Expanded View Figures

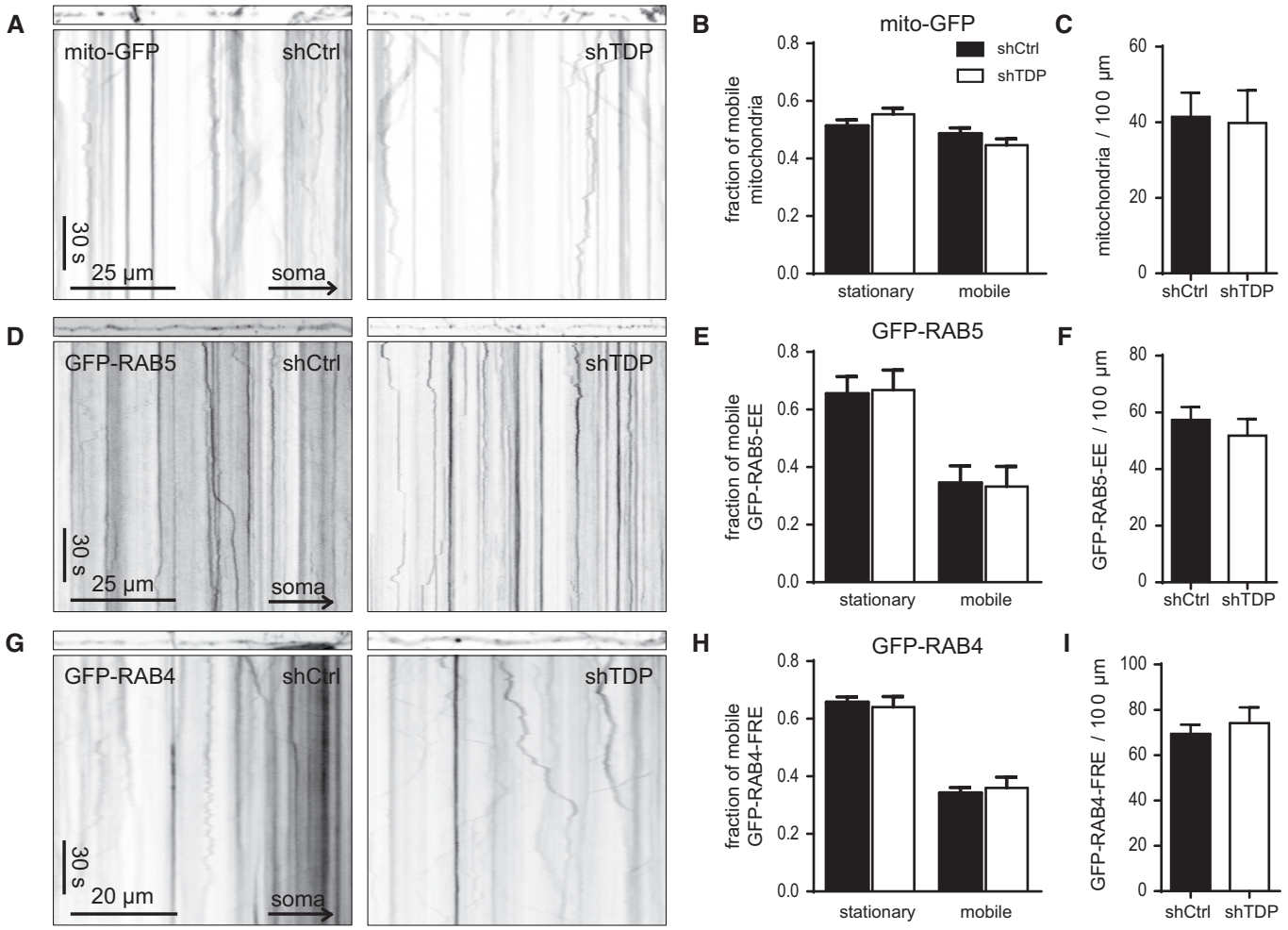

Figure EV1. TDP-43 knockdown has no effect on abundance and trafficking of mitochondria, RAB5-positive early endosomes, or RAB4-positive fast recycling endosomes in dendrites.

Primary hippocampal neurons (DIV6+3) were transfected with either shTDP or shCtrl together with GFP-tagged organelle markers.

A–C Mito-GFP visualizes trafficking of mitochondria. At least four dendrite segments per neuron were live-imaged with 2 Hz for 2.5 min. Quantitative analysis of relative vesicle movement (B) and vesicle number (C) from kymographs.

D–F GFP-RAB5 visualizes early endosomes. At least four dendrite segments per neuron were live-imaged with 2 Hz for 2.5 min. Quantitative analysis of relative vesicle movement (E) and vesicle number (F) from kymographs.

G–I GFP-RAB4 visualizes fast recycling endosomes. At least four dendrite segments per neuron were live-imaged with 2 Hz for 2.5 min. Quantitative analysis of relative vesicle movement (H) and vesicle number (I) from kymographs.

Data information: Mean ± s.e.m., $n = 3$, unpaired, two-tailed t-test: no significant differences were detected.

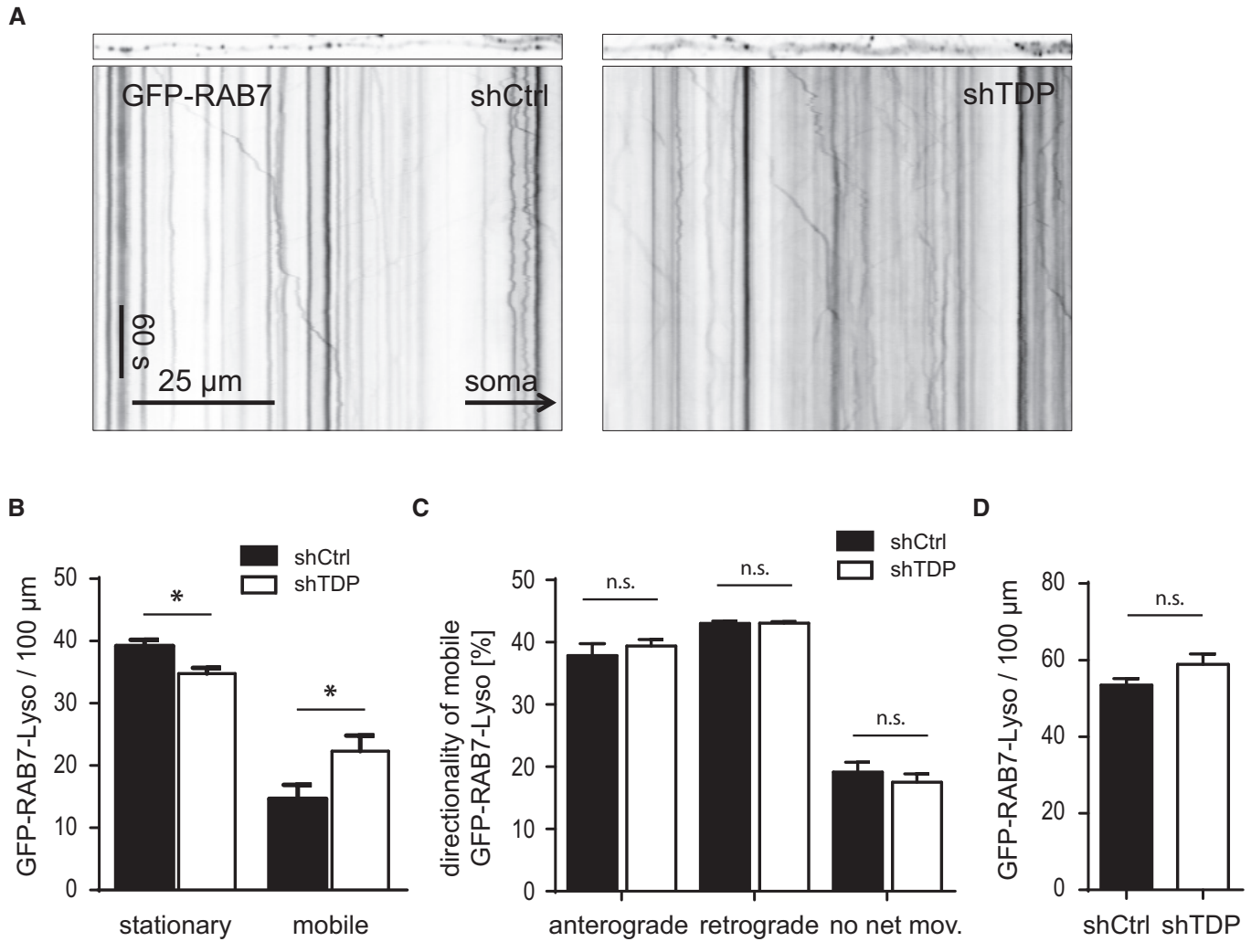

Figure EV2. TDP-43 knockdown slightly increases lysosomal trafficking in primary neurons.

A Primary hippocampal neurons (DIV6+3) were transfected with either shTDP or shCtrl together with GFP-RAB7 to visualize late endosomes/lysosomes. At least four dendrite segments per neuron were live-imaged with 1 Hz for 5 min to analyze the lysosomal transport. Representative dendrite segments and kymographs depict GFP-RAB7 vesicle movement.

B–D Quantitative analysis of vesicle motility (B), directionality (C), and vesicle number (D) from kymographs in (A). Mean ± s.e.m., $n = 4$, unpaired, two-tailed t-test: $^*P < 0.05$.

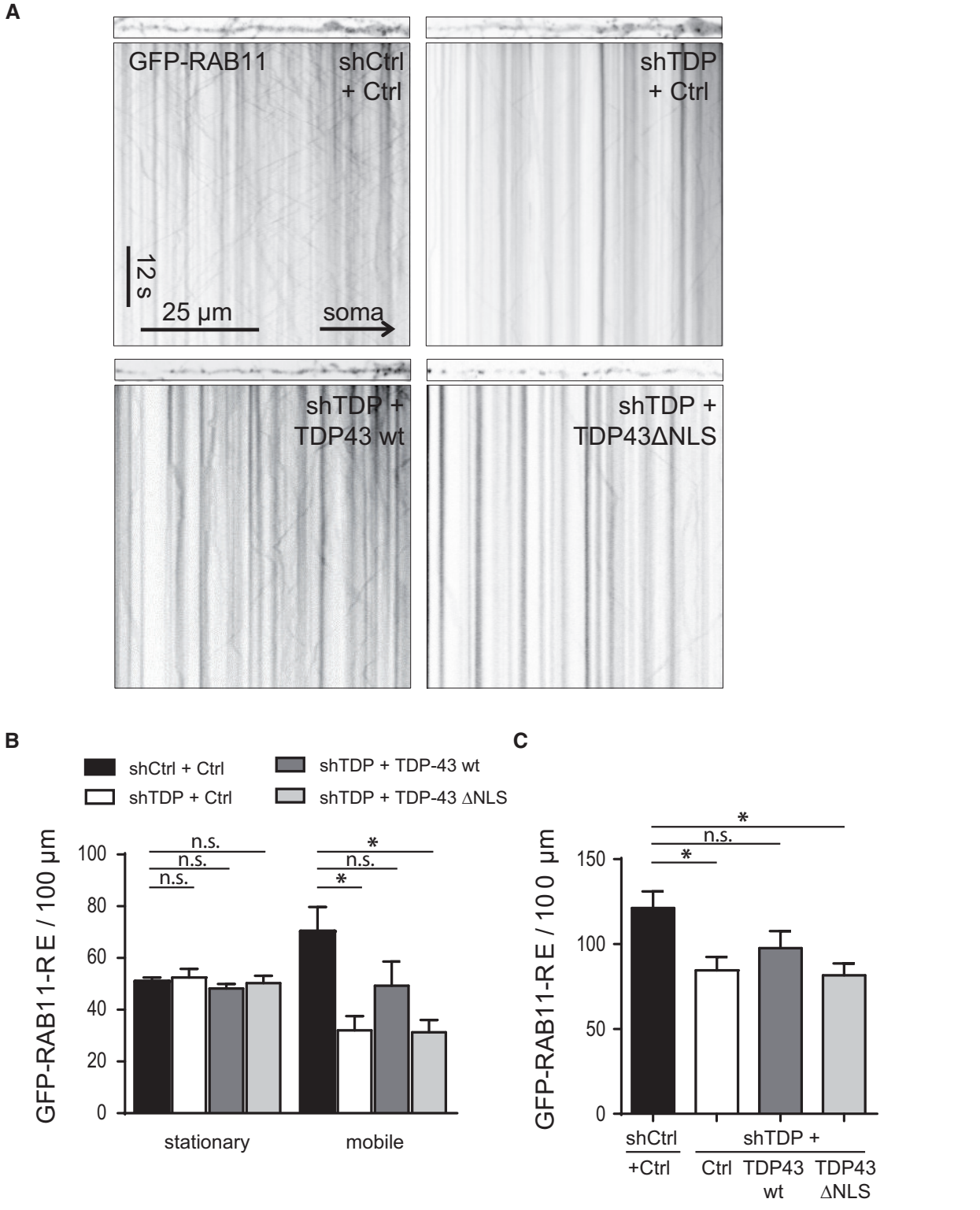

Figure EV3. Only nuclear, but not cytoplasmic, TDP-43 rescues TDP-43-knockdown phenotype.

A Primary hippocampal neurons (DIV6+3) were transfected with shCtrl and empty vector control or shTDP with either empty vector control, TDP-43 wild type, or the ΔNLS variant together with GFP-RAB11 to visualize recycling endosomes. Representative dendrite segments and kymographs of GFP-RAB11 vesicle movement. Scale bar represents 12 s (vertical) and 25 µm (horizontal).

B, C Quantitative analysis of vesicle motility (B) and the number (C) from kymographs in (A). Mean ± s.e.m., $n = 5$, one-way ANOVA: $^*P < 0.05$.

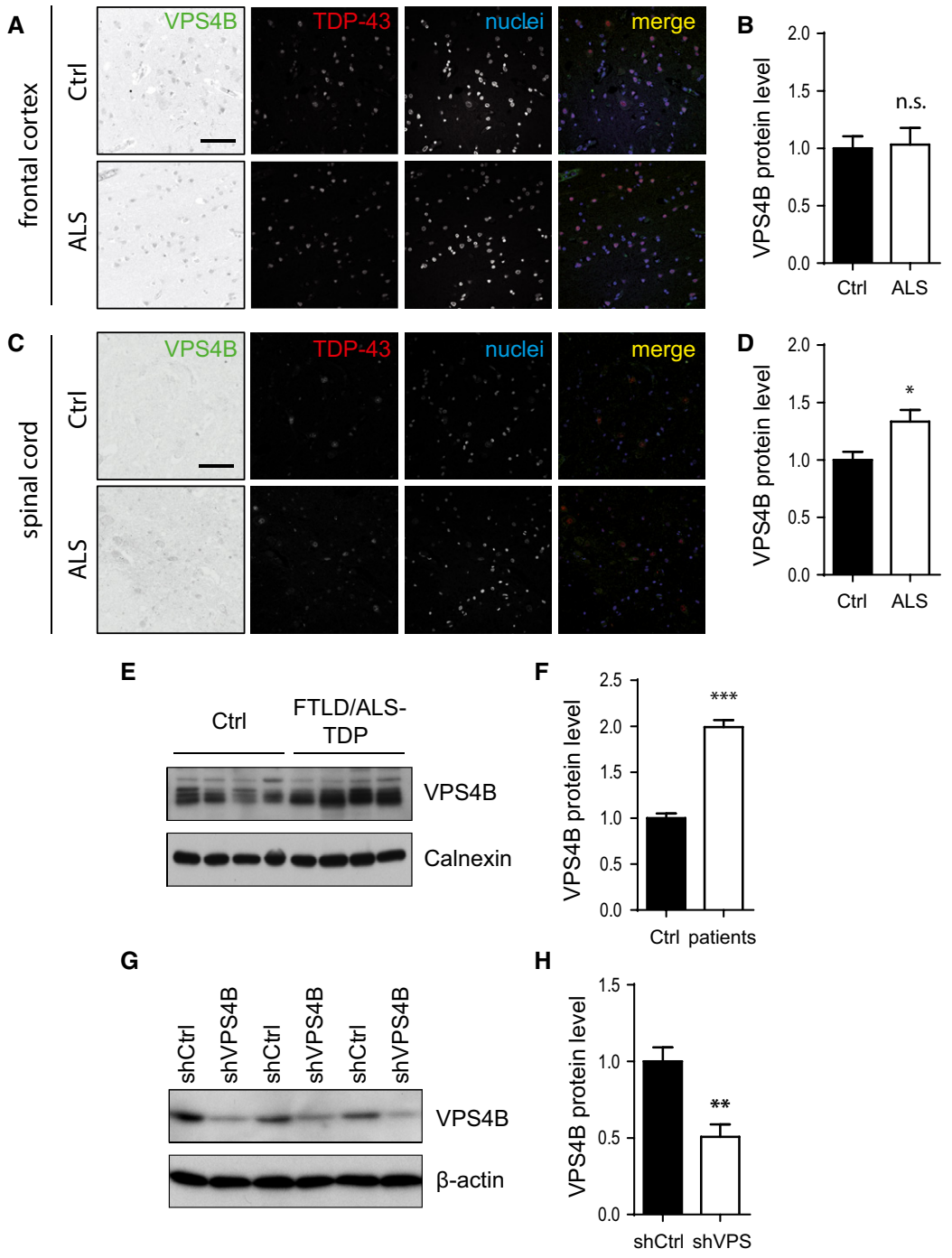

Figure EV4. VPS4B is upregulated in patients with TDP-43 pathology.

A–D Analysis of VPS4B in the tissue of patients with neuropathological diagnosis of ALS and healthy controls. (A, C) Immunofluorescence with the indicated antibodies in the superior frontal gyrus (five patients and four healthy controls) or spinal cord (two patients and healthy controls). Scale bar represents 50 μm. (B, D) Quantification of cellular VPS4B levels. At least 150 cells per case were analyzed.

E Immunoblot with the indicated antibodies of lysates from the frontal superior gyrus of FTLD/ALS-TDP patients and controls.

F Quantification of VPS4B protein levels normalized to calnexin using densitometry (n = 4).

G, H Validation of VPS4B shRNA. Primary hippocampal neurons (DIV9+3) were transduced with shRNA targeting VPS4B (shVPS4B) or a control shRNA (shCtrl). (G) Immunoblot with the indicated antibodies. (H) Quantification of VPS4B protein level using densitometry. Expression was normalized to β-actin (n = 4).

Data information: Mean ± s.e.m., unpaired, two-tailed t-test: *P < 0.05, **P < 0.01, ***P < 0.001.

Source data are available online for this figure.

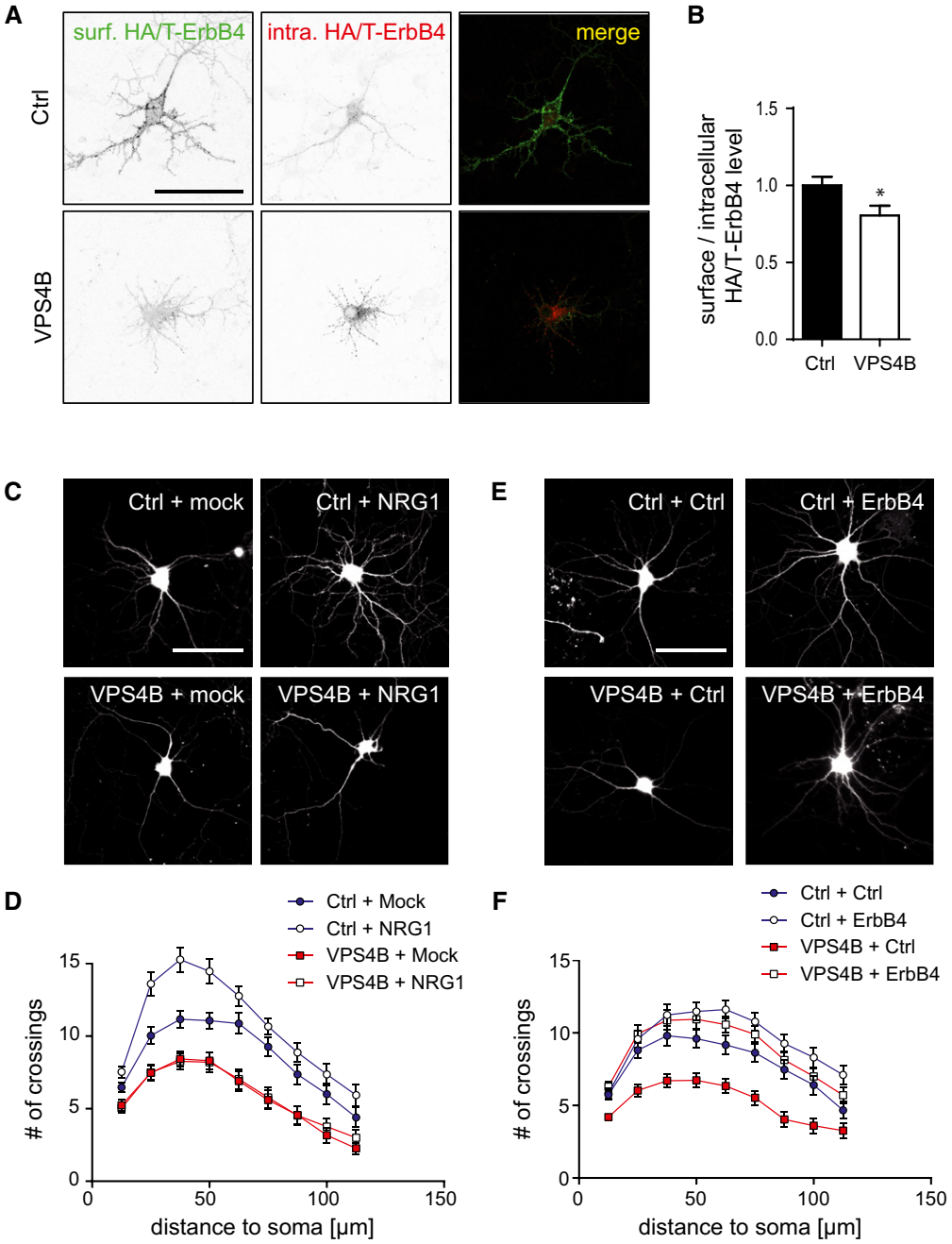

Figure EV5. VPS4B overexpression decreases ErbB4 surface targeting and blunts dendritic complexity and response to NRG1.

A, B Hippocampal neurons were transfected with ErbB4 containing a luminal HA-tag (HA/T-ErbB4) and VPS4B or empty vector (DIV9+3). (A) Surface and intercellular HA/T-ErbB4 was stained in living cells and intracellular HA/T-ErbB4 was stained after fixation and permeabilization with HA antibodies. Scale bar represents 100 µm. (B) For quantification of HA/T-ErbB4 surface levels, at least 10 images per condition per experiment were analyzed in three independent experiments. Mean ± s.e.m., unpaired, two-tailed *t*-test: $*P < 0.05$.

C, D Primary hippocampal neurons (DIV6) were transfected with VPS4B or empty vector and GFP to visualize neuron morphology. Neurons were treated with 1 nM NRG1-beta 1 (NRG1) or vehicle 2 days later; dendritic morphology from at least 30 neurons per condition per experiment was quantified by Sholl analysis and statistically evaluated using two-way ANOVA with Tukey's post-test. Scale bar represents 100 µm. Mean ± s.e.m.. Ctrl vs. VPS4B: from 25 to 37.5 µm radius $P < 0.05$, at 50 µm and from 87.5 to 100 µm radius $P < 0.01$, from 62.5 to 75 µm radius $P < 0.001$. Ctrl vs. Ctrl + NRG1: from 25 to 50 µm radius $P < 0.001$. VPS4B vs. VPS4B + NRG1: no significant difference.

E, F Transfection of the indicated combinations of VPS4B, ErbB4 and vector control (DIV6+5). Dendritic morphology was quantified as above. Scale bar represents 100 µm. Mean ± s.e.m.. Ctrl + Ctrl vs. VPS4B + Ctrl: at 25, 50, 62.5 and 100 µm radius $P < 0.01$, at 37.5, 75 and 87.5 µm radius $P < 0.001$. VPS4B + Ctrl vs. VPS4B + ErbB4: at 12.5 and 112.5 µm radius $P < 0.05$, from 25 to 100 µm radius $P < 0.001$. Ctrl + Ctrl vs. VPS4B + ErbB4: no significant difference.