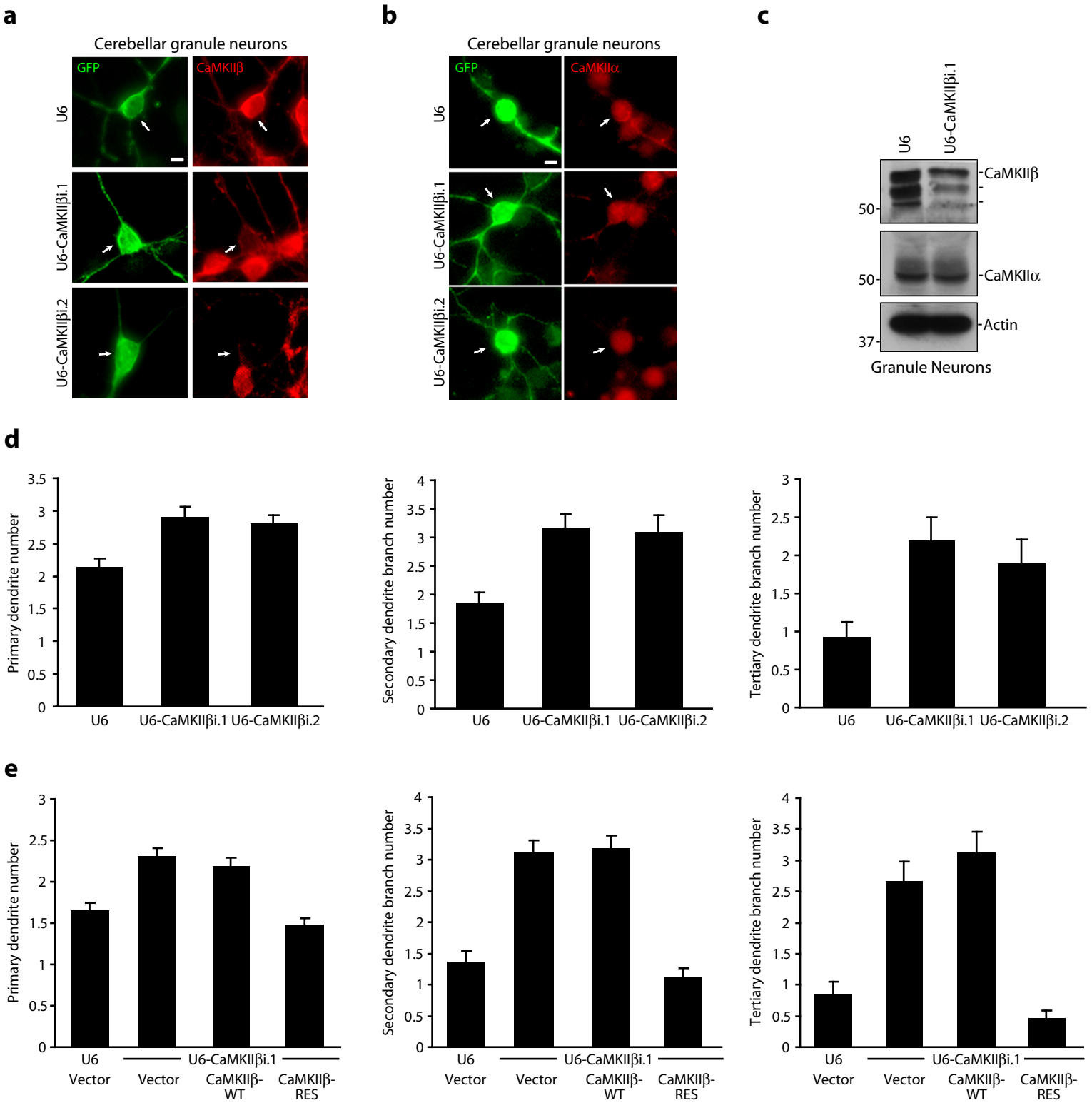
SUPPLEMENTAL INFORMATION

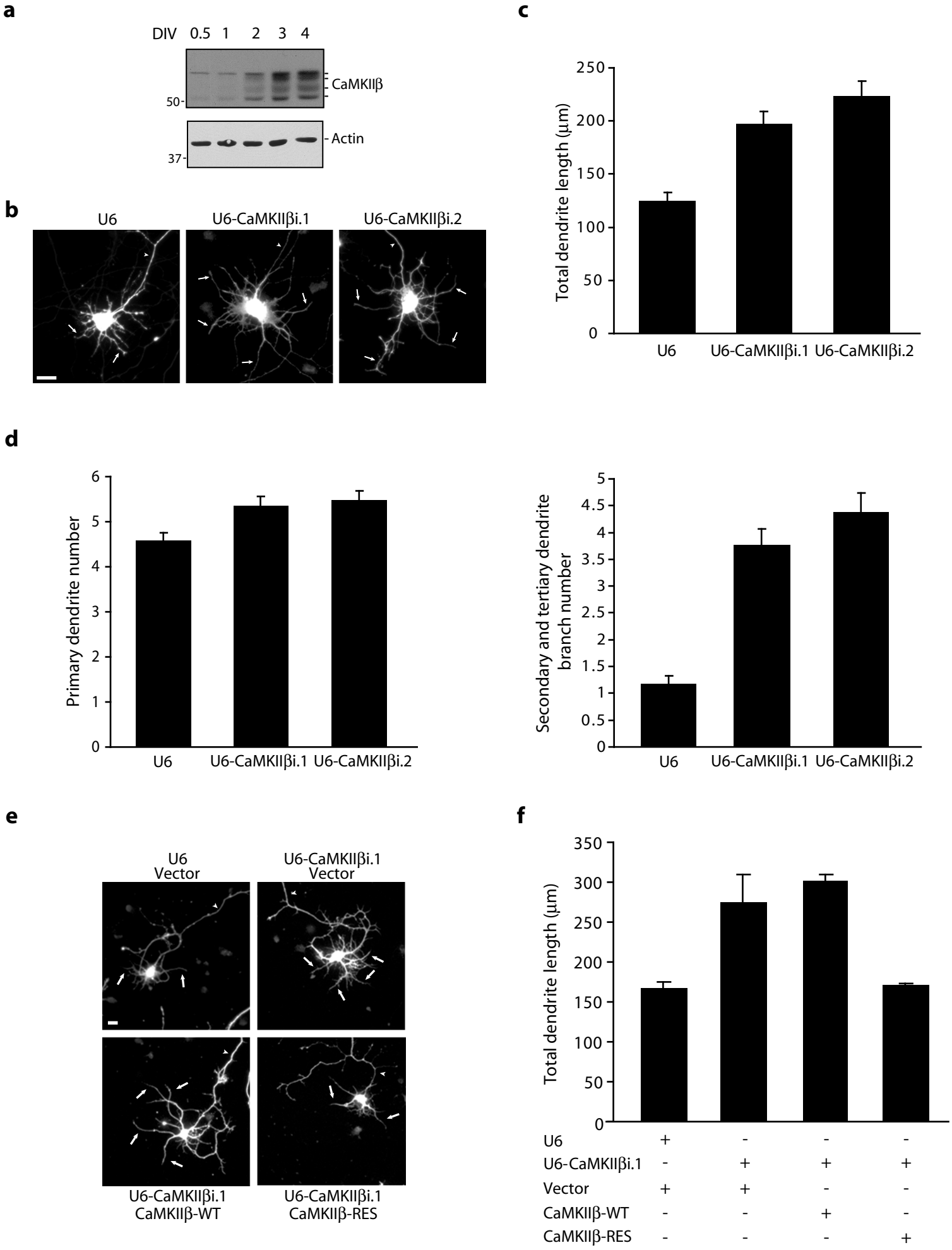
A Unique CaMKIIβ Signaling Pathway at the Centrosome Regulates Dendrite Patterning in the Brain

Sidharth V. Puram, Albert H. Kim, Yoshiho Ikeuchi, Joshua T. Wilson-Grady, Andreas Merdes, Steven P. Gygi, and Azad Bonni

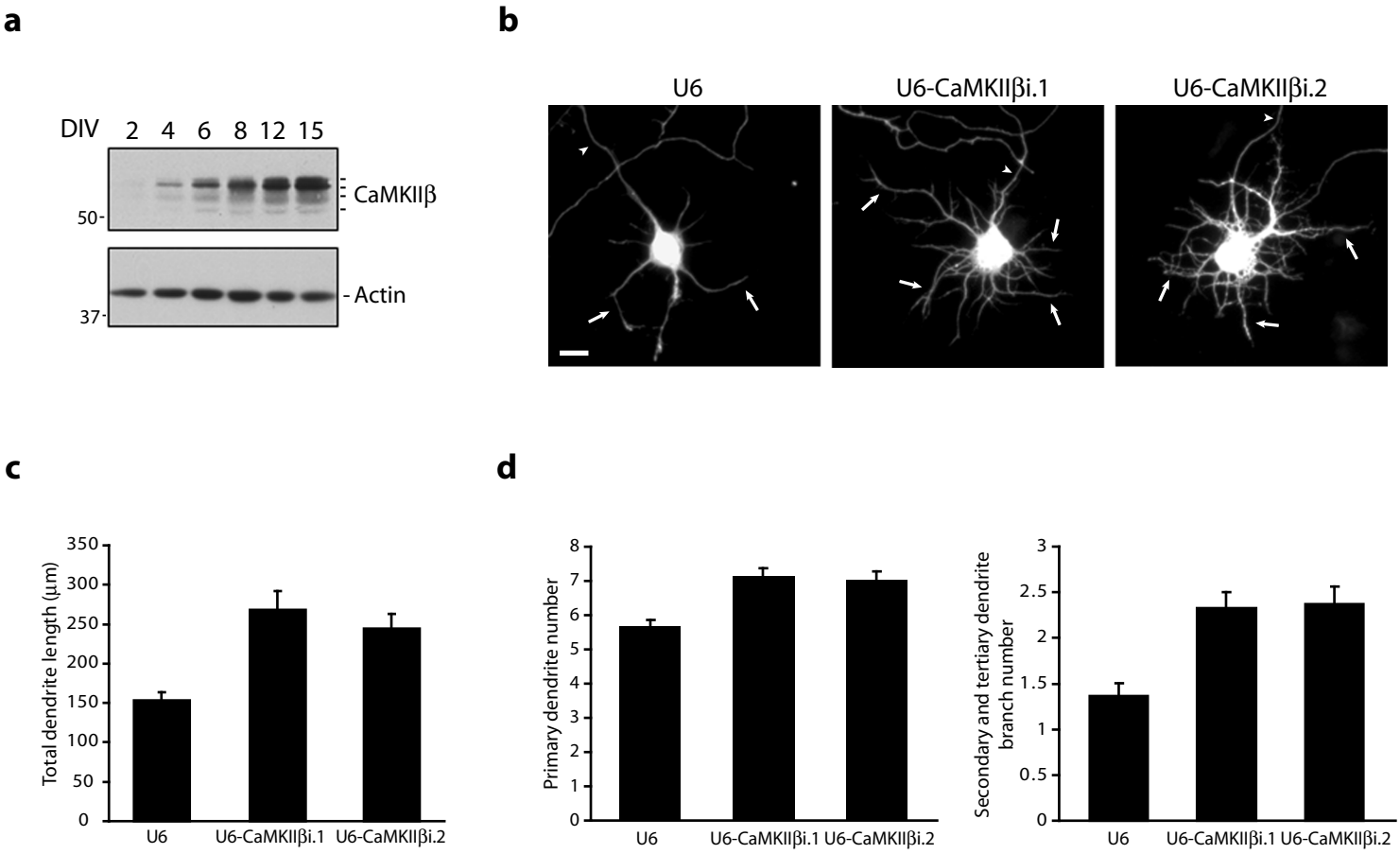


Supplementary Figure 1. CaMKIIβ specifically controls dendrite arborization in granule neurons.
a, Granule neurons transfected with one of two different CaMKIIβ RNAi plasmids (U6-CaMKIIβi) or the control U6 plasmid together with the GFP expression plasmid were subjected to immunocytochemistry using the GFP or CaMKIIβ antibody. Representative neurons are shown. Arrows indicate transfected neurons. CaMKIIβ knockdown significantly reduced CaMKIIβ immunoreactivity in neurons. Scale bar = 5 μm.
b, Granule neurons transfected as in (a) were subjected to immunocytochemistry using the GFP or monoclonal CaMKIIα antibody. CaMKIIβ knockdown had little or no effect on the CaMKIIα immunoreactive signal. Scale bar = 5 μm.

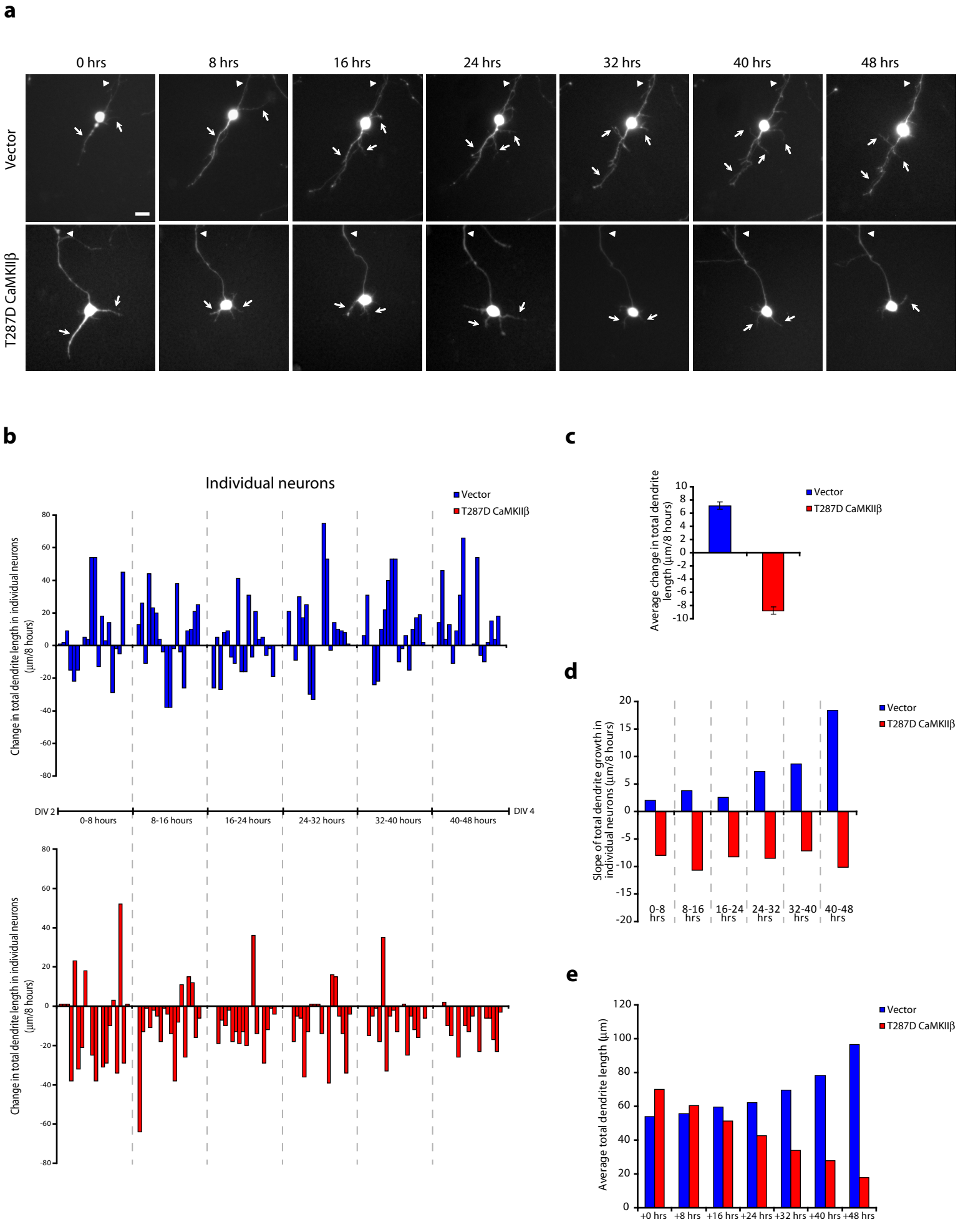
Supplementary Figure 1 (continued).
c, Lysates of granule neurons electroporated with the CaMKIIβ RNAi or control U6 plasmid were immunoblotted with the CaMKIIβ, polyclonal CaMKIIIα, or Actin antibody.
d, Granule neurons transfected with one of two different CaMKIIβ RNAi plasmids (U6-CaMKIIβi) or the control U6 plasmid together with the GFP expression plasmid were analyzed as in Fig. 1d and quantified for primary dendrite number and secondary and tertiary dendrite branch number. Primary dendrite number was modestly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.0005$). Secondary and tertiary dendrite branch number were significantly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.005$). 266 neurons were analyzed.
e, Granule neurons transfected with the CaMKIIβ RNAi or control U6 plasmid together with the expression plasmid encoding CaMKIIβ-WT, CaMKIIβ-RES, or control vector and the GFP expression plasmid were analyzed as in (d). Expression of CaMKIIβ-RES, but not CaMKIIβ-WT, significantly reduced primary dendrite number as well as secondary and tertiary dendrite branch number compared to control vector in the background of CaMKIIβ RNAi (ANOVA, $p < 0.0001$). 348 neurons were analyzed.

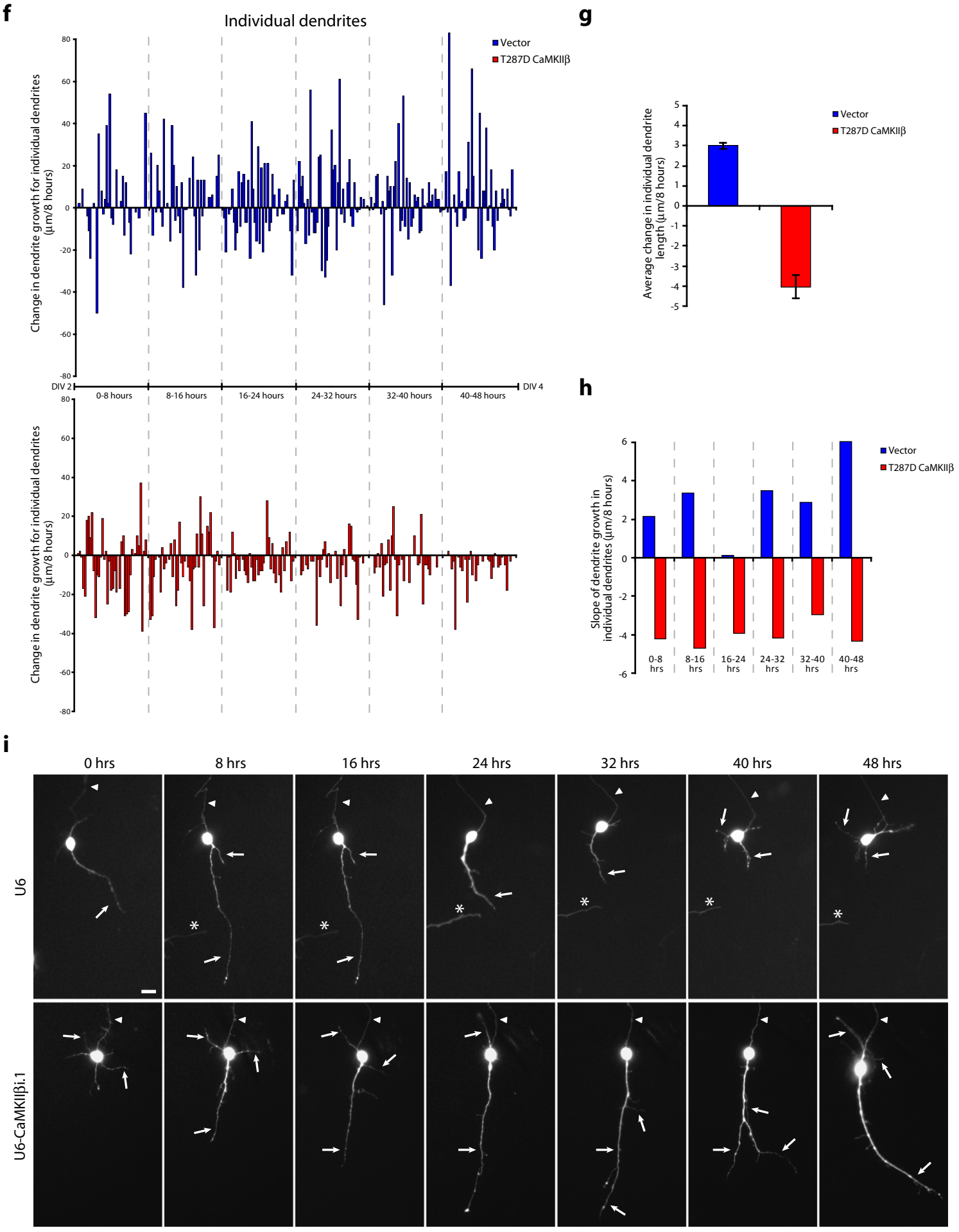

a
DIV 0.5 1 2 3 4
CaMKIIβ
50-
Actin
37-
b
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
c
Total dendrite length (μm)
250
200
150
100
50
0
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
d
Primary dendrite number
6
5
4
3
2
1
0
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
Secondary and tertiary dendrite branch number
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
e
U6
Vector
U6-CaMKIIβi.1
Vector
U6-CaMKIIβi.1
CaMKIIβ-WT
U6-CaMKIIβi.1
CaMKIIβ-RES
f
Total dendrite length (μm)
350
300
250
200
150
100
50
0
U6 + - - -
U6-CaMKIIβi.1 - + + +
Vector + + - -
CaMKIIβ-WT - - + -
CaMKIIβ-RES - - - +

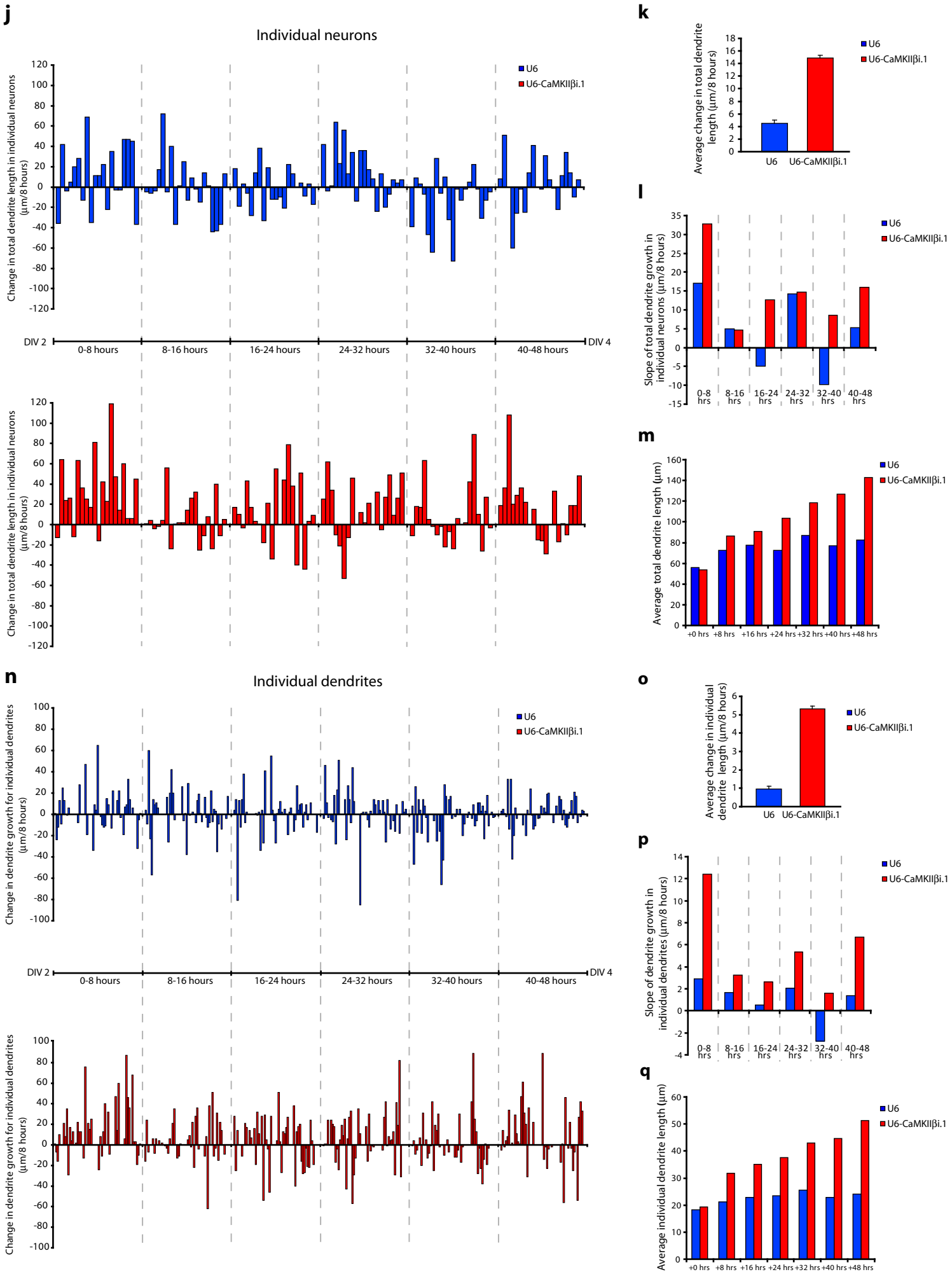
Supplementary Figure 2. CaMKIIβ restricts dendrite elaboration in hippocampal neurons.
a, Lysates of primary E18 hippocampal neurons were immunoblotted with the CaMKIIβ or Actin antibody.
b, E18 hippocampal neurons transfected with one of two different CaMKIIβ RNAi plasmids (U6-CaMKIIβi) or the control U6 plasmid together with the GFP expression plasmid were analyzed as in Fig. 1d. CaMKIIβ knockdown led to longer, more highly branched dendrites. Scale bar = 20 μm.
c, Total dendrite length for E18 hippocampal neurons treated as in (b) was quantified. Dendrite length was significantly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA; $p < 0.0001$). 204 neurons were measured.
d, Primary dendrite number and secondary and tertiary dendrite branch number for E18 hippocampal neurons treated as in (b) were quantified. Primary dendrite number was modestly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.005$). Secondary and tertiary dendrite branch number was significantly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.0001$). 204 neurons were analyzed.
e, E18 hippocampal neurons transfected with the CaMKIIβ RNAi or control U6 plasmid together with the expression plasmid encoding CaMKIIβ-WT, CaMKIIβ-RES, or control vector and the GFP expression plasmid were analyzed as in (b). Expression of CaMKIIβ-RES, but not CaMKIIβ-WT, reduced hippocampal neuron dendrite growth and arborization compared to control vector in the background of CaMKIIβ RNAi. Scale bar = 20 μm.
f, Total dendrite length for E18 hippocampal neurons treated as in (e) was quantified. Expression of CaMKIIβ-RES, but not CaMKIIβ-WT, significantly reduced total dendrite length compared to control vector in the background of CaMKIIβ RNAi (ANOVA; $p < 0.005$). 240 neurons were measured.



Supplementary Figure 3. CaMKIIβ restricts dendrite elaboration in cerebral cortical neurons.
a, Lysates of primary E18 cortical neurons were immunoblotted with the CaMKIIβ or Actin antibody.
b, E18 cortical neurons transfected with one of two different CaMKIIβ RNAi plasmids (U6-CaMKIIβi) or the control U6 plasmid together with the GFP expression plasmid were analyzed as in Fig. 1d. CaMKIIβ knockdown led to longer, more highly branched dendrites. Scale bar = 20 μm.
c, Total dendrite length for E18 cortical neurons treated as in (b) was quantified. Dendrite length was significantly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA; $p < 0.05$). 270 neurons were measured.
d, Primary dendrite number and secondary and tertiary dendrite branch number for E18 cortical neurons treated as in (b) were quantified. Primary dendrite number was modestly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.001$). Secondary and tertiary dendrite branch number was significantly increased in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (ANOVA, $p < 0.001$). 270 neurons were analyzed.


a
0 hrs
8 hrs
16 hrs
24 hrs
32 hrs
40 hrs
48 hrs
Vector
T287D CaMKIIβ
b
Individual neurons
Vector
T287D CaMKIIβ
Change in total dendrite length in individual neurons (μm/8 hours)
DIV 2
DIV 4
0-8 hours
8-16 hours
16-24 hours
24-32 hours
32-40 hours
40-48 hours
c
Average change in total dendrite length (μm/8 hours)
d
Slope of total dendrite growth in individual neurons (μm/8 hours)
0-8 hrs
8-16 hrs
16-24 hrs
24-32 hrs
32-40 hrs
40-48 hrs
e
Average total dendrite length (μm)
+0 hrs
+8 hrs
+16 hrs
+24 hrs
+32 hrs
+40 hrs
+48 hrs


f
Individual dendrites
Vector
T287D CaMKIIβ
Change in dendrite growth for individual dendrites (µm/8 hours)
DIV 2
DIV 4
0-8 hours
8-16 hours
16-24 hours
24-32 hours
32-40 hours
40-48 hours
g
Average change in individual dendrite length (µm/8 hours)
h
Slope of dendrite growth in individual dendrites (µm/8 hours)
0-8 hrs
8-16 hrs
16-24 hrs
24-32 hrs
32-40 hrs
40-48 hrs
i
0 hrs
8 hrs
16 hrs
24 hrs
32 hrs
40 hrs
48 hrs
U6
U6-CaMKIIβi.1


j
Individual neurons
U6
U6-CaMKIIβi.1
Change in total dendrite length in individual neurons (µm/8 hours)
DIV 2
DIV 4
0-8 hours
8-16 hours
16-24 hours
24-32 hours
32-40 hours
40-48 hours
k
Average change in total dendrite length (µm/8 hours)
l
Slope of total dendrite growth in individual neurons (µm/8 hours)
0-8 hrs
8-16 hrs
16-24 hrs
24-32 hrs
32-40 hrs
40-48 hrs
m
Average total dendrite length (µm)
+0 hrs
+8 hrs
+16 hrs
+24 hrs
+32 hrs
+40 hrs
+48 hrs
n
Individual dendrites
Change in dendrite growth for individual dendrites (µm/8 hours)
o
Average change in individual dendrite length (µm/8 hours)
p
Slope of dendrite growth in individual dendrites (µm/8 hours)
q
Average individual dendrite length (µm)

Supplementary Figure 4. CaMKIIβ stimulates retraction of dendrites in neurons.

a, Granule neurons were transfected on DIV1 with the T287D CaMKIIβ expression plasmid or control vector together with the GFP expression plasmid. Starting at DIV2, granule neuron dendrites were assessed by live fluorescent imaging every eight hrs over a 48 hr interval. Expression of T287D CaMKIIβ inhibited dendrite growth and stimulated dendrite retraction compared to control vector-transfection. Scale bar = 10 μm.

b, Change in total dendrite length during each eight hour interval for individual neurons analyzed as in (a) was quantified. T287D CaMKIIβ-expressing neurons had fewer periods of growth and more active retraction of dendrites compared to control vector-transfected neurons.

c, Average change in total dendrite length for all neurons analyzed as in (b) was significantly lower in T287D CaMKIIβ-expressing neurons compared to control vector-transfected neurons (t-test, $p < 0.0001$). 40 neurons were measured.

d, Slope of total dendrite growth for all neurons analyzed as in (b) demonstrated a negative rate of growth in T287D CaMKIIβ-expressing neurons, consistent with dendrite retraction, while control vector-transfected neurons had a positive growth rate over each eight hour interval.

e, Average total dendrite length for all neurons analyzed as in (b) at each timepoint consistently decreased in T287D CaMKIIβ-expressing neurons as compared to control vector-transfected neurons, which displayed consistent growth over the identical period of 48 hrs.

f, Change in individual dendrite length (including secondary and tertiary dendrite branches) during each eight hour interval for neurons analyzed as in (a) was quantified. T287D CaMKIIβ-expressing neurons had fewer periods of growth and more active retraction of individual dendrites compared to control vector-transfected neurons.

g, Average change in individual dendrite length for all individual dendrites analyzed as in (f) was significantly lower in T287D CaMKIIβ-expressing neurons as compared to control vector-transfected neurons (t-test, $p < 0.0001$). 80 individual dendrites were measured.

h, Slope of individual dendrite growth for all primary dendrites analyzed as in (f) demonstrated a negative rate of growth in T287D CaMKIIβ-expressing neurons, consistent with dendrite retraction, while control vector-transfected neurons had a positive growth rate for individual dendrites over each eight hour interval.

i, Granule neurons were transfected on DIV0 with the CaMKIIβ RNAi or control U6 plasmid together with the GFP expression plasmid and analyzed as in (a). Asterisk indicates process from another neuron. CaMKIIβ knockdown led to increased dendrite growth and reduced dendrite retraction compared to control U6-transfection. Scale bar = 10 μm.

j, Change in total dendrite length during each eight hour interval for individual neurons analyzed as in (i) was quantified. CaMKIIβ knockdown neurons had more periods of growth and less active retraction of dendrites compared to control U6-transfection.

k, Average change in total dendrite length for all neurons analyzed as in (j) was significantly greater in CaMKIIβ knockdown neurons as compared to control U6-transfected neurons (t-test, $p < 0.01$). 40 neurons were measured.

l, Slope of total dendrite growth for all neurons analyzed as in (j) demonstrated a robustly positive rate of growth in CaMKIIβ knockdown neurons, consistent with a role for endogenous CaMKIIβ in dendrite retraction, while control U6-transfected neurons had a more limited rate of growth over each eight hour interval.

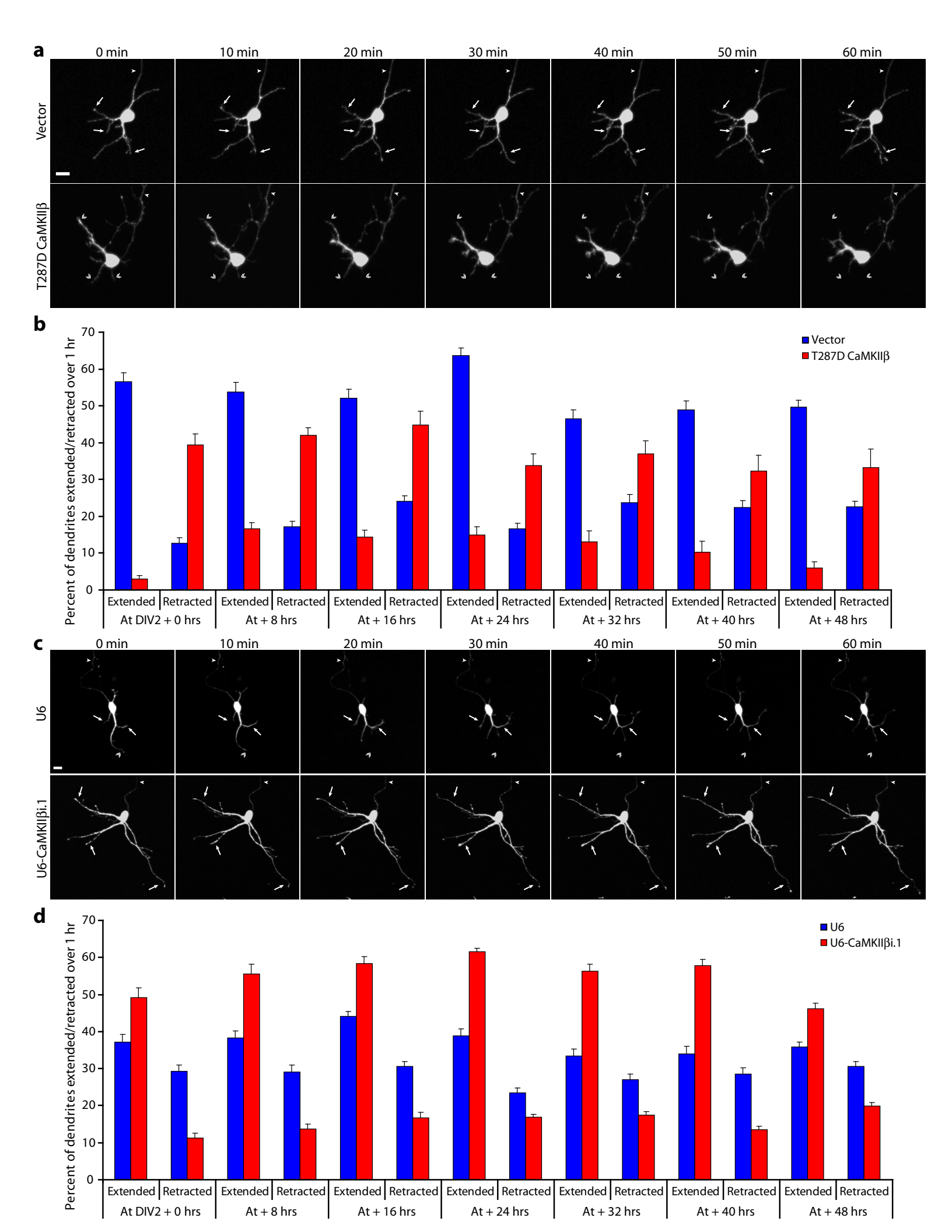
m, Average total dendrite length for all neurons analyzed as in (j) at each timepoint increased more substantially in CaMKIIβ knockdown neurons compared to control U6-transfected neurons over the identical period of 48 hrs.

n, Change in individual dendrite length (including secondary and tertiary dendrite branches) during each eight hour interval was quantified for individual primary dendrites of neurons analyzed as in (i). Individual dendrites of CaMKIIβ knockdown neurons had increased periods of growth and less active retraction compared to individual dendrites of control U6-transfected neurons.

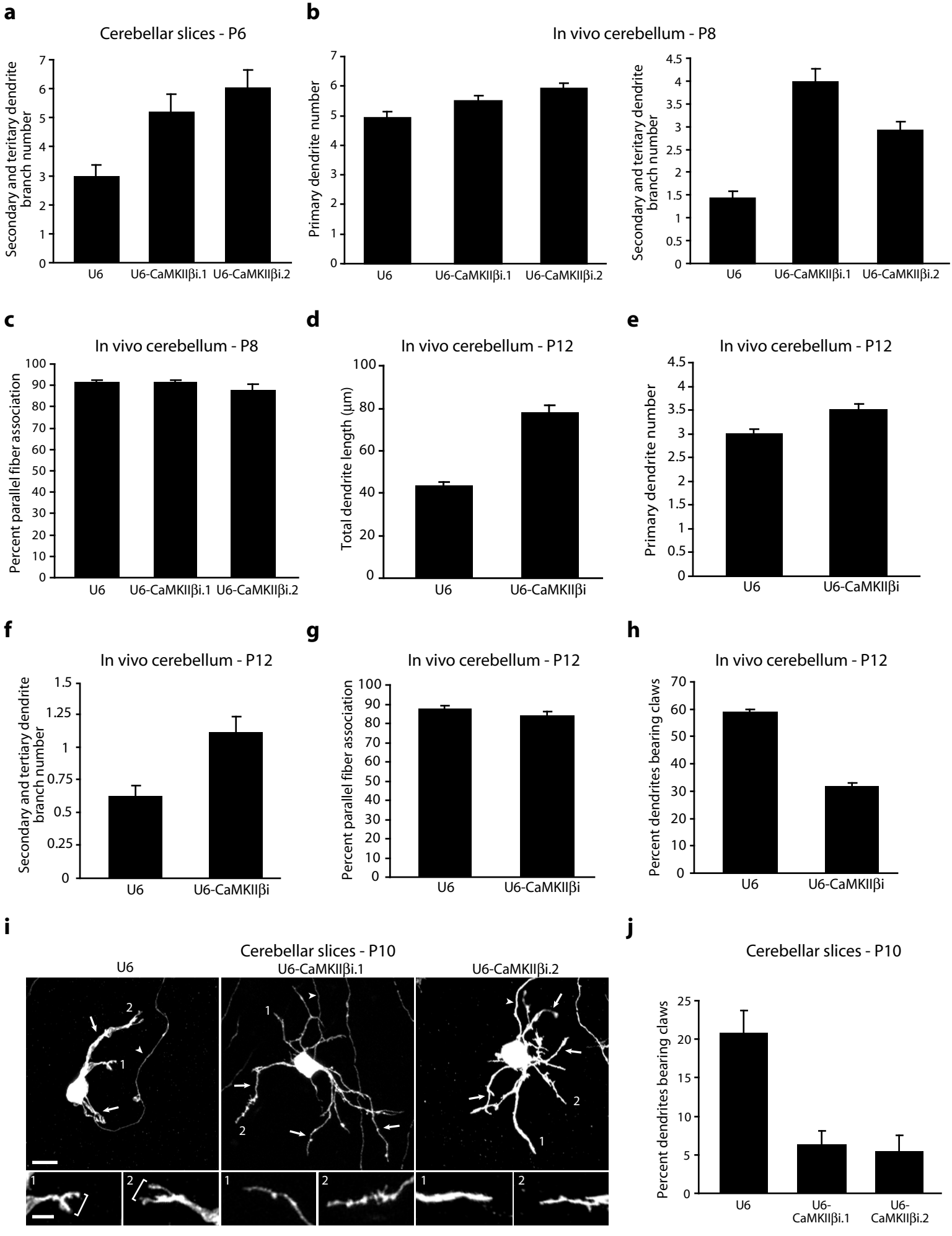
o, Average change in individual dendrite length for all individual dendrites analyzed as in (n) was significantly greater in CaMKIIβ knockdown neurons as compared to control U6-transfected neurons (t-test, $p < 0.01$). 100 individual dendrites were measured.

p, Slope of individual dendrite growth for all primary dendrites analyzed as in (n) demonstrated a robustly positive rate of individual dendrite growth in CaMKIIβ knockdown neurons, consistent with the role of endogenous CaMKIIβ in dendrite retraction, while individual dendrites of control U6-transfected neurons had a more limited rate of growth over each eight hour interval.

Supplementary Figure 4 (continued).
q, Average individual dendrite length for all primary dendrites analyzed as in (n) at each timepoint increased more substantially in CaMKIIβ knockdown neurons compared to control U6-transfected neurons over the identical period of 48 hrs.


a
0 min
10 min
20 min
30 min
40 min
50 min
60 min
Vector
T287D CaMKIIβ
b
Percent of dendrites extended/retracted over 1 hr
Vector
T287D CaMKIIβ
Extended
Retracted
At DIV2 + 0 hrs
At + 8 hrs
At + 16 hrs
At + 24 hrs
At + 32 hrs
At + 40 hrs
At + 48 hrs
c
U6
U6-CaMKIIβi.1
d
U6
U6-CaMKIIβi.1

Supplementary Figure 5. CaMKIIβ regulates minute, dynamic changes in dendrite extension and retraction.
a, Granule neurons were transfected on DIV1 with the T287D CaMKIIβ expression plasmid or control vector together with the GFP expression plasmid. Starting at DIV2, granule neuron dendrites were assessed by live spinning disk confocal imaging every eight hours over a 48 hr interval in an environment-controlled chamber. At each time point, neurons were imaged every ten minutes for a one hour period. Representative neurons at DIV 2 + 24 hrs are shown. Arrows and chevrons indicate dendrite extension and retraction, respectively, and both are in a constant, fixed position across all images. Arrowheads indicate axons. Expression of T287D CaMKIIβ reduced dendrite extension events and promoted dendrite retraction events compared to control vector-transfection. Scale bar = 10 μm.
b, The percentage of dendrites that extended or retracted in length greater than 2 μm over each one hour period of analysis for neurons analyzed as in (a) was quantified. T287D CaMKIIβ-expressing neurons had a significant reduction in the percentage of dendrite extension events with a significant increase in the percentage of dendrite retraction events compared to control vector-transfected neurons at each timepoint analyzed (ANOVA, $p < 0.0001$). 46 neurons were analyzed.
c, Granule neurons were transfected on DIV0 with the CaMKIIβ RNAi or control U6 plasmid together with the GFP expression plasmid and analyzed as in (a). Representative neurons at DIV 2 + 24 hrs are shown. Arrows and chevrons indicate dendrite extension and retraction, respectively, and both are in a constant, fixed position across all images. Arrowheads indicate axons. CaMKIIβ knockdown promoted dendrite extension events and reduced dendrite retraction events compared to control U6-transfection. Scale bar = 10 μm.
d, The percentage of dendrites that extended or retracted in length greater than 2 μm over each one hour period of analysis for neurons analyzed as in (c) was quantified. CaMKIIβ knockdown neurons had a significant increase in the percentage of dendrite extension events with a significant reduction in the percentage of dendrite retraction events compared to control U6-transfected neurons (ANOVA, $p < 0.0001$). 50 neurons were analyzed.


a
Cerebellar slices - P6
Secondary and teritary dendrite branch number
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
b
In vivo cerebellum - P8
Primary dendrite number
Secondary and teritary dendrite branch number
c
In vivo cerebellum - P8
Percent parallel fiber association
d
In vivo cerebellum - P12
Total dendrite length (µm)
U6-CaMKIIβi
e
In vivo cerebellum - P12
Primary dendrite number
f
In vivo cerebellum - P12
Secondary and tertiary dendrite branch number
g
In vivo cerebellum - P12
Percent parallel fiber association
h
In vivo cerebellum - P12
Percent dendrites bearing claws
i
Cerebellar slices - P10
U6
U6-CaMKIIβi.1
U6-CaMKIIβi.2
j
Cerebellar slices - P10
Percent dendrites bearing claws
U6-CaMKIIβi.1
U6-CaMKIIβi.2

Supplementary Figure 6. CaMKIIβ controls the patterning of dendrite arbors.

a, Secondary and tertiary dendrite branch number for IGL granule neurons in cerebellar slices analyzed as in Fig. 2a was quantified. CaMKIIβ knockdown in cerebellar slices significantly increased the number of secondary and tertiary dendrite branches in IGL granule neurons (ANOVA, $p < 0.0005$). 199 neurons were analyzed.

b, Rat pups electroporated in vivo with a U6-CaMKIIβi/CMV-GFP RNAi or control U6/CMV-GFP plasmid were sacrificed at P8 and analyzed as in Fig. 2d. Morphometric analyses revealed that primary dendrite number was modestly increased, and secondary and tertiary dendrite branch number were significantly increased in IGL granule neurons in CaMKIIβ knockdown animals compared to control U6 animals (ANOVA, $p < 0.005$). 275 neurons were analyzed.

c, Granule neurons analyzed as in (b) were counted in the IGL and axons were counted in the molecular layer. 270 neurons were analyzed.

d, Rat pups electroporated in vivo with a U6-CaMKIIβi/CMV-GFP RNAi or control U6/CMV-GFP plasmid were sacrificed at P12 and analyzed as in (b). Morphometric analyses revealed that total dendrite length was significantly increased in IGL granule neurons in CaMKIIβ knockdown animals compared to control U6 animals (t-test, $p < 0.0001$). 240 neurons were measured.

e, IGL granule neurons analyzed as in (d) were subjected to morphometric analyses. Primary dendrite number was modestly increased in IGL neurons in CaMKIIβ knockdown animals compared to control U6 animals (t-test, $p < 0.005$). 240 neurons were analyzed.

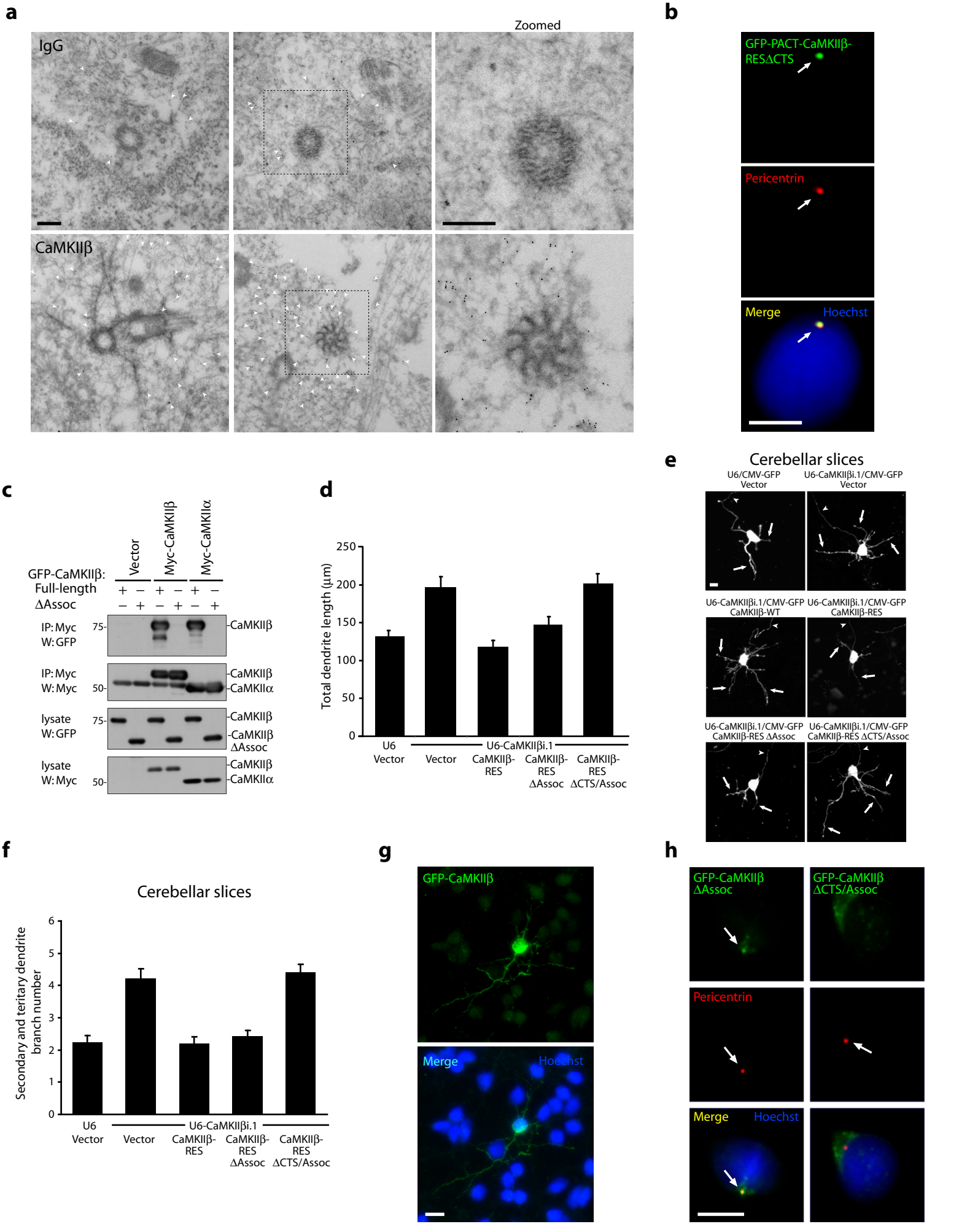
f, IGL granule neurons analyzed as in (d) were subjected to morphometric analysis. Secondary and tertiary dendrite branch number was significantly increased in IGL granule neurons in CaMKIIβ knockdown animals compared to control U6 animals (t-test, $p < 0.005$). 240 neurons were analyzed.

g, Granule neurons analyzed as in (d) were counted in the IGL and axons were counted in the molecular layer as described[1-2]. 542 neurons were analyzed.

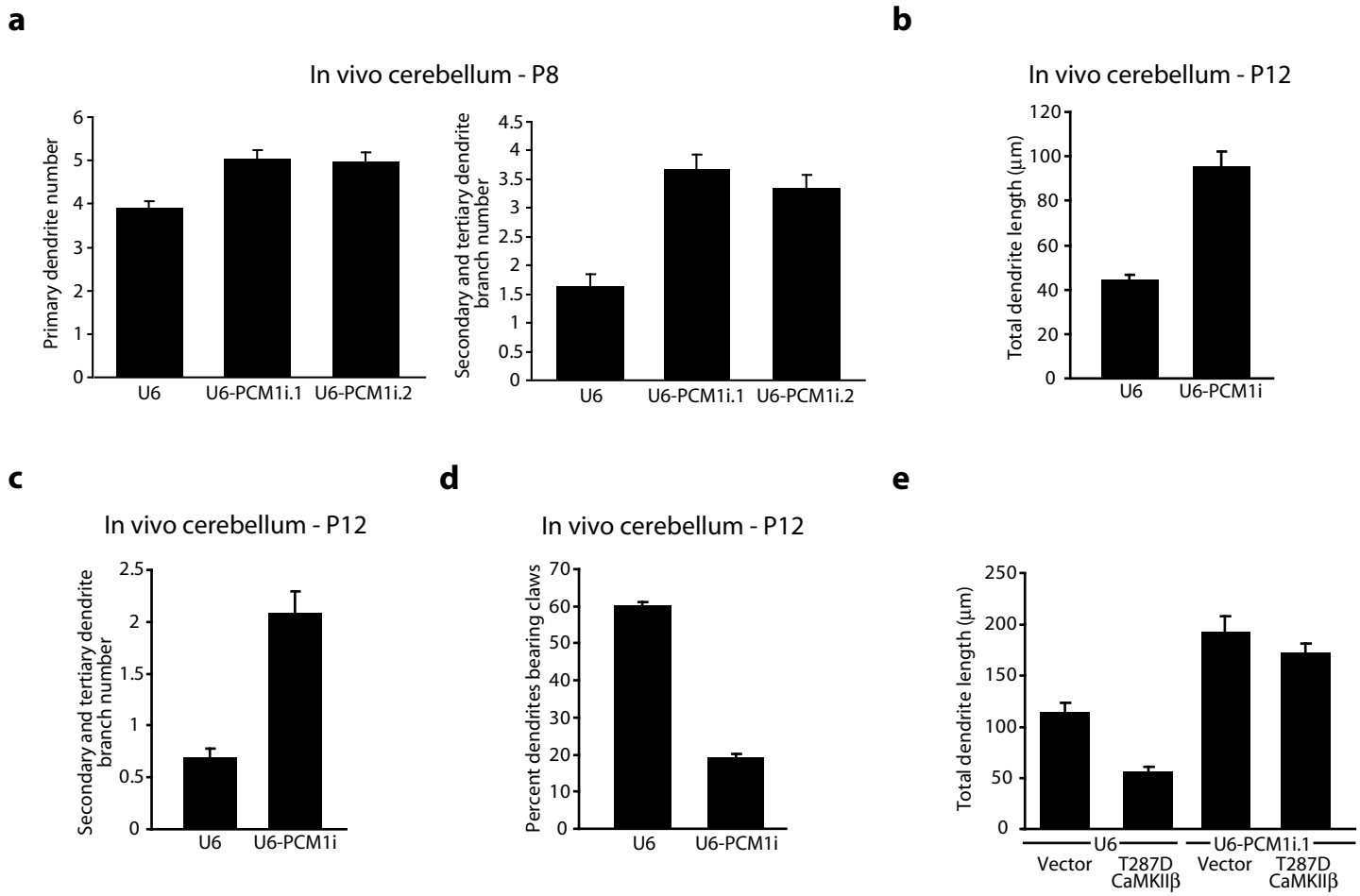
h, IGL granule neurons analyzed as in (b) were subjected to morphometric analysis. The percentage of dendrites bearing claws was significantly decreased in IGL granule neurons in CaMKIIβ knockdown animals compared to control U6 animals (t-test, $p < 0.0001$). 240 neurons were analyzed.

i, P10 rat cerebellar slices transfected by a biolistics method with the CaMKIIβ RNAi or control U6 plasmid together with the GFP expression plasmid were analyzed as in (a). Representative granule neurons are shown. Scale bar = 10 μm. Inset: Zoomed view of dendritic tips of individual neurons. Scale bar = 5 μm. Bracket identifies dendritic claws. CaMKIIβ knockdown in cerebellar slices led to longer, more highly branched dendrites with fewer dendritic claws in IGL granule neurons.

j, The percentage of dendrites bearing claws for IGL granule neurons in P10 cerebellar slices analyzed as in (i) was quantified. CaMKIIβ knockdown in cerebellar slices significantly reduced the percentage of dendrites bearing claws in IGL granule neurons (ANOVA, $p < 0.0001$). 105 neurons were analyzed.


a
IgG
Zoomed
CaMKIIβ
b
GFP-PACT-CaMKIIβ-RESΔCTS
Pericentrin
Merge
Hoechst
c
GFP-CaMKIIβ:
Full-length
ΔAssoc
Vector
Myc-CaMKIIβ
Myc-CaMKIIα
IP: Myc
W: GFP
IP: Myc
W: Myc
lysate
W: GFP
lysate
W: Myc
75-
50-
CaMKIIβ
CaMKIIα
CaMKIIβ ΔAssoc
d
Total dendrite length (μm)
U6 Vector
Vector
U6-CaMKIIβi.1
CaMKIIβ-RES
CaMKIIβ-RES ΔAssoc
CaMKIIβ-RES ΔCTS/Assoc
e
Cerebellar slices
U6/CMV-GFP Vector
U6-CaMKIIβi.1/CMV-GFP Vector
U6-CaMKIIβi.1/CMV-GFP CaMKIIβ-WT
U6-CaMKIIβi.1/CMV-GFP CaMKIIβ-RES
U6-CaMKIIβi.1/CMV-GFP CaMKIIβ-RES ΔAssoc
U6-CaMKIIβi.1/CMV-GFP CaMKIIβ-RES ΔCTS/Assoc
f
Cerebellar slices
Secondary and teritary dendrite branch number
g
GFP-CaMKIIβ
Merge
Hoechst
h
GFP-CaMKIIβ ΔAssoc
GFP-CaMKIIβ ΔCTS/Assoc
Pericentrin
Merge
Hoechst

Supplementary Figure 7. CaMKIIβ controls dendrite morphogenesis independently of CaMKIIα.
a, Granule neurons were subjected to immunocytochemistry using the CaMKIIβ or control (IgG) antibody and 5 nm gold labeling for analysis by electron microscopy. Neurons immunostained with control antibody had few gold particles near the centrosome, while CaMKIIβ staining revealed numerous gold particles in the pericentriolar region (indicated by white arrowheads; also see zoomed image). Scale bar = 100 nm.
b, Granule neurons transfected with the GFP-PACT-CaMKIIβ-RESΔCTS expression plasmid were analyzed as in Fig. 3d. Arrows indicate co-localization of GFP-PACT-CaMKIIβ-RESΔCTS with the centrosomal marker pericentrin. Scale bar = 5 μm.
c, Lysates of 293T cells transfected with the GFP-CaMKIIβ or GFP-CaMKIIβΔAssoc expression plasmid together with the Myc-CaMKIIβ or Myc-CaMKIIα expression plasmid or control vector were immunoprecipitated using the Myc antibody and immunoblotted with the GFP or Myc antibody.
d, Granule neurons transfected with the CaMKIIβ RNAi or control U6 plasmid together with the expression plasmid encoding CaMKIIβ-RES, CaMKIIβ-RESΔAssoc, CaMKIIβ-RESΔCTS/Assoc, or control vector and the GFP expression plasmid were analyzed as in Fig. 1d. Expression of CaMKIIβ-RES and CaMKIIβ-RESΔAssoc, but not CaMKIIβ-RESΔCTS/Assoc, significantly reduced total dendrite length compared to control vector in the background of CaMKIIβ RNAi (ANOVA; $p < 0.0001$). 450 neurons were measured.
e, Rat cerebellar slices biolistically transfected with the U6-CaMKIIβi/CMV-GFP RNAi or control U6/CMV-GFP plasmid together with the expression plasmid encoding CaMKIIβ-RES, CaMKIIβ-RESΔAssoc, CaMKIIβ-RESΔCTS/Assoc, or control vector were analyzed as in Fig. 2a. Representative granule neurons are shown. CaMKIIβ-RES and CaMKIIβ-RESΔAssoc, but not CaMKIIβ-WT or CaMKIIβ-RESΔAssoc/CTS, increased dendrite retraction compared to control vector in the background of CaMKIIβ RNAi. Scale bar = 10 μm.
f, Cerebellar slices were analyzed as in (e) and subjected to morphometric analysis. CaMKIIβ-RES and CaMKIIβ-RESΔAssoc, but not CaMKIIβ-RESΔCTS/Assoc, significantly reduced secondary and tertiary dendrite branch number compared to control vector in the background of CaMKIIβ RNAi (ANOVA; $p < 0.0001$). 479 neurons were analyzed.
g, Granule neurons transfected with the GFP-CaMKIIβ expression plasmid were subjected to immunocytochemistry using the GFP antibody. GFP-CaMKIIβ localized throughout the soma and processes. Scale bar = 10 μm.
h, Granule neurons transfected with the GFP-CaMKIIβΔAssoc or GFP-CaMKIIβΔCTS/Assoc expression plasmid were analyzed as in (b). Arrows identify the centrosome based on pericentrin immunofluorescence. GFP-CaMKIIβΔAssoc but not GFP-CaMKIIβΔCTS/Assoc was enriched at the centrosome. Scale bar = 5 μm.



Supplementary Figure 8. PCM1 regulates dendrite morphogenesis in vivo.

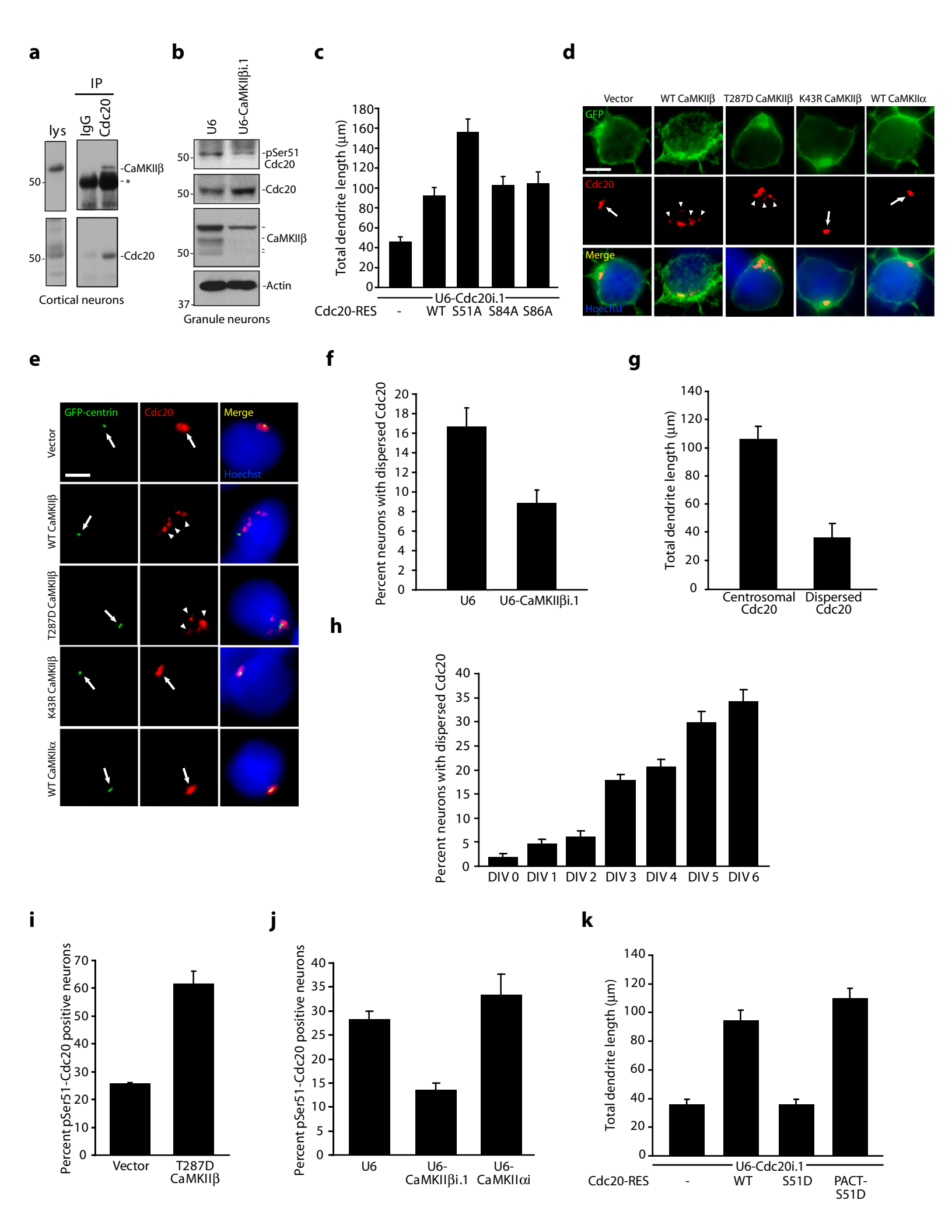
a, Rat pups electroporated in vivo with a U6-PCM1i/CMV-GFP RNAi or control U6/CMV-GFP plasmid were sacrificed at P8 and analyzed as in Fig. 2d. Morphometric analysis revealed that primary dendrite number was modestly increased (ANOVA, $p < 0.05$), and secondary and tertiary dendrite branch number was significantly increased in IGL granule neurons in PCM1 knockdown animals compared to control U6 animals (ANOVA, $p < 0.0001$). 239 neurons were analyzed.

b, Rat pups electroporated in vivo with a U6-PCM1i/CMV-GFP RNAi or control U6/CMV-GFP plasmid were sacrificed at P12 and analyzed as in (a). Morphometric analysis revealed that total dendrite length was significantly increased in IGL granule neurons in PCM1 knockdown animals compared to control U6 animals (t-test, $p < 0.0001$). 150 neurons were measured.

c, IGL granule neurons analyzed as in (b) were subjected to morphometric analysis. Secondary and tertiary dendrite branch number was significantly increased in IGL granule neurons in PCM1 knockdown animals compared to control U6 animals (t-test, $p < 0.0001$). 150 neurons were analyzed.

d, IGL granule neurons analyzed as in (b) were subjected to morphometric analysis. The percentage of dendrites bearing claws was significantly decreased in IGL granule neurons in PCM1 knockdown animals compared to control U6 animals (t-test, $p < 0.0001$). 150 neurons were analyzed.

e, Granule neurons transfected with the T287D CaMKIIβ expression plasmid or control vector together with PCM1 RNAi or control U6 plasmid and the GFP expression plasmid were analyzed as in Fig. 1d. Expression of T287D CaMKIIβ significantly reduced total dendrite length compared to control. PCM1 RNAi significantly increased total dendrite length in neurons in the presence or absence of T287D CaMKIIβ expression (ANOVA, $p < 0.0001$). 236 neurons were measured.


a
IP
lys
IgG
Cdc20
-CaMKIIβ
-*
50-
50-
-Cdc20
Cortical neurons
b
U6
U6-CaMKIIβi.1
50-
-pSer51 Cdc20
50-
-Cdc20
-
-CaMKIIβ
50-
-
-Actin
37-
Granule neurons
c
Total dendrite length (μm)
180
160
140
120
100
80
60
40
20
0
U6-Cdc20i.1
Cdc20-RES
-
WT
S51A
S84A
S86A
d
Vector
WT CaMKIIβ
T287D CaMKIIβ
K43R CaMKIIβ
WT CaMKIIα
GFP
Cdc20
Merge
Hoechst
e
GFP-centrin
Cdc20
Merge
Hoechst
Vector
WT CaMKIIβ
T287D CaMKIIβ
K43R CaMKIIβ
WT CaMKIIα
f
Percent neurons with dispersed Cdc20
20
18
16
14
12
10
8
6
4
2
0
U6
U6-CaMKIIβi.1
g
Total dendrite length (μm)
140
120
100
80
60
40
20
0
Centrosomal Cdc20
Dispersed Cdc20
h
Percent neurons with dispersed Cdc20
40
35
30
25
20
15
10
5
0
DIV 0
DIV 1
DIV 2
DIV 3
DIV 4
DIV 5
DIV 6
i
Percent pSer51-Cdc20 positive neurons
70
60
50
40
30
20
10
0
Vector
T287D CaMKIIβ
j
Percent pSer51-Cdc20 positive neurons
40
35
30
25
20
15
10
5
0
U6
U6-CaMKIIβi.1
U6-CaMKIIαi
k
Total dendrite length (μm)
140
120
100
80
60
40
20
0
U6-Cdc20i.1
Cdc20-RES
-
WT
S51D
PACT-S51D

Supplementary Figure 9. CaMKIIβ promotes Cdc20 phosphorylation and dispersion from the centrosome in neurons.

a, Lysates of cortical neurons were immunoprecipitated with the Cdc20 or control (IgG) antibody and immunoblotted with the CaMKIIβ or Cdc20 antibody. Asterisk indicates IgG heavy chain.

b, Lysates of granule neurons electroporated with the CaMKIIβ RNAi or control U6 plasmid were immunoblotted with the CaMKIIβ, Cdc20, phosphoSer51-Cdc20, or actin antibody.

c, Granule neurons transfected with the Cdc20 RNAi or control U6 plasmid together with an expression plasmid encoding WT Cdc20-RES, S51A Cdc20-RES, S84A Cdc20-RES, S86A Cdc20-RES, or control vector and the GFP expression plasmid were analyzed as in Fig. 1d. S51A Cdc20-RES, but not S84A Cdc20-RES or S86A Cdc20-RES, significantly increased total dendrite length compared to WT Cdc20-RES in the background of Cdc20 RNAi (ANOVA, $p < 0.0001$). 360 neurons were measured.

d, Granule neurons transfected with the expression plasmid encoding WT CaMKIIβ, T287D CaMKIIβ, K43R CaMKIIβ, CaMKIIα, or control vector together with the fGFP expression plasmid were analyzed as in Fig. 6a. Arrows indicate Cdc20 localized to the centrosome, while arrowheads denote dispersed Cdc20. Expression of WT or T287D CaMKIIβ, but not K43R CaMKIIβ or CaMKIIα, induced dispersion of the centrosomal perinuclear Cdc20 punctum. Scale bar = 5 μm.

e, Granule neurons transfected with the expression plasmid encoding WT CaMKIIβ, T287D CaMKIIβ, K43R CaMKIIβ, CaMKIIα, or control vector together with an expression plasmid encoding GFP-centrin were analyzed as in (d). Arrows indicate the location of the centrosome based on GFP-centrin immunofluorescence, while arrowheads denote dispersed Cdc20. Expression of WT or T287D CaMKIIβ, but not K43R CaMKIIβ or CaMKIIα, induced dispersion of endogenous Cdc20 away from the centrosome as marked by GFP-centrin immunofluorescence. Scale bar = 5 μm.

f, Granule neurons transfected with the CaMKIIβ RNAi or control U6 plasmid together with the fGFP expression plasmid were analyzed as in (d). The percentage of neurons displaying dispersed endogenous Cdc20 was significantly reduced in CaMKIIβ knockdown neurons compared to control U6-transfected neurons (t-test; $p < 0.01$). 334 neurons were analyzed.

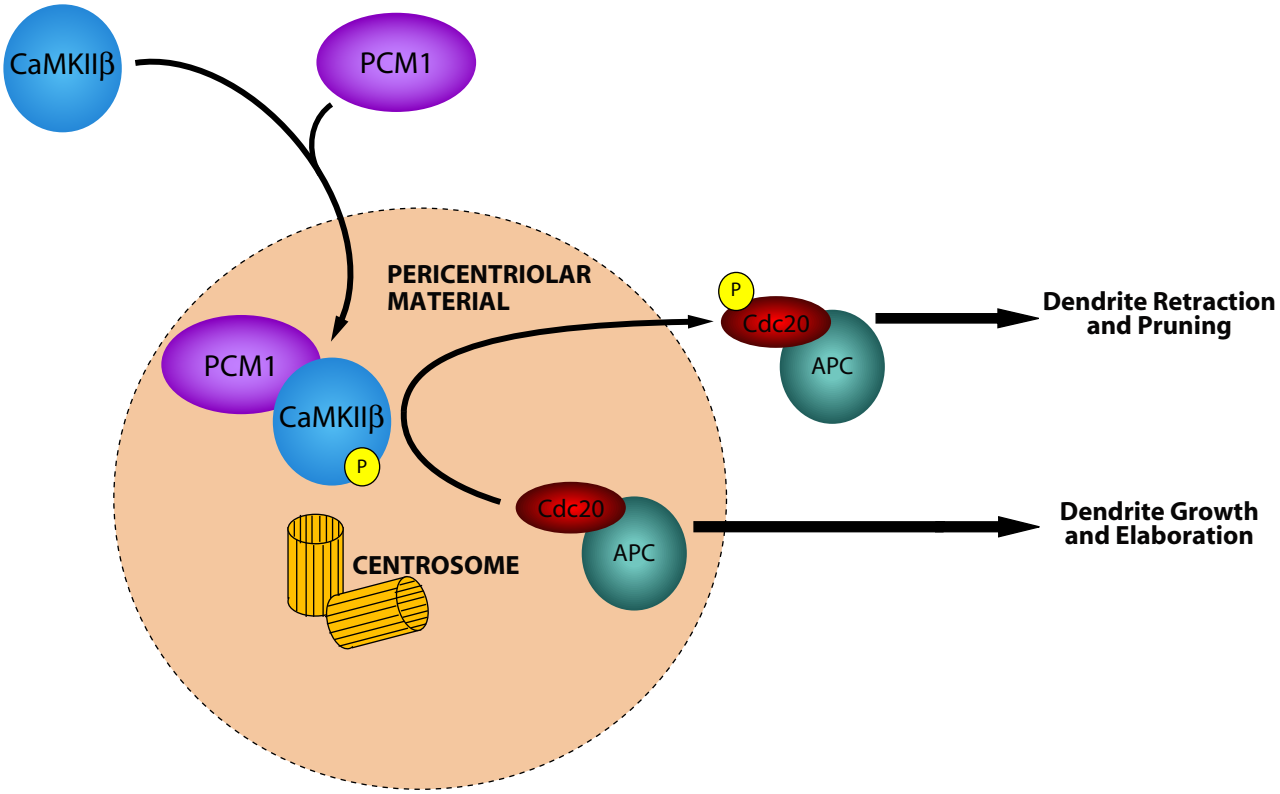
g, Granule neurons transfected with the T287D CaMKIIβ expression plasmid together with the fGFP expression plasmid were analyzed as in (d). Total dendrite length was significantly reduced in neurons with dispersed endogenous Cdc20 compared to neurons with centrosomally-enriched endogenous Cdc20 (t-test, $p < 0.01$). 180 neurons were measured.

h, Granule neurons transfected with the GFP-centrin expression plasmid were fixed on DIV0-6 and analyzed as in (e). The percentage of neurons displaying dispersed endogenous Cdc20 was significantly increased with maturation of neurons (ANOVA; $p < 0.0001$). 360 neurons per condition were analyzed.

i, Granule neurons transfected with the T287D CaMKIIβ expression plasmid or control vector together with the fGFP expression plasmid were analyzed as in Fig. 6g. The percentage of neurons with phosphoSer51-Cdc20 immunofluorescence was significantly increased in T287D CaMKIIβ-expressing neurons compared to control vector-transfected neurons (t-test, $p < 0.005$). 121 neurons were analyzed.

j, Granule neurons transfected with the CaMKIIβ or CaMKIIα RNAi or control U6 plasmid together with the GFP-centrin expression plasmid were analyzed as in (i). The percentage of neurons with phosphoSer51-Cdc20 immunofluorescence was significantly reduced in CaMKIIβ knockdown neurons compared to control U6-transfected neurons. In contrast, CaMKIIα knockdown had little or no effect on the percentage of neurons with phosphoSer51-Cdc20 immunofluorescence compared to control U6 transfection (ANOVA, $p < 0.001$). 414 neurons were analyzed.

k, Granule neurons transfected with the Cdc20 RNAi or control U6 plasmid together with an expression plasmid encoding WT Cdc20-RES, S51D Cdc20-RES, PACT-S51D Cdc20-RES, or control vector and the GFP expression plasmid were analyzed as in (c). WT Cdc20-RES and PACT-S51D Cdc20-RES, but not S51D Cdc20-RES, significantly increased total dendrite length compared to control vector in the background of Cdc20 RNAi (ANOVA, $p < 0.0001$). 360 neurons were measured.



Supplementary Figure 10. Model for the centrosomal CaMKIIβ signaling pathway in the control of dendrite patterning in the mammalian brain.

Fig. 1a

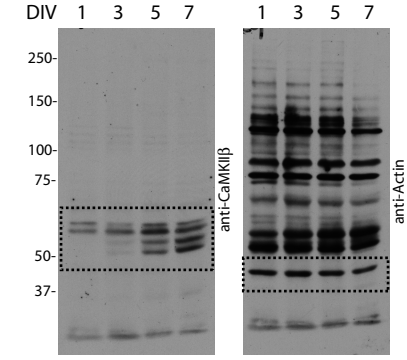


Fig. 1b

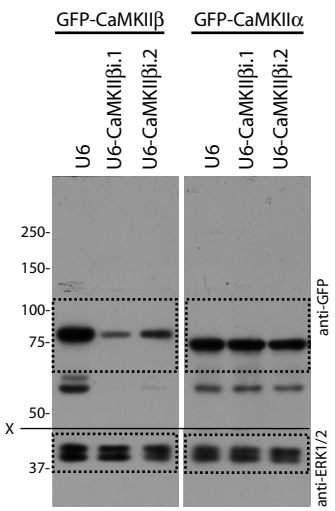


Fig. 1c

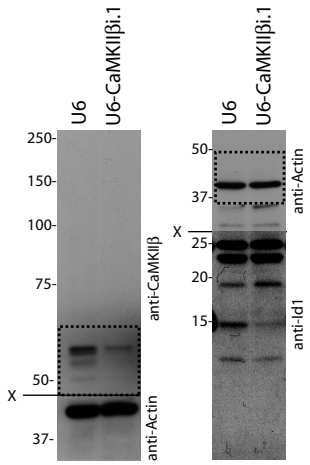


Fig. 1h

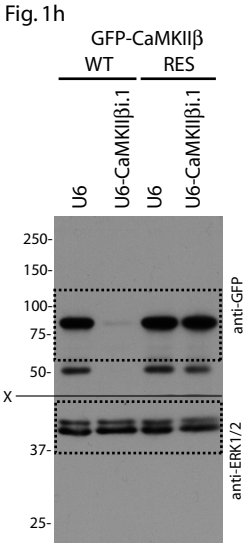


Fig. 3e

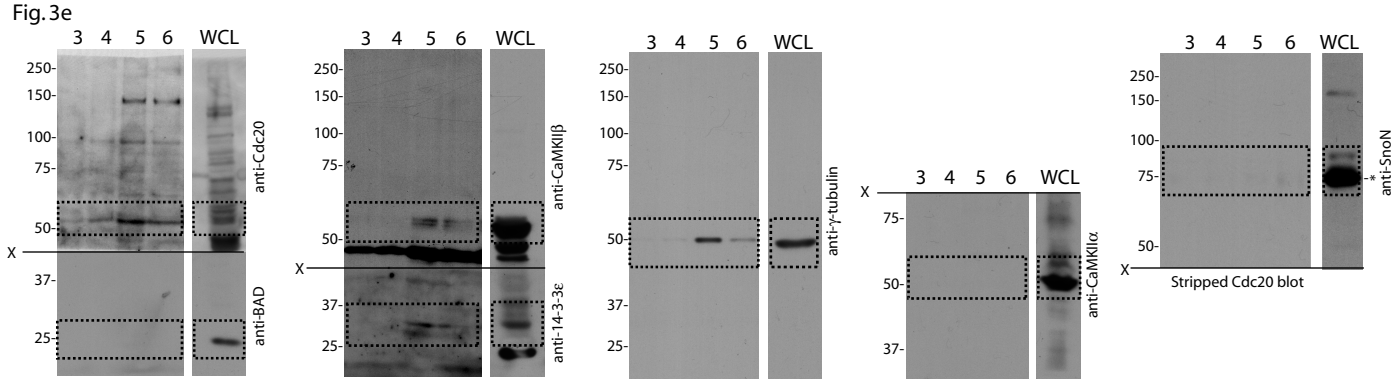


Fig. 4a

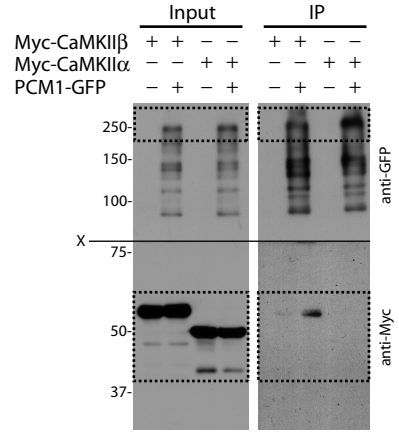


Fig. 4b

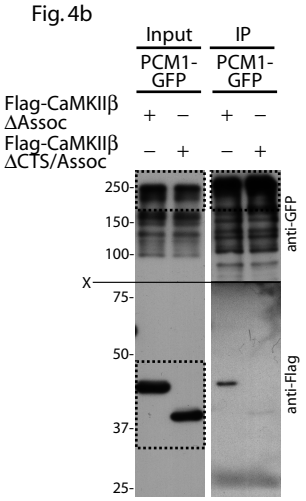


Fig. 4c

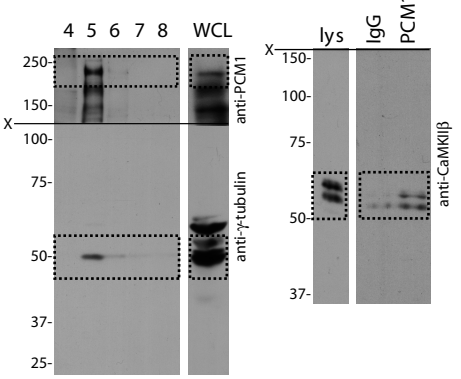


Fig. 4d

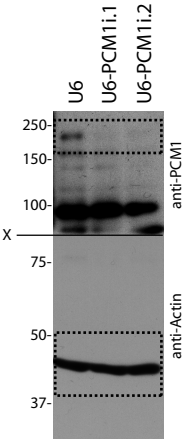


Fig. 5a

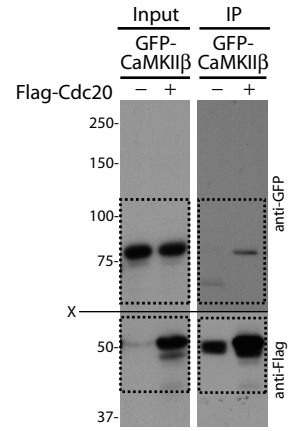


Fig. 5b

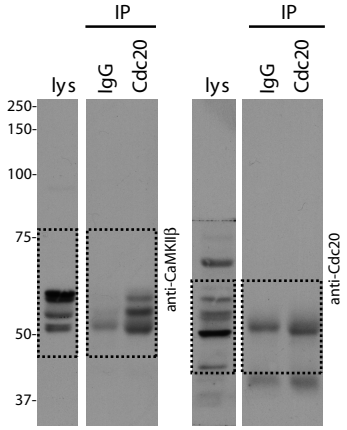


Fig. 5c

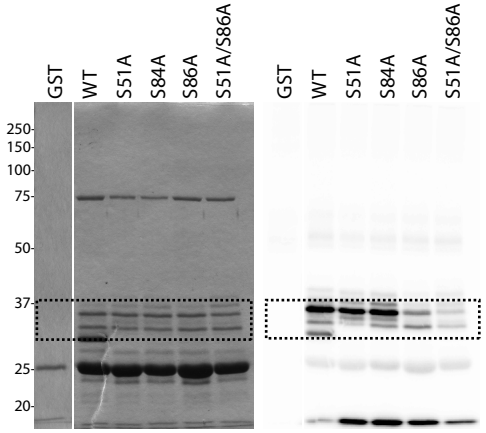


Fig. 5d

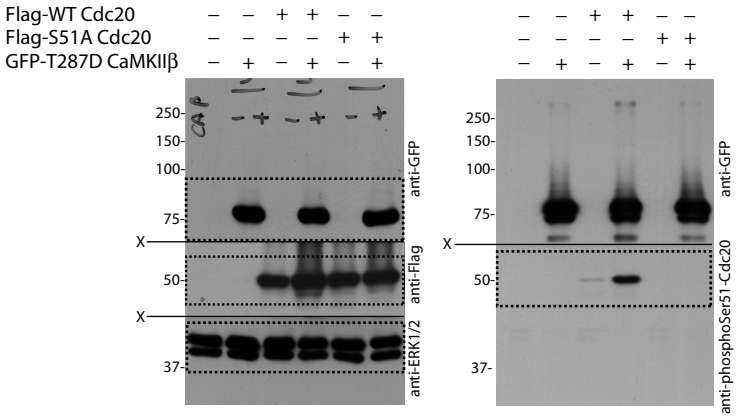
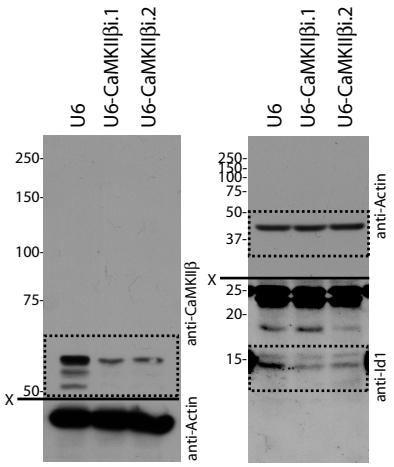


Fig. 5f



Supplementary Figure 11. Full-length blots for all cropped immunoblots shown in the main figures. Blots were incubated with primary antibody as indicated. Dashed boxes denote the cropped regions shown in figures, X's mark point where blots were cut, and asterisk indicates non-specific band.

Supplemental References

1. Konishi, Y., Stegmuller, J., Matsuda, T., Bonni, S. & Bonni, A. Cdh1-APC controls axonal growth and patterning in the mammalian brain. *Science* **303**, 1026-1030 (2004).
2. Stegmuller, J.*, et al.* Cell-intrinsic regulation of axonal morphogenesis by the Cdh1-APC target SnoN. *Neuron* **50**, 389-400 (2006).