Expanded View Figures

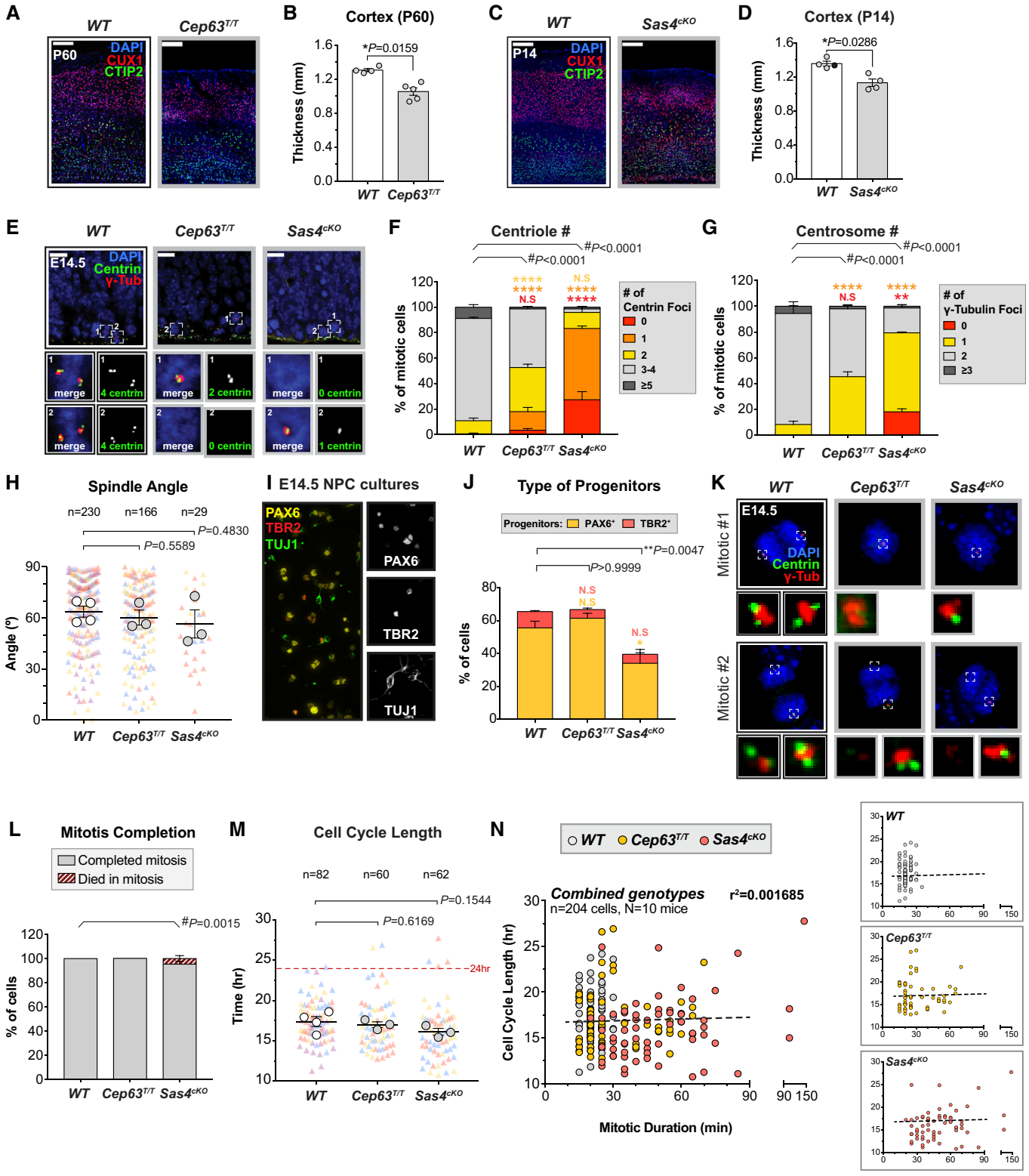


Figure EV1.

Figure EV1. Depletion of SAS4 or CEP63 leads to centrosome loss and reduced cortical size in adult mice. Related to Fig 1.

A *WT* and *Cep63*$^{T/T}$ cortices at P60 stained with antibodies against the deep layer marker CTIP2 (green), superficial layer marker CUX1 (red) and DAPI (blue). Scale bar = 200 μm.

B Cortical thickness of P60 brains of the indicated genotypes. *WT* littermates $N = 4$, *Cep63*$^{T/T}$ $N = 5$; two-tailed Mann–Whitney test.

C *WT* and *Sas4*^{cKO} cortices at P14 stained with antibodies against the deep layer marker CTIP2 (green), superficial layer marker CUX1 (red) and DAPI (blue). Scale bar = 200 μm.

D Cortical thickness of P14 brains of the indicated genotypes. *WT* littermates $N = 4$, *Sas4*^{cKO} $N = 4$; two-tailed Mann–Whitney test.

E *WT*, *Cep63*$^{T/T}$ and *Sas4*^{cKO} cortices at E14.5 stained with antibodies against centrin (green), γ-tubulin (red) and DAPI (blue). Insets showing zoomed in view of 2 representative cells. Scale bar = 25 μm.

F, G Quantification of the number of (F) centrin foci and (G) γ-tubulin foci in mitotic cells in the VZ of E14.5 cortices. *WT* $n = 175$ cells, $N = 4$ embryos; *Cep63*$^{T/T}$ $n = 150$ cells, $N = 3$ embryos; *Sas4*^{cKO} $n = 150$ cells, $N = 3$ embryos; #, chi-square test with *, *post hoc* analysis, comparisons are made to *WT*.

H Plot showing the spindle angle of dividing NPCs relative to the ventricular surface in *WT*, *Cep63*$^{T/T}$ and *Sas4*^{cKO} at E14.5. Triangles represent individual cells; triangles of the same color represent cells derived from the same embryo. Circles represent the average spindle angle of mitotic cells from each embryo. *WT* $n = 230$ cells, $N = 4$ embryos; *Cep63*$^{T/T}$ $n = 166$ cells, $N = 3$ embryos; *Sas4*^{cKO} $n = 29$ cells, $N = 3$ embryos; two-tailed Welch's *t*-test. Note that the frequency of bipolar spindle formation is lower in *Cep63*$^{T/T}$ and *Sas4*^{cKO} brains.

I Representative image from a disassociated NPC culture derived from an E14.5 *WT* brain stained with antibodies against the radial glial cell marker PAX6 (yellow), intermediate progenitor marker TBR2 (red) and differentiated neuron marker TUJ1 (green).

J Graph showing the percentage of radial glial cells (PAX6^{+}, yellow) and intermediate progenitor cells (TBR2^{+}, red) present in NPC cultures derived from E14.5 brains of the indicated genotypes. *WT* $N = 6$, *Cep63*$^{T/T}$ $N = 3$, *Sas4*^{cKO} $N = 5$; multiple t-tests, comparisons are made to *WT*.

K Representative images of mitotic cells in disassociated NPC cultures derived from E14.5 *WT*, *Cep63*$^{T/T}$ and *Sas4*^{cKO} brains stained with antibodies against centrin (green), γ-tubulin (red) and DAPI (blue). Insets showing zoomed in view of the spindle poles.

L Graph showing the fate of mitotic NPCs of the indicated genotypes. *WT* $n = 165$ cells, $N = 4$ embryos; *Cep63*$^{T/T}$ $n = 130$ cells, $N = 3$ embryos; *Sas4*^{cKO} $n = 117$ cells, $N = 3$ embryos. #, chi-square test.

M Graph showing the total cell cycle length in *WT*, *Cep63*$^{T/T}$, and *Sas4*^{cKO} NPCs. The timing of the cell cycle began at NEBD of the mother cell and finished at NEBD in one of the two daughter cells. Triangles represent individual cells; triangles of the same color represent cells derived from the same embryo. Circles represent the average cell cycle length of cells from each embryo. Dashed line is set at 24 h. *WT* $n = 82$ cells, $N = 4$ embryos; *Cep63*$^{T/T}$ $n = 60$ cells, $N = 3$ embryos; *Sas4*^{cKO} $n = 62$ cells, $N = 3$ embryos; two-tailed Welch's *t*-test.

N Graphs showing cell cycle length as a function of mitotic duration in individual NPCs dissociated from *WT* (gray), *Cep63*$^{T/T}$ (yellow), and *Sas4*^{cKO} (red) brains. The dashed line represents the best-fit linear regression function ($r^2 = 0.0017$) for all genotypes combined.

Data information: All data represent the means ± SEM. *$P < 0.05$; **< 0.01; ****< 0.0001 and not significant indicates $P > 0.05$.

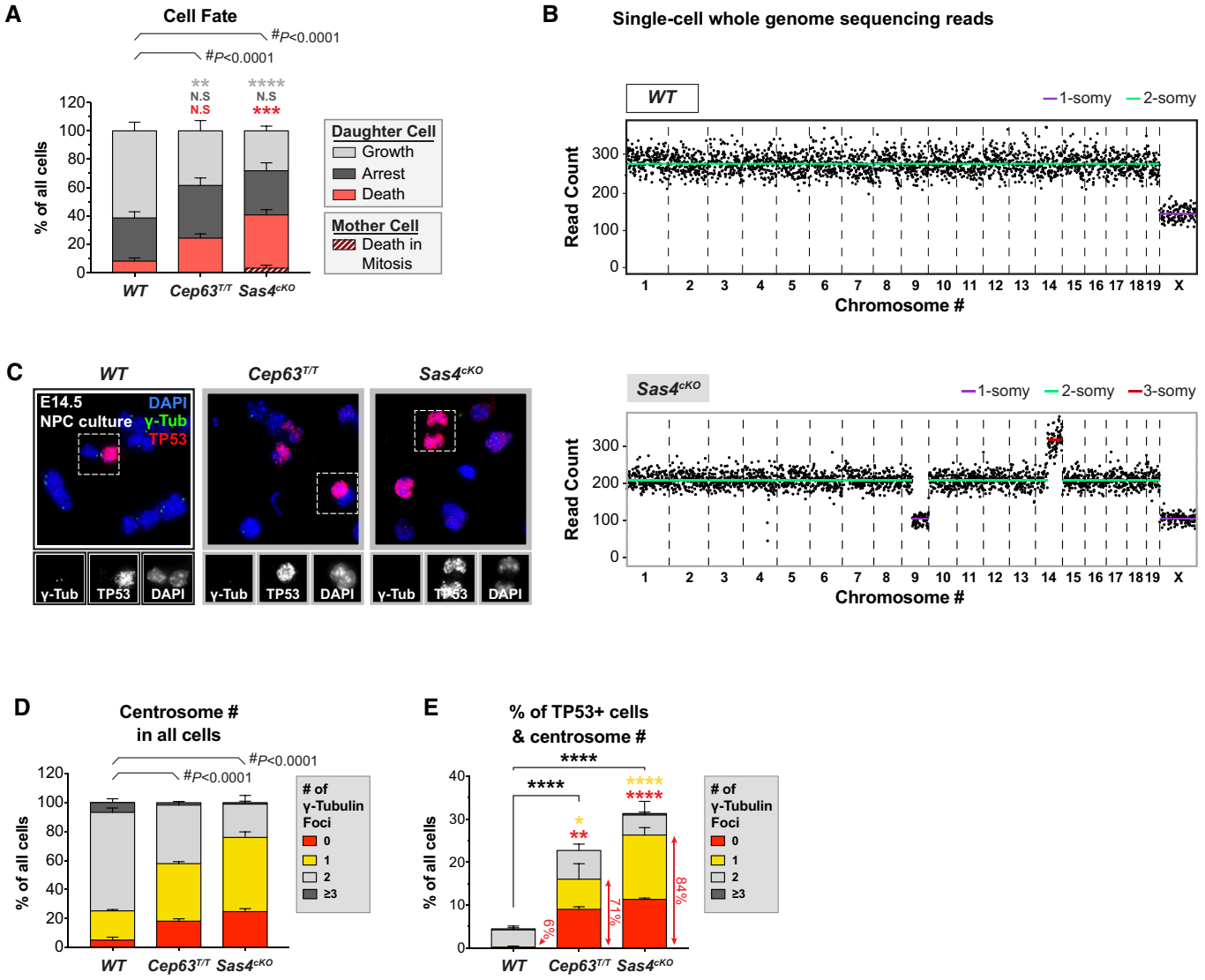


Figure EV2. *Sas4*^{cKO} and *Cep63*$^{T/T}$ NPCs with centrosome loss upregulate TP53. Related to Fig 2.

A Percentage of proliferating, arrested and apoptotic progeny within each genotype, calculated from data shown in Fig 2A–C; #, chi-square test with *, *post hoc* analysis, comparisons are made to *WT*.

B Single-cell whole genome sequencing data of a representative *WT* (top) and *Sas4*^{cKO} (bottom) cell dissociated from E14.5 brains. Each black dot indicates the number of reads per 1 Mb sequence; copy number states indicated by colored lines.

C Representative images of NPCs dissociated from E14.5 *WT*, *Cep63*$^{T/T}$, and *Sas4*^{cKO} brains stained with antibodies against γ-tubulin (green), TP53 (red), and DAPI (blue). Insets showing zoomed in view of TP53-positive cells.

D, E Quantification of the number of γ-tubulin foci in interphase NPCs dissociated from E14.5 cortices. Graphs show centrosome number in all cells (D) or TP53-positive cells (E). Red arrowheads highlight the fraction of cells with centrosome loss among all TP53-positive cells. *WT* n = 399 cells, N = 4 embryos; *Cep63*$^{T/T}$ n = 299 cells, N = 3 embryos; *Sas4*^{cKO} n = 300 cells, N = 3 embryos; #, chi-square test and *, multiple t-tests, comparisons are made to *WT*.

Data information: All data represent the means ± SEM. *P < 0.05; **< 0.01; ***< 0.001; ****< 0.0001 and not significant indicates P > 0.05.

Figure EV3. Ablation of the mitotic surveillance pathway restores brain size and increases survival but does not rescue cilia defects in *Sas4^{cKO}* animals. Related to Fig 3.

A Telencephalon area of P60 brains of the indicated genotypes. *WT* littermates $N = 6$, *Trp53bp1*$^{-/-}$ $N = 5$, *Usp28*$^{-/-}$ $N = 5$, *Trp53*$^{-/-}$ $N = 4$; one-way ANOVA with *post hoc* analysis. 4 *WT* data points are from Fig 1B and shown alongside for comparison.

B Cortical thickness of P60 brains of the indicated genotypes. *WT* littermates $N = 4$, *Trp53bp1*$^{-/-}$ $N = 3$, *Usp28*$^{-/-}$ $N = 4$, *Trp53*$^{-/-}$ $N = 3$; one-way ANOVA with *post hoc* analysis. *WT* data are from Fig EV1B and shown alongside for comparison.

C Representative histology images of P14 brains of the indicated genotypes. Arrows indicate the enlarged ventricles caused by the lack of motile cilia. Scale bar = 0.2 cm.

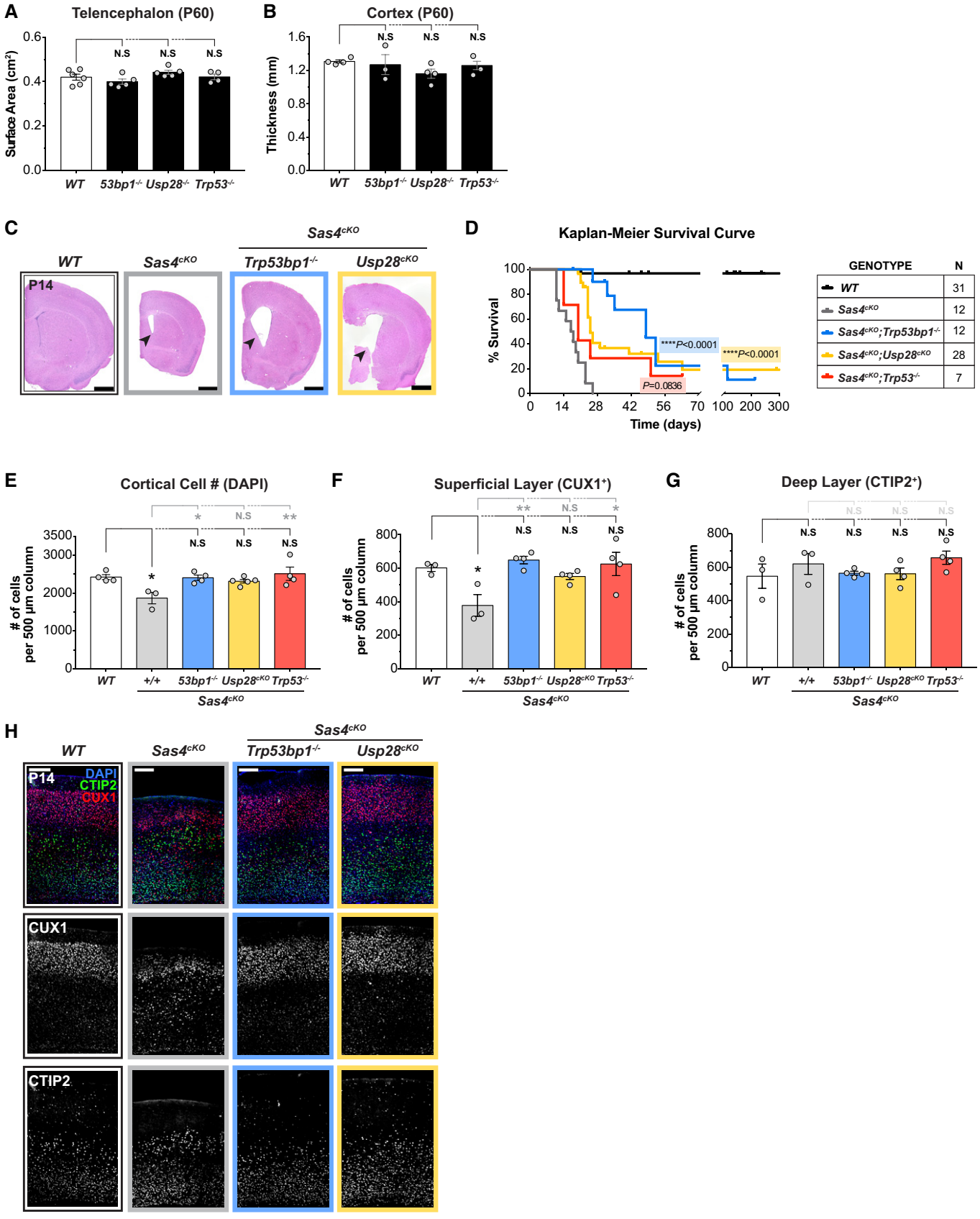
D Kaplan–Meier survival analysis of *Sas4*^{cKO}, *Sas4*^{cKO};*Trp53bp1*$^{-/-}$, *Sas4*^{cKO};*Usp28*^{cKO}, *Sas4*^{cKO};*Trp53*$^{-/-}$ animals compared to *WT* littermates. *P* values were calculated using the log-rank test.

E Quantification of total number of cells within a 500 μm-width column of cortices at P14. *WT* littermates $N = 4$, *Sas4*^{cKO} $N = 3$, *Sas4*^{cKO};*Trp53bp1*$^{-/-}$ $N = 4$, *Sas4*^{cKO};*Usp28*^{cKO} $N = 5$, *Sas4*^{cKO};*Trp53*$^{-/-}$ $N = 4$; one-way ANOVA with *post hoc* analysis.

F, G Quantification of the number of cells in the (F) superficial layer (CUX1^{+}) and (G) deep layer (CTIP2^{+}) within a 500 μm-width column of P14 cortices. *WT* littermates $N = 3$, *Sas4*^{cKO} $N = 3$, *Sas4*^{cKO};*Trp53bp1*$^{-/-}$ $N = 4$, *Sas4*^{cKO};*Usp28*^{cKO} $N = 4$, *Sas4*^{cKO};*Trp53*$^{-/-}$ $N = 4$; one-way ANOVA with *post hoc* analysis.

H P14 cortices of the indicated genotypes stained with antibodies against the deep layer marker CTIP2 (green), superficial layer marker CUX1 (red) and DAPI (blue). Scale bar = 200 μm.

Data information: All data represent the means ± SEM. *$P < 0.05$; **< 0.01; ****< 0.0001 and not significant indicates $P > 0.05$.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Surface Area (cm²)
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0.4
0.2
0.0
N.S
N.S
N.S
WT
53bp1-/-
Usp28-/-
Trp53-/-
B
Cortex (P60)
Thickness (mm)
1.6
1.2
0.8
0.4
0.0
N.S
N.S
N.S
WT
53bp1-/-
Usp28-/-
Trp53-/-
C
Sas4cKO
WT
Sas4cKO
Trp53bp1-/-
Usp28cKO
P14
D
Kaplan-Meier Survival Curve
% Survival
100
80
60
40
20
0
0
14
28
42
56
70
100
200
300
Time (days)
****P<0.0001
****P<0.0001
P=0.0836
GENOTYPE
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WT
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Sas4cKO
12
Sas4cKO;Trp53bp1-/-
12
Sas4cKO;Usp28cKO
28
Sas4cKO;Trp53-/-
7
E
Cortical Cell # (DAPI)
# of cells per 500 µm column
3500
3000
2000
1000
0
*
N.S
**
N.S
N.S
N.S
WT
+/+
53bp1-/-
Usp28cKO
Trp53-/-
Sas4cKO
F
Superficial Layer (CUX1+)
# of cells per 500 µm column
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600
400
200
0
**
N.S
*
N.S
N.S
N.S
WT
+/+
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Trp53-/-
Sas4cKO
G
Deep Layer (CTIP2+)
# of cells per 500 µm column
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400
200
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N.S
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N.S
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+/+
53bp1-/-
Usp28cKO
Trp53-/-
Sas4cKO
H
Sas4cKO
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Usp28cKO
P14
DAPI
CTIP2
CUX1
CUX1
CTIP2

Figure EV3.

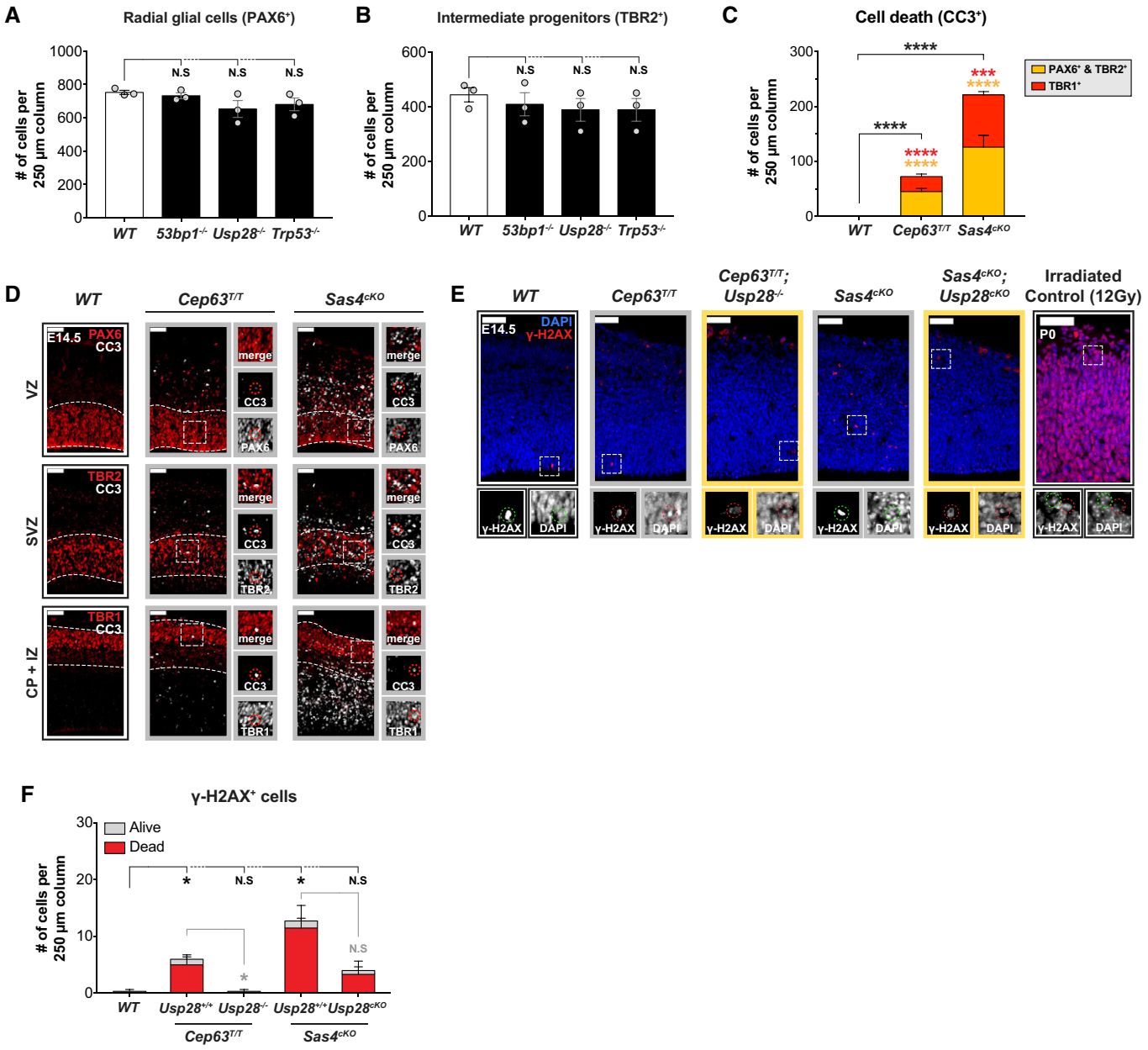


Figure EV4. *Sas4*^{cKO} and *Cep63*$^{T/T}$ brains show elevated cell death in NPCs and neurons without increased DNA damage. Related to Fig 4.

A, B Quantification of the number of (A) radial glial cells (PAX6$^+$) and (B) intermediate progenitors (TBR2$^+$) within a 250 µm-width column of E14.5 cortices. *WT* $N = 3$, *Trp53bp1*$^{-/-}$ $N = 3$, *Usp28*$^{-/-}$ $N = 3$, *Trp53*$^{-/-}$ $N = 3$; one-way ANOVA with *post hoc* analysis.

C Quantification of the number of apoptotic cells (CC3$^+$) co-stained with PAX6-TBR2 or TBR1 within a 250 µm-width column of E14.5 cortices. *WT* $N = 3$, *Cep63*$^{T/T}$ $N = 2$, *Sas4*^{cKO} $N = 3$; multiple *t*-tests, comparisons are made to *WT*.

D Cortices from E14.5 embryos stained with antibodies against cleaved-caspase 3 and markers for radial glial cells (PAX6, red), intermediate progenitors (TBR2, red), and neurons (TBR1, red). Insets showing zoomed in view of the cortical plate (CP), the intermediate zone (IZ), the sub-ventricular zone (SVZ), and the ventricular zone (VZ), demarcated by the white dashed lines. Scale bar = 50 µm.

E E14.5 cortices of indicated genotypes stained with antibodies against γ-H2AX (red) and DAPI (blue). A P0 brain section from an irradiated animal is shown alongside as a positive control for DNA damage signal. Insets showing zoomed in view of representative γ-H2AX$^+$ cells. Red dashed circles indicate dead cells; green dashed circles indicate live cells. Scale bar = 50 µm.

F Quantification of the number of cells stained positive for γ-H2AX within a 250 µm-width column of E14.5 cortices; cells are categorized as dead or alive based on DAPI morphology. *WT* $N = 3$, *Cep63*$^{T/T}$ $N = 4$, *Cep63*$^{T/T}$;*Usp28*$^{-/-}$ $N = 3$, *Sas4*^{cKO} $N = 4$, *Sas4*^{cKO};*Usp28*^{cKO} $N = 3$; multiple *t*-tests.

Data information: All data represent the means ± SEM. *$P < 0.05$; ***< 0.001; ****< 0.0001 and not significant indicates $P > 0.05$.

Figure EV5. Prolonging mitosis results in USP28-dependent cell death in the progeny of NPC divisions. Related to Fig 5.

A, B Quantification of the number of (A) centrin foci and (B) γ-tubulin foci in mitotic cells in the VZ of E14.5 cortices. *WT* $n = 175$ cells, $N = 4$ embryos; *Usp28*$^{-/-}$ $n = 137$ cells, $N = 3$ embryos; *Cep63*$^{T/T}$;*Usp28*$^{-/-}$ $n = 150$ cells, $N = 3$ embryos; *Sas4*^{cKO};*Usp28*^{cKO} $n = 150$ cells, $N = 3$ embryos. #, chi-square test, with * *post hoc* analysis, comparisons are made to *WT*. *WT* data are from Fig EV1F and G and shown alongside for comparison.

C Ratio of mitotic ($PH3^+$) to cycling ($Ki67^+$) cells in E14.5 cortices. *WT* $N = 5$, *Trp53bp1*$^{-/-}$ $N = 3$, *Usp28*$^{-/-}$ $N = 3$, *Trp53*$^{-/-}$ $N = 3$; one-way ANOVA with *post hoc* analysis. *WT* data are from Fig 1H and shown alongside for comparison.

D Percentage of proliferating, arrested, and apoptotic progeny in *Sas4*^{cKO};*Usp28*^{cKO} dissociated NPCs calculated from data shown in Fig 5F. #, chi-square test with *, *post hoc* analysis, comparisons are made to *WT*. *WT* and *Sas4*^{cKO} data are from Fig EV2A and shown alongside for comparison.

E Genome-wide copy number plots of single cells sequenced from NPCs dissociated from *Sas4*^{cKO};*Usp28*^{cKO} E14.5 brains. Individual cells are represented in rows with copy number states indicated by colors; arrow on the zoomed in view (bottom) indicates an aneuploid cell. *Sas4*^{cKO};*Usp28*^{cKO} $n = 41$ cells, $N = 2$ embryos.

F Percentage of aneuploid cells from the single-cell sequencing data shown in (E).

G Schematic of the prolonged mitosis assay.

H Percentage of proliferating, arrested and apoptotic progeny in *WT* and *Usp28*^{cKO} dissociated NPCs with delayed mitosis (nocodazole delayed; *WT* $n = 45$ cells, $N = 3$ embryos; *Usp28*^{cKO} $n = 56$ cells, $N = 3$ embryos) and normal mitosis (After Washout; *WT* $n = 90$ cells, $N = 3$ embryos; *Usp28*^{cKO} $n = 100$ cells, $N = 3$ embryos) calculated from data shown in (I–L); #, chi-square test with *, *post hoc* analysis, comparisons are made to *WT*.

I, J Graph showing the mitotic duration and fate of (I) *WT* and (J) *Usp28*^{cKO} E14.5 NPCs that were delayed in mitosis using nocodazole (0.08 μM). Each bar represents one cell; its height represents the amount of time the mother cell spent in mitosis; bar color represents the fate of the progeny. *WT* $n = 45$ cells, $N = 3$ embryos; *Usp28*^{cKO} $n = 56$ cells, $N = 3$ embryos.

K, L Graph showing the mitotic duration and fate of (K) *WT* and (L) *Usp28*^{cKO} NPCs from the same experiment in I and J that entered mitosis after nocodazole washout. *WT* $n = 90$ cells, $N = 3$ embryos; *Usp28*^{cKO} $n = 100$ cells, $N = 3$ embryos.

Data information: All data represent the means ± SEM. *$P < 0.05$; **< 0.01; ***< 0.001; **** < 0.0001 and not significant indicates $P > 0.05$.

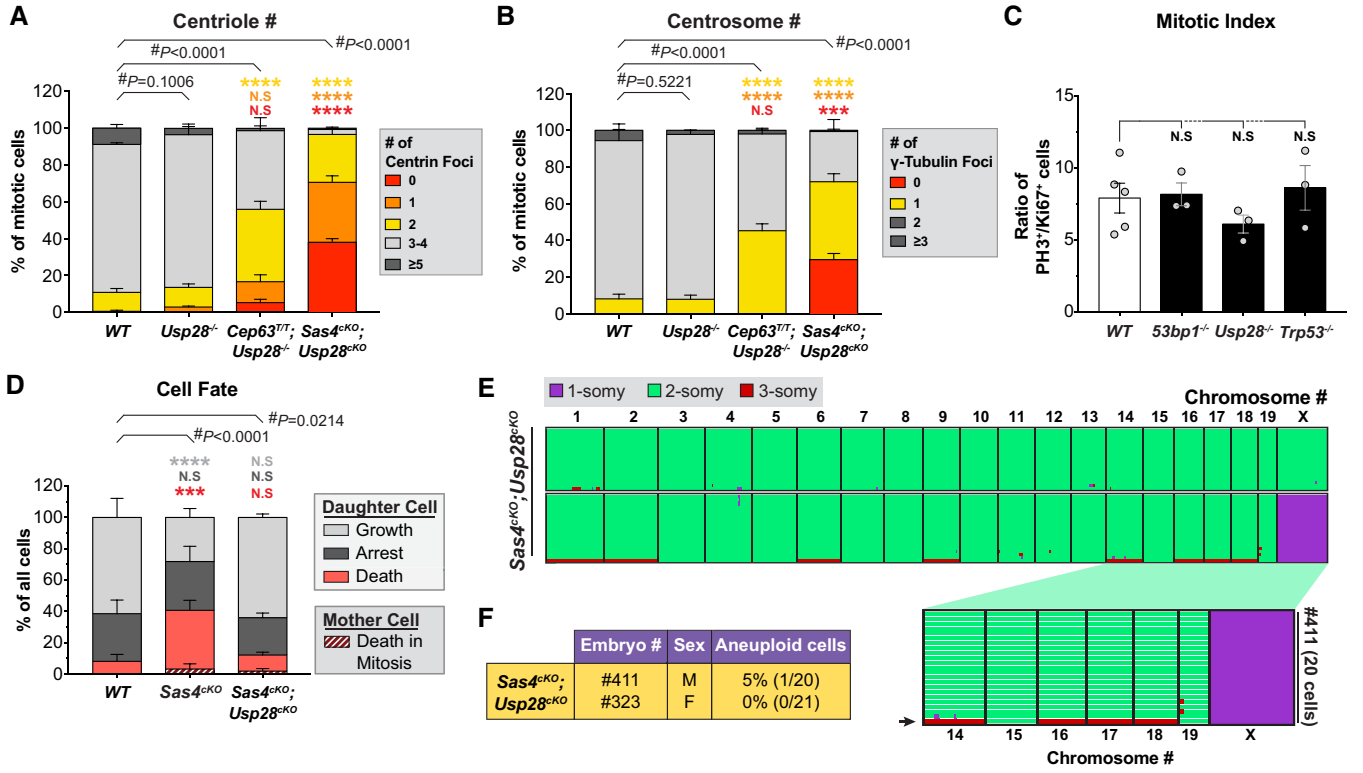
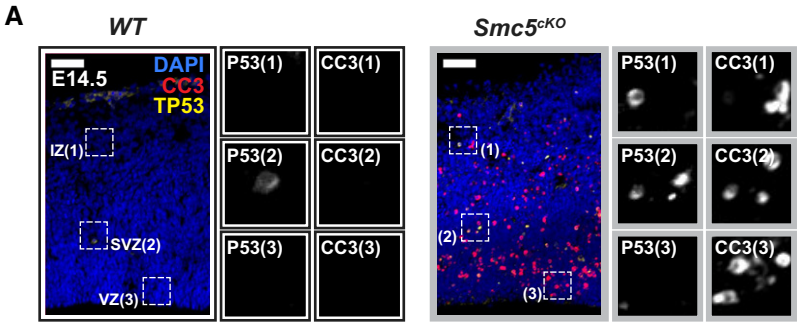


Figure EV5.



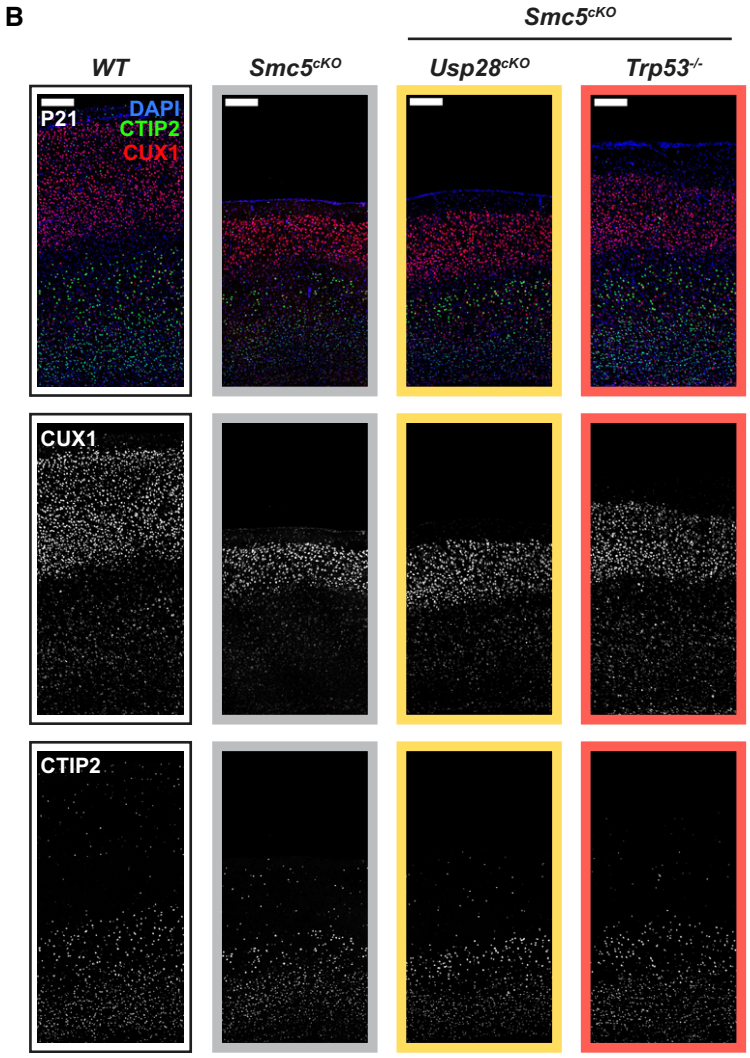


Figure EV6. *Smc5*^{cKO} embryonic brains show TP53 upregulation. Related to Fig 6.

A Cortices from E14.5 embryos stained with antibodies against cleaved-caspase 3 (CC3, red), TP53 (yellow), and DAPI (blue). Insets showing zoomed in view of the intermediate zone (IZ), the sub-ventricular zone (SVZ), and ventricular zone (VZ). Scale bar = 50 μm.

B P21 cortices of the indicated genotypes stained with antibodies against the deep layer marker CTIP2 (green), superficial layer marker CUX1 (red) and DAPI (blue). Scale bar = 200 μm.