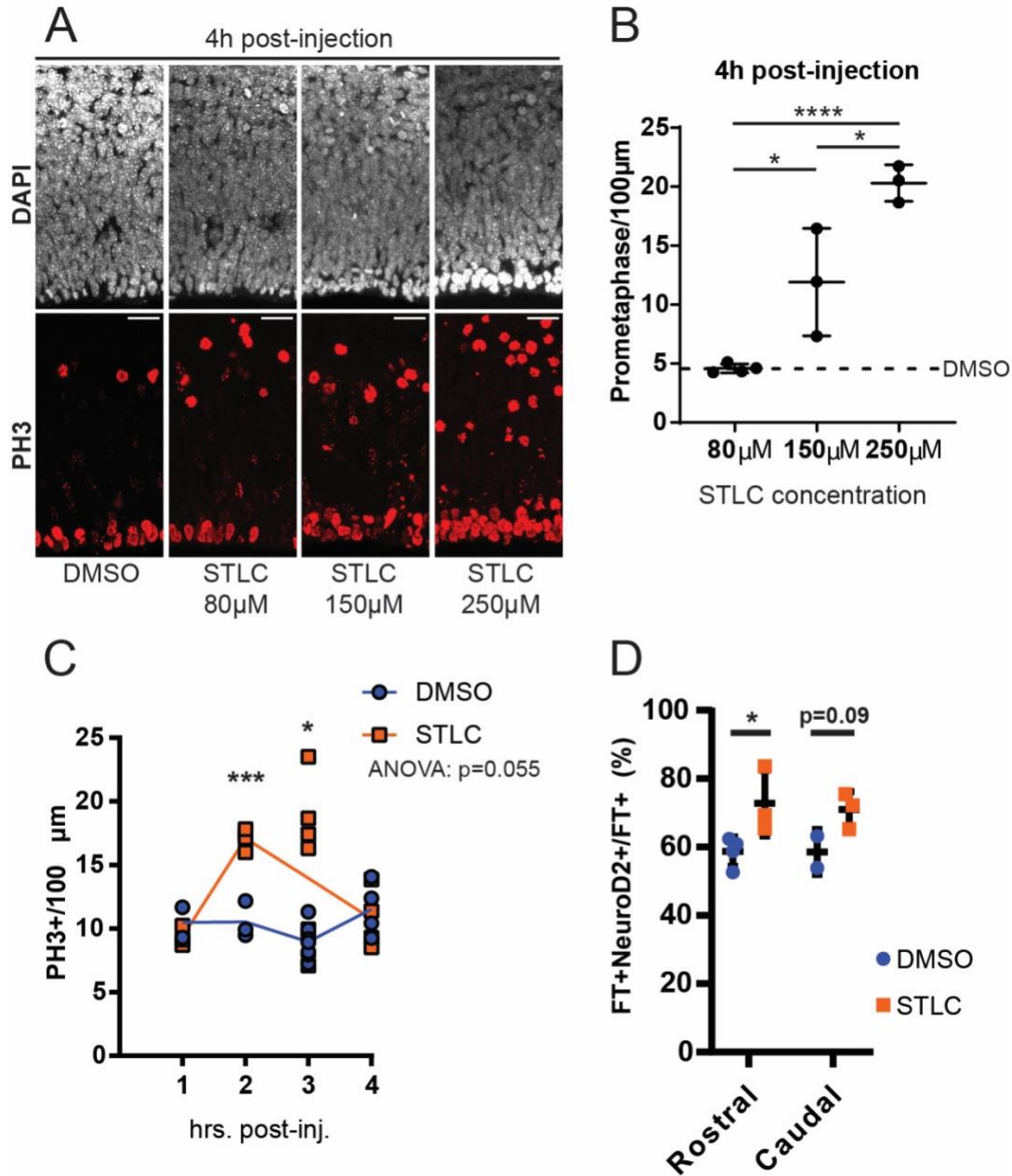


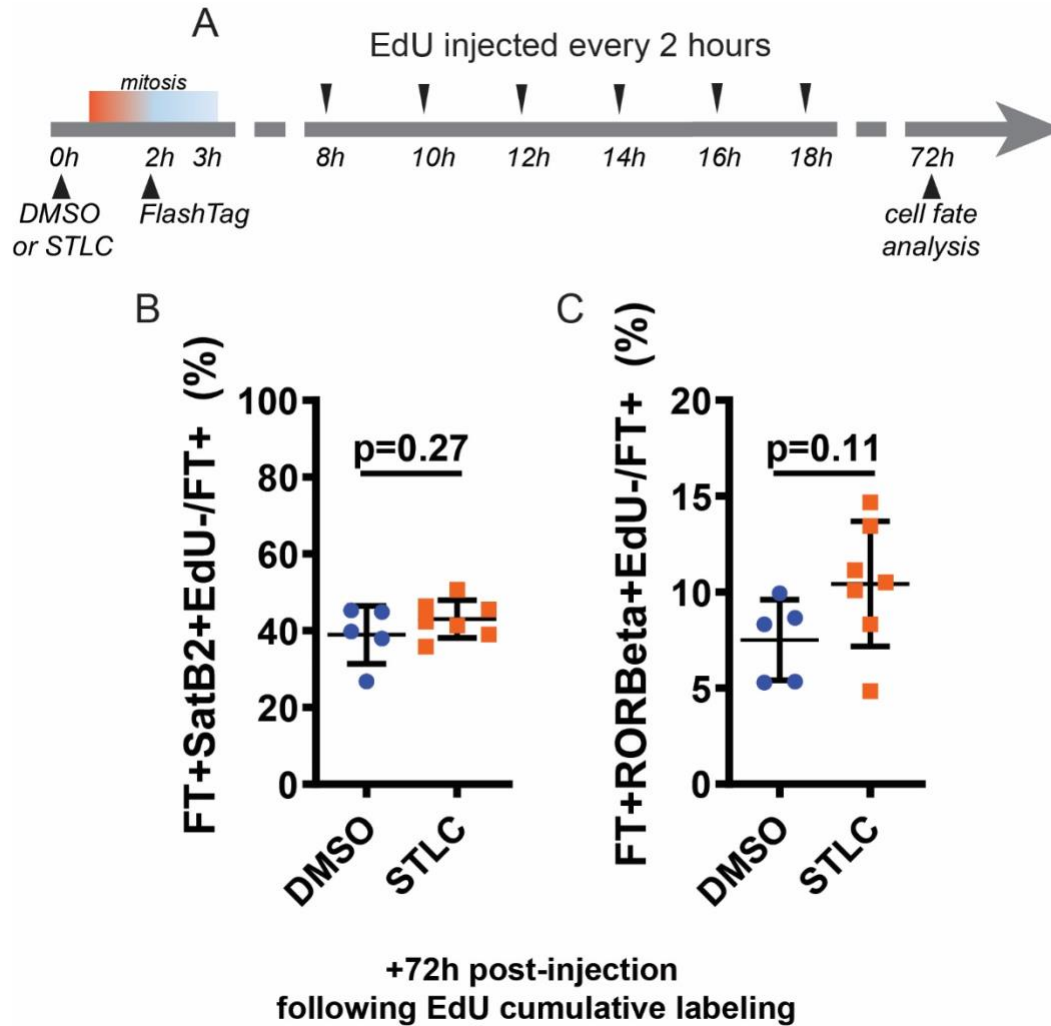
Supplemental Figures and Figure Legends:



Supplemental Figure 1. Effects of STLC injection *in vivo*.

A) Images depicting immunofluorescence of Phospho-Histone H3 (PH3, red) and DAPI (greyscale) at the ventricle, E14.5, 4h following injection of various concentrations of STLC. Scale bar: 25 µm. Student's t-test. 250µM v 80µM ****: $p < 0.0001$, 150µM v 80µM *: $p = 0.02$, 250µM v 150µM *: $p = 0.04$. B) Quantification of the average number of prometaphase cells per 100 µm, along the ventricular

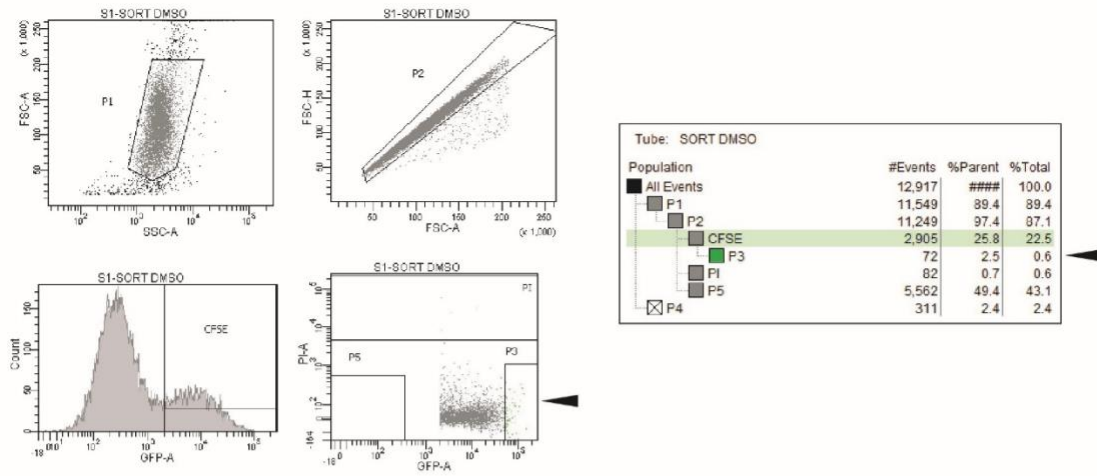
8 surface of the cortex (via PH3 and DAPI morphology) at 3 concentrations of STLC 4h post-
9 injection. Dotted line: averaged DMSO value. C) Quantification of the average number of
10 prometaphase cells per 100 μm , along the ventricular surface of the cortex at different timepoints
11 following injection of DMSO or STLC. Statistics: ANOVA *: $p=0.055$, post-hoc student's t-test. 2h
12 ***: $p=0.0005$, 3h *: $p=0.045$. D) Quantification of the fraction of NeuroD2 cells out of total FT+
13 population, across rostral or caudal sections. Developing hippocampus/hem used to denote
14 Caudal sections v rostral sections. $N \geq 2$ embryos per condition, averages of 2 or more sections
15 per condition. $N=11$ total embryos, Rostral Caudal *: $p=0.04$. Error bar=s.d.



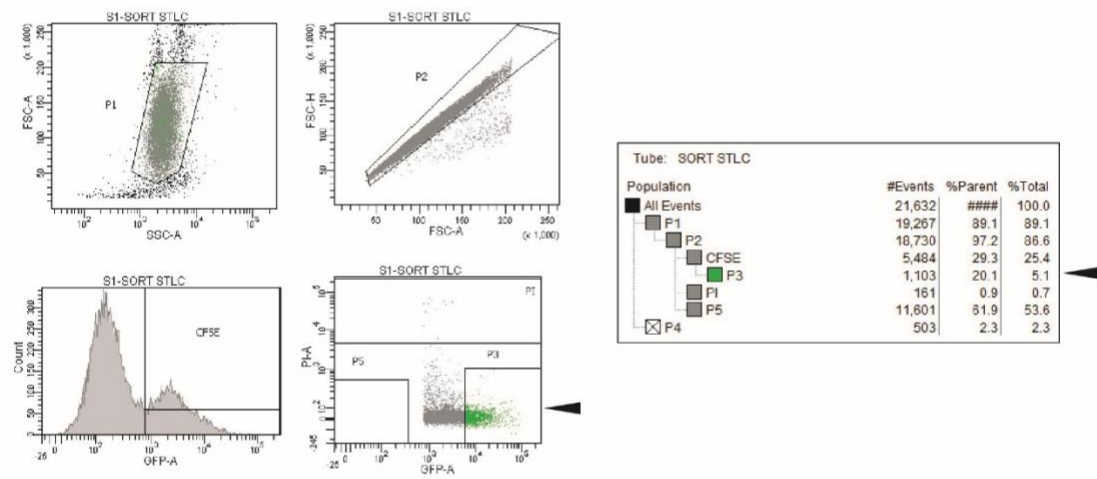
Supplemental Figure 2. Cortical layer marker expression 72h following prolonged mitosis.

A) Scheme of experimental paradigm. B) Proportion of FlashTag cells exiting the cell cycle after the first division (EdU-) that express RORBeta 72h after injection of DMSO or STLC. C) Proportion of FlashTag cells exiting the cell cycle after the first division (EdU-) that express SatB2 72h after injection of DMSO or STLC. Dots represent individual brains. $N \geq 3$ brains per condition, averages of 2 or more sections per brain. Error bar=s.d. Statistics: student's t-test.

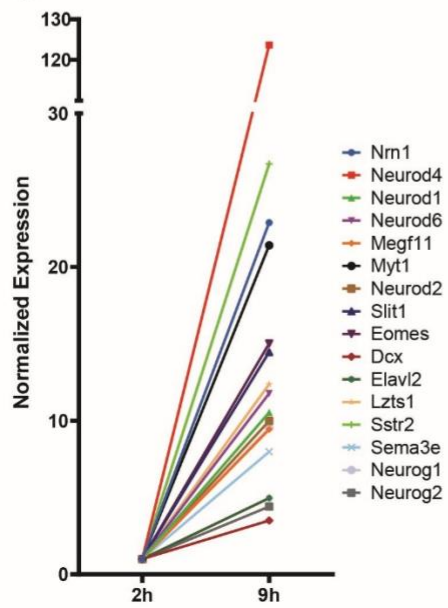
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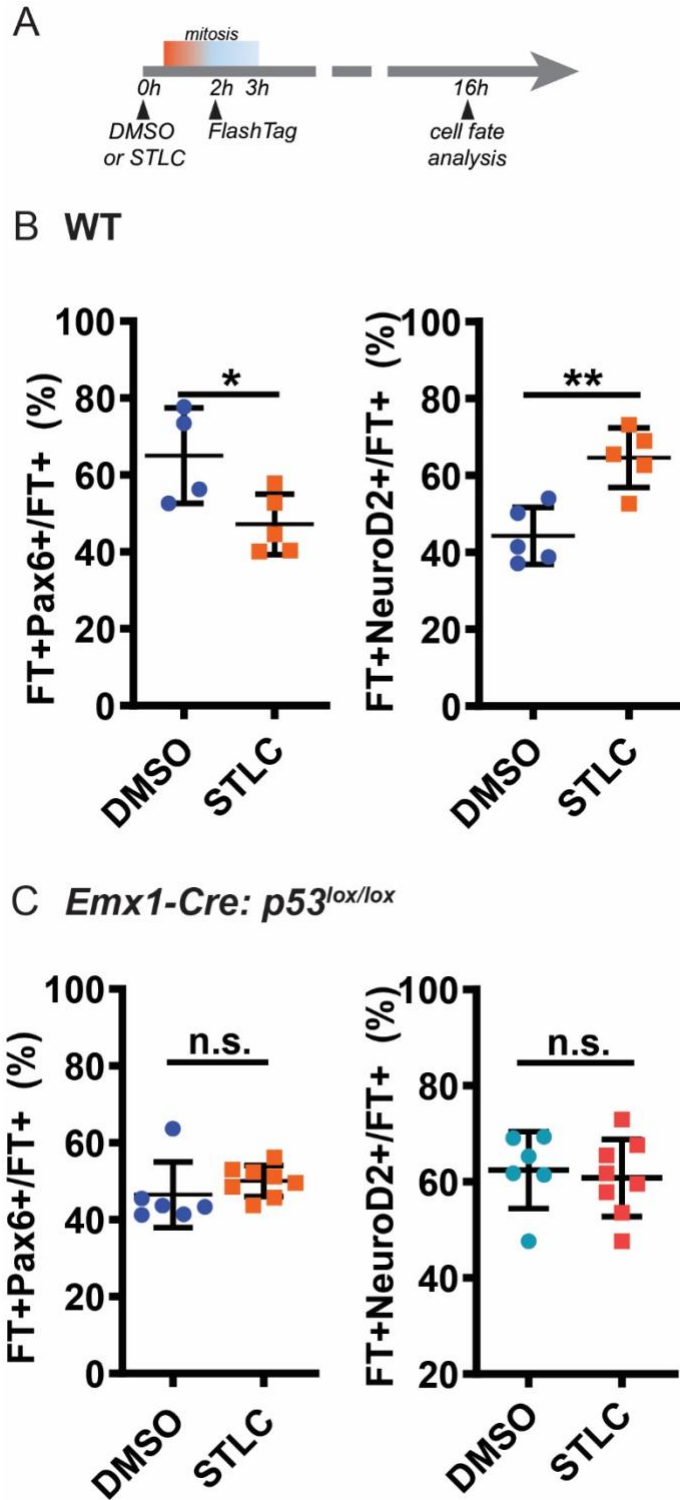
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C



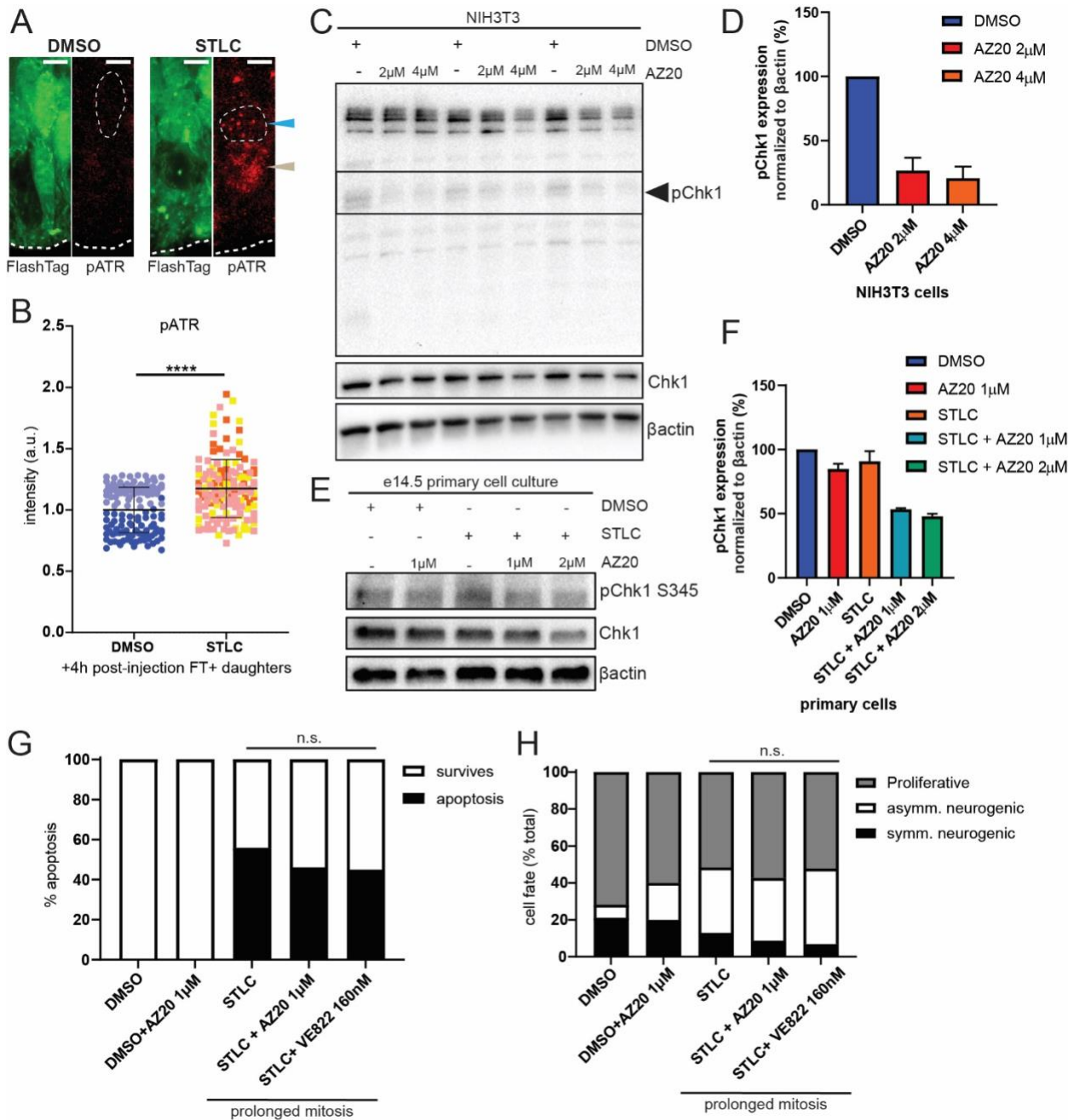
Supplemental Figure 3. Transcriptome analysis following prolonged mitosis. A, B) FACS workflow for purifying live, FT+ cells from the developing cortex in both DMSO and STLC conditions, 2h timepoint. C) Graph depicting RNA-seq values of select neurogenic genes across time, corresponding to timepoints of FT labeled cells at 2 hr and 9h. (2h).



Supplemental Figure 4. Effect of p53 cKO on cell fate after prolonged prometaphase *in vivo*. A) Scheme depicting sequential injection experimental paradigm used in this experiment.

B) Quantification of the fraction of FT+ cells which are Pax6+ and NeuroD2+ in E14.5 WT (C57BL6/J brains). N ≥ 4 brains per condition, 2 or more sections per brain. Dots represent individual brains. statistics: student's t-test. Pax6: p=0.03; NeuroD2: p=0.0029 Error bar =s.d.

C) Quantification of the fraction of FT+ cells which are Pax6+ and NeuroD2+ in E14.5 *Emx-Cre; p53^{lox/lox}* brains. Statistics: student's t-test. Pax6: DMSO p53 cKO: n=6 embryos, averages of 2 or more sections per embryo. STLC p53 cKO: n=8 embryos, n=3 sections each. *NeuroD2*: DMSO p53 cKO: n=6 embryos, n=3 sections each. STLC p53 cKO: n=8 embryos, n=3 sections each. Error bar=s.d. Dots represent individual brains.



Supplemental Figure 5. Inhibition of ATR and ATM signaling has no effect on cell fate following prolonged mitosis *in vitro*. A) Images depicting expression of pATR (red) in FT+ cells (green) 4h after injection of DMSO or STLC. Gray arrowhead: cell in mitosis exhibiting pATR. Blue arrowhead: FT+ daughter cell with pATR expression following STLC. B) Quantification of pATR signal intensity measured in FT cells 4h after injection of DMSO or STLC at e14.5. Dots

69 represent individual cells from 2 embryos. Student's t-test. ****: $p < 0.0001$. C, E) Western Blots
70 of pChk1 following ATR inhibition in MEFs (c) and primary e13.5 cultures (E). D, F) Quantification
71 of western blots in C (D) and E (F). G) Quantification of impact of ATR or ATM inhibition on
72 apoptosis via live imaging *in vitro* of E13.5 cortical primary cell culture. H) Quantification of impact
73 of ATR or ATM inhibition on cell fate via live imaging of E13.5 cortical primary progenitors.
74 Statistics: student's t-test. Scale bar=5 μm .

75 **Table S1 RNA seq**

76 **Table S2 GSEA**

77 **Table S3 Antibodies used**