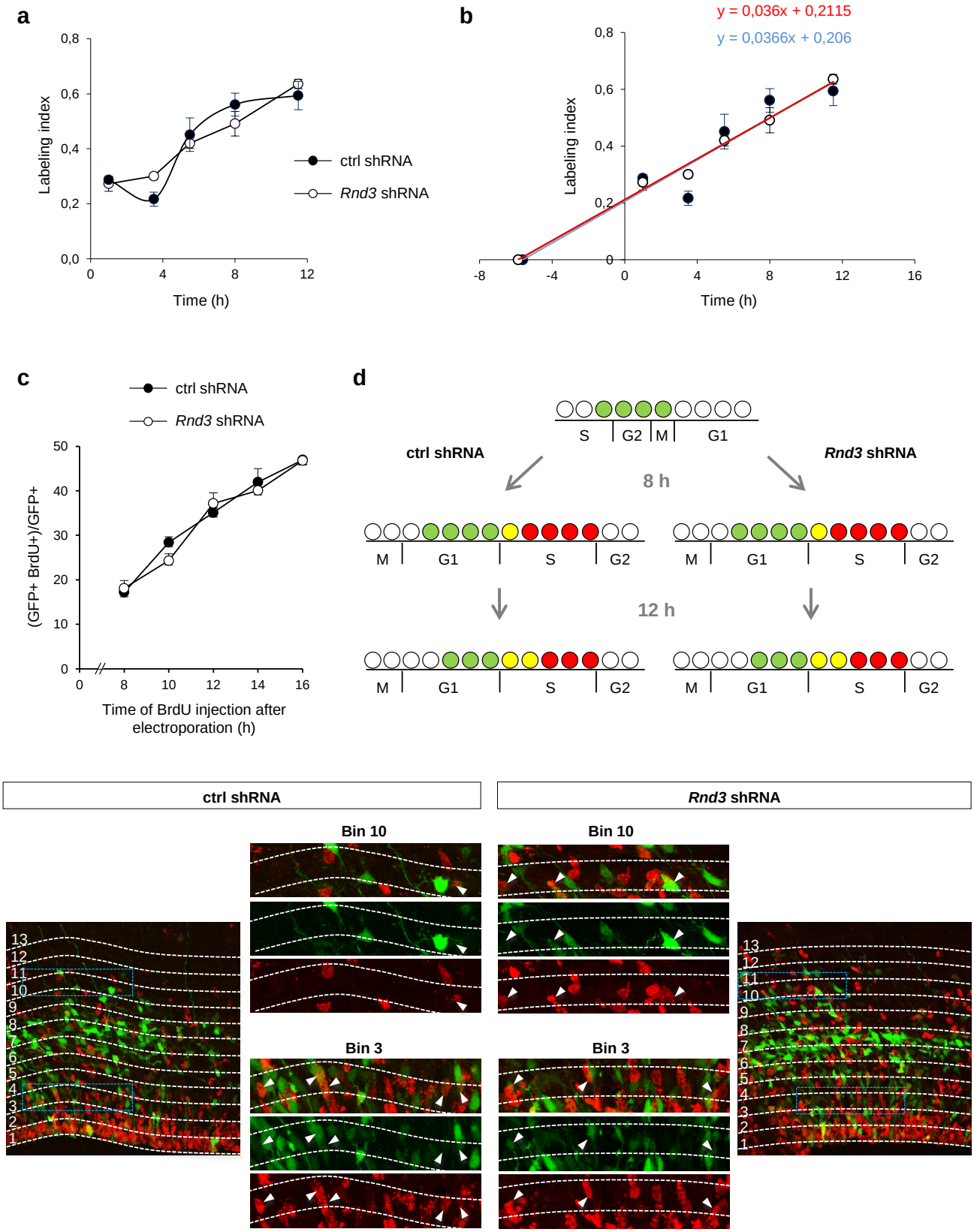
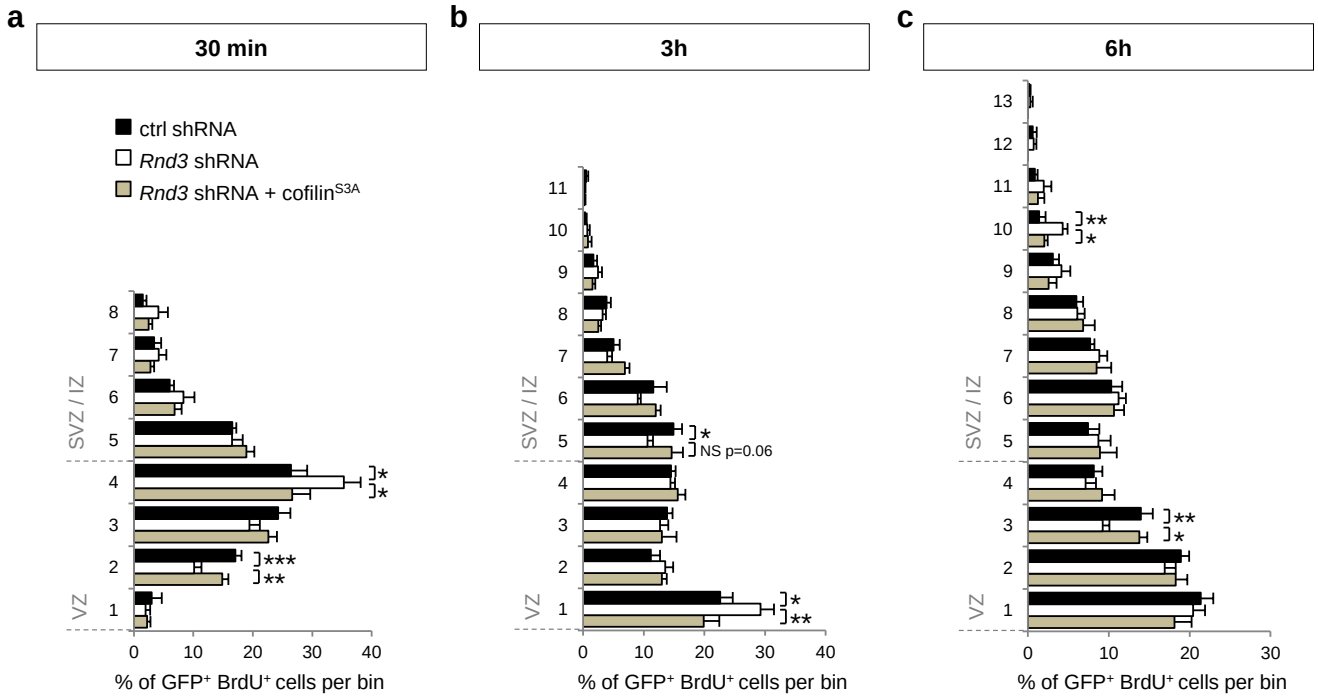
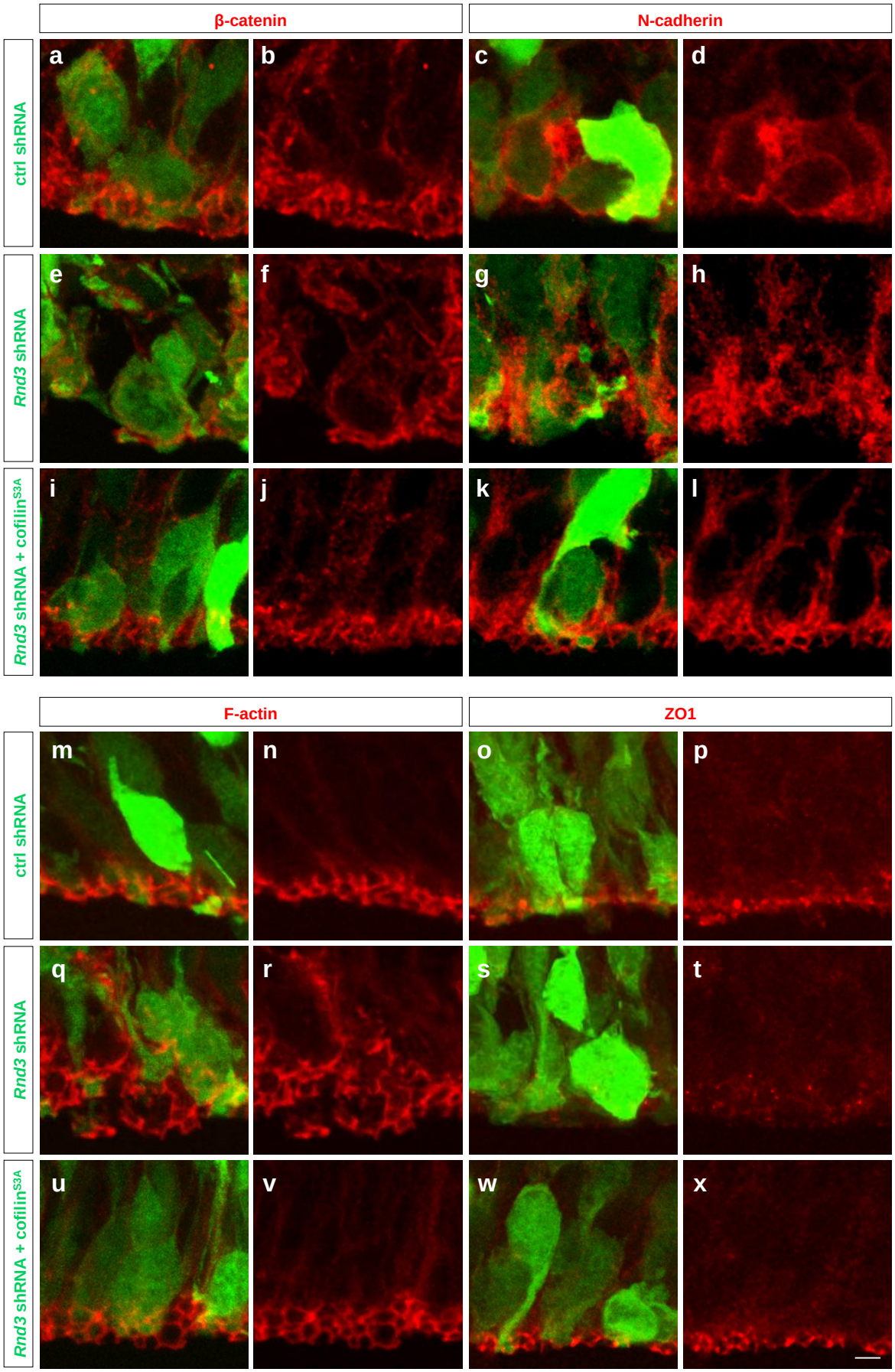

a
Labeling index
Time (h)
ctrl shRNA
Rnd3 shRNA
b
y = 0,036x + 0,2115
y = 0,0366x + 0,206
Labeling index
Time (h)
c
ctrl shRNA
Rnd3 shRNA
(GFP+ BrdU+)/GFP+
Time of BrdU injection after electroporation (h)
d
S
G2
M
G1
ctrl shRNA
Rnd3 shRNA
8 h
12 h
e
ctrl shRNA
Rnd3 shRNA
Bin 10
Bin 3

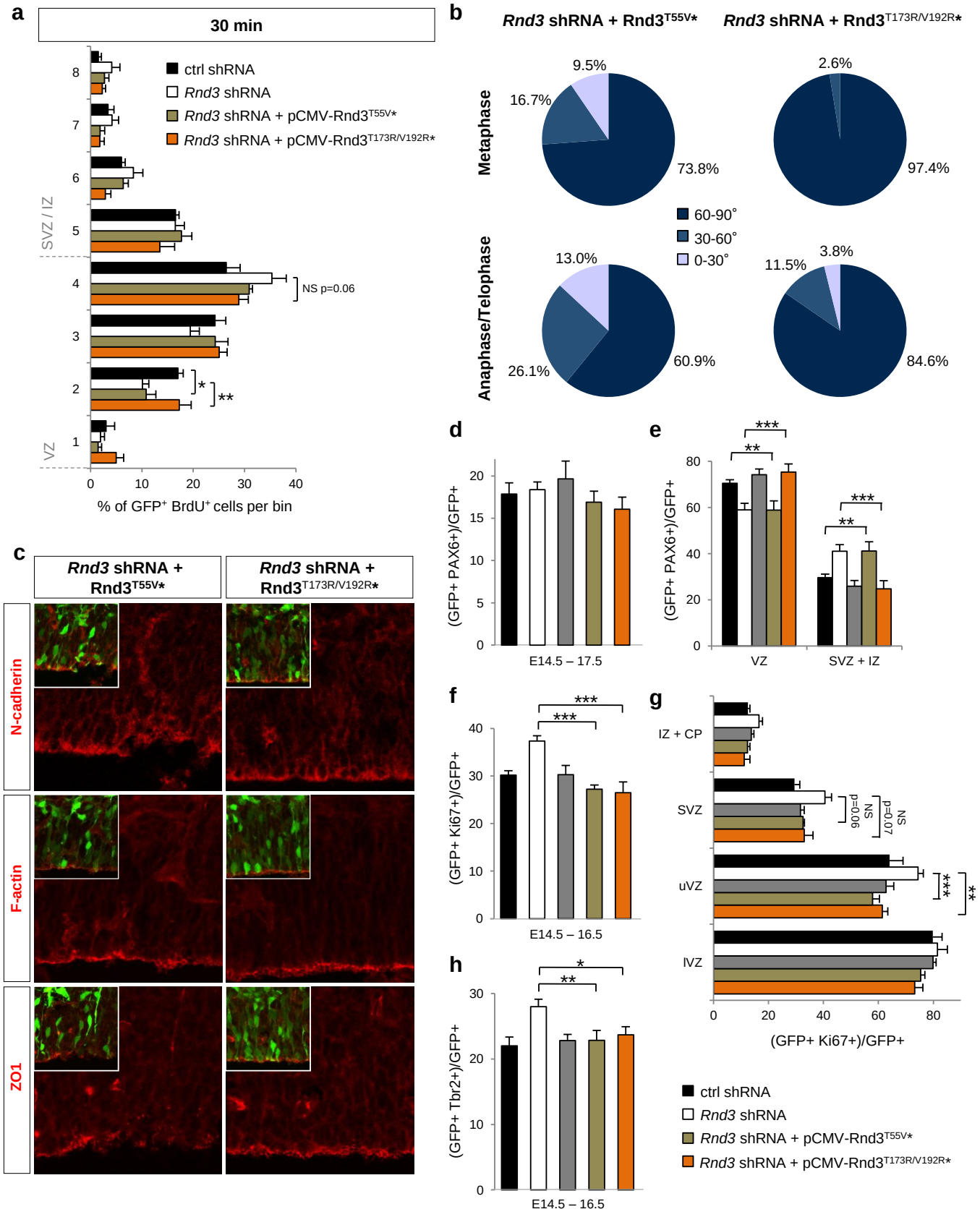
Supplementary Figure S1: *Rnd3* knockdown does not affect cell cycle progression in cortical progenitors. (**a**) We first used the S-phase cumulative labeling technique to analyze *ex vivo* the cell cycle kinetics of control and *Rnd3* deficient cells 2 days after electroporation. However, as previously described [58], the labeling index was not linear suggesting that electroporation in the VZ targets a synchronized cohort of cycling precursors, which precluded the use of BrdU cumulative labeling as a valid technique to measure the cell cycle paramaters of the GFP^+ precursors. (**b**) However, if a linear curve is drawn between the values obtained for the different time points, the equation obtained with control cells (blue) is equivalent to the equation obtained with *Rnd3* knockdown cells (red) suggesting that the cell cycle parameters are similar between these two populations of cells. Data are presented as the mean ± s.e.m. from at least three different sections at each time point. (**c**) G1 duration is similar in control and *Rnd3* deficient cells as the number of GFP^+ cells that had incorporated BrdU is similar between the two conditions at different time points after electroporation. Data are presented as the mean ± s.e.m. from at least six sections prepared from three or four embryos obtained from two or three litters. (**d**) Schematic representation of the results obtained in (c) 8 and 12 h after electroporation. Electroporation is optimal during S, G2 and M phases and thus targets cells preferentially during these phases [58]. The electroporated cells (GFP^+ in green) progress similarly through G1 after electroporation at E14.5 with ctrl shRNA or *Rnd3* shRNA. Yellow cells = GFP^+ $BrdU^+$ cells; Red cells = GFP^- $BrdU^+$ cells; White cells = GFP^- $BrdU^-$ cells. (**e**) Comparison of the distribution of GFP^+ $BrdU^+$ cells between ctrl and *Rnd3* shRNA after 6 h BrdU pulse across the cortex divided into bins as indicated, with bin 1 being at the ventricle surface and bin 4 at the VZ–SVZ boundary. Blue rectangles show the areas enlarged in the insets and white arrowheads indicate double positive cells.



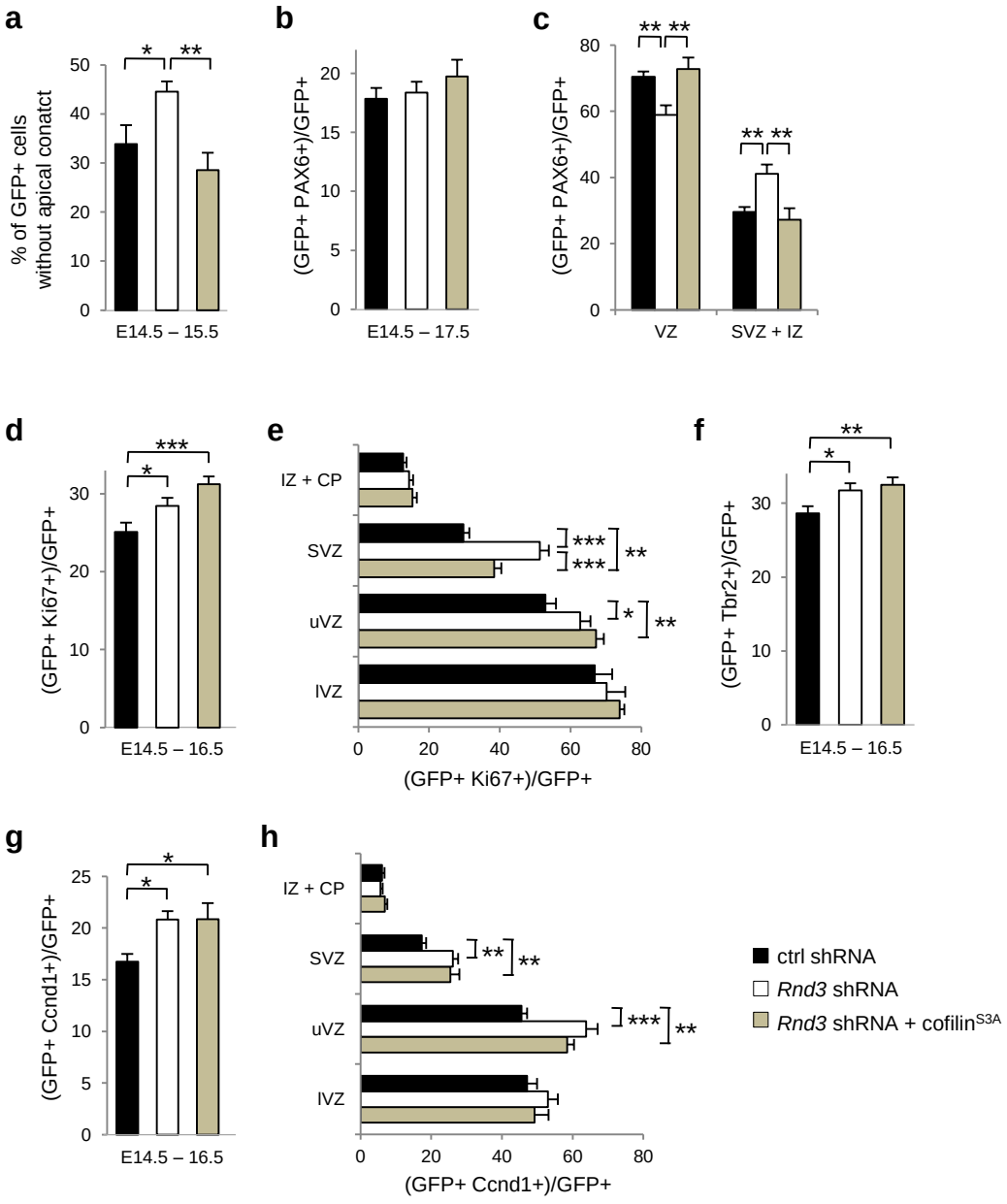
Supplementary Figure S2: Rnd3 regulates interkinetic nuclear migration of cortical neuron progenitors by depolymerizing F-actin. (**a-c**) Coelectroporation of cofilin^{S3A}, a non-phosphorylatable form of cofilin that depolymerizes F-actin, fully rescues the defects observed after *Rnd3* silencing, 30 min (a), 3 h (b) and 6 h (c) after the pulse of BrdU. GFP$^+$ BrdU$^+$ cells in a given bin are expressed as percentage of the total number of GFP$^+$ BrdU$^+$-labeled cells in all bins. Data are presented as the mean ± s.e.m. from seven sections prepared from four embryos obtained from two or three litters. One way ANOVA followed by a Fisher PLSD post hoc test; *p<0.05, **p<0.01, ***p<0.001.


β-catenin
N-cadherin
ctrl shRNA
Rnd3 shRNA
Rnd3 shRNA + cofilinS3A
a
b
c
d
e
f
g
h
i
j
k
l
F-actin
ZO1
ctrl shRNA
Rnd3 shRNA
Rnd3 shRNA + cofilinS3A
m
n
o
p
q
r
s
t
u
v
w
x

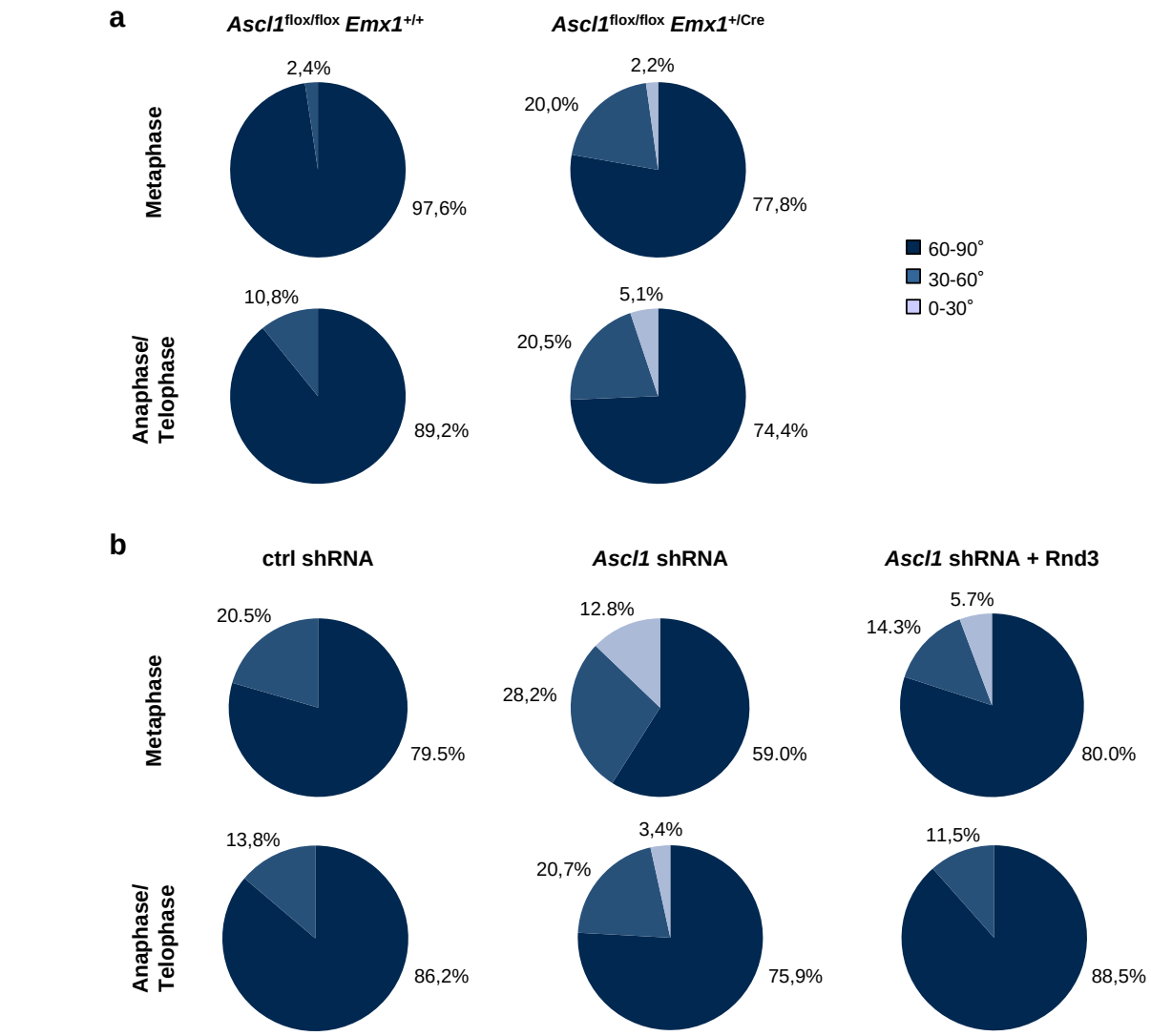
Supplementary Figure S3: *Rnd3* is required for the maintenance of adherens junctions. Close up views of electroporated cells shown in Figure 3. (**a-x**) Cortices immunostained for β-catenin (red in a, b, e, f, i, j), N-cadherin (red in c, d, g, h, k, l), F-actin (red in m, n, q, r, u, v) and ZO1 (red in o, p, s, t, w, x), 24 h after the co-electroporation at E14.5 of GFP (green) and ctrl shRNA (a, b, c, d, m, n, o, p,), *Rnd3* shRNA (e, f, g, h, q, r, s, t) or *Rnd3* shRNA + cofilin^{S3A} (i, j, k, l, u, v, w, x). Scale bar represents 5 µm.

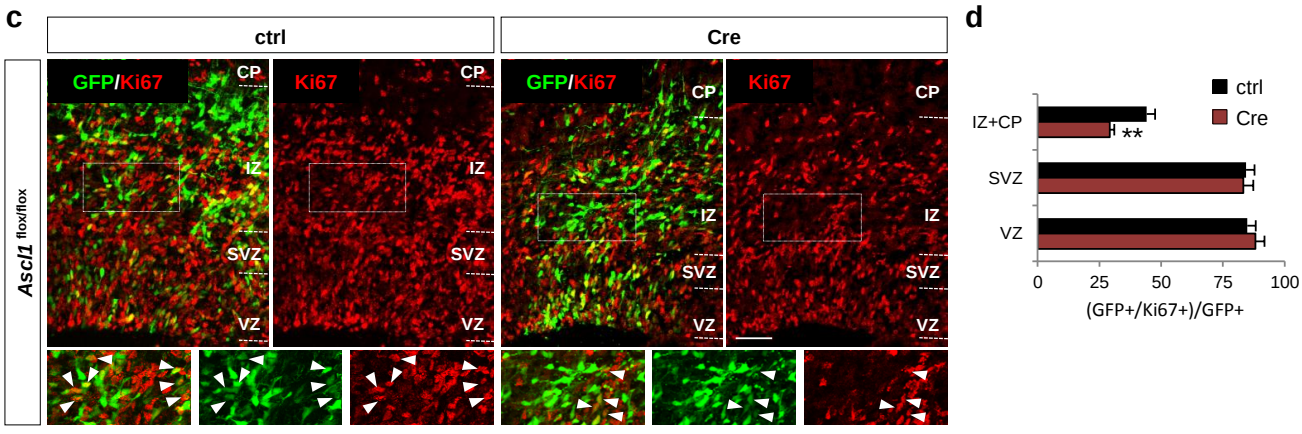

a
30 min
ctrl shRNA
Rnd3 shRNA
Rnd3 shRNA + pCMV-Rnd3T55V*
Rnd3 shRNA + pCMV-Rnd3T173R/V192R*
SVZ / IZ
VZ
NS p=0.06
*
**
% of GFP+ BrdU+ cells per bin
b
Rnd3 shRNA + Rnd3T55V*
Rnd3 shRNA + Rnd3T173R/V192R*
Metaphase
Anaphase/Telophase
9.5%
16.7%
73.8%
2.6%
97.4%
13.0%
26.1%
60.9%
3.8%
11.5%
84.6%
60-90°
30-60°
0-30°
c
Rnd3 shRNA + Rnd3T55V*
Rnd3 shRNA + Rnd3T173R/V192R*
N-cadherin
F-actin
ZO1
d
(GFP+ PAX6+)/GFP+
E14.5 – 17.5
e
(GFP+ PAX6+)/GFP+
VZ
SVZ + IZ
f
(GFP+ Ki67+)/GFP+
E14.5 – 16.5
g
IZ + CP
SVZ
uVZ
lVZ
p=0.06
NS
p=0.07
NS
(GFP+ Ki67+)/GFP+
h
(GFP+ Tbr2+)/GFP+
E14.5 – 16.5
ctrl shRNA
Rnd3 shRNA
Rnd3 shRNA + pCMV-Rnd3T55V*
Rnd3 shRNA + pCMV-Rnd3T173R/V192R*

Supplementary Figure S4: Rnd3 acts in apical progenitors by antagonizing RhoA signaling but not in basal progenitors. The mutations of Rnd3 residues Thr173 and Val192 to arginines disrupts the ability of Rnd3 to bind the RhoA effector ROCKI and to block its kinase activity. Rnd3$^{T173R/V192R}$* was as efficient as wild-type Rnd3* at rescuing the defects induced by *Rnd3* knockdown in cortical progenitors, suggesting that Rnd3 activities in apical and basal progenitors do not require interaction with ROCKI (the star indicates silent point mutations in the sequence recognized by *Rnd3* shRNA). Rnd3 can also bind to and stimulate the activity of the Rho GTPase-activating protein p190RhoGAP and this interaction is disrupted by mutation of residue T55 into valine. Rnd3^{T55V} was partially or totally inactive at rescuing interkinetic nuclear migration, cleavage plane orientation, adherens junctions and radial glia cell displacement defects observed in *Rnd3* silenced apical progenitors indicating that Rnd3 in these progenitors acts by inhibiting RhoA via the stimulation of the Rho GAP activity of p190RhoGAP. However Rnd3^{T55V} was as efficient as wild-type Rnd3 at rescuing basal progenitor proliferation, demonstrating that Rnd3 activity in basal progenitors do not require inhibition of RhoA. See corresponding main figures for details on experiments. (b) Metaphase: *Rnd3* shRNA + Rnd3^{T55V}* n=43 cells, *Rnd3* shRNA + Rnd3$^{T173R/V192R}$* n=39 cells. Anaphase/Telophase: *Rnd3* shRNA + Rnd3^{T55V}* n=23 cells, *Rnd3* shRNA + Rnd3$^{T173R/V192R}$* n=26 cells. Data in a, b, d-h are presented as the mean ± s.e.m; one way ANOVA followed by a Fisher PLSD post hoc test; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

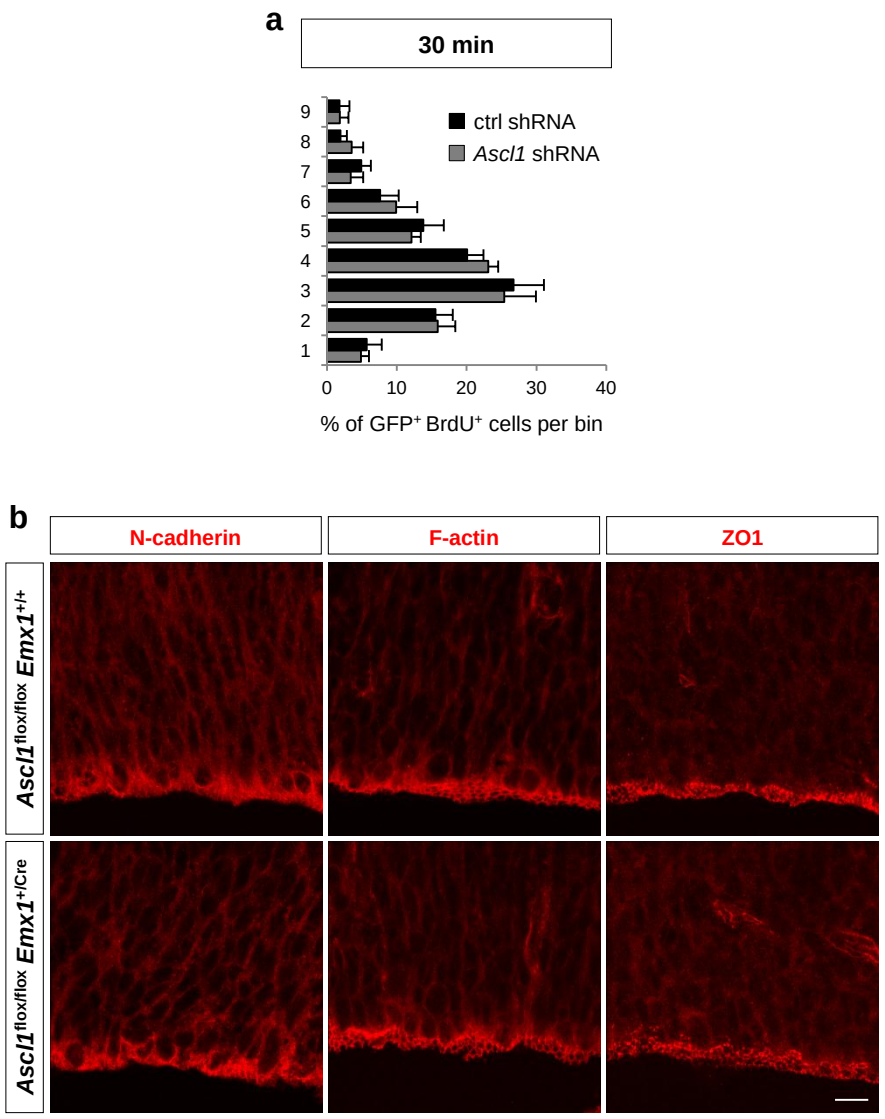


Supplementary Figure S5: Rnd3 regulates cortical neurogenesis through actin dependent and independent mechanisms. (**a-c**) Cofilin^{S3A} expression rescues the loss of the apical endfoot (a) as well as the displacement of radial glia cells (b, c) induced by *Rnd3* silencing. (**d-h**) However, cofilin^{S3A} does not rescue the increase of basal progenitor proliferation observed after *Rnd3* knockdown suggesting that Rnd3 regulates this process through actin-independent mechanism. Data are presented as the mean ± s.e.m. from at least six sections prepared from four embryos obtained from two or three litters. One way ANOVA followed by a Fisher PLSD post hoc test; *p<0.05, **p<0.01, ***p<0.001.


a
Ascl1flox/flox Emx1+/+
Ascl1flox/flox Emx1+/Cre
Metaphase
Anaphase/ Telophase
2,4%
97,6%
2,2%
20,0%
77,8%
10,8%
89,2%
5,1%
20,5%
74,4%
60-90˚
30-60˚
0-30˚
b
ctrl shRNA
Ascl1 shRNA
Ascl1 shRNA + Rnd3
Metaphase
Anaphase/ Telophase
20.5%
79.5%
12.8%
28,2%
59.0%
5.7%
14.3%
80.0%
13,8%
86,2%
3,4%
20,7%
75,9%
11,5%
88,5%


c
ctrl
Cre
Ascl1 flox/flox
GFP/Ki67
Ki67
CP
IZ
SVZ
VZ
d
ctrl
Cre
IZ+CP
SVZ
VZ
**
0
25
50
75
100
(GFP+/Ki67+)/GFP+

Supplementary Figure S6: *Ascl1* regulates the proliferation and cleavage plane orientation of progenitors in the embryonic cerebral cortex. (**a, b**) Cleavage plane orientation is affected in *Ascl1*-deleted (**a**) and *Ascl1*-silenced (**b**) apical progenitors and restored when Rnd3 is co-expressed. Pie charts show the percentage of cells with color coded cleavage angles during metaphase and ana-telophase in *Ascl1*$^{flox/flox}$ Emx1$^{+/+}$ (Metaphase n=42 cells, Ana/telophase n= 37 cells) and *Ascl1*$^{flox/flox}$ *Emx1*$^{+/Cre}$ cortices (Metaphase n=45 cells, Ana/telophase n= 39 cells) (a) or 24 h after the electroporation (E14.5) of ctrl shRNA (Metaphase n=39 cells, Ana/telophase n= 29 cells), *Ascl1* shRNA (Metaphase n=39 cells, Ana/telophase n= 29 cells) or *Ascl1* shRNA + pCMV-Rnd3 (Metaphase n=35 cells, Ana/telophase n= 26 cells) (b). Cells were analyzed from four embryos obtained from two litters in (a) and from at least six embryos obtained from three litters in (b). (**c**, **d**) *Ascl1*$^{flox/flox}$ cortices electroporated at E13.5 with GFP (control panel) or GFP and the recombinase Cre (Cre panel) and immunostained at E15.5 for GFP (green) and Ki67 (red). White rectangles show the areas enlarged in the insets and white arrowheads indicate double positive cells. (**d**) Percentage of electroporated cells that are Ki67^{+} in the different zones of the cortex. Data are presented as the mean ± s.e.m. from five sections prepared from three embryos obtained from two litters. Student's *t*-test; **p<0.01. Scale bar represents 50 µm (c).



Supplementary Figure S7: Deletion of *Ascl1* in the embryonic cerebral cortex does not affect interkinetic nuclear migration and adherens junctions. (**a**) Cerebral cortices of E14.5 embryos were electroporated in utero with *Ascl1* shRNA or ctrl shRNA and subjected to 30 min BrdU pulse labeling 24 h after the electroporation. $GFP^+/BrdU^+$ cells in a given bin are expressed as percentage of the total number of $GFP^+/BrdU^+$-labeled cells in all bins. Data are presented as the mean ± s.e.m. from six sections prepared from five embryos obtained from two or three litters. (**b**) Images of cortices after immunostaining for N-cadherin, F-actin and ZO1 (in red) show no difference between *Ascl1*$^{flox/flox}$ Emx1$^{+/+}$ and *Ascl1*$^{flox/flox}$ Emx1$^{+/Cre}$ embryos. Scale bar represents 10 µm (b).