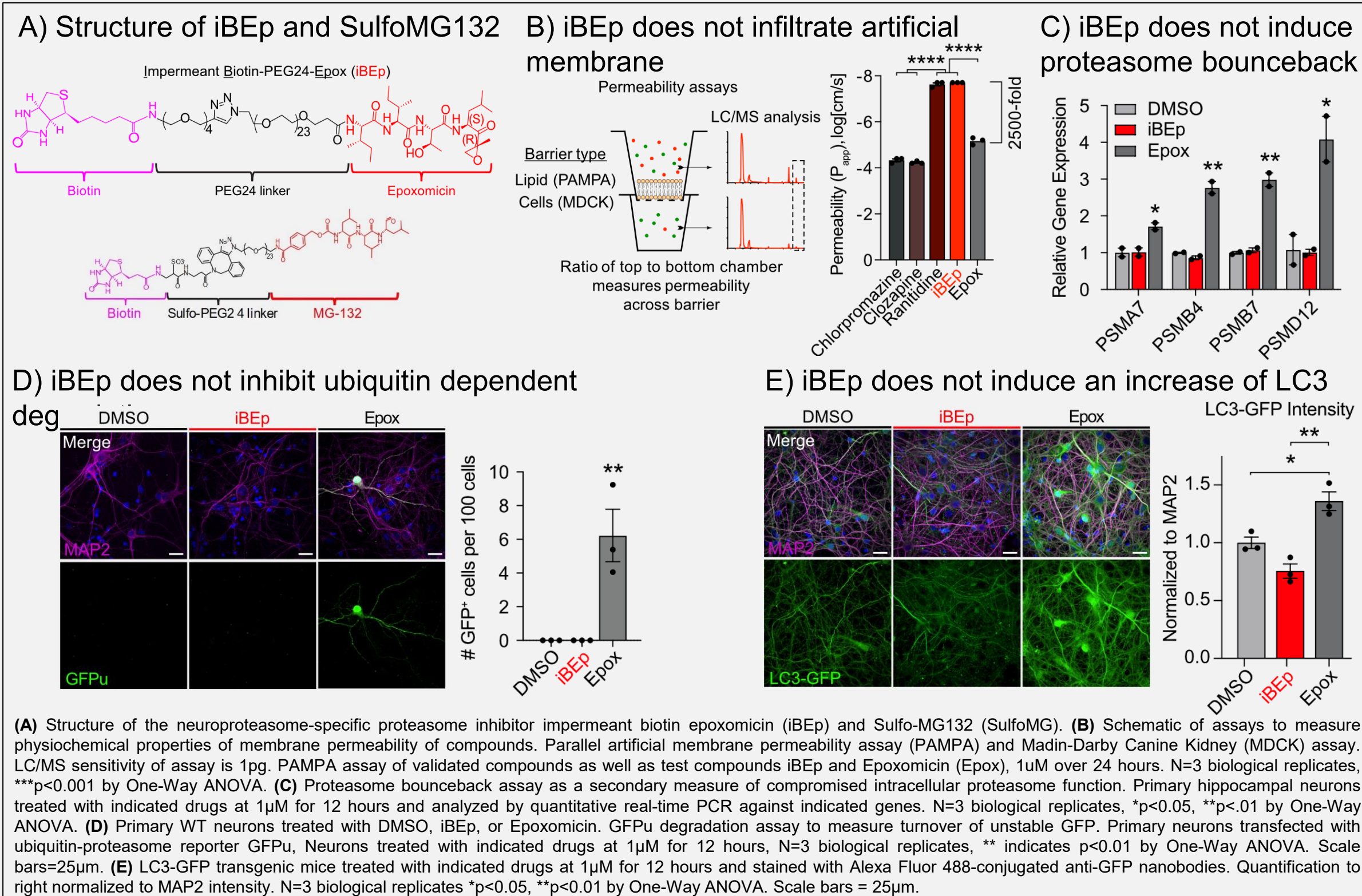


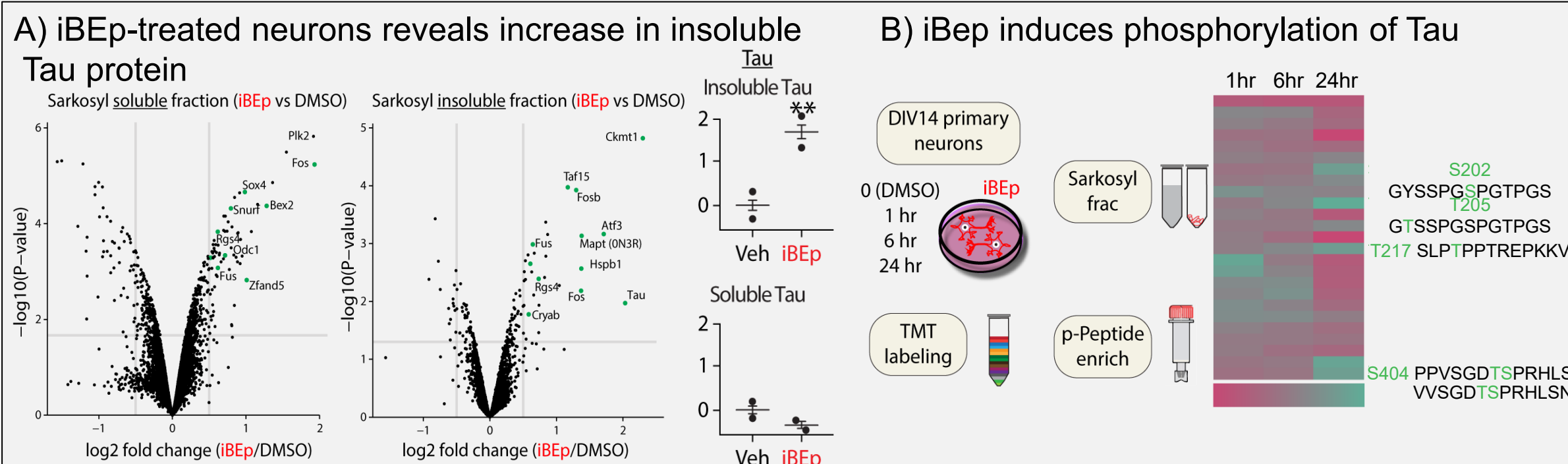
Tau is exclusively synthesized in neuronal dendrites

The diagram illustrates a cell membrane as a phospholipid bilayer. A protein channel, composed of blue and green subunits, is embedded in the membrane. A red dashed line representing a signal sequence extends from the extracellular space, through the protein channel, into the cytoplasmic space. The extracellular space is labeled 'extracellular' and the cytoplasmic space is labeled 'cytoplasmic'.

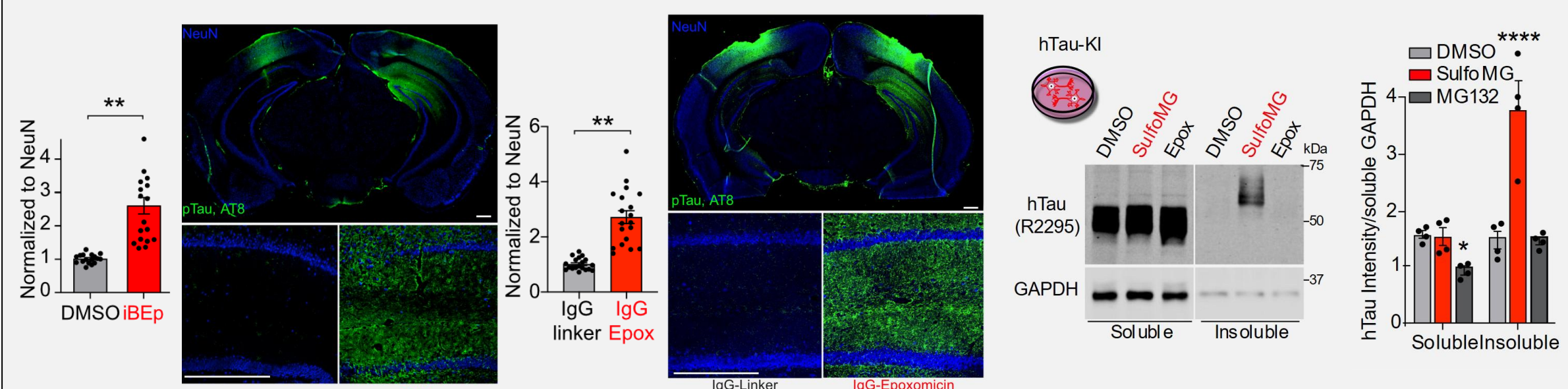
Development of selective cell impermeable neuroprotease inhibitors



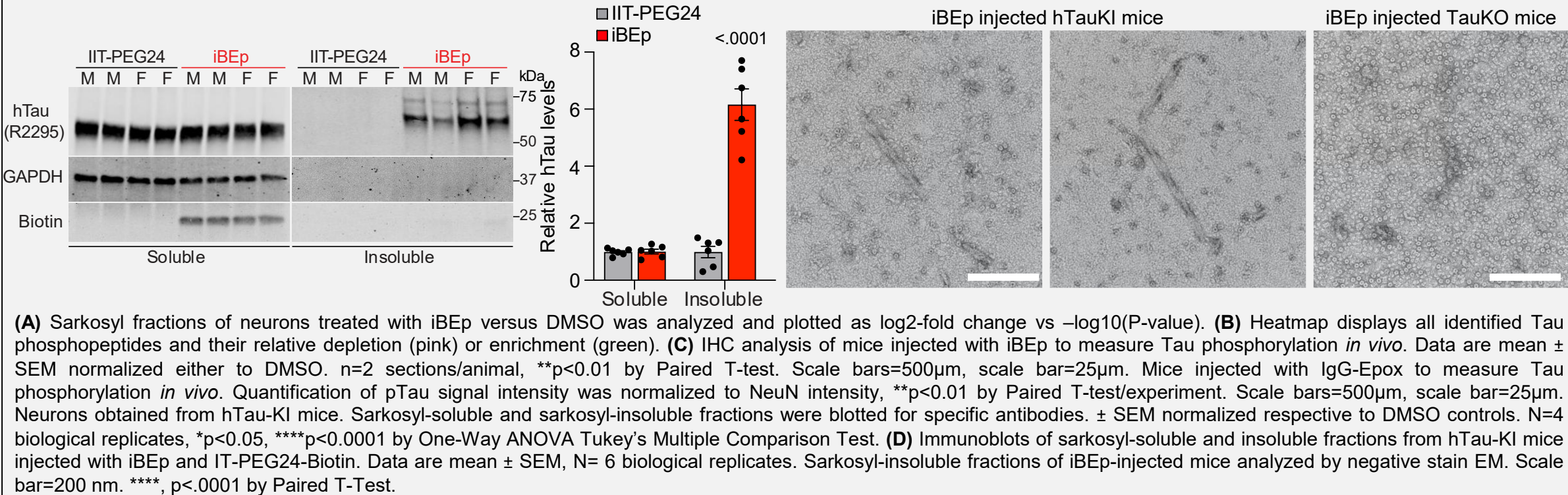
Neuroproteasome inhibition induces aggregation of endogenous Tau into PHFs



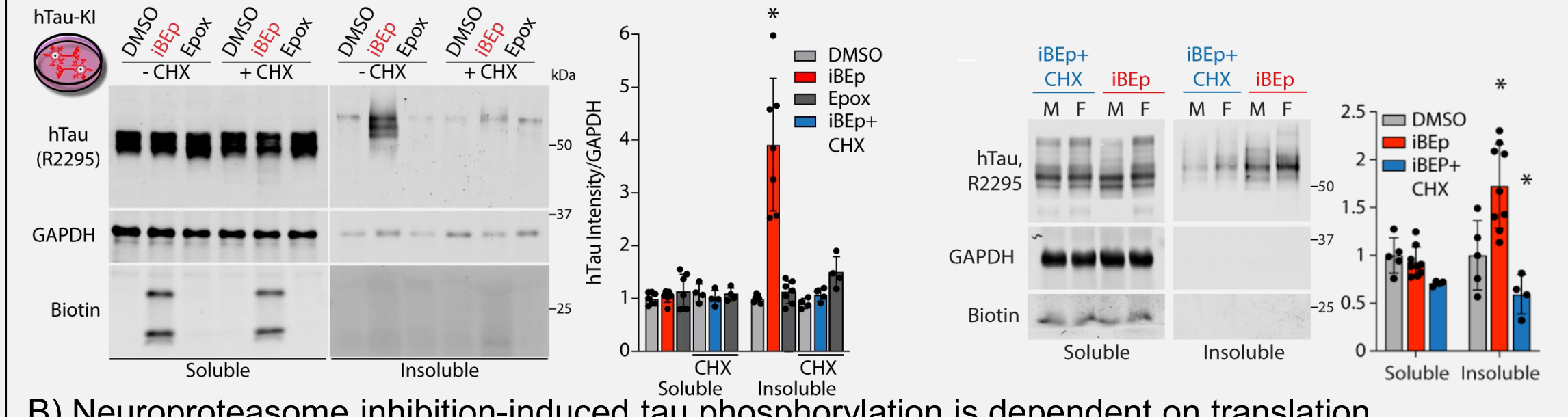
C) IgG-EpoX and SulfoMG phenocopy effects on phosphorylation of Tau and aggregation



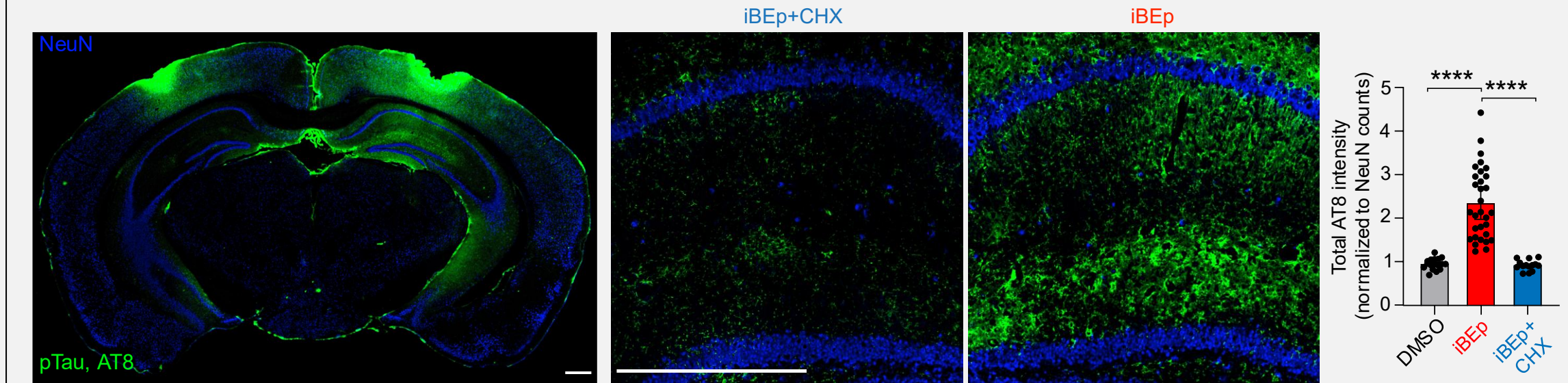
D) Neuroproteasome inhibition drives formation of endogenous Tau PHFs *in vivo*



A) iBEP-induced Tau aggregates are dependent on translation in neurons and *in vivo*

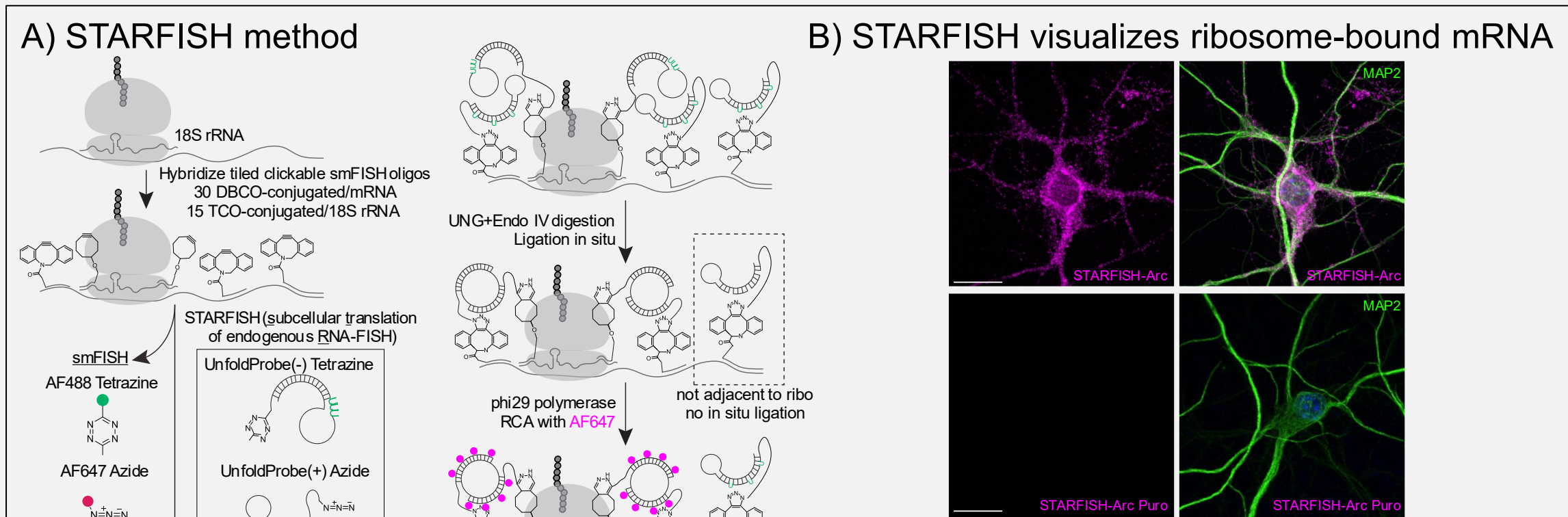


B) Neuroproteasome inhibition-induced tau phosphorylation is dependent on translation

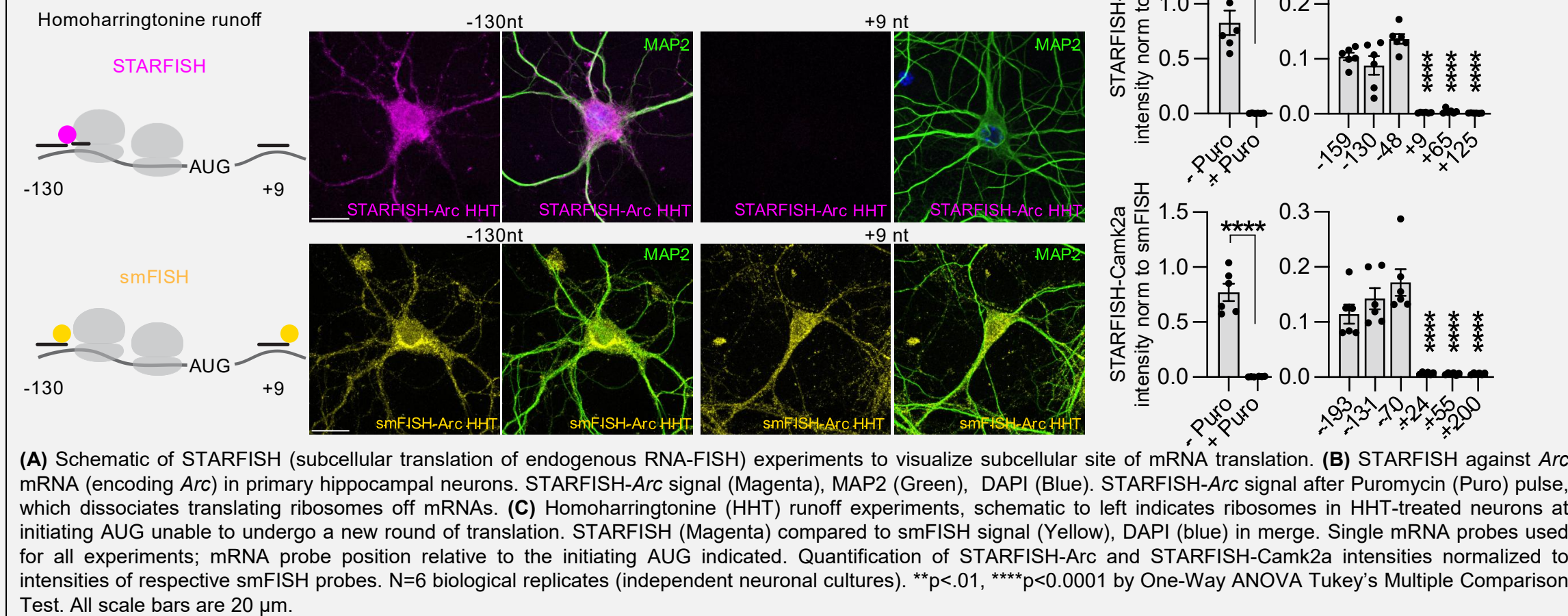


(A) Sarkosyl-soluble (Soluble) and sarkosyl-insoluble (Insoluble) fractions were immunoprecipitated using indicated antibodies and fluorescent Streptavidin (Biotin). Quantification of soluble and insoluble Tau intensities were normalized to soluble GAPDH. Data (right) are mean $\pm$ SEM normalized respective to DMSO controls. N=4 biological replicates. Hippocampi were subjected to sarkosyl fractionation 72 hours post injection. The sarkosyl-soluble and sarkosyl-insoluble fractions were immunoprecipitated using indicated antibodies and fluorescent streptavidin (Biotin). N=4 biological replicates (2M, 2F). Effect of protein synthesis inhibition on neuroproteasome-dependent Tau aggregation in mice. Mice were subjected to sarkosyl fractionation 72 hours post injection. Quantification of soluble and insoluble Tau intensities normalized to soluble GAPDH. Data shown (right) are mean $\pm$ SEM normalized to DMSO. N=4 biological replicates (2M, 2F). * p < 0.05, *** p < 0.001 by one-way ANOVA Tukey's Multiple Comparison Test. (B) tTau-K1 mice were stereotactically injected (schematic) with iBEP (red) and iBEP + CHX (blue) contralaterally (N=6, 3M, 3F). Mice were collected 72 hours post injection and sections were stained using indicated antibodies. Quantification of soluble and insoluble tTau-K1 intensities were normalized to soluble GAPDH. Data shown (right) are mean $\pm$ SEM normalized to DMSO condition. Data are mean $\pm$ SEM normalized to DMSO. N=3 sections/animal, N=3 mice. *** p < 0.001 by one-way ANOVA. Scale bar=500 μ m.

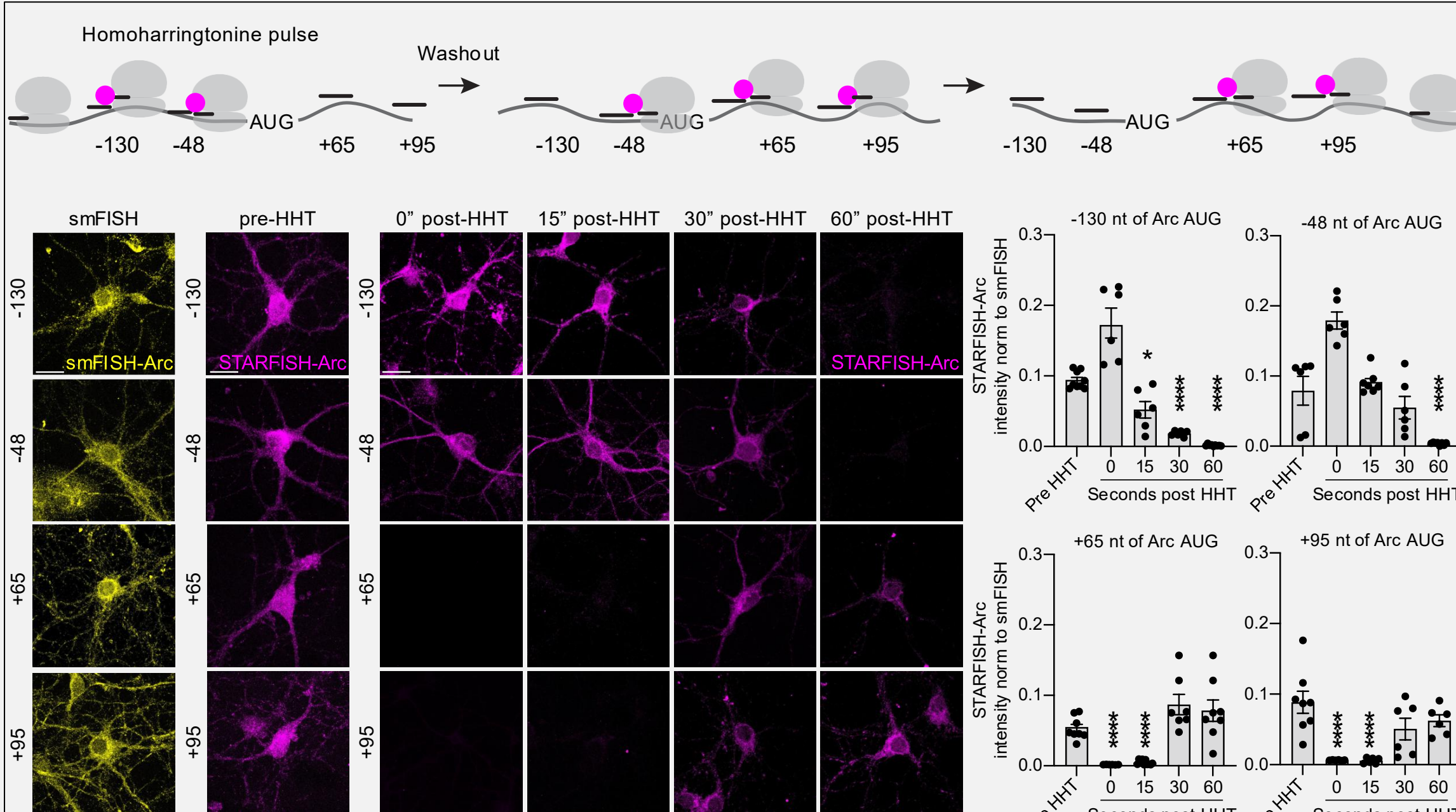
Spatially-resolved translation of endogenous RNA FISH (STARFISH) Method



C) STARFISH exhibits near codon resolution for specific transcripts

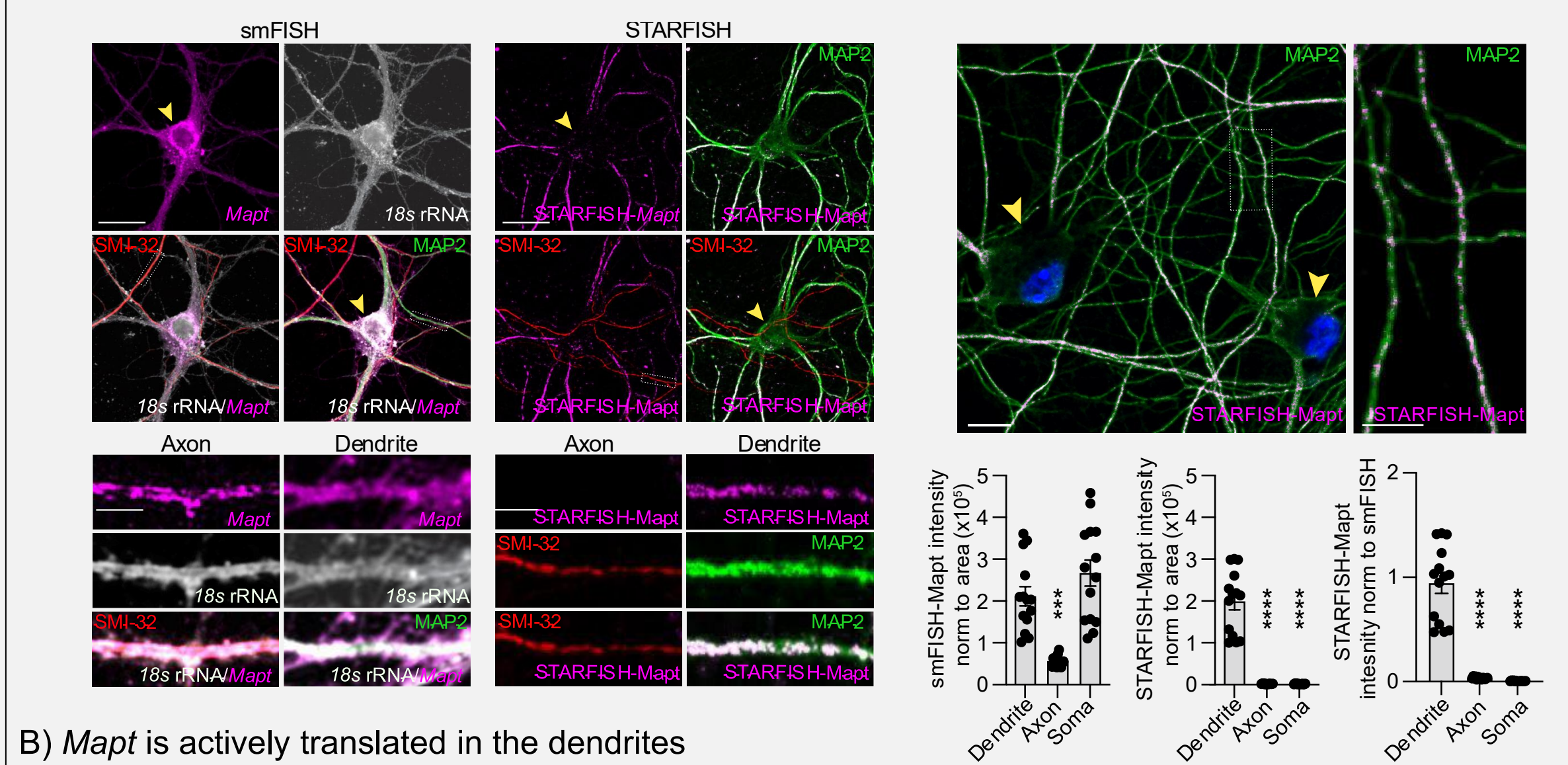


STARFISH visualizes the sites of active translation

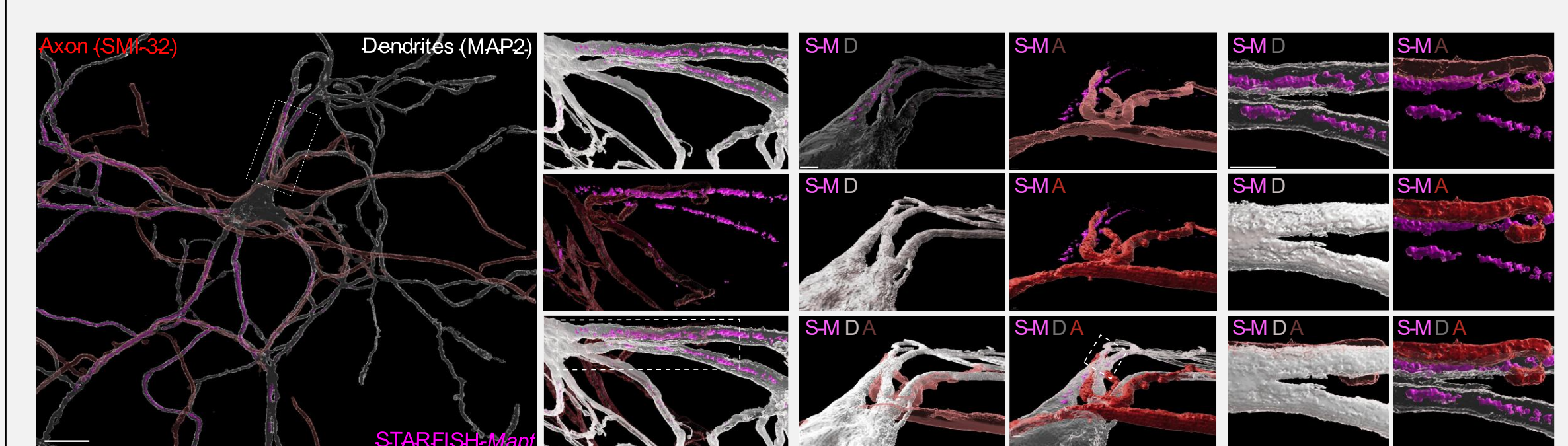


Homoharringtonine pulse-chase experiments; schematic indicates how ribosomes stacked at initiating AUG after HHT incubation and translation initiation and movement down mRNA following HHT washout. Single Arc probes at indicated positions (-130, -48, +65, +95) used for all experiments. smFISH of each position shown to left after HHT incubation. STARFISH pre-HHT and at indicate times post-HHT indicated. HHT runoff experiments, STARFISH (Magenta) compared to smFISH signal (Yellow). Single mRNA probes used for all experiments; mRNA probe position relative to the initiating AUG indicated. Quantification of STARFISH-Arc intensities normalized to intensities of respective smFISH probes. Puromycin (Puro) and HHT experiments indicated, tint CDS probes used for Puro, single probes used for HHT bound to indicated positions relative to AUG. N=6 biological replicates (independent neuronal cultures). N=3 independent neuronal cultures, n=6 neurons quantified per condition, **p<.01, ****p<.0001 by One-Way ANOVA Tukey's Multiple Comparison Test. All scale bars are 20 μ m.

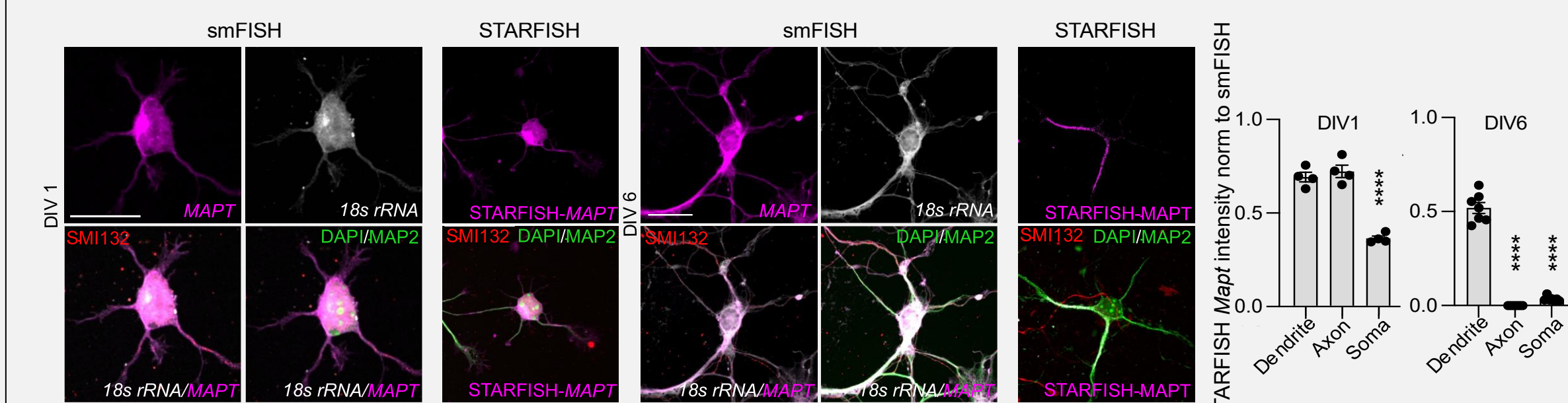
A) STARFISH reveals dendritic translation of *Mapt* (Tau) despite broad expression of *Mapt* mRNA



B) *Mapt* is actively translated in the dendrites

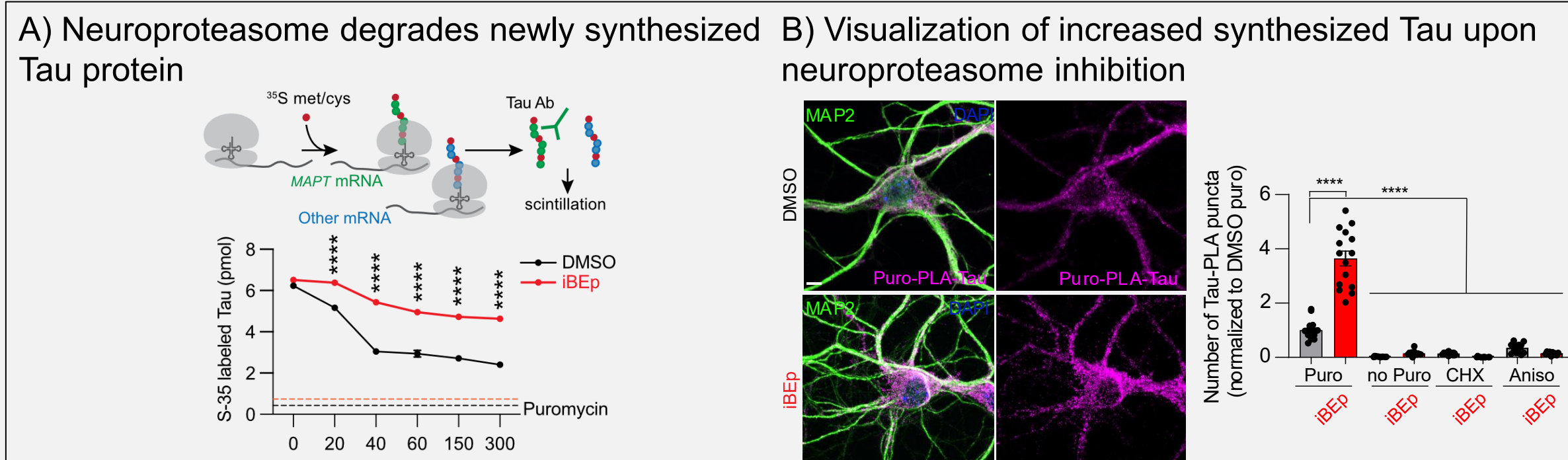


C) Spatial and temporal differences in *Mapt* STARFISH during neuronal maturation

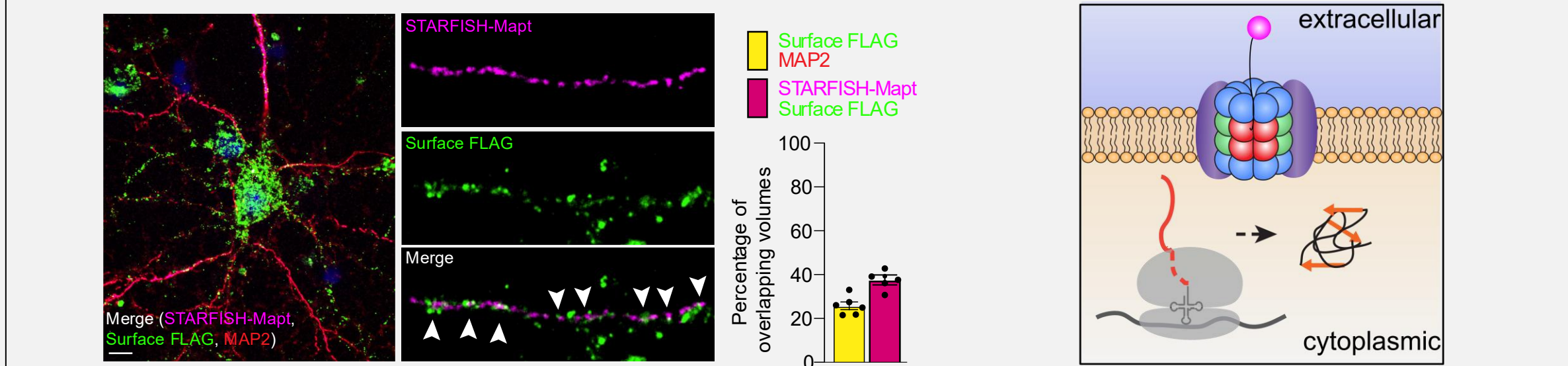


(A) smFISH experiment against *Mapt* in primate neurons. *Mapt* mRNA (magenta), 18S rRNA (white), MAP2 dendrites (green), SM1-32 axons (red), STARFISH against *Mapt* mRNA (enriching Tau). STARFISH-*Mapt* signal (Magenta), *Mapt* (Green), SM1-32. Small inset from to exemplify STARFISH-*Mapt* signal relative to MAP2+ dendrite and SM1-32 axons. Lower magnification image of STARFISH-*Mapt* signal to demonstrate co-localization with MAP2+ dendrites. Quantification of STARFISH-*Mapt* overlap with MAP2+ dendrites and SM1-32 smFISH-*Mapt* (left) and STARFISH-*Mapt* (middle) raw intensities and STARFISH-*Mapt* normalized to smFISH intensities (right) separated by neuronal compartment. N=4 biological replicates, 14 neurons quantified per condition. ****p*<0.001 by One-Way ANOVA Tukey's Multiple Comparison Test (B) 3D reconstruction in Imaris of primate neurons stained with SM1-32 (red, S), *Mapt* (gray, M), and STARFISH-*Mapt* (magenta, S-M). Insets to right show all signals rendered as 3D objects. Inset scale bars are 5 μ m. (C) Quantification of STARFISH-*Mapt* in DIV1 and DIV6 primary hippocampal neurons. smFISH probing *Mapt* and 18S rRNA in DIV1 axons and MAP2+ dendrites. Quantification of STARFISH-*Mapt* by compartment normalized to smFISH signal by smFISH probe (****p*<0.001 by One-Way ANOVA Tukey's Multiple Comparison Test). Unless specified, scale bars are 20 μ m.

Dendritically synthesized Tau is a substrate of neuroproteasomes



C) Neuroproteasomes are colocalized with actively translated Tau



(A) 35S methionine (35S radiolabel) (red circle) incorporated for 30 seconds onto neurons from WT animals pre-treated with either DMSO or IBeP (30 minutes). Tau protein (green) or other proteins (blue) immunoprecipitated after indicated chase times and quantified by liquid scintillation. Identical experiments performed after puromycin pulse (10µM) concomitant with 35S pulse (dashed black line in graph) or out of Primary cortical neurons from Tau KO mice (dashed red line in graph). N=3 biological replicates, ****p<0.0001 by One-way ANOVA. (B) Neurons from WT mice treated with DMSO or IBeP (red) were puromycylated for 10 minutes. Tau-PLA-Puro labeling (pink) denotes newly synthesized Tau puncta. Scale bar = 5µm. (C) Immunofluorescence images of neurons from WT mice treated with DMSO or IBeP (red) and stained for Tau (green) and DAPI (blue). Scale bar = 5µm. (D) Quantification of the percentage of neurons with newly synthesized Tau puncta. ****p<0.0001 by Paired T-test. Scale bar = 5µm. (E) Visualization of subcellular site of Tau synthesis adjacent to neurotomesomes. Magneta stain indicates STARFISH-*Mapt* signal, green indicates FLAG surface neurotomesome signal. Primary hippocampal neurons from FLAG-20S mice transduced with AAVs to express tau and visualize neurotomesomes. Probes against 18S rRNA and *Mapt* surface FLAG-neurotomesomes (Surface FLAG), immediately conjugated to Alexa Fluor 488 and briefly fixed to visualize neurotomesomes. Probes against 18S rRNA and *Mapt* surface FLAG were hybridized and carried through STARFISH protocol to visualize sites of Tau translation. White arrows indicate colocalization of neurotomesomes and neurotomesomes. Scale bar = 5µm. (F) Neurons taken from WT mice were treated with DMSO or Puro-PLA assays as well as the degradation evidence is present regarding neurotomesome formation, we conclude that neurotomesomes are functionally degrade newly synthesized Tau and failure in this degradation results in the formation of endogenous Tau aggregates that form into PFHs of Tau protein without mutations within the Tau gene.

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