Supplementary Information

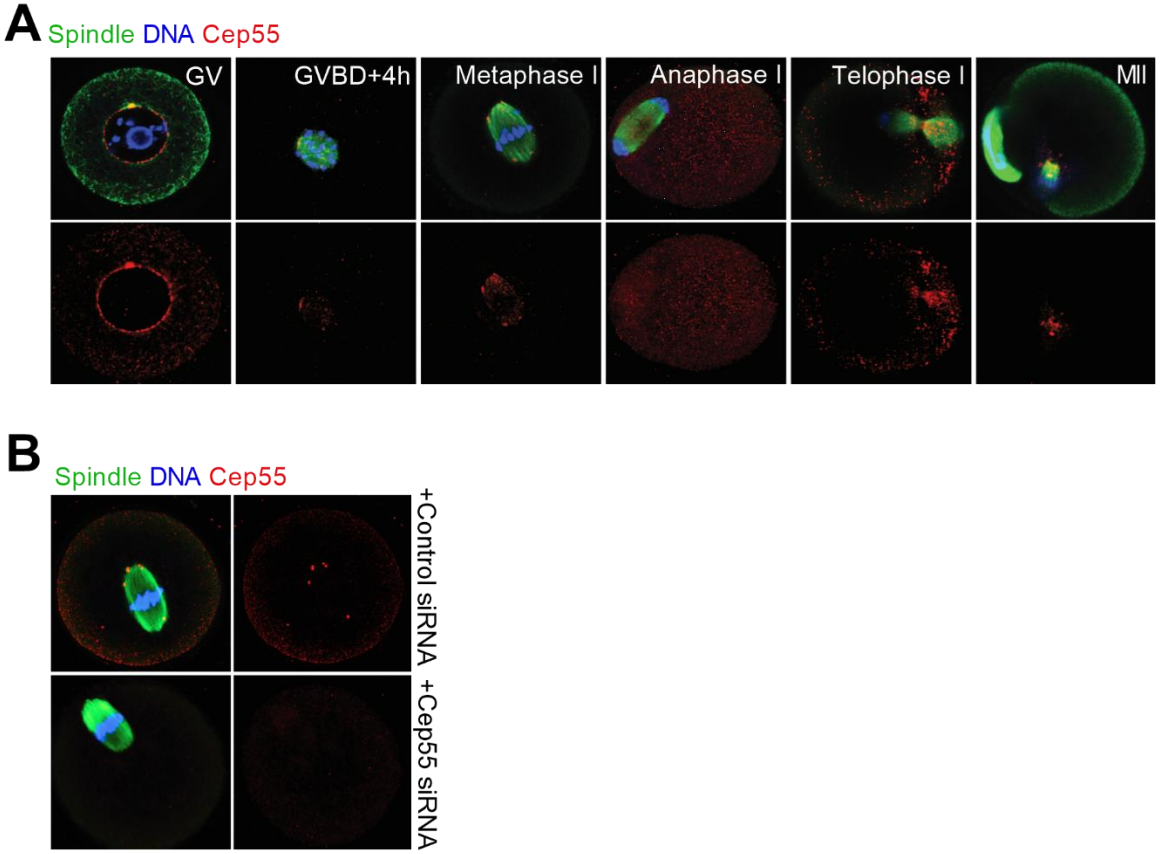


Fig. S1. Cep55 localisation during MI and following Cep55 siRNA microinjection. (A) Localization of endogenous Cep55 in mouse oocytes during meiotic maturation. Shown are representative images of oocytes at the stages shown immunostained for Cep55, spindle microtubules and chromosomes. (B) Cep55 spindle pole labelling is lost in oocytes injected with Cep55 siRNA whilst spindle assembly and chromosome alignment remain intact. Shown are representative images of mock-depleted (n = 45) and Cep55-depleted (n = 23) oocytes immunostained for Cep55, spindle microtubules and chromosomes. Note that following assembly of the bipolar spindle, Cep55 localises as discrete foci in the region of spindle poles in mock-depleted oocytes. Note also that spindle assembly and chromosome alignment remain intact whilst spindle pole labelling is lost in oocytes injected with Cep55 siRNA. Results are representative of at least three independent experiments.

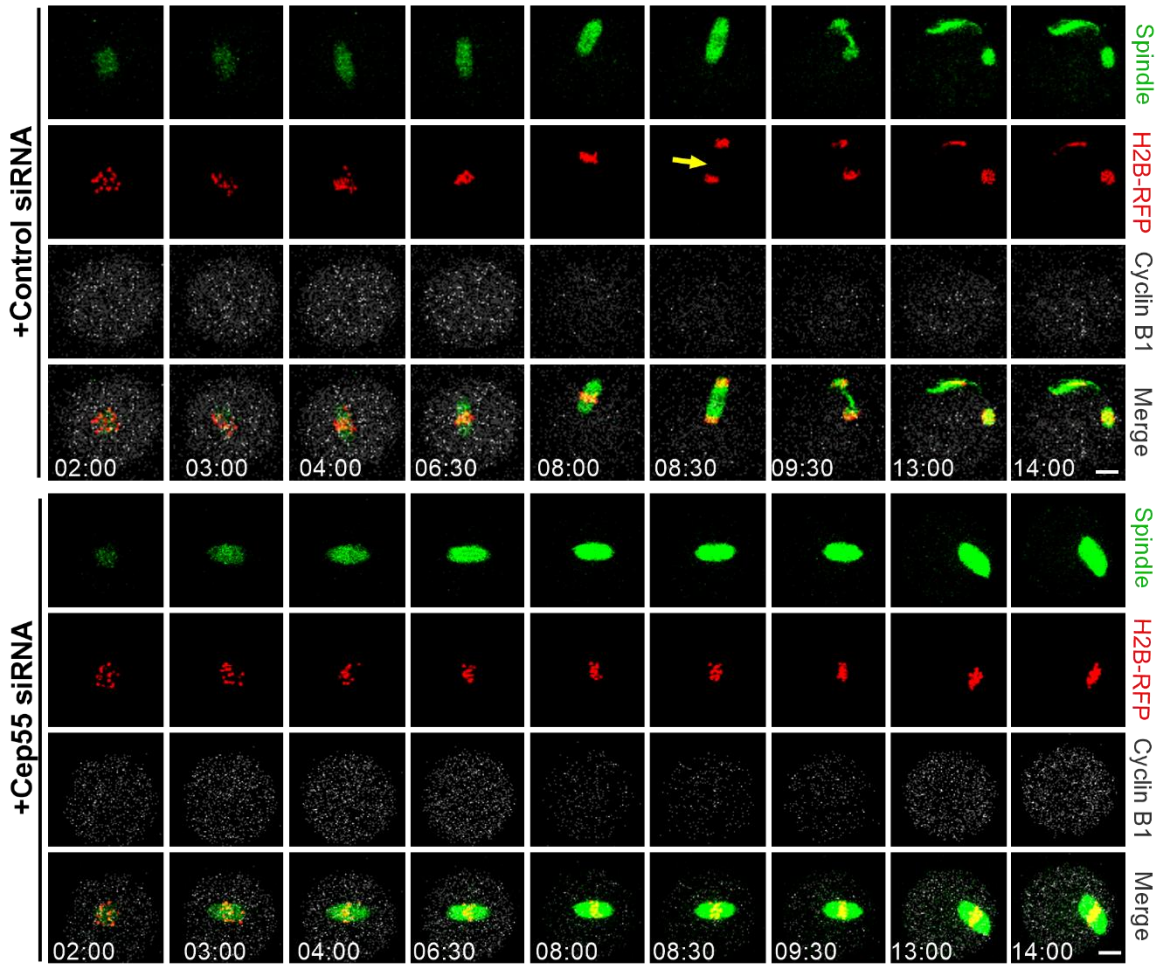


Fig. S2. Cep55-depletion induces anaphase-failure without preventing cyclin B1 proteolysis. Shown are images from representative timelapse series of mock-depleted (*n* = 12) and Cep55-depleted (*n* =15) oocytes expressing H2B-RFP and cyclin B1-GFP and stained with SiR-tubulin for labelling spindles. Note that in both groups of oocytes, cyclin B1-GFP fluorescence declines to a nadir around 8-8.5 h post-GVBD (reflecting proteolysis) and partially recovers around 13 h post-GVBD but anaphase only occurs in mock-depleted oocytes. Yellow arrow, anaphase I. Time is hh:mm post-GVBD. Scale bars, 10 µm. Results are representative of at least three independent experiments.

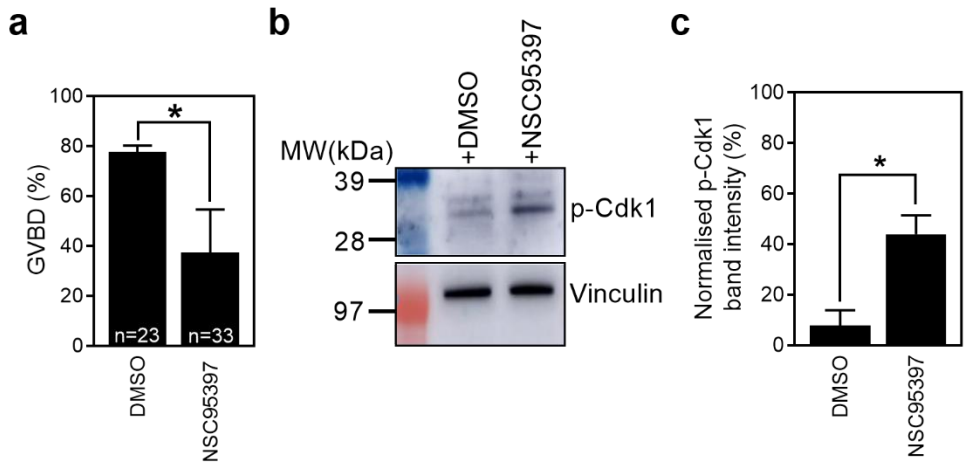


Fig. S3. The Cdc25B inhibitor, NSC95397, inhibits Cdk1 activity in mouse oocytes by promoting inhibitory Cdk1 phosphorylation. (A) GVBD is significantly inhibited in GV-stage oocytes treated with NSC95397. Note that GVBD is reduced by half following treatment with NSC95397. (B and C). NSC95397 significantly increases p-Cdk1 levels in GV-stage oocytes. Shown is a representative Western blot of p-Cdk1. Vinculin served as a loading control. $n = 50$ oocytes per lane (B). Graph shows the average p-Cdk1 band intensity from three experiments (C). Data are mean ± s.e.m. $*p < 0.05$ (Student's t test). Results are representative of at least three independent experiments.

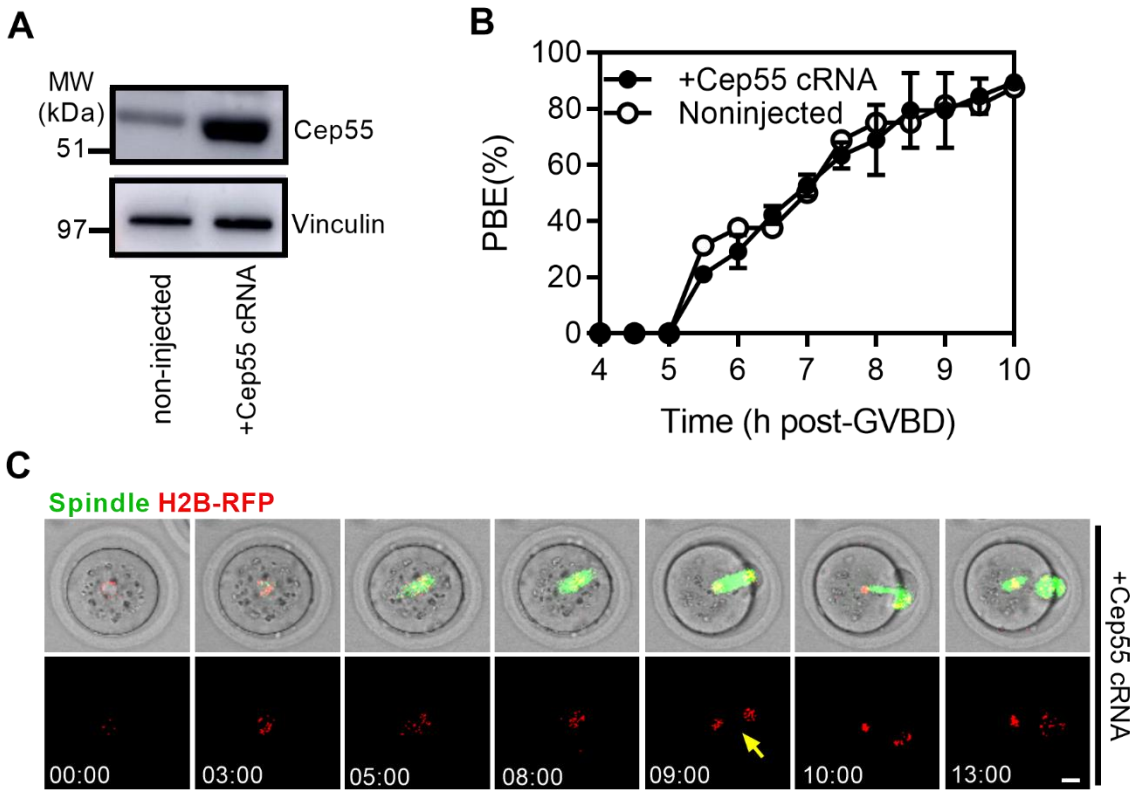


Fig. S4. Cep55 overexpression has no effect on the timing of anaphase I or PBE. (A) Shown is a representative Western blot of oocytes that were microinjected with Cep55 cRNA. Vinculin served as a loading control. *n* = 30 oocytes per lane. (B) Rates of polar body extrusion (PBE) in non-injected and Cep55 cRNA injected oocytes. (C) Representative timelapse images of spindles and chromosomes during meiotic maturation in live oocytes over-expressing Cep55 (*n* = 25 oocytes). Yellow arrow, anaphase. Time is hh:mm post-GVBD. Scale bar, 10 µm.

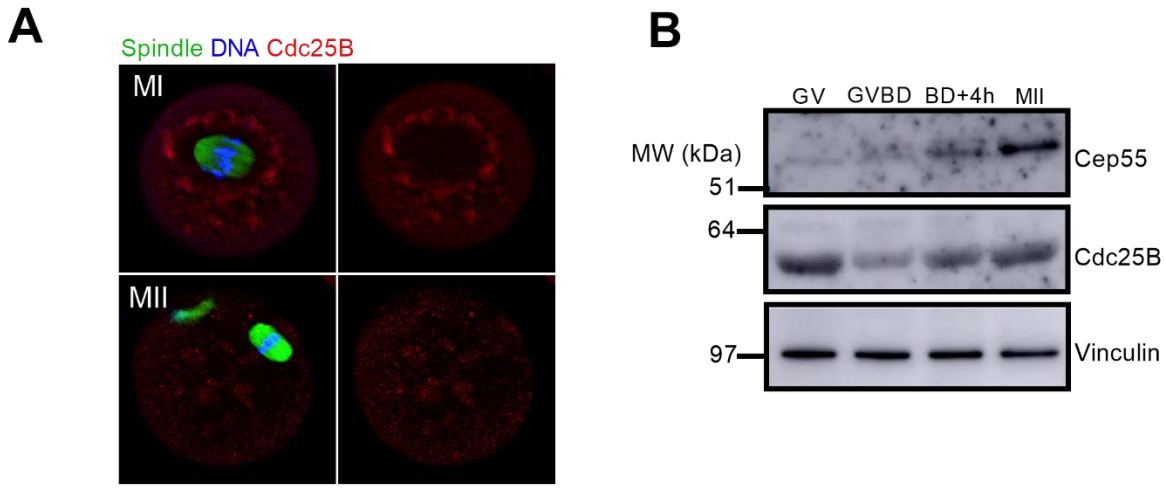
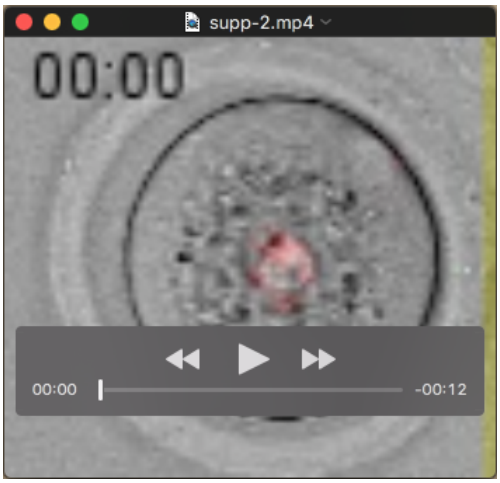
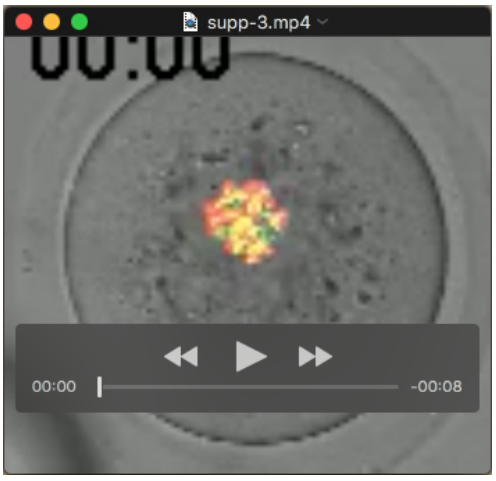


Fig. S5. Cdc25B localisation and expressing level during MI and MII. (A) Shown are representative images of oocytes at the stages shown immunostained for Cdc25B, spindle microtubules and chromosomes (n = 12 oocytes). Note the diffuse cytoplasmic localisation. (B) Endogenous levels of Cep55 and Cdc25B throughout meiotic maturation in mouse oocytes. Shown is a representative Western blot of Cep55 and Cdc25b. Vinculin served as a loading control. n = 30 oocytes per lane.

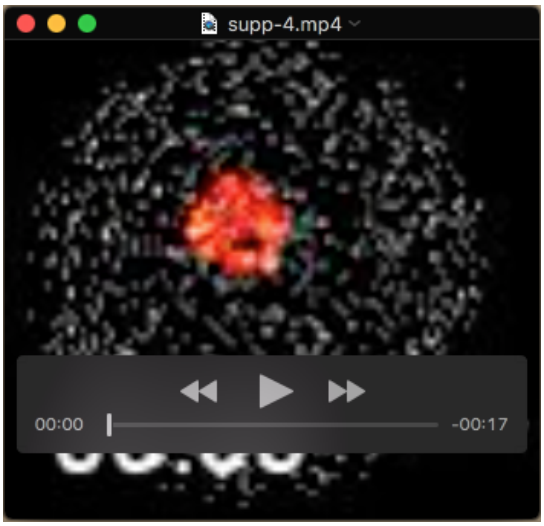
Movies



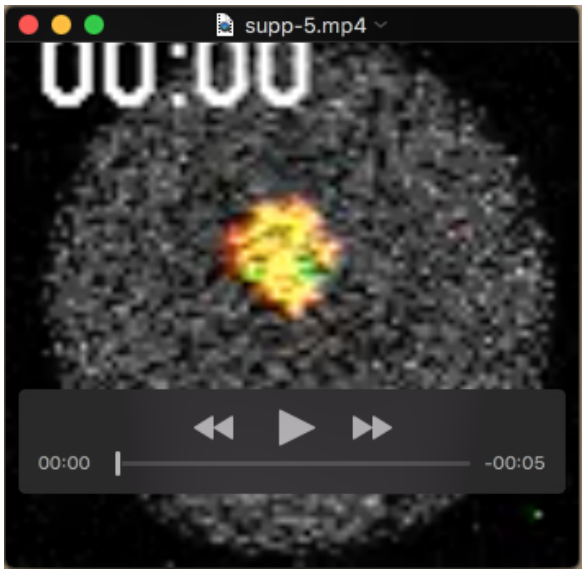
Movie 1. Spindle assembly, chromosome alignment, anaphase I and PBE in a control oocyte. Timelapse imaging of an oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles slowly close to the centre of the oocyte prior to migrating to the oocyte cortex and undergoing anaphase I around 9 h post-GVBD followed accompanied very shortly after by PBE.



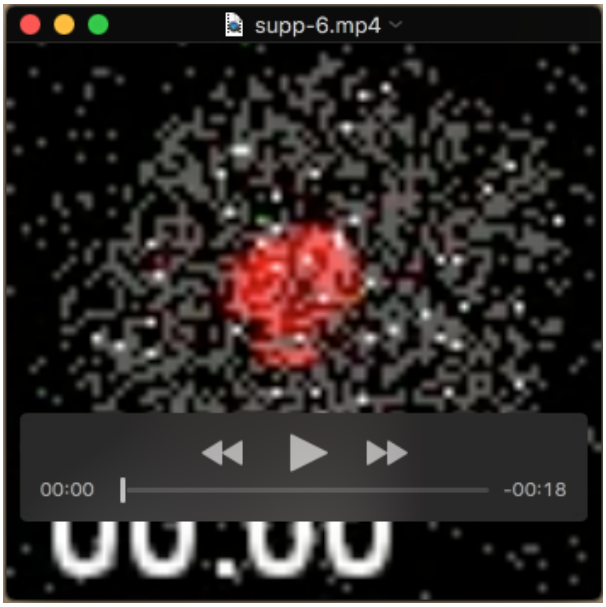
Movie 2. Spindle assembly and chromosome alignment without anaphase I in a Cep55-depleted oocyte. Timelapse imaging of a Cep55-depleted oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles and chromosomes align normally but that anaphase I fails to occur even by 19 h post-GVBD.



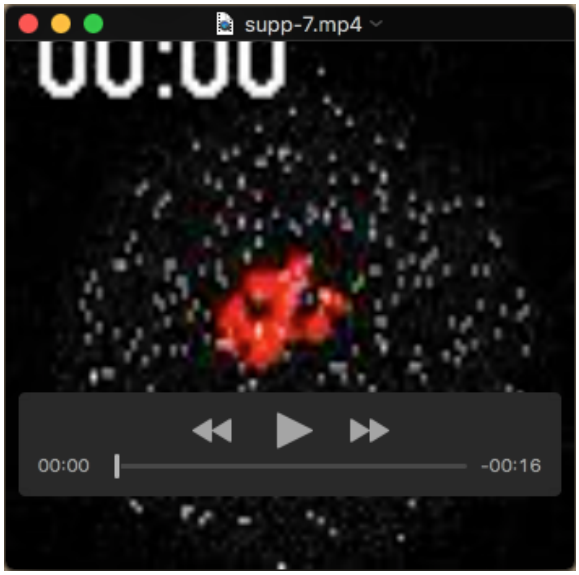
Movie 3. Spindle assembly, chromosome alignment, securin proteolysis, anaphase I and PBE in a mock-depleted oocyte. Timelapse imaging of an oocyte expressing H2B-RFP (red) and securin-GFP (monochrome) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles, chromosomes align and anaphase I occurs around 8 h post-GVBD concurrently with which, securin-GFP levels reach a nadir followed very shortly after by PBE, entry into MII and partial recovery of securin-GFP levels.



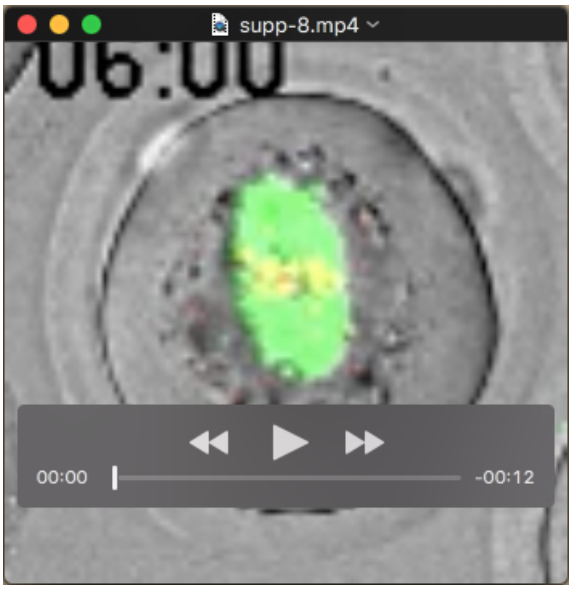
Movie 4. Spindle assembly, chromosome alignment and securin proteolysis without anaphase I or PBE in a Cep55-depleted oocyte. Timelapse imaging of an oocyte expressing H2B-RFP (red) and securin-GFP (monochrome) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles, chromosomes align and securin-GFP reaches a nadir around 8-8.5 h post-GVBD followed by recovery but that neither anaphase I nor PBE occur.



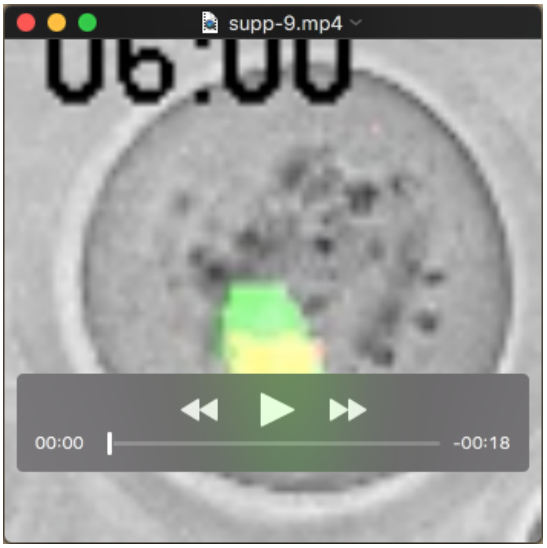
Movie 5. Spindle assembly, chromosome alignment, cyclin B1 proteolysis, anaphase I and PBE in a mock-depleted oocyte. Timelapse imaging of an oocyte expressing H2B-RFP (red) and cyclin B1-GFP (monochrome) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles, chromosomes align and anaphase I occurs around 9 h post-GVBD concurrently with which, cyclin B1-GFP levels reach a nadir followed very shortly after by PBE, entry into MII and partial recovery of cyclin B1-GFP levels.



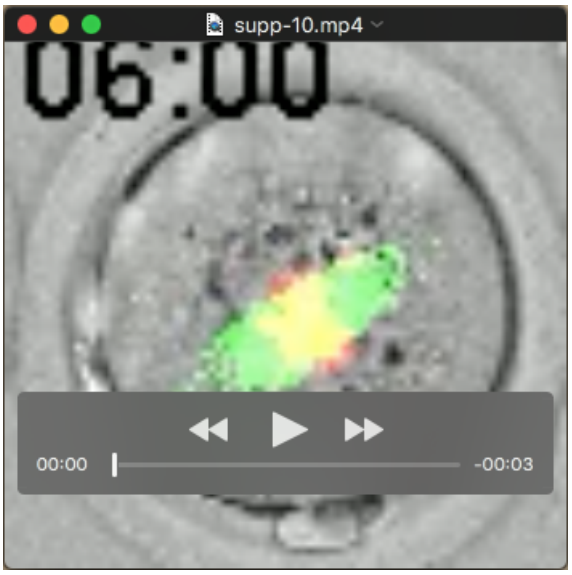
Movie 6. Spindle assembly, chromosome alignment and cyclin B1 proteolysis without anaphase I or PBE in a Cep55-depleted oocyte. Timelapse imaging of an oocyte expressing H2B-RFP (red) and cyclin B1-GFP (monochrome) and stained with SiR-tubulin dye (green). Time scale is shown in hh:mm. Note that a bipolar spindle assembles, chromosomes align and securin-GFP reaches a nadir around 8.5 h post-GVBD followed by partial recovery but that neither anaphase I nor PBE occur.



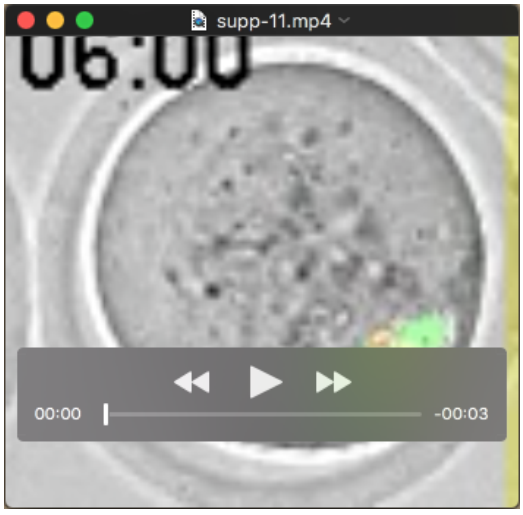
Movie 7. Forced Cdk1 inactivation by flavopiridol induces anaphase I and PBE in Cep55-depleted oocytes. Timelapse imaging of a Cep55-depleted oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with flavopiridol from 6 h post-GVBD. Time scale is shown in hh:mm. Note that anaphase I occurs by 30 min following treatment and is followed by PBE that is apparent by 1.5 hours. Note also that chromosomes eventually decondense due to persistent Cdk1 inactivation induced by flavopiridol.



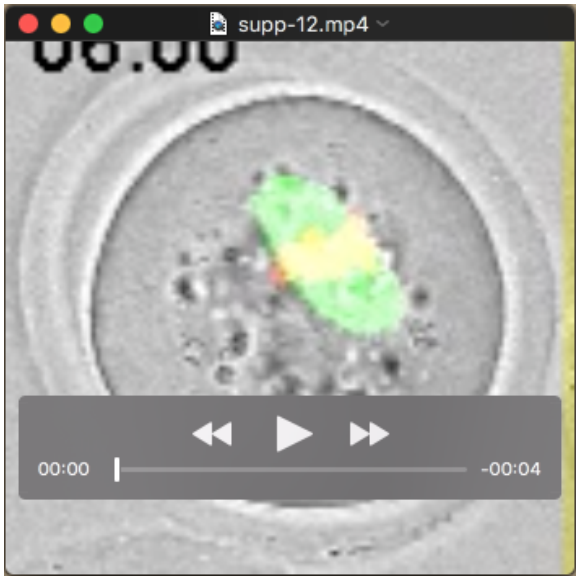
Movie 8. Treatment with DMSO solvent does not induce anaphase I or PBE in Cep55-depleted oocytes. Timelapse imaging of a Cep55-depleted oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with DMSO solvent from 6 h post-GVBD. Time scale is shown in hh:mm. Note that neither anaphase nor PBE occur.



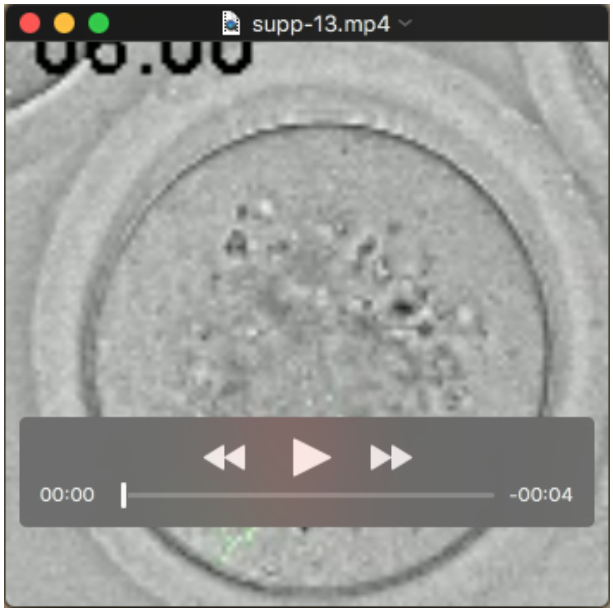
Movie 9. DMSO does not prevent anaphase I and PBE in control oocytes. Timelapse imaging of a control oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with DMSO solvent from 6 h post-GVBD. Time scale is shown in hh:mm.



Movie 10. The Wee1 inhibitor, MK1775, prevents anaphase I and PBE in control oocytes. Timelapse imaging of a control oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with MK1775 from 6 h post-GVBD. Time scale is shown in hh:mm. Note that anaphase I and PBE do not occur even by 15 h post-GVBD.



Movie 11. DMSO does not induce anaphase I and PBE in Cep55-depleted oocytes. Timelapse imaging of a Cep55-depleted oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with DMSO solvent from 6 h post-GVBD. Time scale is shown in hh:mm. Note that anaphase I and PBE do not occur even by 18 h post-GVBD.



Movie 12. The Cdc25B inhibitor, NSC95397, induces anaphase and PBE in Cep55-depleted oocytes. Timelapse imaging of a Cep55-depleted oocyte expressing H2B-RFP (red) and stained with SiR-tubulin dye (green) treated with NSC95397 from 6 h post-GVBD. Time scale is shown in hh:mm. Note that anaphase I and PBE occur by 10 h post-GVBD.