Lkb1 regulates granule cell migration and cortical folding of the cerebellar cortex

Kaitlyn E. Ryan, Patrick S. Kim, Jonathan T. Fleming, Emily Brignola, Frances Y. Cheng, Ying Litingtung and Chin Chiang

Supplementary Figures

Figure S1. Histological analysis of *Lkb1^{cko}* cerebella at additional stages. A. Hematoxylin and eosin staining of control and *Lkb1^{cko}* mid-vermal cross-sections at the indicated stages. Scalebar 500 µm. B. H and E staining of 3 control and 3 *Lkb1^{cko}* cerebella at P14. C. H and E staining of control and *Lkb1^{cko}* cerebella at embryonic day 16.5 (E16.5).

Figure S2. Cross sectional area and IGL area do not differ between control and *Lkb1^{cko}* cerebella at P30. A-B Representative mid-sagittal cross-sections of P30 control and *Lkb1^{cko}* cerebella. Lower panels illustrate how IGL area was measured. C. Quantification of mid-saggttal cross-sectional area reveals no difference between control and *Lkb1^{cko}*. D. Quantification of perimeter reveals a persistent increase in perimeter in *Lkb1^{cko}*. E. Quantification of mid-sagittal IGL area reveals that there is no significant difference at P30. n=4, *, $p<0.05$, Student's t-test. Con = control.

Figure S3. Cilia length and Gli1 RNA are not altered in GCPs lacking *Lkb1*. A. Representative staining for cilia marker ARL13B and DNA in P6 control and *Lkb1^{cko}* cerebella. Dashed line denotes pial surface. Scalebar 10 µm. B. Quantification of average cilium length using ARL13B staining at P6. n=3, ns, Student's t-test. C-D. *In situ* hybridization for *Gli1* in Control (C) and *Lkb1^{cko}* cerebella at birth (P0).

Figure S4. Validation of automated cell counting using CellProfiler. Example of automated cell counting using the program CellProfiler. (A) Sample image showing BrdU/Ki67 labeled raw

image (left) and 8 bit images of BrdU (middle top) and Ki67 (middle bottom). Far right panels show cell outlines after being processed by CellProfiler. Green outlines denote counted cells, yellow and magenta outlines indicate discarded cells due to size. (B) Comparison of handcounting and CellProfiler (CP) counted images for BrdU and Ki67. n=3, no significant difference, student's paired t-test.

Figure S5. *Lkb1*^{cko} do not exhibit difference in the proportion of Ki67+BrdU+ cells, Calbindin+ Purkinje cells, or Pax2+ Interneurons. (A) Quantification of proliferation using Ki67/BrdU co-labeling at P6. (B) Quantification of total number of Calbindin+ Purkinje cells in the P8 cerebellum. (C) Quantification of Pax2+ interneurons at P8. (D) Quantification of proliferation using Ki67/BrdU co-labeling at P3 after BrdU chase for 24 hours. n=3, no significant difference, student's paired t-test.

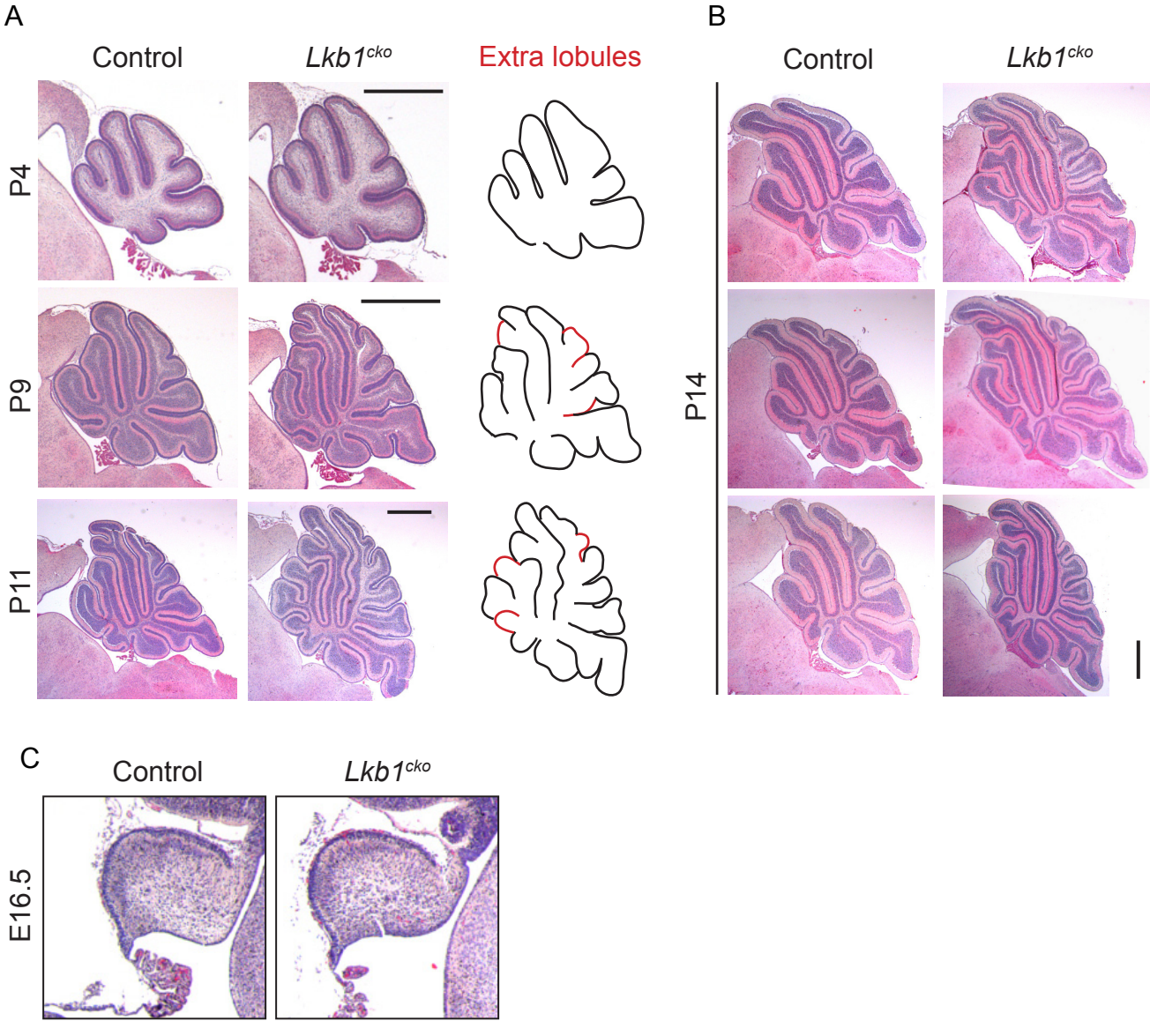
Figure S6. Loss of *Lkb1* does not alter outer or inner EGL area. A-B. P7 control (A) and *Lkb1*^{cko} (B) sections stained for Ki67 and p27Kip1 to mark proliferating and differentiated GCPs, respectively. C. Quantification of the proportion of proliferative (Ki67+, p27Kip1-), differentiated (Ki67-, p27Kip1+) or double-positive (Ki67+, p27Kip1+) GCPs in the EGL of control and *Lkb1*^{cko} cerebella. n=3, p=ns, Student's paired t-test. Note: images shown are representative images; quantification was done over the entire cerebellum using automated cell counting in CellProfiler D. Quantification of average cell size in the inner and outer EGL. E-F. Representative images of P7 Ki67 staining of control and *Lkb1*^{cko} cerebella to mark the outer EGL. G. Quantification of Ki67+ outer EGL area revealed oEGL area was not significantly different between control and *Lkb1*^{cko} cerebella, either when measured by lobe (left) or across the entire cerebellum (right). H-I. p27Kip1 staining of P7 control and *Lkb1*^{cko} cerebella to mark the iEGL. J. Quantification of p27Kip1+ inner EGL area revealed iEGL area was not significantly different between control

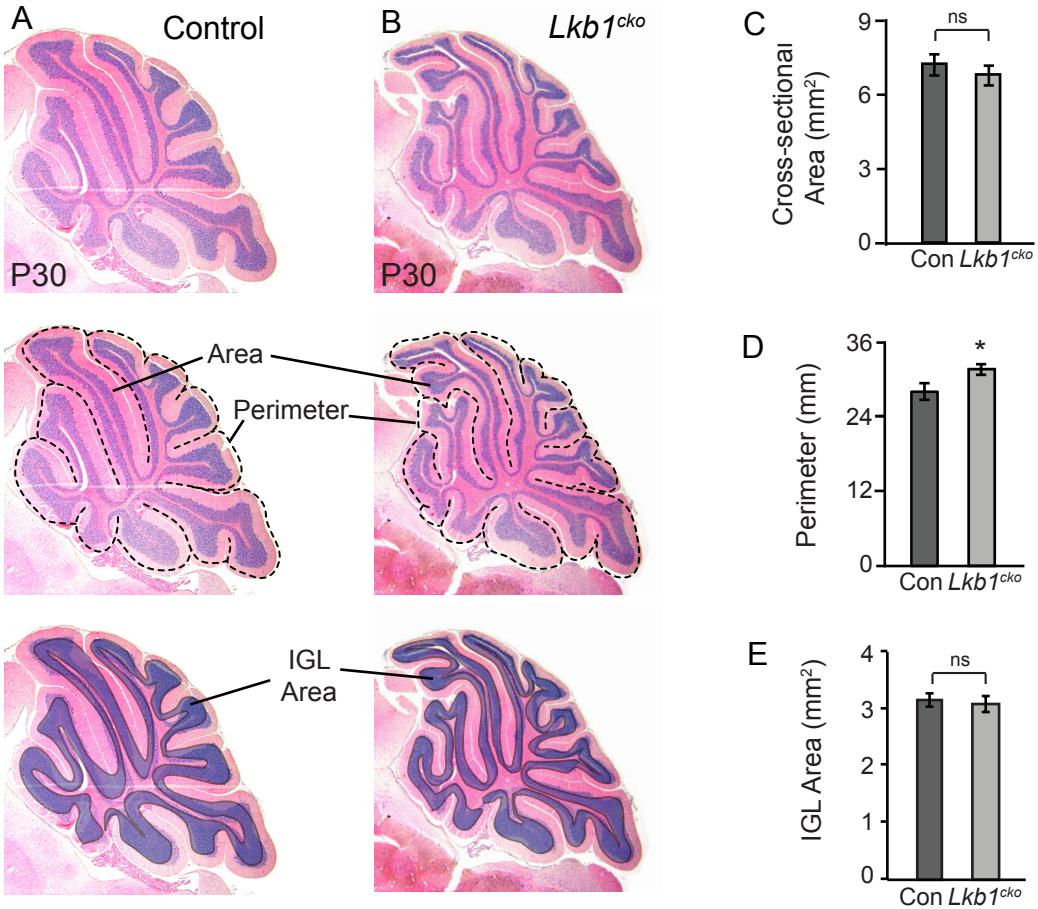
and *Lkb1*^{cko} cerebella, either when measured by lobe (left) or across the entire cerebellum (right). For all analysis, n=3, p=ns, Student's paired t-test.

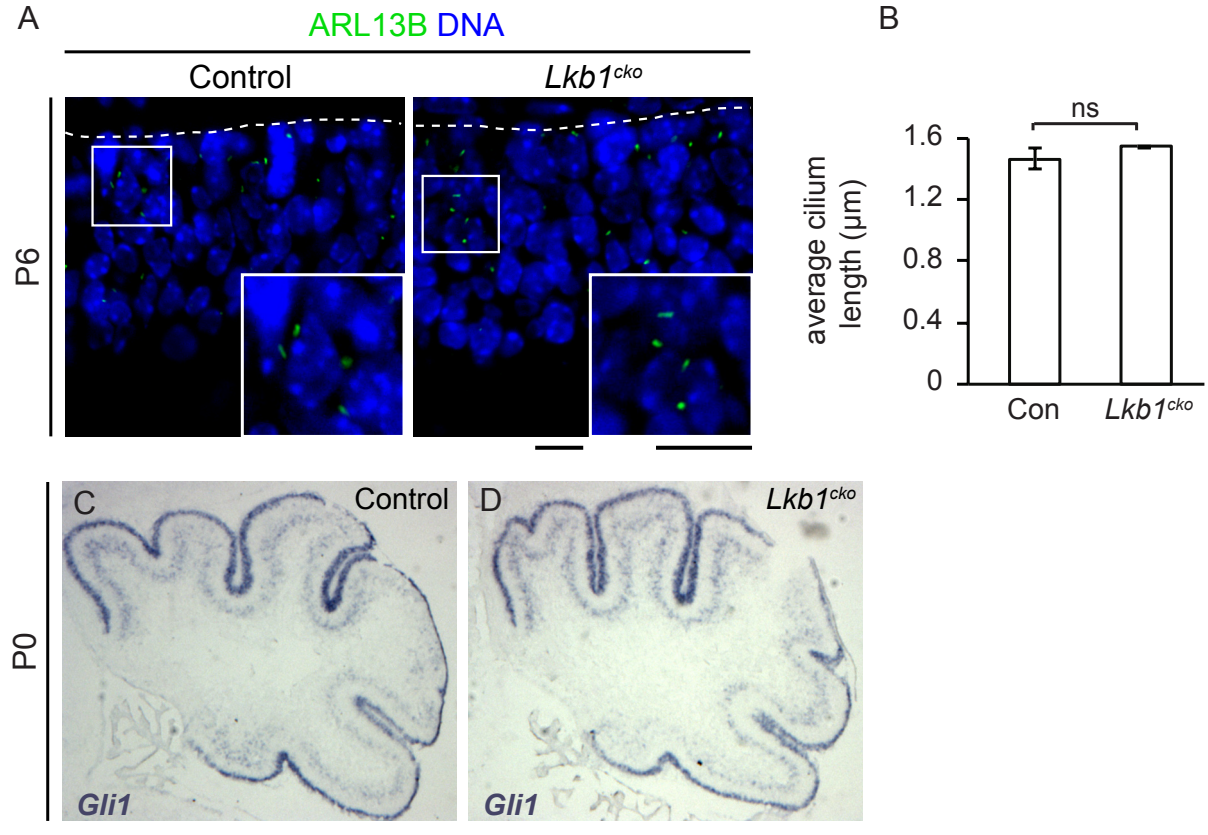
Figure S7. Loss of *Lkb1* does not affect the plane of GCP division. A. Orientation of GCP divisions relative to cell surface. B-C. Aurora B, phospho-histone H3, and Dapi co-staining at postnatal day 2 (P2) labels mitotic DNA, spindle-associated microtubules and DNA, respectively. Dashed line denotes pial surface. Arrows indicate cells enlarged in neighboring panels. C. Distribution of GCP division angles at P2. D. Distribution of GCP division angles at P4. E. Distribution of division angles at P6. n=3, * = $p<0.05$, Student's t-test. Scalebar 5 µm.

Figure S8. *TSC1*^{cko} develop normally. A-B. Hematoxylin and eosin staining of P60 control and *TSC1*^{cko} cerebella reveals that *TSC1*^{cko} cerebella develop normally.

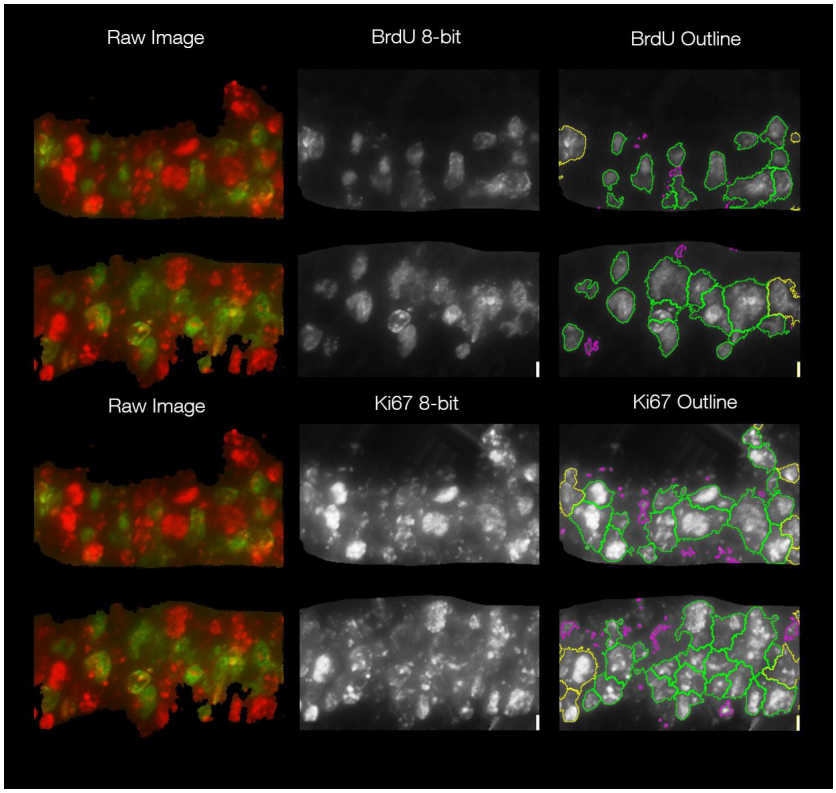
Figure S9. Full-length blots of main figures.


A
Control
Lkb1cko
Extra lobules
P4
P9
P11
B
Control
Lkb1cko
P14
C
Control
Lkb1cko
E16.5

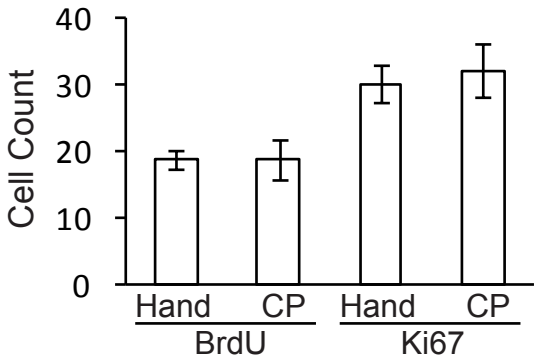

A
Control
B
Lkb1cko
P30
P30
Area
Perimeter
IGL
Area
C
Cross-sectional
Area (mm2)
9
6
3
0
ns
Con Lkb1cko
D
Perimeter (mm)
36
24
12
0
*
Con Lkb1cko
E
IGL Area (mm2)
4
3
2
1
0
ns
Con Lkb1cko

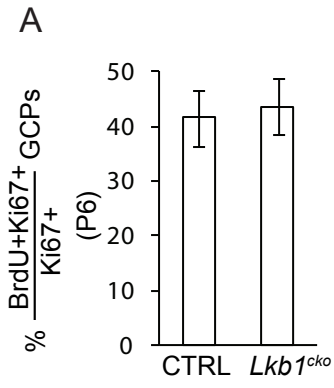

A
ARL13B DNA
Control
Lkb1cko
P6
B
ns
average cilium length (μm)
1.6
1.2
0.8
0.4
0
Con
Lkb1cko
P0
C
Control
Gli1
D
Lkb1cko
Gli1

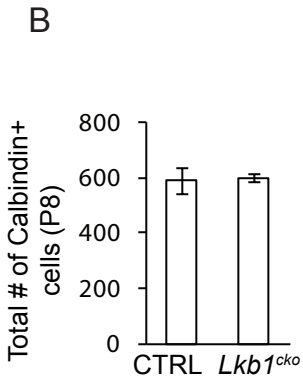
A

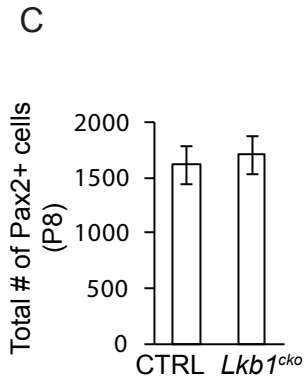

Raw Image
BrdU 8-bit
BrdU Outline
Raw Image
Ki67 8-bit
Ki67 Outline

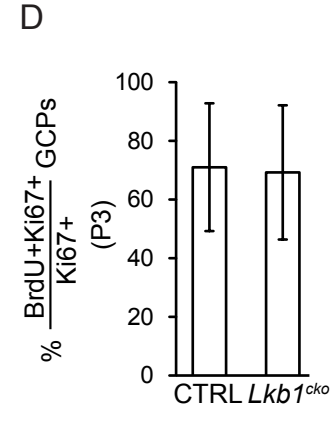
B

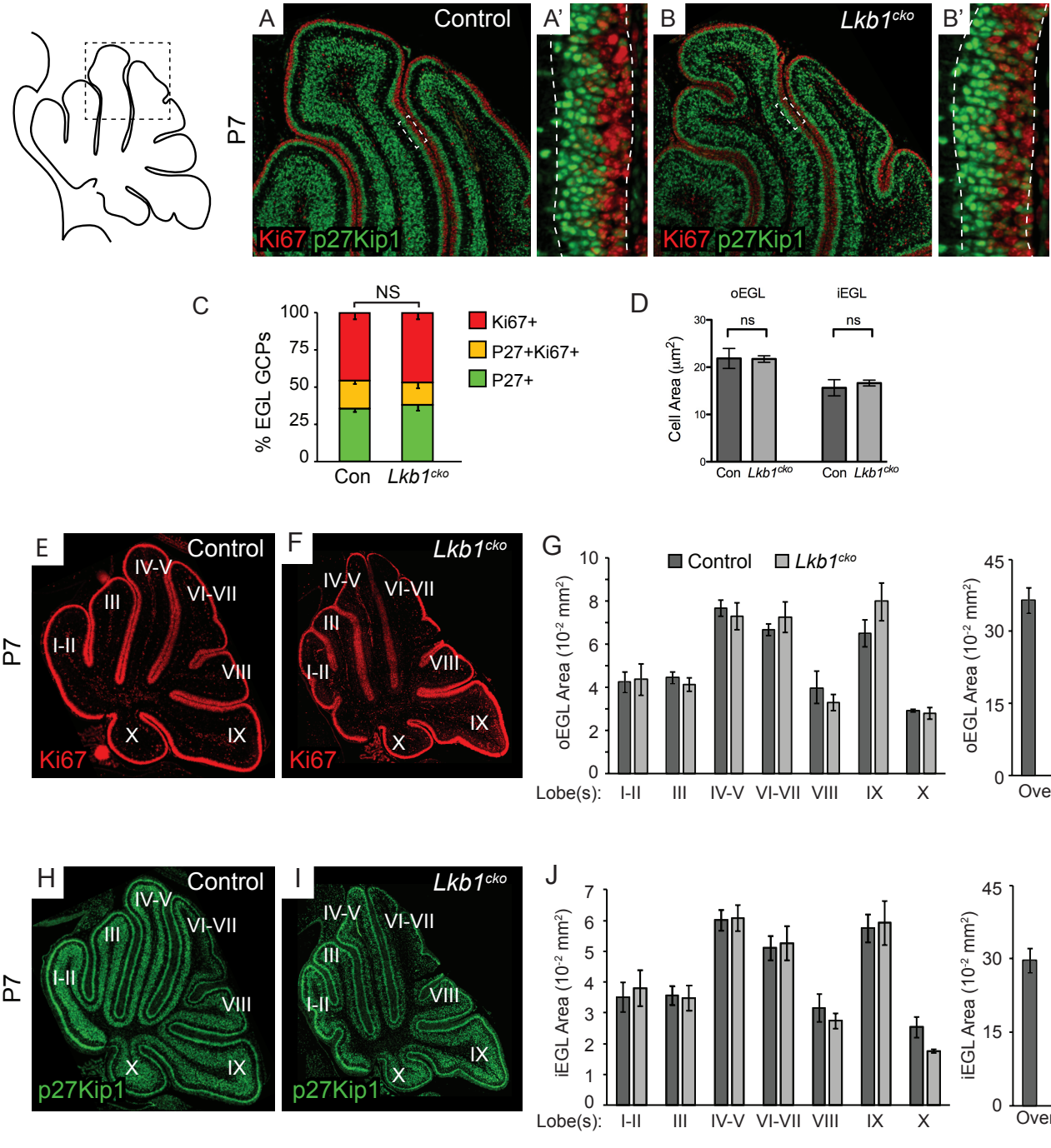

Cell Count
40
30
20
10
0
Hand
CP
Hand
CP
BrdU
Ki67

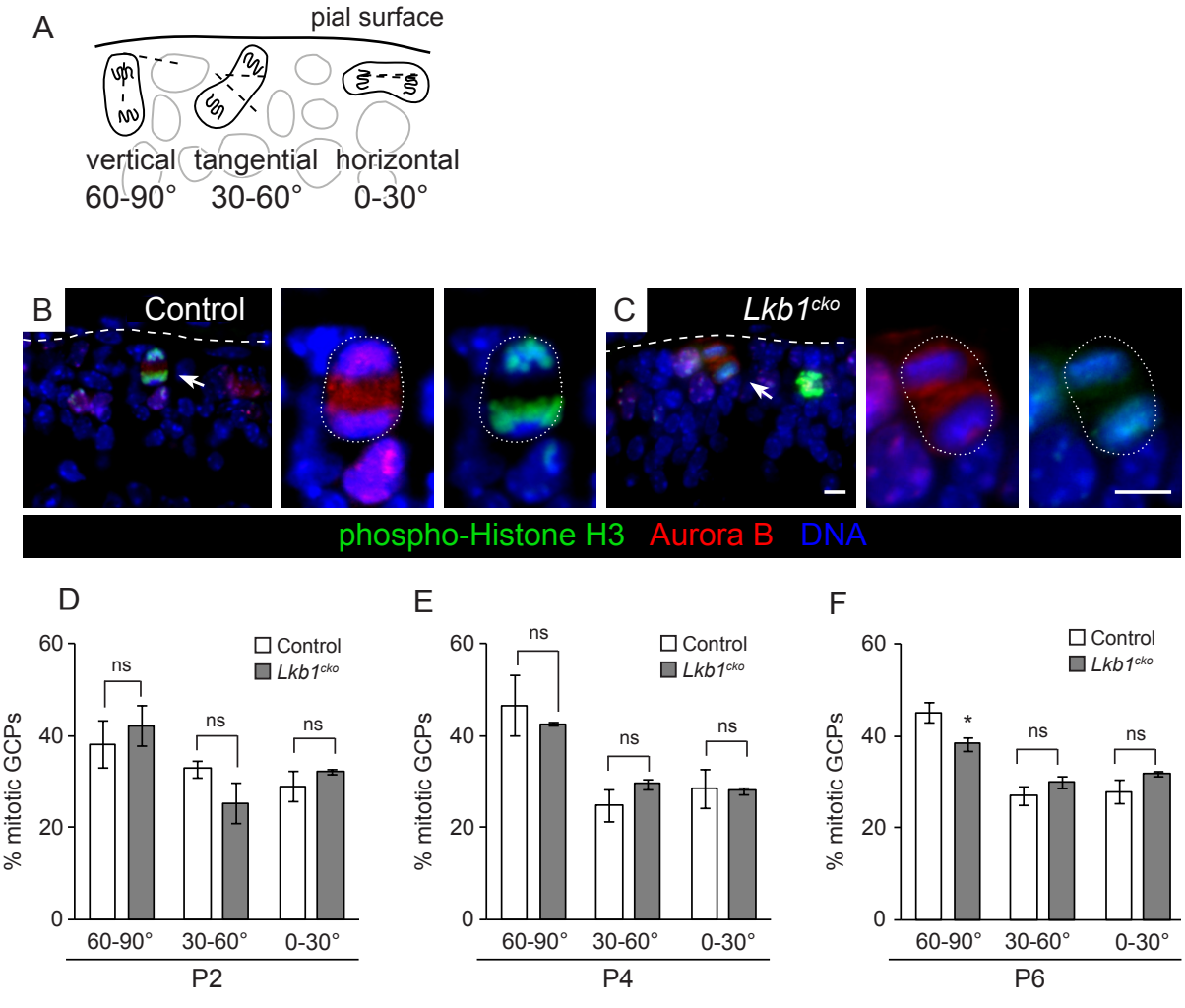

A
% BrdU+Ki67+ / Ki67+ GCPs (P6)
50
40
30
20
10
0
CTRL
Lkb1cko


B
Total # of Calbindin+ cells (P8)
800
600
400
200
0
CTRL
Lkb1cko


C
Total # of Pax2+ cells (P8)
2000
1500
1000
500
0
CTRL
Lkb1cko


D
% BrdU+Ki67+ / Ki67+ GCPs (P3)
100
80
60
40
20
0
CTRL
Lkb1cko


A
Control
A'
B
Lkb1cko
B'
P7
Ki67 p27Kip1
Ki67 p27Kip1
C
NS
100
75
50
25
0
% EGL GCPs
Ki67+
P27+Ki67+
P27+
Con
Lkb1cko
D
oEGL
iEGL
30
20
10
0
Cell Area (μm2)
ns
ns
Con
Lkb1cko
Con
Lkb1cko
E
Control
F
Lkb1cko
IV-V
VI-VII
III
I-II
VIII
X
IX
Ki67
Ki67
G
Control
Lkb1cko
10
8
6
4
2
0
oEGL Area (10-2 mm2)
Lobe(s):
I-II
III
IV-V
VI-VII
VIII
IX
X
45
30
15
0
oEGL Area (10-2 mm2)
Overall
H
Control
I
Lkb1cko
p27Kip1
p27Kip1
J
7
6
5
4
3
2
1
0
iEGL Area (10-2 mm2)
Lobe(s):
I-II
III
IV-V
VI-VII
VIII
IX
X
45
30
15
0
iEGL Area (10-2 mm2)
Overall


A
pial surface
vertical
60-90°
tangential
30-60°
horizontal
0-30°
B
Control
C
Lkb1cko
phospho-Histone H3
Aurora B
DNA
D
E
F
% mitotic GCPs
Control
Lkb1cko
ns
*
60-90°
30-60°
0-30°
P2
P4
P6

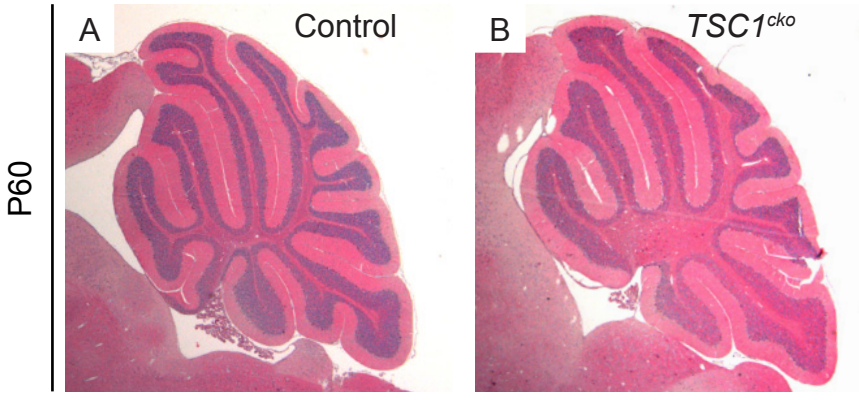

A
Control
B
TSC1cko
P60

Figure 1D

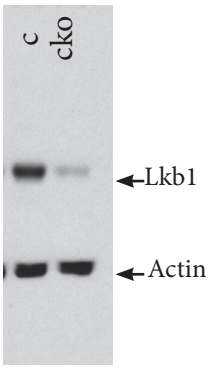
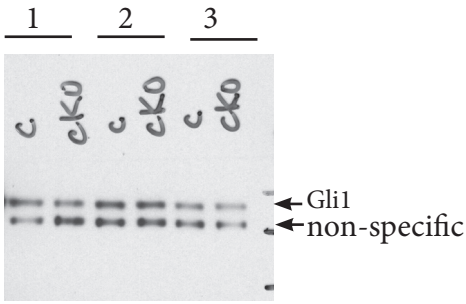


Figure 3C



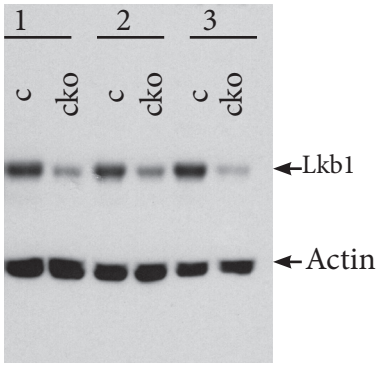
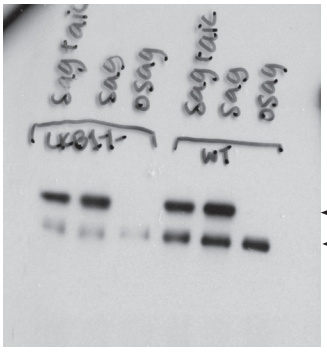


Figure 3F



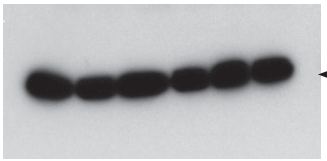


Figure 3G

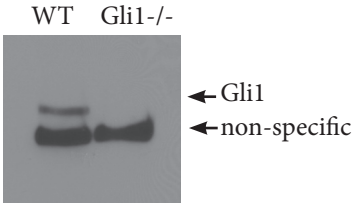


Figure 6C

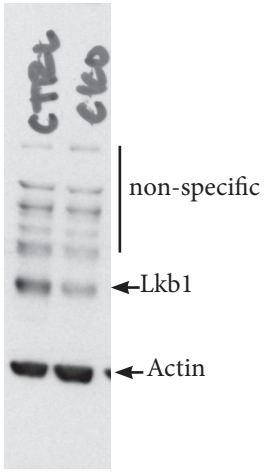
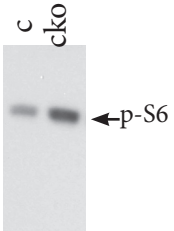


Figure 6D



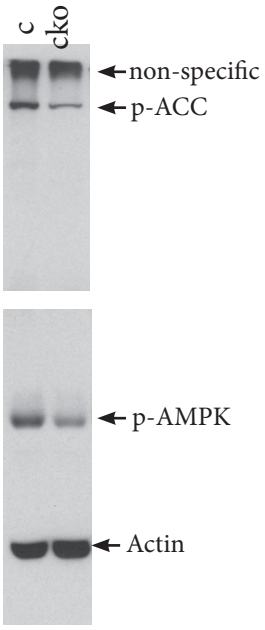


Figure 6E