

**Figure S1: Low levels of DSBs cause segregation problems, but do not delay APC/C activation and anaphase onset.**

**A)** Time-lapse imaging of oocytes expressing H2B-mCHERRY and securin-EGFP. Oocytes were untreated (control) or treated with 10 or 100 ng/ml NCS in GV stage and then matured in normal medium. Maximum z-projection of EGFP and mCHERRY channels and single bright field section is shown. Time in hh:mm. **B)** Anaphase onset in hours of oocytes expressing H2B-mCHERRY and securin-EGFP. Untreated (control), NCS treated (10 and 100 ng/ml) groups are subdivided into the subgroups with and without segregation errors (n=10, 9, 1, 6, 10). **C)** Quantification of securin-EGFP level during meiotic maturation. Securin-EGFP level was normalized to 1 in the time of resumption of meiosis. Means with SE are shown. Time is in hours. Note that only one control oocyte exhibited segregation error (n = 10, 9, 1, 6, 10).

**Figure S2: ATR but not ATM is responsible for H2AX phosphorylation during meiotic maturation.**

**A)** Immunofluorescence of control and KU55933 treated GV-stage and metaphase I oocytes labeled with  $\gamma$ H2AX and MDC1 antibodies. Maximum z-projection of confocal sections across chromatin region is shown. KU55933 treated oocytes were initially incubated with 10  $\mu$ M KU55933 together with milrinone for 1 h in the GV-stage and fixed or after transfer to milrinone-free medium and then cultured for 7 h in the presence of KU55933 before fixation. **B)** Quantification of  $\gamma$ H2AX foci in GV-stage oocytes non-treated or treated with KU55933, NCS or both for 1 h in milrinone-supplemented medium before fixation (n= 23, 28, 10, 10). **C)** Quantification of  $\gamma$ H2AX intensity in control and KU55933 treated oocytes in metaphase I (n = 10, 43). **D)** Immunofluorescence of control, KU60019, and VE821 treated metaphase I oocytes labeled with  $\gamma$ H2AX antibody. Maximum z-projection of confocal sections across chromatin region is shown. KU60019 and VE821 treated oocytes were initially incubated with 1  $\mu$ M KU60019 or 1  $\mu$ M VE821 together with milrinone for 1 h in the GV-stage and after transfer to milrinone-free medium were cultured for 7 h in the presence of inhibitors before fixation. **E)** Quantification of  $\gamma$ H2AX intensity in control, KU60019 and VE821 treated oocytes in metaphase I (n = 12, 14, 9). **F)** Quantification of chromosome segregation errors of H2B-EGFP control, KU55933 and VE821 treated oocytes analysed by live confocal imaging (movies not shown) (n= 20, 11, 11). **G)** Percentage of oocytes with at least one  $\gamma$ H2AX focus matured into the metaphase II in control, KU55933, KU60019 or VE821 supplemented medium (n=11, 14, 8, 13). **H)** Immunofluorescence of control, NCS (10 ng/ml) and NCS+KU55933 (10  $\mu$ M) treated NIH3T3 cells labelled with  $\gamma$ H2AX antibody. **I)** Quantification of  $\gamma$ H2AX fluorescence in control, NCS and NCS+KU55933 treated NIH3T3 cells (n= 27, 44, 33).

**Figure S3: MRE11 is not required for H2AX phosphorylation in meiosis II.**

**A)** Quantification of  $\gamma$ H2AX foci number in *in vivo* matured metaphase II oocytes non-treated (control) or treated with mirin, NCS or both for 1 h in metaphase II (n = 7, 17, 23, 29). **B)** Percentage of oocytes (from A) with at least three  $\gamma$ H2AX foci. **C)** Quantification of  $\gamma$ H2AX foci number in metaphase II oocytes treated or not with NCS in GV stage for 1 h before meiotic maturation in control or mirin-supplemented medium (n=31, 58, 72, 51). **D)** Percentage of oocytes (from C) with at least one  $\gamma$ H2AX focus.

**Figure S4: Spindle assembly is not affected by Mirin treatment**

**A)** Timing of resumption of meiosis in control oocytes and oocytes treated with Mirin (n = 41, 36). **B)** Anaphase onset normalized to onset of NEBD in control oocytes and oocytes matured in Mirin (n = 38, 28). **C)** Percentage of oocytes arrested in GV, MI or MII

stage upon maturation in control medium or upon addition of mirin (n = 41, 36). Data in A) - C) are from live time-lapse imaging of control and mirin treated H2B-EGFP oocytes as shown in Fig. 5A). **D)** Time-lapse imaging of oocytes expressing MAP4-EGFP and H2B-mCHERRY in control or mirin-supplemented medium. Maximum intensity z-projection images are shown. Time is in hh:mm. Arrowhead shows chromosome segregation problems. **E)** The volume of the spindle ( $\mu\text{m}^3$ ) during oocyte maturation quantified from 3D reconstructed data. Time t=0 corresponds to the time of NEBD (n=18, 31).