Supplemental Data
Supplemental Figure 1. Related to Figure 1.

SENP2 promotes DNA damage signalling and DNA repair.

A. Quantification of γH2AX foci (% of cells with >10 foci /nucleus) in WT or SENP2 KO HAP1 post 4 Gy IR. n=100 cells from three experiments.

B. IR colony survival of WT and SENP2 KO HAP1 cells, n=3

C. CPT colony survival assay as for B) but using a 2 hour treatment with CPT, n=3.

D. Western blots of SENP2 protein expression levels in HAP1.

E. Cartoon schematic of human SENP2 domain locations, the N terminal 65 amino acids encompass the NLS (nuclear localisation signal) which also directs binding to the NUP153 nuclear pore component (Zhang, Saitoh, and Matunis 2002). An amphipathic helix between amino acids 1-18 directs SENP2 to cellular membranes (Odeh et al. 2018). The NES (nuclear export signal) directs SENP2 shuttling between the nucleus and cytoplasm. Interaction with NUP107 is through amino acids 143-350 although this interaction has not been as finely mapped as for NUP153. The NP mutant of SENP2 is illustrated below.

F. Western blot related to figure 1A. Note the SENP2NPm migrates at a similar molecular weight to a lower band that cross reacts with the SENP2 antibody.

G. Colony survival in HeLa depleted with indicated siRNA using CPT (1 μM) or Olaparib (10 μM) for 2 hr. Stable expression of SENP2 mutants was induced with doxycycline. n=3.

H. IF images related to figure 1D. Scale bar = 10 μm

I. Quantification of MDC1/γH2AX foci in HAP1 cells fixed at indicated times post 4 Gy IR, n=100 cells from a total of three experiments.

J. Representative images related to S1H. Note HAP1 have smaller nuclei. Scale bar = 5 μm.
Supplemental Figure 2. Related to Figure 1.

RNF4-VCP is responsible for defective DNA damage signalling in SENP2 depleted cells

A. Western blots of SUMO1 and SUMO2/3 in HeLa treated with siRNA as indicated. Lysates were made at indicated time points following 4 Gy IR.

B-C. Quantification of % change in SUMO conjugates (relative to non-irradiated SUMO conjugates) following 4 Gy IR related to S2A n=4

D. Top; cartoon of workflow of S2E, bottom; representative images related to S2E.

E. HeLa transfected with myc-ubiquitin and depleted with siNTC or siSENP2, irradiated with 4 Gy, 0.5 hr later cells were treated with DMSO or VCPi CB-5083 (0.1µM). Cells were fixed at 4 hours post IR and scored for MDC1 foci / cell, n=100 from three experiments.

F. Quantification of MDC1 co-localising with γH2AX post 4 Gy IR in cells treated with specified siRNAs. Top shows western blots to indicate knockdown, n=100

G. MDC1 / γH2AX foci quantification in HeLa depleted with indicated siRNA for 72 hr prior to irradiation (4 Gy) and fixation (4 hr post IR), n=100 cells.
Supplemental Figure 3. Related to Figure 2.

MDC1 is a SENP2 substrate and hypo-SUMOylation of MDC1 permits DDR signalling.

A. Prevalence of detected SUMO conjugation sites on MDC1 given as fraction of protein intensity as described in (Hendriks et al. 2017).

B. Representative images related to figure 2A. HeLa^FlpIn myc-MDC1^WT or MDC1^K1840R^ cells transfected with either siNTC or siSENP2 and induced with the addition of Dox for 72 hours prior to irradiation (4 Gy). Cells were fixed at indicated times. Cells are immunostained for myc. Scale bar = 10 μm.

C. Ni^{2+} pulldown in HAP1 WT or SENP2 KO cells transiently transfected with 6xHis-myc-SUMO2 for 72 hours prior to irradiation (10 Gy). Western blots are probed with MDC1 antibody.

D. Quantification of the MDC1 purified by Ni^{2+} pulldowns in HEK293^FlpIn 6xHis-myc-SUMO1 cells treated with indicated siRNAs and either untreated or treated with 10 Gy IR. Cells were allowed to recover for 1 hour post IR before harvesting. Relative enrichment in SUMO-MDC1 conjugates was determined by densitometry with the untreated siNTC sample being set as 1 (4 experiments). Error bars show s.e.m.

E. Recombinant His-MDC1^{1818-2094} fragments were SUMOylated in vitro with SUMO2, the product was divided in two with half incubated with SENP2 catalytic domain for 30 minutes and the other untreated. DeSUMOylation reactions were stopped with 2X Lamelli buffer, divided in half again and separated by SDS-PAGE. Reaction products were probed with His to detect MDC1 and SUMO2/3 to detect SUMOylated products. SUMO mix contains SUMO E1 and E2 enzymes. * denotes non-specific contaminants of the MDC1^K1840R^ fragment.
Supplemental Figure 4

A

B

C

D

E

F
Supplemental Figure 4. Related to Figure 2.

A conserved coiled-coil region of SENP2 contributes to MDC1 regulation

A. Prediction of SENP2 coiled coil domain using NPS® coiled coil prediction (Combet et al. 2000) using the MTIDK scoring matrix with no weight for the 2.5 patterns a and d. Data shown is the coil-coil probability with a 14 amino acid window. Predicted CCs that map to the catalytic domain were ignored as they identify as known helices. Secondary structure output was from the PredictProtein server (Rost, Yachdav, and Liu 2004). Conservation is shown as a heatmap using the 1-9 scores from Consurf (Ashkenazy et al. 2016) with higher amino acid conservation shown in red. The black region in the N terminus was unscored as too few SENP2 species contain this region.

B. Sequence alignment of SENP2 using ClustalOmega centring on human SENP2 amino acids 178-237.

C. Representative images depicting the localisation of FLAG-SENP2 variants in HeLa<sup>FlpIn</sup>.

D. Quantification of SENP2 localisation in 100 cells related to C.

E. SUMO1 and SUMO2/3 high molecular weight (HMW) conjugates in FLAG-SENP2 expressing HEK293 cells. FLAG-SENP2 was titrated to determine relative effects on SUMO conjugates versus SENP2 expression levels.

F. Quantification of (E). The intensity of the HMW SUMO conjugates was divided by the expression of FLAG-SENP2 to account for differences in expression levels. This was then shown relative to the intensity of SUMO HMW conjugates in mock transfected cells (set at 1.0). Data from three experiments.
Supplemental Figure 5

A

![Graph showing number of MDC1 foci/cell over time post IR (4Gy/hr) for siNTC, siSENP2, siATXN3, and siSENP2 + siATXN3.

B

![Bar graph showing percentage of colony survival for siNTC, siSENP2, siATXN3, and siSENP2 + siATXN3.

C

![Bar graph showing 53BP1 foci/cell for siNTC, siSENP2, siATXN3, and siSENP2 + siATXN3.

D

![Graph showing 53BP1 foci/cell with conditions siNTC, siUBC13, siSENP2, and siBRCC36.

E

![Images showing siRNA: NTC, UBC13, SENP2, BRCC36, and SENP2 + BRCC36 effects on 53BP1.

F

![Bar graphs showing percentage of colony survival for different conditions including siNTC, siSENP2, siATXN3, and siSENP2 + siATXN3.

G

![Graph showing RNA levels of BRCC36 with conditions siNTC, siSENP2, and siBRCC36.

H

![Table showing Input and SIM PD for WT and KO conditions.

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Supplemental Figure 5. Related to Figure 3.

Requirement for SENP2 can be bypassed by increased K63-Ub signalling.

A. Quantification of MDC1 foci co-localising with γH2AX staining in HeLa depleted with indicated siRNA. n=100 cells from three experiments. Graph shows mean number of co-localising foci per cell error bars are s.e.m.

B. IR colony survival of cells treated with indicated siRNA for 72 hours prior to irradiation (2 Gy). n=3

C. Quantification of 53BP1 foci 2 hours after 4 Gy IR in cells treated with specified siRNAs and transfected with GFP-RNF8, n=50.

D. Quantification of 53BP1 foci 2 hours after 4 Gy IR in cells treated with specified siRNAs n=100

E. Representative images related to (D). Scale bar = 10 μm

F. IR colony survival of a cells treated with indicated siRNA for 72 hours prior to irradiation (2 Gy), n=3.

G. RPA foci number in HeLa treated with indicated siRNA for 72 hours prior to irradiation (4 Gy) and 2 hour recovery. Cells were pulsed with 10 μM EdU 30 minutes prior to irradiation. The number of RPA70 foci per EdU positive cell is shown for a minimum of 100 cells across three experiments.

H. SIM (SUMO Interacting Motif) peptide pulldowns of HAP1 cell lysates (WT and SENP2 KO) from cells treated with CPT (1μM / 2hr) or DMSO. Pulldowns were probed with indicated antibodies to detect SIM dependent enrichment as a proxy for SUMOylation / SUMO association. Blots were re-probed with SUMO2/3 to illustrate pulldown of total SUMO. SIMm denotes a mutant peptide with a disrupted SIM to confirm specificity of pulldown.
**Supplemental Figure 6. Related to Figure 6.**

**High levels of SENP2 disrupts DSB repair.**

A. Illustration of Chromosome 3, indicating the location of *USP13, SENP2 and RNF168*. A selection of candidate oncogenic driver genes within the 3q amplicon are also shown.

B. The extent of SENP2 amplification in the top six 3q amplified cancer types, data is adapted from Cbioportal (August 2018).

C. Correlation between SENP2 mRNA and copy number in the LUSC (Lung Squamous Cell Carcinoma) dataset from the TCGA, data is adapted from Cbioportal (August 2018). Pearson correlation co-efficient = 0.85, Spearman co-efficient = 0.86.

D. Kaplan-Meier survival plot of overall survival generated via KM Plotter (August 2018) (Gyorffy et al. 2013). Patients (n=1928) were split at the median SENP2 expression as determined by microarray (Affymetrix probe ID 218122_s_at).

E. Oncoprints adapted from Cbioportal TCGA datasets (August 2018) for USP13, SENP2 and RNF168 genomic amplification (red) in indicated cancer types. Values in parenthesis indicate % of samples with amplification.

F. Western blot of USP13, RNF168 and FLAG-SENP2 expression in inducible HeLa<sup>FlpIn</sup> over-expressing USP13, SENP2 or RNF168 cell lines.

G-H Colony survival after IR (2 Gy) or CPT (1 μM 2 hr) in HeLa<sup>FlpIn</sup> over-expressing USP13, SENP2 or RNF168, n=3.

I. HeLa cells, transfected with expression constructs for SENP1, SENP2, SENP3, SENP5, SENP6 and SENP7 for 48 hr before exposure to 4 Gy IR. Non transfected cells were either allowed to recover for 1 hour, or 2 hours. SENP expressing cells were allowed to recover for 2 hours, n=100, Graph shows mean number of MDC1 foci per cell, error bars are s.e.m.

J. Representative images for I illustrating SENP expression. Scale bar = 5 μm
Supplemental Figure 7

A  Non S phase

Recruit  Amplify  Clear

SENPP2

B  S phase

Untreated  DSB HR

SENPP2
Supplemental Figure 7.

Model of SENP2 action in promoting DSB repair.

A. SENP2 interacts with MDC1 and constitutively cleaves SUMO from it. On damage interaction with SENP2 is lost. MDC1 recruited to chromatin is modified by PIAS4 SUMO E3 ligase and recruits RNF8/RNF168 which lay down K63-Ub marks leading to 53BP1 and BRCA1-A complex recruitment. RNF4 engages with SUMO-MDC1 once sufficient SUMO is conjugated to MDC1, resulting in ubiquitination and VCP engagement.

Without SENP2, MDC1 is recruited to chromatin bearing SUMO moieties and is engaged by RNF4-VCP before sufficient K63-Ub is generated by RNF8/RNF168.

Note although polySUMO of MDC1 is illustrated here, we do not discount that the single SUMO site required encourages multi-mono-SUMOylation that engages RNF4.

B. SENP2 cleaves SUMO from cellular conjugates and processes immature SUMO so that on the induction of DSBs sufficient SUMO is available for incorporation into the multiple interactions required for HR, group modification and or specific SUMO-mediated interactions. Without SENP2 SUMO remains in conjugates and immature SUMO is less efficiently processed so that insufficient SUMO is available for HR.
Supplemental Materials and Methods

Overexpression and purification of MDC1\textsuperscript{WT} and MDC1\textsuperscript{K1840R} (aa 1818-2094) C-terminal domains.

The expression of His-SUMO MDC1\textsuperscript{WT} and His-SUMO-MDC1\textsuperscript{K1840R} in BL21(DE3)/pCA528-MDC1 was induced by the addition of 1 mM Isopropyl-β-d-thiogalactopyranoside (IPTG), and the proteins were produced in LB medium containing 100 μg/ml of kanamycin overnight at 18°C. For purification of the His-SUMO MDC1\textsuperscript{WT} and His-SUMO MDC1\textsuperscript{K1840R} products, the cells were harvested and re-suspended in 20 mM HEPES potassium salt, pH 7.4, 50 mM Imidazole, 500 mM NaCl, 1.0 mM TCEP [tris(2-carboxyethyl)phosphine], complete EDTA-free protease inhibitor cocktail tablet (Roche). Cells were lysed using an Emulsiflex-C3 homogenizer (Avestin) and broken by three passages through the chilled cell. The lysate was centrifuged at 75,000 xg using a JA 25 rotor (Beckman Coulter) and filtered through a 0.45-μm filter. The clarified lysate was applied onto a 5-ml HisTrap HP column (GE Healthcare). The column was washed extensively using the same buffer, and the protein was eluted using buffer containing 500 mM imidazole.

Fractions containing a band of the correct size were concentrated using a Vivaspin 20-ml concentrator (10,000 molecular weight cut-off [MWCO]) (GE Healthcare) and gel purified using an Akta Pure 25 (GE Healthcare LS) with a prepacked Hi-Load 10/300 Superdex 200 PG column.

For removal of the His-SUMO tag, 1 ul of ULP-1 (20mg/ml) was added to 5ml of His-SUMO MDC1\textsuperscript{WT} and His-SUMO-MDC1\textsuperscript{K1840R} and left overnight at 4°C. The samples were concentrated to 500μl using a Vivaspin 4-ml concentrator (10,000 molecular weight cut-off [MWCO]) (GE Healthcare) and gel purified on a Hi-Load 10/300 Superdex 75 PG column in order to separate the untagged proteins from the ULP-1 protease and the cleaved His-SUMO tag.

\textit{In vitro SUMOylation assay}

\textit{In vitro} SUMOylation assay reactions were performed in a total volume of 20 μl with 200 ng recombinant Human SUMO E1 (SAE1/UBA2) (R&D Systems), 100 ng of Ubc9 (Boston Biochem), 1 μg of SUMO2, (Boston Biochem), 1 μg of recombinant untagged-MDC1 (aa1818–2094) or untagged MDC1\textsuperscript{K1840R}. Reaction buffer (50 mM HEPES, 50 mM MgCl\textsubscript{2}, 0.5 mM DTT) was added to a final 1x concentration and supplemented with 4 mM ATP-Mg. Reactions were incubated at 30°C for 1 hr and stopped by addition of 2x Laemmli loading buffer.

\textit{In vitro deSUMOylation assay.}

For de-SUMOylation; the \textit{in vitro} SUMOylation reaction was divided in two and SENP2 catalytic domain (Boston Biochem) was added to a final concentration of 50 nM. Reactions were incubated at 30°C for 0.5 hr and stopped by addition of 2x Laemmli loading buffer.
Cloning

SENP2 was cloned with an N terminal FLAG tag into the KpnI and EcoRV sites in pCDNA5/FRT/TO vector (Invitrogen). Synonymous mutations were made in the SENP2 cDNA to generate siRNA resistance (see table 4). To generate FLAG-RFP-SENP2, SENP2 cDNA (without siRNA resistance) was cloned into pCDNA3.1 mRFP vector using Clal. All site directed mutagenesis was performed using Pfu polymerase (Promega) and mutations were confirmed by Sanger sequencing (Source Biosciences Nottingham). To generate a nuclear pore binding mutant of SENP2 we truncated amino acids 1-65 and mutated the SENP2 NES to prevent nuclear export. The coiled coil deletion mutant was generated using the megaprimer method with primers that flank the deleted region and external primers to generate the megaprimer. The PCR product was then used for site directed mutagenesis. MDC1, the longest isoform of human MDC1 (NM_014641.2) was used to generate synthetic MDC1 cDNA that was extensively codon optimised by GenScript to remove repetitive DNA sequences to enable gene synthesis. The optimised cDNA has an N terminal myc tag, synonymous mutations to enable resistance to two siRNA targeting Exon 11 and multiple silent mutations that disrupt restriction enzyme recognition sites. The myc-MDC1 cDNA was cloned into AfII and BamHI sites in pCDNA5/FRT/TO. The K1840R mutation was made by GenScript. To generate the MDC1 fragments for in vitro SUMOylation / deSUMOylation, WT and K1840R MDC1 were and cloned into pCA528 containing a His-SUMO N terminal tag using Bsal and BamHI sites. RNF4, human RNF4 (NM_002938.4) cDNA was synthesised by GenScript to contain resistance to two siRNA sequences, an N terminal HA tag, and cloned into pCDNA5/FRT/TO HindIII and BamHI sites. Site directed mutagenesis was used to generate the RNF4 mutants. The SIM mutant of RNF4 was generated by SDM of SIM2 and SIM3 followed by the megaprimer method using a forward primer that contained mutations in SIM1 and a reverse primer that contained mutations in SIM4. RNF168 was cloned from pEGFP-RNF168 (a kind gift of Grant Stewart, University of Birmingham). The two BamHI sites were silenced with synonymous mutations by site directed mutagenesis, and the resulting cDNA was sub-cloned into pCDNA5/FRT/TO using BamHI Xhol sites.

SUMO1 and SUMO2 (NM_003352.4, NM_006937.3) cDNA (both in their processed forms) were cloned into pCDNA5/FRT/TO with an N terminal 6x Histidine - myc tag. GA mutations that prevent SUMO conjugation were generated by incorporating mismatches in the cloning primers. USP13 (NM_003940) was synthesised by GenScript to incorporate an N terminal HA tag, two sites of siRNA resistance and loss of BamHI and BgIII sites by synonymous mutations. The cDNA was cloned into BamHI Xhol sites. The following plasmids were from Addgene FLAG-SENP1 (#17357, Edward Yeh (Cheng et al. 2007)) GFP-SENP3, GFP-SENP5 (#34554, #34555 Mary Dasso, (Yun et al. 2008)) and FLAG-SENP6 (#18065, Edward Yeh, (Dou et al. 2010)).

His-SUMO Pulldown

HEK293Fip20 6xHis-myc-SUMO1 or SUMO2 were seeded on 10 cm plates in the presence of doxycycline (1 µg/mL) for 24 hr prior to knockdown with indicated siRNA for a further 48 hr. Cells were treated with 10 Gy IR and pelleted 1 hr later in cold PBS. Cell pellets were lysed in 8 M Urea buffer (8 M urea, 0.1 M Na2HPO4/NaH2PO4, 0.01 M Tris-HCl, pH 6.3, 10 mM β-mercaptoethanol, 5 mM imidazole plus 0.2% Triton-X-100) with vigorous pipetting. Lysates were left on ice for 30 minutes prior to sonication and clarification at 12,000 rpm for 10 minutes. Cleared lysates (0.9 mL) were incubated with Nickel-agarose (HIS-Select, Sigma) (30 µL packed bead volume) at 4°C with rotation for 16 hr. Beads were washed 3x with 8 M Urea buffer before elution with 4X Lamelli buffer.
**SIM peptide pulldown**

Streptavidin dynabeads (Invitrogen) were incubated with biotin conjugated SETDB1 peptides (GenScript, SETDB1\textsuperscript{SIM\textsc{WT}} Biotin-RDSSSEDESSRPTEEIIPDEDDD or SETDB1\textsuperscript{SIM\textsc{Mut}} Biotin-RDSSSEDESSRPTEAAEAPDEDDD (100 ng per µl of bead slurry) for 1 hr in PBS supplemented with BSA. HAP1 (1x15 cm plate/condition) were treated with CPT (1 µM 2 hr) or DMSO prior to lysis (150 mM NaCl, 10mM Tris pH 7.5, 1.5mM MgCl\textsubscript{2}, 10% Glycerol, 0.2 mM EDTA, 0.1% Triton, Protease and Phosphatase inhibitor cocktails, 50 mM NEM, 50 mM IAA, 5 mM 1-10 Phenanthroline, 1U/mL DNaseI and 1 µg/mL RNaseA). Lysates were sonicated and clarified prior to overnight incubation with peptide conjugated beads at 4°C. Beads were separated on a magnetic rack and washed 3x with PBS-T prior to elution in 4X Lamelli buffer.

**Metaphases**

HeLa\textsuperscript{FlpIn} or HeLa\textsuperscript{FlpIn} SENP2\textsc{WT} cells were plated on 60 mm plates in the presence of doxycycline for 48 hr prior to irradiation at 2 Gy. 18 hr later cells were incubated with Colcemid (0.05 µg/ml) 6 hr. Cells were then trypsinized and centrifuged at 1200 rpm for 5 minutes. Supernatant was discarded and cells re-suspended. 5 ml of ice-cold 0.56% KCl solution was then added and incubated at room temperature for 15 min before centrifuging at 1200 rpm for 5 min. Supernatant was discarded and cell pellet broken before fixation. Cells were then fixed in 5 ml of ice-cold methanol: glacial acetic acid (3:1). Fixation agents were removed and 10 µl of cells suspension was dropped onto alcohol cleaned slide. Slides were allowed to dry at least 24 hr and then stained with Giemsa solution (Sigma) diluted 1:20 for 20 min. Slide mounting was performed with Eukitt (Sigma).

**Cell cycle synchronisation.**

Cells were synchronized at various stages as described previously (Somyajit et al. 2015). HAP1 WT and SENP2 KO cells were arrested at G0/G1 phase by serum starvation for 24 hrs. For S-phase, cells were kept in thymidine (2 mM) supplemented media for 14 hrs. Cells were washed twice with serum free media and kept in 10 % serum supplemented media for 11 hours. Later cells were treated with aphidicolin (1 µg/ml) for 12 hrs. For synchronization at M-phase, cells were cultured in media supplemented with nocadazole (150 ng/ml) for 14 hrs. After treatment, detached cells were collected, washed and re-plated for 1 hr. Finally cells were lysed in 8M Urea lysis buffer (8M Urea, 0.1M Na\textsubscript{3}PO\textsubscript{4}/NaH\textsubscript{2}PO\textsubscript{4}, 0.01M Tris-HCl, pH8, 10 mM β-mercaptoethanol) supplemented with 50 mM NEM and 50 mM IAA.
### Supplemental table 1. Antibodies

| Target | Host / clonality | Vendor | Use | Catalogue number | RRID |
|--------|------------------|--------|-----|------------------|------|
| 53BP1  | Goat polyclonal  | R & D Systems | 1:2000 (IF) | AF1877 | AB_2206635 |
| 53BP1  | Rabbit polyclonal | DAKO   | 1:2000 (IF) | ab36823 | AB_722497 |
| BLM    | Goat polyclonal  | Abcam  | 1:1000 (WB) | ab5446 | AB_304894 |
| ATXN3  | Rabbit polyclonal | Abcam  | 1:1000 (WB) | ab96316 | AB_10680570 |
| β-actin| Rabbit polyclonal | Abcam  | 1:2000 (WB) | ab8227 | AB_2305186 |
| BRCA1 (MS110) | Mouse monoclonal | Calbiochem | 1:500 (WB) | OP92 | AB_564282 |
| BRCA1 (D9)  | Mouse monoclonal | Santa Cruz | 1:500 (IF) | sc6954 | AB_626761 |
| Cyclin A1 | Mouse monoclonal | Abcam  | 1:1000 (WB) | ab38 | AB_304084 |
| EXO1   | Rabbit polyclonal | Bethyl | 1:1000 (WB) | A302-640 | AB_10567122 |
| FLAG M2 | Mouse monoclonal | Sigma  | 1:2000 (WB/IF) | F1804 | AB_262044 |
| GAPDH  | Mouse monoclonal | Abcam  | 1:2000 (WB) | ab8245 | AB_2107448 |
| H-sis  | Mouse monoclonal | Sigma  | 1:2000 (WB) | H1029 | AB_260015 |
| H2AX-pSer139 | Mouse monoclonal | Abcam  | 1:2000 (IF) | ab2893 | AB_303388 |
| H2AX-pSer139 | Rabbit polyclonal | Abcam  | 1:2000 (IF) | ab22551 | AB_447150 |
| KAP1   | Goat polyclonal  | Abcam  | 1:1000 (WB) | ab3831 | AB_304099 |
| Lamin B1 | Rabbit polyclonal | Abcam  | 1:1000 (WB) | ab16048 | AB_443298 |
| MDC1   | Rabbit polyclonal | Abcam  | 1:1000 (WB) | ab11169 | AB_297807 |
| MDC1   | Rabbit polyclonal | Bethyl | 1:1000 (WB) | PLA-0016 | AB_203282 |
| MDC1   | Mouse monoclonal | Abcam  | 1:1000 (WB) | ab50003 | AB_881103 |
| MYC    | Mouse monoclonal | Abcam  | 1:2000 (WB) | ab32 | AB_303599 |
| NUP107 | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab178399 | AB_2620147 |
| NUP153 | Mouse monoclonal | Abcam  | 1:1000 (WB) | ab24700 | AB_2154467 |
| PIA51  | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab109388 | AB_10867435 |
| PIA54  | Mouse monoclonal | Abcam  | 1:500 (WB) | ab211625 | AB_350399 |
| RAD51  | Rabbit polyclonal | Santa Cruz | 1:200 (IF) | sc8349 | AB_2253533 |
| RFP    | Rabbit polyclonal | Abcam  | 1:1000 (WB) | ab62341 | AB_945213 |
| RNF168 | Rabbit polyclonal | Calbiochem | 1:1000 (WB) | AB18587 | AB_11205761 |
| RNF4   | Goat polyclonal  | R & D Systems | 1:1000 (WB) | AF7964-100 | AB_2620147 |
| RPA70  | Mouse monoclonal | Calbiochem | 1:100 (IF) | NA18 | AB_213121 |
| RPA70  | Mouse monoclonal | Abcam  | 1:1000 (WB) | ab176467 | AB_2620147 |
| SENP1  | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab108981 | AB_10862449 |
| SENP2  | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab124724 | AB_10972485 |
| SMARCAD1 | Rabbit polyclonal | Bethyl | 1:1000 (WB) | A301-593A | AB_1078386 |
| SUMO1  | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab32058 | AB_778173 |
| SUMO1  | Rabbit polyclonal | Santa Cruz | 1:200 (IF) | FL-101 | AB_661458 |
| SUMO2/3 | Mouse monoclonal | Abcam  | 1:2000 (WB) | ab32058 | AB_1658424 |
| SUMO2/3 | Rabbit polyclonal | Santa Cruz | 1:200 (IF) | FL-103 | AB_2286984 |
| Ub K63 Apu3 | Rabbit monoclonal | Calbiochem | 1:200 (IF) | 05-1308 | AB_1587580 |
| USP13  | Rabbit polyclonal | Sigma  | 1:1000 (WB) | HAP004827 | AB_1080497 |
| Vinculin| Rabbit monoclonal | Abcam  | 1:2000 (WB) | ab129002 | AB_11144129 |
| WRN    | Rabbit monoclonal | Abcam  | 1:1000 (WB) | ab124673 | AB_10972871 |

- Goat α Mouse AF 488  | Goat polyclonal | Life Tech | 1:2500 (IF) | A11001 | AB_2534069 |
- Goat α Rabbit AF 488  | Goat polyclonal | Life Tech | 1:2500 (IF) | A11008 | AB_143165 |
- Goat α Mouse AF 555  | Goat polyclonal | Life Tech | 1:2500 (IF) | A21422 | AB_141822 |
- Goat α Rabbit AF 555  | Goat polyclonal | Life Tech | 1:2500 (IF) | A21428 | AB_141784 |
- Donkey α Mouse AF488 | Donkey polyclonal | Life Tech | 1:2500 (IF) | A21202 | AB_141607 |
- Donkey α Rabbit AF555 | Donkey polyclonal | Life Tech | 1:2500 (IF) | A31572 | AB_162543 |
- Donkey α Goat AF488  | Donkey polyclonal | Life Tech | 1:2500 (IF) | A11055 | AB_2534102 |
- Donkey α Rabbit AF488 | Donkey polyclonal | Life Tech | 1:2500 (IF) | A2106 | AB_141708 |
- Rabbit α Mouse HRP   | Rabbit polyclonal | DAKO  | 1:5000 (WB) | P0161 | AB_2687969 |
- Swine α Rabbit HRP   | Pig polyclonal  | DAKO  | 1:5000 (WB) | P0217 | AB_278719 |
- Rabbit α Goat HRP    | Rabbit polyclonal | DAKO  | 1:5000 (WB) | P0449 | AB_2617143 |
- Goat α Rabbit LC HRP | Goat polyclonal  | Millipore | 1:5000 (WB) | AP2009 | AB_2617143 |
| Cell Line               | Growth Media        | Originator     | Notes                  |
|------------------------|---------------------|----------------|------------------------|
| HEK293 TRex-FlipIn     | DMEM + 10% FBS      | Invitrogen     | CVCL_U427              |
| HeLa-TRex-FlipIn       | DMEM + 10% FBS      | Grant Stewart  |                        |
| U2OS HR Reporter (DR3)| DMEM + 10% FBS      | Jeremy Stark   |                        |
| U2OS NHEJ Reporter (EN5)| DMEM + 10% FBS  | Jeremy Stark   |                        |
| HAP1 Parental          | IMEM + 10% FBS      | Horizon        | CVCL_Y019              |
| HAP1 SENP2 KO          | IMEM + 10% FBS      | Horizon        | CVCL_TK57              |
| HeLa-TRex-FlipIn FLAG  | DMEM + 10% FBS*     | This paper     |                        |
| SENP2 WT               |                     |                |                        |
| HeLa-TRex-FlipIn FLAG  | DMEM + 10% FBS*     | This paper     |                        |
| SENP2 C546A            |                     |                |                        |
| HeLa-TRex-FlipIn FLAG  | DMEM + 10% FBS*     | This paper     |                        |
| SENP2 NpM              |                     |                |                        |
| HeLa-TRex-FlipIn FLAG  | DMEM + 10% FBS*     | This paper     |                        |
| SENP2 ΔCC              |                     |                |                        |
| HEK293-TRex-FlipIn     | DMEM + 10% FBS*     | This paper     |                        |
| FLAG SENP2 WT          |                     |                |                        |
| HAP1-SENP2 KO (128bp   | DMEM + 10% FBS      | Horizon        | CVCL_TK57              |
| deletion)              |                     |                |                        |
| HeLa-TRex-FlipIn       | DMEM + 10% FBS*     | This paper     |                        |
| FLAG SENP2 ΔCC         |                     |                |                        |
| HeLa-TRex-FlipIn       | DMEM + 10% FBS*     | This paper     |                        |
| FLAG SENP2 WT          |                     |                |                        |
| HeLa-TRex-FlipIn MYC   | DMEM + 10% FBS*     | This paper     |                        |
| MDC1 K1840R            |                     |                |                        |
| HeLa-TRex-FlipIn       | DMEM + 10% FBS*     | This paper     |                        |
| MYC MDC1 WT            |                     |                |                        |
| HeLa-TRex-FlipIn 6x-HIS| DMEM + 10% FBS*     | This paper     |                        |
| MYC SUMO1 WT           |                     |                |                        |
| HeLa-TRex-FlipIn 6x-HIS| DMEM + 10% FBS*     | This paper     |                        |
| MYC SUMO1 GA           |                     |                |                        |
| HeLa-TRex-FlipIn 6x-HIS| DMEM + 10% FBS*     | This paper     |                        |
| MYC SUMO2 WT           |                     |                |                        |
| HeLa-TRex-FlipIn 6x-HIS| DMEM + 10% FBS*     | This paper     |                        |
| MYC SUMO2 GA           |                     |                |                        |
| HeLa-TRex-FlipIn 6x-HIS| DMEM + 10% FBS*     | This paper     |                        |
| MYC-RNF168 WT          |                     |                |                        |
| HeLa-TRex-FlipIn HA-RNF| DMEM + 10% FBS*     | This paper     |                        |
| 4 Y189H                |                     |                |                        |
| HeLa-TRex-FlipIn HA-RNF| DMEM + 10% FBS*     | This paper     |                        |
| 4 I188A                |                     |                |                        |
| HeLa-TRex-FlipIn HA-RNF| DMEM + 10% FBS*     | This paper     |                        |
| 4 ASIM                 |                     |                |                        |
| HeLa-TRex-FlipIn HA-USP| DMEM + 10% FBS*     | This paper     |                        |
| 13 WT                  |                     |                |                        |
| HeLa-TRex-FlipIn MYC-RNF| DMEM + 10% FBS*   | This paper     |                        |
| 168 WT                 |                     |                |                        |

* Supplemented with 100 μg/mL Hygromycin B (Invitrogen).
1) SENP2 cDNA is resistant to siRNA siSENP2 Exon13
2) MDC1 cDNA is resistant to siRNA siMDC1 Exon11A and Exon11B
3) RNF4 cDNA is resistant to siRNA siExon4 and Exon11
4) USP13 cDNA is resistant to siRNA siExon5 and Exon6
| siRNA       | Sequence                                      |
|------------|-----------------------------------------------|
| 53BP1 Ex13 | GGACCUUGGACAAACCUAA[dT][dT]                   |
|            | [Phos]UUUACCUUGGAACCA[dT][dT]                |
| ATXN3 Ex11 | GCAGGCUAUCACGUAGU[dT][dT]                    |
|            | [Phos]UCAGUUGGUACCAUGCG[dT][dT]              |
| ATXN3 UTR  | CGUCGGUGUAGCAGCAAUUU[dT][dT]                 |
|            | [Phos]UAUUACGUUCACACACAG[dT][dT]             |
| BRCC36     | SMARTPool L-005798-00-0005                   |
| CtBP       | GGACCUUGGACAAACCUAA[dT][dT]                   |
|            | [Phos]UUUACCUUGGAACCA[dT][dT]                |
| Luciferase (siNTC) | CUUAACGUAGUACUCGA[dT][dT]           |
|            | [Phos]UCAGUUGGUACCAUGCG[dT][dT]              |
| MDC1 Exon11A | ACAGUUGCUCCACACACG[dT][dT]          |
|            | [Phos]GGGCCUGUGGGAACACUG[dT][dT]             |
| MDC1 Exon11B | GUCUCCGAGAAGACAGUG[dT][dT]               |
|            | [Phos]CAUGUCUCUGGGGAGAC[dT][dT]              |
| NUP107 Ex24 | GUAUUGACGUUGGUAUUU[dT][dT]                   |
|            | [Phos]AAUACCAAGCAGCAAACAC[dT][dT]            |
| NUP153 Ex19 | CCCAGUUCUAAUCAUUG[dT][dT]                   |
|            | [Phos]AUUGGAGUAAAGACUG[dT][dT]               |
| PIAS1 UTR  | CCGAAUACGUACGAA[dT][dT]                     |
|            | [Phos]UUUCUCACACAGAUCC[dT][dT]               |
| PIAS4      | SMARTPool L-006445-00-0005                   |
| RNF168 UTR | CAAGUGCCGUGCGUAA[dT][dT]                    |
|            | [Phos]UUUACGCGCUCUACUUG[dT][dT]             |
| RNF4 Ex11  | GAUUGGACGUCAUCGUU[dT][dT]                    |
|            | [Phos]AACGAGAAGACGUCAU[dT][dT]               |
| RNF4 Ex9   | GACAGAGACTGAUAUGAG[dT][dT]                   |
|            | [Phos]UACCAUAAGACGUCAU[dT][dT]               |
| SENP1 Ex6  | CCGAAAGACCAUUGGAA[dT][dT]                    |
|            | [Phos]AAUCACUAUGGCUUUC[dT][dT]               |
| SENP2 Ex13 | GAGGAGAUAACACAUU[dT][dT]                    |
|            | [Phos]AAUGUCUAAACAGCUUCC[dT][dT]             |
| SENP2 Ex4  | ACAACACUCGUACGUGUU[dT][dT]                   |
|            | [Phos]CAAACCUUCGUACGUA[dT][dT]               |
| SENP3      | SMARTPool L-006034-00-0005                   |
| SENP5 Ex2  | CCUACAGACACGUUACUG[dT][dT]                   |
|            | [Phos]UAGAAGCUUGGCUUAAUGG[dT][dT]           |
| SENP6 Ex9  | CAGAGAUAAACACAGAA[dT][dT]                    |
|            | [Phos]UCUCAGCCGUGAGUAA[dT][dT]               |
| SENP7 Ex14 | GAAUGAAGACGAAAGAUA[dT][dT]                   |
|            | [Phos]AAUAUCGUUCAGCAUAA[dT][dT]              |
| SUMO2      | SMARTPool L-016450-00-0005                   |
| SUMO3      | SMARTPool L-019730-00-0005                   |
| UBE2N      | SMARTPool L-003920-00-0005                   |
| USP13 Ex5  | CGAUUUAAUGCCAGAUAU[dT][dT]                   |
|            | [Phos]UAUACGCGUACUUAUUAAUGG[dT][dT]          |
| USP13 Ex6  | GCCGAGUACAAAUAUGCCAA[dT][dT]                 |
|            | [Phos]UUGGCAUUAUAAGAUGCG[dT][dT]             |
### Supplemental table 4. DNA Oligonucleotides

| pCDNA5/FRT/TO 6x-His SUMO | 6xHis myc SUMO1 Fwd | AAAAAGCTTATGCATCATCATCATCATCATGAACAAAAACTCATCTCAGAAGAGGATCTGTCTGACCAGGAGG |
|---------------------------|---------------------|--------------------------------------------------------------------------|
|                           | 6xHis myc SUMO1 WT Rev | AAAGGATCTAAGCGCCGGCTTGGTTC |
|                           | 6xHis myc SUMO1 G97/98A Rev | AAAGGATCTAAGCGCCGGCTTGGTTC |
|                           | 6xHis myc SUMO2 Fwd | AAAAAGCTTATGCATCATCATCATCATCATGAACAAAAACTCATCTCAGAAGAGGATCTGTCTGACCAGGAGG |
|                           | 6xHis myc SUMO2 WT Rev | AAAAAGCTTATGCATCATCATCATCATCATGAACAAAAACTCATCTCAGAAGAGGATCTGTCTGACCAGGAGG |
|                           | 6xHis myc SUMO2 G92/93A Rev | AAAAAGCTTATGCATCATCATCATCATCATGAACAAAAACTCATCTCAGAAGAGGATCTGTCTGACCAGGAGG |

#### HA-RNF4 mutagenesis

- **RNF4 I188A Fwd**: CAAACGGTACCACCCCGCTTATATAACGCGTAC<br>- **RNF4 I188A Rev**: GTACGCCGTATTATAAGGGGCGATGCCGTTTG<br>- **RNF4 Rev**: CATATATAATGGG<br>- **RNF4 SIM1 Fwd**: CTTTGGAAGCAGACCCGCAGAAGCCGCGGAAACTGCTGGAGATG<br>- **RNF4 SIM2 Fwd**: GAAACTGCTGGAGATGAAGCTGCGGACGCCACTTGTGAATCTTTAGAG<br>- **RNF4 SIM2 Rev**: CTCTAAAGATTCACAAGTGGCGTCCGCAGCTTCATCTCCAGCAGTTTC<br>- **RNF4 SIM3 Fwd**: RNF4 SIM3 Rev: AAAGGATCCCTAAGCCGCCGTTTGTTCC<br>- **RNF4 SIM4 Rev**: AAAGGATCCCTAAGCCGCCGTTTGTTCC<br>- **RNF4 Y189H Fwd**: GGTACCACCCCATTCATATAACGCGTACG<br>- **RNF4 Y189H Rev**: CGTACGCCGTATTATAATGGGCGATGCCGTTTG<br>- **SENP2 FLAG Fwd**: GATCGCTAGCGGTACCATGGACTACAAGGACGACGATGACAAGATCGATGCTGCCAGCTTATTTGG<br>- **SENP2 FLAG Rev**: GATCGATATCATCGATTGACAGCAACTGCTGATGAAG<br>- **SENP2 siResistance Fwd**: CGTAAGTGTCGGCCCGAGCCCGCGGGCAGTGGAAG<br>- **SENP2 siResistance Rev**: GTACGCCGTATTATAATGGGCGATGCCGTTTG<br>- **SENP2 C548A F**: GAATGGGAGTGATGCTGGAATGTTTAC<br>- **SENP2 C548A R**: GTAAACATTCCAGCATCACTCCCATTC<br>- **SENP2 D65 Fwd**: GATCGGTACCATGGACTACAAGGACGACGATGACAAGATCGATGCTGCCAGCTTATTTGG<br>- **SENP2 NESm Fwd**: GGAAGTGTCGGCCCGAGCCCGCGGGCAGTGGAAG<br>- **SENP2 NESm Rev**: CTTCACGCCCGGCGGCGCTGCGGGCCGACCTTC<br>- **SENP2 CC Fwd**: GAGGCGTCCCCATTGTGGAAACTCTGTCTGTCC<br>- **SENP2 CC Rev**: GGACAGACAGATTTCCACACATGGGACGCGCTC<br>- **RNF168 BamH1 MutF**: CCGTGGAAGCAGCGACTACAAGGACGACGATGACAAGATCGATGCTGCCAGCTTATTTGG<br>- **RNF168 BamH1 MutR**: GTAAAGTGACTACATCGGCTGCGGGGAGCCTTATTTGGCG<br>- **RNF168 myc F BamH1**: AAAAGGATCATGGGAAACAAAAACTCATCTCAGAAGAGGATCTGCGGTCTCCTACCCAAAGACGCGCATCC<br>- **RNF168 R Xhol**: AAACCTCGAGTTACTGGCATCTCCTGAAAAACTCATCATGAAACAC<br>- **MDC1 BsaI F**: CCATCGGTCTCAGGTGGTAGCTTCCACACACAGGAAG<br>- **MDC1 BamHI R**: GGGGGATGGTCCAGCATCCTCATTTCCAGTGCGG
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