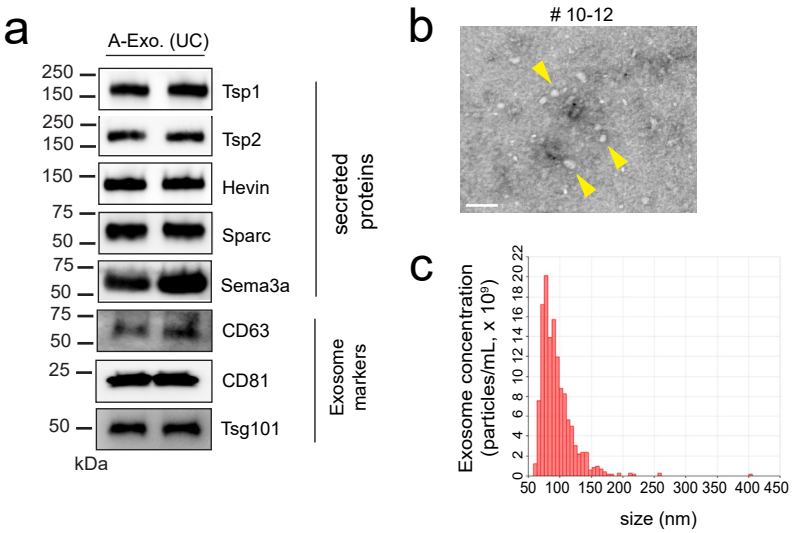
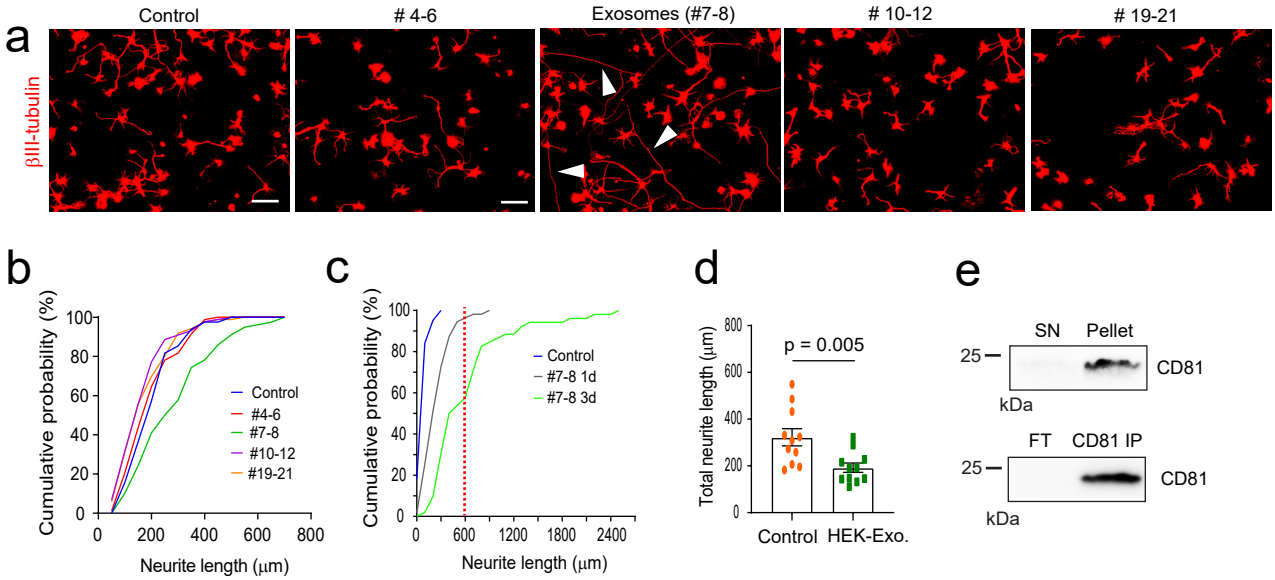


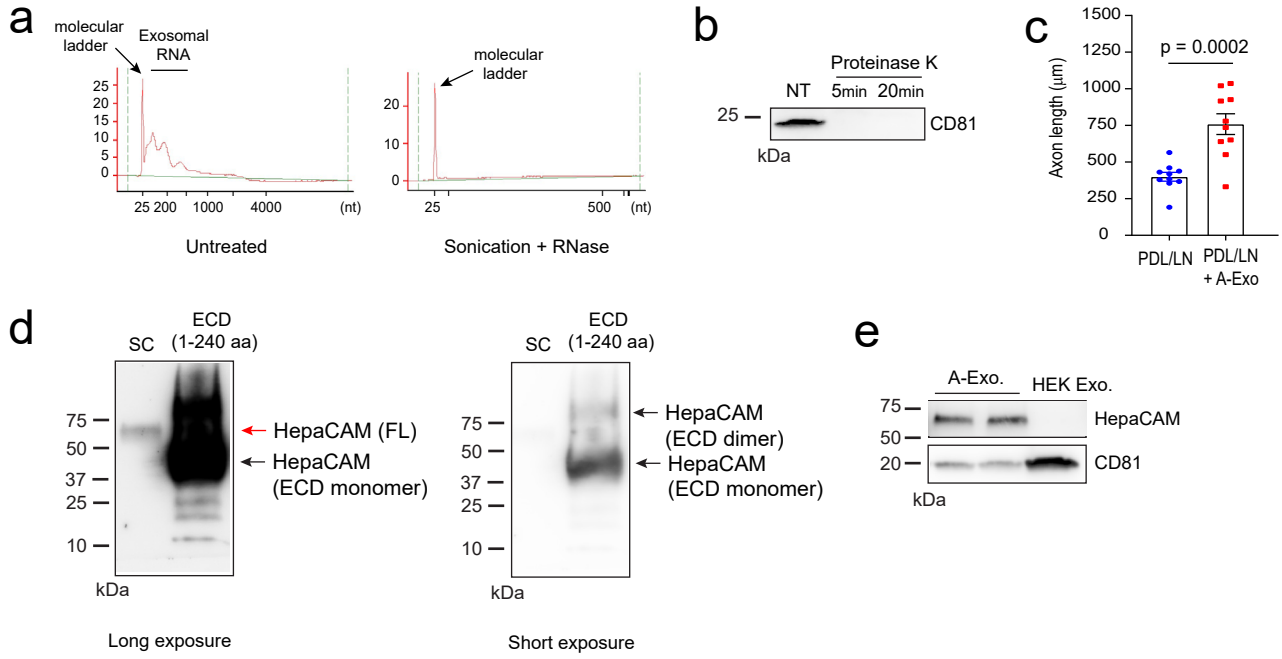
Supplementary figure 1

Supplementary Fig. 1 a, Representative immunoblots of astroglia secreted proteins and exosome markers in A-Exo. isolated from ACM (20 mL/sample) by ultracentrifυγation (UC). **b,** Representative immunoEM images of CD63 labeling in SEC eluted fractions #10-12; yellow arrows: $CD63^{-}$ small vesicles; scale bar: 100 nm. **c**, Representative size distribution analysis of WT A-Exo measured by the qNano particle analyzer.



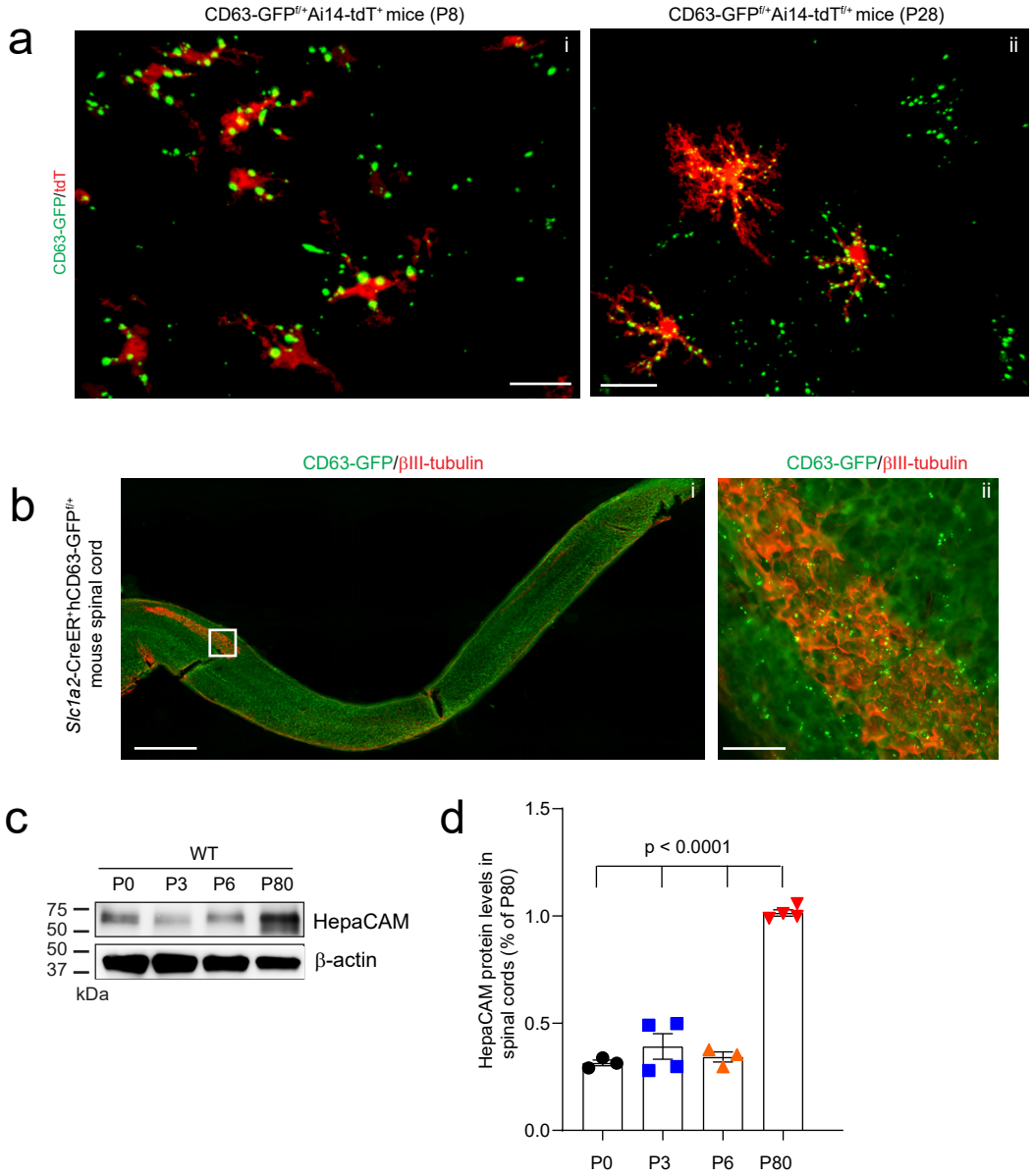
Supplementary figure 2

Supplementary Fig. 2 a, Representative images of βIII-tubulin$^+$ cortical neurons following treatment with eluted fractions (pooled as indicated) #4-6, #7-8, #10-12, or #19-21 from the SEC. Scale bar: 100 μm; **b**, Quantification of total neurite length of cortical neurons following treatment with eluted fractions (pooled as indicated, 100 μl). #4-6 (no protein detected), #10-12, and #19-21 (1μg/μl) from the SEC of ACM (initial 100 mL). 1μg exosomes (#7-8) were used in treatment, n = 78-88 neurons (> 3 biological replicates)/group; **c**, Quantification of total neurite length of cortical neurons following treatment with fractions #7-8 (5μl, 0.2 μg/μl) for 1 or 3 d. n = 52-82 neurons (> 3 biological replicates)/group; **d**, Quantification of total neurite length of cortical neurons following treatment with HEK exosomes isolated by SEC. n = 11-13 neurons (2 biological replicates)/group; **e**, Representative immunoblot of CD81 in the supernatant (SN) or pellet of SEC fractions #7-8 (1 mL, from initial 10 mL ACM) following an additional 24 h ultracentrifugation (UC, 100,000 x g), or in the flowthrough (FT) or CD81 immunoprecipitation (IP) pellet of SEC fractions #7-8 after CD81 pull-down. **f**, Quantification of dendrite (Map2$^+$βIII-tubulin$^+$) length of cortical neurons following A-Exo. treatment. n = 51-55 neurons (> 3 biological replicates)/group; **g**, Representative images of Map2 and Tau staining on cortical neurons following A-Exo treatment. Scale bar: 50 μm; **h**, Sholl analysis of cortical neurons following A-Exo treatment. n = 10 neurons (2 biological replicates)/group; 1 μg exosome was used in **b-c, f,** and **h**. p values in **d** and **f** determined from two-tailed t test.



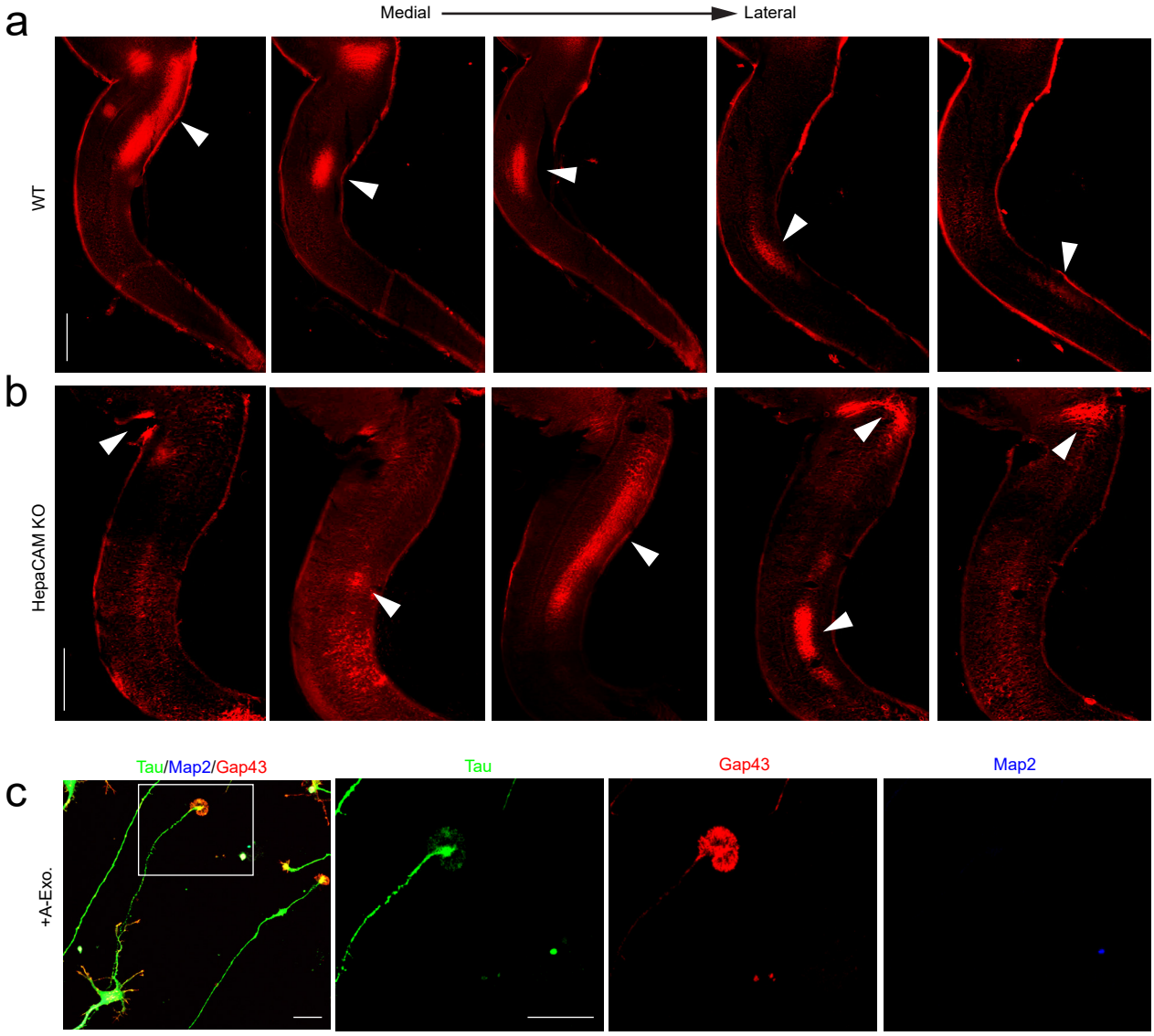
Supplementary figure 3

Supplementary Fig. 3 a, Representative bioanalyzer tracer of exosomal RNA with and without RNase treatment (5 minutes) following sonication. Sufficient small RNA was observed in untreated A-Exo. **b**, Representative immunoblot of CD81 following proteinase K treatment. 0.5 μg A-Exo. was treated with proteinase K for either 5 or 20 minutes. NT: not treated A-Exo; CD81 immunoreactivity disappeared from the immunoblot as a result of the proteinase K digestion; **c**, Quantification of axon length of cortical neurons plated on either PDL/laminin (LN) coated or PDL/LN/A-Exo. coated coverslips. n = 10 neurons (2 biological replicates)/group; 1μg A-Exo. was used in each treatment. p value in **c** determined from two-tailed t test; **d**, Representative HepaCAM immunoblot with spinal cord (sc) lysate (20 μg) and recombinant human HepaCAM extracellular domain (ECD) protein (1-240 aa, 1 μg). HepaCAM antibody (Proteintech) is able to detect mouse HepaCAM full-length (sc lane) and human ECD (monomer and dimer). **e**, Representative HepaCAM immunoblot in A-Exo. and HEK exosomes.



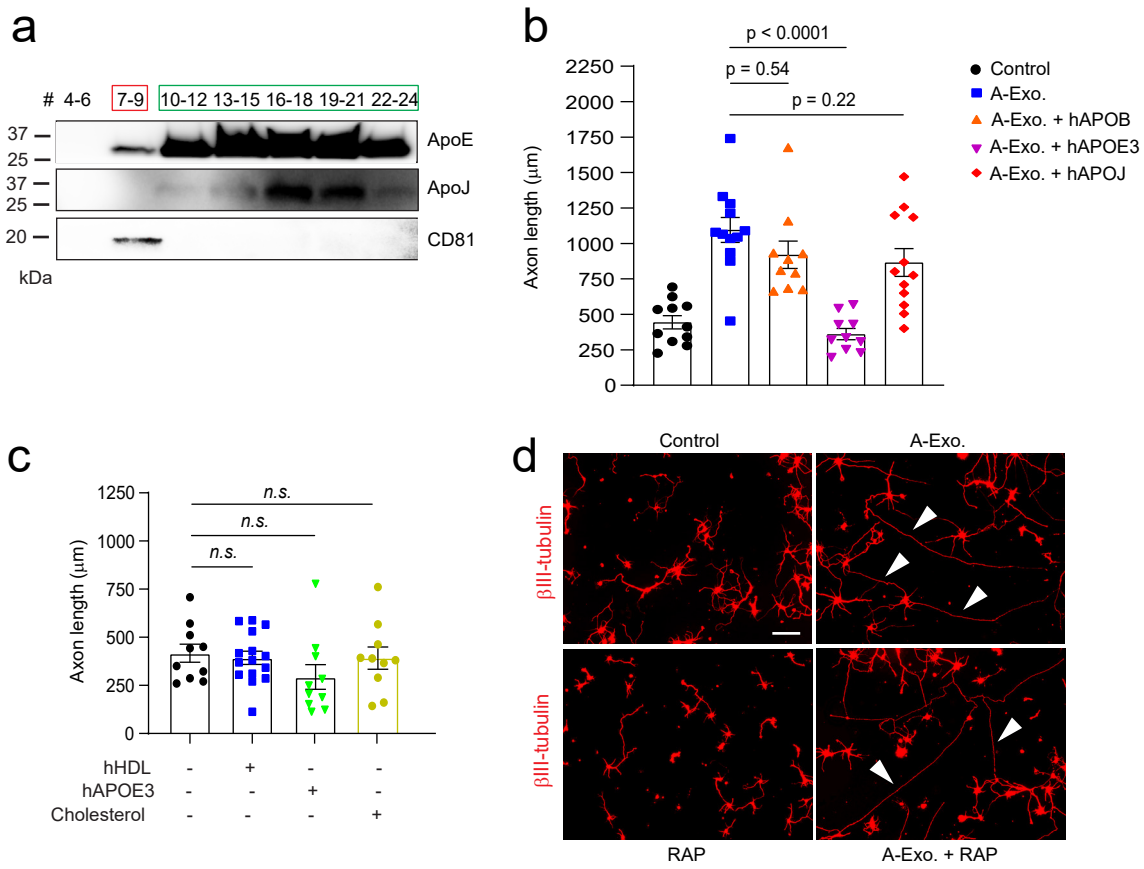
Supplementary figure 4

Supplementary Fig. 4 a, Representative images of tdT$^+$ astroglia and astroglia-derived CD63-GFP$^+$ puncta from the motor cortex of AAV5-mCherry-*Gfap*-Cre-injected CD63-GFP$^{f/+}$Ai14-tdT$^{f/+}$ mice at P8 (i) and P28 (ii). Scale bar: 20 μm; **b**, Representative longitudinal image of βIII-tubulin staining and astroglia-δεριϖεδ CD63-GFP$^+$ puncta along the spinal cord from 4-OHT-injected *Slc1a3*-CreER$^+$ mice at P8. Subpanel i: the longitudinal image of the spinal cord; Subpanel ii: a magnified view of the box in the subpanel i; Scale bar: 1mm (subpanel i); 100 μm (subpanel ii); Representative HepaCAM immunoblot (**c**) and quantification (**d**) of HepaCAM expression in spinal cords during postnatal development. n = 3-4 mice/time point; p values determined by one-way ANOVA followed by post-hoc Tukey's test.



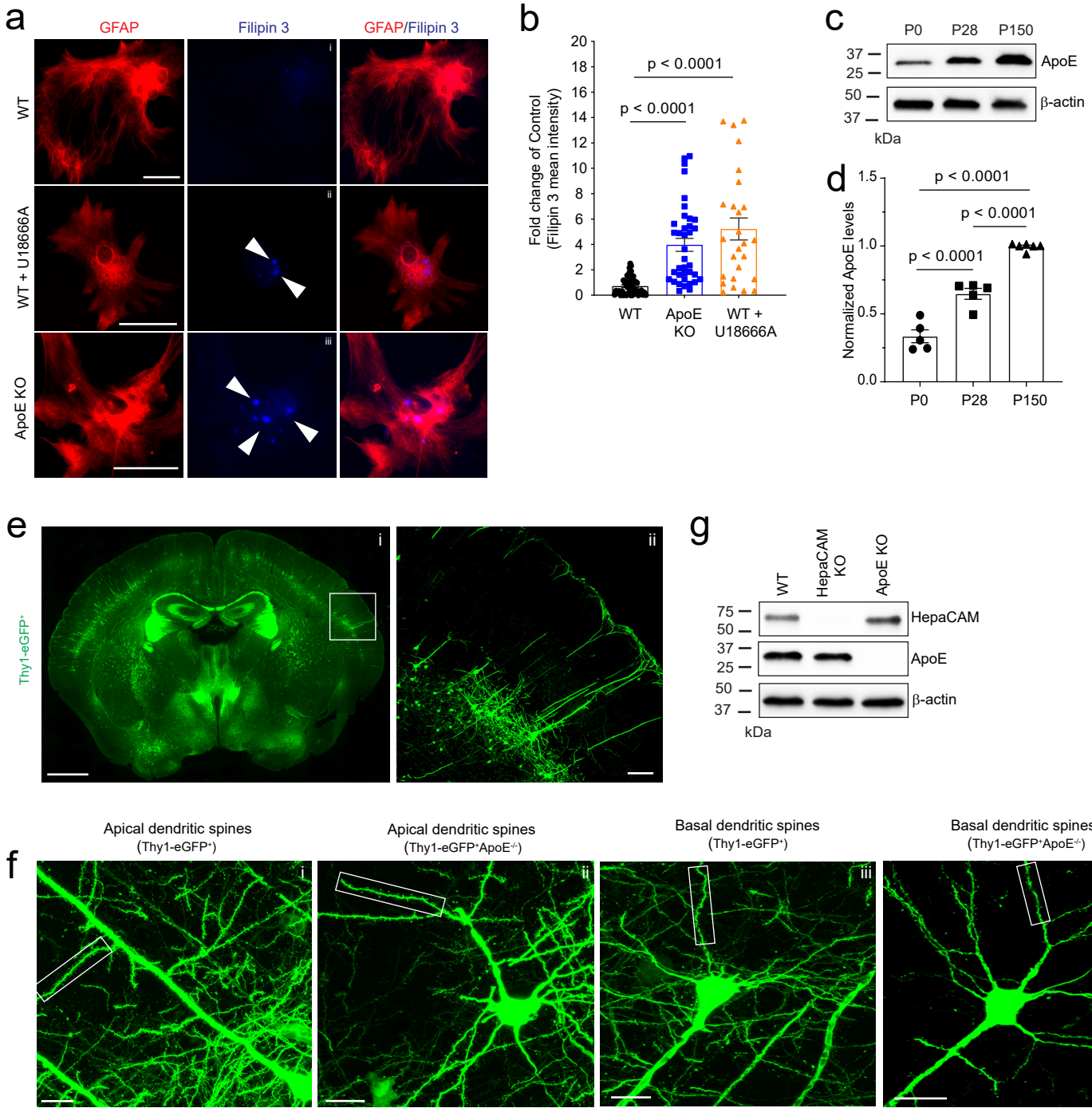
Supplementary figure 5

Supplementary Fig. 5 Representative original set of longitudinal images from CM-DiI-injected WT (**a**) and HepaCAM KO (**b**) mouse spinal cords that were superimposed into the continuous CST axon growth image shown in Fig. 5C. Images of longitudinal spinal cord sections were taken from lateral to medial orientation at P3. White arrows: CM-DiI labeling; Scale bar: 1mm; **c**, Representative image of Tau, Map2, and Gap43 immunostaining of A-Exo-treated cultured cortical neurons to illustrate axonal growth cones and axons; Scale bar: 20 μm.



Supplementary figure 6

Supplementary Fig. 6 a, Representative immunoblot of ApoE and ApoJ in all eluted fractions (500 μl/fraction, pooled as indicated) of ACM (100 mL) from SEC with oversaturated exposure. 15 μl unconcentrated elution was run on immunoblot. **b**, Quantification of βIII-tubulin$^+$ neuronal axon length following co-treatment of A-Exo. with hAPOEB, hAPOEJ, or hAPOE3, respectively. 1μg A-Exo. was used in treatment. hAPOEB, hAPOEJ, or hAPOE3 each was at 10 μg/mL dose. n = 11-12 neurons (2 biological replicates)/group; **c**, Quantification of βIII-tubulin$^+$ neuronal axon length following treatment of hHDL (10 μg/mL), hApoE3 (20 μg/mL), and cholesterol (1 μg/mL), respectively. n = 10-15 neurons (2 biological replicates)/group; Representative images (**d**) and quantification (**e**) of βIII-tubulin$^+$ neuronal axon (white arrows) length following co-treatment of A-Exo. and ApoE competitive receptor associated protein (RAP, 50 μg/mL). Scale bar: 100 μm; **f**, Representative ApoE immunoblot from WT or ApoE ACM (50μg proteins), and ApoE A-Exo (2 μg proteins). **g**, Detection of HepaCAM but not Apoe following HepaCAM immunoprecipitation from astrocyte lysates (50 μg proteins). 1μg A-Exo. was used in **b, d**, **e,** and **f**. p values in **b, c,** and **e** determined from one-way ANOVA followed by a Tukey post-hoc test.



Supplementary figure 7

Supplementary Fig. 7 Representative images (**a**) of cultured astrocytes and cholesterol labeling and quantification (**b**) of cholesterol in astrocytes based on Filipin 3 fluorescent intensity. Scale bar: 50 μm; White arrows: Filipin 3^+ cholesterol labeling; n = 26-35 astrocytes (3 biological replicates)/group; Representative images (**c**) of ApoE immunoblot and quantification (**d**) of ApoE expression in the cortex during postnatal development; n = 5-6 mice/group; **e**, eGFP labeling of neurons and neurites in Thy1-eGFP$^+$ mice. Subpanel i: Representative image of coronal section of the Thy1-eGFP$^+$ mouse brain (scale bar: 1mm); ii: a magnified view of the motor cortex (white box) in the subpanel i (scale bar: 100 μm); **f**, Representative images of eGFP$^+$ neurons and their dendritic spines. Subpanel i: apical dendritic spines from Thy1-eGFP$^+$ mice; ii: apical dendritic spines from Thy1-eGFP$^+$ApoE$^{-/-}$ mice; iii: basal dendritic spines from Thy1-eGFP$^+$ mice; iv: basal dendritic spines from Thy1-eGFP$^+$ApoE$^{-/-}$ mice; Scale bars: 20 μm; a magnified view of the highlighted box is shown in Fig. 7d-e; **g**, Representative HepaCAM and ApoE immunoblots from cortex of ApoE KO and HepaCAM KO mice at P30.

Supplementary Table 1. Transmembrane proteins identified from A-Exo. by LC/MS/MS. Each identified protein has at least 3 peptide hits with 95% confidence threshold; The mean iBAQ value is greater than 1×10^5.

Supplementary Movies Live imaging of control and A-Exo (1μg). -induced axon growth in primary cortical neuronal cultures.