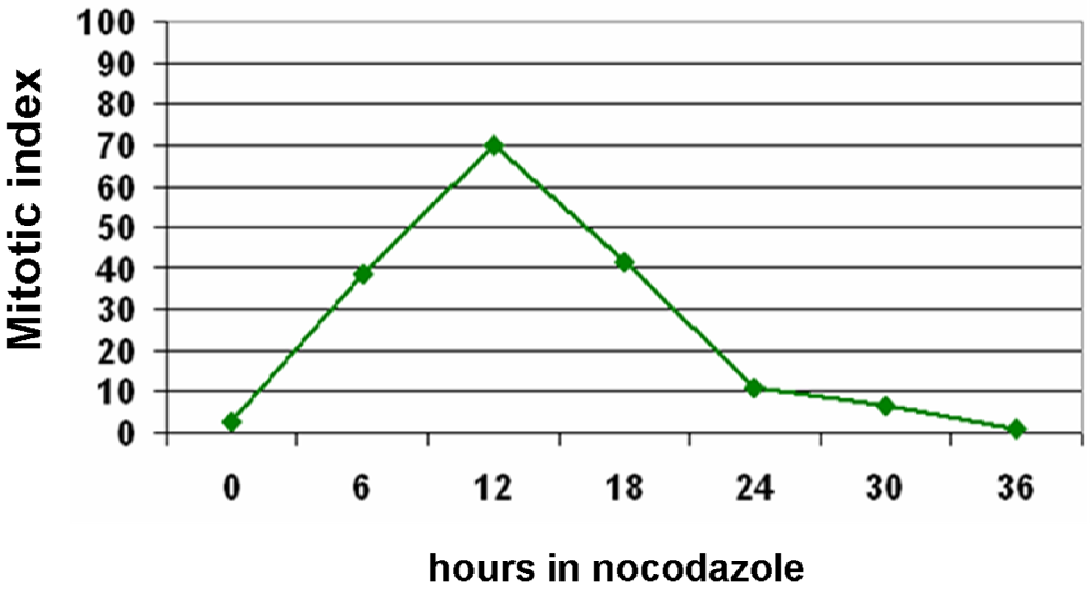
Supplementary Methods

Antibodies

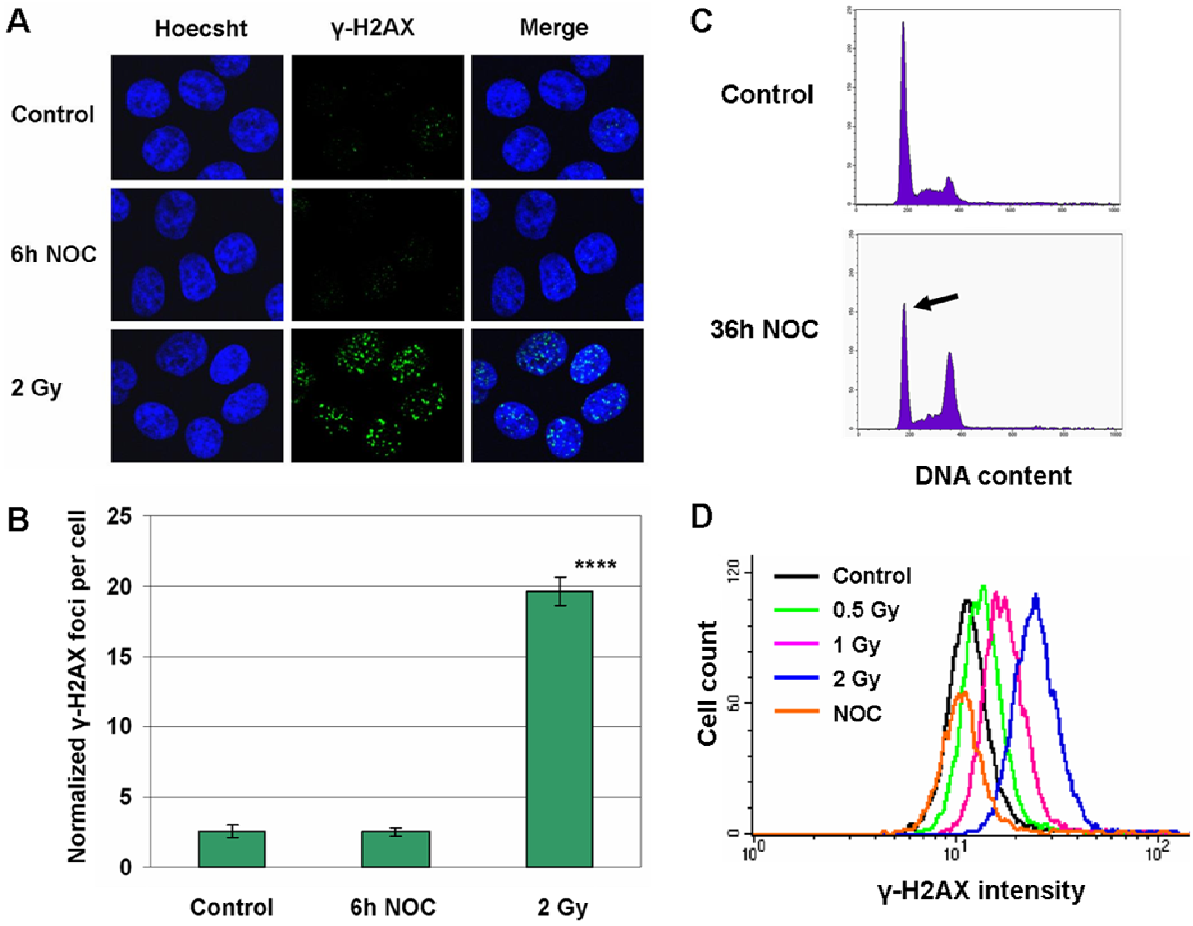
For immunocytochemistry, the following antibodies were used: mouse anti-γ-H2AX (Upstate), rabbit anti-γ-H2AX (Abcam), rabbit anti-53BP1 (Novus), mouse anti-ATM-phosphoserine1981 (Upstate), rabbit anti-cleaved caspase 3 (Cell Signaling), mouse anti-cytochrome c (Cell Signaling), mouse anti-activated-Bax (Cell Signaling), rabbit anti-γ-tubulin (Sigma), and rat anti-α-tubulin (Chemicon). For flow cytometry, mouse anti-MPM-2 (Upstate) and mouse anti-γ-H2AX-FITC (Upstate) antibodies were used. All fluorescent secondary antibodies were Alexa-conjugates (Molecular Probes). For immunoblotting, rabbit anti-CENP-E (Sigma), rabbit anti-RNA Polymerase II (Santa Cruz), mouse anti-γ-H2AX (Upstate), and rabbit anti-actin (Sigma) antibodies were used. To confirm specificity of the mouse anti-γ-H2AX (Upstate) antibody, immunoblotting was performed on control and nocodazole-treated cells (Supplementary Fig. S5).

Image Quantification of γ-H2AX

Confocal stacks of individual nuclei stained with mouse anti-γ-H2AX antibody and Hoechst were obtained at 1-micron intervals. For interphase nuclei, maximum projection images were created, and γ-H2AX foci number was quantified using Metamorph (Molecular Devices). For prometaphase nuclei, total γ-H2AX intensity was quantified by summation of pixel intensity of each plane, as mitotic compaction of chromatin produced too much foci overlap for discrimination of foci number. Raw intensity values were then divided by the mean intensity value of control cells, to set the baseline intensity value at 1.



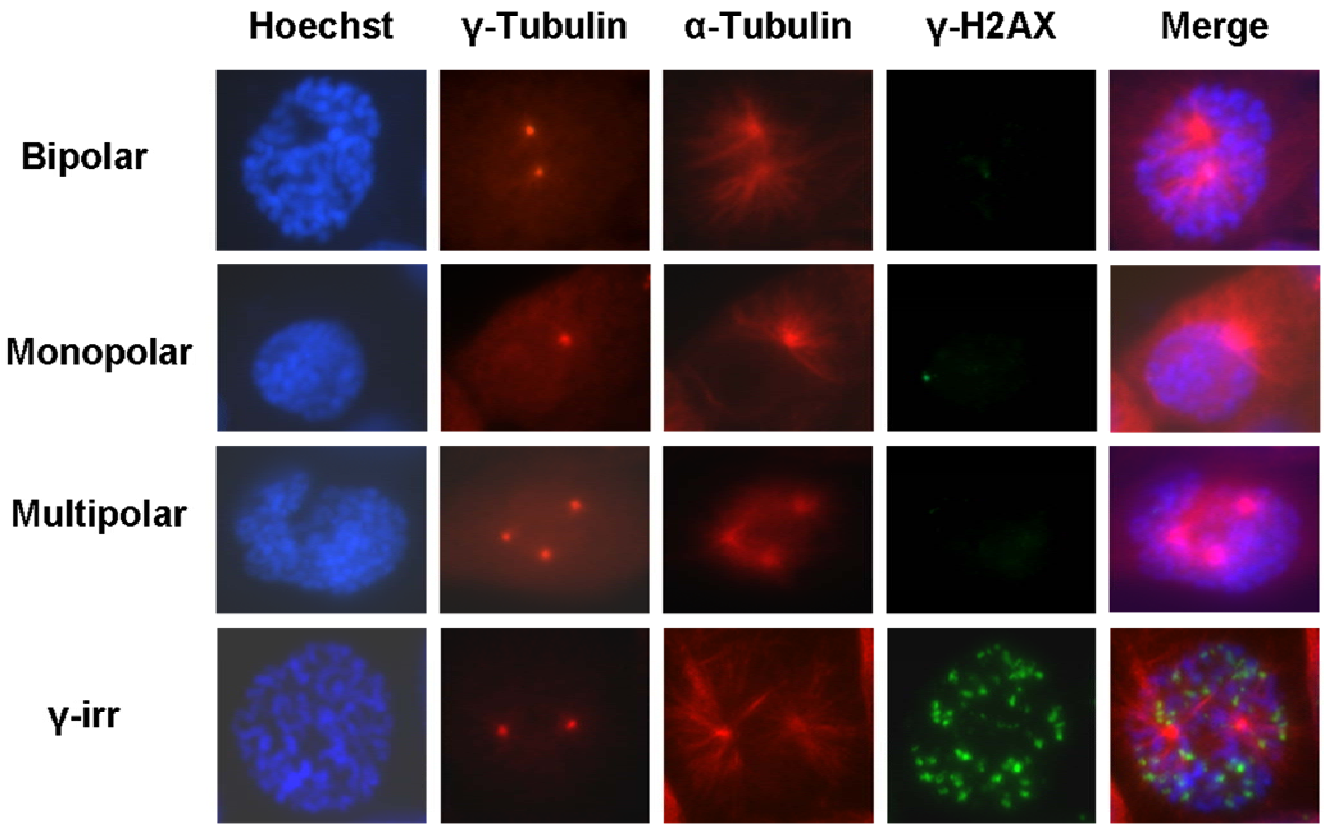
Supplementary Figure S1. Nocodazole induces transient mitotic arrest in HCT116 cells. Mitotic index was measured using flow cytometric analysis of cells stained for the mitotic phosphoepitope MPM-2. DNA was counterstained with propidium iodide.



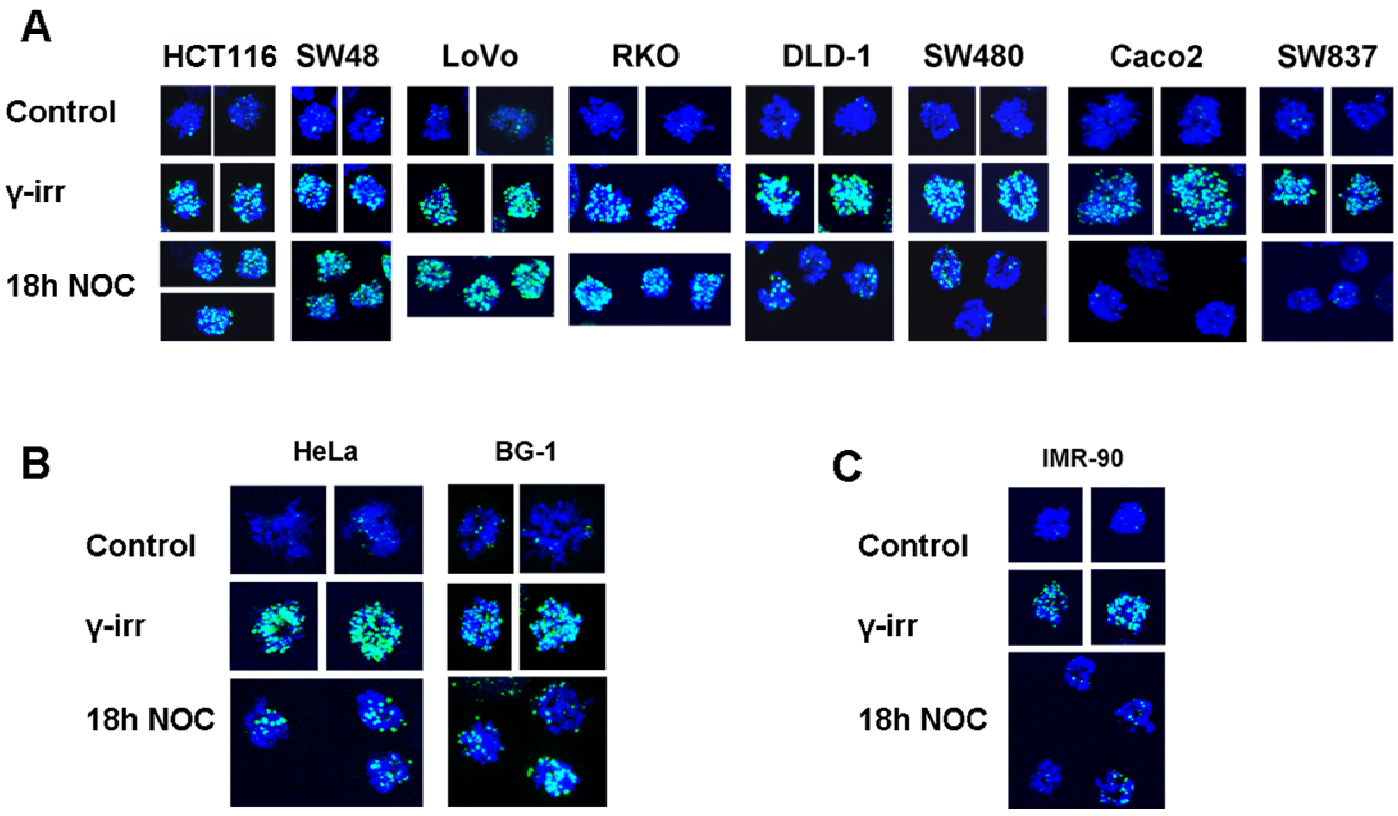
Supplementary Figure S2. Nocodazole does not induce γ-H2AX foci in premitotic HCT116 cells. *A*, Maximum projection images of stacks of interphase cells stained for γ-H2AX after 6h nocodazole, at which point all interphase cells are premitotic. *B*, Quantification of γ-H2AX foci per interphase cell after 6h nocodazole, normalized to DNA content of a 2N cell. Means and SEMs are from at least 98 cells per sample, taken from two independent experiments. **** = $p<0.0001$, for Mann-Whitney tests, as compared to control. *C*, Cells were contact-inhibited by growing to confluency over 6 days, exposed to either 36h nocodazole or 36h drug-free medium, and stained with propidium iodide. The cell cycle distribution at the end of treatment is shown, and arrows indicate cells which remain in G_1 due to contact inhibition despite nocodazole treatment *D*, γ-H2AX intensity was then measured by gating on the contact-inhibited G_1 cells, which had also been stained for γ-H2AX. Positive control cells were irradiated 30 min before the end of the final 36h incubation in drug-free medium. Data are representative of two independent experiments.

	Cells	Chrom	Chromosome-type		Chromatid-type		Total breaks per chrom	Cells with at least 1 break
			CSB per chrom % (n)	CSE per chrom % (n)	CTB per chrom % (n)	CTE per chrom % (n)	% (n)	% (n)
con	**124**	**5593**	**0.43 (24)**	**0.018 (1)**	**0.14 (8)**	**0.036 (2)**	**0.68 (38)**	**17 (21)**
γ-irr	**135**	**6121**	**1.8 (108)**	**0.28 (17)**	**0.36 (22)**	**0.41 (22)**	**3.5 (214)******	**59 (80)******
noc	**106**	**8075**	**2.4 (193)**	**0.26 (21)**	**0.50 (40)**	**0.35 (28)**	**4.1 (331)******	**66 (70)******

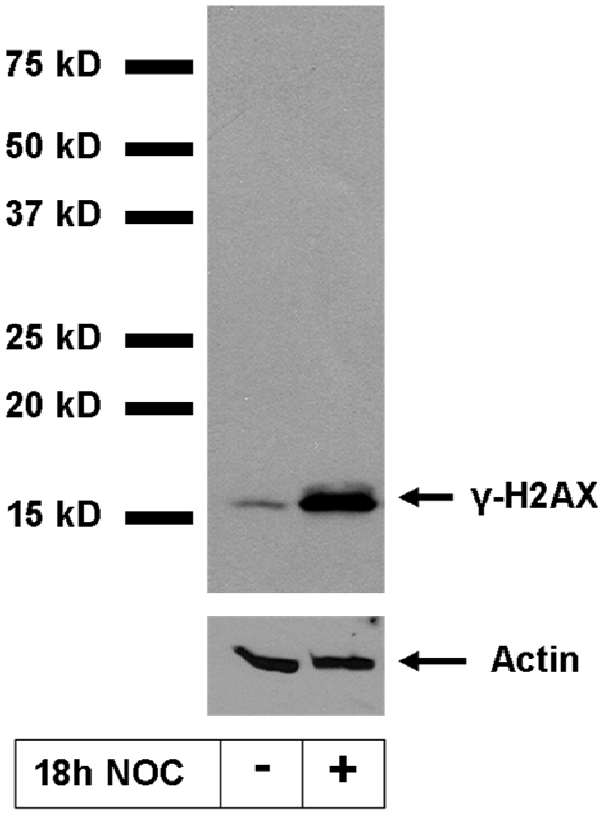
Supplementary Table. Quantification of chromosome aberrations. Treated cells were exposed to 18h nocodazole, washed, and released into drug-free medium for 19h. Positive control cells were exposed to 2 Gy of γ-irradiation and incubated for 19h, and negative control cells were incubated in drug-free medium for 19h. All cells were then exposed to nocodazole for 1h to disassemble spindles for spreading, and chromosome spreads were prepared. Con = control. γ-irr = γ-irradiated. Noc = pooling of both the tetraploid (70%) and near-diploid (30%) chromosome spreads produced through drug washout. Cells = number of cells analyzed from two independent experiments. Chrom = total number of chromosomes contained in analyzed cells. CSB = chromosome break. CSE = chromosome exchange. CTB = chromatid break. CTE = chromatid exchange. Total breaks = CSB + CTB + 2(CSE + CTE), as two breaks produce exchanges. **** = $p<0.0001$, for chi square tests, as compared to control.



Supplementary Figure S3. Prophase HCT116 cells with spindle abnormalities do not contain elevated γ-H2AX. Single focal planes of prophase cells stained for γ-H2AX, γ-tubulin, and α-tubulin, indicating that monopolar or multipolar cells do not have elevated γ-H2AX. γ-irr = 30 min after 2 Gy γ-irradiation.



Supplementary Figure S4. Acquisition of γ-H2AX foci during mitotic arrest is a common occurrence in human cell lines. Maximum projection images of stacks of prometaphase cells stained with Hoechst (blue) and anti-γ-H2AX (green) after 18h nocodazole. γ-irr = 30 min after 2 Gy γ-irradiation. *A*, Colorectal cancer cell lines. *B*, HeLa cervical cancer cells and BG-1 ovarian cancer cells. *C*, IMR90 primary human fibroblasts. Acquisition settings were constant between treatments within a given cell line, but settings were optimized for each cell line to account for differences in background staining.



Supplementary Figure S5. Anti-γ-H2AX antibody recognizes a single protein in HCT116 cells. Cells treated with or without nocodazole for 18h were subjected to immunoblotting with mouse anti-γ-H2AX antibody (Upstate). Control and nocodazole-arrested cells were also co-treated with the Q-VD-OPh caspase inhibitor to prevent apoptotic γ-H2AX from diluting the signal of non-apoptotic cells. Actin was used as a loading control.

Supplementary Movie 1. Mitotic HCT116 cells released from nocodazole divide and continue proliferating. Cells were treated with nocodazole for 6h, washed, and incubated for 30h in drug-free medium. Filming of this video starts immediately following washout (after 6h nocodazole) and continues for 30h.

Supplementary Movie 2. A fraction of HCT116 cells dies during continuous treatment in nocodazole. Cells were treated with nocodazole for 6h, mock washed in drug-containing medium, and incubated for an additional 30h in nocodazole. Filming of this video starts immediately following mock washout (after 6h nocodazole) and continues for 30h.