Figure S1

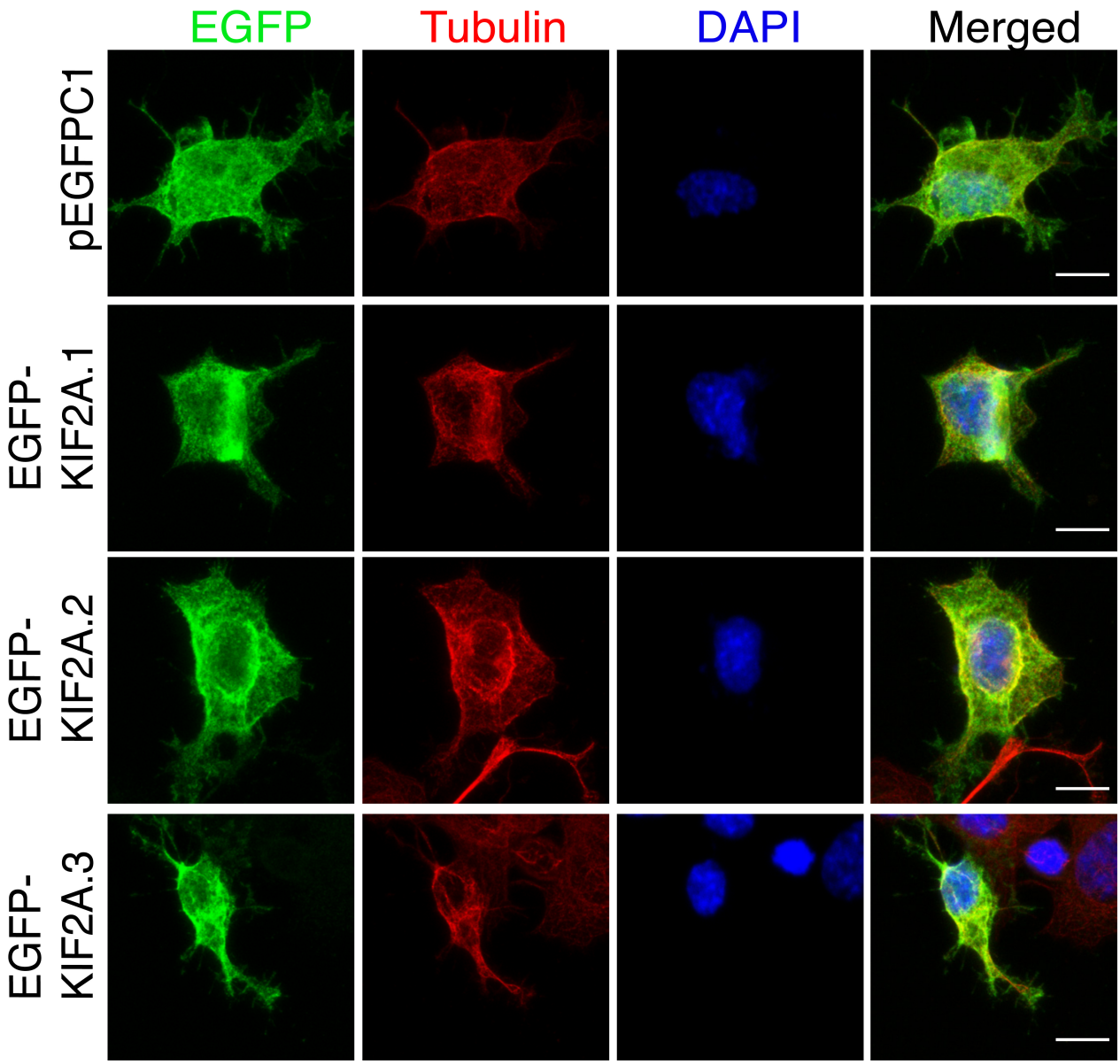

Figure S1. All KIF2A isoforms have diffused localization to cytoplasm and nucleus in Neuro2A cells. Neuro2A cells transfected with plasmids expressing pEGFPC1-tagged KIF2A isoforms (KIF2A.1, KIF2A.2 and KIF2A.3) 2 days after plating, fixed with 4% PFA 2 days after transfection and immunostained with anti-GFP (green). Anti-αTubulin visualized microtubules (red) and DAPI stained DNA. Scale bar, 10 μm.

Figure S2

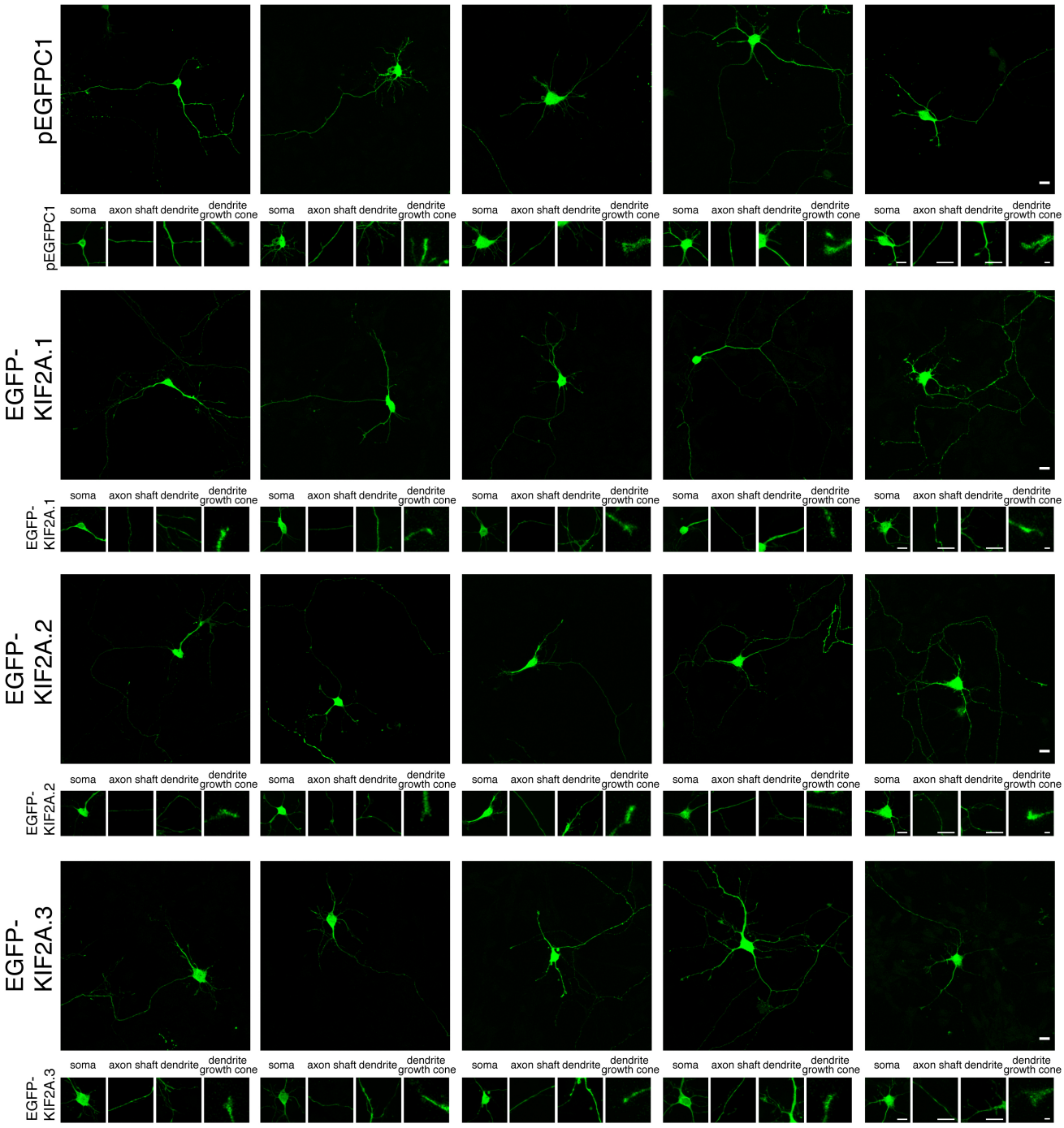

Figure S2. All KIF2A isoforms are localized to soma, axon and dendrites in primary cortical neurons. E14.5 primary cortical neurons were transfected with pEGFPC1 backbone or one of the EGFP expressing KIF2A isoforms at 2 DIV, fixed and immunostained against EGFP at 4 DIV. Digital zoom was applied to visualize localization of KIF2A isoforms in soma, axon shaft, dendrite and dendrite growth cone of each transfected neuron. 5 representative images for each condition were shown in the figure. Scale bar, 10 μm; scale bar for dendrite growth cone, 1 μm.

Figure S3

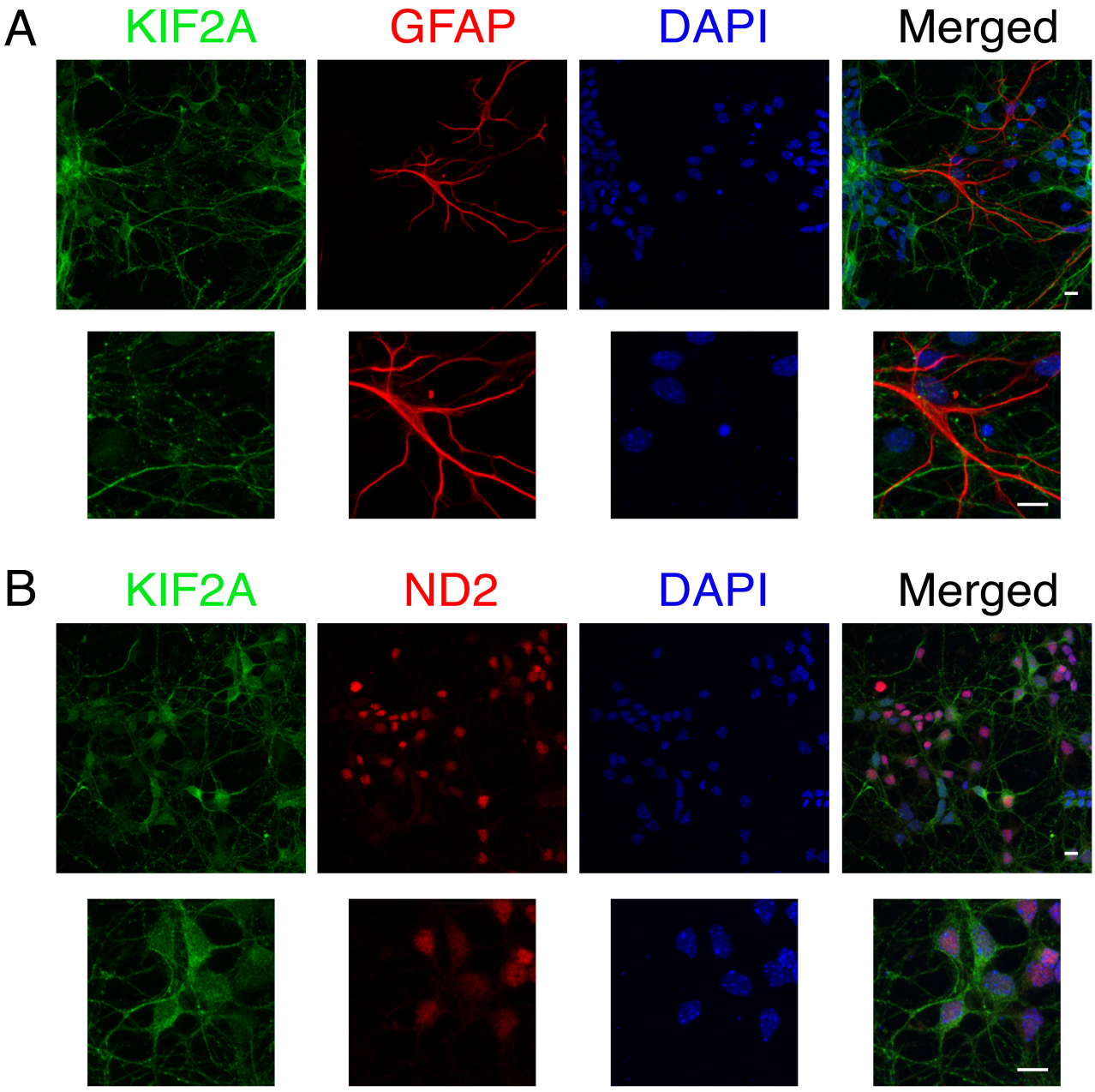

Figure S3. KIF2A is expressed predominantly by neurons but not by glia cells. A and B. E14.5 primary cortical culture was fixed at 4 DIV and stained with KIF2A (green) either anti-GFAP antibody (red) to stain glia cells (A) or anti-NEUROD2 antibody (red) to label neurons (B). Digital zoom was applied to visualize localization of KIF2A. Scale bar, 10 μm.

Figure S4

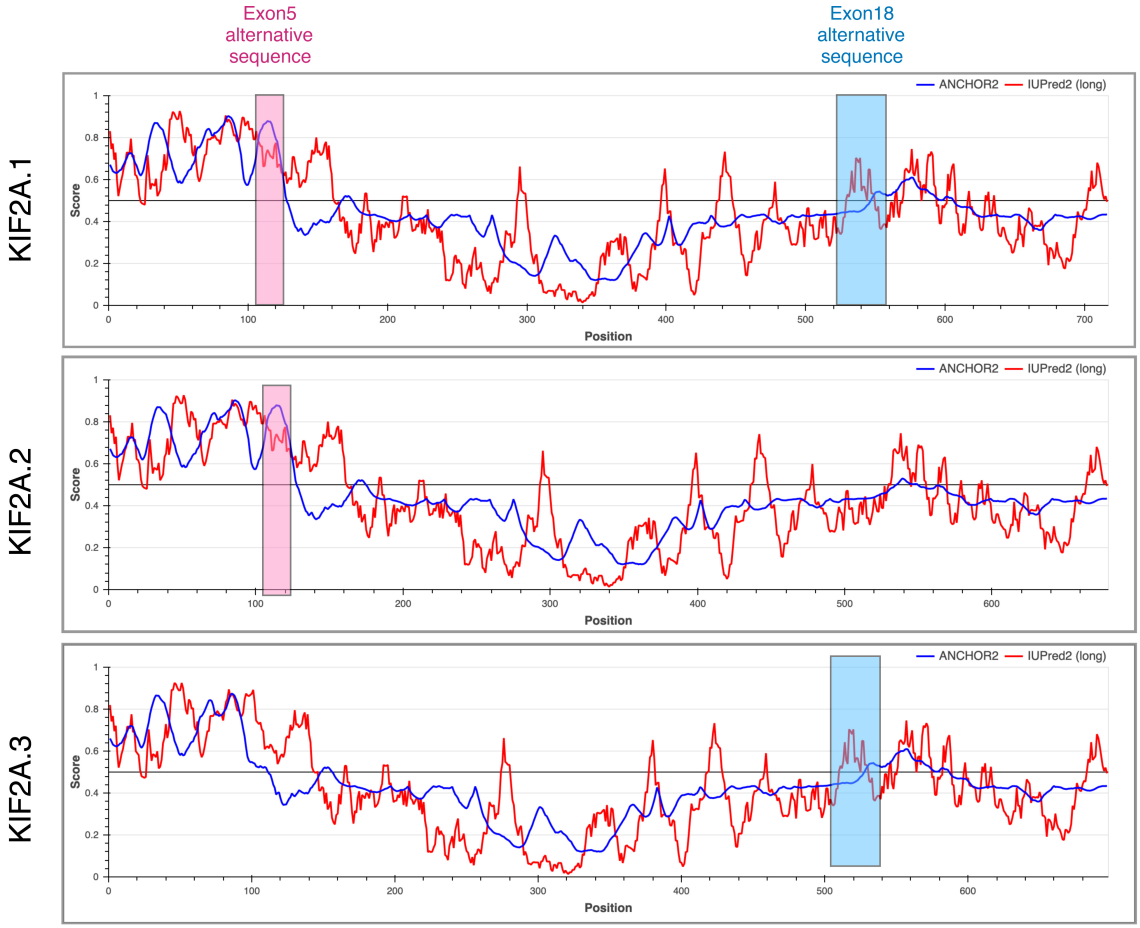

Figure S4. Measure of disorder in protein regions of individual KIF2A isoforms. A measure of protein disorder is plotted as a function of amino acid sequence of individual KIF2A isoforms. The IUPred2 and ANCHOR2 algorithms are used (Dosztányi, 2018). Alternative sequences are labeled in pink (exon5) and blue (exon18).

Figure S5

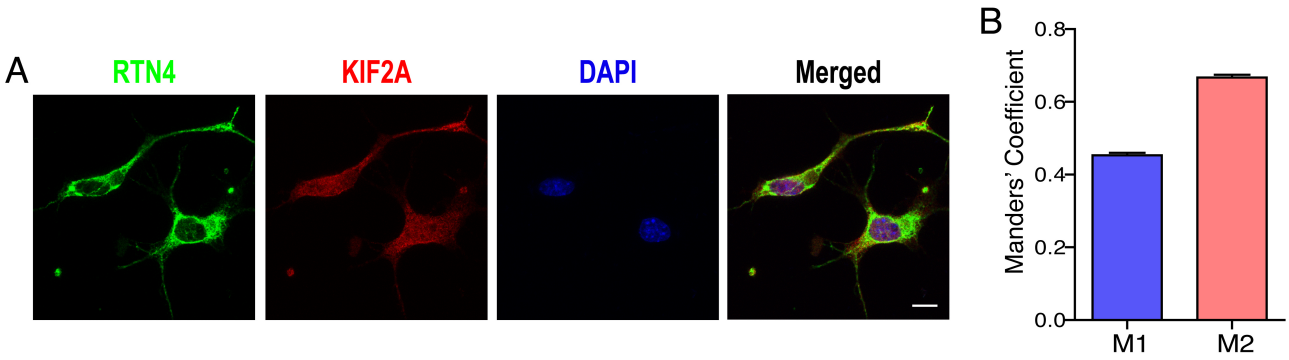

Figure S5. KIF2A and RTN4 are partially co-localized in Neuro2A cells. A. Immunofluorescence staining of RTN4 (green), KIF2A (red), DNA (blue) in Neuro2A cells. Scale bar, 10µm. **B.** Quantification of co-localization of KIF2A with RTN4A using Manders' coefficient (Dunn et al., 2011; Manders et al., 1993; Zinchuk et al., 2008). M1: Fraction of KIF2A fluorescence overlapped with RTN4 fluorescence. M2: Fraction of RNT4 fluorescence overlapped with KIF2A fluorescence. n=219, data is represented as bar graphs. Lines represent S.E.M.

Figure S6

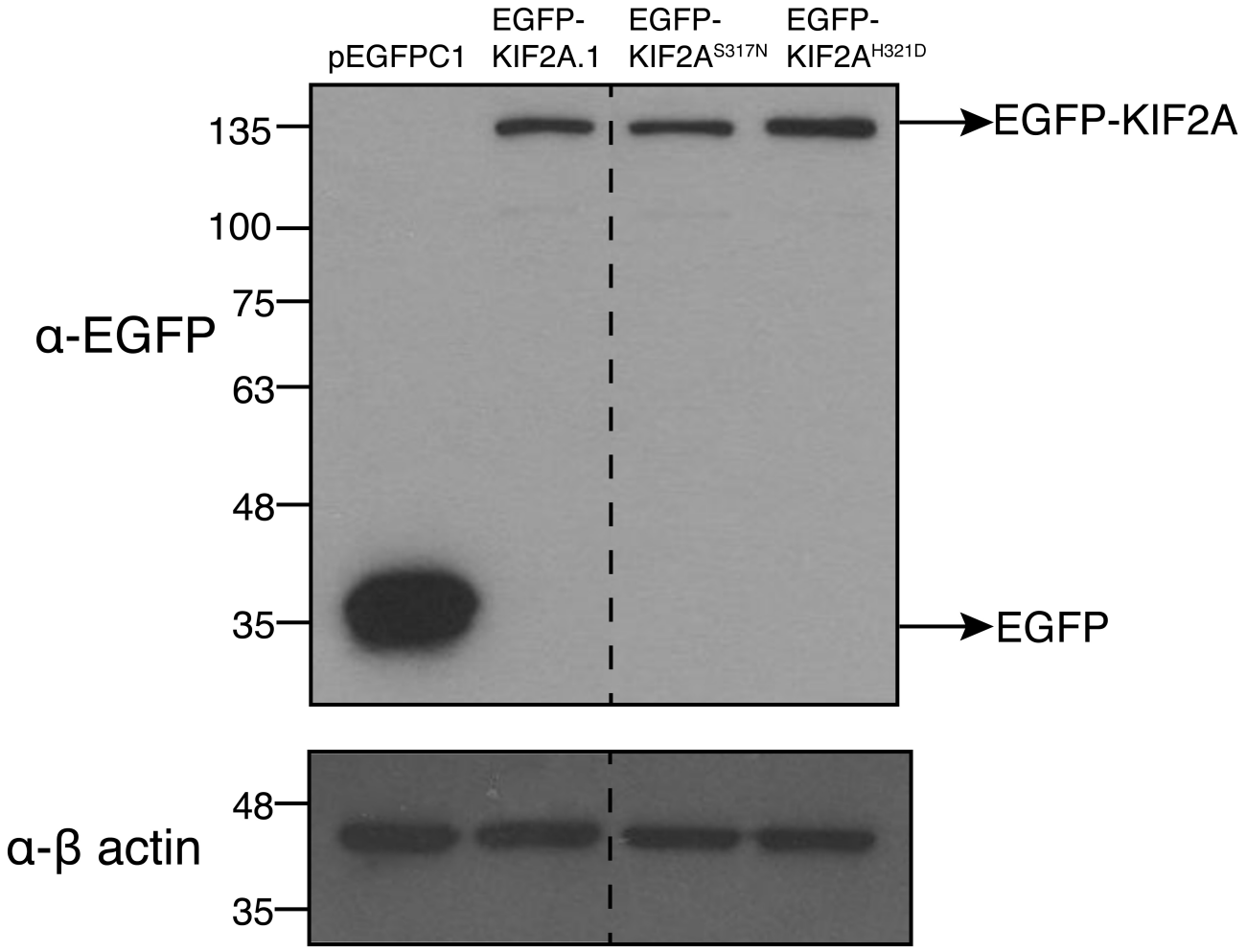

Figure S6. KIF2A mutants (*Kif2a*^{S317N} or *Kif2a*^{H321D}) expressions are similar with wild-type KIF2A and mutants do not affect protein instability. Immunoblotting with anti-EGFP demonstrating the levels of EGFP-tagged KIF2A isoforms and KIF2A mutants. B-ACTIN is used as a loading control.

Figure S7

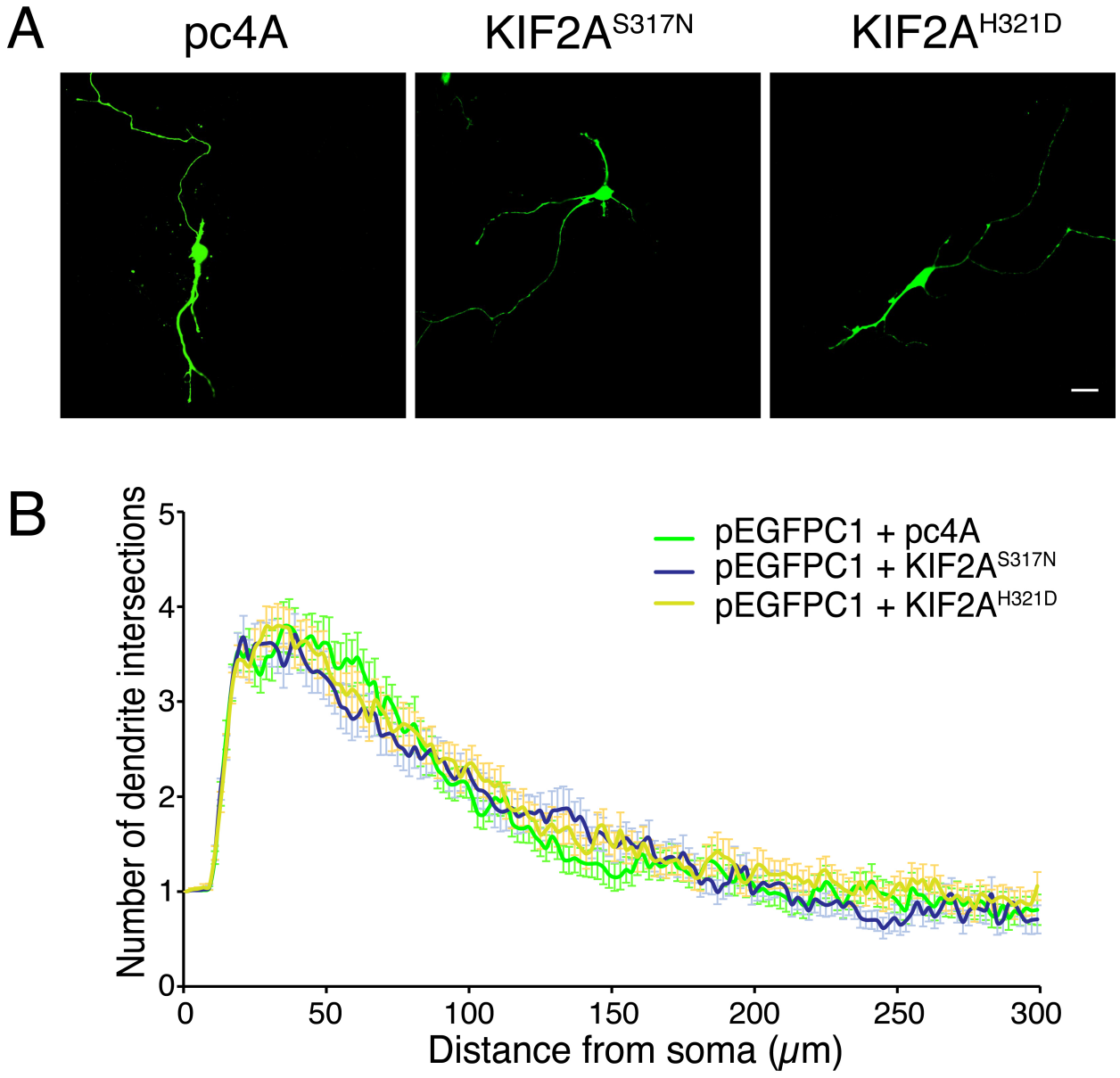

Figure S7. Expression *Kif2a*^{S317N} or *Kif2a*^{H321D} in wild-type neurons do not affect dendrite arborization. A. Representative images of E14.5 primary cortical neurons co-transfected with pEGFPC1 along with *Kif2a* mutants. Scale bar, 10 μm. **B.** Dendrite development was quantified by Sholl analysis (Ferreira et al., 2014; Schindelin et al., 2012). n=70 for each condition derived from three separate neuronal cultures. Bars represent S.E.M. Unpaired two-tailed *t* test determined the p value. $p<0.05$.

Figure S8

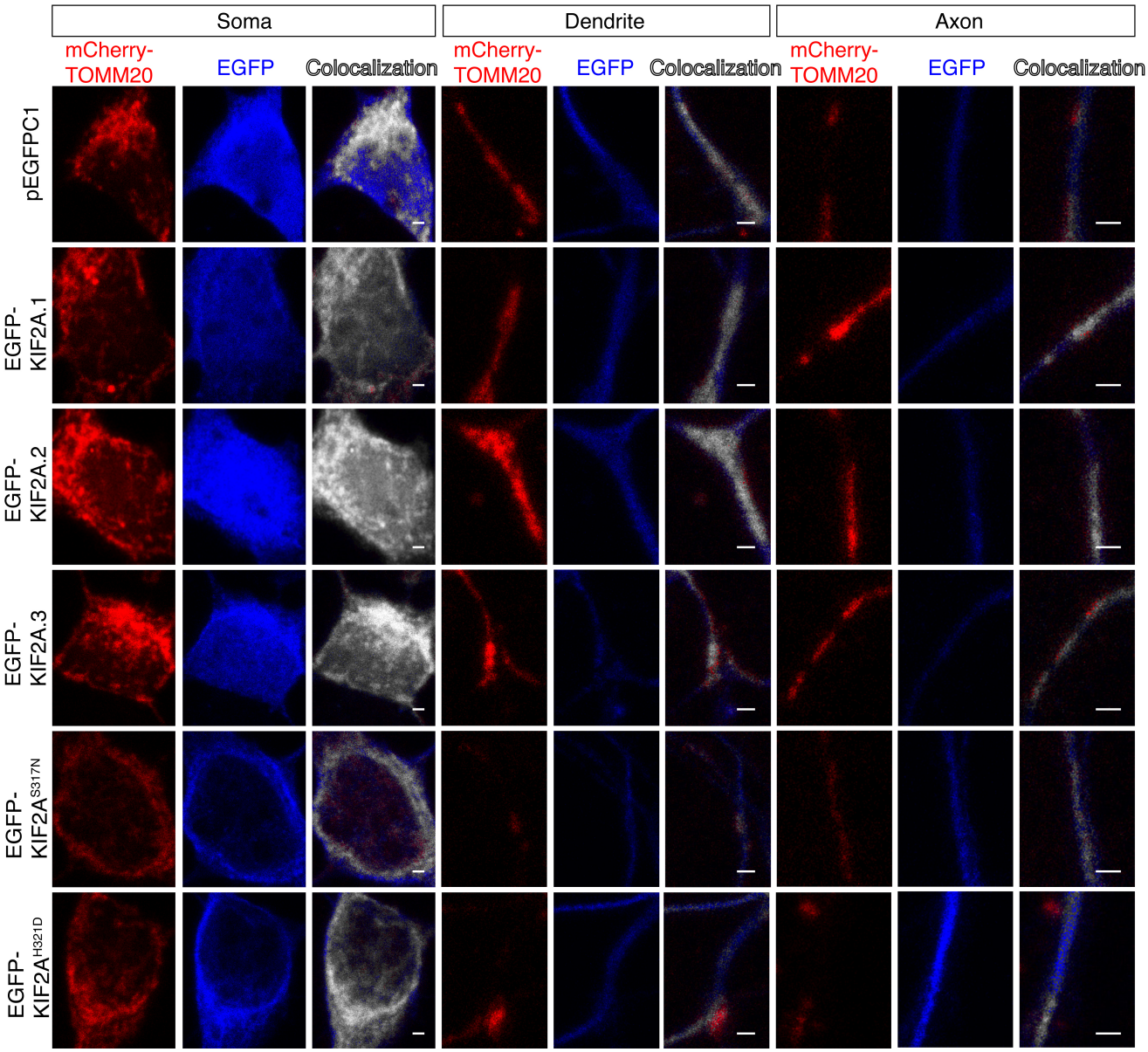

Figure S8. Co-localization of different KIF2A isoforms and *Kif2a* patient mutants with mitochondria in soma, axon and dendrites of primary cortical neurons. Magnified images of primary cortical neurons co-transfected with pEGFPC1-tagged three *Kif2a* isoforms and *Kif2a* disease mutants (*Kif2a*^{S317N} or *Kif2a*^{H321D}) along with mCherry-TOMM20-N-10. Co-localization (white) of KIF2A (EGFP, blue) with mitochondria (mCherry, red) in soma, axon and dendrite was shown in separate images by co-localization threshold tool in ImageJ. Scale bar, 1 μm.

Figure S9

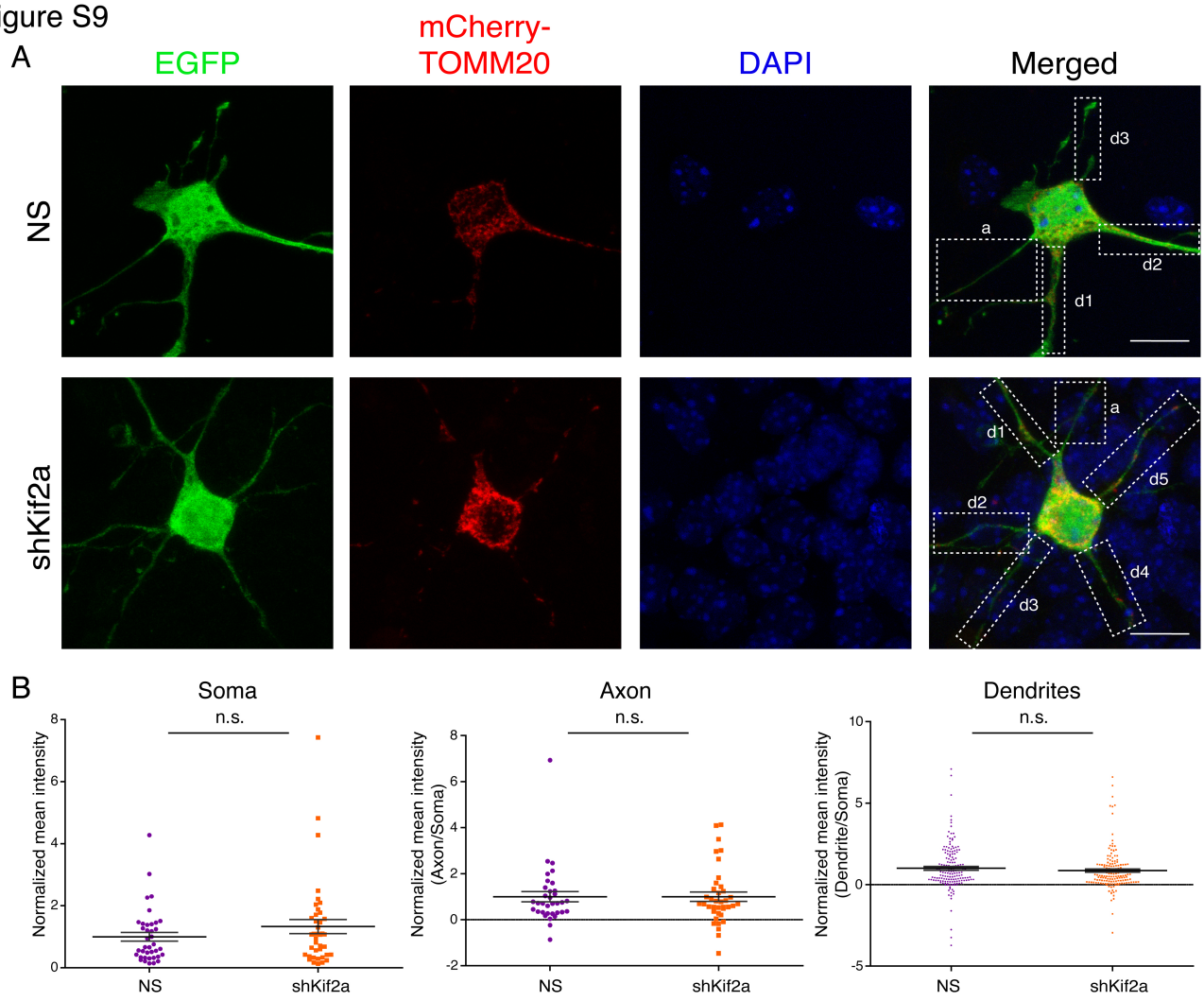

Figure S9: Silencing of endogenous *Kif2a* does not change mitochondrial localization. A. NS or shKif2a plasmids were co-transfected with mCherry-TOMM20 plasmid in E14.5 primary cortical neurons. Transfected neurons were fixed at 4 DIV and immunostained against EGFP and mitochondria were quantified based on the mCherry signal. Axon (a) and dendrites (d1-n) used for quantification were indicated with white dashed rectangles in merged channel. Scale bar, 10 µm. **B.** Quantification of images displayed in (A). Mean gray value of mCherry-TOMM20 was quantified in soma, axon and dendrites. Data of shKif2a was normalized to NS in soma. shKif2a data in axon and dendrites were normalized to soma first and then NS. n=38 for NS-mCherry-TOMM20 and n=40 for shKif2a-mCherry-TOMM20 derived from two separate neuronal cultures. Data is represented as scattered plot, lines indicate the mean number and bars represent S.E.M. Unpaired two-tailed *t* test. n.s. non-significant.

Table S1. Peptide Spectra Match Counts for BioID-mass spectrometry hits for individual KIF2A isoforms

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