Expanded View Figures

Figure EV1. Maternally expressed CHK1 regulates early embryonic development.

A Schematic depiction of Cre-recombinase expression specifically in growing oocytes under *zona pellucida 3 (Zp3)* promoter.

B Schematic depiction of *Chk1* gene with indicated protein domains. Exon 2 of *Chk1*, which contains a translation initiation site and is flanked by LoxP sites, is excised by Cre-recombinase, thereby preventing translation initiation of full-length *Chk1* mRNA.

C Female mice carrying floxed *Chk1* exon 2 and Cre-recombinase under oocyte-specific *Zp3* promoter are mated with *Chk1* wild-type (WT) male producing maternal *Chk1* knock-out embryos with present paternal allele (*Chk1 mKO*).

D CHK1 protein level detected by immunoblot from positive controls (10 germinal vesicle (GV) stage oocytes microinjected with full-length 300 ng/μl of *Chk1* cRNA), 10 GV oocytes (GV WT), and sperm (0.22×10^6). kDa—kilodalton.

E CHK1 protein levels detected by immunoblot in *Chk1* WT and *Chk1* mKO zygotes at 26 h post-hCG administration.

F Proportion of zygotes developed to blastocysts after pharmacological CHK1 inhibition in C57J/BL6 or CD-1 females. The numbers in brackets denote the number of analyzed embryos. Error bars denote the 95% confidence interval. ***$P = 0.00000$ (Fisher's exact test, two-sided).

G Data from bright-field time-lapse imaging (Fig 1B) were used to quantify the division time (h) from zygote to two-cell in *Chk1* WT, *Chk1* mKO *class I*, and *Chk1* mKO *class II* embryos categorized retrospectively based on their division timing to the three-cell stage, related to Fig 1B–F. The numbers in parentheses show the number of embryos analyzed for each genotype. The median is shown. ns, non-significant, $P = 0.135868$, **$P = 0.00808$, and ***$P = 0.000026$ (Mann–Whitney *U*-test).

H Time of the two-cell to three-cell division after hCG administration relative to the zygote to two-cell division, related to Fig 1B–F. *Chk1* mKO *class I* embryos show a positive correlation between the division time from zygote to two-cell and two-cell to three-cell (Pearson's correlation coefficient of 0.7932, $P < 0.0000$). *Chk1* WT embryos show a positive correlation (Pearson's correlation coefficient of 0.4214, $P = 0.0001$) but with some substantial outliers. *Chk1* mKO *class II* shows a non-significant negative correlation (Pearson's correlation coefficient −0.3080, $P = 0.0812$).

I CHK1 protein levels detected by immunoblot from two- or three- to four-cell embryos collected 47 h post-hCG administration. $n = 79$, 79, and 78 embryos for *Chk1* WT, *Chk1* mKO *class I*, and *Chk1* mKO *class II*, respectively.

J Single-cell PCR analysis of Cre-mediated DNA recombination status in individual two-cell stage *Chk1* WT, two-cell stage *Chk1* mKO *class II*, and three-cell stage *Chk1* mKO *class I* embryos. 1 kb—1 kb ladder, *Lox*—Chk1 lox/lox, PC—positive control, NC—negative control.

K Proportion of *class I* embryos indicated for individual mice. The embryos were scored at 46–47 h post-hCG administration. The numbers in brackets denote the number of mice. Data are shown as box and whisker plots, where central band represents median, upper and lower quartiles are the ends of the box, and whiskers box edges ±15%. ***$P = 0.000002$ (Mann–Whitney *U*-test).

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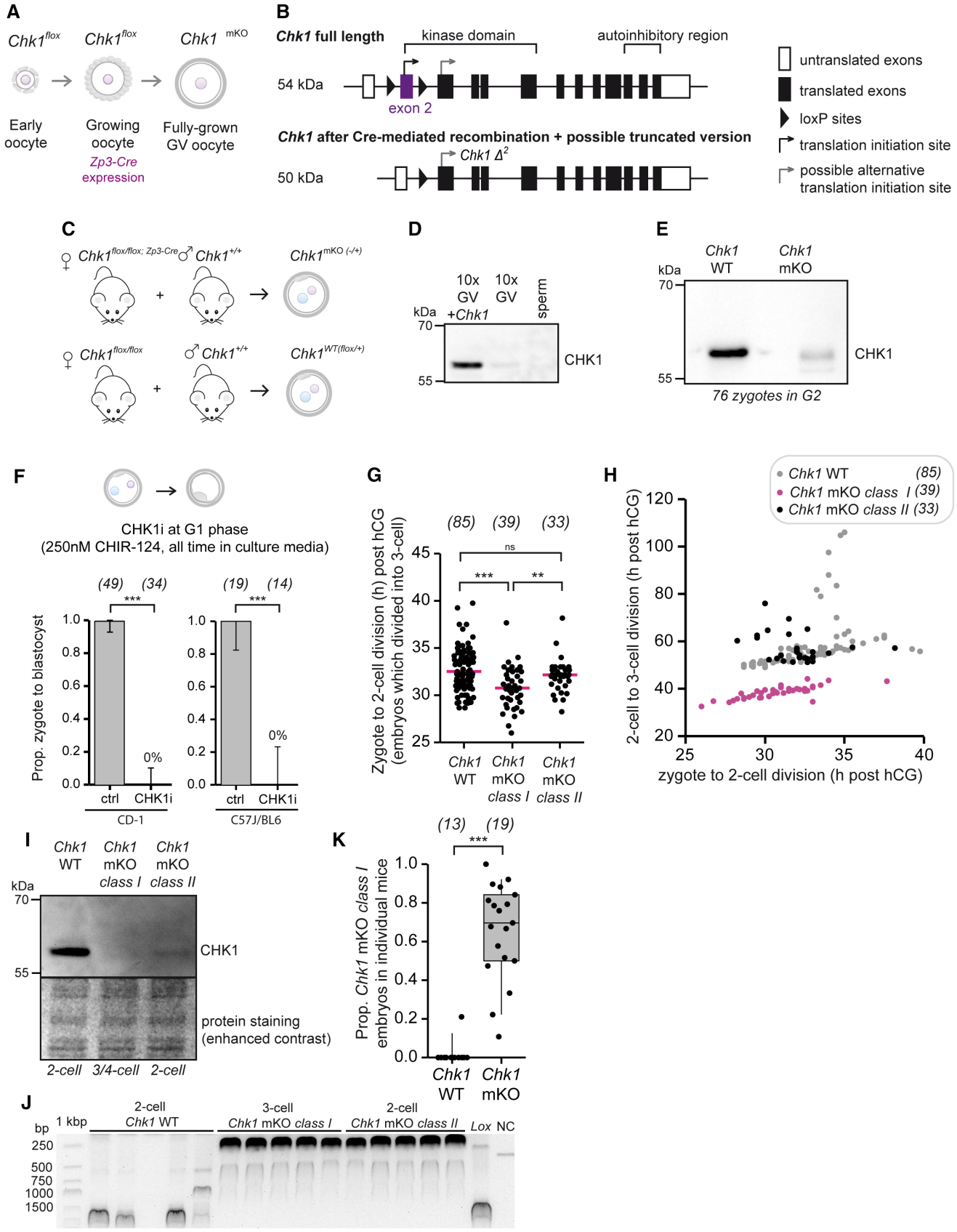

Figure EV1.

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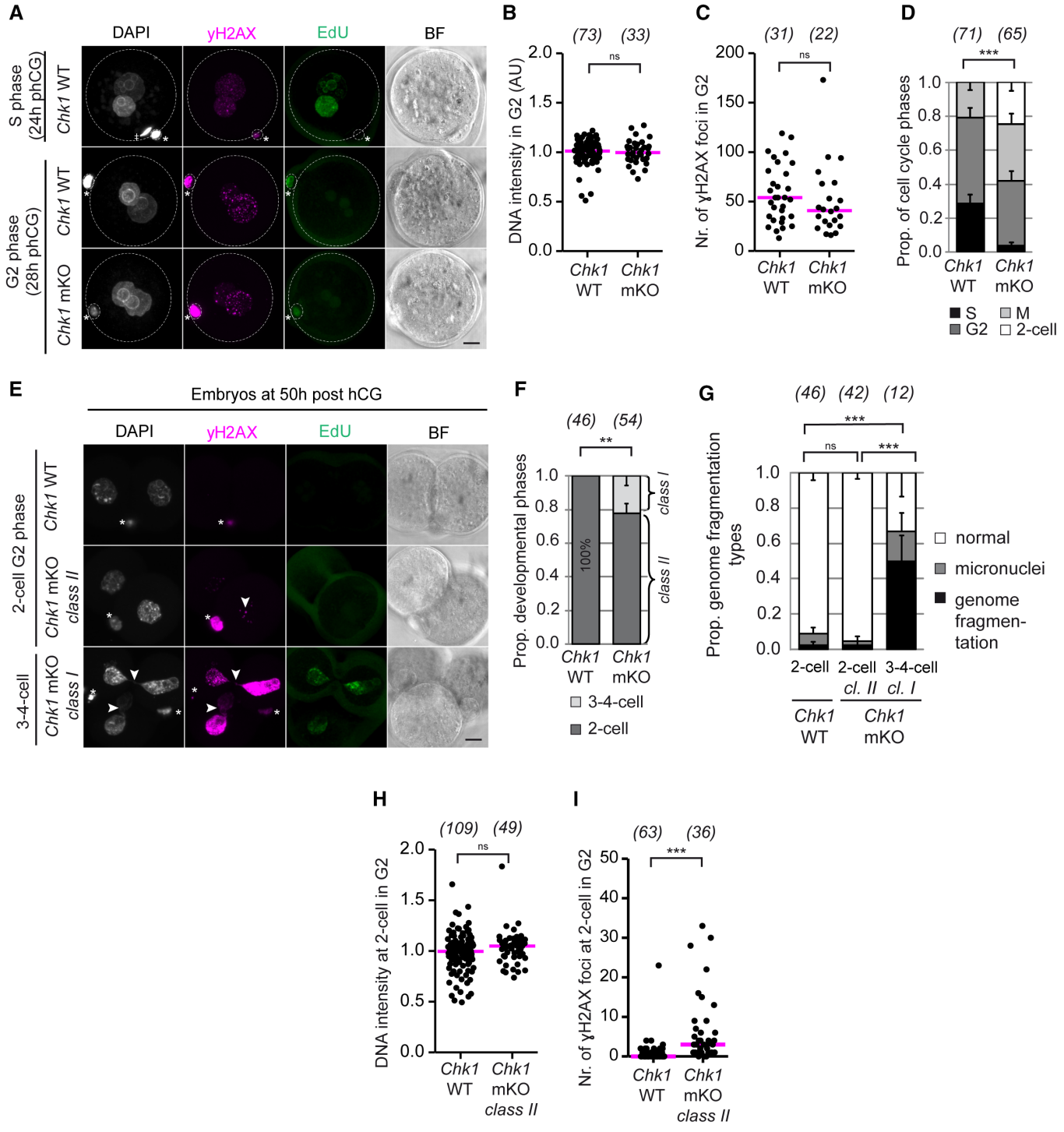
A
DAPI
γH2AX
EdU
BF
S phase (24h phCG)
Chk1 WT
G2 phase (28h phCG)
Chk1 WT
Chk1 mKO
B
(73) (33)
ns
DNA intensity in G2 (AU)
Chk1 WT
Chk1 mKO
C
(31) (22)
ns
Nr. of γH2AX foci in G2
D
(71) (65)
***
Prop. of cell cycle phases
S
G2
M
2-cell
E
Embryos at 50h post hCG
2-cell G2 phase
Chk1 mKO class II
3-4-cell
Chk1 mKO class I
F
(46) (54)
**
Prop. developmental phases
100%
class I
class II
3-4-cell
2-cell
G
(46) (42) (12)
***
ns
Prop. genome fragmentation types
normal
micronuclei
genome fragmentation
2-cell
2-cell cl. II
3-4-cell cl. I
H
(109) (49)
ns
DNA intensity at 2-cell in G2
Chk1 mKO class II
I
(63) (36)
***
Nr. of γH2AX foci at 2-cell in G2
Chk1 mKO class II

Figure EV2.

Figure EV2. CHK1 kinase regulates cell cycle progression.

A Representative still images from immunofluorescence analysis of cell cycle distribution in *Chk1* WT and *Chk1* mKO embryos combined with γH2AX foci and DNA content analysis. Zygotes in the S phase (24 h post-hCG administration) and G2 phase (28 h post-hCG administration) were fixed after a 60 min EdU pulse to identify zygotes that completed DNA replication. Embryos with γH2AX signal (magenta), DAPI (chromatin, gray), EdU (green), and BF (bright field) are shown. Asterisk marks polar bodies. Crosses mark the sperm head at the membrane. Scale bar 10 μm.

B, C Data from immunofluorescence analysis (A) were used to quantify DNA content (B) and γH2AX foci number (C) in zygotes in the G2 phase. The median is shown. DNA content was measured by DAPI intensity. The data were normalized to the mean in each group. AU (arbitrary units). The data were pooled from two independent experiments. ns, non-significant, $P = 0.727857$ (B), $P = 0.190458$ (C) (Mann–Whitney *U*-test, two-sided).

D Data from immunofluorescence analysis (A) were used to quantify the proportion of cell cycle phases in embryos. Error bars denote the standard error of the mean. ***$P = 0.00000$ (Cochran–Armitage trend test, two-sided).

E Representative still images from immunofluorescence analysis of cell cycle distribution in *Chk1* WT and *Chk1* mKO embryos combined with γH2AX foci and DNA content analysis. Embryos at 50 h post-hCG administration (G2 phase) were fixed after a 60 min EdU pulse to identify zygotes that completed DNA replication. Embryos with γH2AX signal (magenta), DAPI (chromatin, gray), EdU (green), and BF (bright field) are shown. Asterisk marks polar bodies. Arrowhead points to DNA damage visualized by γH2AX or DAPI. Scale bar 10 μm.

F, G Data from immunofluorescence analysis (E) were used to quantify the proportion of developmental phase (F) and genome fragmentation types (G) in embryos. Error bars denote the standard error of the mean. The data were pooled from two independent experiments. ns, non-significant, $P = 0.84592$, **$P = 0.00194$, ***$P = 0.00000$ (Cochran–Armitage trend test, two-sided).

H, I Data from immunofluorescence analysis (E) were used to quantify DNA content (H) and γH2AX foci number (I) in embryos at 50 h post-hCG administration. Median is shown. DNA content was measured by DAPI intensity. The data were normalized to the mean in each group. AU (arbitrary units). The data were pooled from two independent experiments. ns, non-significant, $P = 0.135607$, ***$P = 0.00000$ (Mann–Whitney *U*-test, two-sided).

Data information: The numbers in parentheses show the number of embryos analyzed for each genotype.

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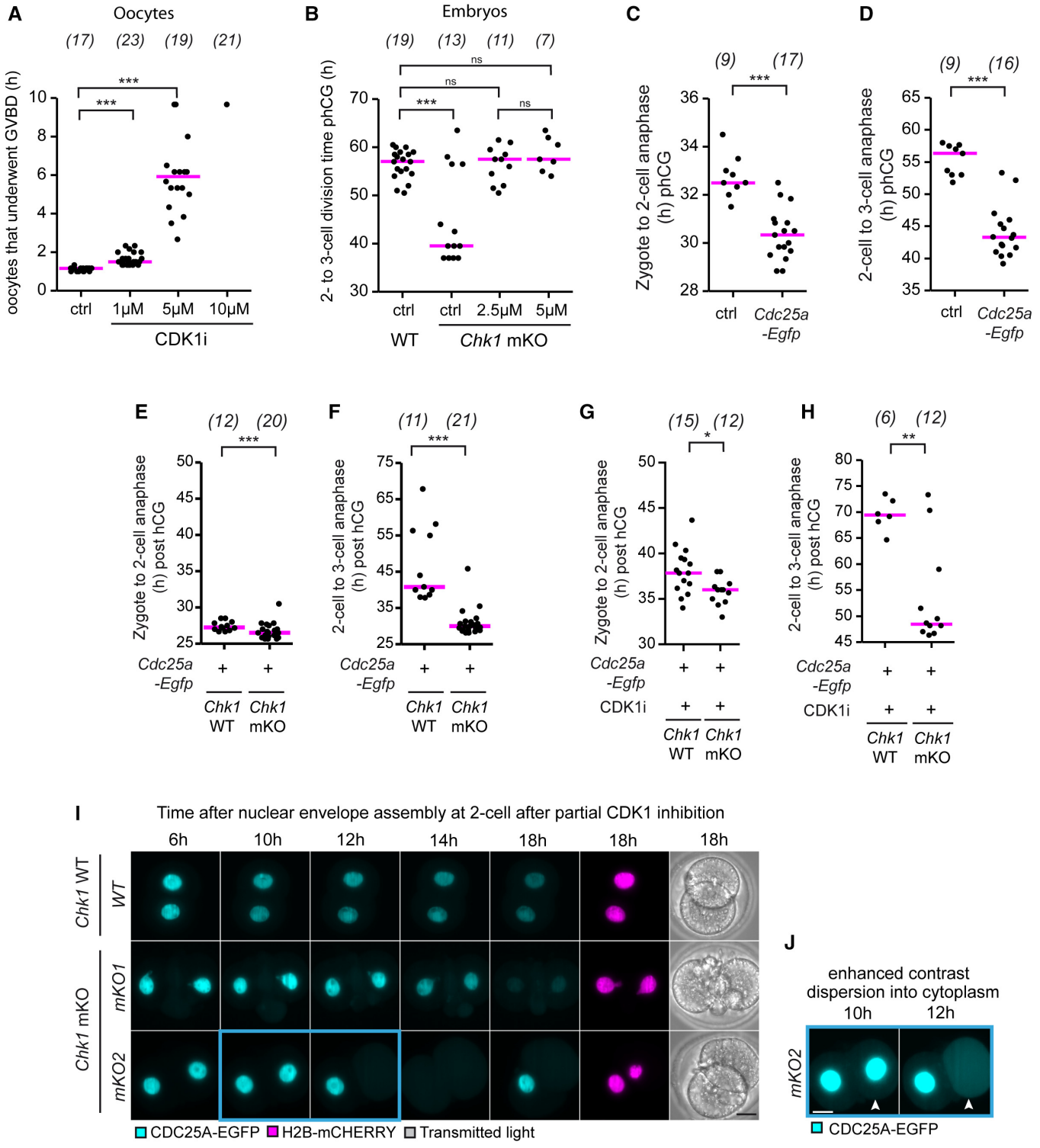
A
Oocytes
(17) (23) (19) (21)
oocytes that underwent GVBD (h)
ctrl 1μM 5μM 10μM
CDK1i
B
Embryos
(19) (13) (11) (7)
2- to 3-cell division time phCG (h)
ctrl ctrl 2.5μM 5μM
WT Chk1 mKO
C
(9) (17)
Zygote to 2-cell anaphase (h) phCG
ctrl Cdc25a -Egfp
D
(9) (16)
2-cell to 3-cell anaphase (h) phCG
ctrl Cdc25a -Egfp
E
(12) (20)
Zygote to 2-cell anaphase (h) post hCG
Cdc25a -Egfp
Chk1 WT Chk1 mKO
F
(11) (21)
2-cell to 3-cell anaphase (h) post hCG
Cdc25a -Egfp
Chk1 WT Chk1 mKO
G
(15) (12)
Zygote to 2-cell anaphase (h) post hCG
Cdc25a -Egfp
CDK1i
Chk1 WT Chk1 mKO
H
(6) (12)
2-cell to 3-cell anaphase (h) post hCG
Cdc25a -Egfp
CDK1i
Chk1 WT Chk1 mKO
I
Time after nuclear envelope assembly at 2-cell after partial CDK1 inhibition
6h 10h 12h 14h 18h 18h 18h
Chk1 WT
WT
Chk1 mKO
mKO1
mKO2
CDC25A-EGFP H2B-mCHERRY Transmitted light
J
enhanced contrast dispersion into cytoplasm
10h 12h
mKO2
CDC25A-EGFP

Figure EV3.

Figure EV3. CHK1 kinase regulates the long G2 phase in two-cell embryos by restraining CDK1 activity via CDC25A degradation.

A Dot-plot graph of GVBD time (h) analyzed from time-lapse wide-field imaging of oocytes after transfer to milrinone-free medium and treated with CDK1 kinase inhibitor (1, 5, and 10 μM RO3306). In the 10 μM RO3306 group, only 1 oocyte from 21 resumes meiosis. The data are from one experiment. ***P = 0.00000 (Mann–Whitney U-test).

B Dot-plot graph of division time (h) from two-cell to three-cell stage analyzed from time-lapse wide-field imaging of wild-type (WT) and *Chk1 mKO* embryos. The *Chk1 mKO* embryos were treated with 2.5 or 5 μM RO3306. Strain background: CD-1 with small part C57J/BL6 and C57J/BL6 with small part CD-1. ns, non-significant, ***P = 0.00034 (Kolmogorov–Smirnov test).

C, D Data from time-lapse light-sheet imaging were used to quantify the initiation of anaphase (h) from zygotes to two-cell stage embryos (C) and from two-cell to three-cell embryos (D) in WT embryos microinjected with *Cdc25a-Egfp* cRNAs. The data were pooled from two independent experiments. ***P = 0.000178 (C), P = 0.000133 (D) (Mann–Whitney U-test, two-sided).

E, F Data from time-lapse light-sheet imaging (Fig 3B) were used to quantify initiation of anaphase (h) from zygote to two-cell (E) and from two-cell to three-cell (F) in *Cdc25a*-microinjected *Chk1 WT* and *Cdc25a*-microinjected *Chk1 mKO*. Median is shown. ***P = 0.00000 (Mann–Whitney U-test, two-sided).

G, H Data from time-lapse light-sheet imaging (Fig 3E) were used to quantify initiation of first anaphase time (h) (G) and time of entry into the second mitosis (H) in *Chk1 WT* and *Chk1 mKO* embryos microinjected with *Cdc25a-Egfp* cRNA and treated with CDK1 inhibitor. Due to experimental procedures, not all embryos underwent cytokinesis after the first mitosis. Median is shown. *P = 0.015593, **P = 0.005921 (Mann–Whitney U-test, two-sided).

I Representative still images from time-lapse light-sheet imaging (Fig 3E) of *Chk1 WT* and *Chk1 mKO* embryos treated with CDK1 inhibitor. Embryos were microinjected, as shown in Fig 3E. Embryos expressing H2B-mCHERRY (magenta, chromosomes) and CDC25A-EGFP (cyan) are shown. Scale bar 20 μm. See also related Movie EV3 (*Chk1 WT* + CDC25A-EGFP + CDK1i, *Chk1 mKO (mKO1)* + CDC25A-EGFP + CDK1i, *Chk1 mKO (mKO2)* + CDC25A-EGFP + CDK1i).

J Enhanced contrast of still images of mKO2 at 10 h and 12 h. Arrowheads mark CDC25A-EGFP signal in cytoplasm. Note that cytoplasmic signals increase from 10 to 12 h, indicating that there is CDC25A-EGFP dispersed from the nucleus into cytoplasm (not degradation). Scale bar 20 μm. See also related Movie EV3 (*Chk1 WT* + CDC25A-EGFP + CDK1i, *Chk1 mKO (mKO1)* + CDC25A-EGFP + CDK1i, *Chk1 mKO (mKO2)* + CDC25A-EGFP + CDK1i).

Data information: The numbers in parentheses denote the number of analyzed oocytes per group. Median is shown.

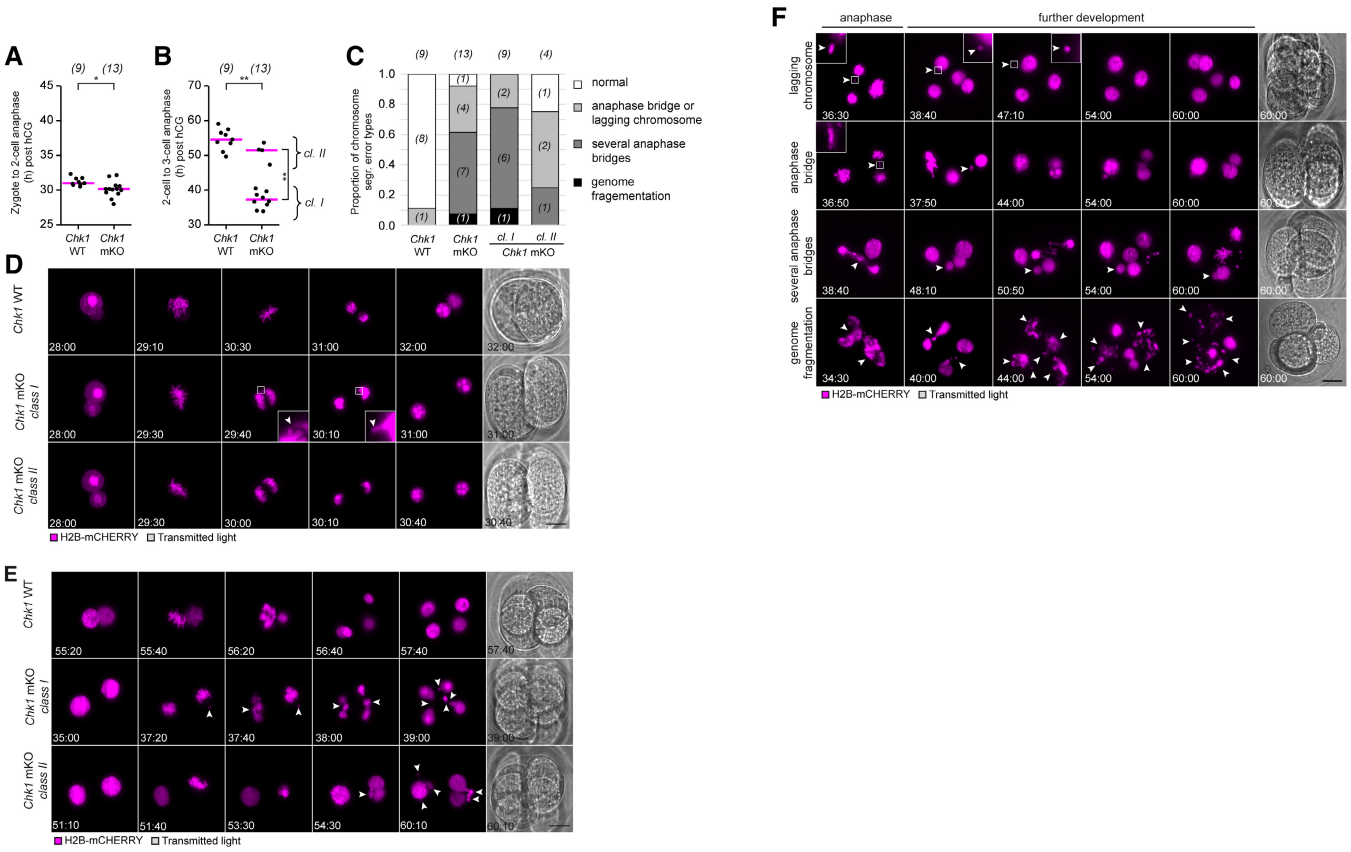

Figure EV4.

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Figure EV4. CHK1 is essential for DNA integrity protection in the second embryonic division.

A, B Data from time-lapse light-sheet imaging (Fig 5B) were used to quantify initiation of anaphase (h) from zygote to two-cell (A) and time of division (h) from two-cell to three-cell (B) in *Chk1 WT* and *Chk1 mKO* embryos. A subgroup of *Chk1 mKO* embryos (*Chk1 mKO class I*) shows substantially accelerated second division. The median is shown. The data were pooled from two independent experiments. *$P = 0.01745$ (equal-variance *T*-test, two-sided). **$P < 0.01$ relative to *class I* (Kolmogorov–Smirnov test, two-sided), **$P < 0.01$ relative to *Chk1 WT* (Mann–Whitney *U*-test).

C Data from time-lapse light-sheet imaging (Fig 5B) were used to quantify the proportion of different types of chromosome segregation errors in embryos.

D–F Representative still images from time-lapse light-sheet imaging (Fig 5B) of the first embryonic division (D), second embryonic division (E), and different chromosome segregation error types (F) in wild-type (*Chk1 WT*), *Chk1 mKO class I*, and *Chk1 mKO class II* embryos. Embryos were microinjected, as shown in Fig 5A. Embryos expressing H2B-mCHERRY (magenta, chromosomes) are shown. Arrowheads mark different chromosome segregation error types. Scale bar 20 μm. See also related Movie EV6 (lagging chromosome, anaphase bridge, several anaphase bridges, and genome fragmentation).

Data information: The numbers in brackets denote the number of analyzed embryos per genotype.

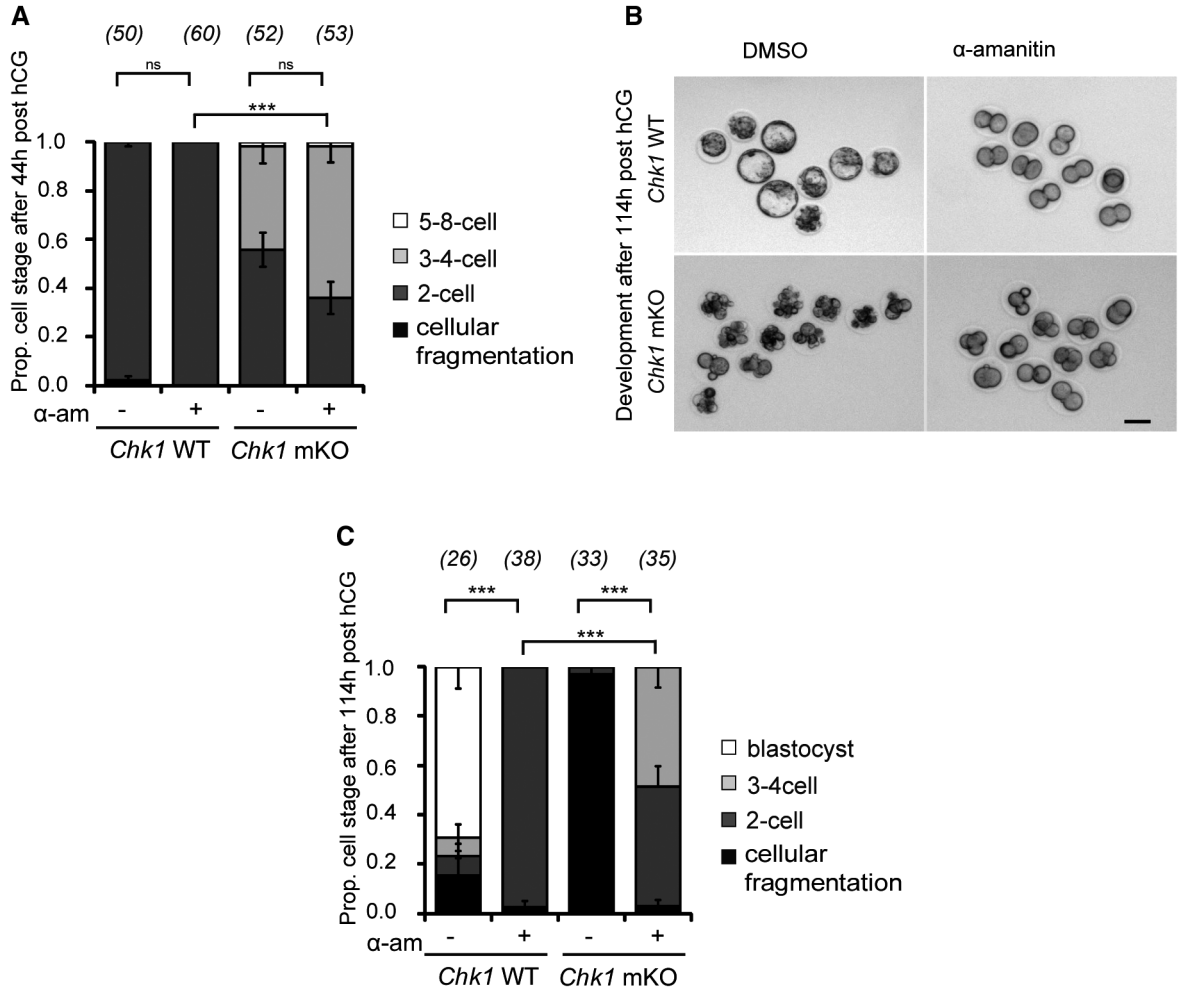

Figure EV5. CHK1 is necessary for the two-cell stage transcription block.

A Developmental analysis of *Chk1* WT and *Chk1* mKO embryos treated with α-amanitin (24 μg/ml) or DMSO. The embryos were scored at 44 h post-hCG administration. The numbers in parentheses denote the number of analyzed embryos per group. The data were pooled from two independent experiments. Strain background: CD-1 with small part C57J/BL6 and C57J/BL6 with small part CD-1. Error bars denote the standard error of the mean. ns, non-significant $P = 0.27113$ (*Chk1* WT), $P = 0.05605$ (*Chk1* mKO), ***$P = 0.00000$ (Cochran–Armitage trend test, two-sided).

B Representative images from developmental analysis (C) of developmental stages at 114 h post-hCG administration. Scale bar 60 μm.

C Developmental analysis of *Chk1 WT* and *Chk1 mKO* embryos treated with α-amanitin (24 μg/ml) or DMSO. The embryos were scored at 114 h post-hCG administration. The numbers in parentheses denote the number of analyzed embryos per group. The data were pooled from two independent experiments. Error bars denote the standard error of the mean. ***$P = 0.000$ (Cochran–Armitage trend test, two-sided).

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