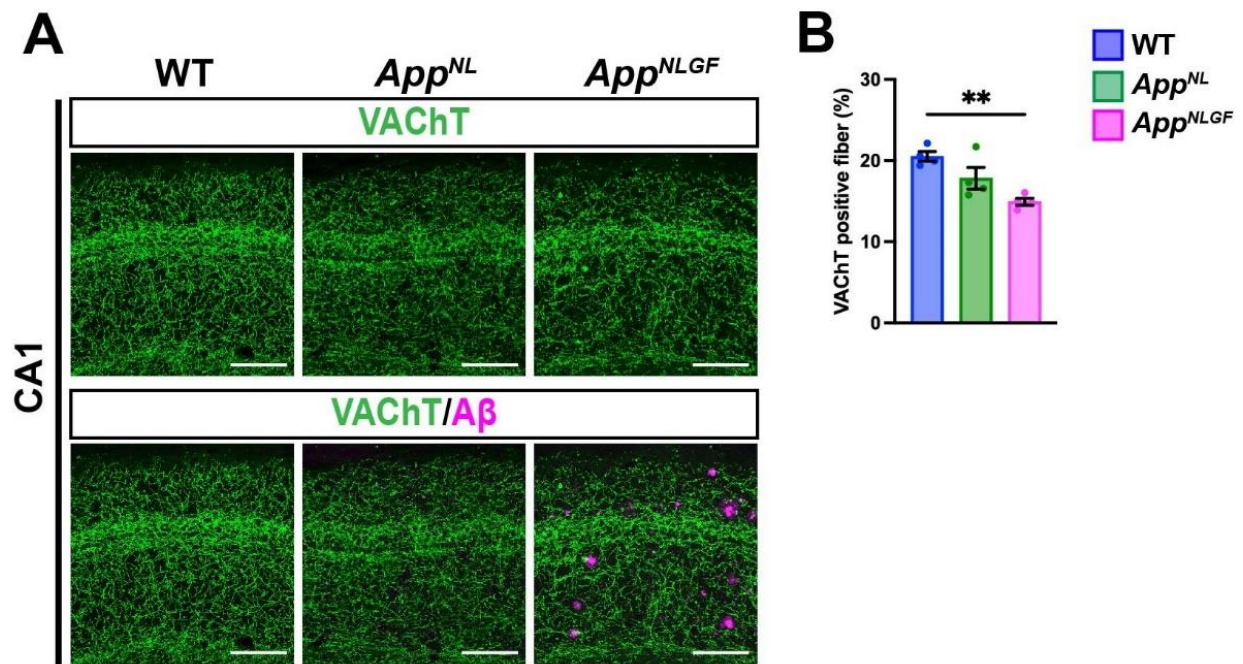
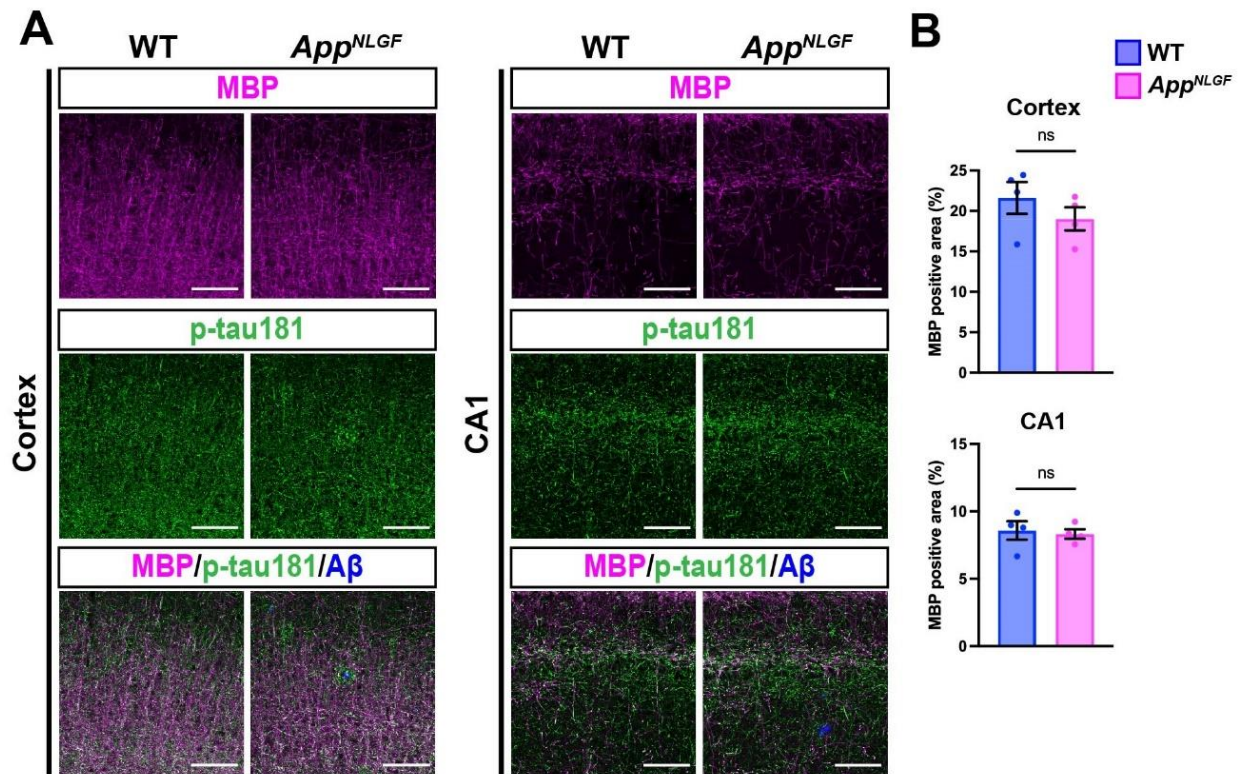


# Supplementary Material

Alzheimer's Disease-Related Phospho-Tau181 Signals Are Localized to Demyelinated Axons of Parvalbumin-Positive GABAergic Interneurons in an *App* Knock-In Mouse Model of Amyloid- $\beta$  Pathology



**Supplementary Figure 1.** The density of cholinergic axons is significantly reduced in the hippocampal CA1 subfield of *App*<sup>NLGF</sup> mice. A) Representative images of the hippocampal CA1 subfield from frozen coronal brain sections immunostained with antibodies against VChT (green). FSB was used for detecting A $\beta$  plaques (magenta). Scale bars, 100  $\mu$ m. B) VChT immunoreactivity was quantified and expressed as positive area (%).  $n = 4$  /group. \*\* $p < 0.01$ .



**Supplementary Figure 2.** Myelin sheaths of p-tau181-positive axons are not disrupted in 6-month-old *App*<sup>NLGF</sup> mice. A) Representative images of the cortex and hippocampal CA1 subfield from frozen coronal brain sections immunostained with antibodies against MBP (magenta) and p-tau181 (green). FSB was used for detecting A $\beta$  plaques (blue). Scale bars, 100  $\mu$ m. B) MBP immunoreactivity in the cortex or hippocampal CA1 subfield was quantified and expressed as positive area (%). n = 4 /group. ns; not significant.

**Supplementary Table 1. Antibodies used in the present study**

REAGENT or RESOURCE	SOURCE	IDENTIFIER	RRID	Dilution
<b><i>Primary antibodies</i></b>				
Rabbit polyclonal anti-VACht	Frontier Institute	VACht-Rb-Af1000	AB_2571850	1:300
Mouse monoclonal anti-ChAT [CL3169]	Sigma	AMAB91129	AB_2665811	1:500
Mouse monoclonal anti-p-tau181 [AT270]	Thermo Fisher	MN1050	AB_223651	1:500
Rabbit polyclonal anti-NET	Synaptic systems	260003	AB_2619974	1:300
Rabbit polyclonal anti-VGLUT1	Synaptic systems	135303	AB_887875	1:1000
Rabbit polyclonal anti-VGAT	Millipore	AB5062P	AB_2301998	1:300
Rat monoclonal anti-MBP [12]	Abcam	Ab7349	AB_305869	1:200
Rabbit polyclonal anti-A $\beta$ -N	IBL	18584	AB_10705431	1:200
Rabbit monoclonal anti-Parvalbumin [EPR13091]	Abcam	ab181086	AB_2924658	1:200
<b><i>Secondary antibodies</i></b>				
Goat polyclonal anti-Mouse IgG H&L Alexa Fluor 488	Abcam	ab150117	AB_2688012	1:500
Goat polyclonal anti-Mouse IgG H&L Alexa Fluor 594	Abcam	ab150120	AB_2631447	1:500
Goat polyclonal anti-Rabbit IgG H&L Alexa Fluor 488	Abcam	ab150081	AB_2734747	1:500
Goat polyclonal anti-Rabbit IgG H&L Alexa Fluor 594	Abcam	ab150084	AB_2734147	1:500
Goat polyclonal anti-Rabbit IgG H&L Alexa Fluor 647	Abcam	ab150083	AB_2714032	1:500
Goat polyclonal anti-Rat IgG H&L Alexa Fluor 594	Abcam	ab150168	N/A	1:500
<b><i>Counterstain</i></b>				
DAPI (4',6-diamidino-2- phenylindole, dihydrochloride)	Thermo Fisher	D1306	AB_2629482	5.7 $\mu$ M
PI (propidium iodide)	Thermo Fisher	P1304MP	AB_2629482	1.5 $\mu$ M
FSB (1-Fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene, DMSO solution)	Dojindo	F308	N/A	23.8 $\mu$ M

**Supplementary Movie 1. Related to Figure 1 A5 and C5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against VACHT (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 2. Related to Figure 1 D5 and F5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against NET (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 3. Related to Figure 4 A5 and C5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against MBP (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 4. Related to Figure 5 A5 and C5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against VGLUT1 (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 5. Related to Figure 6 A5 and C5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against VGAT (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 6. Related to Figure 6 D5 and F5**

Representative movie of the hippocampal CA1 subfield from frozen coronal brain sections immunostained with antibodies against VGAT (magenta) and p-tau181 (green). Scale bars, 2.5  $\mu\text{m}$ .

**Supplementary Movie 7. Related to Figure 7 A5 and C5**

Representative movie of the cortex from frozen coronal brain sections immunostained with antibodies against parvalbumin (magenta), p-tau181 (green), and MBP (cyan). Scale bars, 2.5  $\mu\text{m}$ .