Mosammaparast et al., http://www.jcb.org/cgi/content/full/jcb.201302092/DC1

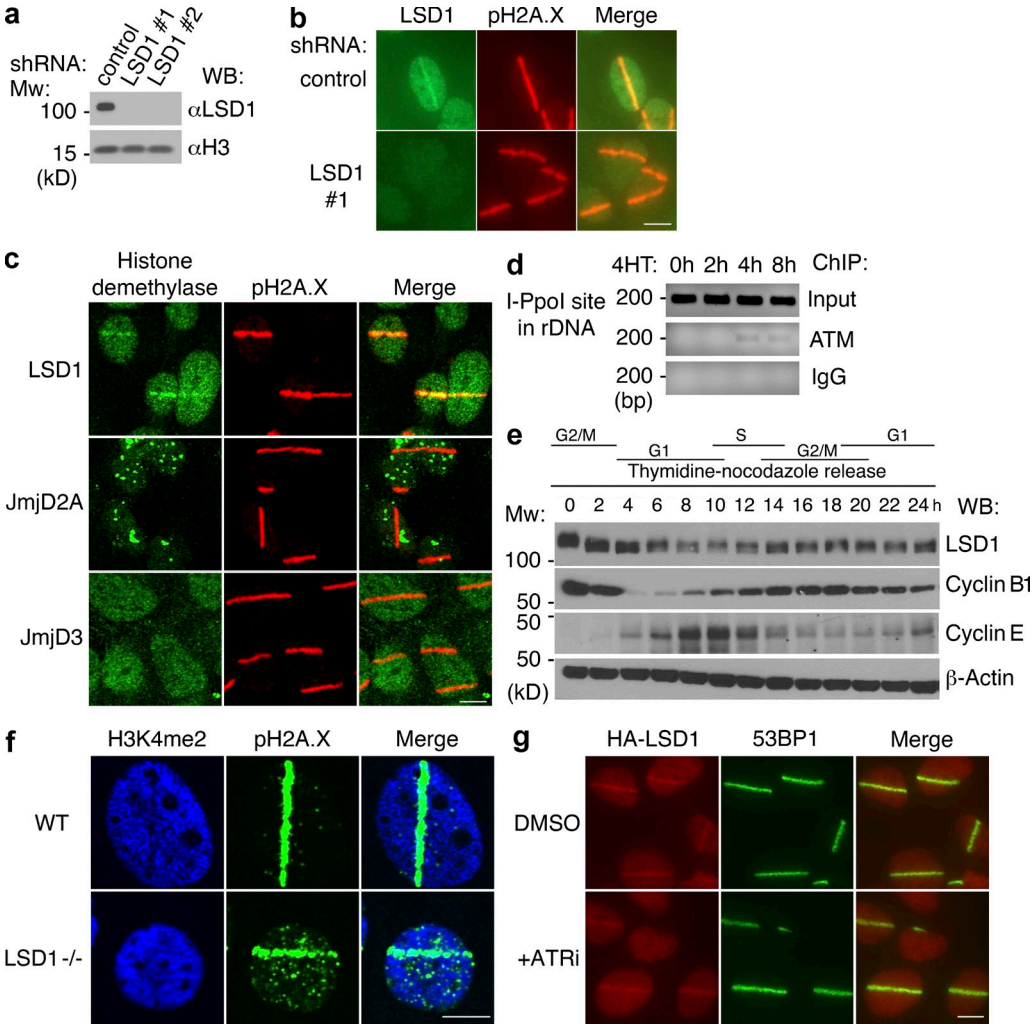

Figure S1. **Evidence for a direct role of LSD1 at DNA damage sites.** (a) Knockdown was performed in U2OS cells using the indicated lentiviral shRNAs and confirmed by Western blotting using an anti-LSD1 antibody. Histone H3 was used as a loading control. (b) U2OS cells were infected with the indicated shRNAs, and UV microirradiation was performed after knockdown. The cells were subsequently stained for LSD1 and pH2A.X and analyzed by fluorescence microscopy. (c) UV laser microirradiation was performed on U2OS cells followed by incubation at 37°C for 10 min. The cells were subsequently stained for the indicated histone demethylases and pH2A.X, and then analyzed by confocal microscopy. (d) Cells transduced with I-PpoI vector were treated with 4-OH-tamoxifen for the indicated time period. ChIP was then performed using antibodies against ATM with IgG as a control, followed by PCR on the ChIP material using the rDNA primer set. (e) HeLa cells were synchronized in late G2 using sequential thymidine-nocodazole block, released, and then collected every 2 h. Whole cell lysates were Western blotted using the indicated antibodies. (f) Wild-type or LSD1-deficient MEFs were microirradiated, stained with the indicated modification-specific histone antibodies, and analyzed by confocal microscopy. (g) U2OS cells stably expressing HA-tagged LSD1 were laser microirradiated after treatment with the ATR inhibitor ETP-46464 (5 µM) or DMSO. The cells were subsequently stained for HA-LSD1 and pH2A.X. Bars, 10 µm.

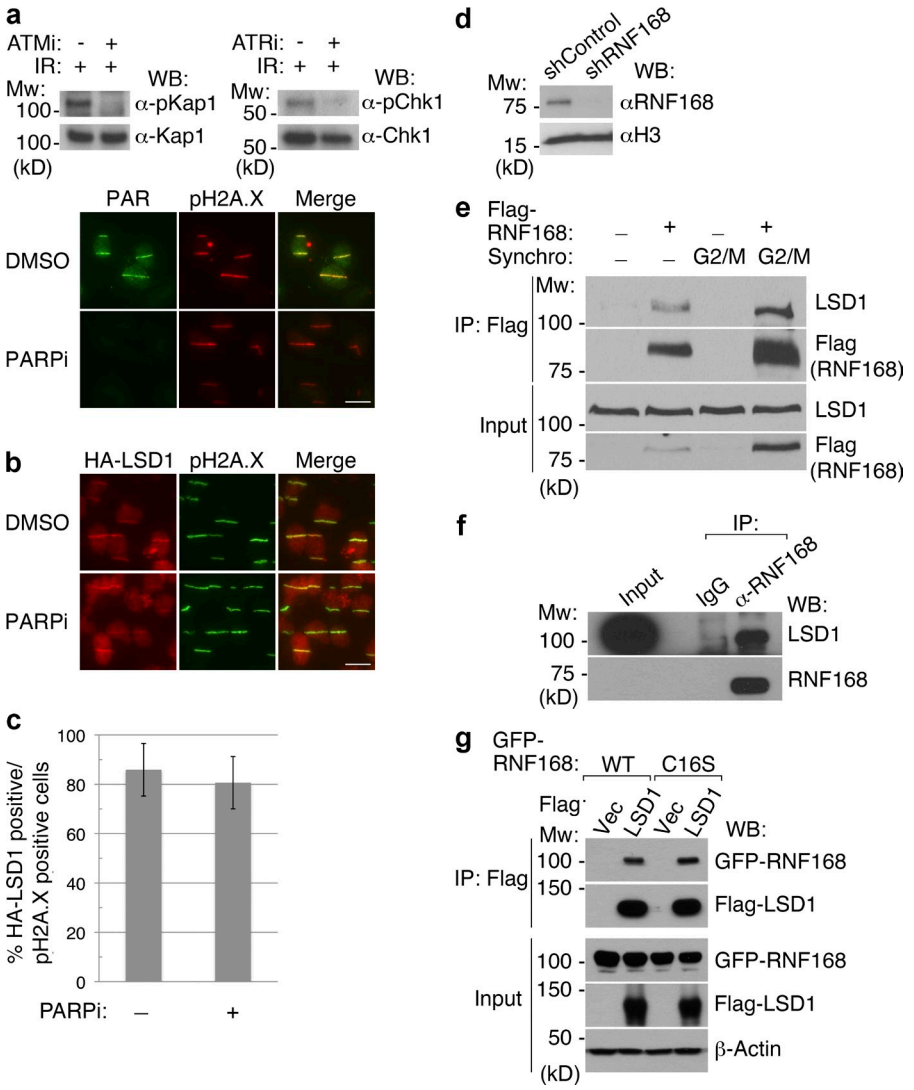

Figure S2. **Elucidating the interaction of LSD1 and the DNA damage machinery.** (a, top) U2OS cells were treated with the ATM inhibitor KU55933 (15 μM), the ATR inhibitor as in Fig. S1 g, or with DMSO for 1 h. The cells were then irradiated (5 Gy) and incubated at 37°C for 1 h, and whole cell extracts were Western blotted for phosphorylated Kap1 (an ATM target) or phosphorylated Chk1 (an ATR target). (bottom) U2OS cells were treated with the PARP inhibitor KU58948 (2 μM) or with DMSO for 1 h. The cells were then laser microirradiated and stained for poly-ADP-ribose (PAR) and pH2A.X. (b) U2OS cells stably expressing HA-tagged LSD1 were laser microirradiated after treatment with the PARP inhibitor KU58948 (2 μM) or with DMSO for 1 h. The cells were subsequently stained for HA-LSD1 and pH2A.X. Bars, 20 μm. (c) Quantitation of panel b, with error bars representing the SD of triplicate experiments. (d) U2OS cells were infected with the indicated lentiviral shRNAs and whole cell extracts were Western blotted with the indicated antibodies. (e) Immunoprecipitation was performed from 293T cells mock infected or stably expressing Flag-HA-RNF168, with or without synchronization in G2/M with nocodazole, followed by Western blotting as indicated. (f) Immunoprecipitation was performed from HeLa nuclear extract using equal amounts of an antibody against RNF168 or a control IgG. Input and immunoprecipitated material were analyzed by Western blotting using the indicated antibodies. (g) Immunoprecipitation was performed from 293T cells expressing Flag-vector or Flag-LSD1, in combination with GFP-tagged wild-type RNF168 or a catalytically inactive mutant form (C16S) of RNF168 as indicated. Input and immunoprecipitated material were analyzed by Western blotting as indicated.

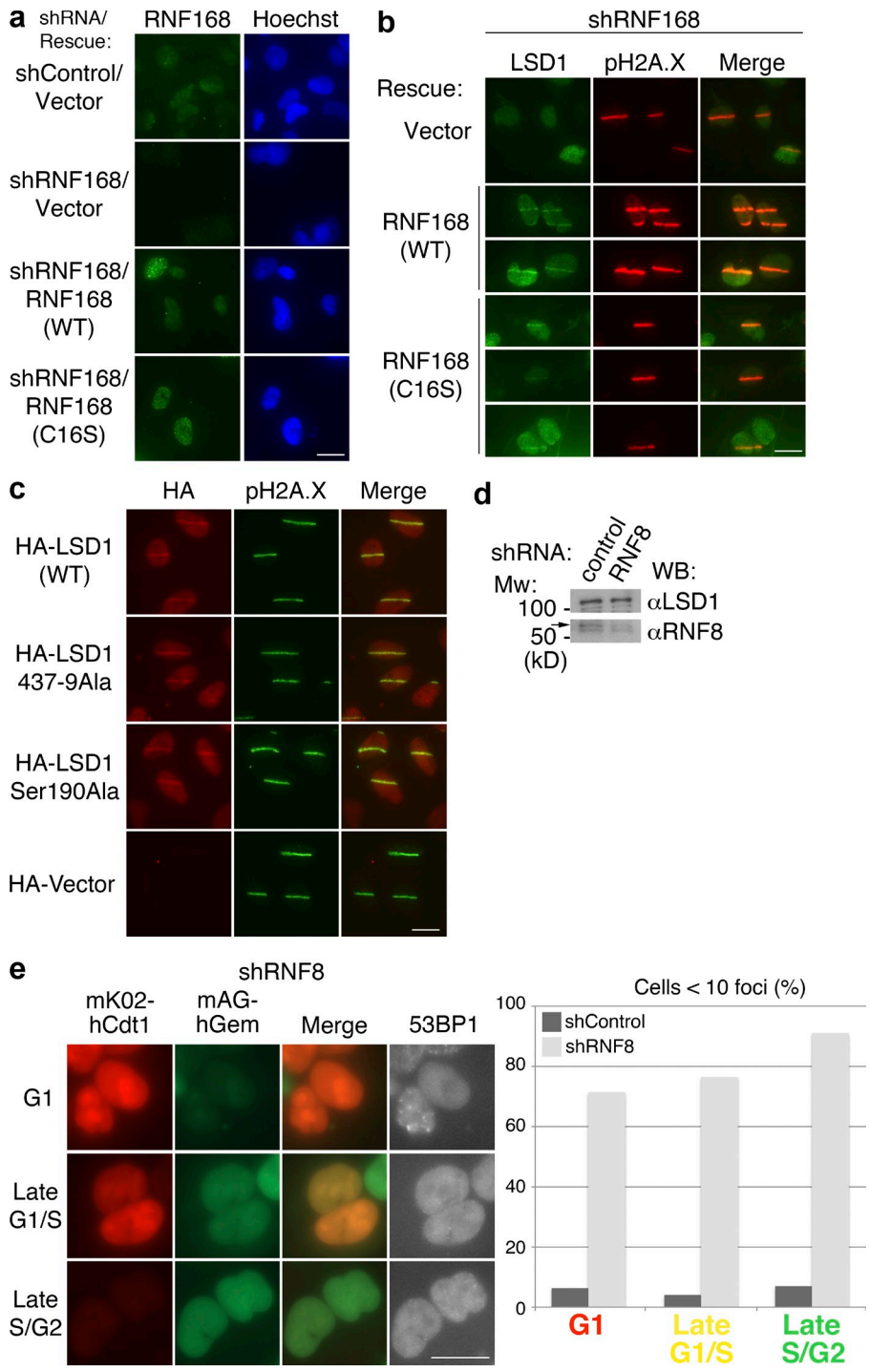

Figure S3. **Recruitment of LSD1 to DNA damage sites by RNF168 and functional consequence of RNF8 knockdown.** (a) U2OS cells were infected with vectors expressing a control or RNF168-specific shRNA, in combination with the shRNA-resistant versions of RNF168 as indicated. The cells were then stained for RNF168 and nuclei were stained with Hoechst. (b) Cells from panel a were microirradiated and subsequently stained for LSD1 and pH2A.X. (c) Targeted mutations made in tagged LSD1 were stably expressed in U2OS cells, laser microirradiated, and stained as indicated. A sample of two mutations are shown, including one targeting a conserved central region (amino acids 437–439) not involved in CoREST binding, as well as a putative ATM/ATR phosphorylation site (serine 190). (d) U2OS-FUCCI cells were infected with the indicated shRNAs, selected, and analyzed by Western blot using the indicated antibodies. (e) The cells from panel d were then exposed to 10 Gy IR, incubated at 37°C for 1 h, and processed for immunofluorescence using an antibody against 53BP1. (e) Foci quantitation of d. The data shown are from a single representative experiment out of two repeats. $n > 150$ total cells quantified for each shRNA. Bars, 20 µm.

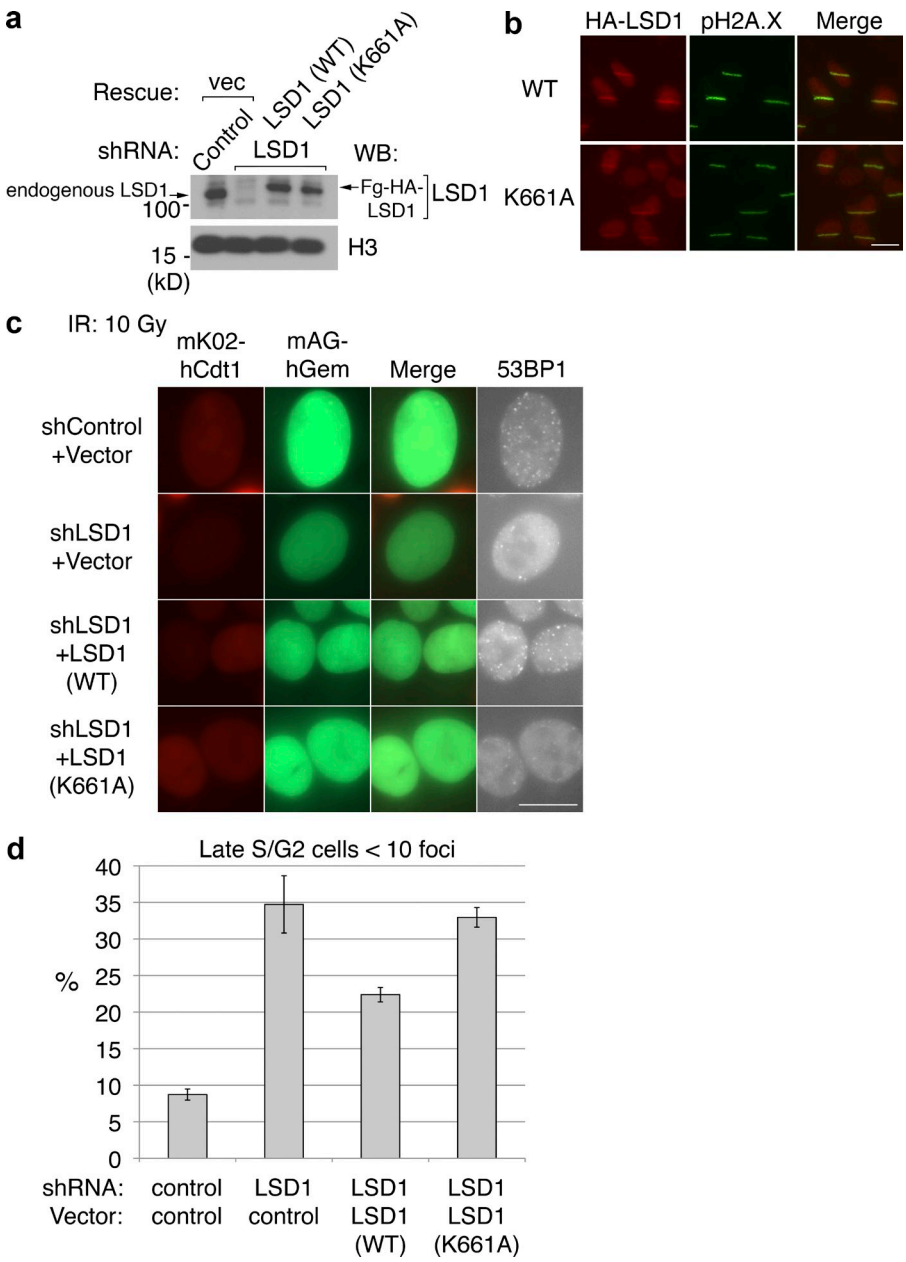

Figure S4. **Catalytic activity of LSD1 promotes 53BP1 IRIF formation in late S/G2 cells.** (a) U2OS-FUCCI cells were infected with retroviral, shRNA-resistant Flag-HA-LSD1 (wild type or catalytically inactive K661A) or with control vector, as well as the indicated shRNA vectors. After selection, whole cell extracts were analyzed by Western blot as shown. (b) U2OS cells expressing Flag-HA-LSD1 (wild type or catalytically inactive K661A) were laser microirradiated and stained as indicated. (c) Cells from panel a were exposed to 10 Gy IR and processed for immunofluorescence using an antibody against 53BP1. (d) The experiment in panel c was performed in duplicate and quantified. Error bars represent ± SD. Bars, 20 µm.

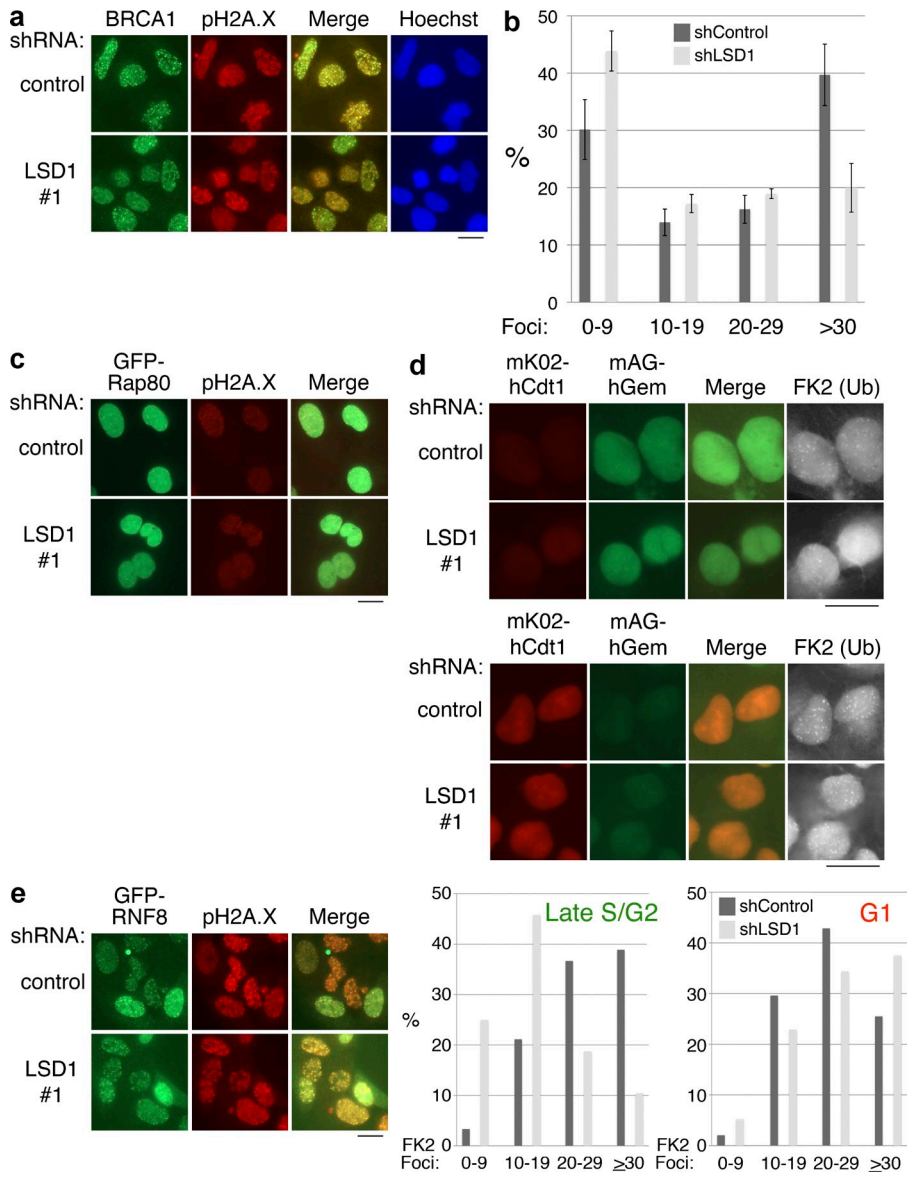

Figure S5. **Functional consequences of LSD1 knockdown.** (a) U2OS cells were infected with the indicated lentiviral shRNAs, irradiated, incubated at 37°C for 1 h, and stained for BRCA1 and pH2A.X. (b) Quantitation of panel a. Error bars represent ± SD. (c) U2OS cells expressing GFP-Rap80 were irradiated as in panel a and stained as indicated. (d) U2OS-FUCCI cells were infected with the indicated shRNAs, irradiated, and stained for ubiquitin conjugates (FK2). Top shows cells in late S/G2 (high mAG-Gem), whereas middle shows cells in G1/early S (high mK02-hCdt1). Quantitation of each is shown below. The data shown are from a single representative experiment out of two repeats. $n > 150$ total cells quantified for each shRNA. (e) U2OS cells expressing GFP-RNF8 were irradiated as in panel a and stained as indicated. Bars, 20 µm.

Table S1. **Peptide numbers of proteins found by MS/MS analysis**

Protein/gene Symbol	Total peptides (−IR)	Total peptides (+IR)
RNF168	57	59
PYR1	38	40
DNA-PKcs	32	25
ADT2	24	22
XRCC6/Ku70	21	20
XRCC5/Ku80	20	21
PRDX1	18	18
IRS4	16	14
ADT3	16	18
PNPO	16	16
ADT1	13	14
RO52	12	13
TIF1B	12	11
SUPT16H	12	11
LSD1/AOF2	10	11
H4	10	11
ADT3A	10	12

Peptide numbers of proteins found by MS/MS analysis of the Flag–RNF168 complex purified from 293T nuclear extract, with or without prior γ-IR (10 Gy). All proteins with ≥10 total peptides for both samples are shown.

Table S2. **Antibodies used in this study**

Protein/antigen	Manufacturer	Used for
53BP1	Santa Cruz Biotechnology, Inc.	Western blot
53BP1	Bethyl Laboratories, Inc.	ChIP
LSD1	Bethyl Laboratories, Inc.	Western blot/immunofluorescence/ChIP
LSD1	Santa Cruz Biotechnology, Inc.	Western blot/ChIP
JMJD2A	Sigma-Aldrich	Immunofluorescence
JMJD3	Gift of S. Chen (Harvard Medical School, Boston, MA)	Immunofluorescence
CoREST	Bethyl Laboratories, Inc.	Western blot/immunofluorescence
Cyclin B1	Santa Cruz Biotechnology, Inc.	Western blot
Cyclin E	Santa Cruz Biotechnology, Inc.	Western blot
BHC80	Gift of S. Iwase (Harvard Medical School, Boston, MA)	Immunofluorescence
MDC1	Bethyl Laboratories, Inc.	Immunofluorescence
BRCA1	Santa Cruz Biotechnology, Inc.	Immunofluorescence
RNF8	Santa Cruz Biotechnology, Inc.	Western blot
RNF168	Gift of D. Durocher (Mount Sinai Hospital, Toronto, Canada)	Immunofluorescence/Western blot/IP
H2A-Ub	EMD Millipore	Immunofluorescence
FK2 (Ub)	Enzo Life Sciences	Immunofluorescence
H2A.X	Active Motif	Western blot
pH2A.X	Abcam	Immunofluorescence/Western blot
pH2A.X	Active Motif	Immunofluorescence/Western blot
H4K5ac	EMD Millipore	ChIP
H3	Abcam	ChIP/Western blot
H3K4me2	EMD Millipore	Immunofluorescence
H3K4me2	Abcam	ChIP
H3K9me2	Abcam	Immunofluorescence
H3K4me1	Abcam	ChIP
H4	Active Motif	Western blot
H4K20me2	Active Motif	Western blot
PAR (Poly-ADP-Ribose)	Trevigen	Immunofluorescence