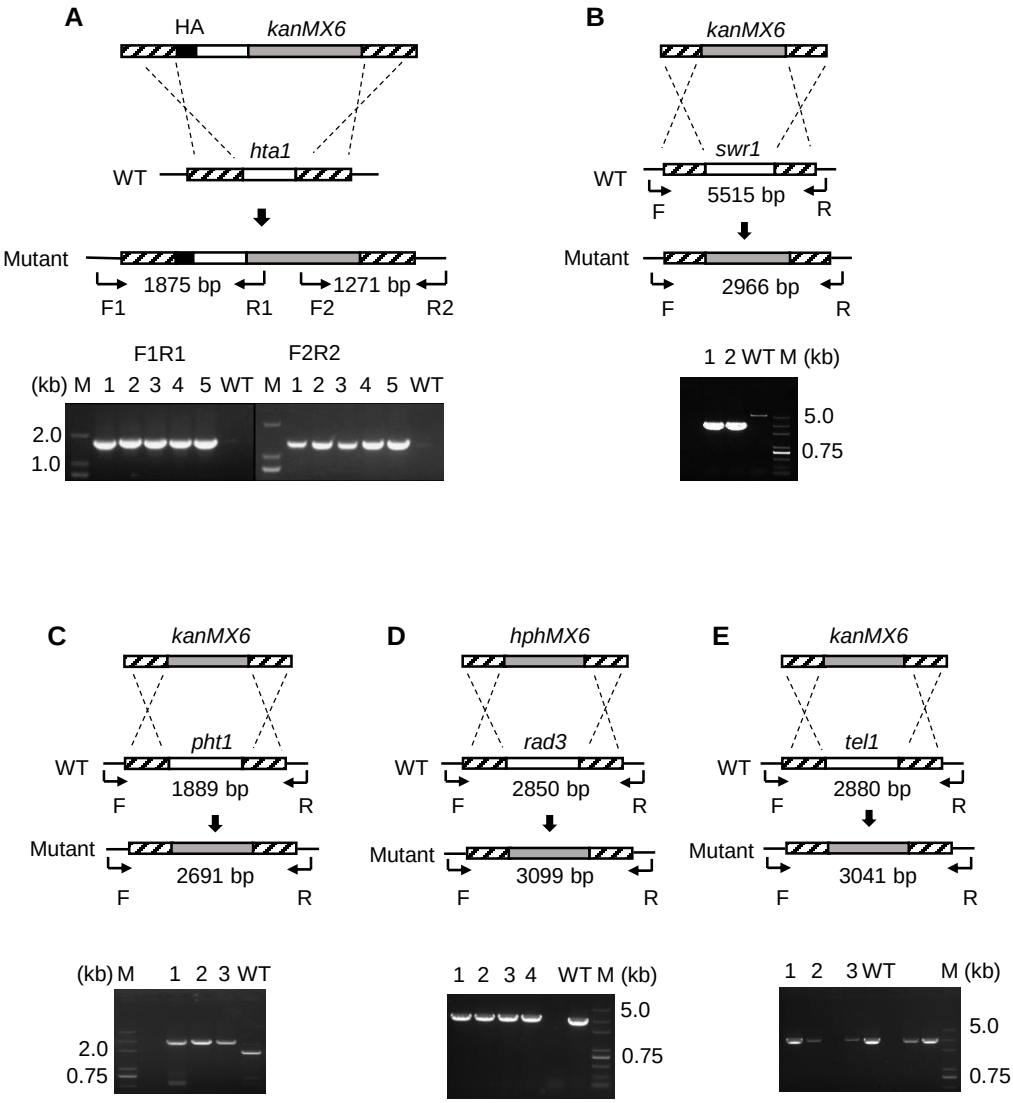

Figure S1. Gene disruption by homologous recombination. (A) *hta1*, (B) *swr1*, (C)*pht1*, (D) *rad3* and (E) *tel1*. Flanking DNA (hatched boxes, upstream of start codon and downstream of stop codon) and coding region of genes were amplified and linked respectively to selection gene *kanMX6* or *hphMX6* by overlapping PCR to yield linear substrate for homologous recombination in WT cells. For *hta1*, 3× HA fragment (black box) included. Positive colonies were selected on YES medium with 75 μg/ml G418 or YES medium with 75 μg/ml hygromycin B and gene disruption mutations were identified by PCR with primers as shown (F1+R1, F2+R2, F+R), M: DNA marker.

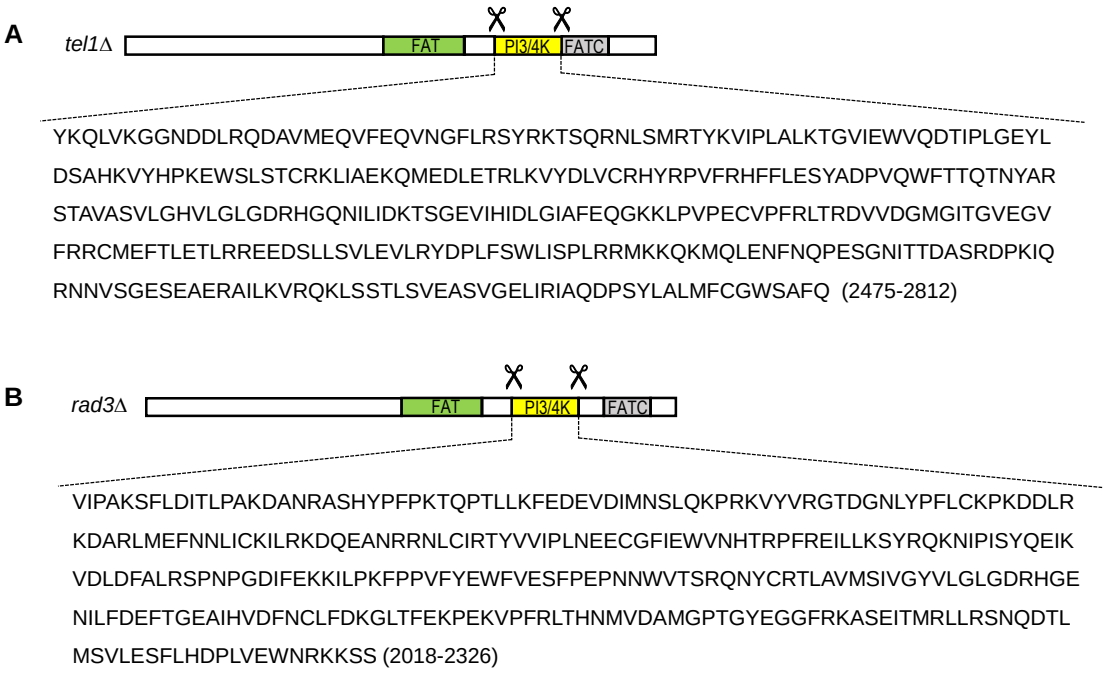

Figure S2. Deletion of P3/4K domain in *tel1*Δ (A) and *rad3*Δ (B). Scissors mark extent of deletion. PI3/4K, Phosphatidylinositol 3-/4-kinase catalytic domain; FAT, FRAP-ATM-TRRAP domain; FATC, FAT-C-terminal domain.

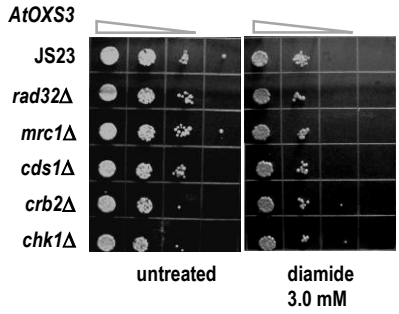

Figure S3. Null mutants on key components from downstream ATM/ATR pathway, *rad32*, *mrc1*, *cds1*, *crb2* and *chk1*, expressing *AtOXS3*, were spotted on EMM selective media without or with 3.0 mM diamide.

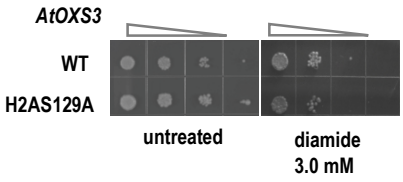

Figure S4. Dilution assay of H2AS129A strain producing AtOXS3 on 3.0 mM diamide, together wild-type control.

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H2A.Z   MSGGGKGKHVGGKGGSIKGERGQMSHSARAGLQFPVGRVRRFLKAKTQNNMRVGAKSAVY   60
H2Aα    MSGGK-------SGGKAAVAKSAQSRSAKAGLAFPVGRVHRLLRKG-NYAQRVGAGAPVY   52
H2Aβ    MSGGK-------SGGKAAVAKSAQSRSAKAGLAFPVGRVHRLLRKG-NYAQRVGAGAPVY   52

H2A.Z   SAAVLEYLTAEVLELAGNAAKDLKVKRITPRHLQLAIRGDEELDTLIR-ATIAGGGVLPH   119
H2Aα    LAAVLEYLAAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGHVTIAQGGVVPN   112
H2Aβ    LAAVLEYLAAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGHVTIAQGGVVPN   112

H2A.Z   INKQLLIRTKEKYPEEEEII*          139
H2Aα    INAHLLPKTSGRTGKPSQEL*          132
H2Aβ    INAHLLPKQSGK-GKPSQEL*          131
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Figure S5. Alignment of *S. pombe* H2A histones. Non-conserved residues are represented with a white background. SQE motifs highlighted in orange, and the red letters indicate the potential phosphosites within the C-terminal tails of H2Aα and H2Aβ besides of SQE motif.

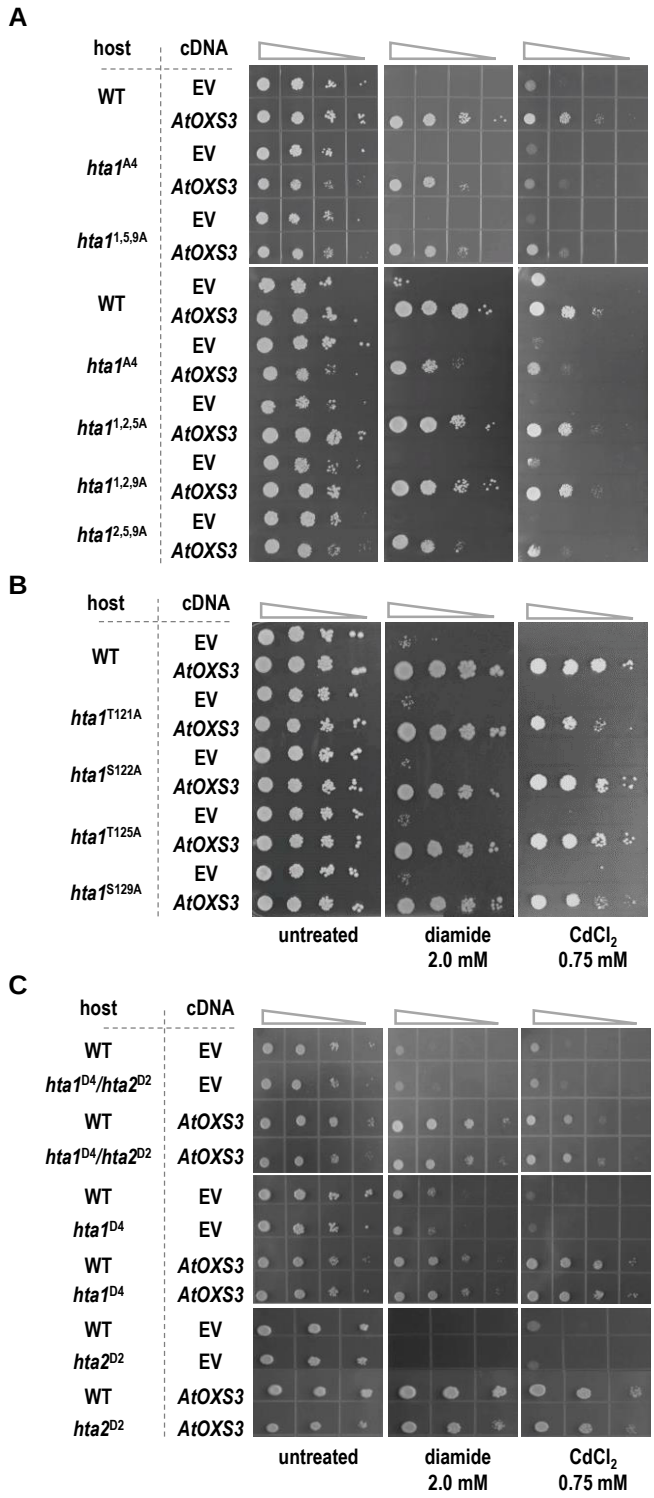

Figure S6. Tolerance assay to diamide and $CdCl_2$ on H2AX mutants. Serial dilution of alanine substitution in (A) four triple mutants *hta1*^{1,5,9A}, *hta1*^{1,2,5A}, *hta1*^{1,2,9A}, and *hta1*^{2,5,9A}; *hta1*^{A4}(*hta1*-T121A,S122A, T125A, S129A) was used as the negative control; (B) four single mutants *hta1*^{T121A}, *hta1*^{S122A}, *hta1*^{T125A} and *hta1*^{S129A}; and aspartic acid substitution in (C) double mutant *hta1*^{D4}*hta2*^{D2}, two single mutants *hta1*^{D4} and *hta2*^{D2}, together with WT control transformed with EV or *AtOXS3* on EMM selective media containing the indicated concentration of diamide or $CdCl_2$. EV, empty vector pART1. Photographs show representative images from three independent experiments.

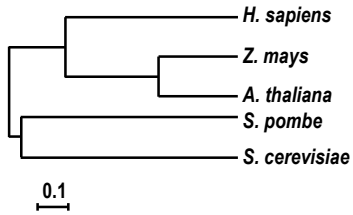

Figure S7. Phylogenetic tree of Swc2 among *S. pombe*, *Saccharomyces. cerevisiae* (*S. cerevisiae*), *Homo. Sapiens* (*H. sapiens*), *Arabidopsis. thaliana* (*A. thaliana*) and *Zea. Mays* (*Z. mays*) from MEGA X by neighbor-Joining method with 1000 bootstrap replicates. *S*cale bar (0.1) indicates the number of nucleotide substitutions per site.