

APPENDIX

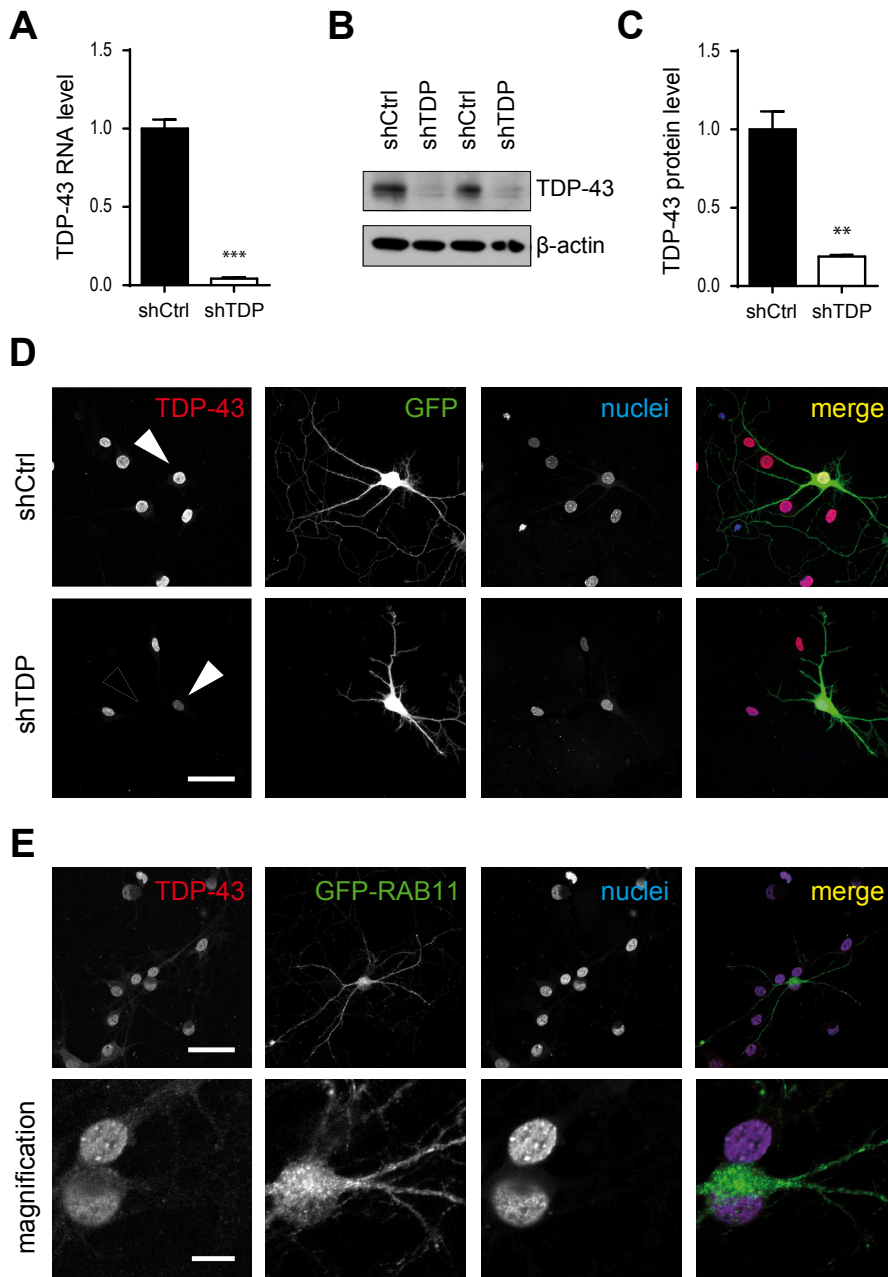
TDP-43 loss of function inhibits endosomal trafficking and alters trophic signaling in neurons

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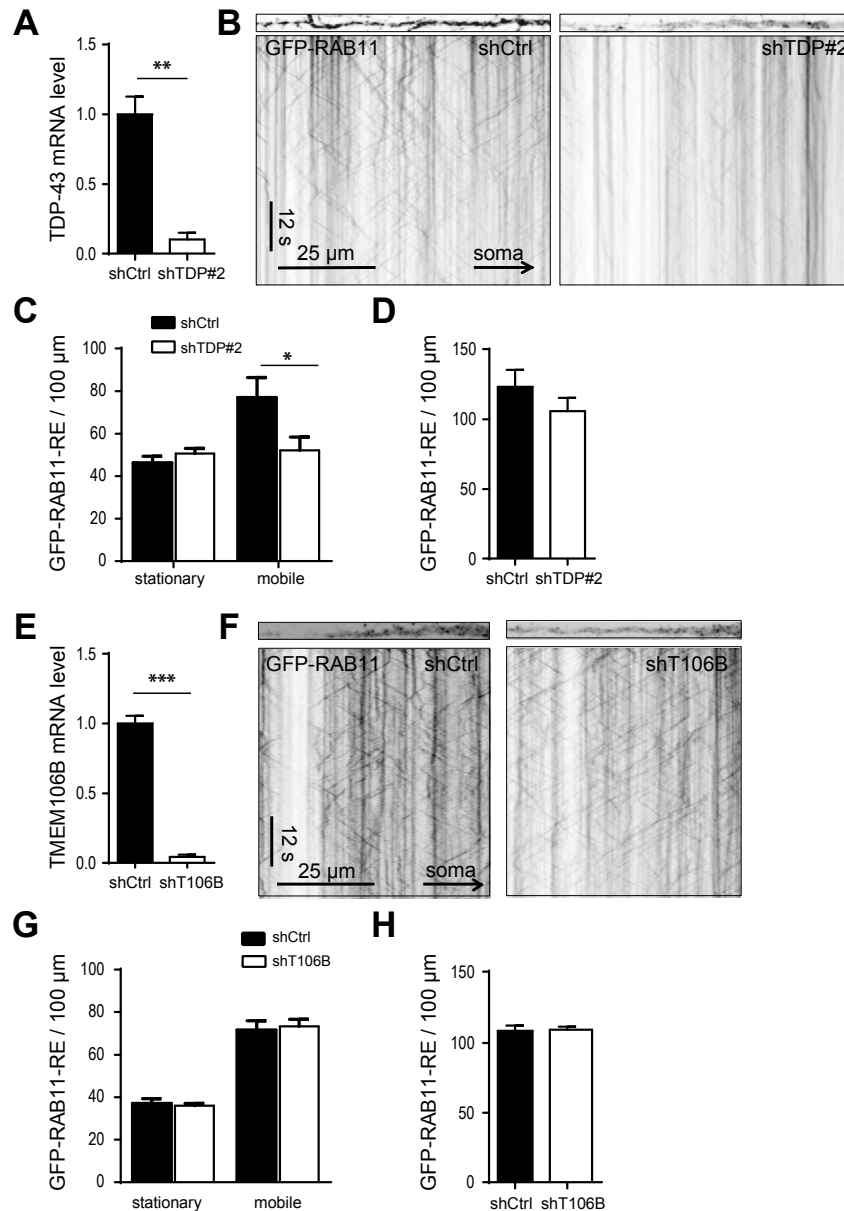
Appendix Figures S1-S4

Appendix Figures:



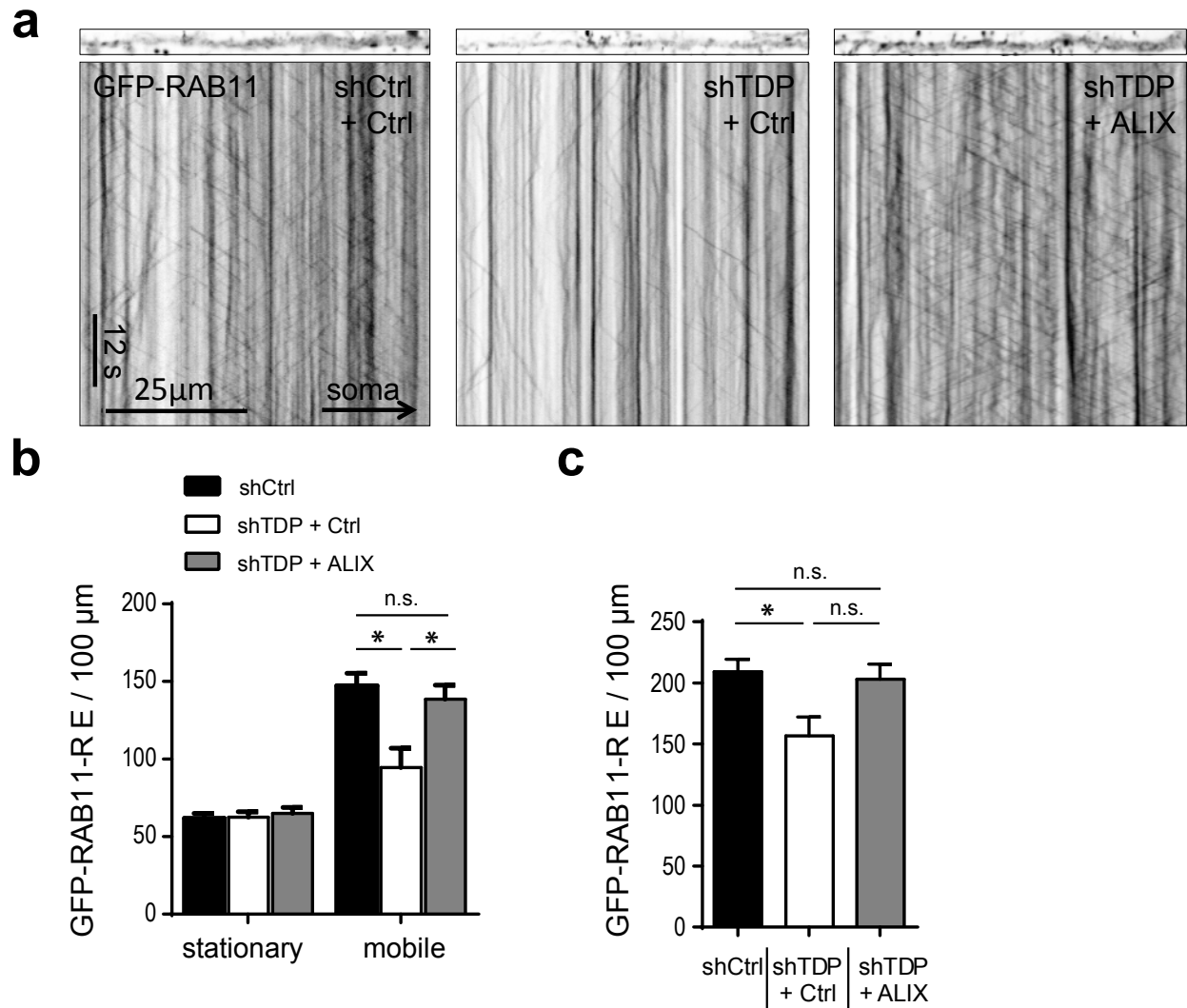
Appendix Figure S1: Validation of TDP-43 shRNA in primary neurons

(A-C) Primary hippocampal neurons (DIV6+4) were transduced with shRNA targeting TDP-43 (shTDP) or control shRNA (shCtrl). (A) TDP-43 mRNA level were analyzed by quantitative RT-PCR. Expression was normalized to the housekeeping genes YWHAZ and PGK1. $n=3$ (B) Immunoblots with the indicated antibodies. $n=6$ (C) Quantification of TDP-43 protein level using densitometry. Expression was normalized to β -actin. (D) Primary hippocampal neurons (DIV6+3) were transfected with either shTDP or shCtrl and GFP as transfection marker. Immunostaining with the indicated antibodies. Arrowheads point to transfected cells. Scale bar represents 50 μ m. (E) Primary hippocampal neurons were transfected with GFP-RAB11. Immunostaining with the indicated antibodies. Scale bar represents 50 μ m and 10 μ m, respectively. mean $\pm$ s.e.m., unpaired, two-tailed t-test: * denotes $p < 0.5$, *** denotes $p < 0.001$.



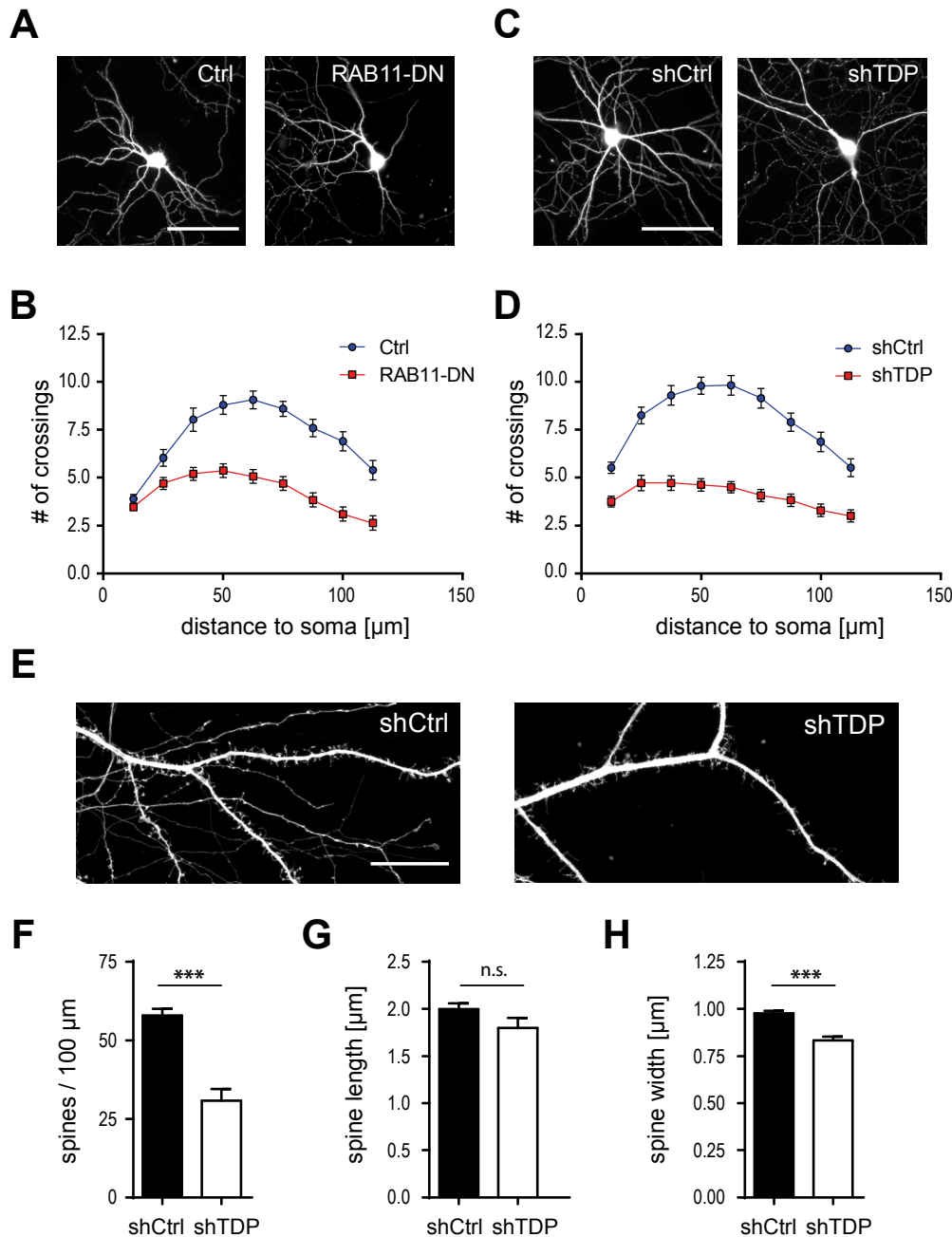
Appendix Figure S2: A second TDP-43 shRNA but not TMEM106B knockdown impairs recycling endosome motility

(A) Primary hippocampal neurons (DIV6+5) were transduced with a second shRNA targeting TDP-43 (shTDP#2) or a control shRNA (shCtrl). TDP-43 mRNA levels were analyzed by quantitative RT-PCR. Expression was normalized to the housekeeping genes YWHAZ and PGK1 (n=3). (B) Primary hippocampal neurons (DIV6+3) were transfected with either shTDP#2 or shCtrl together with GFP-RAB11 to visualize recycling endosomes. Representative dendrite segments and kymographs depict GFP-RAB11 vesicle movement. Quantitative analysis of vesicle motility (C) and vesicle number (D) from kymographs. n=5 (E) Primary hippocampal neurons (DIV6+5) were transduced with shRNA targeting TMEM106B (shT106B) or a control shRNA. TMEM106B mRNA level were analyzed by quantitative RT-PCR and normalized to YWHAZ and PGK1. (F) Primary hippocampal neurons (DIV6+3) were transfected with either shT106B or shCtrl together with GFP-RAB11. Quantitative analysis of recycling endosome motility (G) and number (H) as above (n=). mean $\pm$ s.e.m., unpaired, two-tailed t-test: * denotes p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001.



Appendix Figure S3: ALIX overexpression restores impairment of recycling endosome transport upon TDP-43 knockdown

(A) Primary hippocampal neurons (DIV6+3) were transfected with either shCtrl, shTDP + Ctrl or shTDP + ALIX together with GFP-RAB11 and imaged as in Figure 1A. Quantitative analysis of vesicle motility (B) and number (C) from kymographs (n=4). mean $\pm$ s.e.m., one-way ANOVA (Tukey's post-test): * denotes $p < 0.05$.



Appendix Figure S4: TDP-43 knockdown reduces dendritic spine density and head width

(A) Primary hippocampal neurons were cotransfected with control or dominant negative RAB11 (S25N) and GFP to visualize neuron morphology (DIV6+4). Scale bar represents 100 μm . (B) Quantification of dendrite morphology by Sholl analysis as in Figure 7. Two-way ANOVA (Bonferroni's post-test): Ctrl vs. RAB11-DN: from 50 μm radius $p < 0.001$. (C) Primary hippocampal neurons were cotransfected with shCtrl or shTDP and GFP (DIV14+5). Scale bar represents 100 μm . (D) Quantification of dendritic morphology by Sholl analysis as in Figure 7. Two-way ANOVA (Bonferroni's post-test): shCtrl vs. shTDP: at 12.5 μm radius $p < 0.05$, from 25 μm radius $p < 0.001$. (E) Magnification of neurons transfected as in (C) to visualize spine morphology. Scale bar represents 25 μm . Quantification of spine density (F), spine length (G) and spine head width (H). mean $\pm$ s.e.m., unpaired, two-tailed t-test (G,H) or Mann-Whitney-test (F): *** denotes $p < 0.001$.