Cerqueira et al., http://www.jcb.org/cgi/content/full/jcb.200903033/DC1

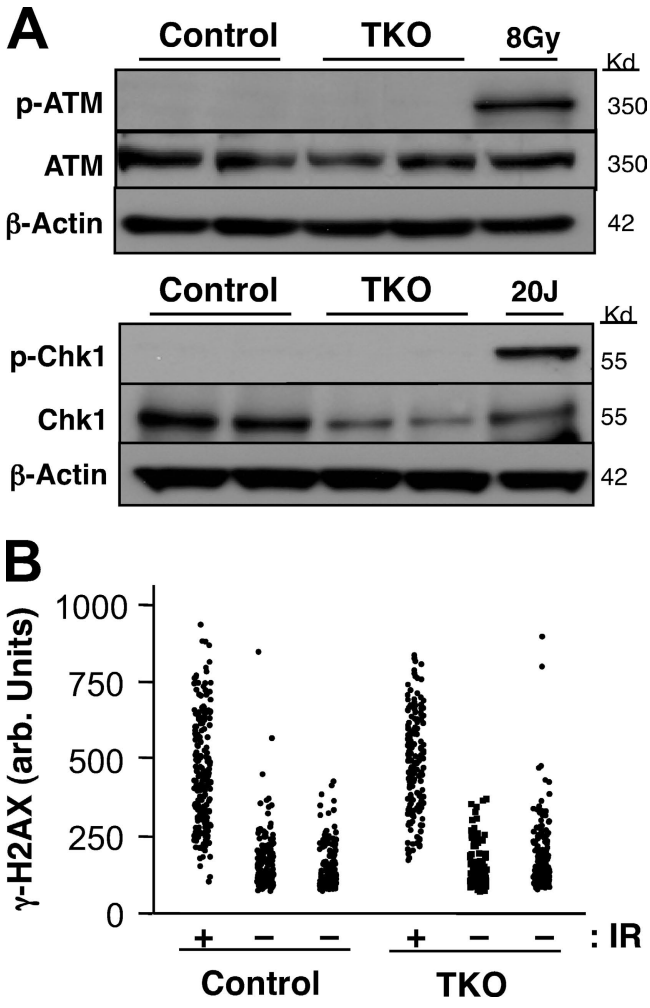

Figure S1. **Absence of DNA damage in unperturbed TKO cells.** (A) Whole cell extracts from *Cdk4*$^{+/+}$;*Cdk2*$^{+/+}$;*Cdk6*$^{-/-}$ control and *Cdk4*$^{-/-}$;*Cdk2*$^{-/-}$;*Cdk6*$^{-/-}$ TKO MEFs were blotted with antibodies against ATM-Ser^{1981}, Chk1-Ser^{345}, ATM, or Chk1 as indicated in Materials and methods. As a control, TKO cells were exposed to 8 Gy of IR or 20 joules (J) of UV light. Extracts were prepared 45 min after the treatment. (B) Distribution of the intensity of the phosphorylated γ-H2AX signal per nucleus in two independent populations of asynchronous *Cdk4*$^{+/+}$;*Cdk2*$^{+/+}$;*Cdk6*$^{-/-}$ control and *Cdk4*$^{-/-}$;*Cdk2*$^{-/-}$;*Cdk6*$^{-/-}$ TKO MEFs. (left) As a control, these cells were also exposed to 3 Gy of IR. Samples were analyzed by high throughput microscopy.

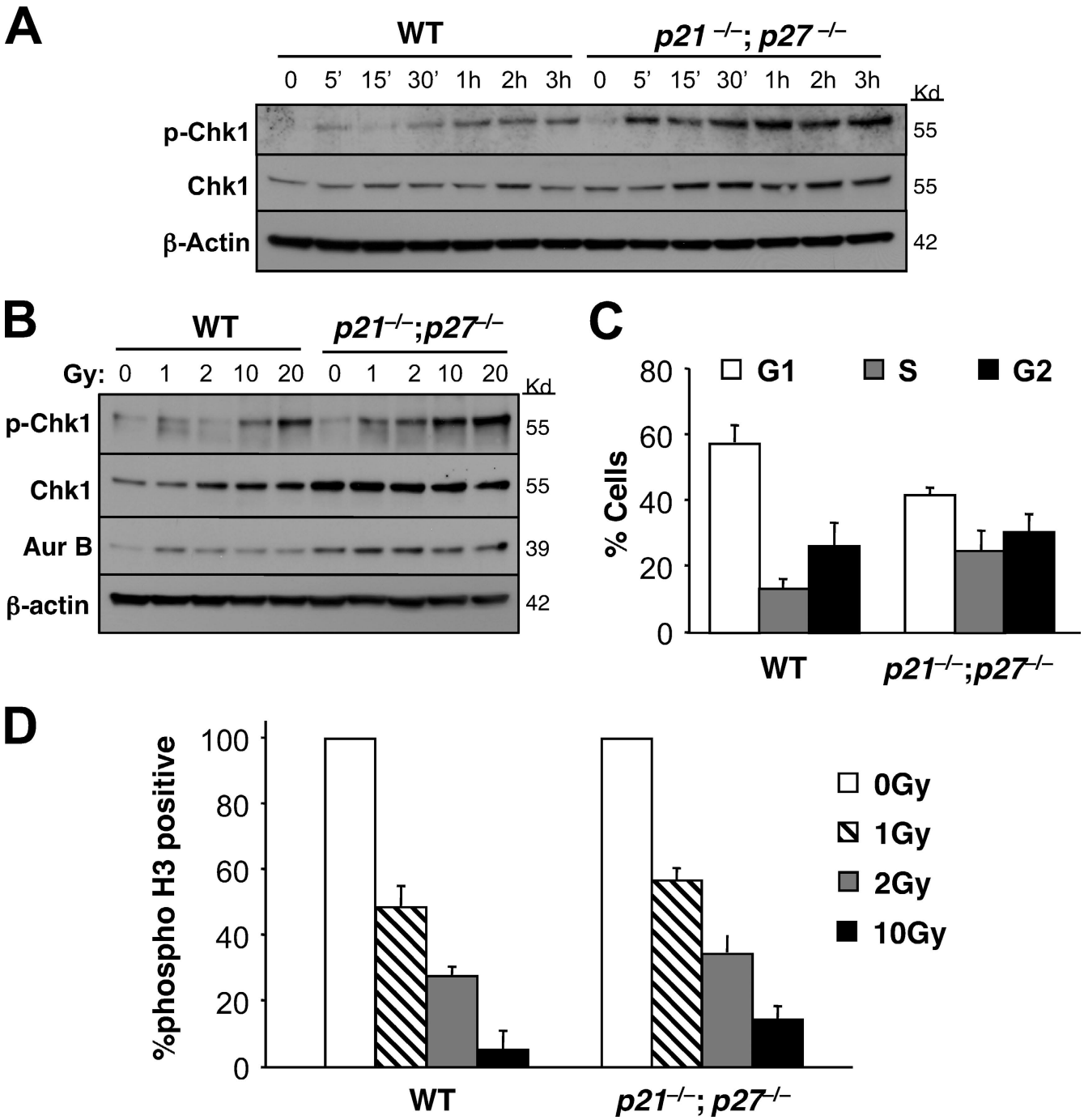

Figure S2. **Increased Cdk activity does not enhance the DDR.** (A) Whole cell extracts from wild-type (WT) and $p21^{-/-};p27^{-/-}$ primary MEFs prepared at the indicated times after a single dose of 20 Gy. $p21^{-/-};p27^{-/-}$ MEFs show increased phosphorylation on Chk1-Ser^{345}. Total levels of Chk1 are shown as a control. (B) Wild-type and $p21^{-/-};p27^{-/-}$ primary MEFs were submitted to IR with the indicated doses, and whole cell extracts were prepared after 45 min. Samples were blotted with a phosphospecific antibody against Chk1-Ser^{345}. Total Chk1 and AurB are shown as controls to measure protein variations associated to cell cycle progression as indicated in Materials and methods. β-Actin is shown as a loading control. (C) Asynchronous cultures of wild-type and $p21^{-/-};p27^{-/-}$ primary at the time of IR were analyzed for cell cycle distribution by FACS. (D) Wild-type and $p21^{-/-};p27^{-/-}$ primary MEFs were submitted to IR with the indicated doses and analyzed by FACS to assess the variation in phospho-H3 positives. Values are represented normalized to untreated controls. Error bars indicate mean ± SD (n = 3).

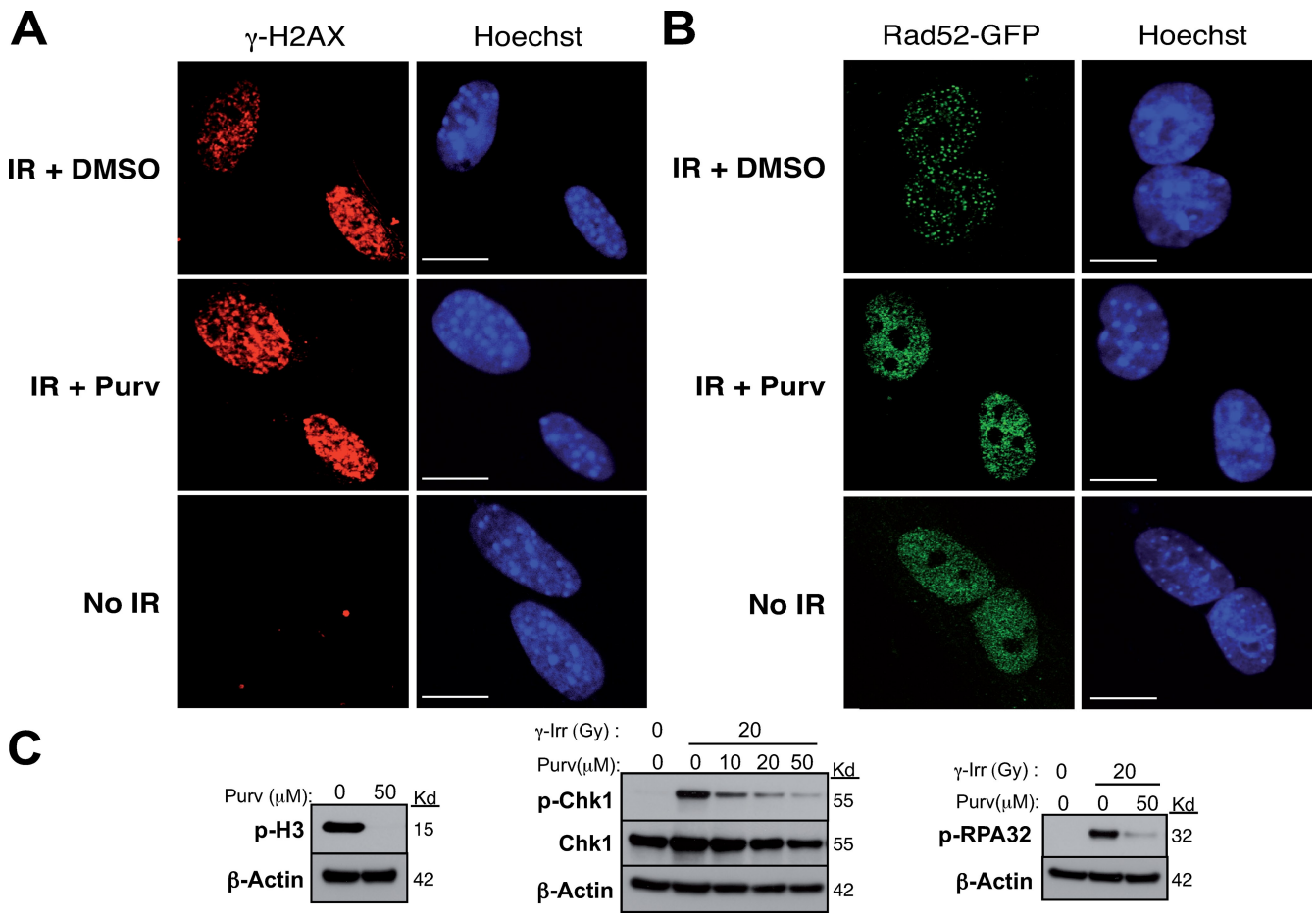

Figure S3. **Purvalanol treatment and DSB end resection.** (A) TKO MEFs were preincubated for 3 h before IR with 50 μM of the Cdk inhibitor purvalanol (Purv) or DMSO as a control. The presence of phosphorylated γ-H2AX was analyzed by confocal microscopy 90 min after IR. (B) TKO MEFs were infected with retroviral particles expressing a Rad52-GFP chimera. The cells were preincubated for 3 h before IR with 50 μM of the Cdk inhibitor purvalanol or DMSO as a control. The formation of Rad52-GFP foci was analyzed by confocal microscopy 90 min after IR. (C) U2OS cells were incubated for 3 h with the indicated concentrations of the Cdk inhibitor purvalanol and exposed to IR where appropriate. Whole cell extracts were prepared after 45 min. Samples were blotted with a phosphospecific antibody against histone H3–Ser^{10} to assess the efficacy of the treatment. Phosphospecific antibodies against Chk1-Ser^{345} and RPA32-Ser$^{4/8}$ were used to measure the effect on DSB end resection. Bars, 10 μM.