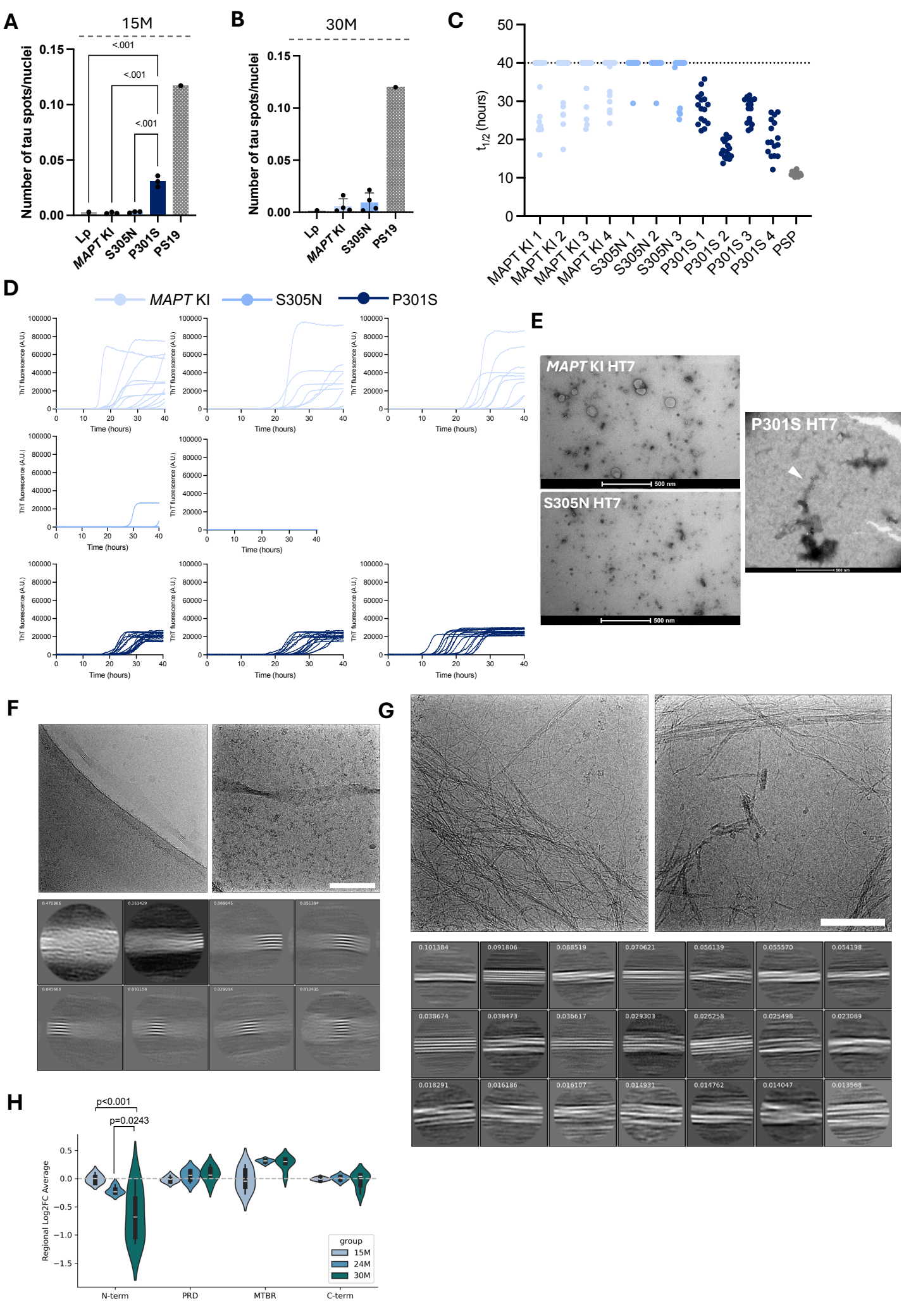
A
15M
Number of tau spots/nuclei
<.001
<.001
<.001
Lp
MAPT KI
S305N
P301S
PS19
B
30M
C
t1/2 (hours)
MAPT KI 1
MAPT KI 2
MAPT KI 3
MAPT KI 4
S305N 1
S305N 2
S305N 3
P301S 1
P301S 2
P301S 3
P301S 4
PSP
D
MAPT KI
S305N
P301S
ThT fluorescence (A.U.)
Time (hours)
E
MAPT KI HT7
S305N HT7
P301S HT7
500 nm
F
G
H
p<0.001
p=0.0243
Regional Log2FC Average
group
15M
24M
30M
N-term
PRD
MTBR
C-term

Fig. S5. (A) Tau seeding activity from 15M posterior cortex *MAPT* KI, S305N and P301S using S305N biosensor cells (N=3 per group; N=2 female and N=1 male), compared to PS19 mouse at 9M and lipofectamine negative control ("Lp"). Data represents mean±S.D. **(B)** Tau seeding activity from 15M posterior cortex *MAPT* KI and S305N using S305N biosensor cells (N=4 per group; sex-matched), compared to PS19 mouse at 9M and lipofectamine negative control ("Lp"). Data represents mean±S.D. **(C)** RT-QuIC aggregates half-way curve ($t_{1/2}$) from the combination of 16 replicates per biological sample from 15M posterior cortex from *MAPT* KI (N=4), S305N (N=3) and P301S (N=4; all sex-matched) and PSP positive control human brain (N=1). **(D)** Raw RT-QuIC ThT reactions from posterior cortex of mice at 15 months, *MAPT* KI (N=4), S305N (N=3) and P301S (N=4). N=1 is represented in Fig. 4E. **(E)** Representative negative-stain EM images of sarkosyl-insoluble tau fibrils isolated from whole-brain homogenates of 30M *MAPT* KI (top) and *S305N* mice (bottom). **(F)** Representative cryoEM micrographs and 2D class averages of sarkosyl-insoluble filaments (crossover distance 280-350 nm) purified from whole-brain P301S KI mouse sarkosyl-insoluble fraction at 30M. Scale bar represents 100 nm. **(G)** Representative cryoEM micrographs and 2D class averages of thin filaments (untwisted) purified from whole-brain P301S KI mouse sarkosyl-insoluble fraction at 30M. Scale bar represents 100nm. **(H)** Eigenprotein values for phospho-tau–associated peptides were derived from principal component analysis of TMT-based phospho-proteomics 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 comparing hippocampal tissue from P301S mice at 15M, 24M and 30M. 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).