SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE S1. Measurement of the total dendrite length of mouse hippocampal neurons. This panels in this figure are close-up views of the upper panels of Fig. 4*A* (GFP; green, MAP2, a dendrite marker; red). The total dendrite length and total dendrite branch tip numbers were determined based on the MAP2 signals (see also *Experimental Procedures*). This neuron had 5 dendrite branch tips (labeled 1 to 5), which we identified by the MAP2-staining (middle panel). The length of each dendrite (broken white lines) from the edge of the cell body (or its branch point; see dendrite number 5) to its tip was measured. Total dendrite length was calculated by adding the lengths of the five dendrites. Bar, 50 μm. The total axon length and total axon branch tip numbers were similarly determined based on the neurofilament-H signals.

SUPPLEMENTAL FIGURE S2. Localization of EGFP-Rab1-43 in hippocampal neurons. At 5 DIV mouse hippocampal neurons were transfected with pEGFP-C1 or pEGFP-C1-Rab1-43. The neurons were fixed at 14 DIV and then subjected to immunocytochemistry with antibodies against neurofilament-H (an axon marker; red) and MAP2 (a dendrite marker; blue). The panels (a) and (b) on the right are magnified views of the boxed areas in the panels on the left. Bars, 10 μm (two panels on the left) and 2.5 μm (four panels on the right).

SUPPLEMENTAL FIGURE S3. Quantification of the Rab17-positive dots in the axons and dendrites of hippocampal neurons. *A*, Quantification of the EGFP-positive dots in the axons and the dendrites of mouse hippocampal neurons expressing EGFP (n=20), EGFP-Rab17 (n=21), or EGFP-Rab3A (n=21) shown in Fig. 1*A* and Supplemental Fig. S2. *B*, Quantification of the Myc-Rab17-positive dots in the axons and dendrites of the hippocampal neurons (n=22) shown in Fig. 1*B*. *C*, Quantification of the endogenous Rab17-positive dots in the axons and dendrites of 3 DIV hippocampal neurons (n=20) (Fig. 2*D*) and 11 DIV hippocampal neurons (n=20) (Fig. 2*E*). The black bars and gray bars indicate the number of dots in the axons and the number of dots in the dendrites, respectively.

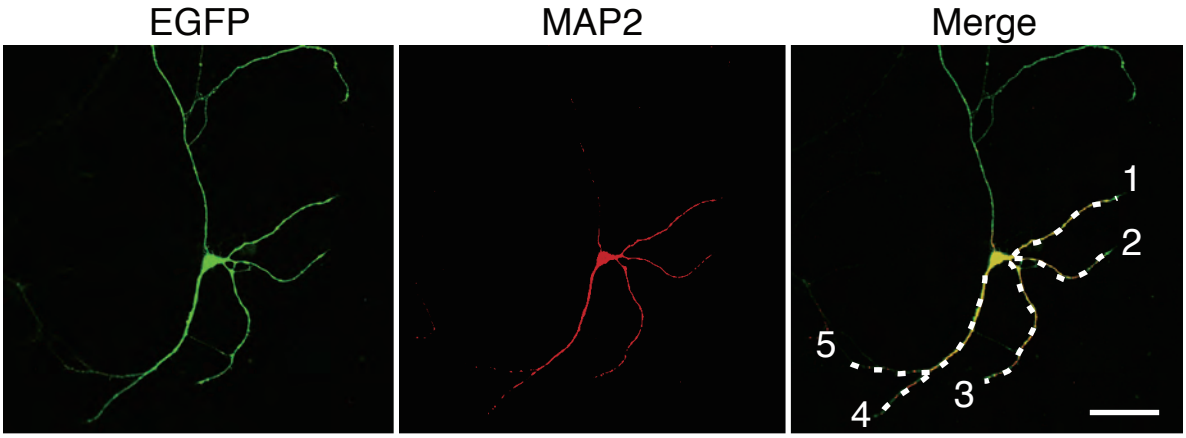
SUPPLEMENTAL FIGURE S4. Tissue distribution of mouse Rab17 protein. Tissue homogenates of mouse brain, lung, heart, liver, kidney, spleen, pancreas, and testis were analyzed by immunoblotting with anti-Rab17 antibody (upper panels) and anti-GAPDH antibody (lower panels). The positions of the molecular mass markers (in kilodaltons) are shown on the left.

SUPPLEMENTAL FIGURE S5. shRNA-mediated knockdown of endogenous Rab17 molecules. *A*, Neuro2A cells were transfected with a vector encoding control-shRNA or *Rab17*-shRNA, and two days after transfection the cells were lysed and subjected to immunoblot analysis with anti-Rab17 antibody (upper panel) and anti-actin antibody (lower panel). The positions of the molecular mass markers (in kilodaltons) are shown on the left. *B,* At 4 DIV hippocampal neurons were transfected with a vector encoding EGFP and control-shRNA (upper panels in *B*) or *Rab17*-shRNA (lower panels in *B*), and the neurons were fixed at 11 DIV

and subjected to immunocytochemistry with antibodies against GFP (green) and Rab17 (red). The arrows and arrowheads indicate transfected neurons and untransfected neurons, respectively. Note the dramatic decrease in Rab17 immunoreactive signals after knockdown of endogenous Rab17 molecules in hippocampal neurons (bottom right panel). Bar, 10 μm.

SUPPLEMENTAL FIGURE S6. Rab17 is necessary for postsynaptic development. *A*, Hippocampal neurons were fixed at 24 DIV and subjected to immunocytochemistry with antibodies against Rab17 (green), PSD95 (a spine marker; red), and MAP2 (blue). Bar, 2.5 μm. *B*, At 4 DIV hippocampal neurons were transfected with a vector encoding EGFP and control-shRNA (upper panels) or *Rab17*-shRNA (lower panels), and at 24 DIV the neurons were fixed and subjected to immunocytochemistry with antibodies against GFP (green), PSD95 (red), and MAP2 (blue). Bar, 5 μm.

SUPPLEMENTAL FIGURE S7. Surface level of NMDAR1 of Rab17-knockdown hippocampal neurons. At 4 DIV hippocampal neurons were transfected with a vector encoding EGFP and control-shRNA (upper panels) or *Rab17*-shRNA (lower panels), and at 24 DIV the neurons were fixed and subjected to immunocytochemistry with antibodies against GFP (green), NMDAR1 (red), and MAP2 (blue). Note that in order to visualize the surface NMDAR1 the anti-NMDAR1 antibody was allowed to react before the permeabilization step. Bar, 1 μm.



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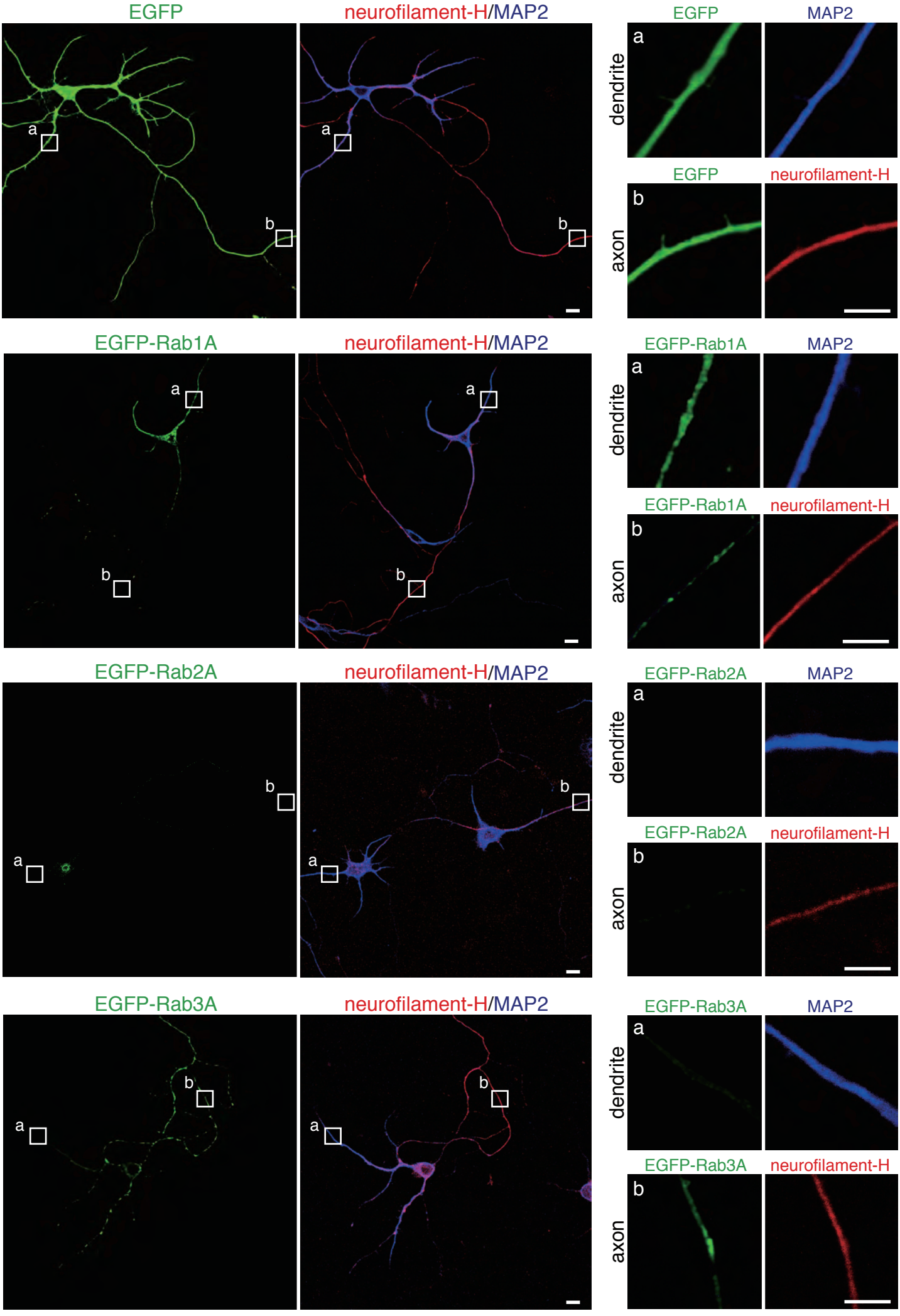

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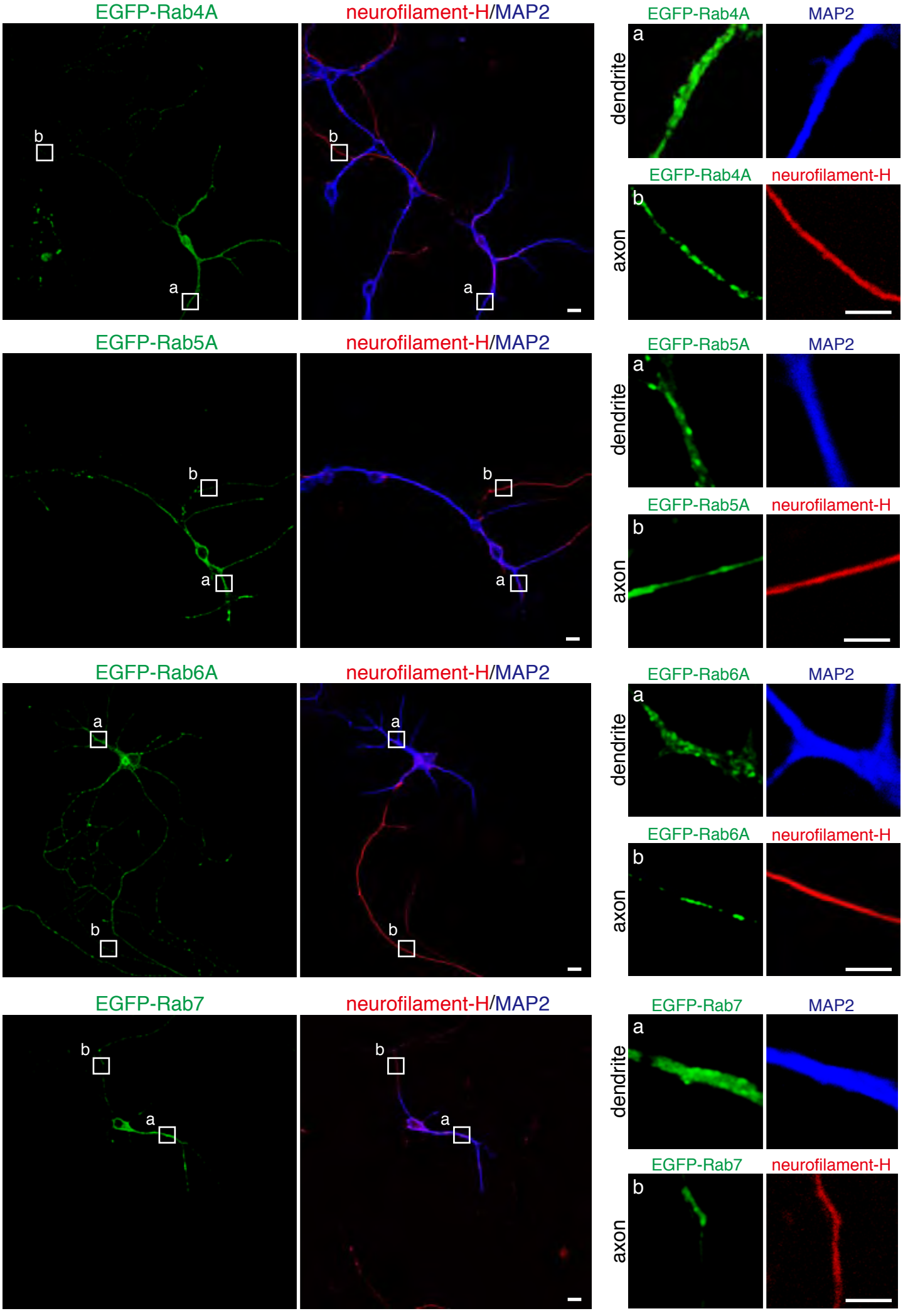

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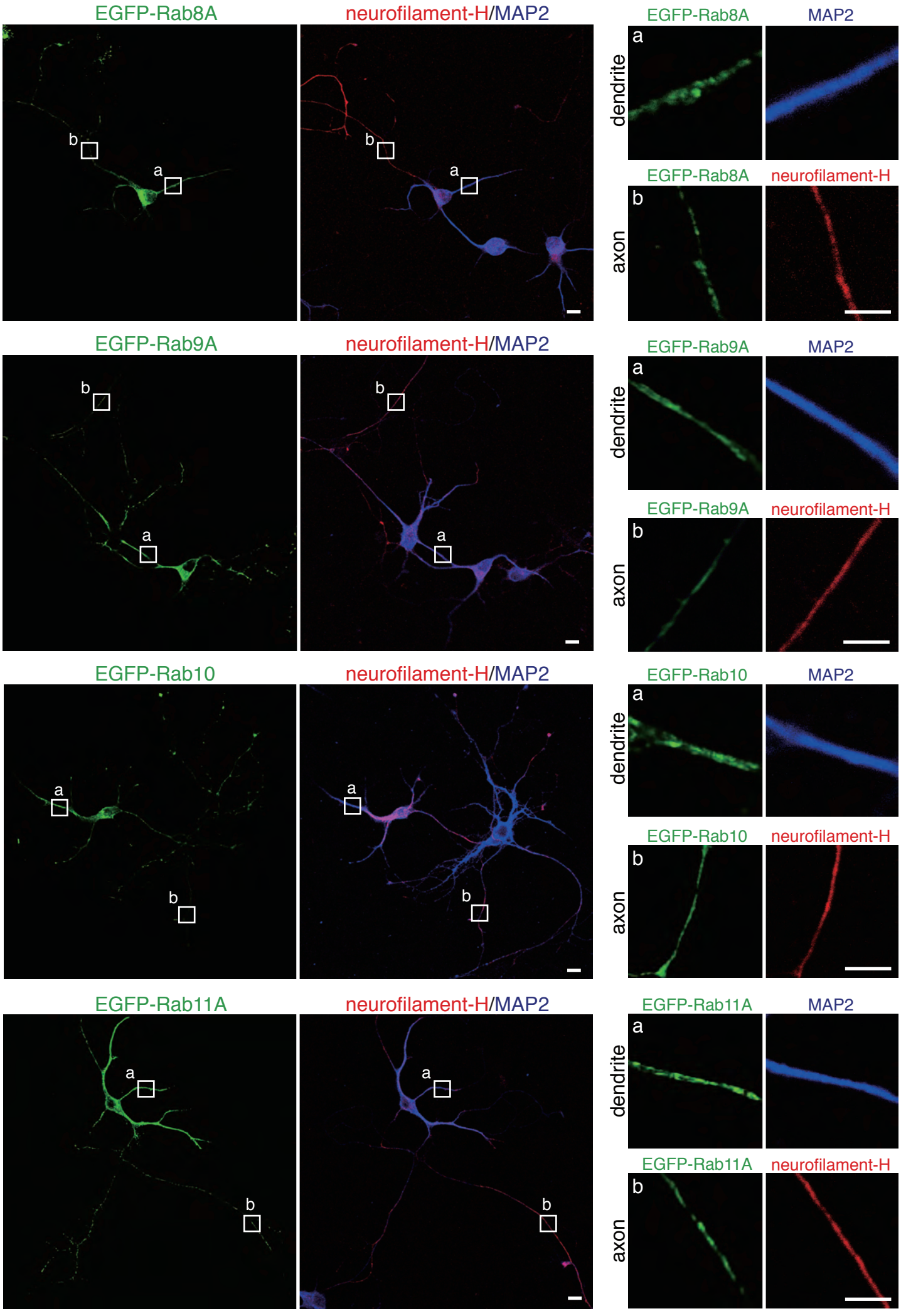

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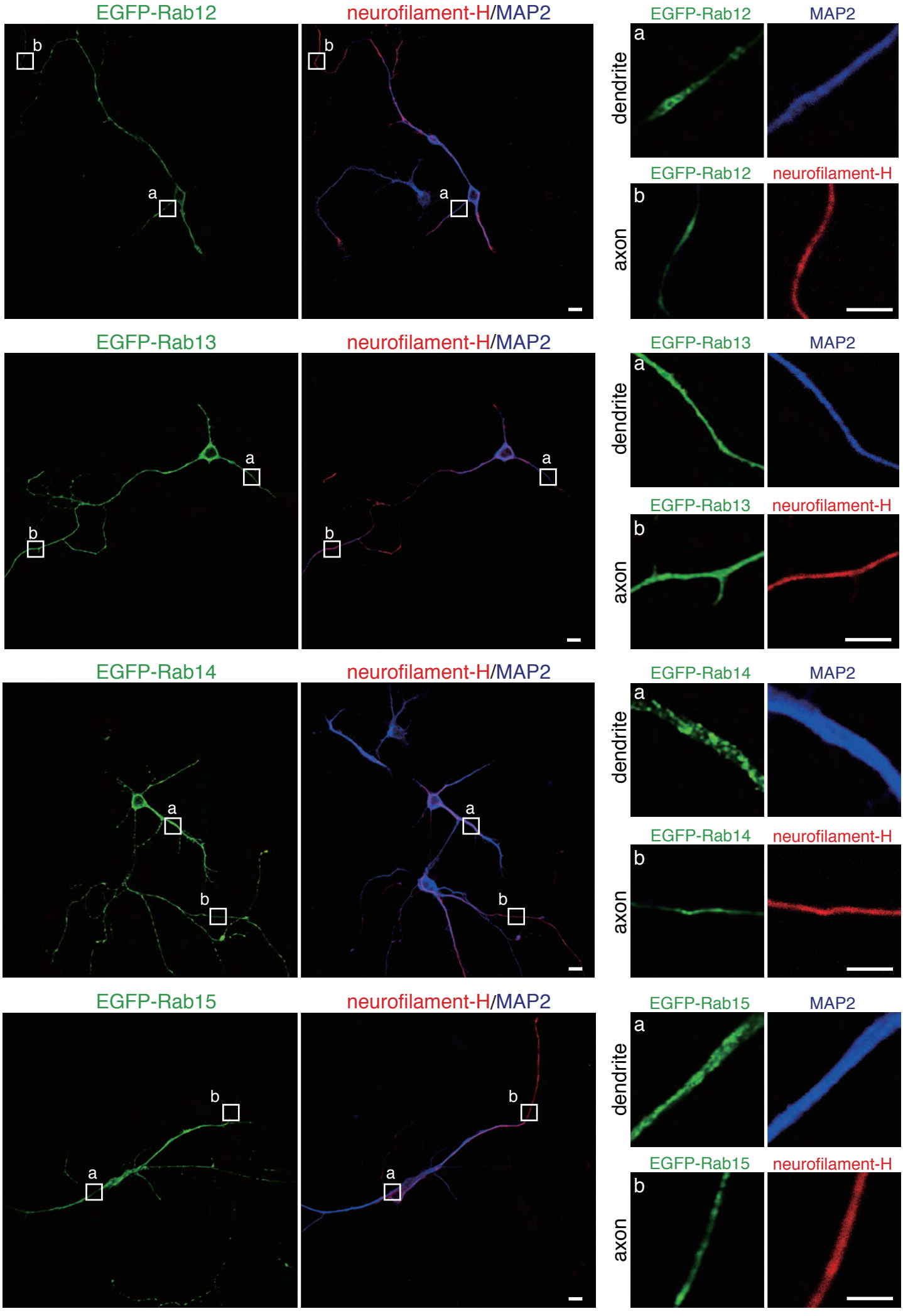

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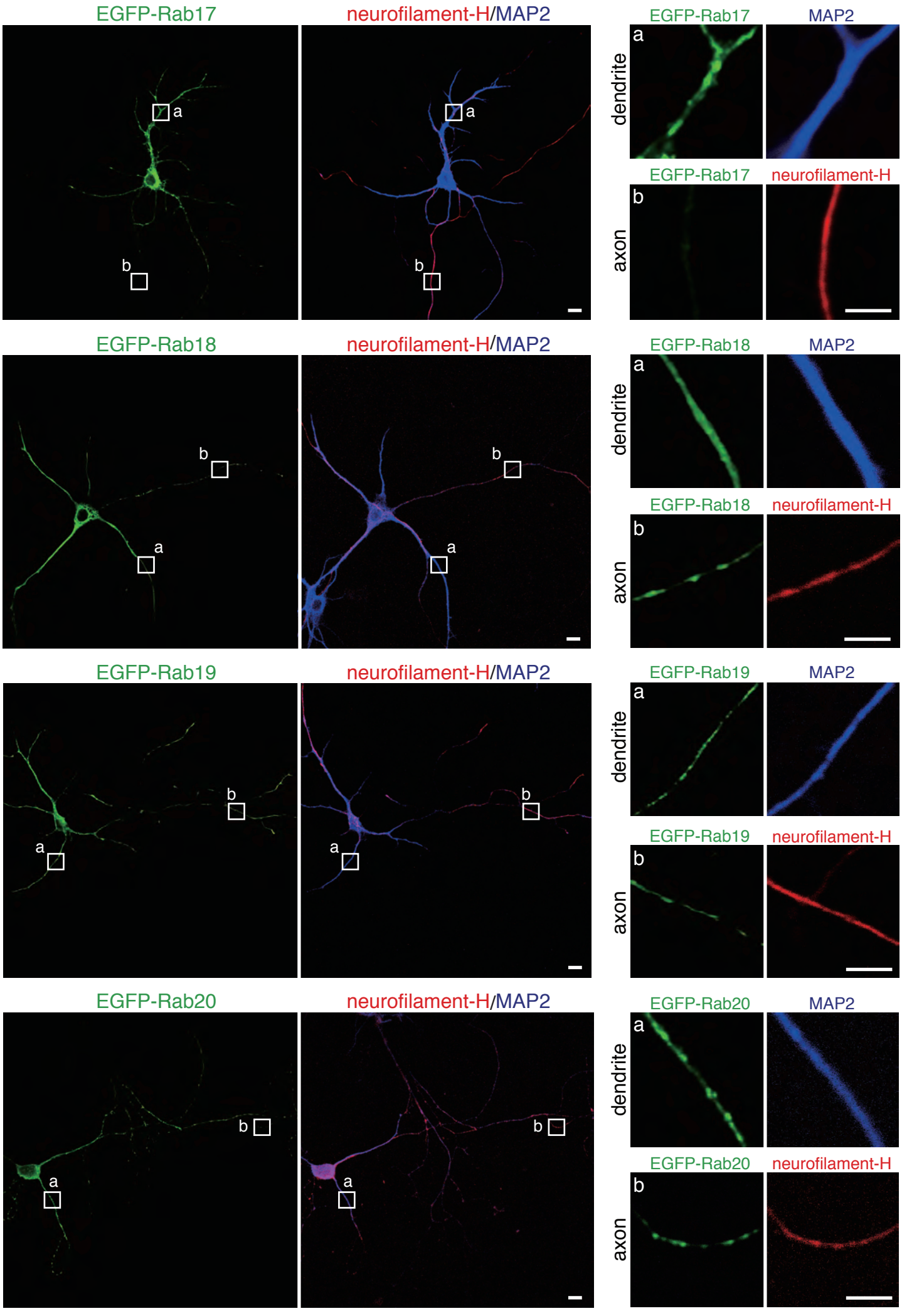

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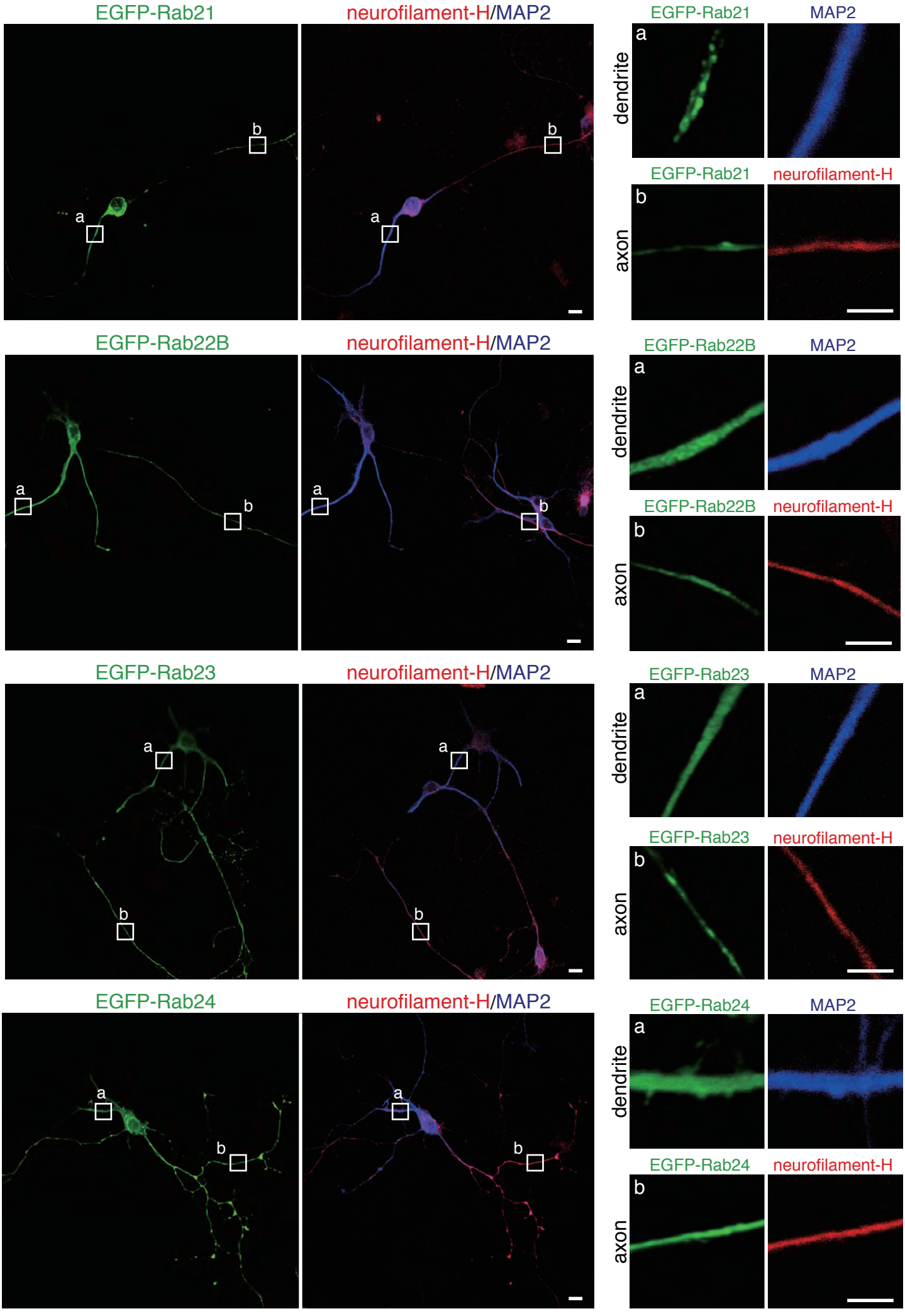

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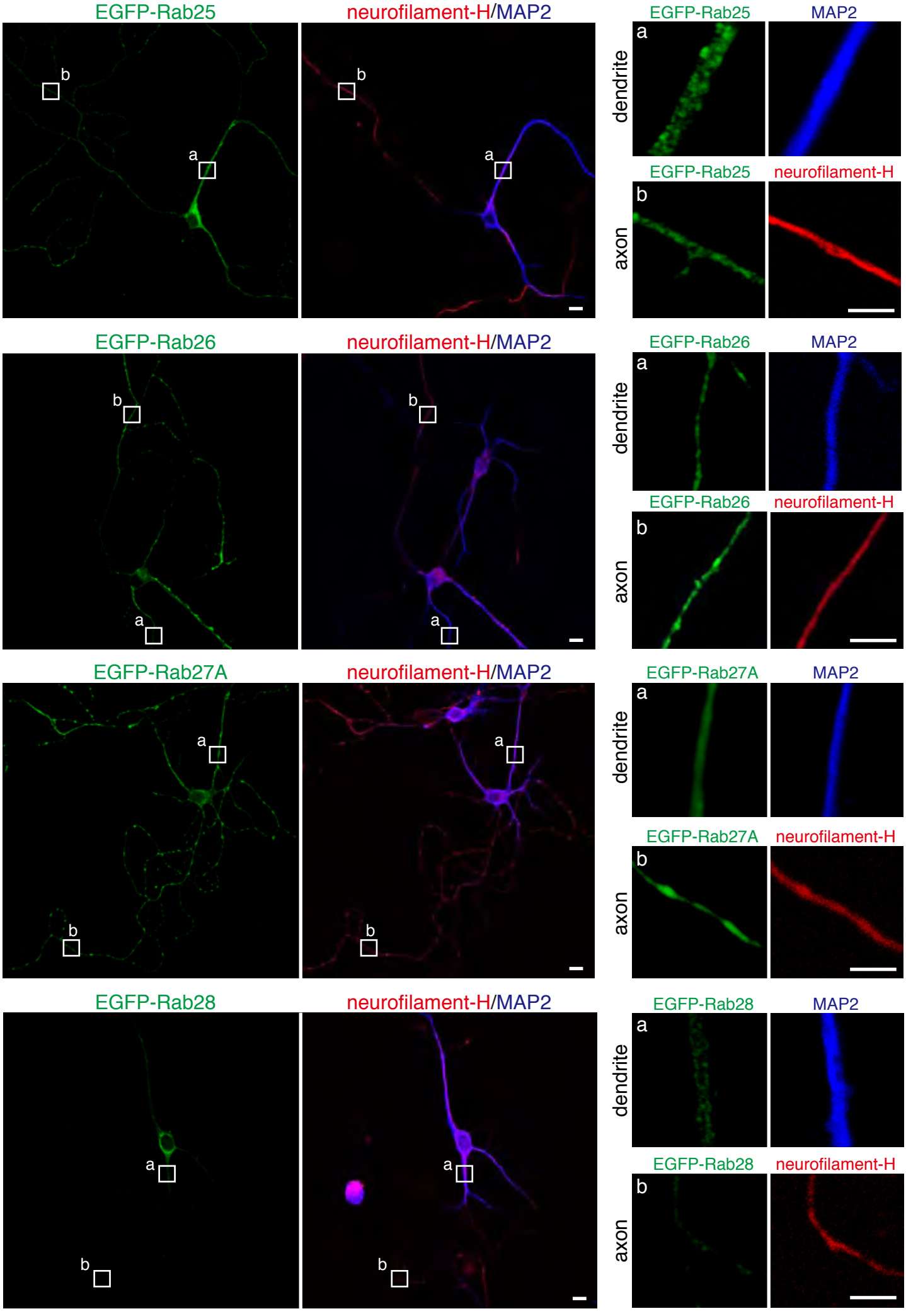

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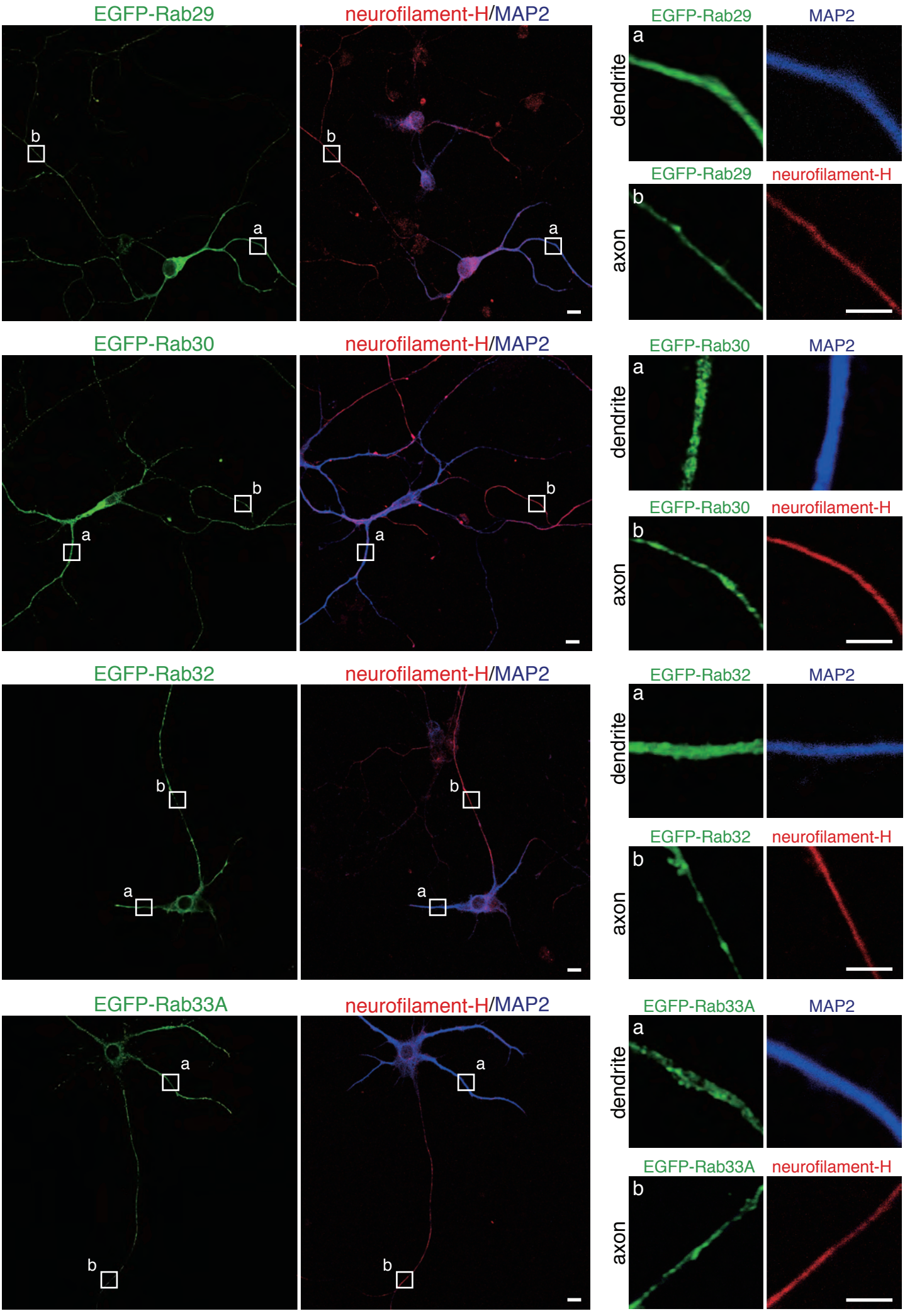

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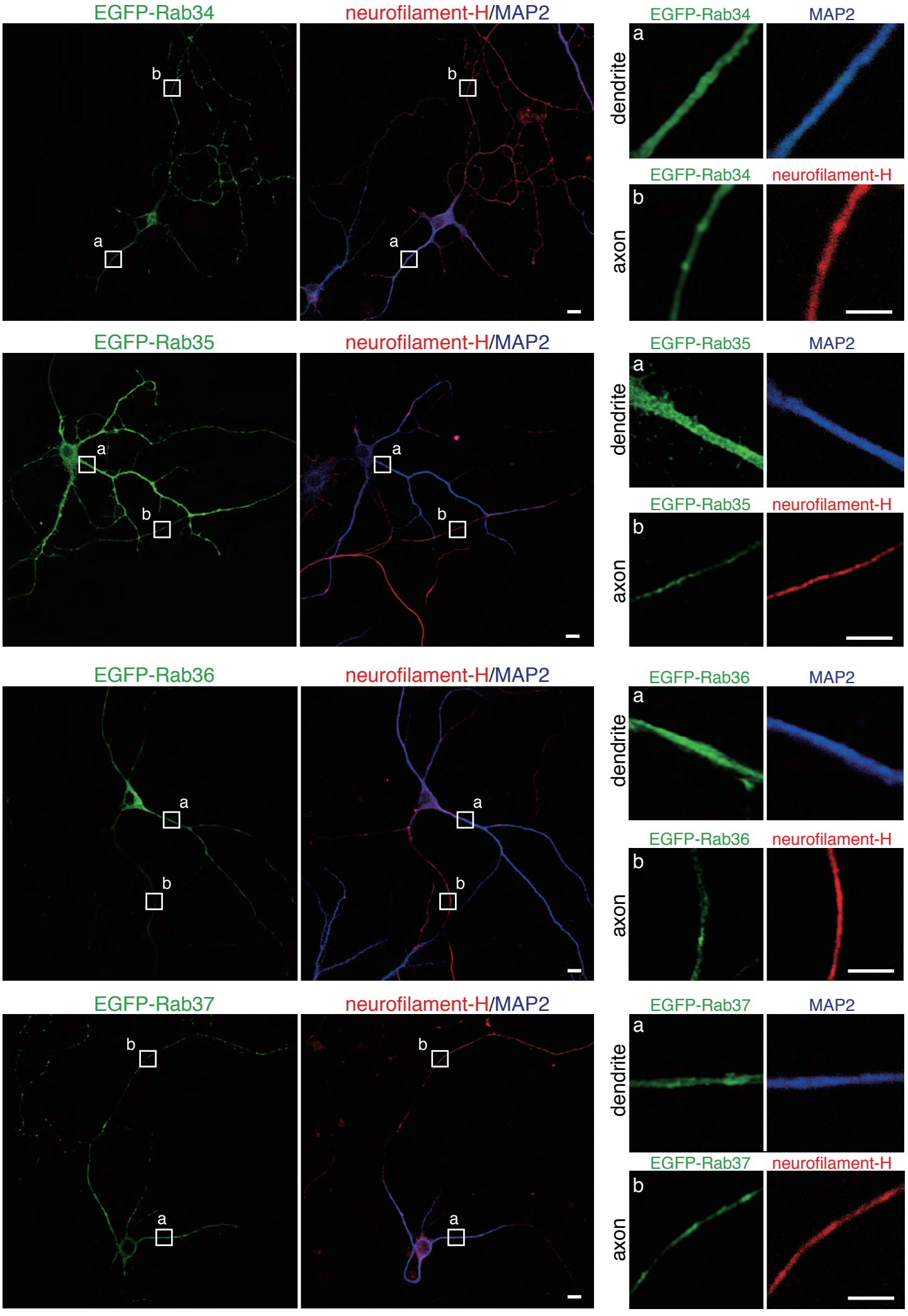

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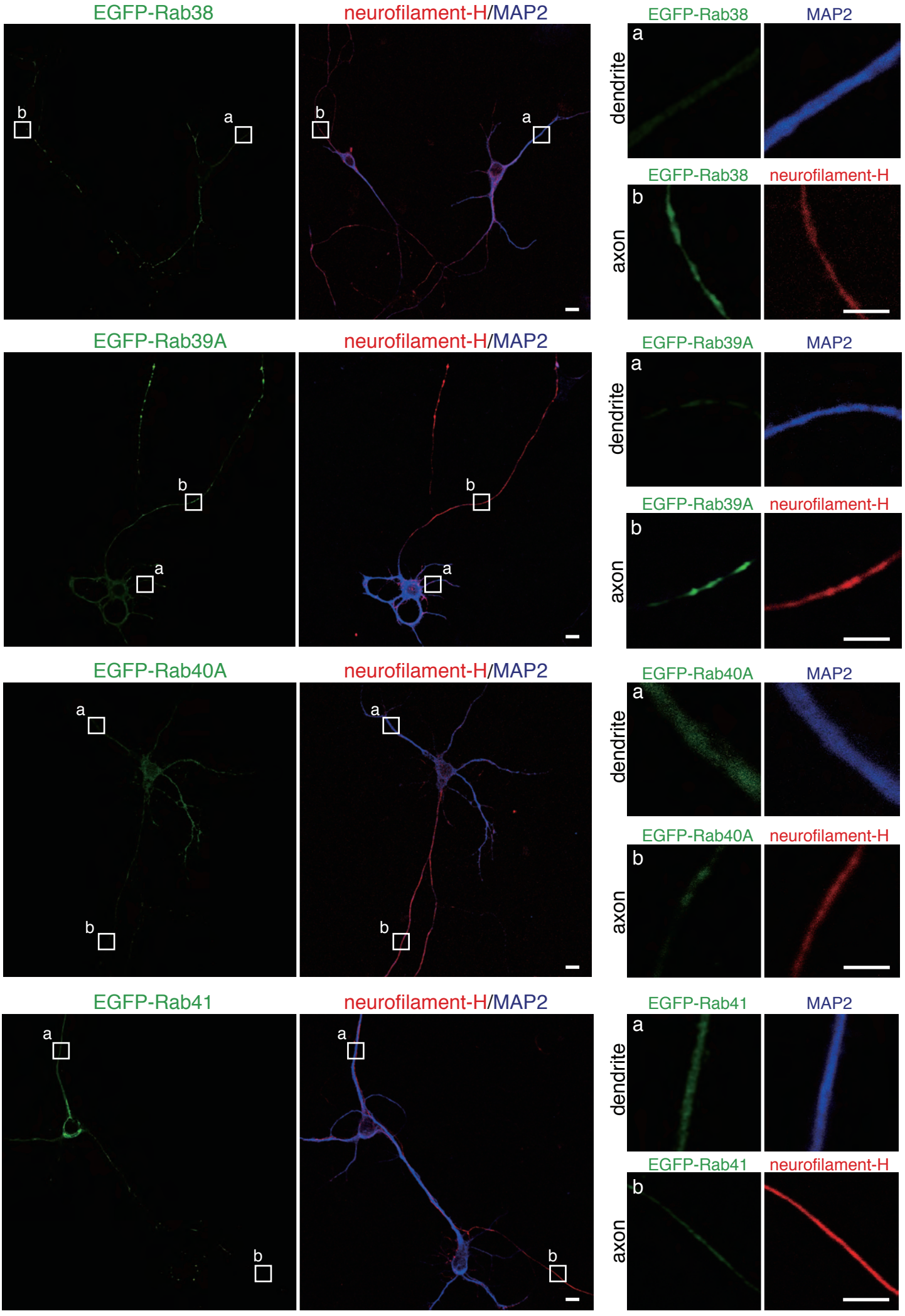

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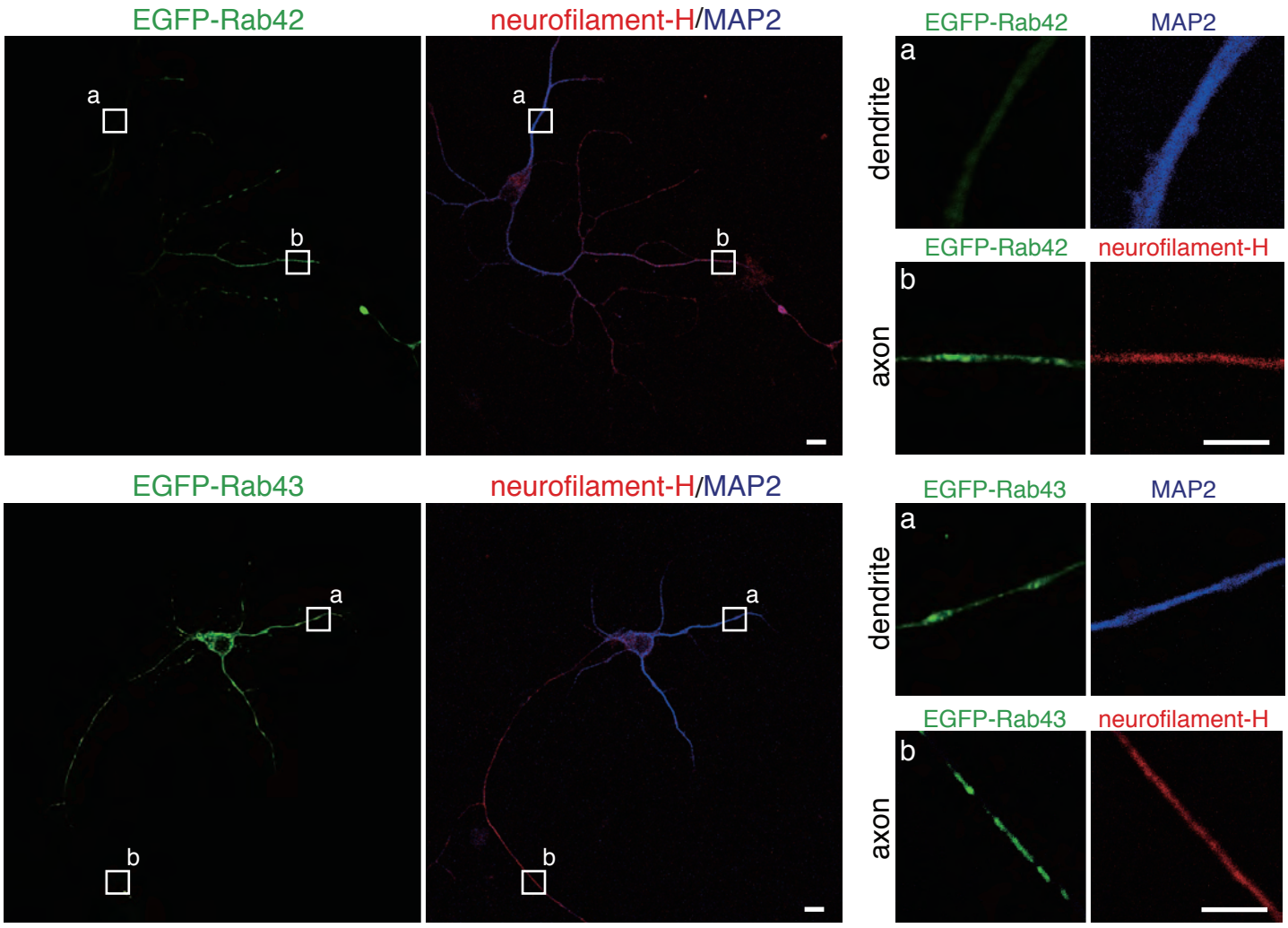

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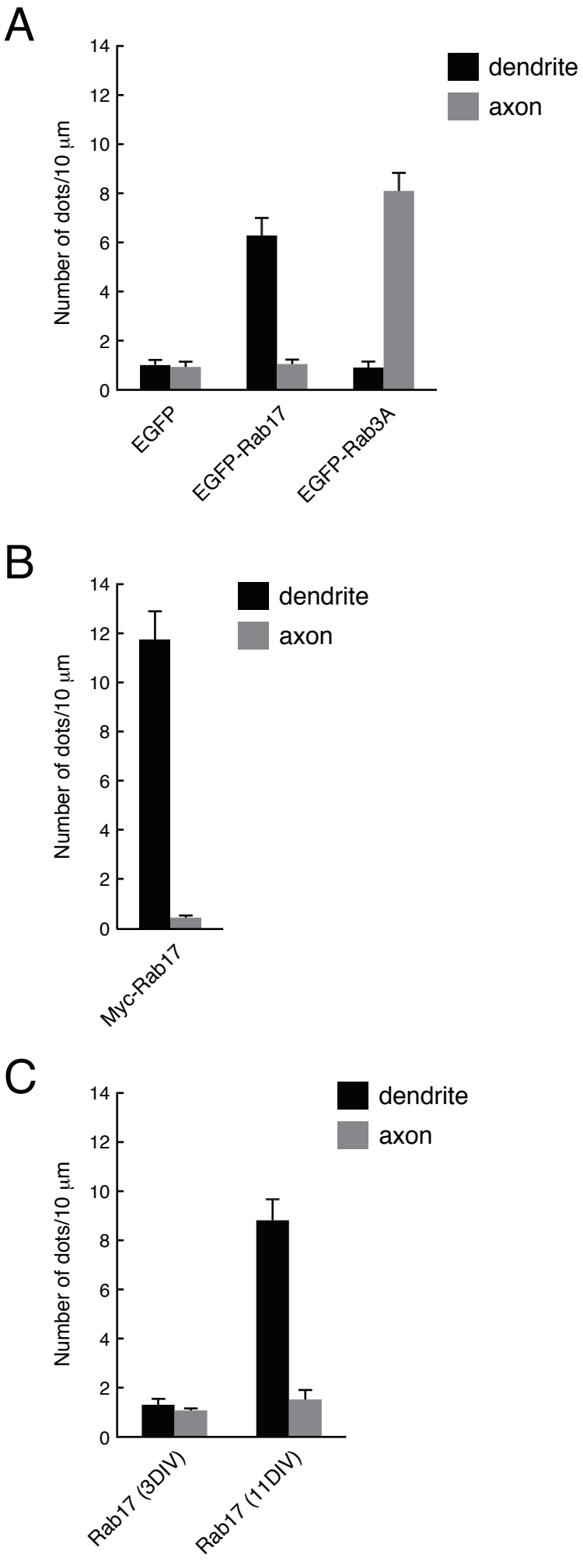

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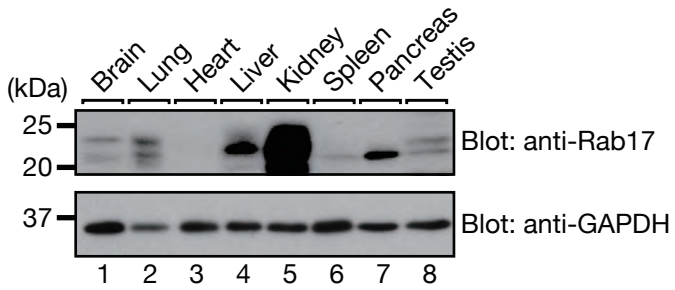

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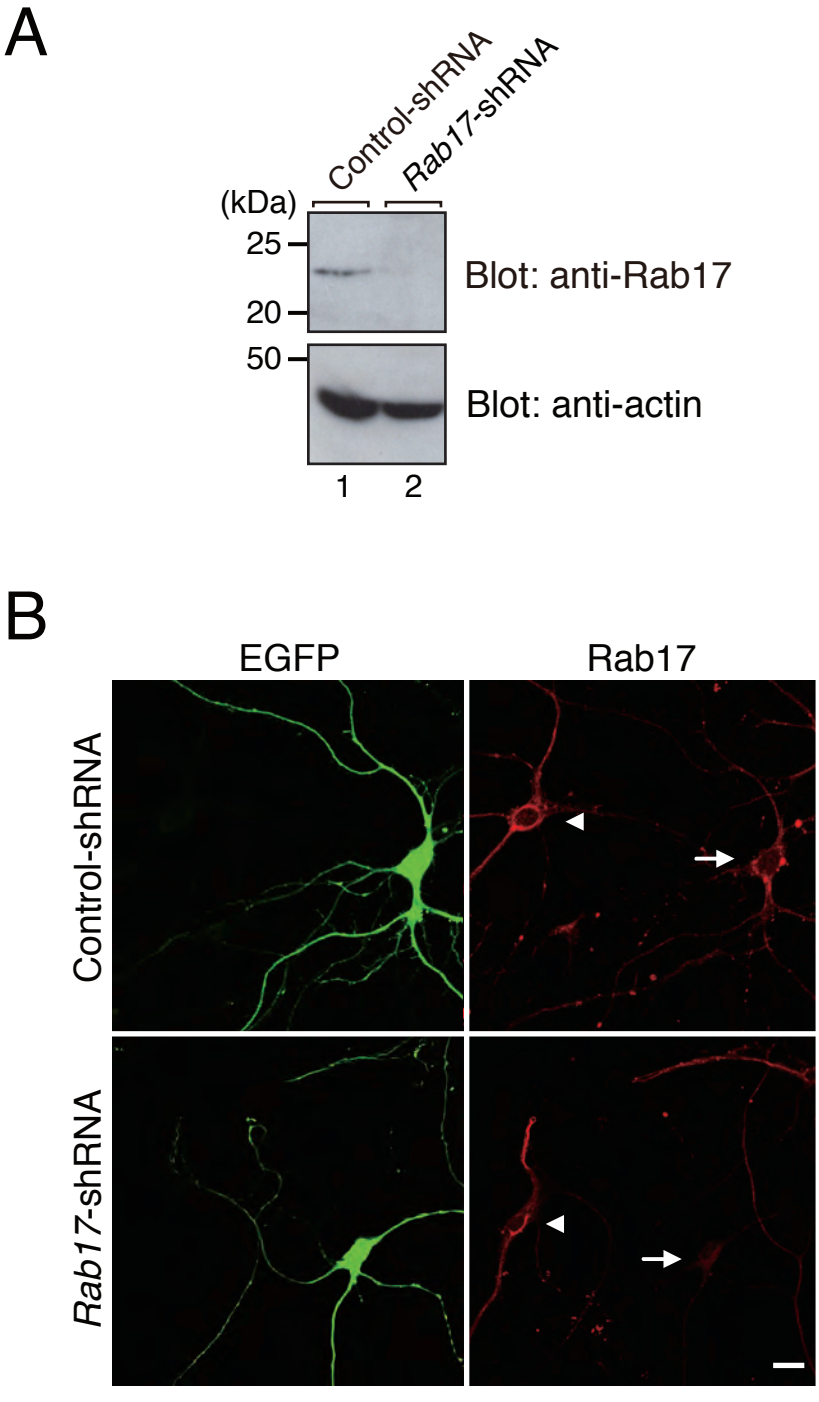

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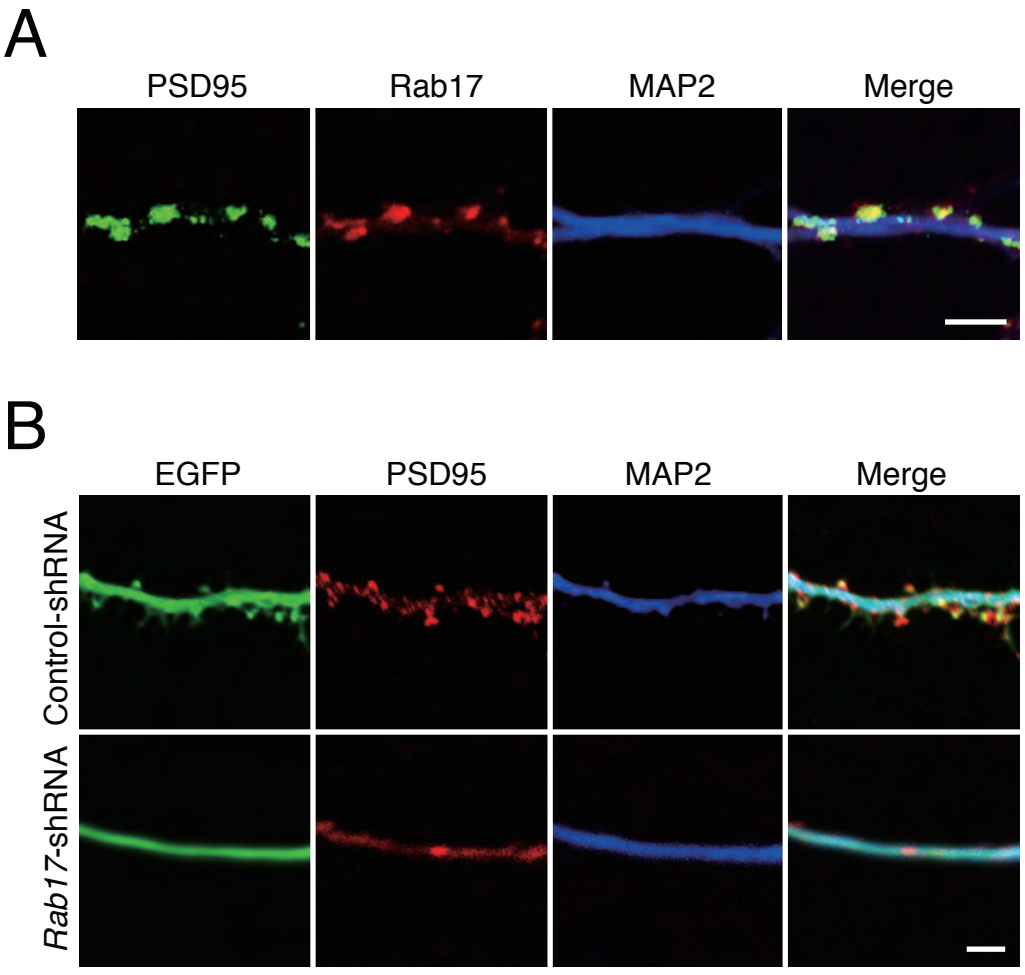

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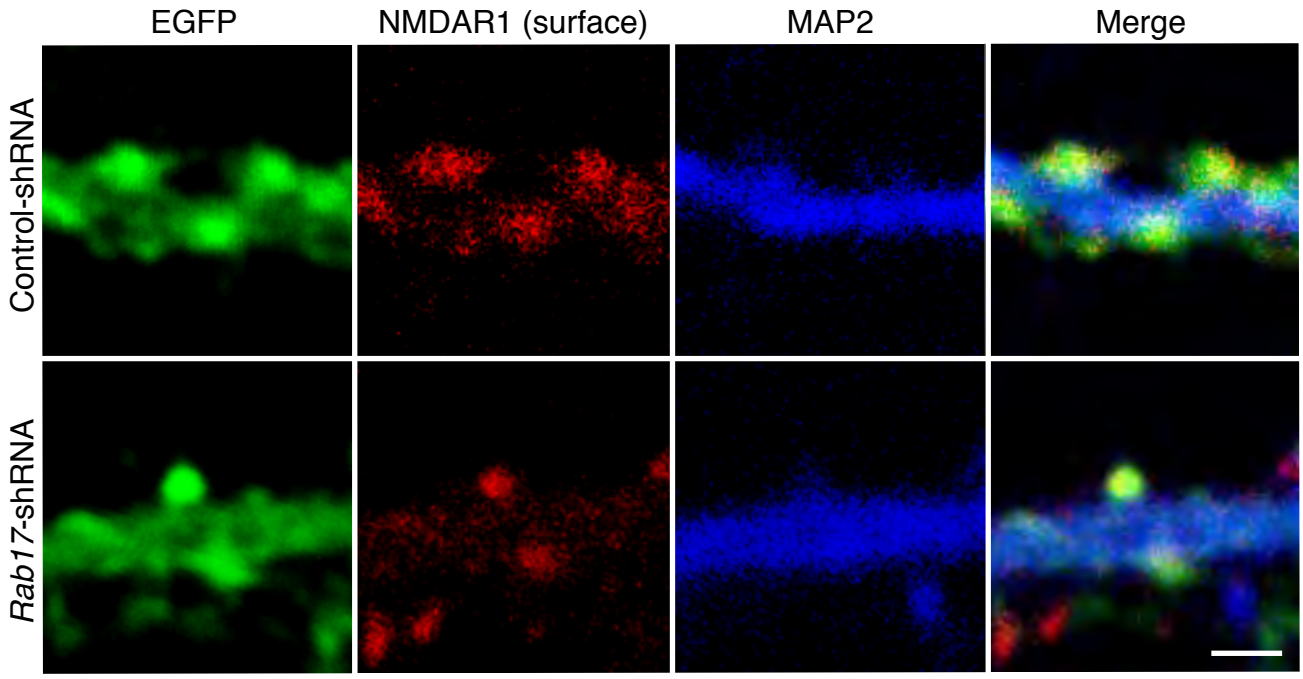

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