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Supplemental Information

Anti-tau scFvs Targeted to the Cytoplasm or Secretory Pathway Variably Modify Pathology and Neurodegenerative Phenotypes

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Supplemental information

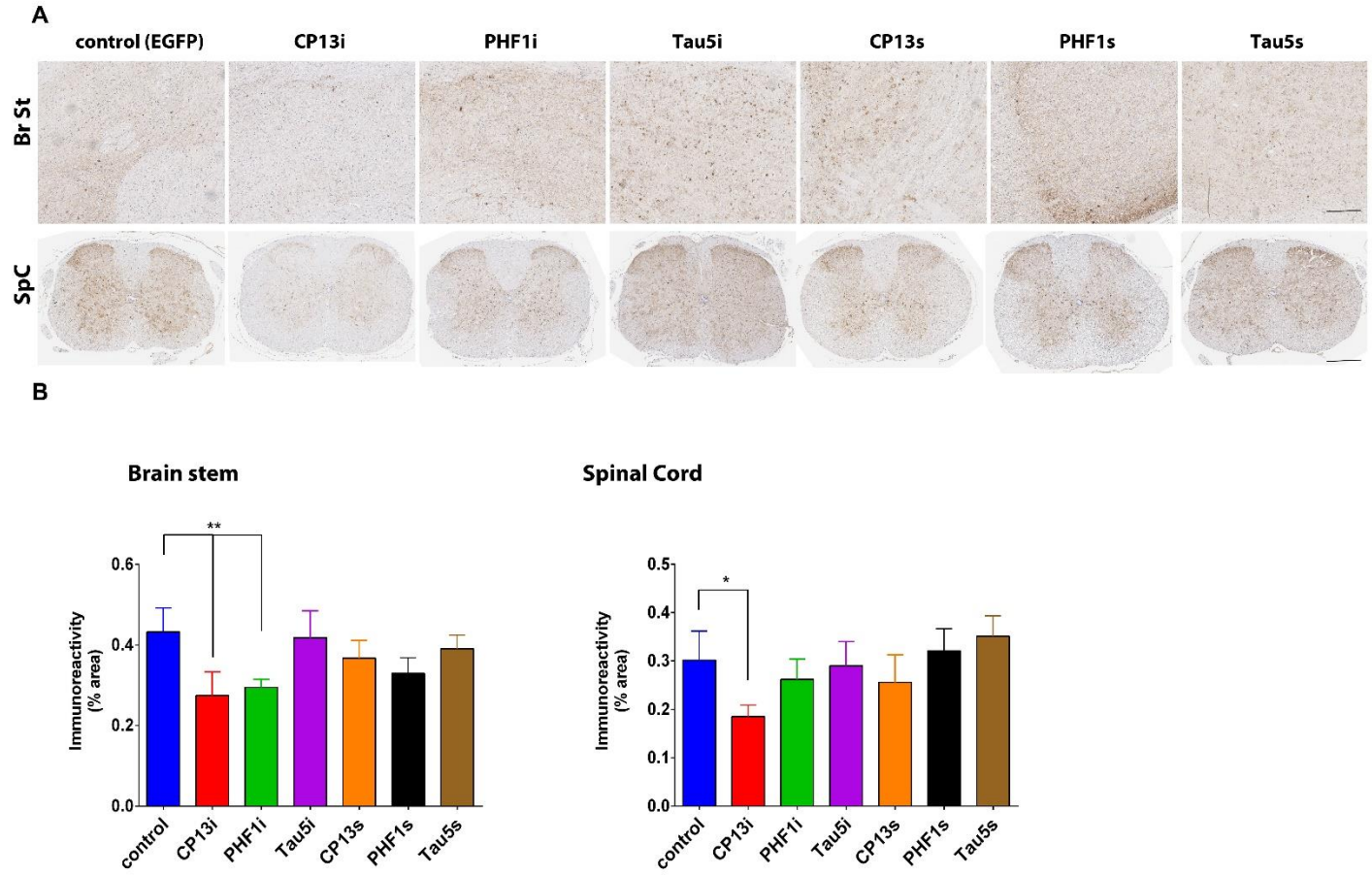

Figure S1: P-tau specific iBs and scFvs significantly reduce misfolded tau in brainstems and spinal cords of homozygous JNPL3 mice at six months.

Homozygous JNPL3 mice were intraspinally injected at P0 with pAAV2/8 (2 x 10^{10} genomes) expressing CP13, PHF1, and Tau5 iBs or scFvs. Control mice were injected with rAAV-EGFP. Mice were harvested at six months and pathology was assessed by staining with ALZ50 mAb (misfolded tau). A. Representative paraffin sections of brainstem (Br St) and spinal cord (SC) stained with ALZ50. Scale bar, 150□□m (Br St) and 200 □m (SpC). B. Immunoreactivity analysis shows reduction in ALZ50 staining in brain stem area of mice expressing CP13i and PHF1i, and in the spinal cord area of mice expressing CP13i. N=4. Data represents mean ±SEM. *$p<0.05$, **$p<0.01$.

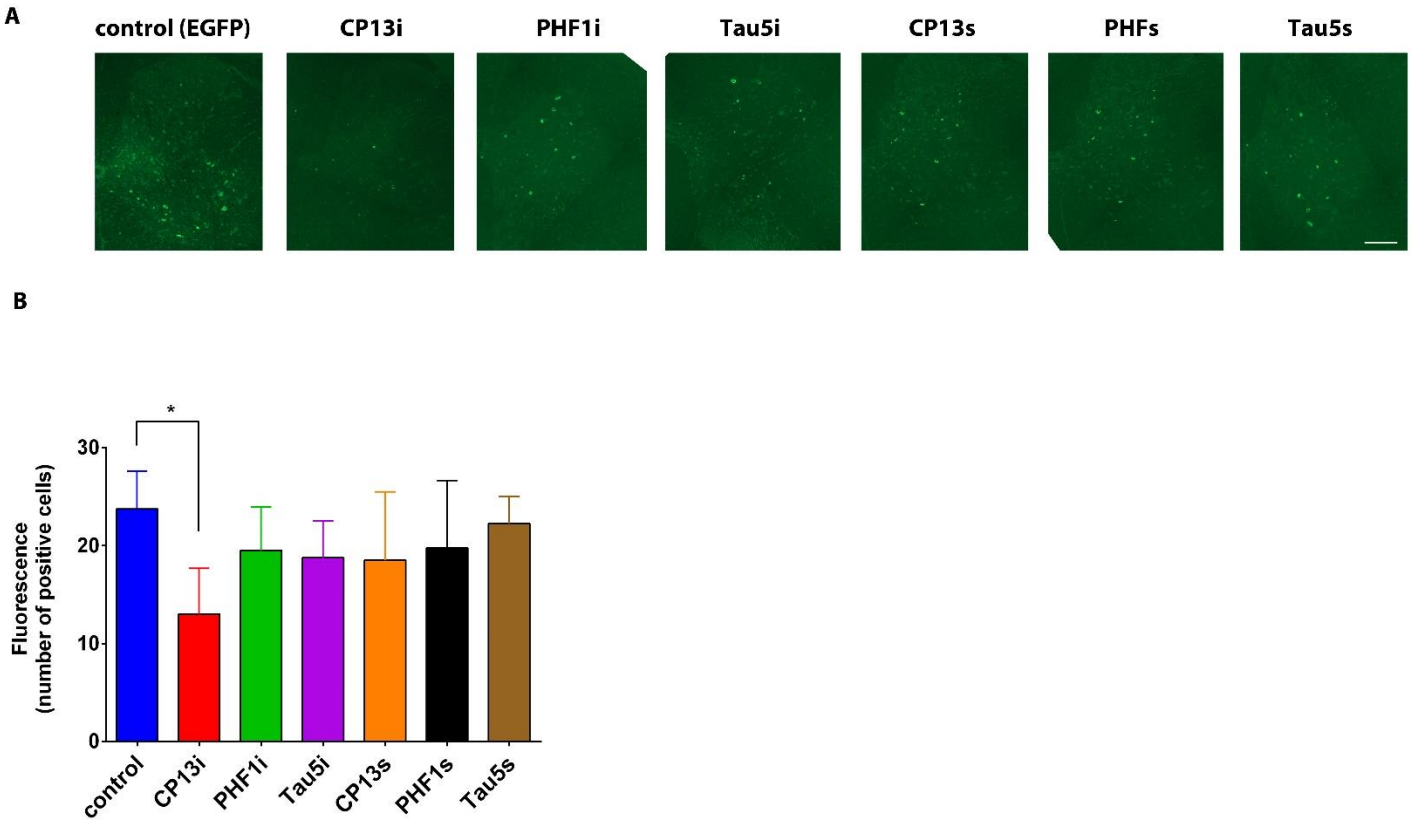

Figure S2: P-tau specific iB CP13i significantly reduces Thio-S staining in spinal cords of homozygous JNPL3 mice at six months.

Homozygous JNPL3 mice injected at P0 with pAAV2/8 (2 x 10^{10} genomes) expressing CP13, PHF1, and Tau5 iBs or scFvs, were harvested at six months and NFT pathology was assessed by staining with thioflavin-S (Thio-S). A. Representative paraffin sections of spinal cord (SC) stained with Thio-S. Scale bar 100 □m. B. Fluorescent cell count analysis shows reduction in Thio-S staining in the spinal cord area of mice expressing CP13i. N=4. Data represents mean ±SEM. *$p<0.05$.

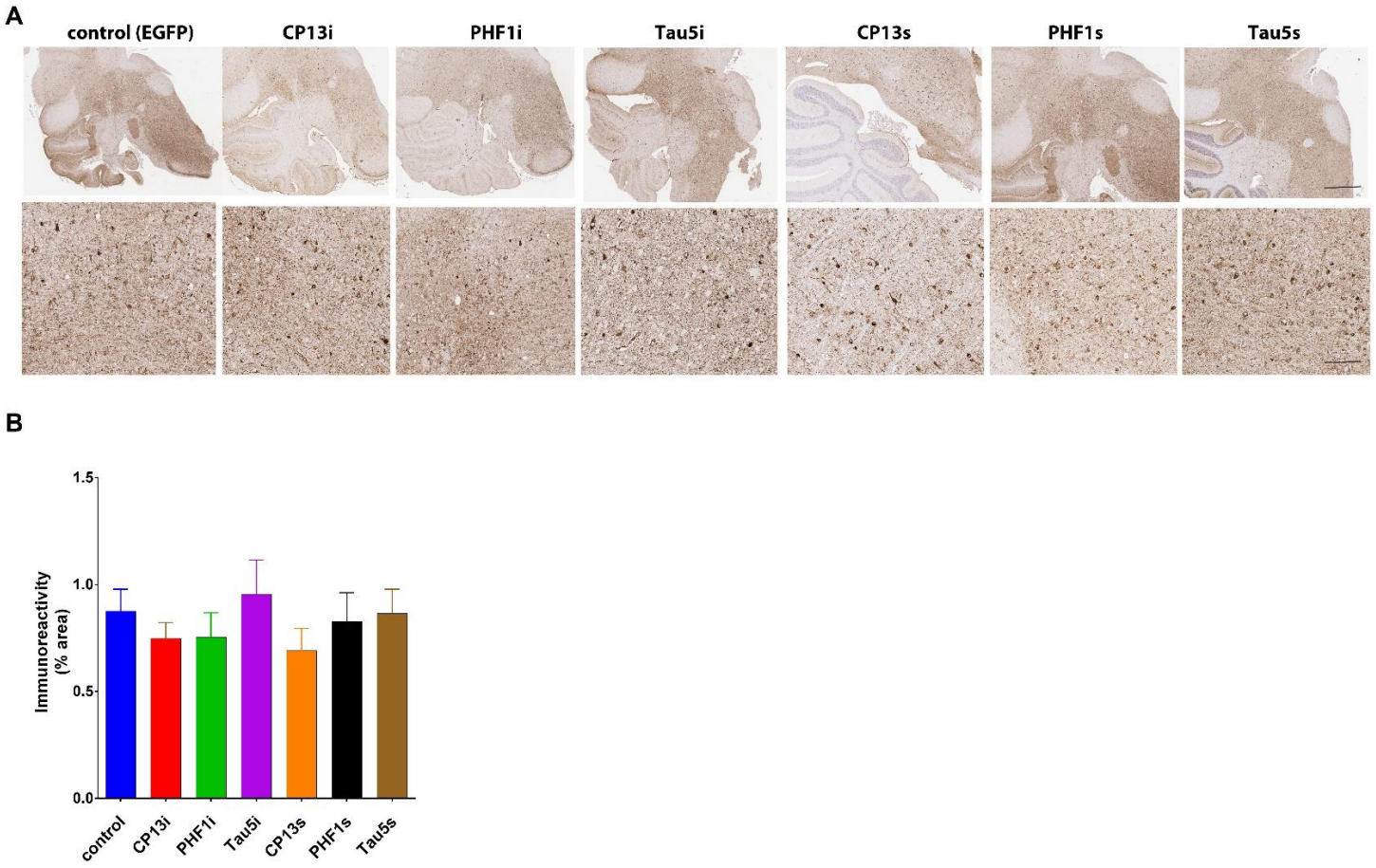

Figure S3. Quantification reveals no significant difference in p-tau staining in the midbrains of paralyzed homozygous P301L mice expressing anti-tau iBs or scFvs.

A. Representative paraffin sections of brainstem of terminal stage mice show no difference in CP13 staining between control mice and mice expressing anti-tau iBs or scFvs. Scale bars, 250 □m and 100 □m. B. Quantification confirms that there is no significant difference in staining between groups. N=4. Data represents mean ±SEM.

Supplemental methods

Thio-S (Sigma-Aldrich) staining was performed on paraffin-embedded spinal cord sections using established protocols. Fluorescently stained sections were captured using the Zeiss Slide scanner and analyzed using ZEN Imaging software. For Thio-S quantification, one section per sample was used by a blinded observer to calculate the number of positive cells per spinal cord in the lumbar area using ImageJ 58.

Supplemental references

58. Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**, 671-675 (2012).