Expanded View Figures

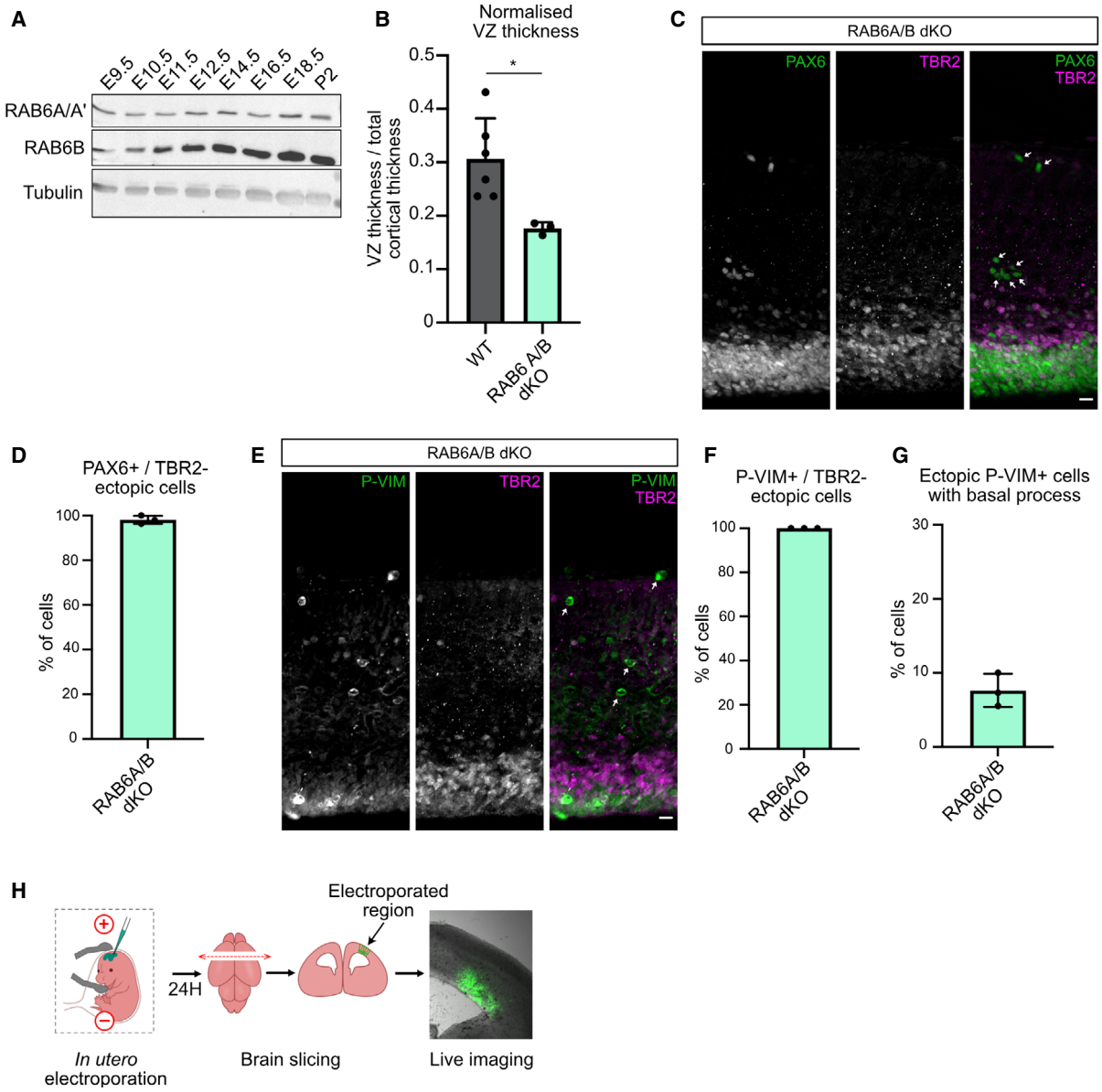

Figure EV1. RAB6 A/B dKO leads to ectopic TBR2-negative basal progenitors.

A RAB6A/A' and RAB6B expression in the developing brain and at P2.
B Ventricular zone (VZ) thickness normalized to total cortical thickness in $N = 6$ WT brains and $N = 3$ *RAB6A/B* dKO. Mann–Whitney *U* test, $*P \leq 0.05$.
C PAX6 and TBR2 staining in *RAB6A/B* dKO E15.5 brains. Arrows indicate detached PAX6+/TBR2− cells. Scale bar = 25 μm.
D Percentage of ectopic PAX6+/TBR2− cells in *RAB6A/B* dKO E15.5 brains. *RAB6A/B* dKO: 470 cells from $N = 3$ brains.
E P-VIM and TBR2 staining in *RAB6A/B* dKO E15.5 brains. Arrows indicate detached P-VIM+/TBR2− cells. Scale bar = 25 μm.
F Percentage of ectopic P-VIM+/TBR2− cells in *RAB6A/B* dKO E15.5 brains. *RAB6A/B* dKO: 99 cells from $N = 3$ brains.
G Percentage of ectopic P-VIM+ cells that maintained a basal process in *RAB6A/B* dKO E15.5 brains. *RAB6A/B* dKO: 99 cells from $N = 3$ brains.
H Schematic representation of *in utero* electroporation and live imaging procedure in the mouse developing cortex.

Data information: All error bars indicate SD.

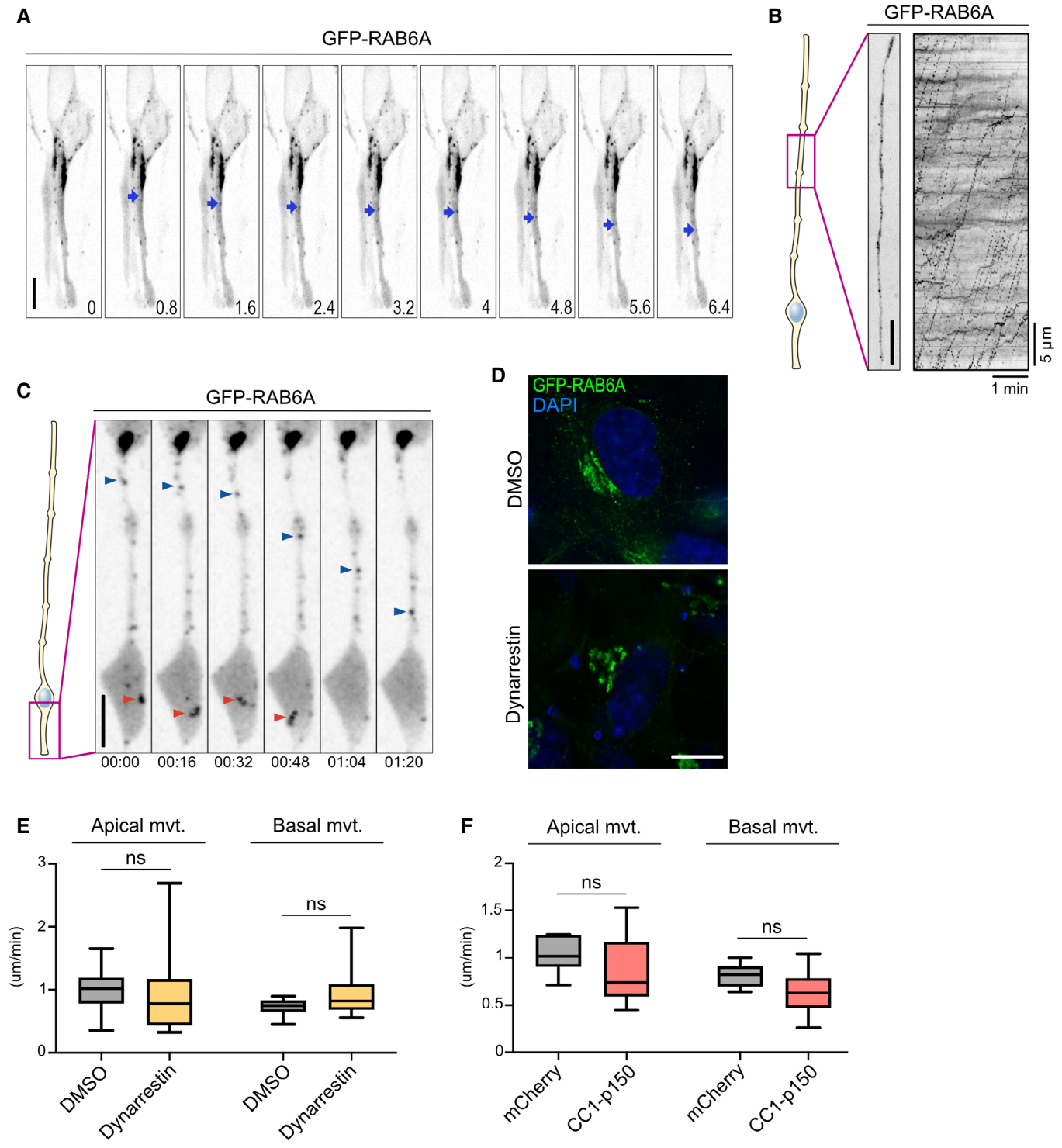
A
GFP-RAB6A
0
0.8
1.6
2.4
3.2
4
4.8
5.6
6.4
B
GFP-RAB6A
5 µm
1 min
C
GFP-RAB6A
00:00
00:16
00:32
00:48
01:04
01:20
D
GFP-RAB6A
DAPI
DMSO
Dynarrestin
E
Apical mvt.
Basal mvt.
ns
ns
(um/min)
3
2
1
0
DMSO
Dynarrestin
DMSO
Dynarrestin
F
Apical mvt.
Basal mvt.
ns
ns
(um/min)
2
1.5
1
0.5
0
mCherry
CC1-p150
mCherry
CC1-p150

Figure EV2.

Figure EV2. RAB6A dynamics in aRG cells and dynarrestin validation.

A Live imaging of GFP-RAB6A in the apical process of an aRG cell at E15.5. At 0.8 s, a tubule is budding from the Golgi, leading to the formation of an apically moving vesicle. Blue arrowhead indicates RAB6A+ vesicle. Scale bar = 5 μm.
B Live imaging of GFP-RAB6A in the basal process of an aRG cell at E15.5. Right: kymograph. Scale bar = 5 μm.
C Live imaging of GFP-RAB6A in the apical process of an aRG cell at E15.5. Red arrowhead: a RAB6A+ vesicle can be seen disappearing in the endfoot, suggesting fusion with the apical membrane. Blue arrowhead: a RAB6A+ vesicle moving apically within the apical process. Scale bar = 10 μm.
D RPE-1 cells transfected with GFP-RAB6A to visualize the Golgi apparatus architecture, and treated for 4 h with 100 μM dynarrestin or DMSO. Scale bar = 10 μm.
E Velocity of apically and basally moving RAB6A vesicles within the apical process of DMSO and dynarrestin-treated aRG cells. In all, 142 vesicles from $N = 7$ cells for DMSO, 74 vesicles from $N = 18$ cells for dynarrestin.
F Velocity of apically and basally moving RAB6A vesicles within the apical process of mcherry control and CC1-p150-expressing aRG cells. In all, 120 vesicles from $N = 17$ cells for mCherry control, 39 vesicles from $N = 11$ cells for CC1-p150.

Data information: (E, F) Mann–Whitney *U* test. Boxplots whiskers indicate min and max, boxes indicate 25^{th} and 75^{th} percentiles, and central band indicates the median.

Figure EV3. CRB3 exits the Golgi within RAB6+ vesicles.

A SBP-CRB3-GFP and GalNacT2-mCherry expression in aRG cells before and 45 min after addition of biotin. SBP-CRB3-GFP relocates from a diffuse perinuclear localization to the Golgi. Scale bar = 5 μm.
B SBP-CRB3-GFP and mCherry-RAB6A localization in HeLa cells before and 40 min after addition of biotin. Scale bar = 5 μm. White arrowheads: colocalizing foci.
C Quantification of SBP-CRB3-GFP and mCherry-RAB6A colocalization away from the Golgi apparatus 40 min after biotin addition. $N = 16$ cells from three independent experiments.
D SBP-CRB3-GFP and mCherry-RAB6A localization in dissociated aRG cells cultivated *in vitro*, 40 min after addition of biotin. Scale bar = 5 μm. Yellow arrowheads: colocalizing foci.
E Quantification of SBP-CRB3-GFP and mCherry-RAB6A colocalization away from the Golgi apparatus 40 min after biotin addition. $N = 14$ cells from three independent experiments.
F SBP-CRB3-GFP and mCherry-RAB6A localization in aRG cells cultivated within brain slices, 40 min after addition of biotin. Scale bar = 5 μm. Yellow arrowheads: colocalizing foci.
G Quantification of SBP-CRB3-GFP and mCherry-RAB6A colocalization away from the Golgi apparatus 40 min after biotin addition. $N = 15$ cells from three independent experiments.

Data information: All error bars indicate SD.

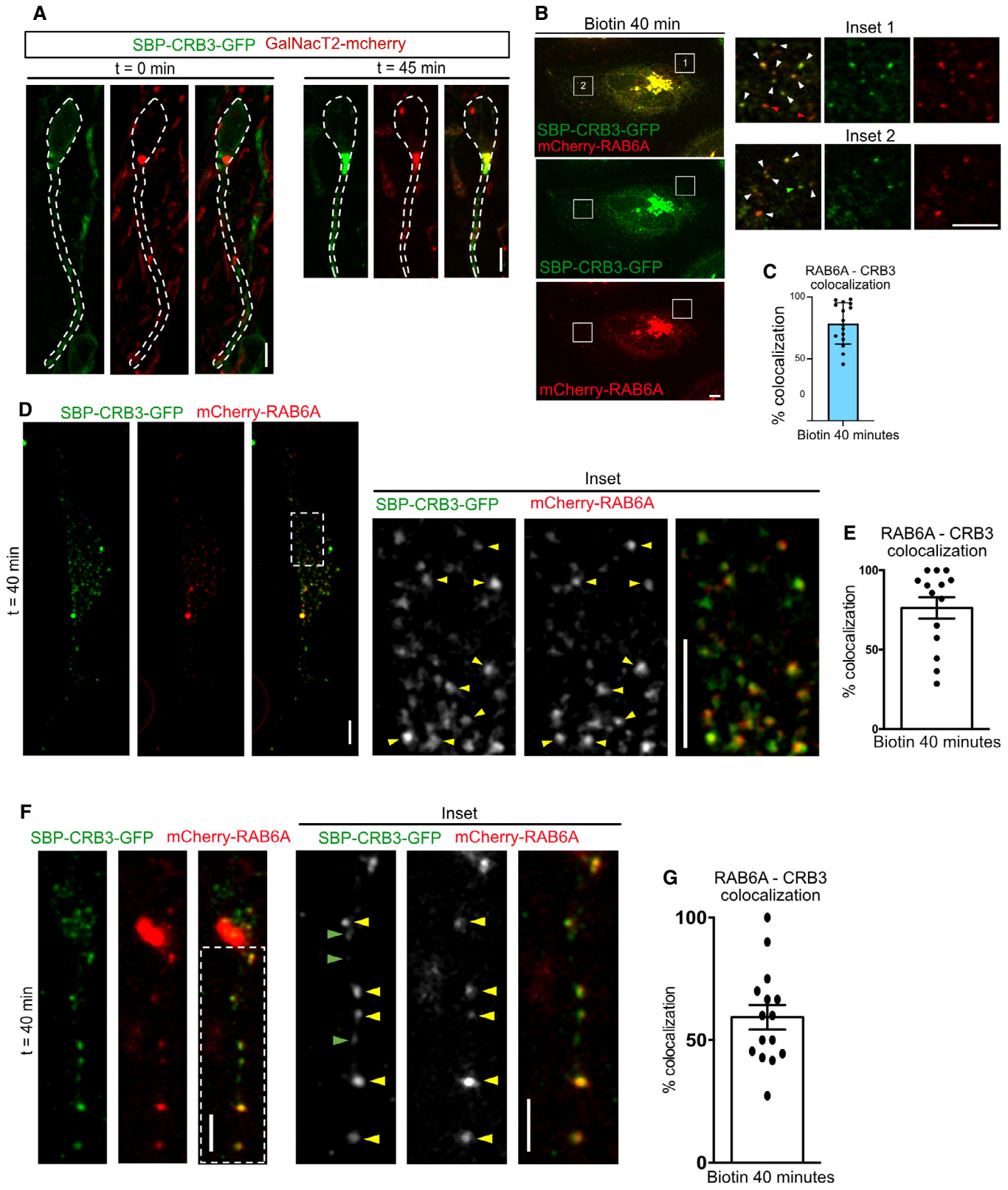
A
SBP-CRB3-GFP GalNacT2-mcherry
t = 0 min
t = 45 min
B
Biotin 40 min
SBP-CRB3-GFP
mCherry-RAB6A
SBP-CRB3-GFP
mCherry-RAB6A
Inset 1
Inset 2
C
RAB6A - CRB3 colocalization
% colocalization
100
50
0
Biotin 40 minutes
D
SBP-CRB3-GFP mCherry-RAB6A
t = 40 min
Inset
SBP-CRB3-GFP mCherry-RAB6A
E
RAB6A - CRB3 colocalization
% colocalization
100
50
0
Biotin 40 minutes
F
SBP-CRB3-GFP mCherry-RAB6A
Inset
SBP-CRB3-GFP mCherry-RAB6A
t = 40 min
G
RAB6A - CRB3 colocalization
% colocalization
100
50
0
Biotin 40 minutes

Figure EV3.

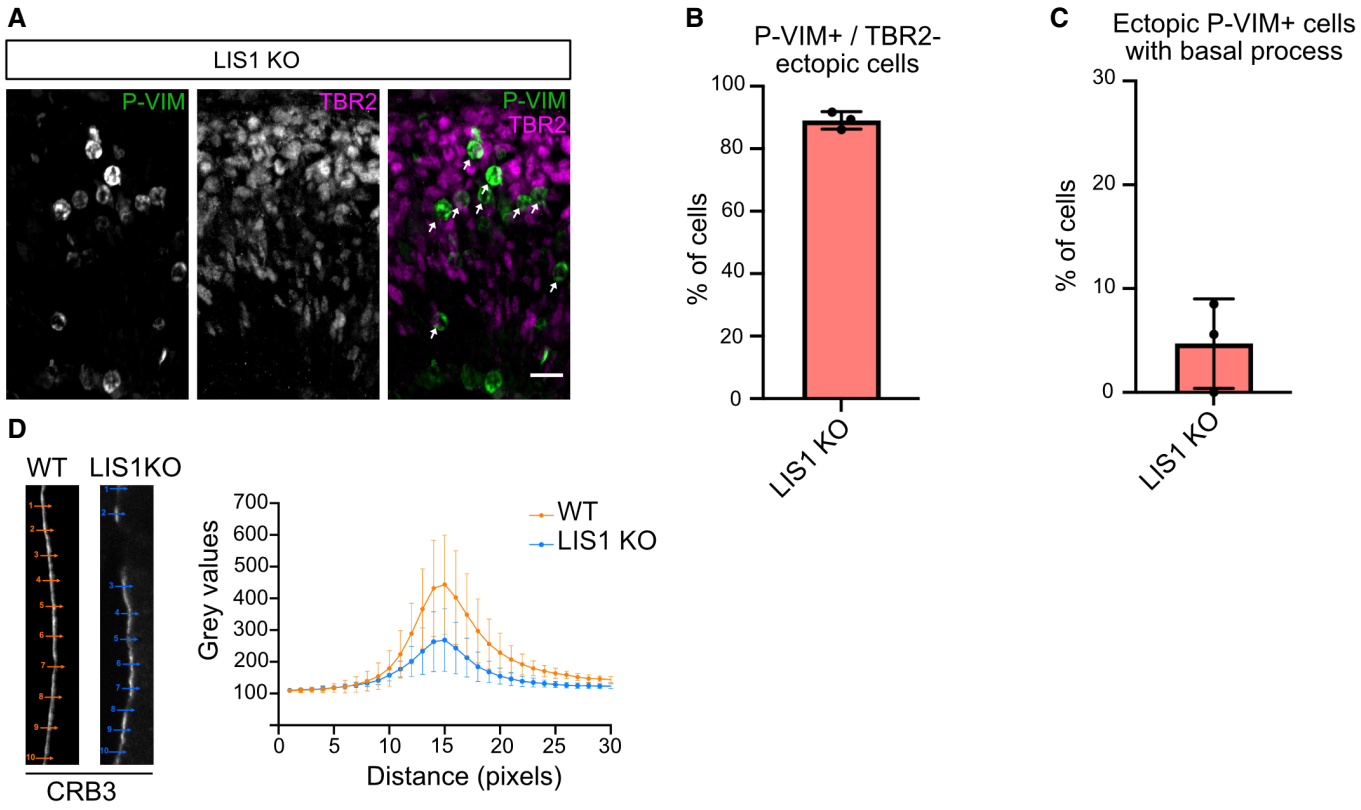

Figure EV4. LIS1 KO leads to ectopic TBR2-negative basal progenitors.

A P-VIM and TBR2 staining in *LIS1 KO* E12.5 brains. Arrows indicate detached P-VIM+/TBR2− cells. Scale bar = 25 μm.

B Percentage of ectopic P-VIM+/TBR2− cells in *LIS1 KO* E12.5 brains. *LIS1 KO*: 301 cells from $N = 3$ brains. Error bars indicate SD.

C Percentage of ectopic P-VIM+ cells that maintained a basal process in *LIS1 KO* E12.5 brains. *LIS1 KO*: 301 cells from $N = 3$ brains. Error bars indicate SD.

D CRB3 average apical signal intensity ± SEM in WT and *LIS1* KO E12.5 brains. $N = 3$ brains per condition.