Supporting Information

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SI Materials and Methods

Cell Culture and Treatment. MEFs were isolated from *E*10.5–*E*12.5 embryos and cultured in DMEM containing 10% FBS. HCT116 cells were cultured in the same media. For the analysis of mitotic index, the cells were treated with nocodazole (100 nM) for 12 h and fixed and stained with propidium iodine, and then the number of cells with condensed chromosomes was counted.

Lentiviral Atm vector was purchased from Open Biosystems and packaged into virions in 293T cells by cotransfection with packaging plasmids. Early passage MEFs were infected and selected with puromycin (5 μg/mL). After the selection, the cells were collected for Western blot analysis and used for growth curve analysis and mitotic index assays. The shRNA against $Cdc20^{AAA}$ (GCGATCCTCGCGCTCTCCGGAA) was constructed in pLKO.1 vector.

To knock down MAD2, BUBR1, and CENP E, HCT116 cells were transfected with siRNA duplexes mixed in lipofactamine 2000 (Invitrogen). The ON-TARGETplus SMARTpool siRNAs were purchased from Thermal Scientific. CENP E siRNA, whose sequence was reported previously (31), was purchased from Sigma.

To assay for the ability to from colonies in soft agar, 1,000 cells were mixed with 0.5% agarose and seeded onto a bottom layer of agarose (0.75%). After 2- to 3-wk culture, the colonies were stained with crystal violet and counted under a microscope.

The measurement of 8-OHdG was carried out with an ELISA-based assay kit (Cell Biolabs). The manufacturer's instruction was followed to obtain the results.

Immunostaining and Western Blot Analysis. For immunostaining, the cells were fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.4% Triton X-100 in PBS for 15 min, blocked with 5% goat serum in PBS, incubated with phospho-p53 (Ser15) and phospho-histone H2A.X (Ser139) (Cell Signaling Technology) overnight at 4 °C, and washed with PBS. The primary antibodies were visualized with fluorochrome-conjugated secondary antibodies (1:500; Jackson ImmunoResearch Laboratories) for 60 min at room temperature. The cells were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The fluorescence image was captured using a Nikon ECLIPSE E800 microscope. The exposure time was set at 100 ms with two-by-two binning for both the mutant and the control cells.

For Western blot analysis, the cells were harvested and lysed in RIPA buffer [50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA plus protease inhibitor mixture (Roche)]. A total of 50 μg total protein was separated in SDS/PAGE and immunoblotted with antibodies against pS18-p53 and p53 (R&D Systems); p21, MAD2, CENP E, and Cdc20 (Santa Cruz Biotechnology); BUBR1 and ATM (Abcam); pS1981 ATM (Rockland); and Phospho-Histone H2A.X (Ser139) (Cell Signaling Technology).

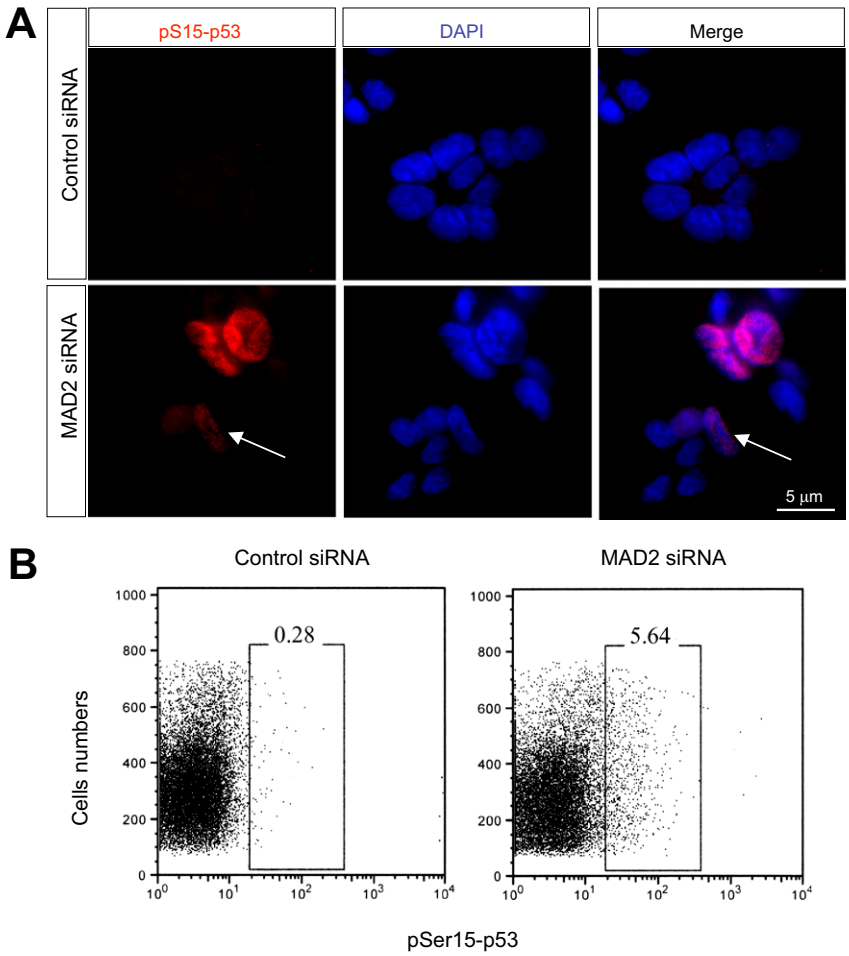

Fig. S1. Analysis of p53 activation in HCT116 cells. (*A*) Immunostaining of Ser15-phosphorylated p53. The arrow indicates a weakly stained cell. (*B*) FACS sorting of the cells in *A*.

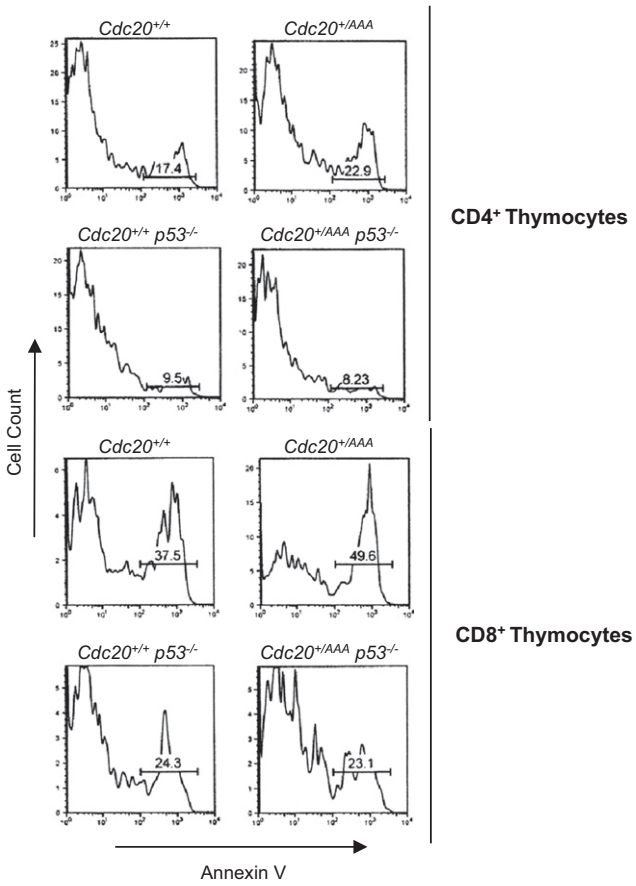

Fig. S2. Analysis of apoptosis in thymocytes. Thymocytes were isolated from adult mice, stained for CD4, CD8, and annexin V, and subjected to FACS analysis.

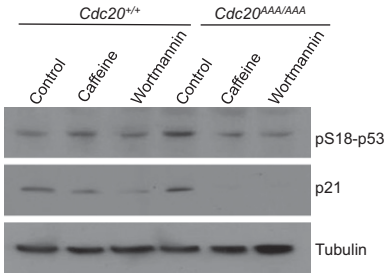

Fig. S3. Western blot analysis of wild-type and Cdc20 mutant MEFs treated with Atm inhibitors.

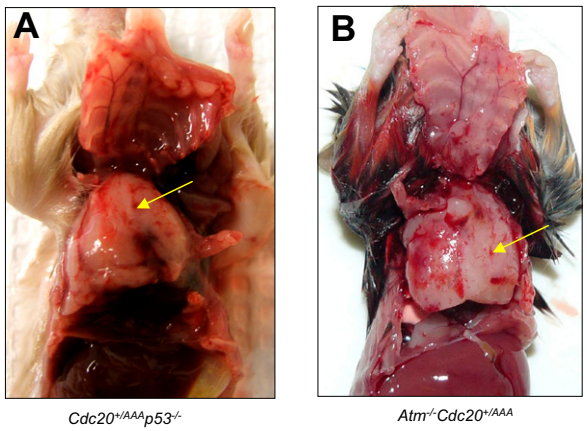

Fig. S4. Gross images of gigantic lymphomas developed in a $Cdc20^{+/AAA}p53^{-/-}$ (*A*) and an $Atm^{-/-}Cdc20^{+/AAA}$ mouse (*B*).

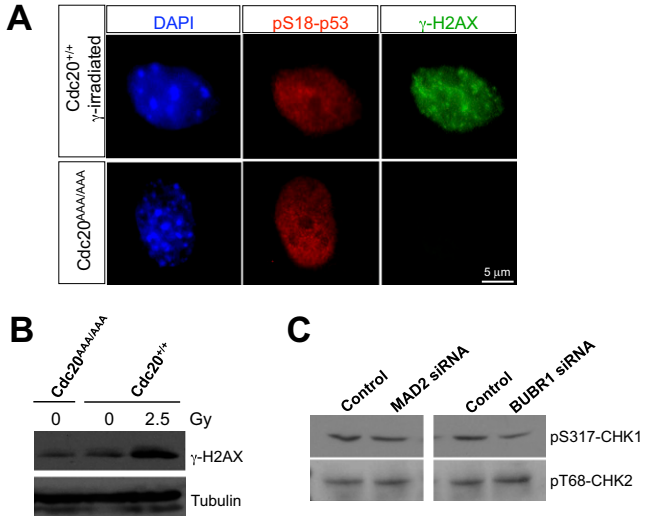

Fig. S5. Canonical DNA damage signaling is undetectable in SAC-deficient cells. (*A*) Immunofluorescent staining of Ser18-phosphorylated p53 and γ-H2AX. The wild-type MEFs were treated with 2.5 Gy γ-irradiation. (*B*) Western blot analysis of γ-H2AX. (*C*) Western blot analysis of HCT116 cells in Fig. 1*D*. For loading control, see Fig. 1*E*.

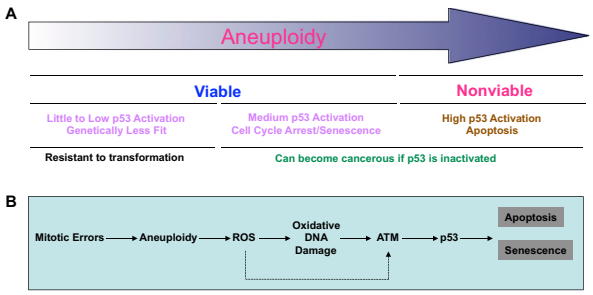

Fig. S6. The cellular response to aneuploidy. (*A*) Potential outcomes of aneuploiy. (*B*) The aneuploidy checkpoint. The dashed arrow indicates the possibility that ROS may directly activate ATM.