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## Supplemental information

# Axonal endoplasmic reticulum tubules control local translation via P180/RRBP1-mediated ribosome interactions

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# Figure S1 (related to Figure 1)

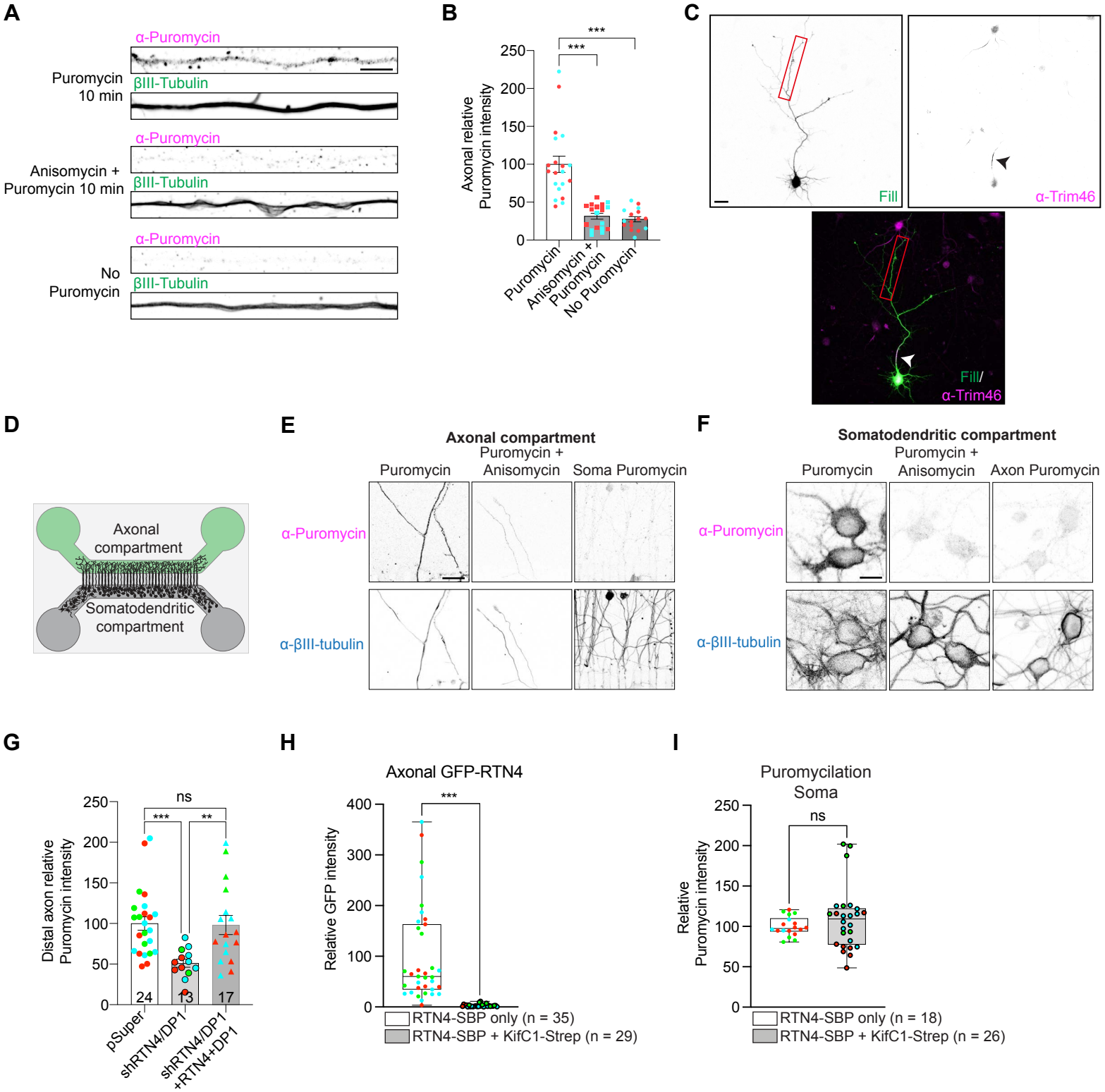

**Fig. S1. (Related to Figure 1). Axonal puromycilation specificity, efficient and selective ER removal from the axon which does not affect somatic puromycilation**

(**A**) Representative images of puromycilated peptides in axons from neurons treated with puromycin for 10 min (top), treated with puromycin for 10 min after anisomycin pre-treatment for 30 min (middle) or without puromycin (bottom). Scale bar represents 5 µm.

(**B**) Quantification of axonal puromycin intensity in neurons relative to puromycin treated neurons.

(**C**) Representative images of a DIV7 neuron transfected with a GFP fill and stained with the axon initial segment marker Trim46. This shows the clearly polarized nature of these neurons with a clearly formed axon. The red box marks an example of a distal axonal region as used in quantifications of Figure 1. Scale bar represents 20 µm.

(**D**) Schematic of a microfluidic chamber showing the somadendritic and axonal compartments

(**E**) Images of anti-puromycin and anti-BIII-Tubulin staining taken from the axons in the axonal compartment treated with puromycin for 10 minutes (left), treated with puromycin for 10 min after anisomycin pre-treatment for 30 min (middle) or treated with puromycin in the somatodendritic compartment (right). Scale bar represents 25 µm.

(**F**) Images of anti-puromycin and anti-BIII-Tubulin staining taken from neurons in the somatodendritic compartment treated with puromycin for 10 minutes (left), treated with puromycin for 10 min after anisomycin pre-treatment for 30 min (middle) or treated with puromycin in the axonal compartment (right). Scale bar represents 25 µm.

(**G**) Quantification of axonal puromycin intensity in distal axons of DIV7 neurons transfected with a fill and a pSuper plasmid (control), pSuper plasmids containing shRNA targeting RTN4 and DP1 or pSuper plasmids containing shRNA targeting RTN4 and DP1 together with RTN4-GFP and DP1-GFP (rescue).

(**H**) Quantification of RTN4-SBP-GFP levels in distal axons of DIV7 neurons expressing a fill plus RTN4-SBP-GFP in absence or presence of Strep-KifC1.

(**I**) Quantification of puromycin intensity in neuronal soma of DIV7 neurons expressing a fill and RTN4-SBP-GFP in absence or presence of Strep-KifC1.

Individual data points each represent a neuron and each color represents an independent experiment. Data are presented as mean values ± SEM in (**B, G, H, I**). ns = non-significant, ***p<0.001 comparing conditions to control using one-way ANOVA with Dunnett's multiple comparison test (B, G) or Mann-Whitney tests (H, I).

# Figure S2 (related to Figure 2)

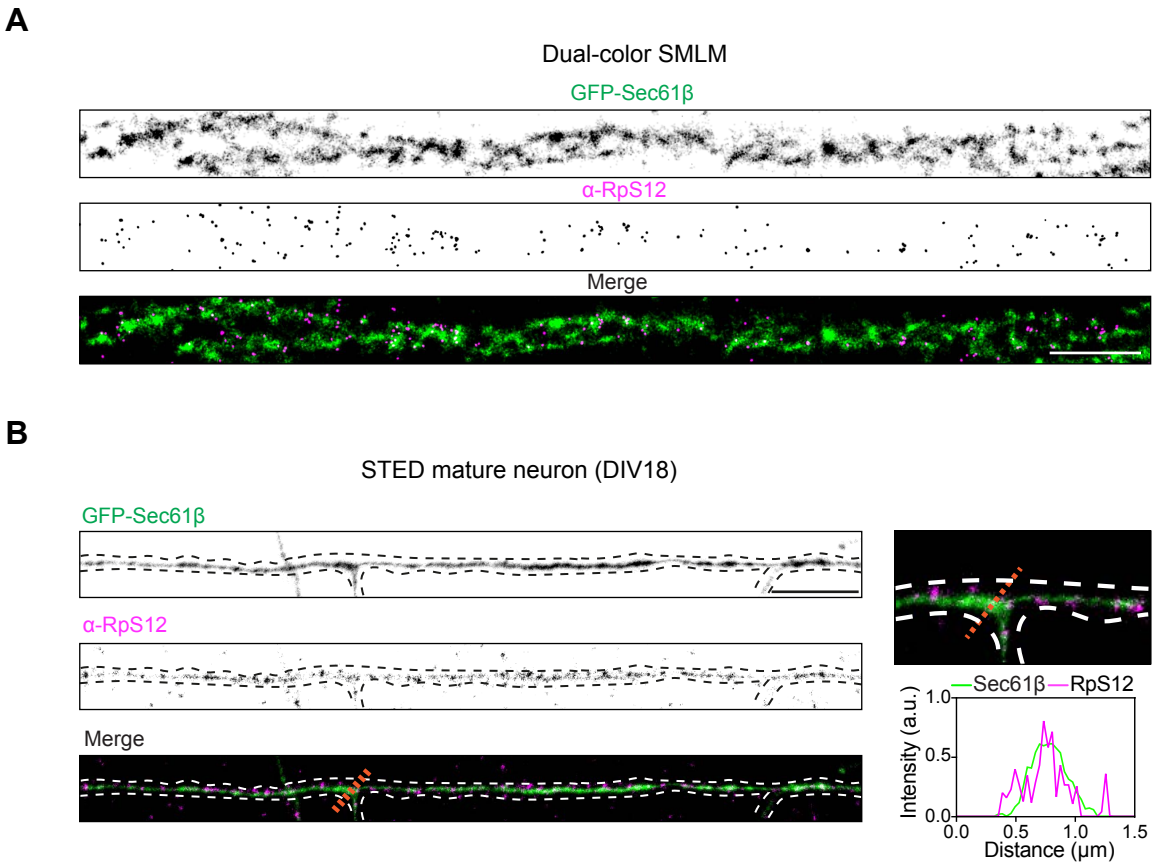


**Fig. S2. (Related to Figure 2). Super-resolution imaging of axonal ER – ribosome interactions**

(**A**) Representative dual-color STORM image of the ER and ribosomes in axons from neurons expressing GFP-Sec61β and stained for RpS12 of a large axonal segment.
(**B**) Representative STED image of the axon of a DIV21 mature neuron expressing GFP-Sec61β and stained for RpS12. A zoomed image and intensity profile plot are shown at the bottom.
Scale bars represent 1μm (**A**) and 5μm in (**B**).

# Figure S3 (related to Figure 3)

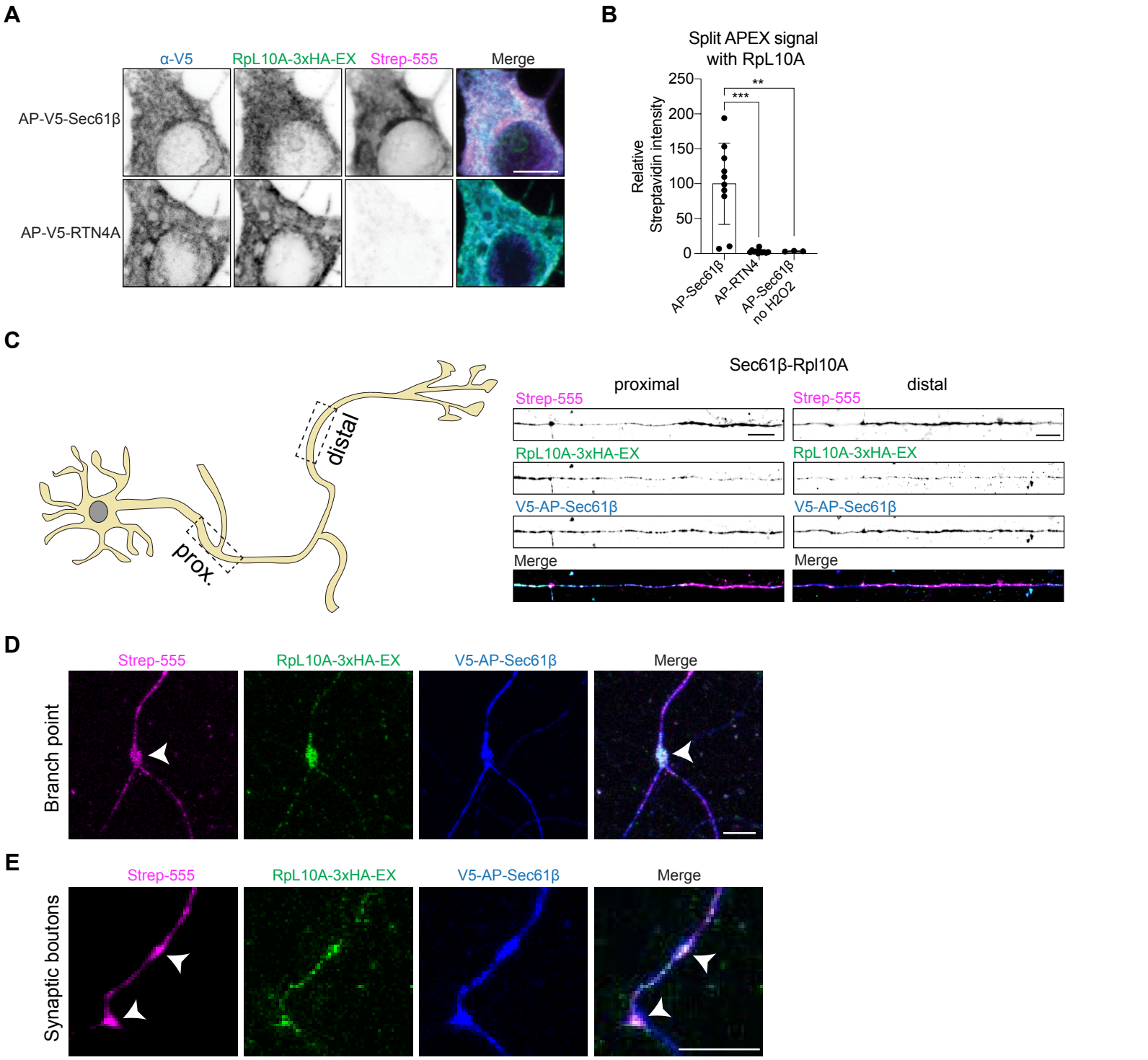


**Fig. S3. (Related to Figure 3). Validation of split APEX assay in neuronal soma and split APEX in mature neurons**

(**A**) Representative images of split APEX assay in soma from neurons expressing Rpl10A-3xHA-EX and V5-AP-Sec61β (top), or V5-AP-RTN4A as a negative control (bottom). Expression of constructs are visualized with V5 and HA antibodies, and biotinylation is detected with conjugated Strep-555. Scale bars represent 5 μm.

(**B**) Quantification of Strep signal in soma from neurons as in (**A**), and without H2O2 as a negative control for the biotinylation reaction.

(**C**) (left) Schematic representation of a DIV18-21 mature neuron. Boxes indicate regions from which images on the right were taken. (right) Representative images of split APEX in mature neurons (DIV18) showing axonal ER – ribosome interactions in the proximal and distal axon.

(**D-E**) Representative split APEX images of in axon of DIV18 neurons showing enriched signal at a branch point (**D**) and possible synaptic boutons (**E**) as indicated with white arrowheads.

Individual data points each represent a neuron and data are presented as mean values ± SD in (**B**). **p < 0.01, ***p<0.001 comparing conditions to V5-AP-Sec61β using ordinary one-way ANOVA tests. Scale bars represent 10μm in (**A**) and 5μm in (**D-E**).

# Figure S4 (related to Figure 4)

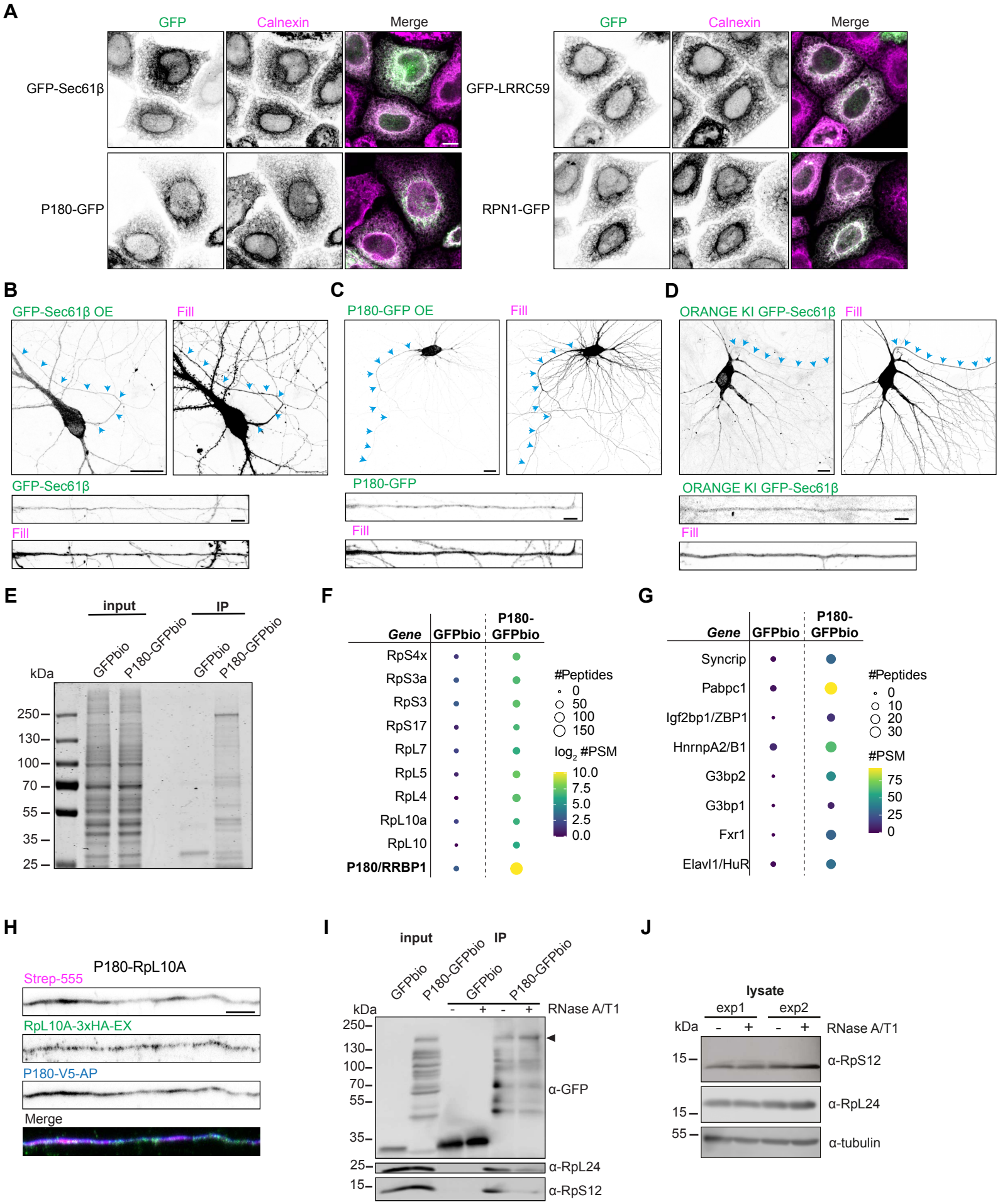

**Fig. S4. (Related to Figure 4). Correct ER localization of GFP-tagged constructs, ER protein distribution in mature neurons and ribosomal protein and RNA-binding protein interactions with P180**

(**A**) Representative images of GFP-tagged ER protein constructs (as indicated) expressed in HEK293T cells and co-stained with an antibody against the ER marker Calnexin. All constructs show a correct ER localization. Scale bar represents 10 µm.

(**B-D**) Representative images of DIV18-21 neurons transfected with (**B**) GFP-Sec61β (**C**) P180-GFP and (**D**) ORANGE knock-in mNeonGreen-Sec61β together with an mCherry fill. Blue arrowheads indicate the axon of which a straightened zoom is shown below each neuron. Scale bars represents 20µm for soma and 5µm for axons.

(**E**) Silver-stained gel from input and pulldown samples of GFPbio and P180-GFPbio.

(**F**) Scaled representation of ribosomal proteins identified with mass spectrometry after pulldown of GFPbio or P180-GFPbio from HEK293T cells. The size and color of each dot reflect the number of PSMs or peptides identified as indicated in the legend.

(**G**) Scaled representation of RNA-binding proteins identified with mass spectrometry after pulldown of GFPbio or P180-GFPbio from adult rat brain extracts. The size and color of each dot reflect the number of PSMs or peptides identified as indicated in the legend.

(**H**) Representative images of split APEX assay for P180-V5-AP and RpL10A-3xHA-EX in the axon. Expression of constructs are visualized with V5 and HA antibodies, and interactions visualized with conjugated Strep-555. Scale bar represents 5 µm.

(**I**) Western blot analysis of ribosomal proteins after GFPbio or P180-GFPbio pulldown with and without RNAseA/T1 treatment.

(**J**) Western blot images showing RpS12, RpL24 and tubulin protein in lysates with and without RNase A/T1 treatment from two independent experiments. No difference in ribosomal protein level is seen after RNAse A/T1 treatment.

Figure S5 (related to Figure 5)

**A**

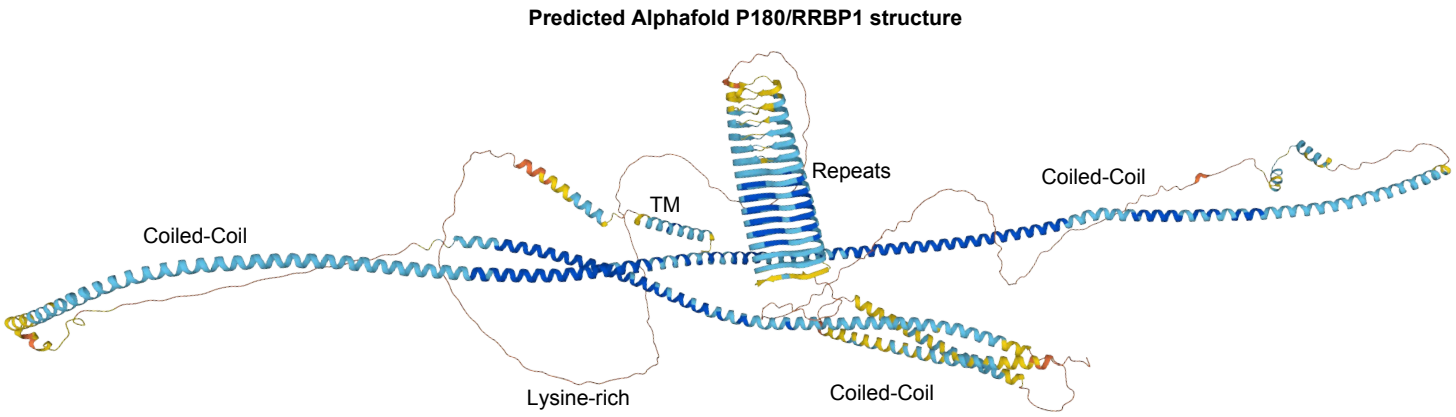


**Fig. S5. (Related to Figure 5). Predicted P180/RRBP1 structure and GO analysis of axonally translated P180-enriched mRNAs.**
(A) Alphafold2 predicted structure of P180/RRBP1 with each domain annotated.