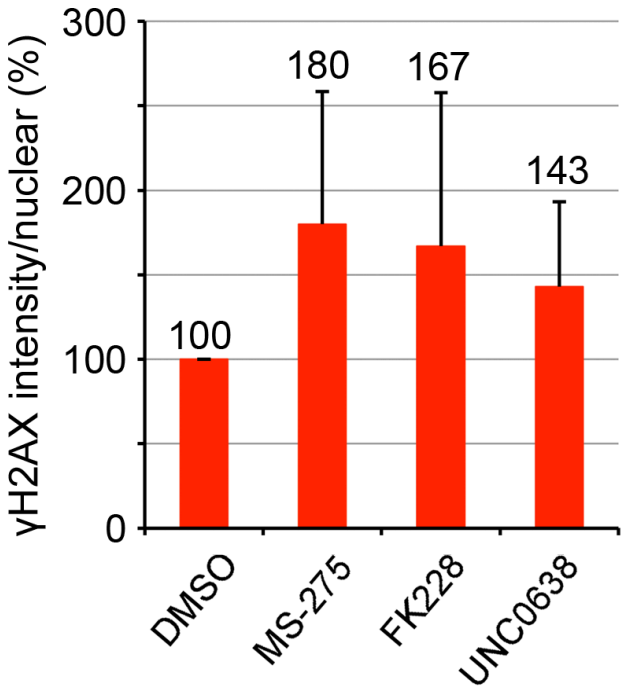
Fukuda, et al., Supporting information

Supplementary Table

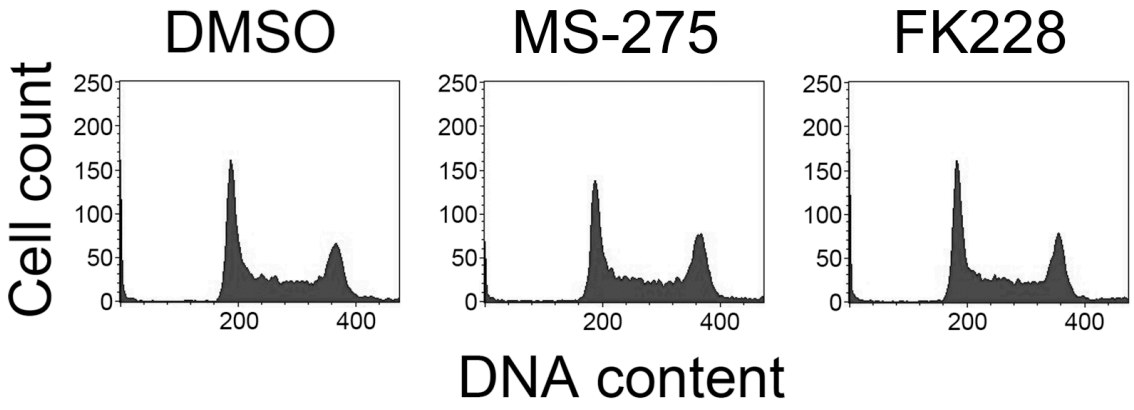
Supplementary Table S1: Summary of the effects of HDACis and UNC0638

		HDACi		HMTi
		MS-275	FK228	(UNC0638)
	H3K9me2	↓	↓	↓
	H3K9ac	↑	↑	→
	H3K56ac	↑	↑	→
	H4ac	↑	↑	→
IRIF formation	BRCA1	↓	↓	↓
IRIF formation	BARD1	↓	↓	↓
IRIF formation	53BP1	↓	↓	→
IRIF formation	RIF1	↓	↓	→
IRIF formation	RAD51	↓	↓	↓
	DR-GFP	↓	↓	↓

Supplementary Figures

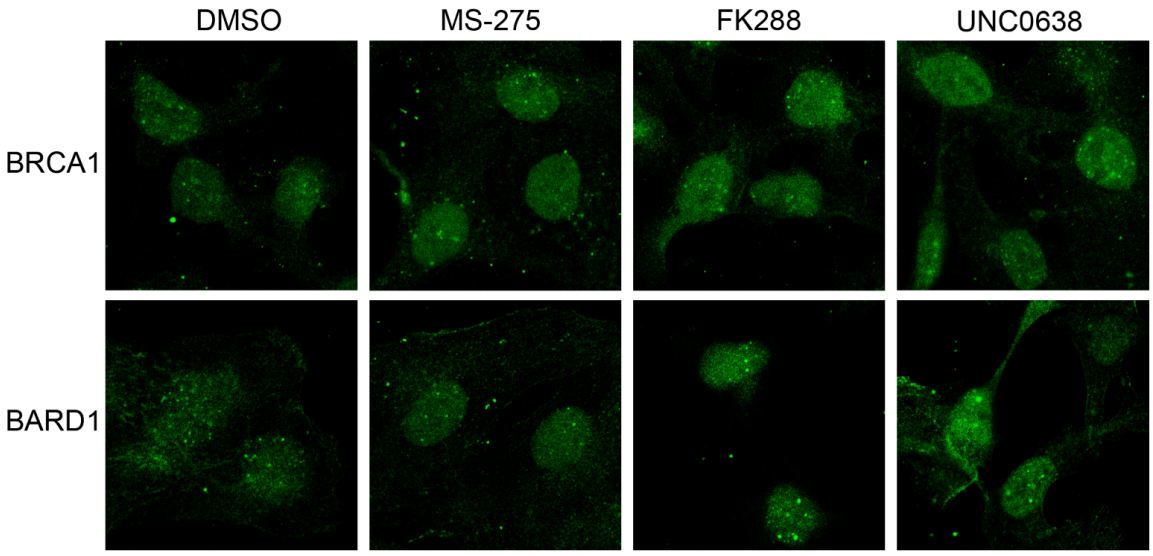


Supplementary Figure S1. MS-275, FK228 and UNC0638 increase the intensity of γH2AX IRIF. U2OS cells treated with DMSO, MS-275, FK228 or UNC0638 for 24 hours were exposed to IR and immunostained with γH2AX antibody one hour after IR. The mean intensity of the γH2AX per nuclear was mechanically analyzed using the Cellomics Image Analyzer (Thermo Fisher). Average ± SD normalized to cells treated with DMSO were derived from duplicate experiments.

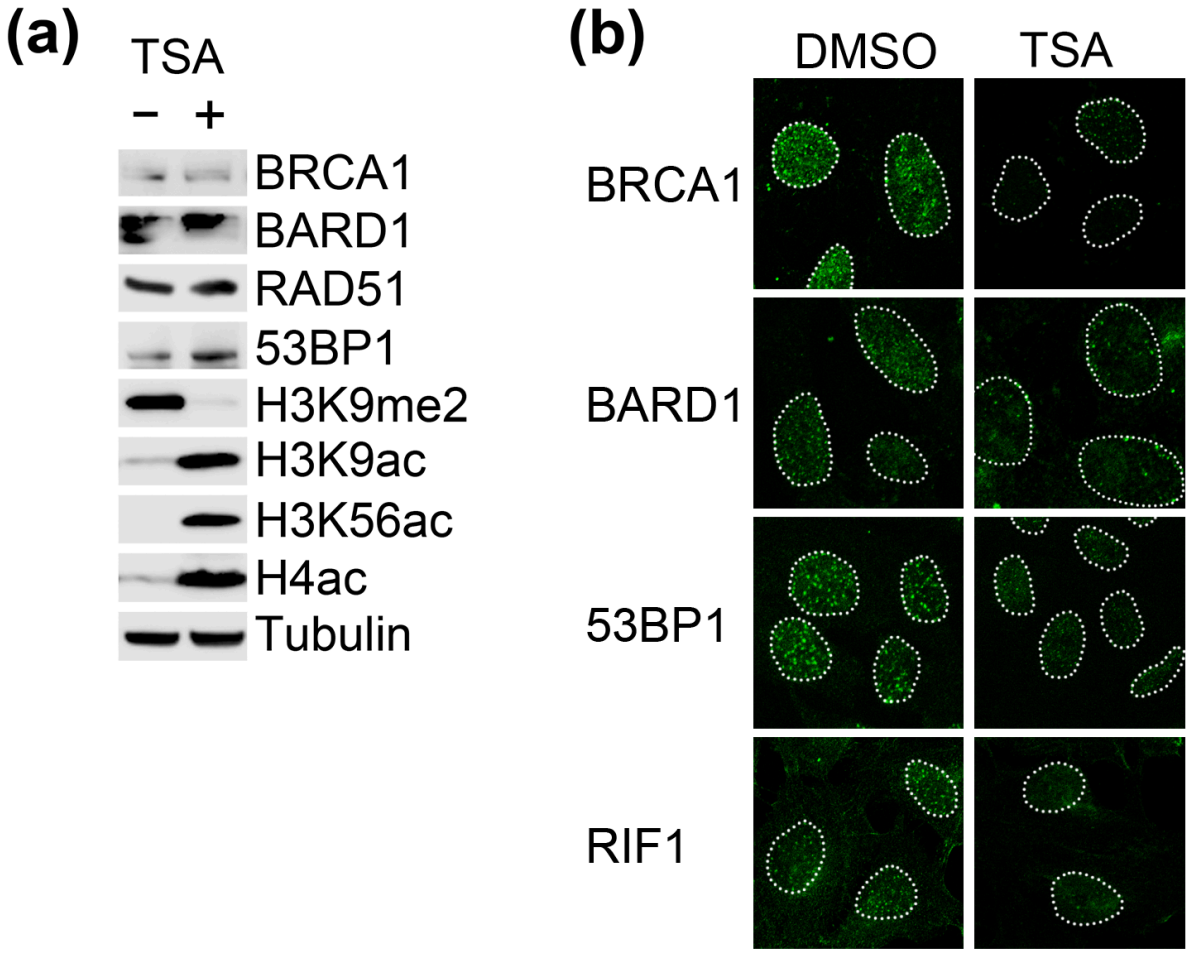


G1	46.1	39.8	46.5
S	32.2	34.8	34.4
G2/M	21.8.	25.4	19.2

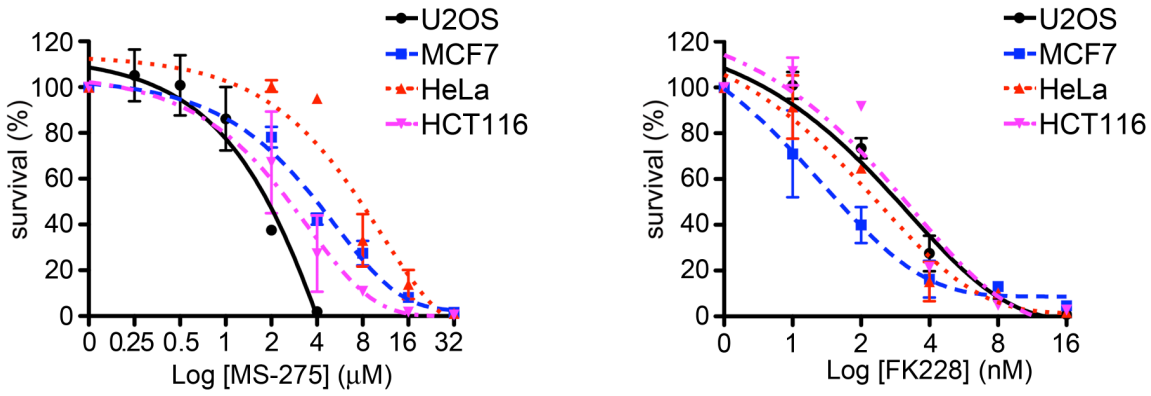
Supplementary Figure S2. Cell cycle progression of cells treated with HDACis. U2OS cells were treated with DMSO, 2.5 μM MS-275, or 2.5 nM FK228 for 24 hours prior to being subjected to cell cycle analysis. The percentage of cells in each phase of the cell cycle is shown at the bottom. The analysis demonstrated comparable distribution of the cell cycle phases in the HDACis-treated cells to that of control cells.



Supplementary Figure S3. Immunostain of BRCA1 and BARD1 without pre-extraction. U2OS cells treated with DMSO or the indicated agents were immunostained for BRCA1 and BARD1 without pre-extraction.



Supplementary Figure S4. Trichostatin A (TSA) inhibits H3K9me2 and suppresses IRIF formation of BRCA1 and BARD1 in addition to 53BP1 and RIF1. U2OS cells were incubated for 5 hours with DMSO (-) or TSA (0.5 μM) and either immunoblotted with the indicated antibodies (a) or exposed to IR and immunostained with γH2AX and indicated antibodies (b). Note that we employed the dose and the time length of treatment that matched with the condition previously reported (Reference 4 in the main text).



Supplementary Figure S5. Clonogenic survival of cells exposed to Class I HDACis. U2OS, HCT116, MCF7 or HeLa cells were seeded at a concentration of 500 cells/well in 6-well plates and, after 6 hours, were treated with the indicated concentrations of MS-275 (left) or FK228 (right). After 24 hours of incubation, the cells were washed and further cultured in fresh medium without the chemicals for 9 days. The cells were then fixed and stained with crystal violet. The colonies were scanned and counted using an ImageQuant LAS-4000 instrument (GE Healthcare). The data are shown with nonlinear regression fit curves for one phase decay (GraphPad Prism). Averages ± S.E.M., normalized to cells without the HDACis, were derived from triplicate experiments.