

## Supplementary information

**S1: BrdU incorporation and asymmetric  $\gamma$ H2A.X pattern during S-phase.** In late PN4 (S-phase) IVF zygotes BrdU is incorporated to same extent in both maternal and paternal pronuclei.  $\gamma$ H2A.X foci are much stronger in the paternal pronuclei at S-phase. Scale bar 20  $\mu$ m.

**S2: Dynamics of  $\gamma$ H2A.X signal during zygotic development (colour version of figure 3).** Representative images of indirect immunostainings using antibodies against  $\gamma$ H2A.X in IVF zygotes at distinct pronuclear stages (PN0 to PN5, Syngamy and Metaphase). (A)  $\gamma$ H2A.X dynamics in untreated controls. (B) IVF Zygotes incubated with aphidicolin for two hours prior to fixation and (C) IVF zygotes incubated with camptothecin for two hours prior the fixation. Scale bar 20  $\mu$ m.

**S3: Equal distribution of H2A.X between paternal and maternal pronuclei.** Indirect immunostainings using antibodies against the histone variant H2A.X: H2A.X is present in maternal and paternal pronuclei at all developmental stages to the same extent. Scale bar 20  $\mu$ m.

**S4: Maternal H3K9me2 and paternal  $\gamma$ H2A.X signal.** To discriminate the parental origin of pronuclei we performed indirect immunostainings using antibodies against H3K9me2 which labels the maternal pronucleus only (Lepikhov et al., 2004). Co-staining with  $\gamma$ H2A.X shows strong signals in the H3K9me2 negative paternal pronucleus (PN3). Scale bar 20  $\mu$ m.

**S5: Aphidicolin does not influence paternal active DNA demethylation.** Representative images of indirect immunostainings using antibodies against 5mC in IVF zygotes at early PN3 stage. Inhibition of DNA polymerases by aphidicolin does not influence the loss of 5mC signal in paternal pronuclei. Scale bar 20  $\mu$ m.

**S6: Aphidicolin enhances  $\gamma$ H2A.X signal only during S-phase in 2-cell embryos.** 2-cell embryos were staged by EdU incorporation for G1-, S- and G2-phase and analyzed for  $\gamma$ H2A.X signal with (two hours prior fixation) or without aphidicolin treatment. Contrary to zygotes, aphidicolin treatment does not enhance the number of  $\gamma$ H2A.X foci outside of S-phase. Scale bar 20  $\mu$ m.

**S7: Induction of DNA damage by MMS induces strong PARP-1 and  $\gamma$ H2A.X signals.** The images shows early PN3 zygote treated with 5 mM MMS for one hour prior to fixation. Both parental pronuclei show strong co-localised immuno-reactivity with antibodies against PARP-1 and  $\gamma$ H2A.X. Scale bar 20  $\mu$ m.

**S8: DNA single strand breaks by nick translation in damage induced early PN3 zygotes.** (A) PolI incorporation of EdCTP after induction of DNA nicks by MMS treatment (one hour prior the fixation) results in strong incorporation in both maternal and paternal PN3 pronuclei. (B) Control treatment with DNase1 to artificially introduce DNA nicks after fixation. PolI incorporates EdCTP in DNA of both pronuclei. Scale bar 20  $\mu$ m, sp - sperm.

**S9: Absence of DNA polymerase  $\beta$  in early PN3 pronuclei.** Representative images of indirect immunostainings using antibodies against DNA polymerase  $\beta$  in IVF zygotes at early PN3 stage. Induction of DNA damage by MMS causes strong appearance of  $\gamma$ H2A.X signal in

pronuclei but not DNA polymerase  $\beta$ ; also the additional inhibition by aphidicolin does not influence DNA polymerase  $\beta$  signal. Scale bar 20  $\mu\text{m}$ .



















