Mass spectrometry identifies tau C-terminal phosphorylation cluster during neuronal hyperexcitation

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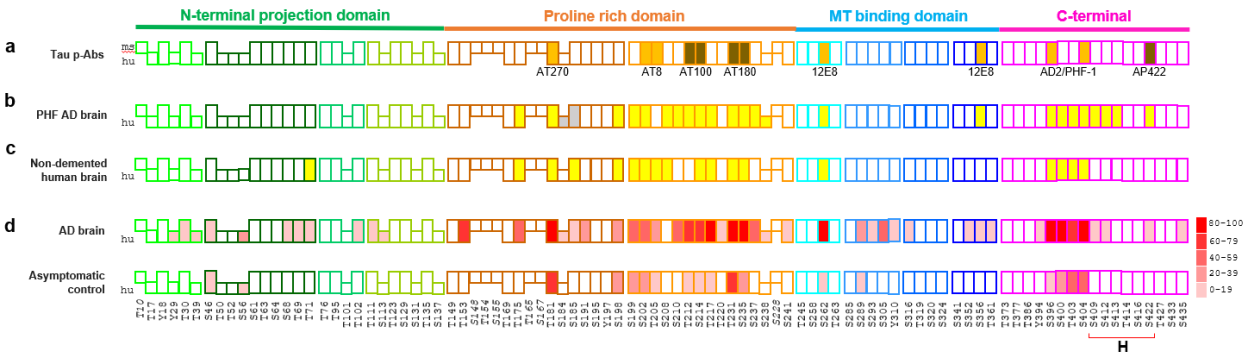
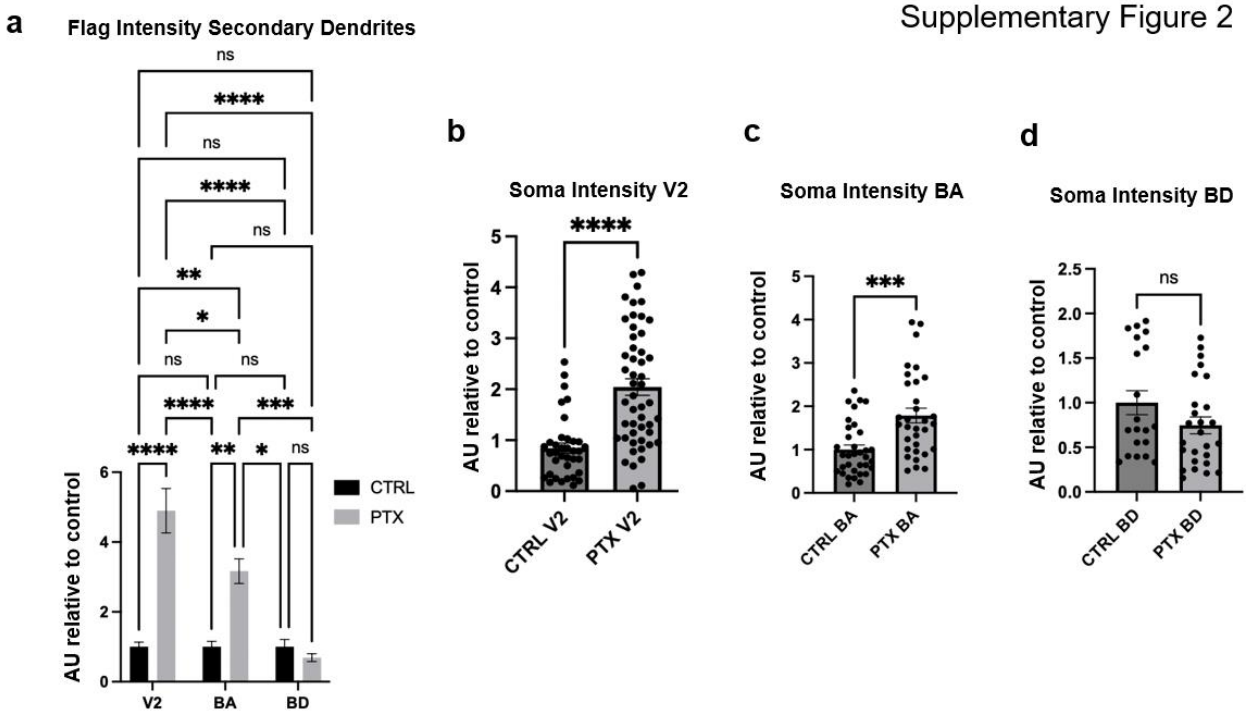
SUPPLEMENTARY FIGURES

Supplementary Figure 1



Supplementary Figure 1. Tau phosphosites identified from selected previously published studies displayed on phosphomaps. (a) Residues identified with tau phosphospecific antibodies (p-Abs) as indicated under each site (Buée *et al.* 2000). Orange fill, sites reported to be enriched in AD. Brown fill, epitopes exclusive to PHFs. **(b)** Yellow fill, phosphosites identified by mass spectrometry in PHF from AD brain (Hanger *et al.* 2002). Light gray fill, ambiguous sites. **(c)** Yellow fill, phosphosites identified by mass spectrometry in normal brain (Funk *et al.* 2014). **(d)** Phosphorylation sites identified by mass spectrometry in AD and asymptomatic control brain (Wesseling *et al.* 2020). Heat map represents frequency % in AD and control samples.

Hyperexcitation-inducible residues are denoted at bottom by bracket and "H." Note phosphorylation within this region has previously been observed only in AD and PHF samples.



Supplementary Figure 2. Effects of C-terminal phosphosite mutations on tau localization in secondary dendrites and soma (a) Two-way ANOVA of Figure 3b. Shapiro Wilks test for normality passed (CTRL, p=0.2530, PTX, p=0.8062). Two-way ANOVA (alpha=0.05) with Šídák's multiple comparisons test was used. V2:CTRL vs. V2:PTX (t=7.348, p<0.0001), V2:CTRL vs. BA:CTRL (t=8.171e-11, p=0.0013), V2:PTX vs. BA:PTX (t=4.017, p=0.0119), V2:CTRL vs. BD:CTRL (t=4.126e-010, p>0.9999), V2:CTRL vs. BD:PTX (t=0.5114, p>0.9999), V2:PTX vs. BA: CTRL (t=7.029, p<0.0001), V2:PTX vs. BA:PTX (t=3.408 p=0.0119), V2:PTX vs BD:CTRL (t=5.828, p<0.0001), V2:PTX vs. BD:PTX (t=7.263 p<0.0001), BA:CTRL vs. BA:PTX (t=3.847, p<0.0001), BA:CTRL vs. BD:CTRL (t=4.688e-10, p>0.9999), BA:CTRL vs.

BD:PTX (t=0.4941, p>0.9999), BA:PTX vs. BD:CTRL (t=3.204, p=0.0236), BA:PTX vs. BD:PTX (t=4.215, p<0.0006), BD:CTRL vs. BD:PTX (t=0.4245, p>0.9999). DF=187 for all comparisons. **(b)** Soma intensity of Figure 3b V2. ROUT outlier test (Q = 1%). D'Afostino and Pearson test (alpha=0.05) was used to test for normality and did not pass; CTRL and PTX, p=0.0044 and p=0.0380, respectively). Mann Whitney test used (****p<0.0001). **(c)** Soma intensity of Figure 3b BA. ROUT outlier test (Q = 1%). D'Afostino and Pearson test (alpha=0.05) was used to test for normality and did pass; CTRL and PTX, p=0.1180 and p=0.1575, respectively). T-test used (****p=0.0002, t=3.993, df=64). **(d)** Soma intensity of Figure 3b BD. ROUT outlier test (Q = 1%). D'Afostino and Pearson test (alpha=0.05) was used to test for normality and did not pass; CTRL and PTX, p=0.0106 and p=0.2157, respectively). Mann Whitney test used (ns, p =0.1244).