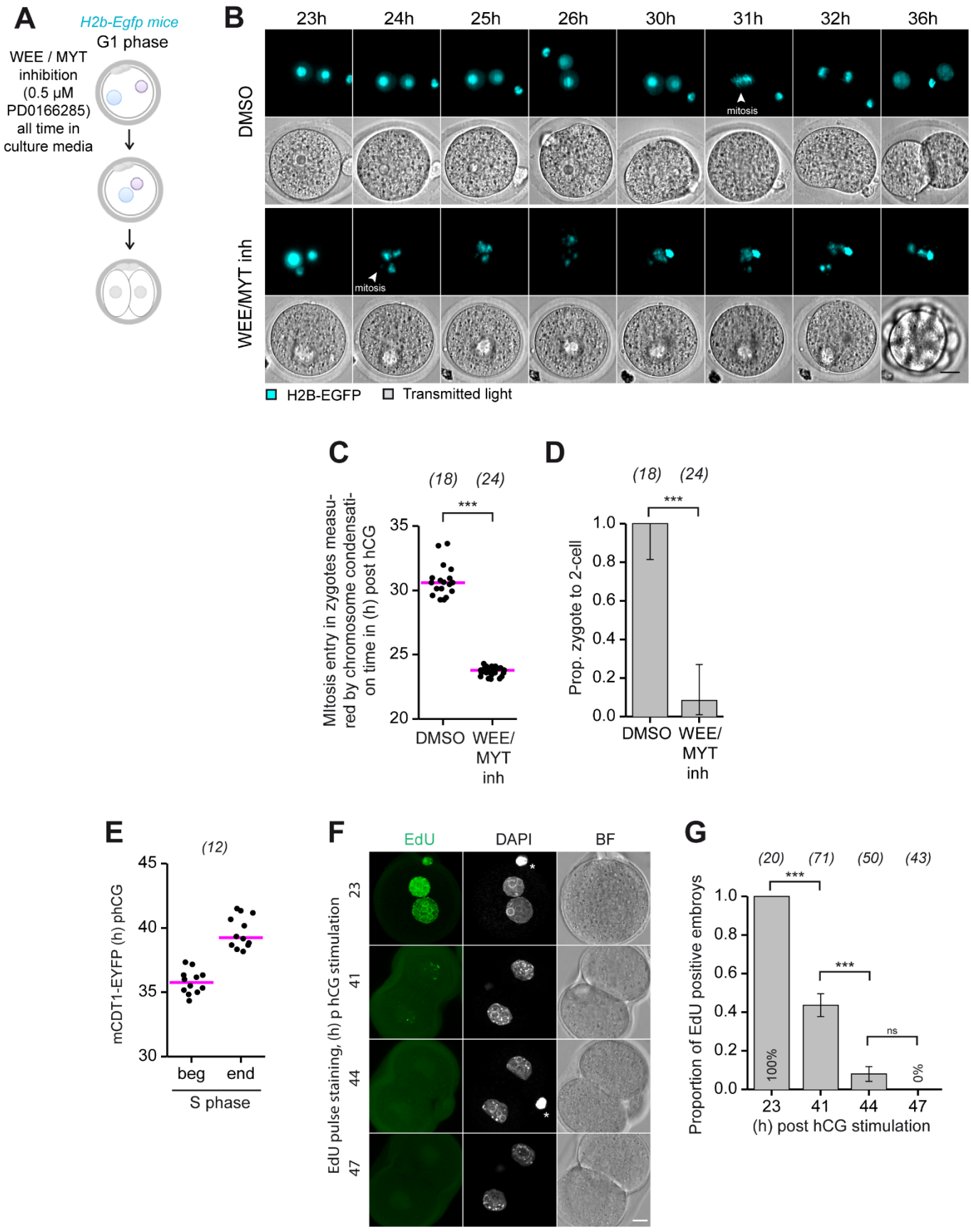
Appendix File Table of Contents

Appendix Figure S1



Appendix Figure S1 Characterisation of completing DNA replication and duration of S-phase

A Schematic of the experimental procedure.

B Representative still images from time-lapse light-sheet imaging of embryos from *H2b-Egfp* transgenic mice from 23 to 36 h post hCG administration after WEE/MYT kinase inhibition (as shown in **A**) or DMSO treatment (21 h post hCG administration). Embryos expressing H2B-EGFP (cyan, chromosomes) are shown. Transmitted light (grays). Arrows indicate mitotic entry. Scalebar 20 µm. See also related Movie EV1 (ctrl, WEE/MYT inhibition)

C Data from time-lapse light-sheet imaging (**B**) was used to quantify chromosome condensation time (h) from zygote to 2-cell stage embryos. The data were pooled from two independent experiments. Median is shown. ***p=0.00000 (Mann-Whitney U Test, two-sided).

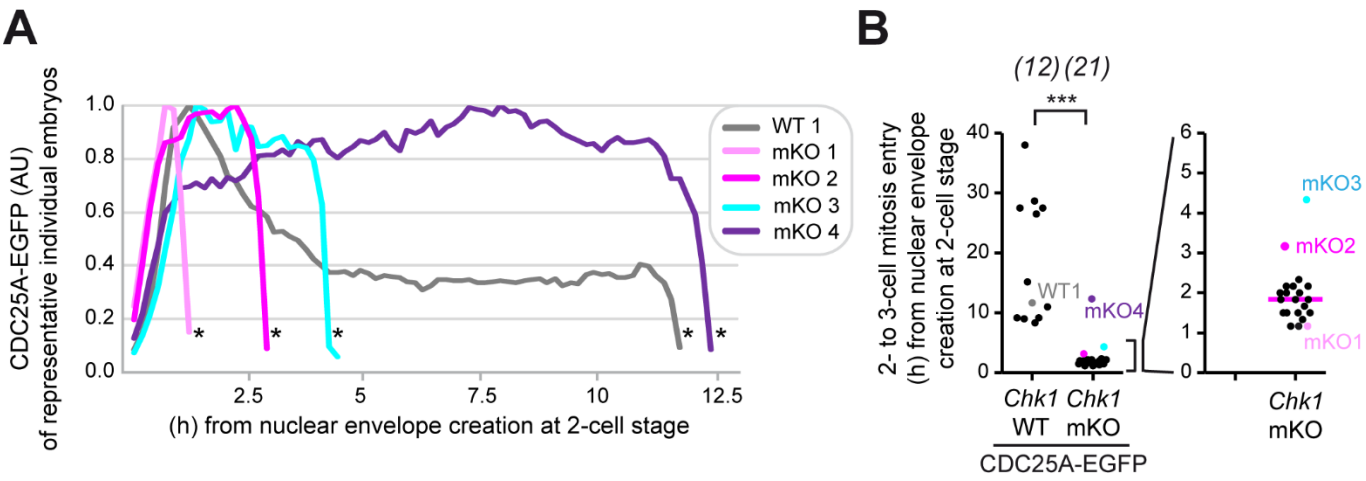
D Data from time-lapse light-sheet imaging (**B**) was used to quantify the proportion of zygote to 2-cell development. The data were pooled from two independent experiments. Error bars denote the 95% confidence interval. ***p=0.0000 (Fisher's Exact Test, two-sided).

E Beginning and ending of S phase measured by mCDT1-EYFP sensor fluorescence signal in wild-type embryos using time-lapse light-sheet imaging. The magenta bar represents the median of the S phase beginning and ending.

F Representative images from analysis of S phase estimated by EdU pulse staining in wild-type embryos at 23, 41, 44, and 47 h post hCG administration. The embryos were fixed after a 60 min EdU pulse (green), and DNA was visualized by DAPI staining (grays, chromatin). BF (bright field). Asterisk mark polar bodies. Scalebar 10 µm.

G Data from immunofluorescence analysis (**F**) was used to quantify the proportion of EdU-positive embryos. Error bars denote the standard error of the mean. The data are from one experiment. ns non-significant p=0.1211, ***p=0.0000 (Fisher's Exact Test).

Appendix Figure S2

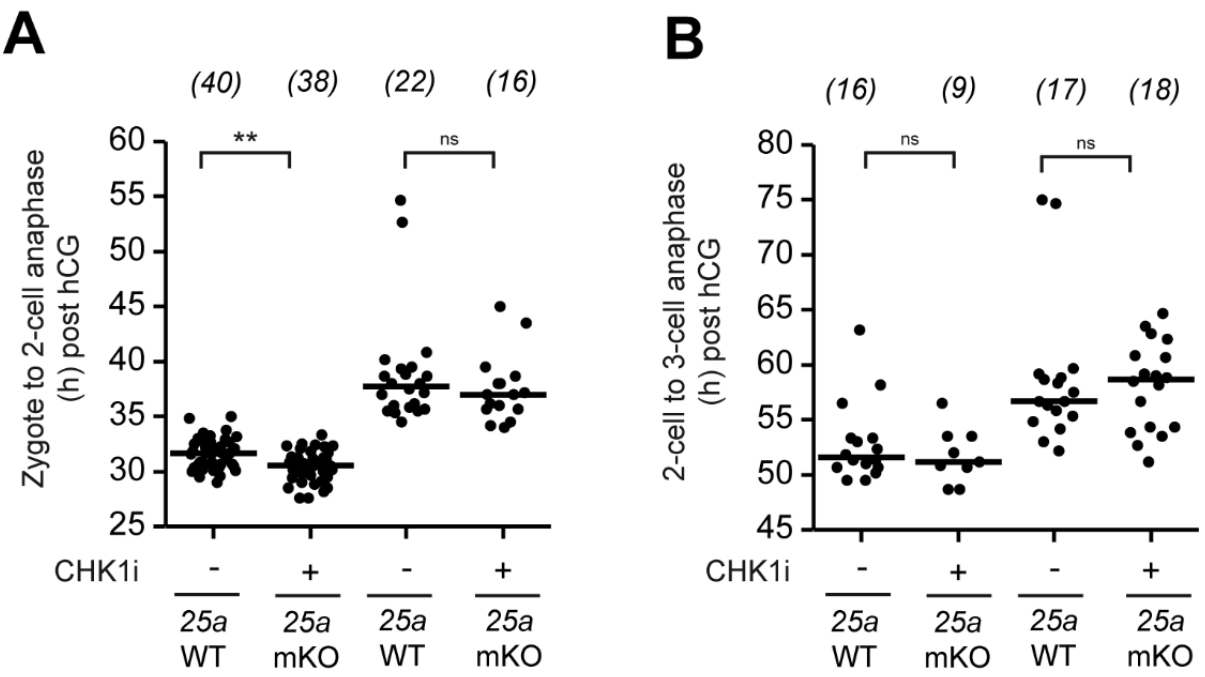


Appendix Figure S2 CHK1 kinase regulates long G2 in mouse 2-cell stage embryos by restraining CDK1 activity via CDC25A degradation

A Line chart of fluorescence signal intensity of CDC25A-EGFP of individual *Chk1 WT* and *Chk1 mKO* 2-cell stage embryos scanned using light-sheet time-lapse microscopy from nuclear envelope to 12,5 h after nuclear envelope formation. Embryos were microinjected as shown in **Fig 3B**. Asterisk mark signal drop after nuclear envelope breakdown (not degradation). The data are from one experiment with two *Chk1 WT* and four *Chk1 mKO* mice treated individually. The line of the *Chk1 WT* average is shown. The 2- to 3-cell anaphase timing is shown in Fig. EV3F.

B Data from time-lapse light-sheet imaging **(Fig 3B)** was used to quantify initiation of anaphase (h) from 2-cell to 3-cell in *Cdc25a*-microinjected *Chk1 WT*, and *Cdc25a*-microinjected *Chk1 mKO.* Median is shown. ***p=0.0000 (Mann-Whitney U test, two-sided).

Appendix Figure S3



Appendix Figure S3 Effect of CHK1 inhibition on the timing of mitosis in *Cdc25a* mKO embryos.

A, B Dot-plot graph of division time (h) from zygote to 2-cell (**A**) and 2-cell to 3-cell stage (**B**) analyzed from time-lapse wide-field imaging of *Cdc25a* WT and *Cdc25a mKO* embryos. The embryos were treated with 250 nM CHIR-124. ns non-significant, **p<0.01 (Mann-Whitney U test, two-sided).