Supplementary Figure Legends

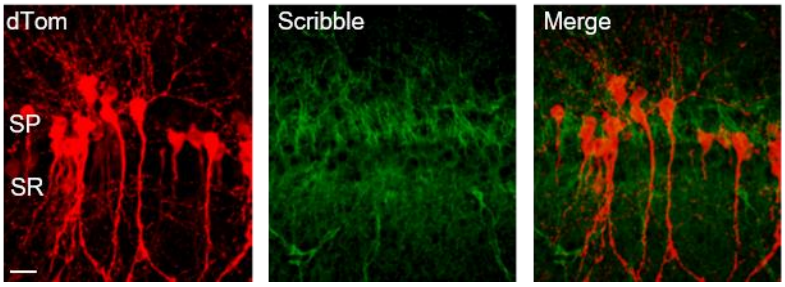

Figure S1. Scribble expression in CA1 pyramidal neurons
Images of Scribble immunofluorescence (green) in representative CA1 pyramidal neurons, at P7, following *in utero* labeling with dTomato marker. SP, stratum pyramidale; SR, stratum radiatum. Scale bar represents 20 µm.
Related to Figure 1 - Separate channels for the images shown in Figure 1C.

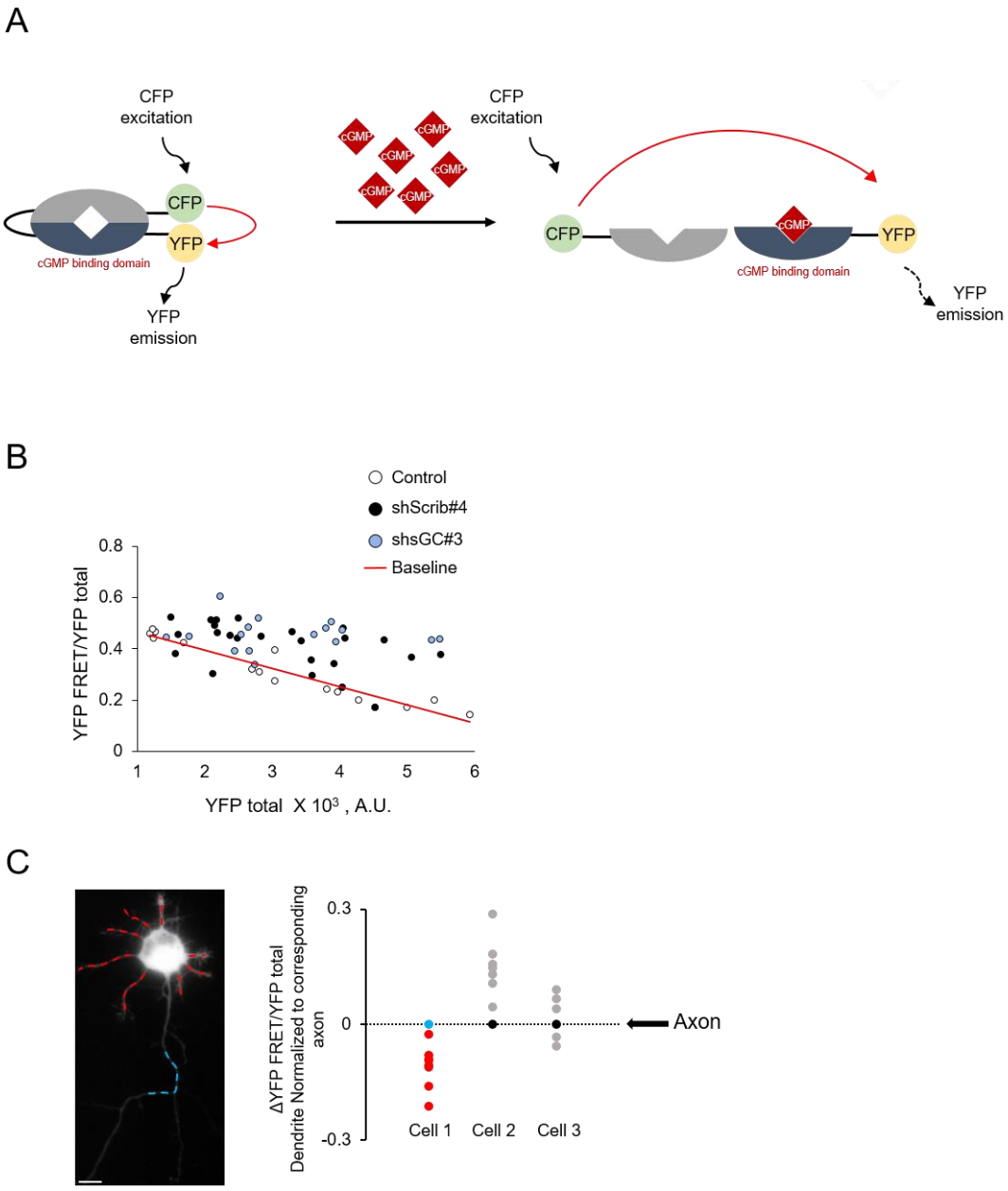

Figure S2. cGMP - FRET measurements in cultured hippocampal neurons

(A) Schematic depiction of the FRET cGMP reporter cGi-500 (Russwurm et al., 2007). The FRET signal was determined by the YPF fluorescence, measured upon CFP excitation (YFP-FRET). Upon cGMP increase and cGMP binding, the cGi-500 sensor relaxes its conformation, resulting in YFP-FRET reduction, whereas cGMP decrease is reflected in YFP-FRET increase.

(B) Measurement of baseline cGMP-levels in cultured hippocampal neurons using FRET cGMP sensor, and effects on cGMP upon Scribble or sGC-β1 knock-down. Neurons co-transfected with FRET cGMP reporter cGi-500 together with control-, Scribble-, or sGC-β1-shRNAs, and somatic FRET measurements performed at 36 - 48 hr after. FRET signal was determined by YPF fluorescence measured upon CFP excitation (YFP-FRET). Because the FRET signal (YFP-FRET) in each cell would depend on the level of probe expression in that cell, FRET values (YFP-FRET), were normalized to total YFP levels determined upon YFP excitation (YFP-total, reflecting the level of probe expression). Baseline cGMP for control-transfected cells was determined by FRET signal in each cell normalized to probe expression level in that cell (YFP-FRET / YFP-total), presented for all cells with varying probe concentration (YFP-total; arbitrary fluorescence units, A.U.) (open circles). A range in which somatic FRET (YFP-FRET / YFP-total) demonstrated linear correlation with levels of probe expression (YFP-total), represents baseline

cGMP-levels in control cells (open circles, linear fit). Normalized FRET signals (YFP-FRET / YFP-total) were determined for ShsGC#3 (blue symbols) or ShScrib#4 (black symbols) transfected cells, which expressed cGi-500 at a level for which control cells showed a linear response. Somatic FRET signals (YFP-FRET / YFP-total) for sGC-β1- or Scribble-shRNA cells were scattered above those for control-shRNA, indicating cGMP decrease compared to control.

(C) Schematic depiction of FRET cGMP measurements in dendrites (as performed in Figure 2I-2J and 7E-7F). Normalized FRET signals (YFP-FRET / YFP-total) were determined for all dendrites in each cell (measurements averaged over the entire dendritic length) and the axon (measurement averaged over a ~50μm axonal segment, at least 30μm away from the soma). Data was presented for each cell (cell #1, #2, and so on), as the difference in normalized FRET signals (ΔYFP-FRET / YFP-total) between dendrites of an individual neuron relative to the axon of that neuron.

Related to Figure 2.

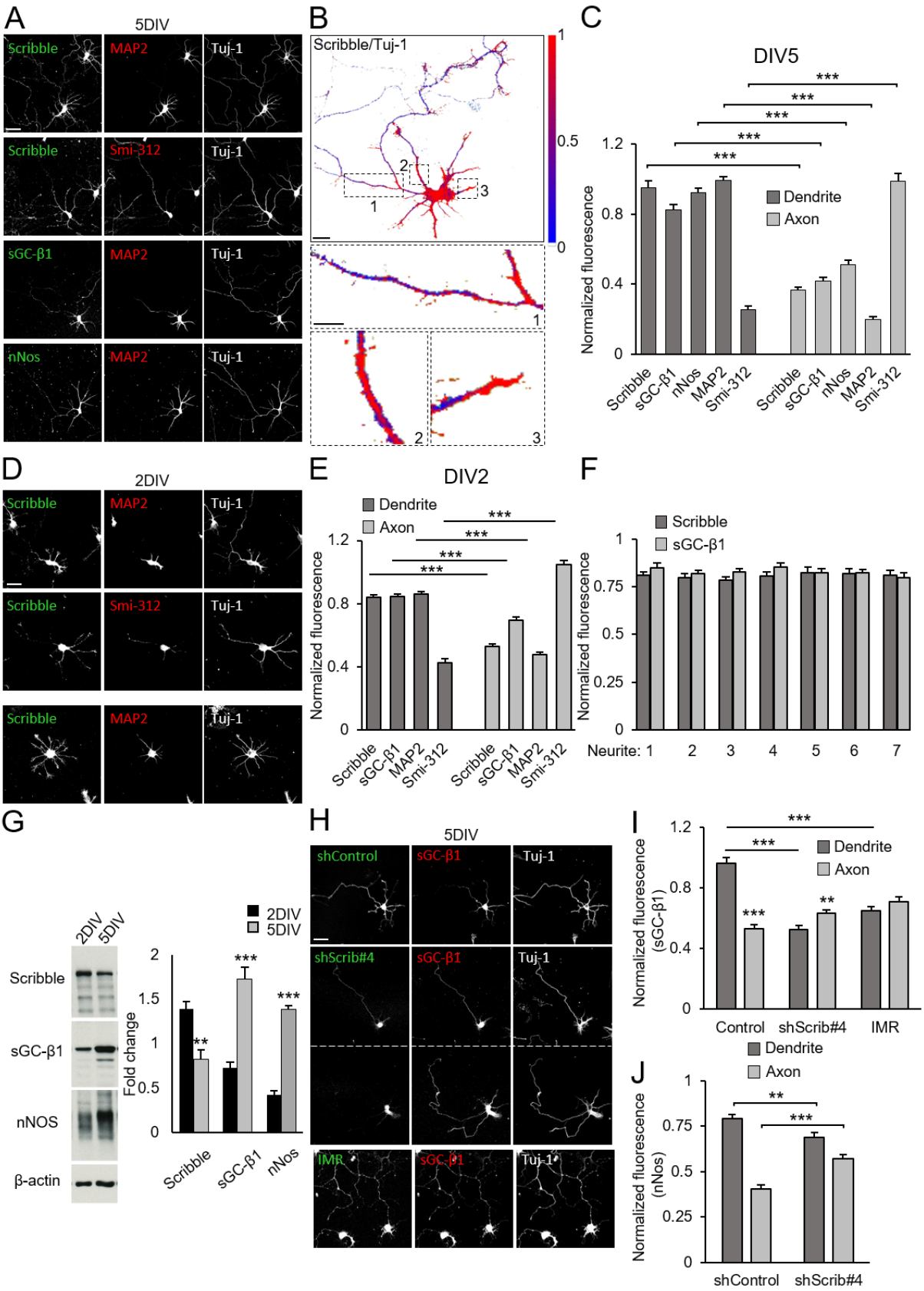
A
5DIV
Scribble
MAP2
Tuj-1
Scribble
Smi-312
Tuj-1
sGC-β1
MAP2
Tuj-1
nNos
MAP2
Tuj-1
B
Scribble/Tuj-1
1
0.5
0
C
DIV5
Normalized fluorescence
Dendrite
Axon
Scribble
sGC-β1
nNos
MAP2
Smi-312
D
2DIV
E
DIV2
F
Scribble
sGC-β1
Neurite: 1 2 3 4 5 6 7
G
2DIV
5DIV
Scribble
sGC-β1
nNOS
β-actin
Fold change
H
5DIV
shControl
shScrib#4
IMR
I
Normalized fluorescence (sGC-β1)
Control
shScrib#4
IMR
J
Normalized fluorescence (nNos)
shControl
shScrib#4

Figure S3. Enrichment of sGC and Scribble expression in dendrites

(A, D, H) Images of representative hippocampal neurons at 5DIV (A, H) or 2DIV (D), immunostained for Scribble, sGC-β1, or nNOS, together with dendrite or axon markers, MAP2 or smi-312, and neuron-specific marker Tuj-1. (H) Neurons were immunostained following transfection with ShScrib#4 or control shRNA, or IMR domain. (D) Shown 2DIV polarized or non-polarized neurons. Scale bar represents 40 μm. Distinctly higher levels of Scribble and sGC were found in soma and dendrites of polarized neurons (A, D). sGC enrichment in dendrites was impaired following Scribble knock-down or IMR expression (H).

(B) Image of normalized Scribble fluorescence (Scribble / Tuj-1) in dendrites vs the axon, in a representative polarized hippocampal neuron, immunostained at 5DIV for Scribble and Tuj-1 (together with MAP2; same cell as in top panel in A). Scribble fluorescence normalized to that of Tuj-1, coded in pseudo-colors. Distinctly higher normalized Scribble fluorescence were found in dendrites. Scale bar represents 50 μm. Bottom, higher magnification images (of boxed regions in the top panel) of normalized Scribble fluorescence in proximal axon (1) or representative dendrites (2 and 3). Scale bar represents 20 μm.

(C, E) Quantification of immunofluorescence for Scribble, sGC-β1 or nNOS, or smi-312 or MAP2, in dendrites vs the axon, in all polarized neurons at 5DIV (C) or 2DIV (E), immunostained for Scribble, sGC-β1 or nNOS, and Tuj-1, together with MAP2 or smi-312. Average fluorescence intensity for Scribble, sGC-β1, nNOS, smi-312 or MAP2, in each dendrite or the axon, for all neurons (n = 50-75 cells, 3 cultures), was normalized to that of Tuj-1. Distinct higher levels of Scribble, sGC-β1 and nNOS were found in dendrites (with high MAP2) than in axons (with high smi-312) at both 2DIV and 5DIV polarized neurons (5DIV; Dendrite: Scribble, 0.95 ± 0.03; sGC-β1, 0.82 ± 0.02; nNOS, 0.92 ± 0.02; MAP2, 0.99 ± 0.02; Smi-312: 0.25 ± 0.02; Axon: Scribble, 0.36 ± 0.01; sGC-β1, 0.41 ± 0.02; nNOS, 0.51 ± 0.02; MAP2, 0.19 ± 0.01; Smi-312, 0.98 ± 0.04; 2DIV; Dendrite: Scribble, 0.84 ± 0.01; sGC-β1, 0.84 ± 0.02; MAP2, 0.86 ± 0.01; Smi-312: 0.42 ± 0.02; Axon: Scribble, 0.52 ± 0.01; sGC-β1, 0.69 ± 0.01; MAP2, 0.47 ± 0.01; Smi-312, 0.94 ± 0.01; Student's t-test, ***p≤0.001).

(F) Quantification of Scribble or sGC-β1 immunofluorescence in undifferentiated neurites of un-polarized neurons at 2DIV, immunostained for Scribble or sGC-β1, together with Tuj-1 and MAP2. Average fluorescence intensity for Scribble or sGC-β1 for each neurite, normalized to that of Tuj-1, was plotted in the order of neurite length for the first seven neurites in each cell, averaged over all cells. No significant differences of Scribble or sGC-β1 fluorescence were found among the neurites (n = 50-60 cells, 3 cultures; One-way ANOVA, Tukey's Multiple Comparison Test).

(G) Immunoblots of total lysates of 2DIV or 5DIV cultured hippocampal neurons with Abs to Scribble, nNOS, or sGC-β1. Expression at 2DIV vs 5DIV was quantified following normalization to β-actin (±SEM, n=4, Student's t-test, **p≤0.01; ***p≤0.001). Scribble expression was higher at 2DIV than at 5DIV.

(I, J) Quantification of sGC-β1 or nNOS immunofluorescence in dendrites vs the axon, following Scribble knock-down or IMR over-expression. Average sGC-β1 (I) or nNOS (J) fluorescence intensity in each dendrite vs the axon, normalized to that of Tuj-1, for all polarized neurons at 5DIV, expressing ShScrib#4 or control-shRNA, or IMR (n = 75 cells, 3 cultures). Distinct sGC or nNOS expression in dendrites (see C, and control) was abolished upon Scribble knock-down or IMR expression (sGC-β1, Dendrite: Control, 0.95 ± 0.04; shScrib#4, 0.52 ± 0.02; IMR, 0.65 ± 0.02; Axon: Control, 0.53 ± 0.03; shScrib#4, 0.63 ± 0.02; IMR, 0.7 ± 0.03; nNOS, Dendrite: shControl, 0.79 ± 0.02; shScrib#4, 0.68 ± 0.02; Axon: shControl, 0.4 ± 0.02; shScrib#4, 0.57 ± 0.02; Student's t-test and One-way ANOVA, Tukey's Multiple Comparison Test, **p≤0.01; ***p≤0.001).

Related to Figure 1C and 1D, Figure 2, and Figure 5.

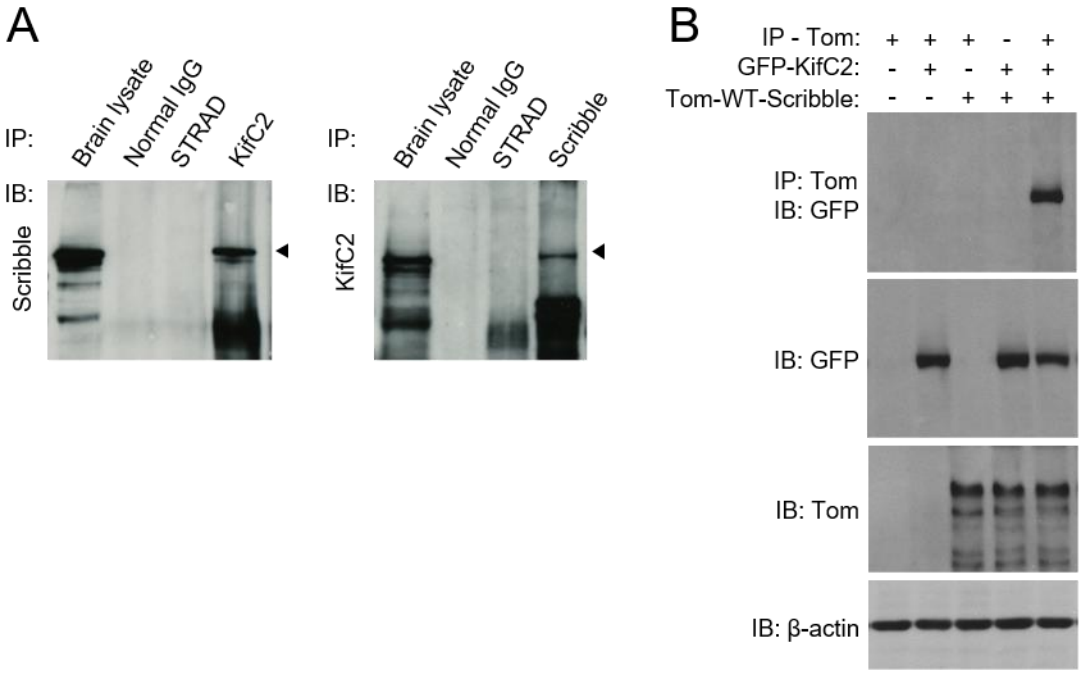

Figure S4. Kinesin KifC2 associates with Scribble

(A) Co-IP of Scribble with KifC2 from the embryonic brain. IP from E18 rat brain lysates with KifC2 or Scribble, and IB with KifC2 or Scribble, to test for reciprocal co-IP with both Abs. "Brain lysate", total brain lysate. Co-IP with normal IgG or with the control protein STRAD, served control (n = 3).

(B) Co-IP of KifC2 with Scribble from HEK-293 cell lysates co-expressing EGFP-KifC2 with dTom-Scribble. IP, with Tom. IB, with GFP. Total cell lysates were subjected to IB with Tom or GFP, to check expression. Co-IP with normal IgG served control. β-actin served control for loading. Scribble co-precipitated KifC2 (n = 3).

Related to Figure 7.

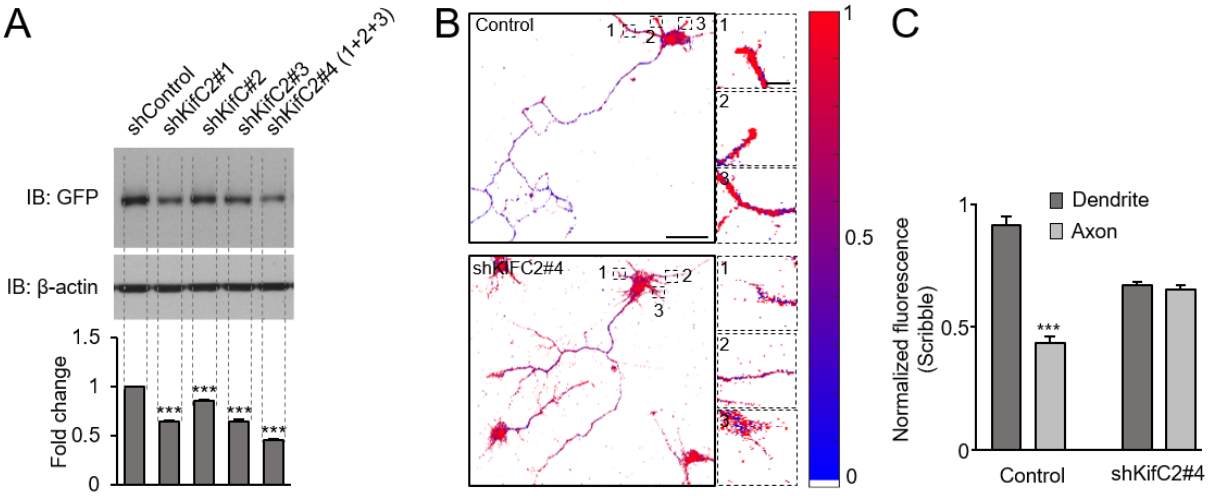

Figure S5. Scribble - KifC2 association is necessary for the dendritic localization of Scribble

(A) Knock-down of KifC2 in HEK-293 cells with shRNAs. Three shRNAs targeting rat KifC2 (ShKifC2#1, #2 and #3; or ShKifC2#4, a combined pool of ShKifC2#1 + #2 + #3) (cloned into pRNAT-U6.3 vector that also drives either EGFP or dTom expression), co-transfected in HEK-293 cells together with targeted EGFP-KifC2. Whole-cell extracts immunoblotted for GFP. "Control", control shRNA. KifC2 levels were quantified as fold reduction (± SEM, n=5; One-way ANOVA; Dunnett's Multiple Comparison Test, ***p≤0.001) relative to control shRNA, normalized to β-actin. Combination of KifC2 shRNAs (ShKifC2#4), most efficiently knocked-down EGFP-KifC2 expression.

(B) Localization of Scribble in axon vs dendrites following KifC2 knock-down. Images of normalized Scribble fluorescence (Scribble / Tuj-1) in dendrites vs the axon, in representative polarized cultured neurons, transfected with ShKifC2#4 or control shRNA, immunostained at 5DIV for Scribble and Tuj-1. Scribble fluorescence normalized to Tuj-1, coded in pseudo-colors. Higher normalized Scribble signal found in dendrites of control-shRNA-transfected cell but not in ShKifC2#4-transfected cell. Scale bar represents 30 µm. Right, higher magnification images (of boxed regions on left) of normalized Scribble fluorescence in three representative dendrites (1, 2, 3). Scale bar represents 5 µm.

(C) Quantification of Scribble immunofluorescence in dendrites vs the axon following KifC2 knock-down. Average Scribble immunofluorescence intensity in each dendrite vs the axon normalized to Tuj-1 fluorescence, for all polarized neurons at 5DIV, transfected with ShKifC2#4 or control-shRNA (n = 75 cells, 3 cultures). Distinct Scribble localization in dendrites was abolished following KifC2 knock-down (Control: Dendrite, 0.91 ± 0.03; Axon, 0.43 ± 0.02; ShKifC2#4: Dendrite, 0.67 ± 0.01; Axon, 0.65 ± 0.01; Student's t-test, ***p≤0.001). Related to Figure 7. Also related to Figure 1C and 1D and Figure 2.

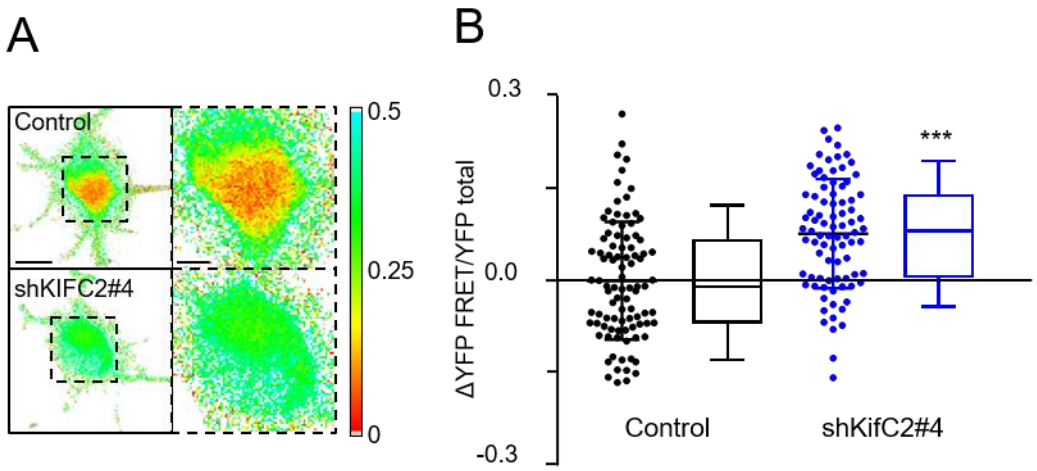

Figure S6. Somatic cGMP levels decrease upon KifC2 knock-down

(A) Representative images of normalized somatic cGMP FRET signals for control- or KifC2-shRNA (ShKifC2#4) transfected cells. Normalized somatic FRET (YFP-FRET / YFP-total) coded in pseudo-colors, for control or ShKifC2#4 cells co-expressing cGi-500. Scale bar represents 10 µm. Right, higher magnification images (of boxed regions on left). Scale bare represents 5 µm. Somatic FRET increase upon KifC2 knock-down reflects cGMP decrease.

(B) Summary of the difference in normalized somatic FRET (ΔYFP-FRET / YFP-total) compared to average of control for all control or ShKifC2#4 transfected cells. Cells expressing ShKifC2#4 exhibited higher FRET than average of control, reflecting somatic cGMP decrease (mean ± SEM; 3 cultures, 20-40 cells each; Control, 0.0 ± 0.010; ShKifC2#4, 0.077 ± 0.010; Student's t-test, ***p≤0.001).

Related to Figure 2 and Figure 7.