A

HIPPOCAMPUS

MAPTKI, S305N, P301S

PRD: p<0.001, p<0.001, p=0.052
C-term: p=0.0147

CORTEX

PRD: p=0.0003, p<0.001, p=0.0694
C-term: p=0.0045

N-term, PRD, MTBR, C-term

B

p181: 0.0062, 0.0023
pTau217: <0.0001, 0.0016
pTau212/217: 0.0406, 0.0299
p231: 0.0082, 0.0092
p231+235: 0.0025, 0.0004
p396+404: 0.0217, 0.0270

MAPT KI, S305N, P301S

C

15M, 30M

MAPT KI, S305N, P301S

AT8, TOC1, CP13, MC1

Fig. S3. (A) Eigenprotein values for phospho-tau–associated peptides were derived from principal component analysis of the TMT-based phospho-proteomics dataset 1 and 3 subdivided by tau regions, *i.e.* N-terminus (aa:1-150), proline-rich domain (PRD; aa:151-243), microtubule binding region (MTBR; aa:244-368) and C-terminus (aa:369-441). Scores are shown for *MAPT* KI controls, S305N, and P301S mice at 15M for the hippocampus (top panel, dataset#1) and the posterior cortex (bottom panel, dataset#2). Group differences reflect the relative burden of tau phosphorylation across genotypes. Data are presented as median and IQR (dashed grey line crosses the median of MAPT KI control). (**B**) Relative levels of phospho-tau peptides by targeted LC–MS analysis in the brains of 15M *MAPT* KI (N = 3), S305N (N = 3;), and P301S (N = 3). Results are expressed in L/H ratio. (**C**) Representative images of coronal hemibrain sections used for the heatmap quantification in Fig. 3B from 6, 12, 15, 24 and 30M *MAPT* KI, S305N and P301S for AT8 IHC staining. Lower panels show zoomed-in boxes from 30M image from S305N and P301S mice.