Ensminger et al., http://www.jcb.org/cgi/content/full/jcb.201312078/DC1

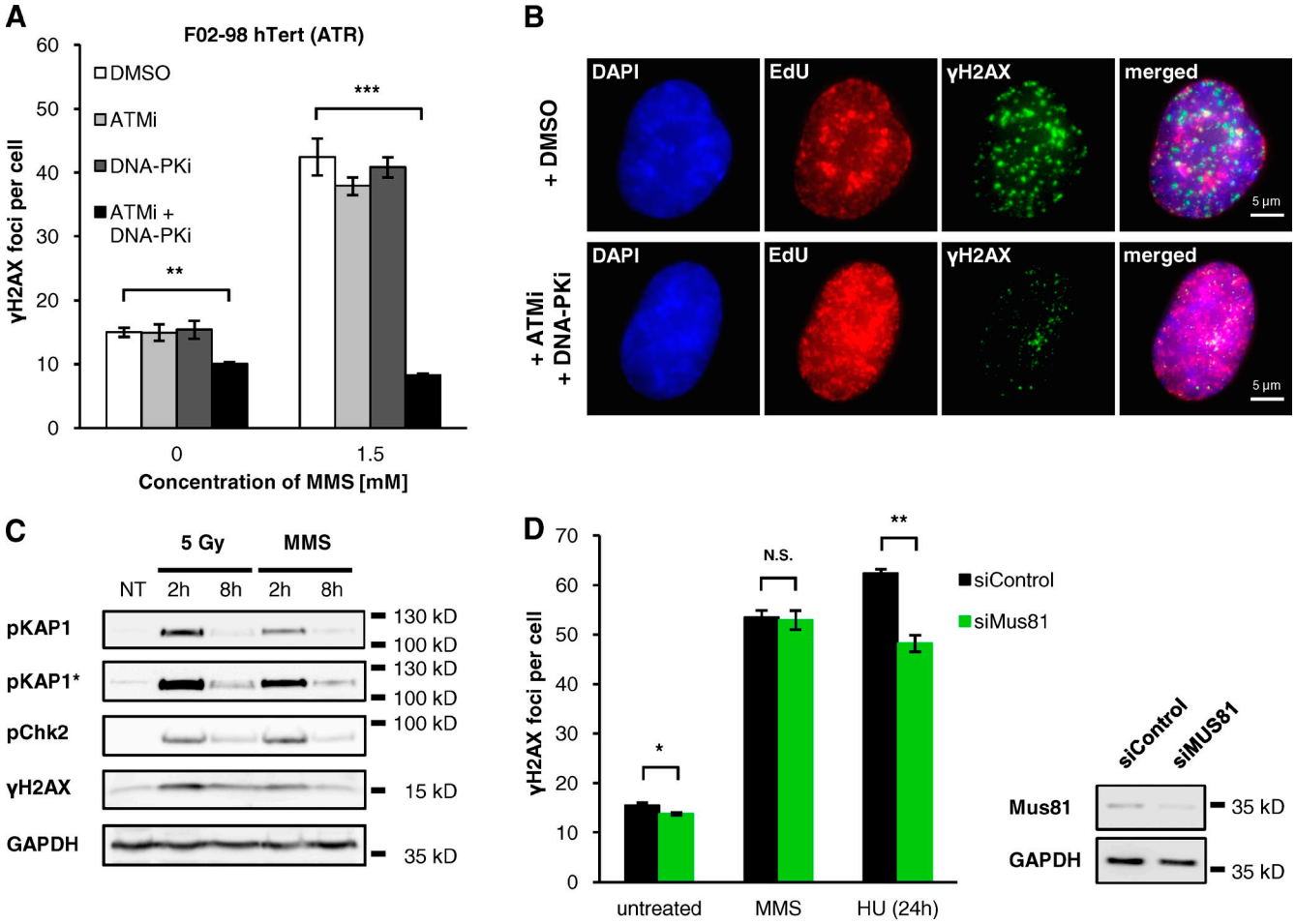

Figure S1. **ATM is activated upon MMS treatment.** (A and B) ATM and DNA-PK contribute to H2AX phosphorylation after MMS. ATR-deficient F02-98 hTert cells were treated with specific inhibitors of ATM (KU55933, 10 µM) or DNA-PK (NU7026, 20 µM), or with both inhibitors together. 1 h later, 10 µM EdU and 1.5 mM MMS were added for 1 h. 15 min after removing the drug, cells were fixed and stained against γH2AX and EdU. The number of γH2AX foci was assessed in EdU-positive S-phase cells (A, ±SEM from three experiments). Representative images of MMS-treated F02-98 hTert cells after pretreatment with ATM and DNA-PK inhibitors are shown in B. (C) ATM activation after MMS treatment or x-irradiation. A549 cells were treated with 1.5 mM MMS or irradiated with 5 Gy. After the indicated times, cells were fixed and phosphorylation of the ATM substrates KAP1, Chk2, and H2AX was determined using Western blotting (pKAP* represents a higher intensity setting). GAPDH was used as a loading control. (D) γH2AX foci in A549 cells after siRNA for 48 h. Cells were treated with EdU and 1.5 mM MMS for 1 h or first with EdU for 1 h and subsequently with 0.5 mM HU for 24 h. 15 min after removing the drugs, cells were fixed and stained against γH2AX and EdU. γH2AX foci were assessed in EdU-positive S-phase cells (±SEM from three experiments). The efficient depletion of Mus81 was confirmed by Western blotting; GAPDH was used as a loading control. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

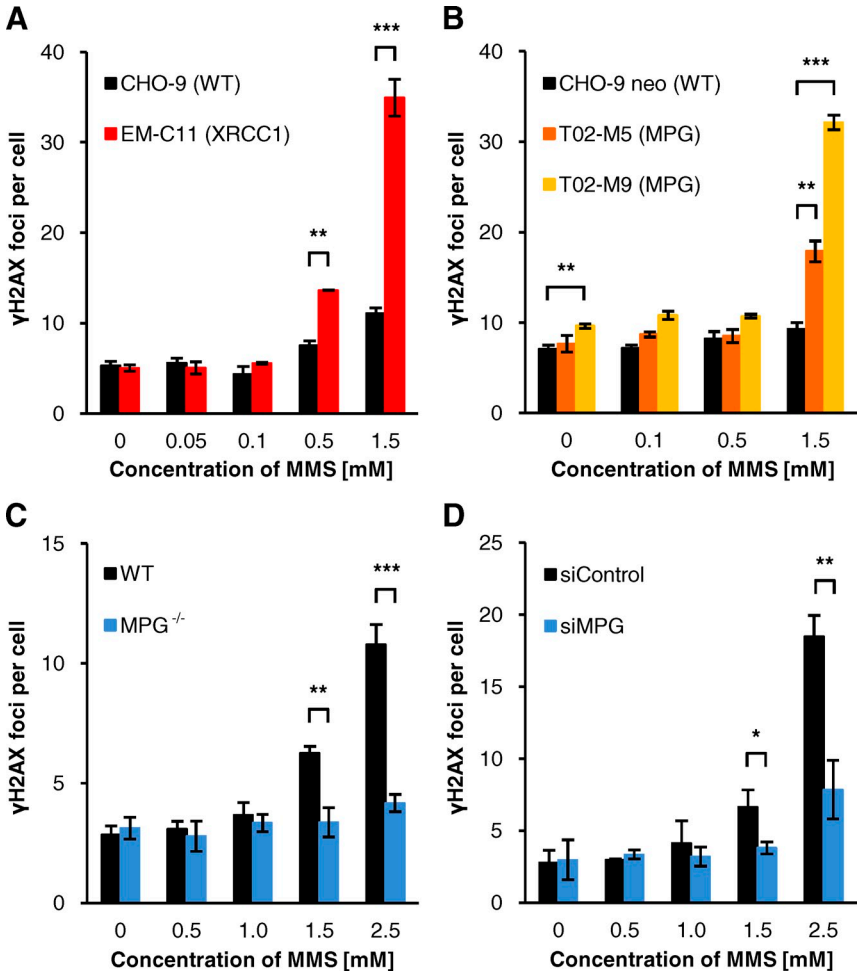

Figure S2. **Formation of γH2AX foci after MMS treatment in G2 phase.** Cells were treated with 10 µM EdU and different concentrations of MMS for 1 h. 15 min after removing EdU/MMS, cells were fixed and stained against γH2AX and EdU. The number of γH2AX foci was assessed in EdU-negative G2-phase cells. Shown are results for (A) CHO-9 (WT) and EM-C11 (XRCC1-deficient) cells, (B) for CHO-9 neo (WT) and MPG-overexpressing T02-M5 and T02-M9 cells, (C) for WT and MPG$^{-/-}$ MEFs, and (D) for MPG-depleted A549 cells (±SEM from three experiments). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

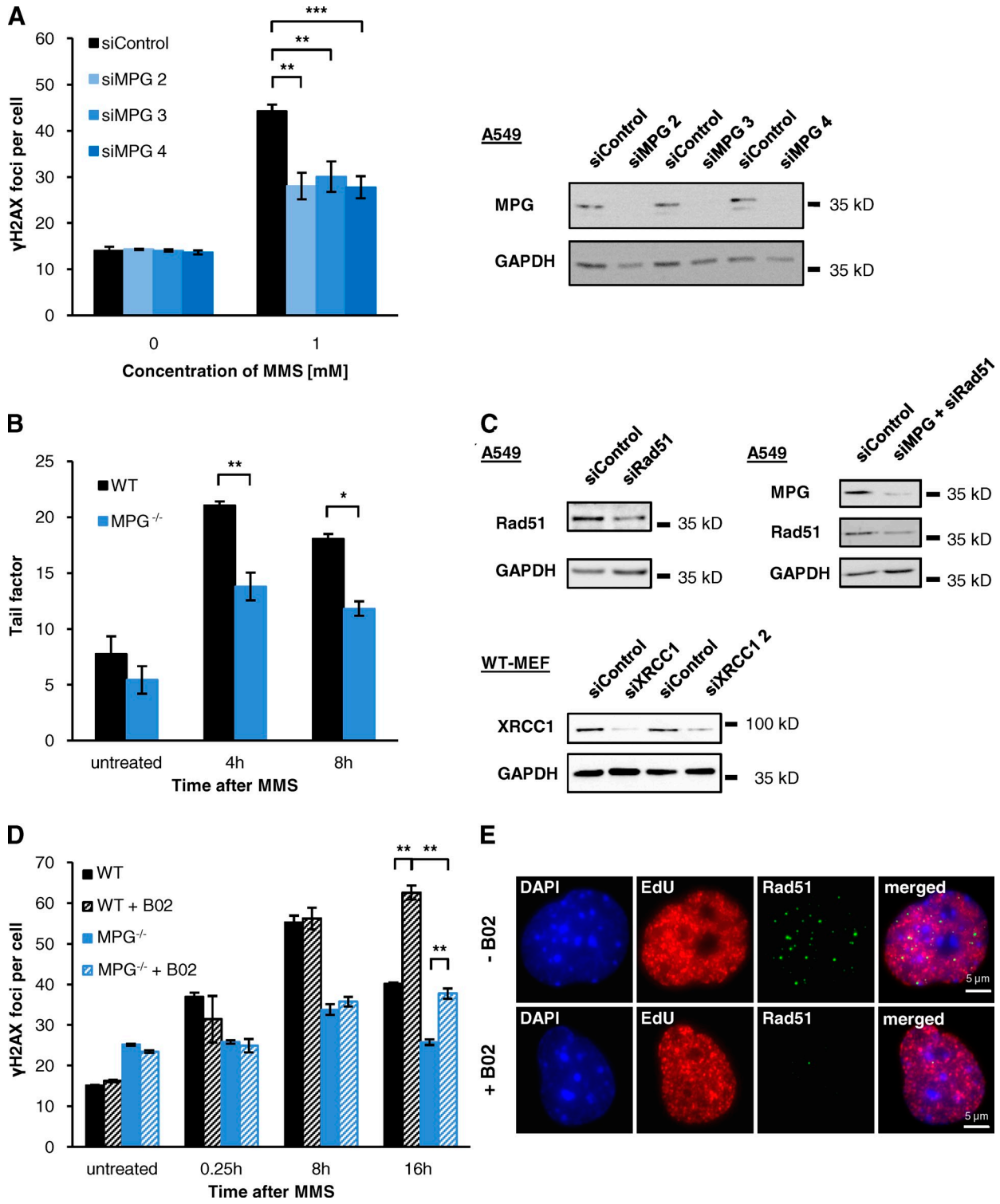

Figure S3. **DSB formation in MPG-deficient cells.** (A) γH2AX foci in MPG-depleted A549 cells. Cells were transfected with different siRNA against MPG. 72 h later, cells were treated with EdU and 1.0 mM MMS for 1 h. 15 min after removing EdU/MMS, cells were fixed and stained against γH2AX and EdU. γH2AX foci were assessed in EdU-positive S-phase cells (±SEM from three experiments). Efficient siRNA depletion was confirmed by Western blotting; GAPDH was used as a loading control. (B) DSB formation measured by the neutral comet assay. WT and MPG$^{-/-}$ MEFs were treated with 1 mM MMS for 1 h. At the indicated times after removing MMS, the neutral comet assay was performed and the tail factor was determined for 50 cells (±SEM from three experiments). (C) Confirmation of efficient Rad51 depletion and co-depletion of MPG and Rad51 in A549 as well as XRCC1 depletion in WT-MEF. Western blotting was performed 48 h after transfection with siRNA; GAPDH was used as a loading control. (D and E) γH2AX foci kinetics in WT and MPG$^{-/-}$ MEFs treated with a specific Rad51 inhibitor. Cells were treated with EdU and 1 mM MMS for 1 h. After removing EdU/MMS, cells were fixed at the indicated times. Where indicated, 60 µM of the Rad51 inhibitor B02 was added 1 h before MMS and was present until fixation. γH2AX foci were analyzed in EdU-positive S/G2 cells (D, ±SEM from two experiments). Representative images of Rad51 foci in MMS-treated WT MEFs (8 h after MMS treatment) with or without B02 are shown in E. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

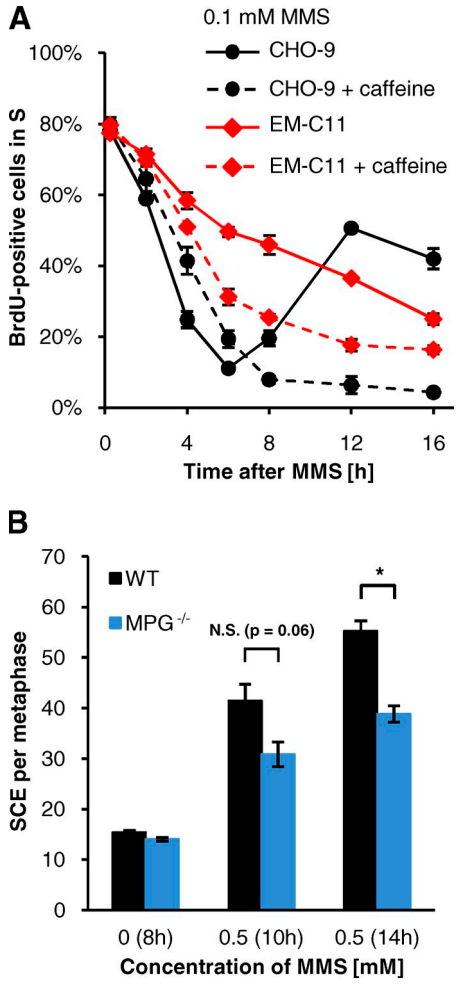

Figure S4. **Cell cycle progression of CHO-9 (WT) and EM-C11 (XRCC1-deficient) cells after 0.1 mM MMS and SCEs in WT and MPG$^{-/-}$ MEFs after 0.5 mM MMS.** (A) Cell cycle progression after 0.1 mM MMS. Cells were treated with 10 µM BrdU and 0.1 mM MMS for 1 h and fixed at the indicated times after removing BrdU/MMS. 5 mM caffeine was added 1 h before MMS addition and was present until fixation. Cell cycle progression was measured by FACS; the frequency of BrdU-positive cells in S phase was calculated (±SEM from three experiments). (B) SCEs in WT and MPG$^{-/-}$ MEFs after 0.5 mM MMS. The data were obtained from the experiments performed for Fig. 5 D (±SEM from two experiments). *, $P < 0.05$.