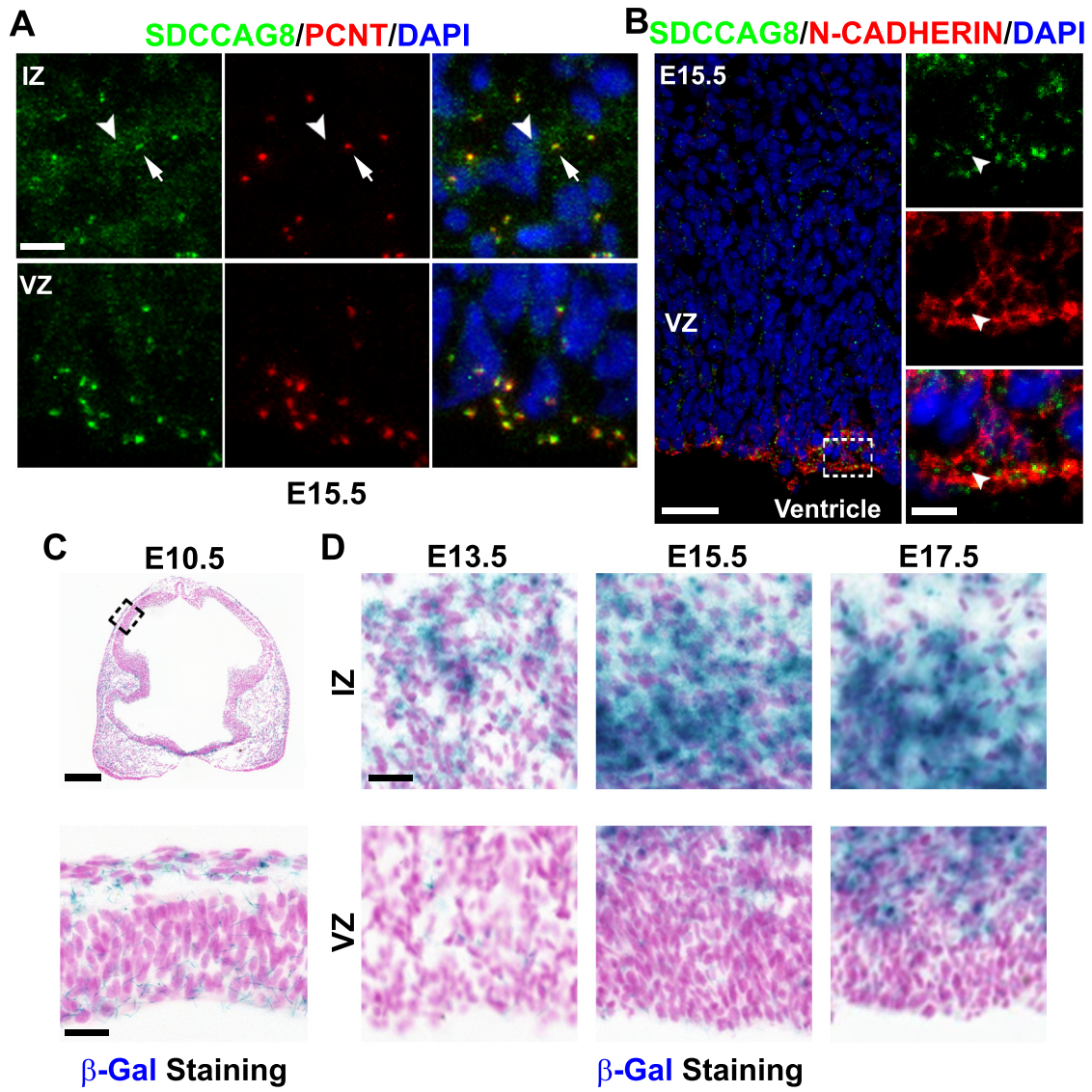**SUPPLEMENTAL FIGURES AND LEGENDS**

Movie S1&S2: Live imaging analysis of bipolar neuron migration, related to Figure 3. Movie 1 shows a bipolar neuron expressing EGFP/Control shRNA (green), and Movie 2 shows a bipolar neuron expressing EGFP/*Sdccag8* shRNA (green). Images were taken every 15 minutes, and the movie is played at a rate of 5 frames/sec.

Movies S3&S4: Live imaging analysis of centrosome movement in bipolar neurons, related to Figure 4. Movie 3 shows a bipolar neuron expressing DsRedex-CETN1 (red) that labels the centrosome and EGFP/Control shRNA (green), and Movie 4 shows a bipolar neuron expressing DsRedex-CETN1 (red) that labels the centrosome and EGFP/*Sdccag8* shRNA (green). Images were taken every 15 minutes, and the movie is played at a rate of 5 frames/sec.
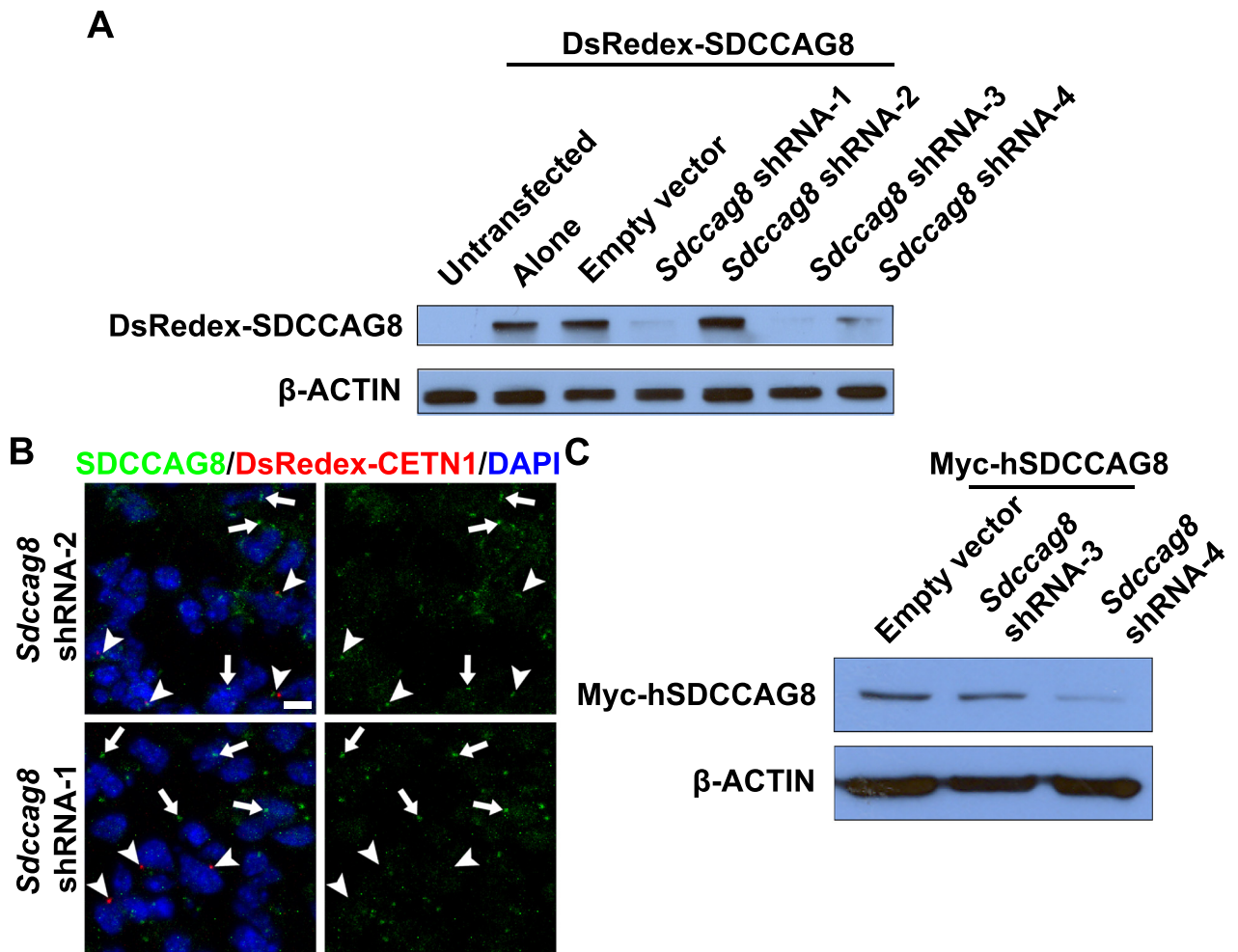
Movie S5: Live imaging analysis of SDCCAG8 and PCM1 co-trafficking in COS7 cells, related to Figure 6. The movie shows a COS7 cell expressing DsRedex-SDCCAG8 (red) and PCM1-EGFP (green) with Hoescht nuclear stain (blue). Images were taken every 2.5 seconds, and the movie is played at a rate of 5 frames/sec.

A SDCCAG8/PCNT/DAPI

IZ

VZ

E15.5

B SDCCAG8/N-CADHERIN/DAPI

E15.5

VZ

Ventricle

C E10.5

β-Gal Staining

D E13.5   E15.5   E17.5

IZ

VZ

β-Gal Staining
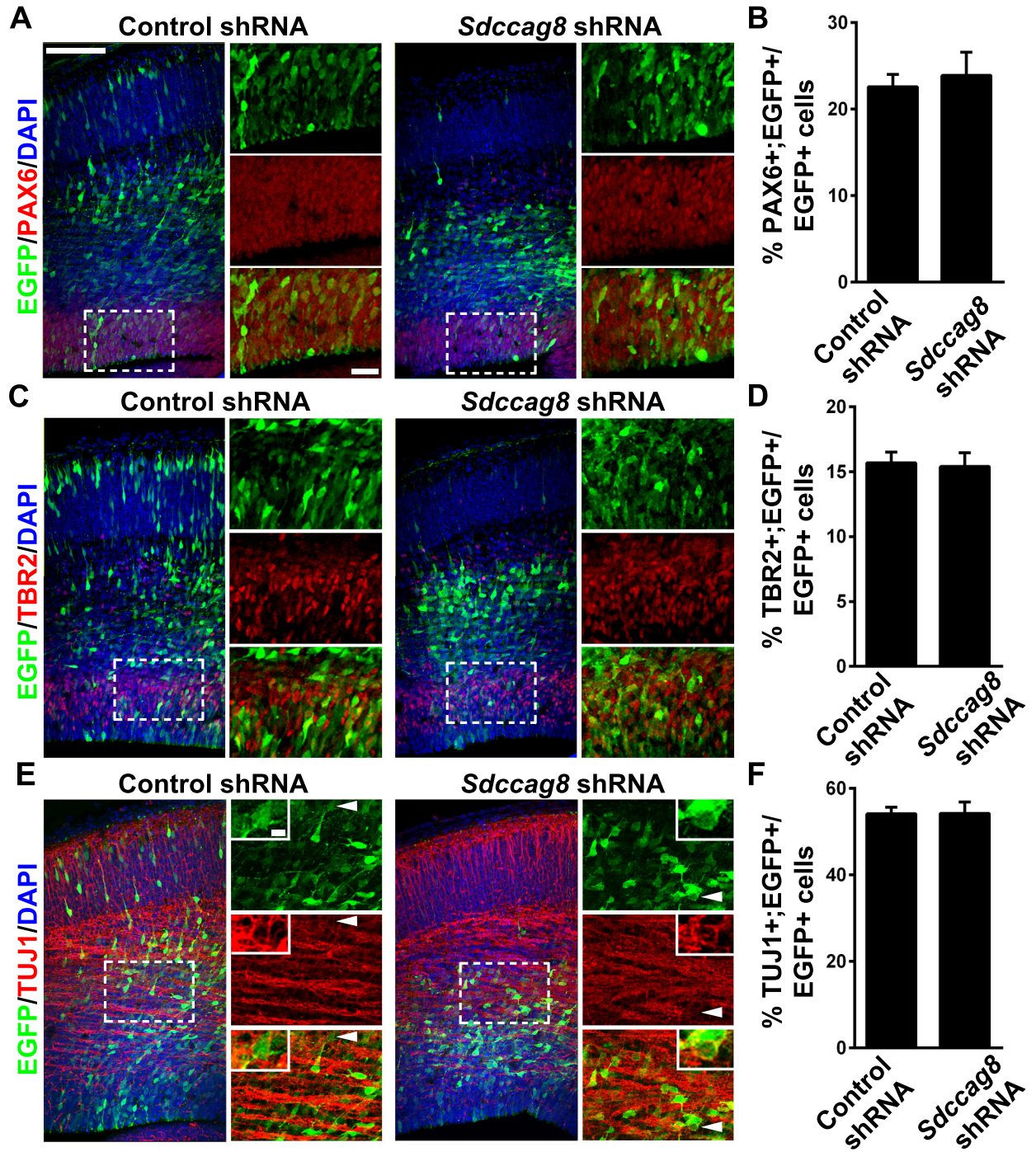
Insolera et al., Figure S1

Figure S1: SDCCAG8 expression in the developing cortex, related to Figure 1. **(A)** Representative high magnification images of the IZ and the VZ surface of an E15.5 cortex stained for SDCCAG8 (green) and PCNT (red), and with DAPI (blue). Arrows indicate the localization of SDCCAG8 at the centrosome and arrowheads indicate the small cytoplasmic puncta. Scale bar: 5 μm. **(B)** Representative images of the VZ of an E15.5 cortex stained for SDCCAG8 (green) and N-CADHERIN (red), a junction marker, and with DAPI (blue). Arrows indicate the localization of SDCCAG8 at the centrosome inside the junction. Scale bars: 50 μm and 5 μm. **(C)** Representative images of an E10.5 brain section of $Sdccag8^{Lacz/+}$ mice, in which a *LacZ* gene was inserted into the *Sdccag8* locus, stained for β-Gal (blue) and with Nuclear Fast Red (magenta). High magnification image of the cortex (broken lines) is shown at the bottom. Scale bars: 200 μm and 20 μm. **(D)** Representative images of the VZ and the IZ of E13.5, E15.5, and E17.5 cortices of $Sdccag8^{LacZ/+}$ mice stained for β-Gal (blue). Note the strong expression in the IZ, but not the VZ. Scale bar: 20 μm.

A

DsRedex-SDCCAG8

Untransfected | Alone | Empty vector | *Sdccag8* shRNA-1 | *Sdccag8* shRNA-2 | *Sdccag8* shRNA-3 | *Sdccag8* shRNA-4

DsRedex-SDCCAG8

β-ACTIN

B
SDCCAG8/DsRedex-CETN1/DAPI

Sdccag8 shRNA-2

Sdccag8 shRNA-1

C

Myc-hSDCCAG8

Empty vector | *Sdccag8* shRNA-3 | *Sdccag8* shRNA-4

Myc-hSDCCAG8

β-ACTIN

Insolera et al., Figure S2

Figure S2: Efficacy of *Sdccag8* shRNAs in suppression of SDCCAG8 expression, related to Figure 2. **(A)** Western blot of COS7 cells expressing DsRedex-SDCCAG8 alone and together with empty vector and four different *Sdccag8* shRNAs (1-4) probed for SDCCAG8 and β-ACTIN. Note shRNA-1, -3, and -4, but not -2, effectively suppress SDCCAG8 expression. **(B)** Representative images of cells in E16.5 cortices electroporated with DsRedex-CETN1 (red) and *Sdccag8* shRNA-2 and shRNA-1 at E13.5 stained for SDCCAG8 (green) and with DAPI (blue). Note the loss of SDCCAG8 expression at the centrosome in cells expressing shRNA-1, but not shRNA-2 (arrowheads), compared to that in non-transfected cells (arrows). Scale bar: 5 μm. **(C)** Western blot showing the resistance of myc-hSDCCAG8 to *Sdccag8* shRNA-3, but not shRNA-4. β-ACTIN is used as a protein loading control.
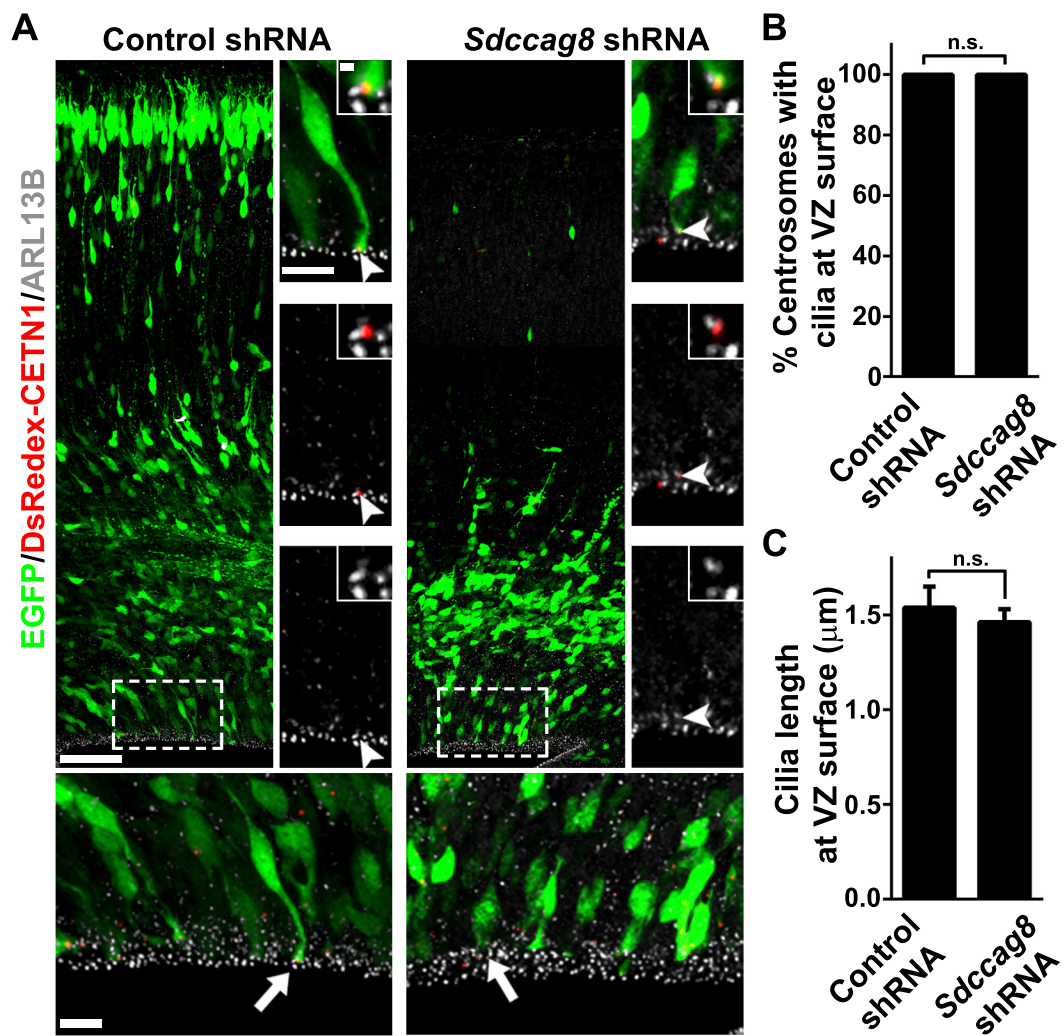
Insolera et al., Figure S3

Figure S3: Expression of *Sdccag8* shRNA does not affect progenitors and neurogenesis, related to Figure 2. **(A)** Representative images of E15.5 cortices electroporated with EGFP/Control or *Sdccag8* shRNA (green) at E13.5 stained for PAX6 (red), an RGP marker, and with DAPI (blue). High magnification images of the VZ (broken lines) are shown to the right. Scale bars, 100 μm and 25 μm. **(B)** Quantification of the percentage of EGFP+ cells expressing PAX6. Data are presented as mean ± s.e.m. (Control, 1,020 cells from 4 brains; *Sdccag8*, 652 cells from 4 brains). **(C)** Representative images of E15.5 cortices electroporated with EGFP/Control or *Sdccag8* shRNA (green) at E13.5 stained for TBR2 (red), an IP marker, and with DAPI (blue). High magnification images of the SVZ (broken lines) are shown to the right. **(D)** Quantification of the percentage of EGFP+ cells expressing TBR2. Data are presented as mean ± s.e.m. (Control, 870 cells from 3 brains; *Sdccag8*, 534 cells from 3 brains). **(E)** Representative images of E15.5 cortices electroporated with EGFP/Control or *Sdccag8* shRNA (green) at E13.5 stained for TUJ1 (red), an immature neuron marker, and with DAPI (blue). High magnification images of the IZ (broken lines) are shown to the right. Representative examples of immature neurons (arrowheads) expressing TUJ1 at the cytoplasm are shown in the inset. Scale bar, 5 μm. **(F)** Quantification of the percentage of EGFP+ cells expressing TUJ1. Data are presented as mean ± s.e.m. (Control, 1,589 cells from 4 brains; *Sdccag8*, 1,614 cells from 4 brains).
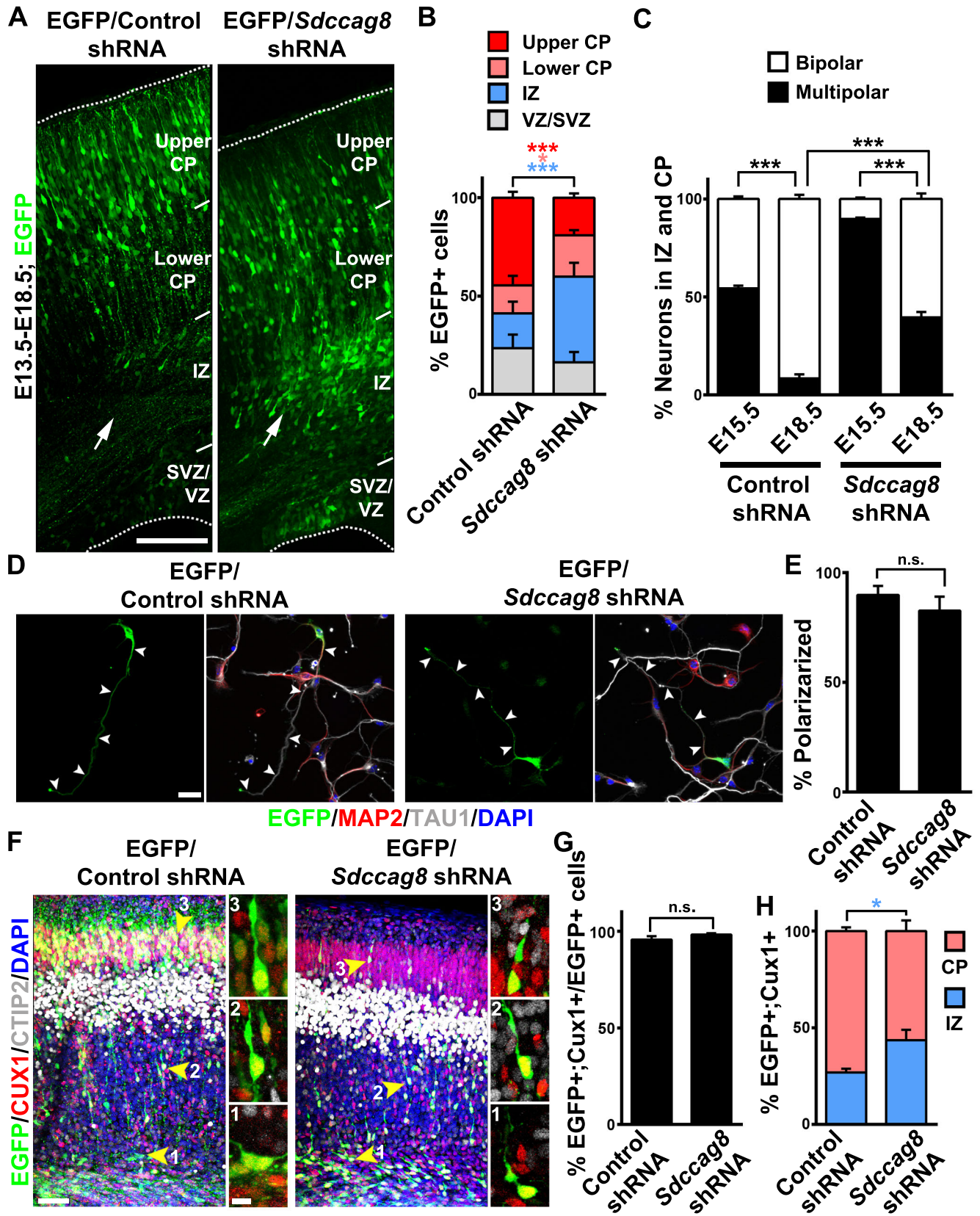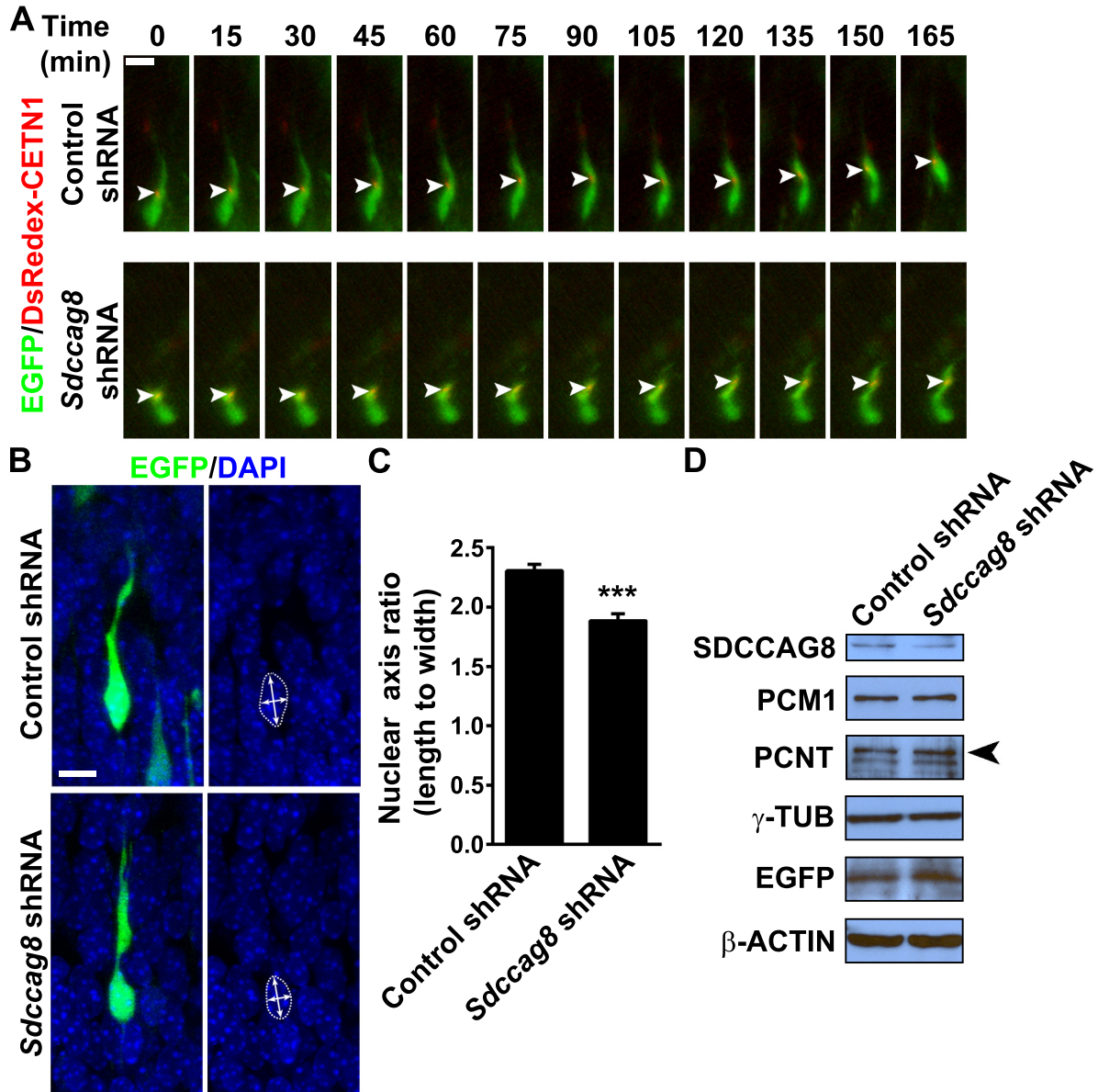
Insolera et al., Figure S4

Figure S4: SDCCAG8 depletion does not impair neuronal polarization and fate specification, but results in persistent defects in neuronal migration, related to Figure 2. **(A)** Representative images of E18.5 cortices electroporated with EGFP/Control or *Sdccag8* shRNA (green) at E13.5. Note the accumulation of cells in the IZ (arrows) and the lower part of the CP in the cortex expressing *Sdccag8* shRNA. Scale bar: 100 μm. **(B)** Quantification of the percentage of EGFP+ cells in different regions of the developing cortex. Data are presented as mean ± s.e.m. (Control, 1,423 cells from 5 brains; *Sdccag8*, 1,624 cells from 5 brains). ***, $p<0.001$; *, $p<0.05$. **(C)** Quantification of the percentage of EGFP+ neurons in IZ and CP that are in a bipolar or multipolar morphology at E15.5 (see Fig 2) and E18.5. Data are presented as mean ± s.e.m. (E18.5: Control, 1,903 cells from 5 brains; *Sdccag8*, 2,522 cells from 5 brains). ***, $p<0.001$. **(D)** Representative images of dissociated cortical neurons cultured in vitro for ~2-3 days expressing EGFP/Control or *Sdccag8* shRNA (green) stained for MAP2 (red) and TAU1 (white), the dendritic and axonal marker, respectively, and with DAPI (blue). Note that neurons expressing Control or Sdccag8 shRNA possess a single axon (arrowheads). Scale bar: 25 μm. **(E)** Quantification of the percentage of neurons polarized with a single axon. Data are presented as mean ± s.e.m. (Control, n = 99 neurons from three independent experiments; *Sdccag8*, n = 125 neurons from three independent experiments). n.s., not significant. **(F)** Representative images of E18.5 cortices electroporated with EGFP/Control or *Sdccag8* shRNA (green) stained for CUX1 (red) and CTIP2 (white), the well-characterized neuronal markers for the superficial and deep layers, respectively, and for DAPI (blue). High magnification images of EGFP+;CUX1+ neurons (arrowheads and numbers) at different regions of the cortex are shown to the right. Note the accumulation of EGFP+;CUX1+ neurons expressing *Sdccag8* shRNA in the IZ. Scale bars: 50 μm and 10 μm. **(G)** Quantification of the percentage of EGFP+ cells that are CUX1+. Data are

presented as mean ± s.e.m. (Control, 1,474 cells from 3 brains; *Sdccag8*, 1,226 cells from 3 brains). n.s., not significant. **(H)** Quantification the percentage of EGFP+/CUX1+ neurons in the CP and the IZ. Data are presented as mean ± s.e.m. (Control, 1,180 cells from 3 brains; *Sdccag8*, 1,450 cells from 3 brains) *, p<0.05.

A   Control shRNA   *Sdccag8* shRNA

EGFP/DsRedex-CETN1/ARL13B

B
% Centrosomes with cilia at VZ surface

n.s.

100 — 80 — 60 — 40 — 20 — 0

Control shRNA   *Sdccag8* shRNA

C
Cilia length at VZ surface (μm)

n.s.

1.5 — 1.0 — 0.5 — 0.0
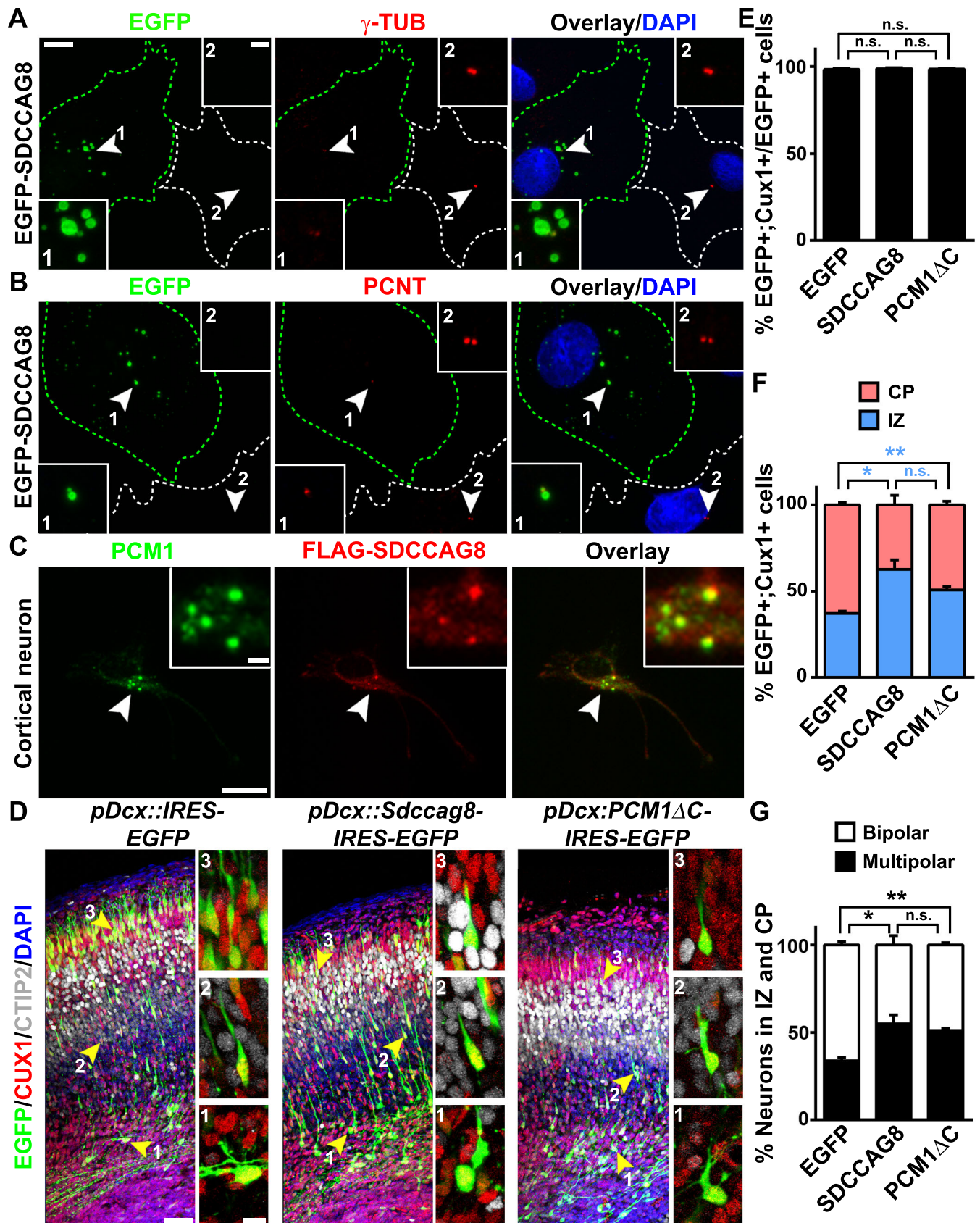
Control shRNA   *Sdccag8* shRNA

Insolera et al., Figure S5

Figure S5: SDCCAG8 depletion does not impair cilia formation in RGPs, related to Figure

2. **(A)** Representative images of E16.5 cortices electroporated with EGFP/Control of *Sdccag8*

shRNA (green) together with DsRedex-CETN1 (red) at E13.5 stained for ARL13B (white), a

well-characterized primary cilium marker. High magnification images of the VZ surface where

RGPs form cilia (broken lines) are shown at the bottom. High magnification images of RGPs

expressing DsRedex-CETN1 (arrows) to label the centrosome (arrowheads) are shown to the

right and in the inset. Note no obvious difference in ciliogenesis in RGPs expressing control or

Sdccag8 shRNA. Scale bars: 50 µm, 10 µm, 10 µm and 1 µm. **(B)** Quantification of the

percentage of the centrosomes at the VZ surface with cilia. Data are presented as mean ± s.e.m.

(Control, n = 113 cells from 4 brains; *Sdccag8*, n= 127 cells from 4 brains). n.s., not significant.

(C) Quantification of the length of cilia at the VZ surface. Data are presented as mean ± s.e.m.

(Control, n = 113 cilia from 4 brains; *Sdccag8*, n=127 cilia from 4 brains). n.s., not significant.

A Time (min)

B EGFP/DAPI

C
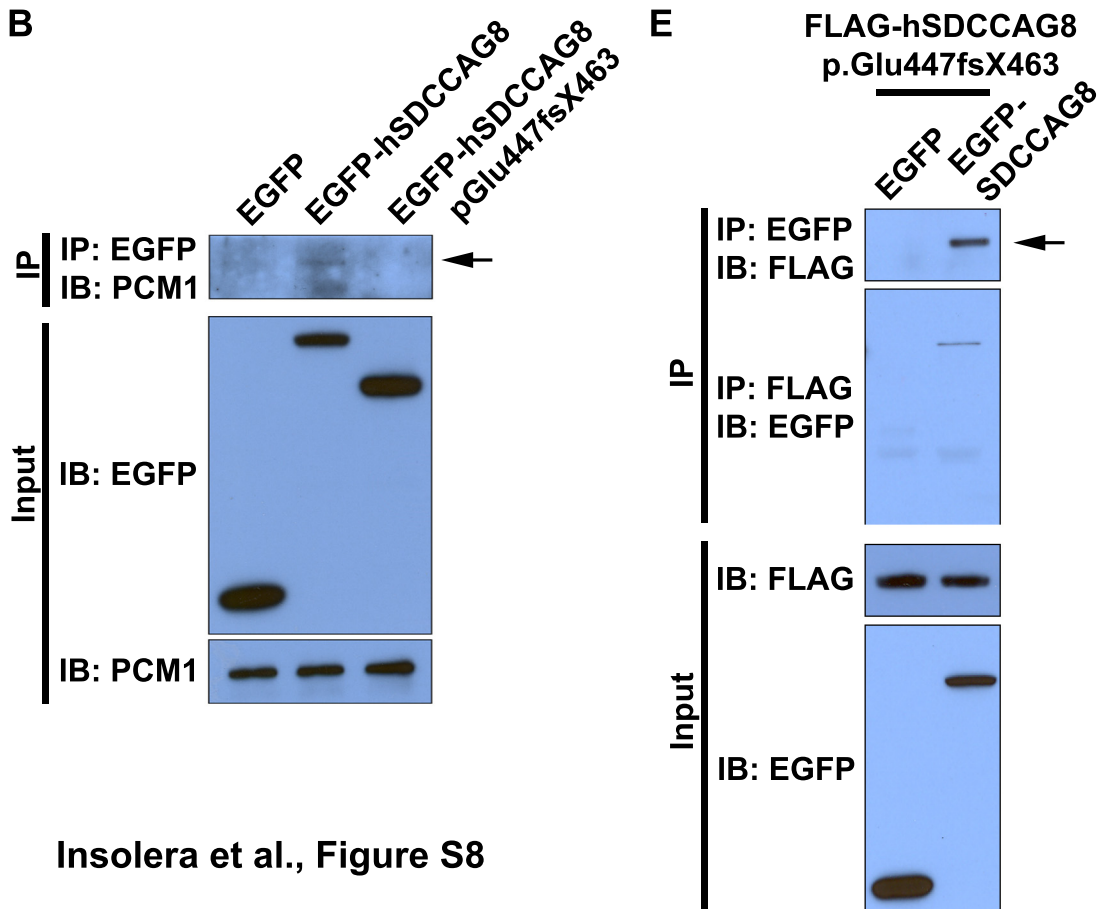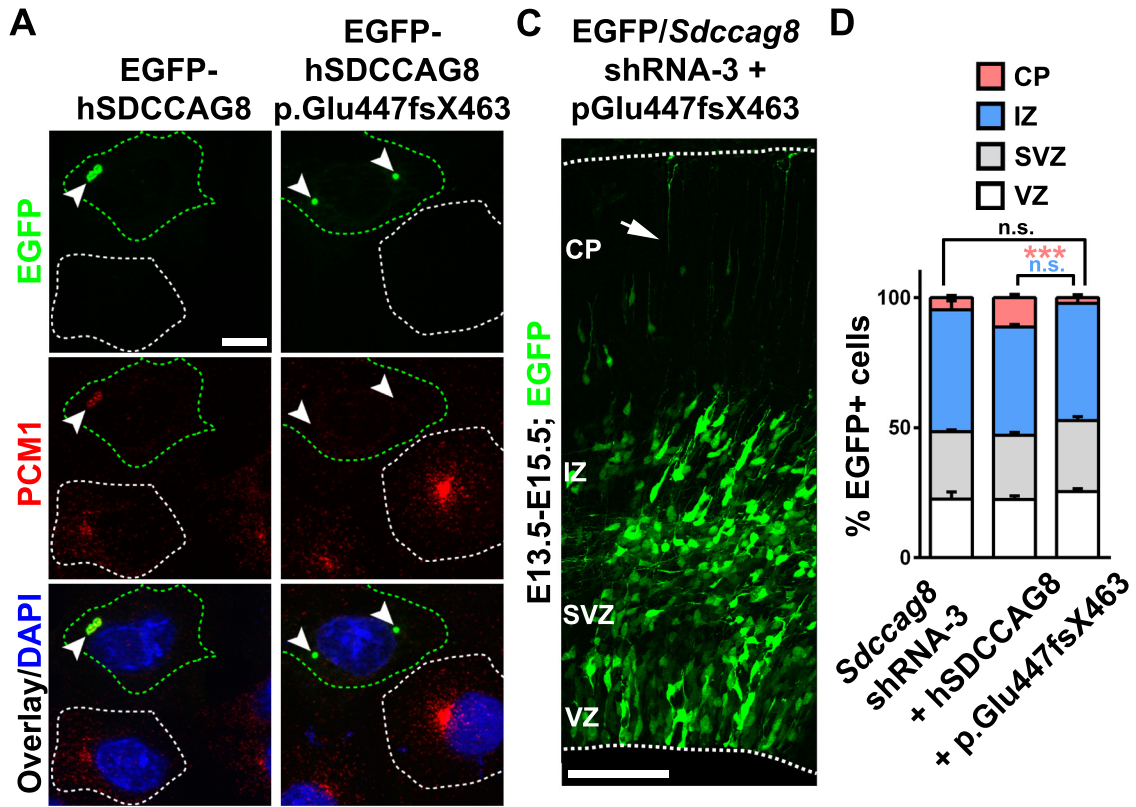
D

Insolera et al., Figure S6

Figure S6: SDCCAG8 depletion does not severely impair forward movement of the

centrosome, but alters nuclear shape, related to Figure 4. **(A)** Representative kymographs of

bipolar migrating neurons expressing DsRedex-CETN1 (red) to label the centrosome and

EGFP/Control (top) or *Sdccag8* (bottom) shRNA. Arrowheads indicate the position of the

centrosome. Note that in the neuron expressing Control or *Sdccag8* shRNA, the centrosome

progressively moves into the dilated region of the leading process. Scale bar: 5 µm. **(B)**

Representative images of bipolar migrating neurons expressing EGFP/Control or *Sdccag8*

shRNA (green) stained with DAPI (blue). Broken lines indicate the contour of the nucleus and

double-headed arrows indicate the axes of the nucleus. Note that while the nucleus of the cell

expressing control shRNA is spindle shaped, the nucleus of the cell expressing *Sdccag8* shRNA

becomes rounded. Scale bar: 10 µm. **(C)** Quantification of the axis ratio of the nucleus. Data are

presented as mean ± s.e.m. (Control, n = 69; *Sdccag8*, n = 49). ***, $p<.001$. **(D)** Western blot

analysis of the lysates of electroporated tissues from E16.5 cortex that received *in utero*

electroporation of EGFP/Control or *Sdccag8* shRNA at E13.5. Note that decreased expression of

SDCCAG8 does not obviously affect the total expression level of γ-TUB, PCNT (arrow), and

PCM1 in the developing cortex.

A EGFP   γ-TUB   Overlay/DAPI

B EGFP   PCNT   Overlay/DAPI

C PCM1   FLAG-SDCCAG8   Overlay

D pDcx::IRES-EGFP   pDcx::Sdccag8-IRES-EGFP   pDcx:PCM1ΔC-IRES-EGFP

EGFP/CUX1/CTIP2/DAPI

E % EGFP+;Cux1+/EGFP+ cells
n.s.
n.s.   n.s.
EGFP   SDCCAG8   PCM1ΔC

F % EGFP+;Cux1+ cells
CP
IZ
**
*   n.s.
EGFP   SDCCAG8   PCM1ΔC

G % Neurons in IZ and CP
Bipolar
Multipolar
**
*   n.s.
EGFP   SDCCAG8   PCM1ΔC

Insolera et al., Figure S7

Figure S7: SDCCAG8 over-expression disrupts PCM accumulation at the centrosome and neuronal migration in the cortex related to Figure 7. **(A, B)** Representative images of COS7 cells expressing EGFP-SDCCAG8 (green) stained for γ-TUB (A) or PCNT (B) (red) and with DAPI (blue). Green broken lines indicate the transfected cell (cell 1) and white broken lines indicate a nearby non-transfected cell (cell 2). High magnification images of the centrosomal region are shown in the inset. Arrowheads indicate the centrosome. Note the reduction of γ-TUB (A) and PCNT (B) at the centrosome in the EGFP-SDCCAG8 expressing cell compared to the non-transfected cell. Scale bars: 10 µm and 2 µm. **(C)** Representative images of dissociated cortical neurons in culture expressing FLAG-SDCCAG8 (red) stained for PCM1 (green). High magnification images of the centrosomal regions (arrows) are shown in the inset. Note the colocalization between FLAG-SDCCAG8 and PCM1. Scale bars: 10 μm and 2 μm. **(D)** Representative images of E16.5 cortices electroporated with *pDcx::IRES-EGFP*, *pDcx::Sdccag8-IRES-EGFP* or *pDcx::PCM1ΔC-IRES-EGFP* (green) at E13.5 stained for CUX1 (red) and CTIP2 (white) and with DAPI (blue). High magnification images of EGFP+;CUX1+ neurons (arrowheads and numbers) at different regions of the cortex are shown to the right. Note the accumulation of EGFP+;CUX1+ neurons expressing SDCCAG8 or PCM1ΔC at the IZ. Scale bars: 50 μm and 10 μm. **(E)** Quantification of the percentage of EGFP+ cells that are CUX1+ in the cortex. Data are presented as mean ± s.e.m. (EGFP, 755 cells from 3 brains; SDCCAG8, 433 cells from 3 brains; PCM1ΔC, 575 cells from 3 brains). n.s., not significant. **(F)** Quantification the percentage of EGFP+/CUX1+ neurons in the CP and the IZ. Data are presented as mean ± s.e.m. (EGFP, 742 cells from 3 brains; SDCCAG8, 427 cells from 3 brains; PCM1ΔC, 566 cells from 3 brains).**, $p<0.01$; *, $p<0.05$; n.s., not significant.**(G)** Quantification of the percentage of EGFP+ neurons with a bipolar or multipolar morphology in the CP and IZ. Data are presented as

mean ± s.e.m. (EGFP, 1,126 cells from 3 brains; SDCCAG8, 427 cells from 3 brains; PCM1ΔC,

566 cells from 3 brains).

A

EGFP-hSDCCAG8

EGFP-hSDCCAG8 p.Glu447fsX463

EGFP

PCM1

Overlay/DAPI

C

EGFP/*Sdccag8* shRNA-3 + pGlu447fsX463

E13.5-E15.5; EGFP

CP

IZ

SVZ

VZ

D

CP
IZ
SVZ
VZ

% EGFP+ cells

n.s.
***
n.s.

Sdccag8 shRNA-3
+ hSDCCAG8
+ p.Glu447fsX463

B

EGFP
EGFP-hSDCCAG8
EGFP-hSDCCAG8 pGlu447fsX463

IP: EGFP
IB: PCM1

IP

IB: EGFP

Input

IB: PCM1

E

FLAG-hSDCCAG8 p.Glu447fsX463

EGFP
EGFP-SDCCAG8

IP: EGFP
IB: FLAG

IP: FLAG
IB: EGFP

IP

IB: FLAG

IB: EGFP

Input

Insolera et al., Figure S8

Figure S8: Human SDCCAG8 truncation mutant fails to interact with PCM1, but binds to full length SDCCAG8, related to Figure 8. **(A)** Representative images of COS7 cells expressing EGFP-hSDCCAG8 wild type (top) or EGFP-hSDCCAG8 p.Glu447fsX463 (bottom) (green) stained for endogenous PCM1 (red) and with DAPI (blue). Green broken lines indicate the transfected cell and white broken lines indicate a nearby non-transfected cell. Arrowheads point to the aggregates formed by EGFP-SDCCAG8 wild type or EGFP-hSDCCAG8 p.Glu447fsX463. Note the accumulation of endogenous PCM1 at hSDCCAG8 wild type, but not the truncated mutant, aggregates. Scale bar: 10 µm. **(B)** Co-IP of EGFP-hSDCCAG8, but not EGFP or EGFP-hSDCCAG8 p.Glu447fsX463 with PCM1 in COS7 cells. **(C)** Representative image of an E15.5 cortex electroporated with EGFP/Sdccag8 shRNA-3 (green) and shRNA-resistant truncated hSDCCAG8 p.Glu447fsX463 at E13.5. Note that truncated hSDCCAG8 p.Glu447fsX463 fails to rescue neuronal migration defect caused by Sdccag8 shRNA (arrow). Scale bar: 100 µm. **(D)** Quantification of the percentage of EGFP+ cells in different regions of the cortex. Data are presented as mean ± s.e.m. (shRNA-3 and +hSDCCAG8, see Fig 2 for n values; + p.Glu447fsX463, 1,531 cells from 5 brains). ***, $p<0.001$; n.s., not significant. **(E)** Reciprocal co-IP of EGFP-SDCCAG8 with FLAG-hSDCCAG8 p.Glu447fsX463 in COS7 cells.
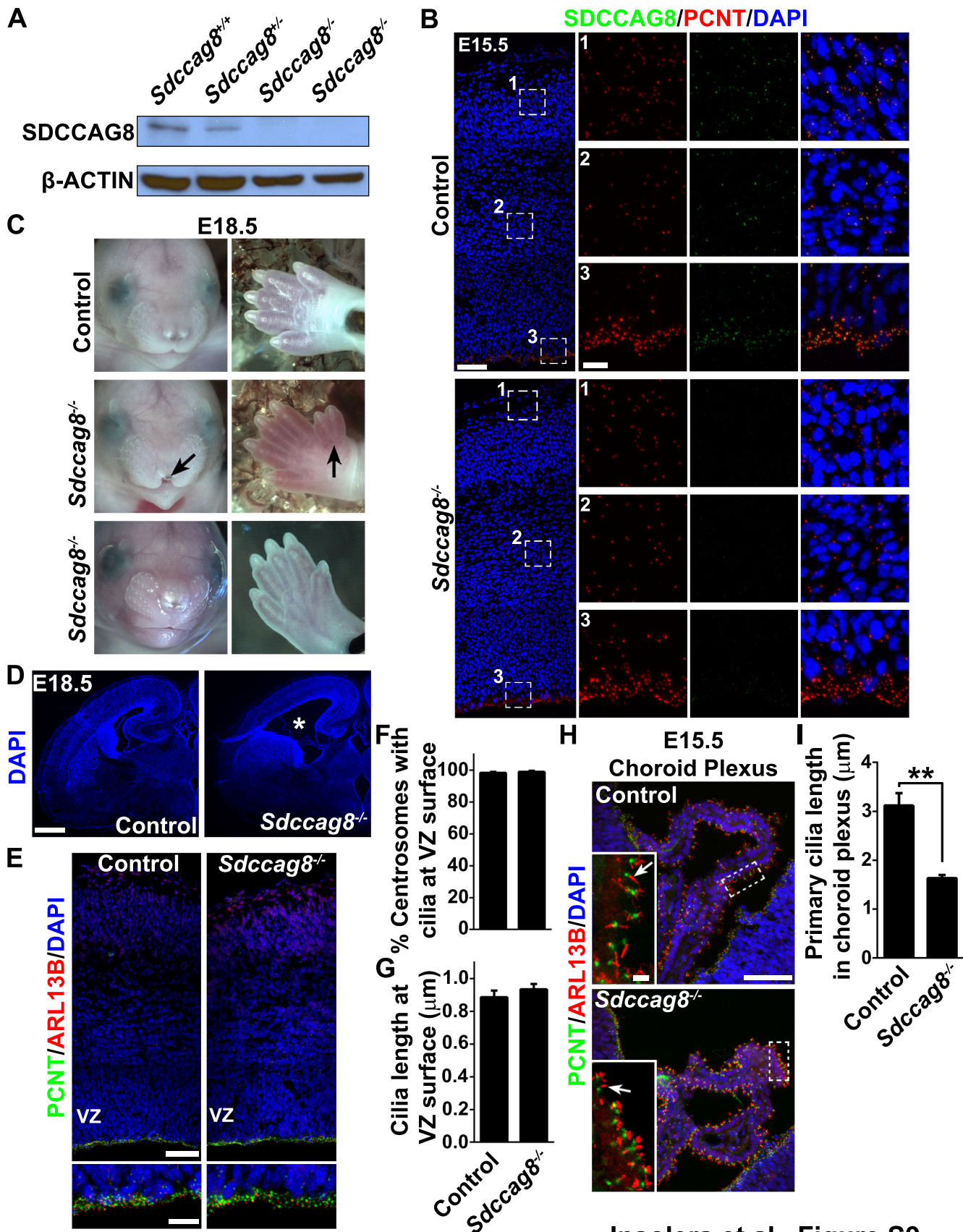
Insolera et al., Figure S9

Figure S9: Characterization of *Sdccag8* knockout mice, related to Figure 9. **(A)** Western blot of E15.5 cortical lysates showing a loss of SDCCAG8 expression in *Sdccag8*$^{-/-}$ brains. **(B)** Representative images of E15.5 Control (*Sdccag8*$^{+/-}$) and *Sdccag8*$^{-/-}$ mice stained for SDCCAG8 (green) and PCNT (red), and with DAPI (blue). High magnification images of different regions of the cortex (broken lines) are shown to the right. Note the loss of SDCCAG8 in the *Sdccag8*$^{-/-}$ cortex. Scale bars: 50 μm and 10 μm. **(C)** Representative images of the face and paw of E18.5 control and *Sdccag8*$^{-/-}$ mice. Arrows indicate the cleft palate and polydactyly in 94% (17 of 18) of *Sdccag8*$^{-/-}$ mice. **(D)** Representative images of E18.5 control and a few (4 out of 18) *Sdccag8*$^{-/-}$ brains that show an enlarged lateral ventricle (asterisk). Scale bar: 500 μm. **(E)** Representative images of E15.5 cortices of control and *Sdccag8*$^{-/-}$ mice stained for PCNT (green) and ARL13B (red), and with DAPI (blue). High magnification images of the VZ surface, where RGPs form cilia, are shown at the bottom. Note no obvious defect in ciliogenesis. Scale bars: 50 μm and 10 μm. **(F)** Quantification of the percentage of the centrosomes at the VZ surface with cilia. Data are presented as mean ± s.e.m. (Control, n = 259 centrosomes from 4 brains; *Sdccag8*$^{-/-}$, n = 248 centrosomes from 4 brains). **(G)** Quantification of the length of cilia at the VZ surface. Data are presented as mean ± s.e.m. (Control, n = 259 cilia from 4 brains; *Sdccag8*$^{-/-}$, n = 248 cilia from 4 brains). **(H)** Representative images of E15.5 choroid plexus from control and *Sdccag8*$^{-/-}$ mice stained for PCNT (green) and ARL13B (red), and with DAPI (blue). High magnification images of the indicated areas (broken lines) are shown in inset. Note the decreased length of primary cilia (arrows) in *Sdccag8*$^{-/-}$ mice. Scale bars: 50 μm and 5 μm. **(I)** Quantification of the primary cilium length in the choroid plexus. Data are presented as mean ± s.e.m. . (Control, n = 203 cilia from 4 brains; *Sdccag8*$^{-/-}$, n = 297 cilia from 4 brains). **, $p<0.01$