iScience, Volume *27*

Supplemental information

The WAVE regulatory complex interacts with Boc and is required for Shh-mediated axon guidance

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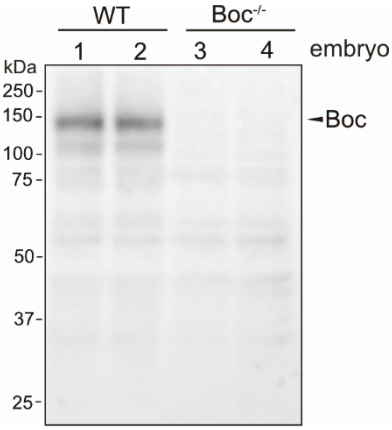

Figure S1. Validation of the Boc antibody, related to Figure 2. Western blot of lysates of brains from two wildtype (WT) and two *Boc*$^{-/-}$ E11.5 mouse embryos immunoblotted with an anti-Boc antibody. The Boc antibody specifically detects only Boc, as there are bands present only in the WT lysates and an absence of signal in the *Boc*$^{-/-}$ lysates.

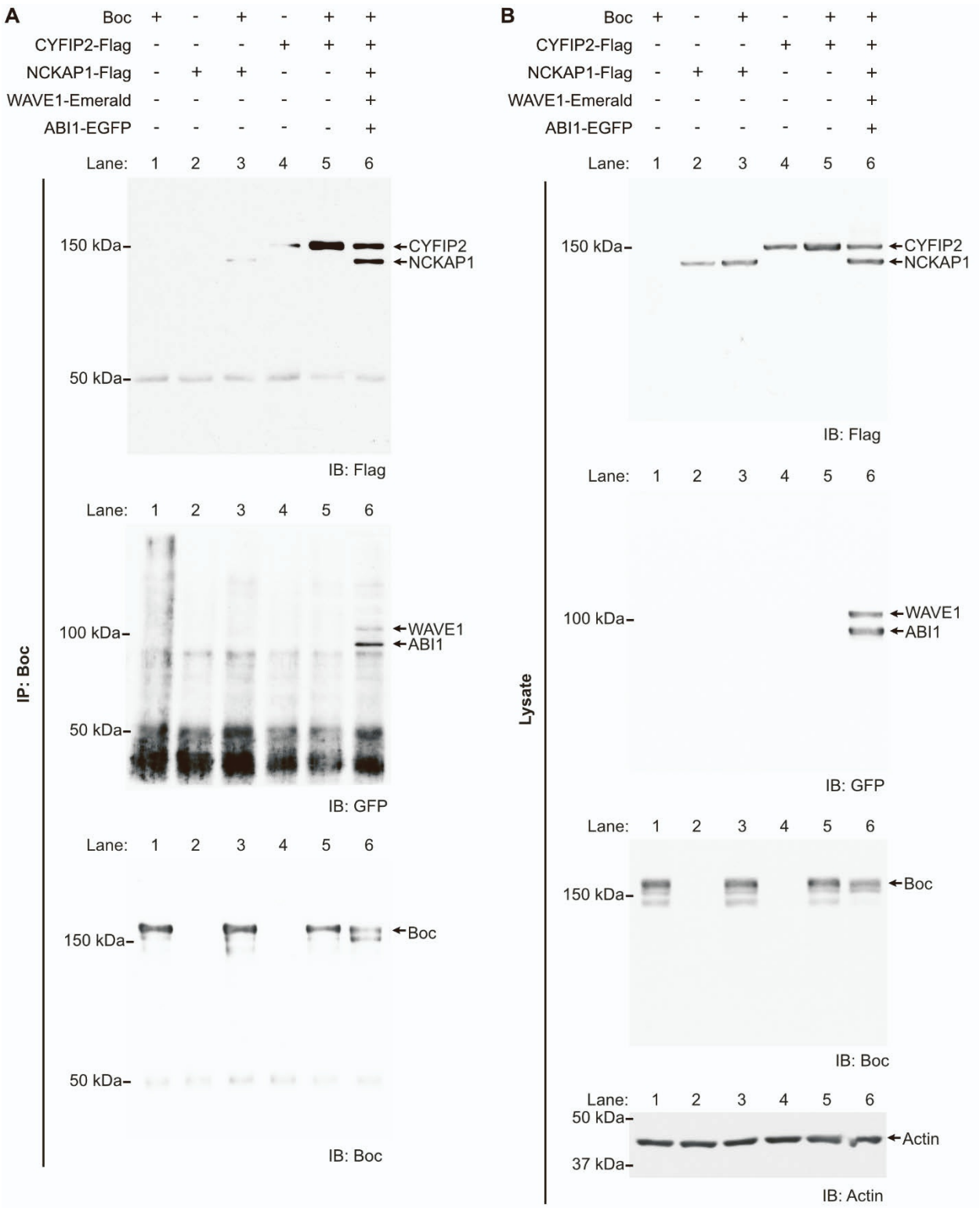

Figure S2. Entire gels from Figure 2A, related to Figure 2. Boc and tagged WRC constructs were expressed in HEK293T cells as indicated. The lysates were immunoprecipitated with an anti-Boc antibody and interacting proteins were analyzed through SDS-PAGE and Western blot. **(A)** Entire uncropped gels

corresponding to the Western blots of the immunoprecipitated proteins in Figure 2A. **(B)** Entire uncropped gels corresponding to the Western blots of the lysate in Figure 2A.

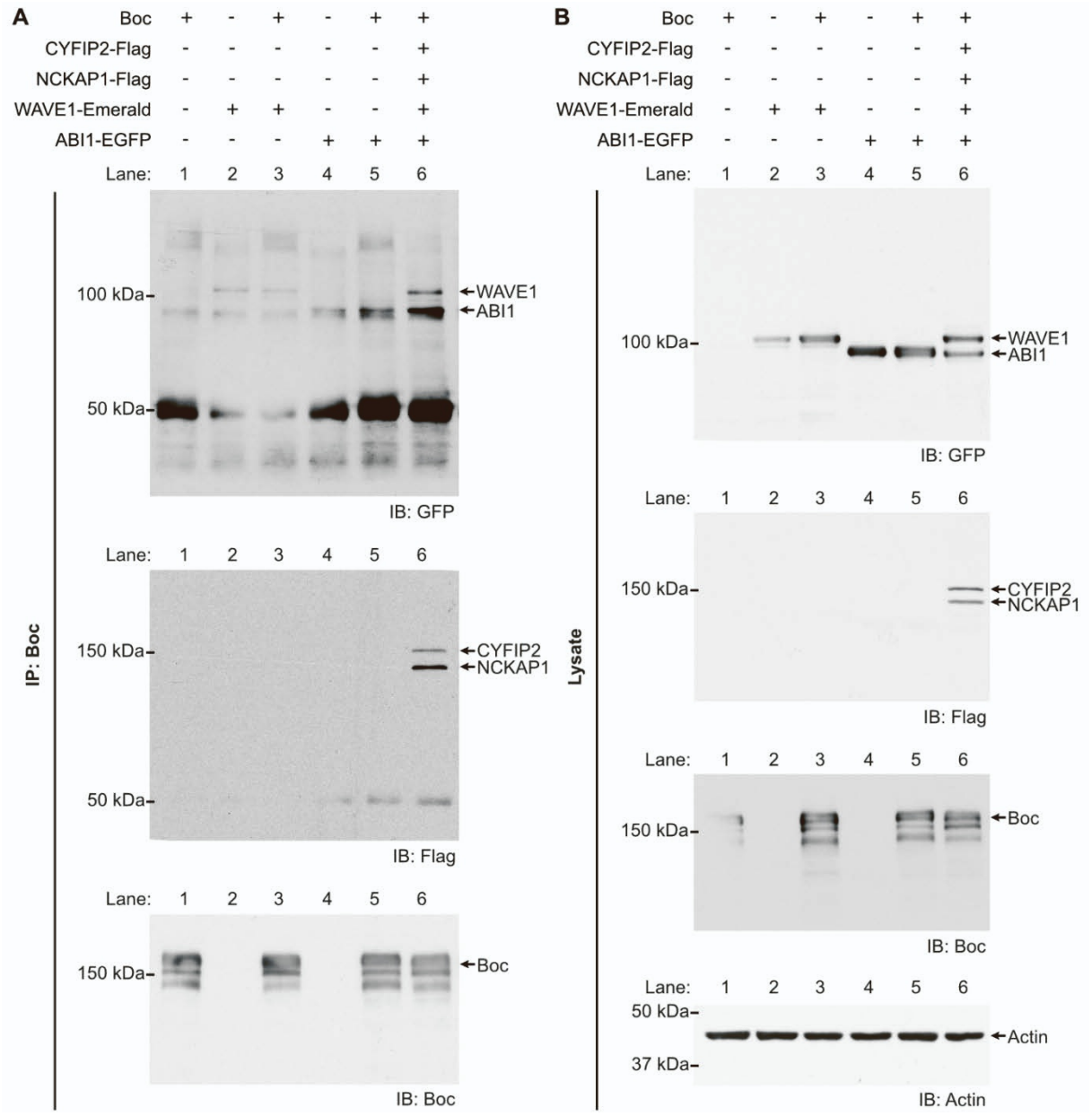

Figure S3. Entire gels from Figure 2D, related to Figure 2. Boc and tagged WRC constructs were expressed in HEK293T cells as indicated. The lysates were immunoprecipitated with an anti-Boc antibody and interacting proteins were analyzed through SDS-PAGE and Western blot. **(A)** Entire uncropped gels corresponding to the Western blots of the immunoprecipitated proteins in Figure 2D. **(B)** Entire uncropped gels corresponding to the Western blots of the lysate in Figure 2D.

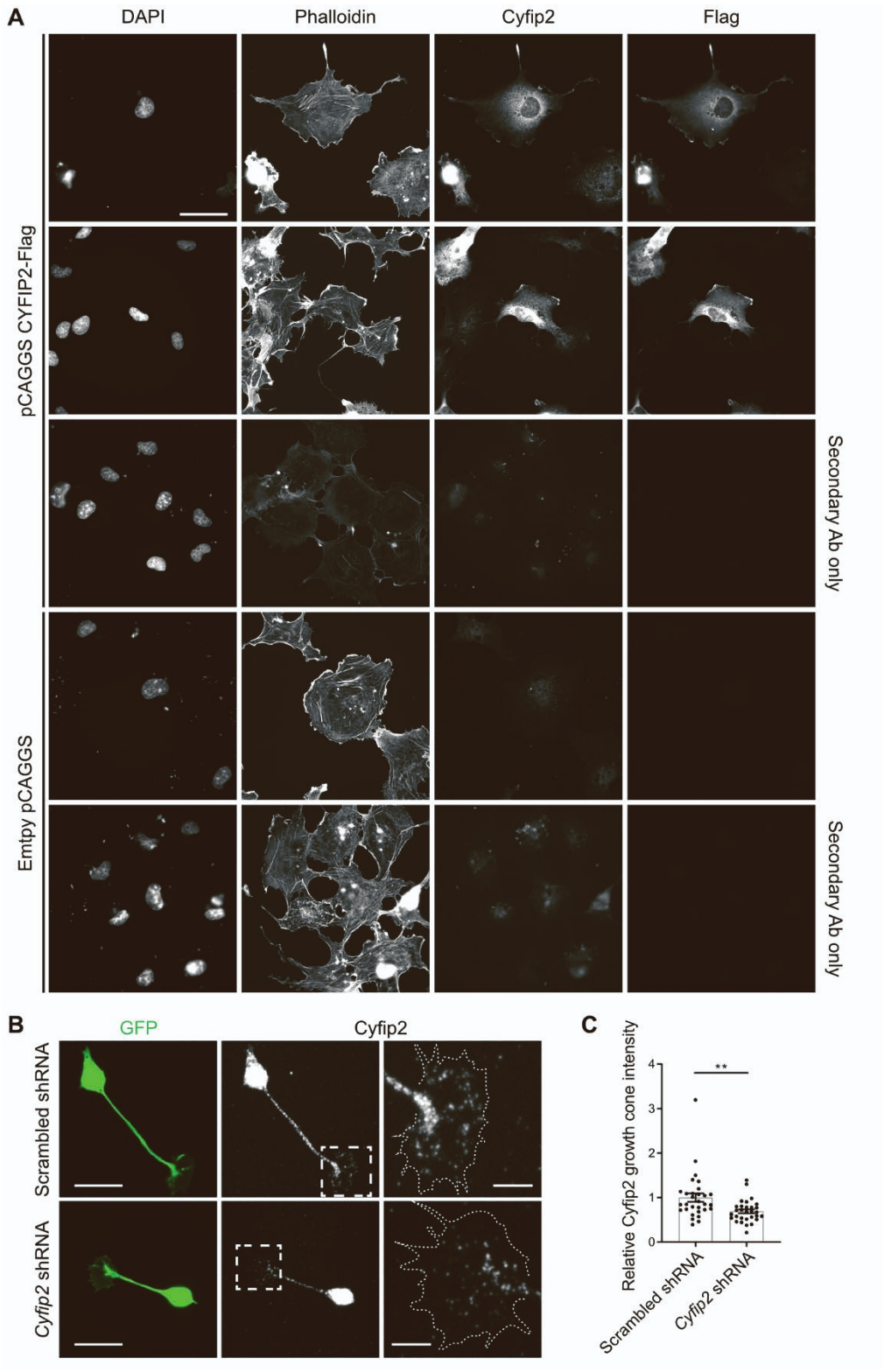

Figure S4. Validation of the CYFIP2 antibody, related to Figures 4 and 5. (A) COS7 cells were transfected to express CYFIP2-Flag or an empty vector control (empty pCAGGS). Cells were immunostained with anti-CYFIP2 and anti-Flag antibody. Nuclei were labeled with DAPI and F-actin was labelled with phalloidin. The CYFIP2 antibody detects overexpressed CYFIP2-Flag, giving a similar

immunostaining pattern to the Flag antibody. Conditions with the secondary antibody only serve as additional controls. Scale bar: 50 µm. **(B, C)** Dissociated E13.5 rat commissural neurons were electroporated with shRNA against *Cyfip2* or with scrambled shRNA as a control. **(B)** Successfully electroporated neurons express GFP. Immunostaining was performed with the CYFIP2 antibody. Scale bar: 20 µm (left), 5 µm (zoomed images, right). **(C)** Relative Cyfip2 fluorescence intensity (±SEM) of commissural neuron growth cones. Cyfip2 immunostaining is significantly reduced in neurons knocked down for *Cyfip2.* Unpaired t-test, n=30 neurons per condition. ** = $p < 0.01$.

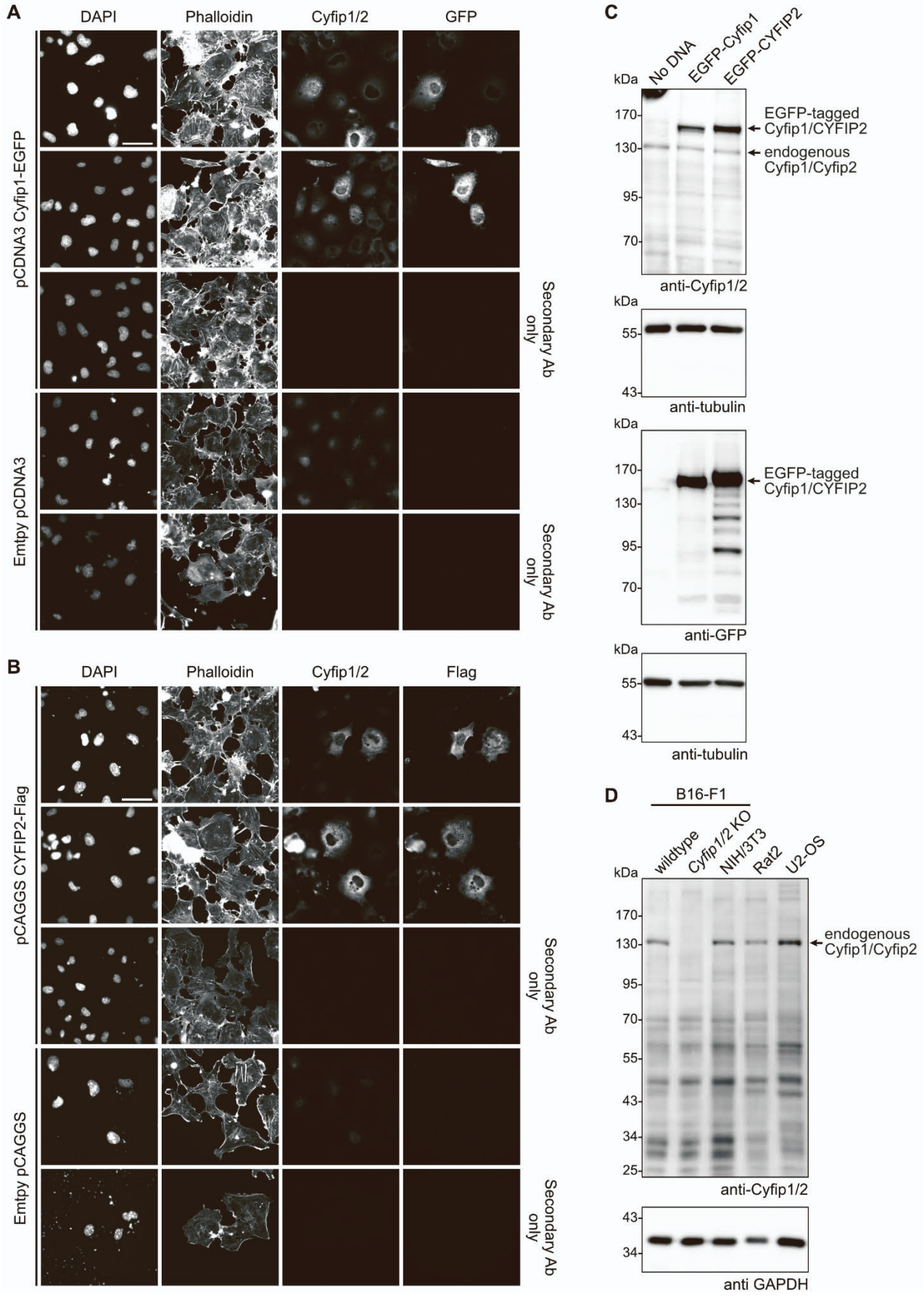
A
DAPI
Phalloidin
Cyfip1/2
GFP
pCDNA3 Cyfip1-EGFP
Emtpy pCDNA3
Secondary Ab only
Secondary Ab only
B
DAPI
Phalloidin
Cyfip1/2
Flag
pCAGGS CYFIP2-Flag
Emtpy pCAGGS
Secondary Ab only
Secondary Ab only
C
No DNA
EGFP-Cyfip1
EGFP-CYFIP2
kDa
170
130
95
70
EGFP-tagged Cyfip1/CYFIP2
endogenous Cyfip1/Cyfip2
anti-Cyfip1/2
kDa
55
43
anti-tubulin
kDa
170
130
95
70
EGFP-tagged Cyfip1/CYFIP2
anti-GFP
kDa
55
43
anti-tubulin
D
B16-F1
wildtype
Cyfip1/2 KO
NIH/3T3
Rat2
U2-OS
kDa
170
130
95
70
55
43
34
25
endogenous Cyfip1/Cyfip2
anti-Cyfip1/2
43
34
anti GAPDH

Figure S5. The Cyfip1/2 antibody recognises both Cyfip1 and Cyfip2, related to Figures 4 and 5. (A) COS7 cells were transfected to express Cyfip1-EGFP or an empty vector control (empty pCDNA3). Cells were immunostained with anti-Cyfip1/2 and anti-GFP antibody. Nuclei were labeled with DAPI and F-actin was labelled with phalloidin. The Cyfip1/2 antibody detects overexpressed Cyfip1-EGFP, which is labelled with the GFP antibody. The Cyfip1/2 antibody also detects endogenous Cyfip1/2. Conditions with the secondary antibody only serve as additional controls. Scale bar: 50 µm. **(B)** COS7 cells were transfected to express CYFIP2-Flag or an empty vector control (empty pCAGGS). Cells were immunostained with anti-Cyfip1/2 and anti-Flag antibody. Nuclei were labeled with DAPI and F-actin was labelled with phalloidin. The Cyfip1/2 antibody detects overexpressed CYFIP2-Flag, which is labelled with the Flag antibody. The Cyfip1/2 antibody also detects endogenous Cyfip1/2. Conditions with the secondary antibody only serve as additional controls. Scale bar: 50 µm. **(C)** Western blot of B16-F1 mouse melanoma cells expressing EGFP-Cyfip1 or EGFP-CYFIP2, immunoblotted with anti-Cyfip1/2 to detect endogenous and overexpressed Cyfip1/2, anti-GFP to detect overexpressed EGFP-Cyfip1 or EGFP-CYFIP2, and anti-tubulin as the loading control for each respective membrane. **(D)** Western blot of B16-F1 (mouse) wildtype and *Cyfip1/2* knockout (KO), NIH/3T3 (mouse), Rat2 (rat) and U2-OS (human) cell lines. The anti-Cyfip1/2 antibody recognises mouse, rat and human Cyfip1/2, with an absence of signal in the *Cyfip1/2* knockout. GAPDH was used as a loading control.

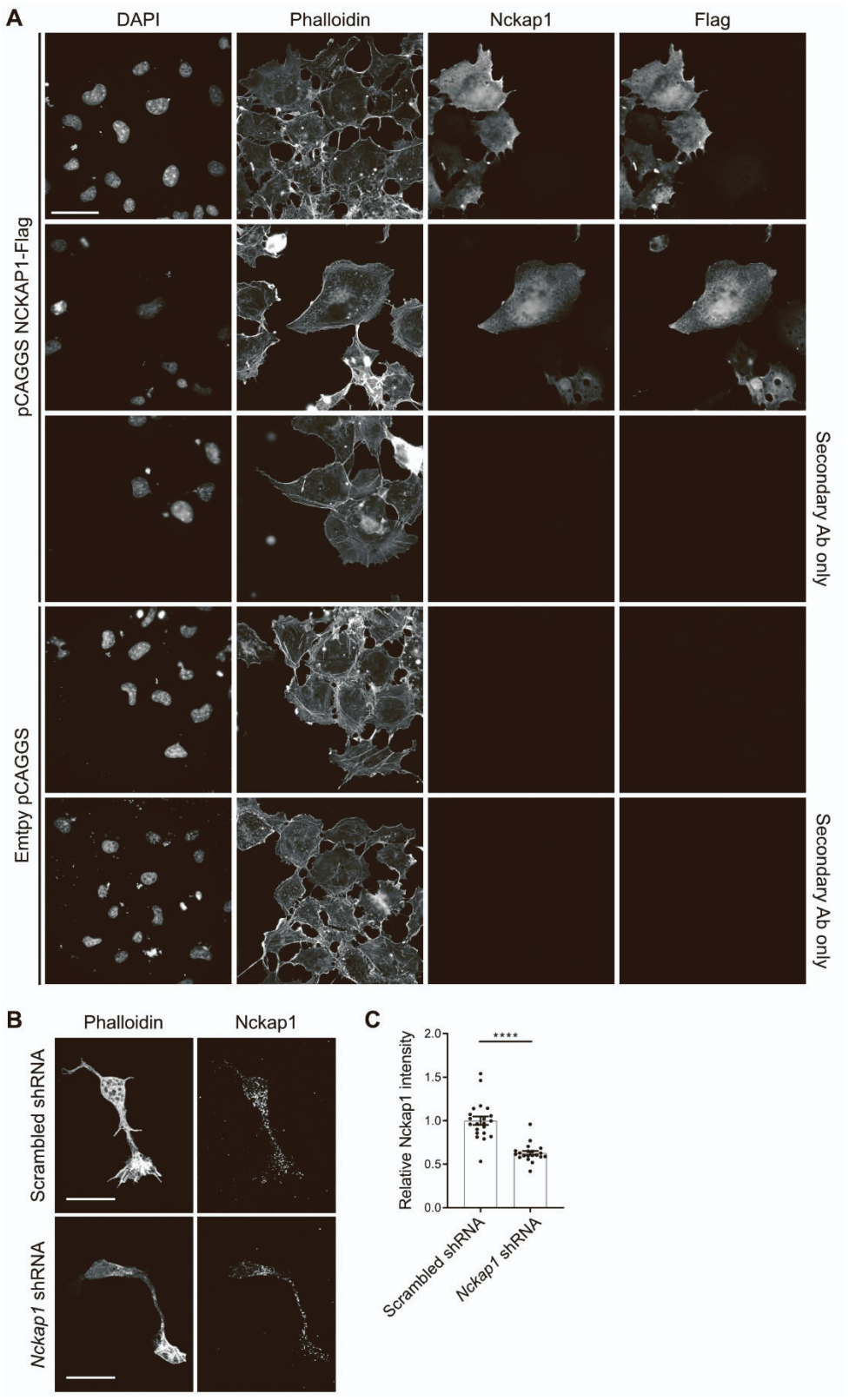
A
DAPI
Phalloidin
Nckap1
Flag
pCAGGS NCKAP1-Flag
Emtpy pCAGGS
Secondary Ab only
Secondary Ab only
B
Phalloidin
Nckap1
Scrambled shRNA
Nckap1 shRNA
C
Relative Nckap1 intensity
2.0
1.5
1.0
0.5
0.0
****
Scrambled shRNA
Nckap1 shRNA

Figure S6. Validation of the Nckap1 antibody, related to Figure 4. (A) COS7 cells were transfected to express NCKAP1-Flag or an empty vector control (empty pCAGGS). Cells were immunostained with the anti-Nckap1 (2391-C) and anti-Flag antibody. Nuclei were labeled with DAPI and F-actin was labelled with phalloidin. The Nckap1 antibody detects overexpressed NCKAP1-Flag, giving a similar immunostaining pattern to the Flag antibody. Secondary antibody only conditions serve as additional controls. Scale bar: 50 µm. **(B, C)** Dissociated E13.5 rat commissural neurons were electroporated with shRNA against *Nckap1* or with scrambled shRNA as a control. **(B)** Neurons were immunostained with the anti-Nckap1 (2391-C) antibody and F-actin was labelled with phalloidin. Scale bar: 20 µm. **(C)** Mean Nckap1 fluorescence intensity (±SEM) of commissural neurons. Nckap1 immunostaining is significantly reduced in neurons knocked down for *Nckap1.* Unpaired t-test, n = 21 and n = 20 neurons for scrambled shRNA and *Nckap1* shRNA electroporated neurons, respectively. Error bars represent SEM. **** = $p < 0.0001$.

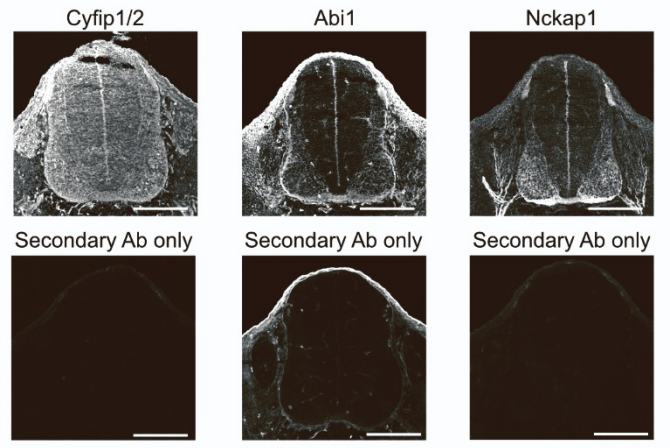

Figure S7. Negative controls for Cyfip1/2, Abi1 and Nckap1 immunostaining in E11.5 mouse spinal cord, related to Figure 4. E11.5 mouse spinal cord cross sections immunostained for Cyfip1/2, Abi1 or Nckap1 (top row) or with secondary antibody alone as controls (bottom row). Scale bar: 200 µm.

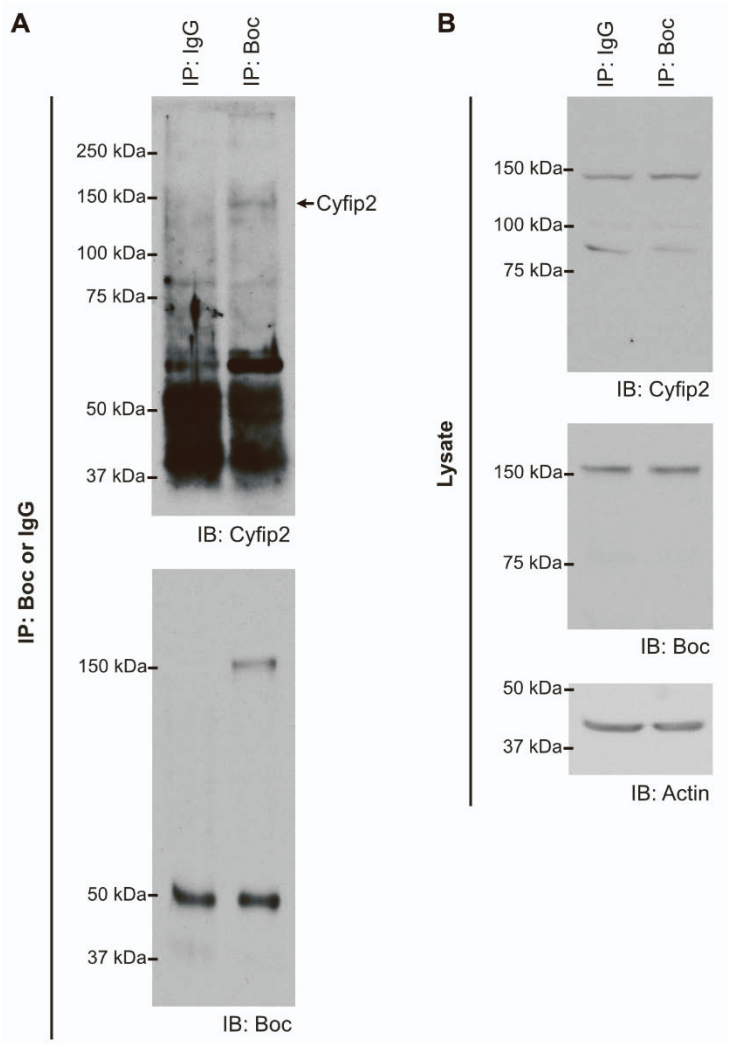

Figure S8. Entire gels from Figure 5B, related to Figure 5. Lysates from spinal cord commissural neurons were immunoprecipitated with an anti-Boc antibody or IgG. Coimmunoprecipitated proteins were analysed through SDS-PAGE and Western blot. **(A)** Entire uncropped gels corresponding to the Western blots of the immunoprecipitated proteins in Figure 5B. **(B)** Entire uncropped gels corresponding to the Western blots of the lysate in Figure 5B.

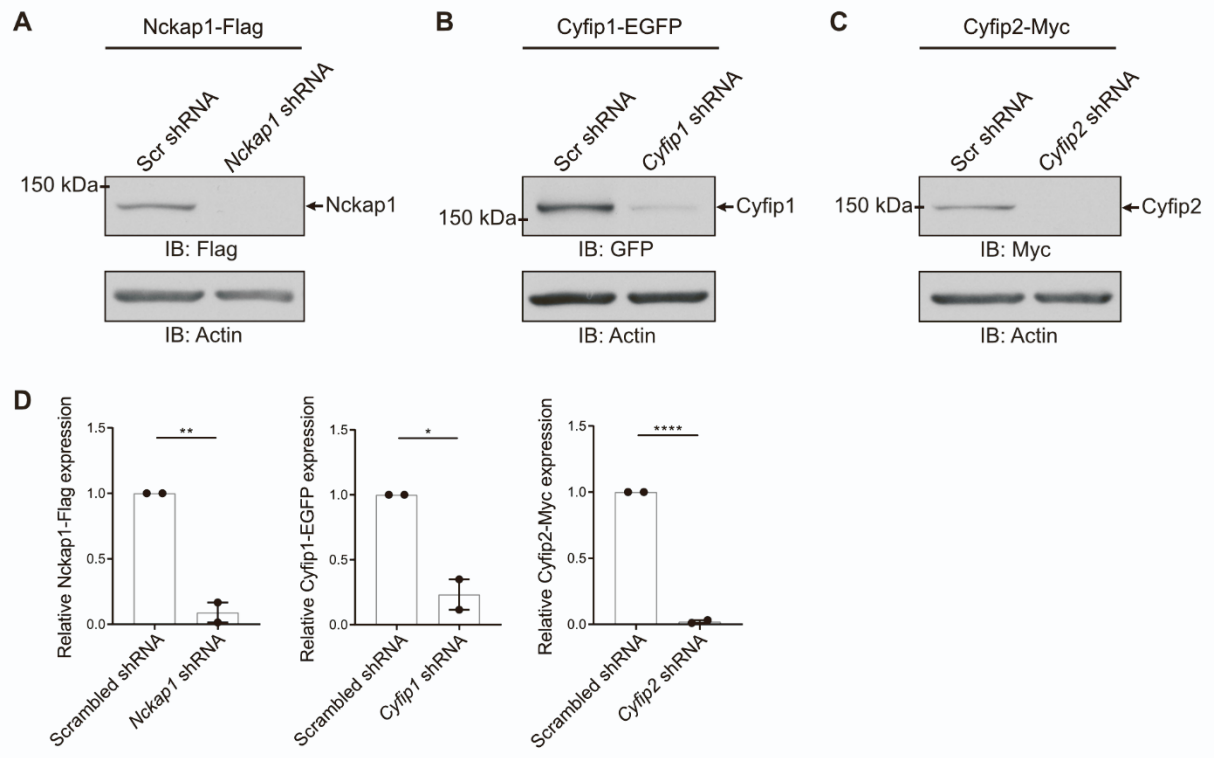

Figure S9. Validation of *Nckap1*, *Cyfip1* and *Cyfip2* shRNAs, related to Figure 6. (A-C) We evaluated the efficiency of each shRNAmir against its target by co-transfecting each shRNAmir or scrambled control shRNA (Scr shRNA) with its target tagged-expression plasmids (mouse) in HEK293T cells. Cell lysates were analyzed with SDS-PAGE and Western blotting. (**D)** The Western blots were quantified, and the expression level of each specified protein was normalised to actin. Graphs indicate the relative expression (mean ± SEM) of tagged Nckap1, Cyfip1 and Cyfip2. Unpaired t-tests, n=2 independent experiments. * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$.

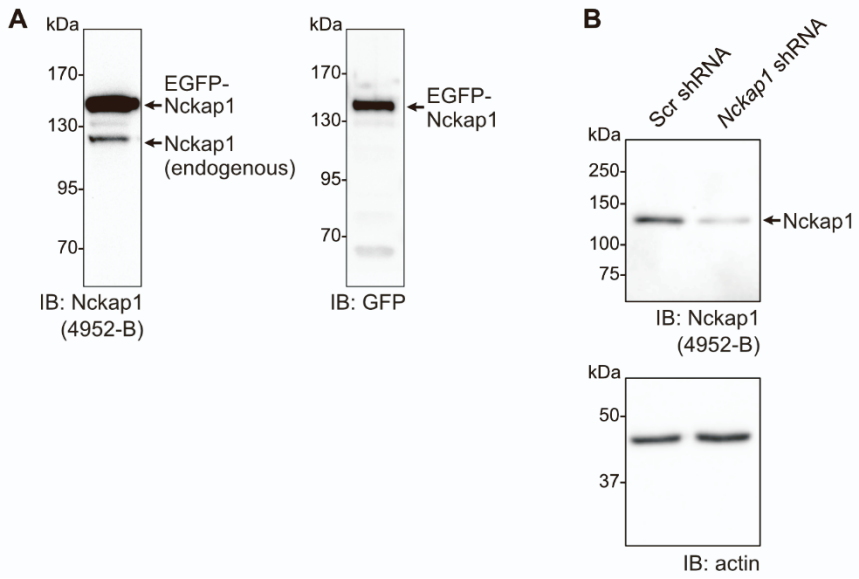

Figure S10. Validation of Nckap1 antibody, related to Figure 6. (A) Western blot of B16-F1 mouse melanoma cells expressing EGFP-Nckap1, immunoblotted with anti-Nckap1 (4952-B) to detect endogenous and overexpressed Nckap1 and anti-GFP to detect overexpressed EGFP-Nckap1. **(B)** Dissociated E13.5 rat commissural neurons were electroporated with shRNA against *Nckap1* or with scrambled shRNA as a control. Cell lysates were analyzed with SDS-PAGE and Western blotting with anti-Nckap1 (4952-B) and anti-actin as a loading control. Anti-Nckap1 specifically detects endogenous Nckap1 in commissural neurons, and Nckap1 expression is reduced by almost 80% in neurons knocked down for *Nckap1.*

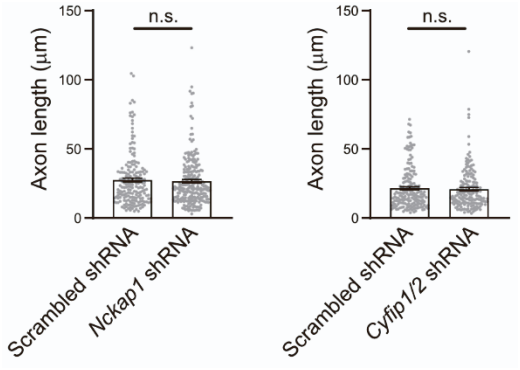

Figure S11. *Nckap1* and *Cyfip1*/2 knockdown do not affect axon length, related to Figure 6. Commissural neurons were electroporated either with scrambled shRNA or shRNA targeting *Nckap1* or *Cyfip1/2.* Axon length of successfully electroporated neurons (which express GFP) was measured after two days of culture *in vitro*. There is no significant difference in axon length between neurons electroporated with control scrambled shRNA and shRNA targeting *Nckap1* or *Cyfip1/2.* Graphs represent the mean ± SEM. n = 167-236 neurons per condition, from 3 independent experiments. Mann-Whitney test. n.s. = not significant.