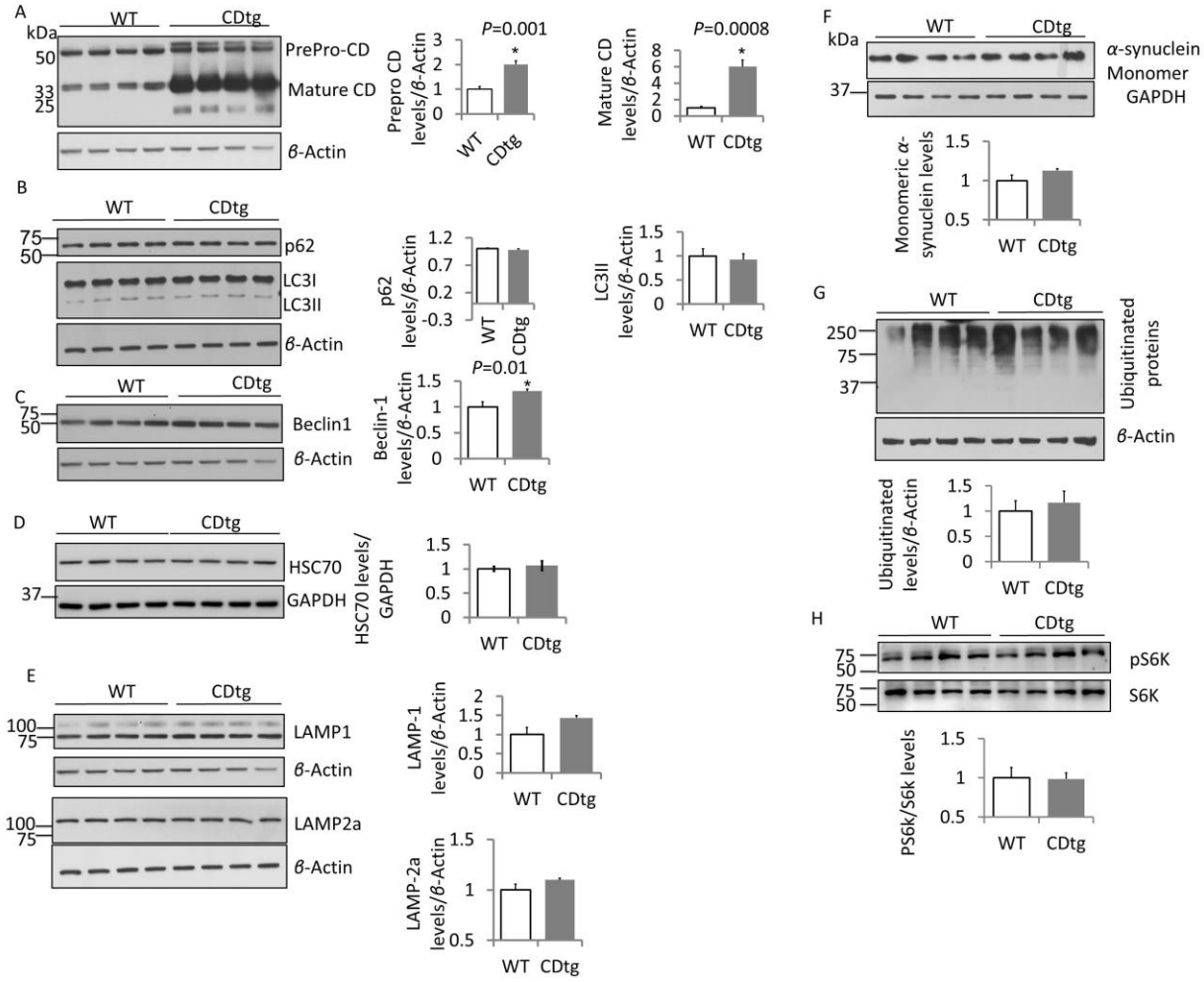


Figure S5 Western blot analysis of cortical lysates from WT and CDtg (2 year old) for indicated proteins. β-Actin or GAPDH was used as a protein loading control. Quantification of the band intensity was done by image J software 1.48e NIH, USA. Data=mean±SEM (n = 4) normalized to WT. Comparison between two groups was performed using unpaired Student t-test. *P < 0.05 was considered statistically significant.

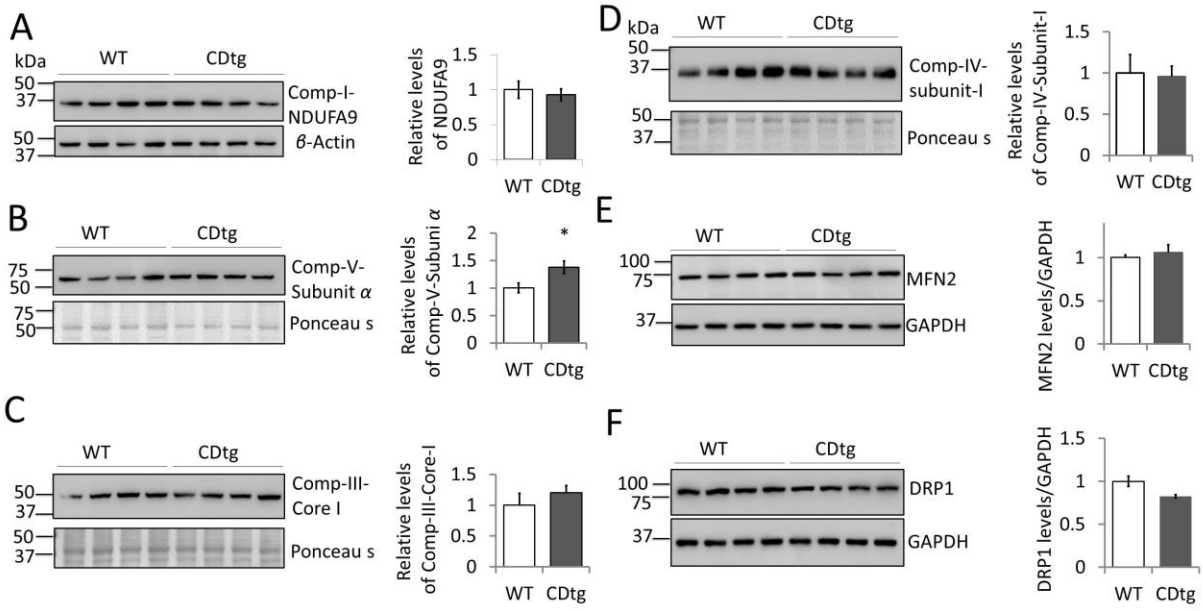


Figure S6 Western blot analysis of cortical lysates from WT and CDtg (2 year old) for indicated proteins. *β*-Actin or GAPDH was used as a protein loading control. Quantification of the band intensity was done by image J software 1.48e NIH, USA. Data=mean±SEM (*n* = 4) normalized to WT. Comparison between two groups was performed using unpaired Student *t*-test. **P*<0.05 compared to WT.

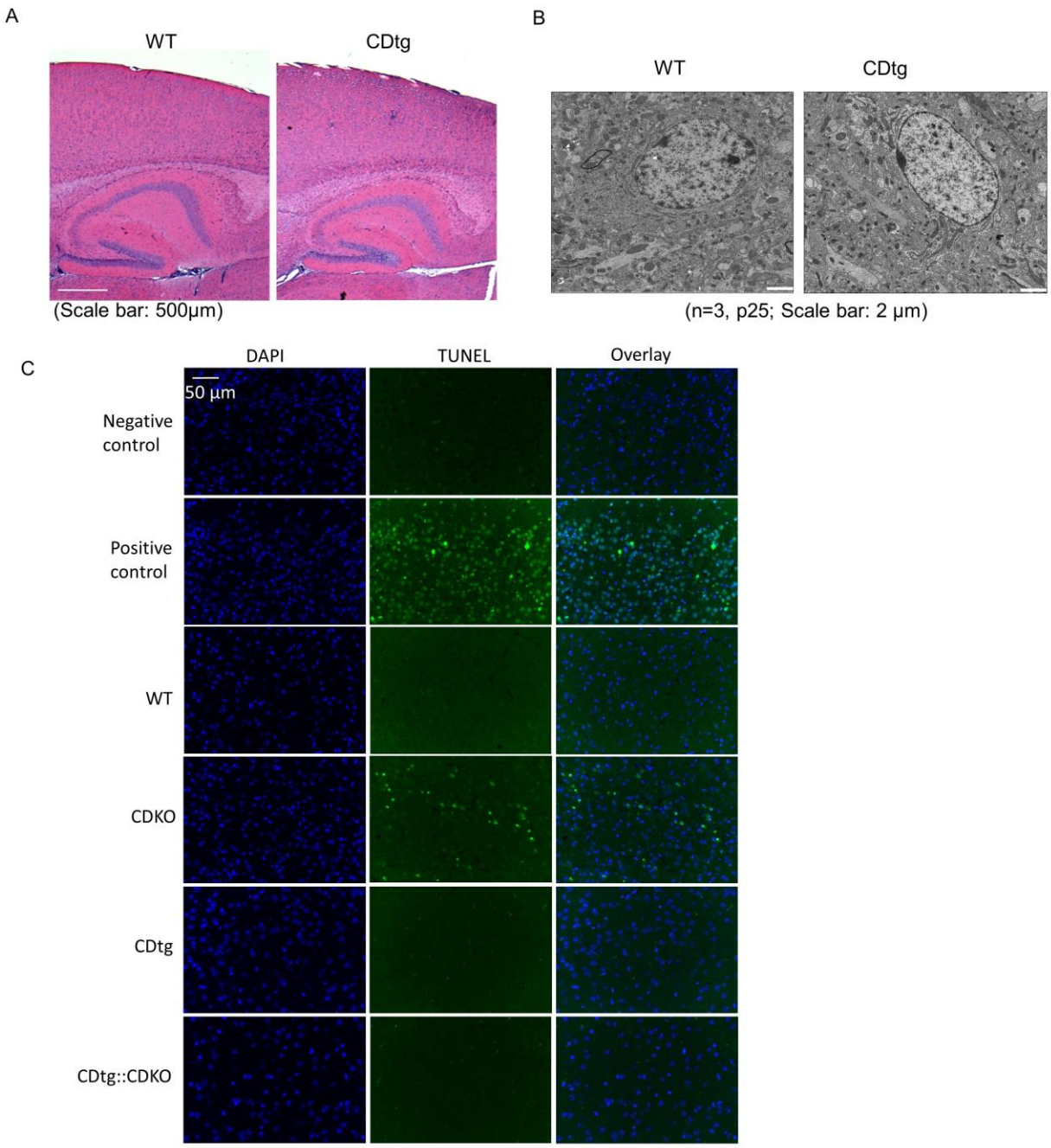


Figure S7 Normal brain structure without overt apoptosis in CDtg mice. A) Normal appearance of cortical regions of CDtg mice compared to wildtype (WT) mice by H&E staining (Scale bar=100 μm). **B)** Normal cortical neuron appearance in CDtg mice at P25 by electron microscopy (Scale bar=2 μm). **C)** TUNEL staining show TUNEL positive cells in the cortex of CDKO mice but not in WT, CDtg, or CDtg::CDKO mice (*n*=3 mice each group). DNase treated sections were used as positive control, no TdT treatment was used as negative control.

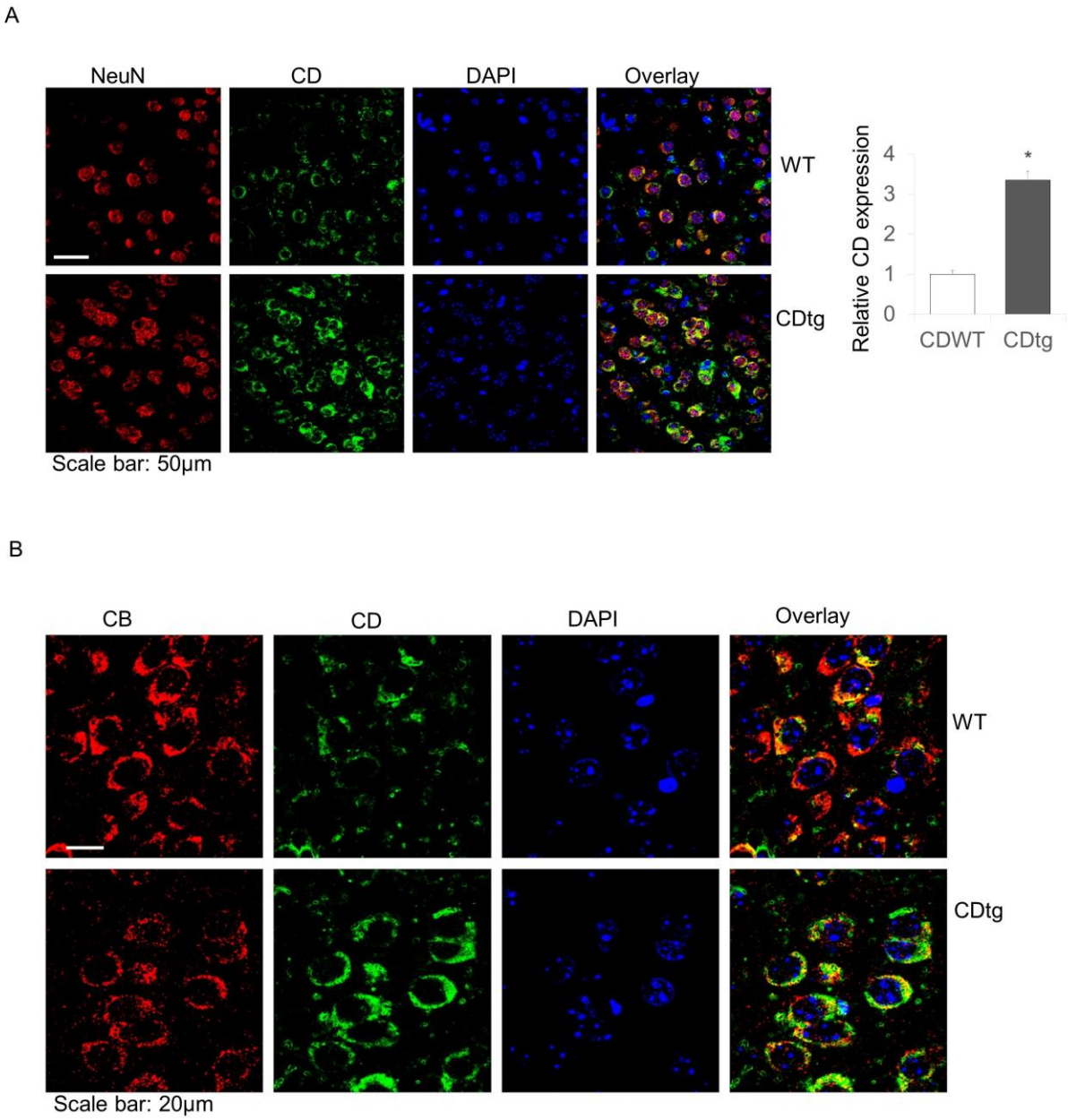
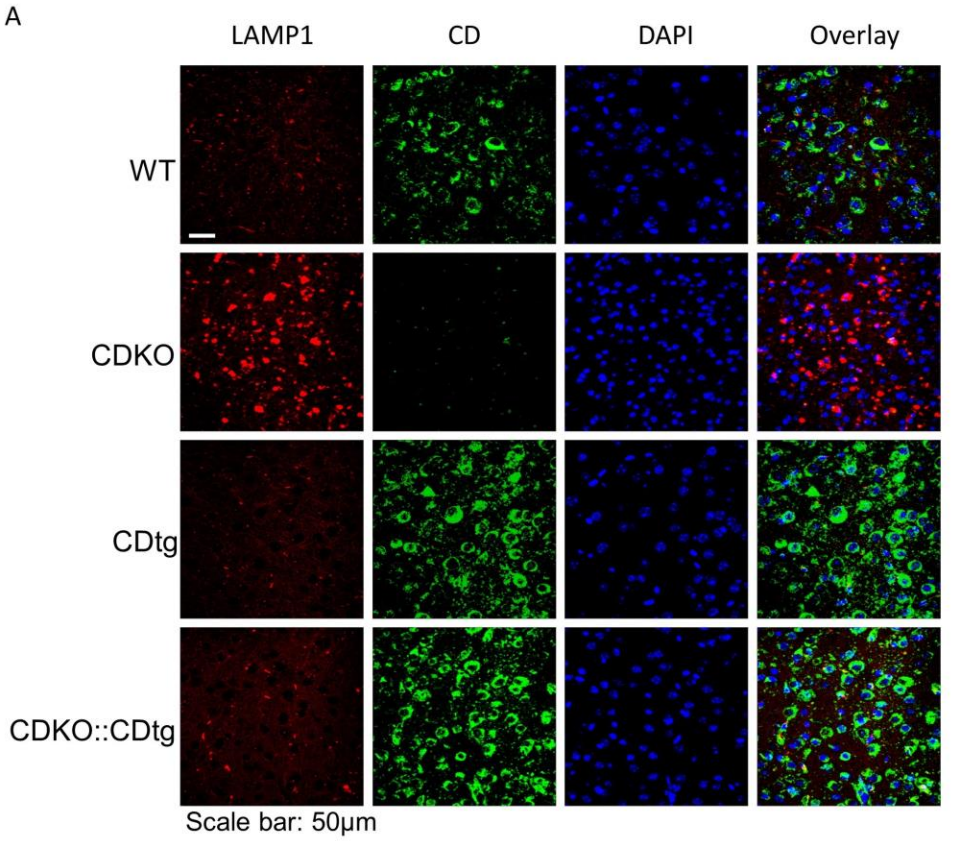


Figure S8 CTSD expression in lysosomes in neurons in CDtg mice. A) By co-immunohistochemistry, we found that Ctsd (Green) is expressed in cells with positive NeuN (Red) immunoreactivity in both wildtype (WT) and CDtg mouse cortex. Scale bar=50 μm. Quantification of immunoreactivity in NeuN positive cells shows that CTSD levels are increase by slightly over 3× fold in neurons. **B)** Ctsd (Green) colocalizes with Ctsb (Red) in both wildtype (WT) and CDtg mouse cortex by co-immunohistochemistry. Scale bar=20 μm. *n*=3 animals per genotype. P25 brains were used.


A
LAMP1
CD
DAPI
Overlay
WT
CDKO
CDtg
CDKO::CDtg
Scale bar: 50μm

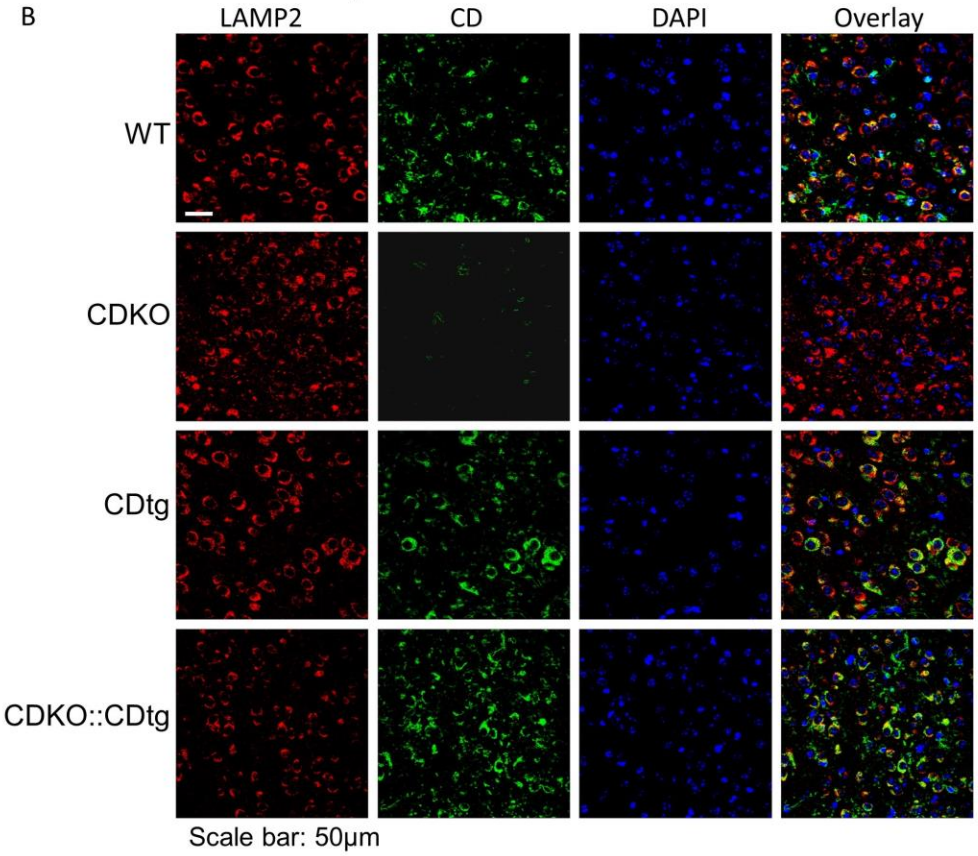

B
LAMP2
CD
DAPI
Overlay
WT
CDKO
CDtg
CDKO::CDtg
Scale bar: 50μm

Figure S9 CTSD overexpression rescued the accumulation of LAMP1 in CDKO mice without changing LAMP2 levels. A) Co-immunohistochemistry of CTSD (Green) with LAMP 1(Red) in mouse cortex (Scale bar=50 µm). **B)**. Co-immunohistochemistry of CTSD (Green) with LAMP2 (Red) in mouse cortex (Scale bar=50µm). *n*=3 animals per genotype. P25 brains were used.

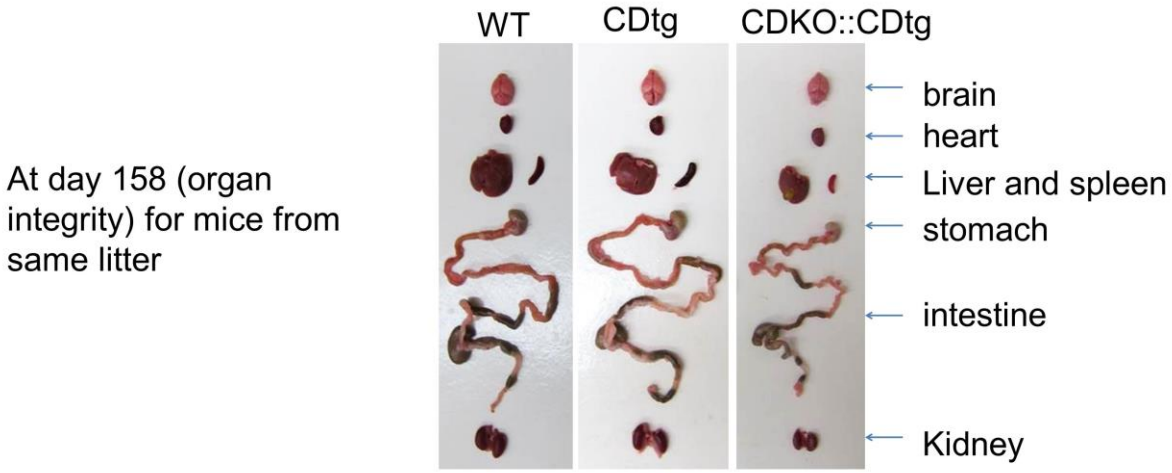


Figure S10 CDtg::CDKO mice succumb to intestinal necrosis at around P150. Tissue appearance is shown from representative dissections of mice of the 3 indicated genotypes. Tissues in CDKO::CDtg mice are smaller than wildtype or CDtg mice at this age. *n*>3.