Supplemental material

Figure S1. **Silencing of p400, p21, ATM, and Rad51 in the various conditions and cell lines used.** Silencing of p400, p21, ATM, and Rad51 in U2OS cells (A), U2OS ER-ISceI cells (B), U2OS ER-AsiS-I cells (C), and 293T cells (D). (E) Cell cycle analysis of U2OS 48 h after siRNA treatment. The mean and standard deviation of the mean from three independent experiments are shown. (F) Cell cycle analysis of 293T cells 48 h after siRNA treatment. Data shown are from a single representative experiment (n = 15,000 events) out of three repeats.
Figure S2. Effect of p400 depletion on γH2AX phosphorylation and 53BP1 foci formation. (A) The Fig. 1 C graph is shown. The inset shows a more precise quantification of γH2AX staining 1 h after irradiation. Cells were separated in five groups according to the percent of the nucleus area stained (0–1 to 75–100%). The mean ± SD from five independent experiments is shown. (B) p400+/+ and p400ΔN/ΔN MEFs were irradiated (8 Gy) and subjected to γH2AX staining after the indicated time. γH2AX-positive cells were then counted. The mean ± SD from three independent experiments is shown. (C) U2OS cells were transfected with the indicated siRNA, irradiated or not 48 h later, and subjected to 53BP1 immunofluorescence after the indicated time. Cells with more than five foci were considered as positive. Representative images and quantification (at least 100 cells per point) are shown. Bar, 10 µm. (D) Expression of p400 and p400dead in 293T cells after transfection with corresponding plasmids (1 µg). p400 expression was quantified by RT-qPCR (relative to P0 mRNA levels) and by Western blot. (E) 293T cells transfected as in D were irradiated (2 Gy) and analyzed for 53BP1 foci (E) or ubiquitin foci (F; using the FK2 antibody) presence after the indicated time. To identify p400 and p400dead overexpressing cells, 100 ng of Histone H3–GFP plasmid was included in the transfection. Pilot experiments using cherry-tagged p400 showed that in these conditions, 100% of GFP-positive cells express exogenous p400. At least 50 cells overexpressing p400 or p400dead (GFP-positive cells) were scored in each sample, as well as three GFP-negative cells immediately adjacent to any transfected cells. Representative images are shown in G. Bar, 10 µm. [H3-GFP plasmid was provided by P. Cooke, University of Oxford, Oxford, UK].
Figure S3. DNA repair activity of DSBs. U2OS cells transfected with control or p400 siRNA were irradiated (20 Gy) or not 48 h later and subjected to a comet assay under neutral conditions after the indicated time. Quantification of the tail moment was performed for each condition and time on 100 cells. (A) Representative images obtained with U2OS cells. Bar, 5 µm. Quantification is shown in Fig. 1 G. (B) p400 wild-type and p400ΔN/ΔN MEFs were irradiated (20 Gy) or not 48 h later and subjected to a comet assay under neutral conditions after the indicated time. Bar, 5 µm. Quantification of the tail moment was performed for each condition and time on 100 cells. Representative images (top) and quantification of a representative experiment with SEM (bottom) are shown.
Figure S4. Characterization of cell lines expressing AsiSI or I-SceI used in this study. (A) Description of the assay for HDR efficiency. Schematic representation of the intracellular substrate stably integrated in the genome of the U2OS cell line used to measure the HR events (U2OS-ER-I-SceI). The substrate is composed of two inactive copies of the GFP gene. The first copy is truncated at the 5′ end, the second is full length with an interruption by an 18-bp sequence recognized by the rare-cutting restriction endonuclease I-SceI. Repair of an I-SceI–induced DSB can generate functional GFP by intrachromatid or interchromatid gene conversion. The amount of cells expressing GFP thus measures HDR and reflects HR efficiency. (B) U2OS-ER-I-SceI cells were treated with OHTam (2 h), and then subjected to immunofluorescence using anti-HA antibody. Note the nuclear relocalization of HA-tagged ER-I-SceI upon OHTam addition. (C) Cleavage efficiency of the AsiSI site analyzed in ChIP experiments. U2OS-ER-AsiSI cells were transfected by the indicated siRNA. 48 h later, cells were treated or not with OHTam. Genomic DNA was prepared 4 h later and subjected to a cleavage efficiency assay as described previously (Iacovoni et al., 2010). The mean and standard deviation from three PCR replicates are shown. Note that cleavage efficiency is higher in cells transfected by the p400 siRNA. [D] U2OS-ER-AsiSI cells were treated with OHTam (4 h). Cells were then subjected to immunofluorescence using anti-γH2AX antibody to reveal the presence of DSB. Note the absence of DSB in untreated cells and the presence of a large number of DSB in OHT-treated cells. Bar, 10 μm. (E) Additional experiment showing the complementation of p400 depletion on HDR of DSB. Experimental conditions are similar to Fig. 9 B. The data shown are from a single experiment (n = 15,000 events) out of three repeats. (F) Cleavage efficiency of the I-SceI site analyzed in HDR experiments. RG37 cells were transfected by the indicated siRNA. 24 h later, cells were transfected or not with I-SceI. Genomic DNA was prepared 24 h later and subjected to a cleavage efficiency assay as described previously (Iacovoni et al., 2010). The mean and standard deviation from three PCR replicates are shown.
Figure S5. **Analysis of p400–Rad51 interaction by FRET/FLIM.** (A) GFP fluorescence lifetime values in RG37 cells transfected with Rad51-GFP plus p400-mCherry (n = 101 from two independent experiments) or Rad51-GFP plus p400dead-mCherry (n = 44 from two independent experiments). (B) Summary of the FRET/FLIM results obtained in RG37 cells. Results are from two independent experiments. (C) Summary of the FRET/FLIM results obtained in U2OS-AsiSI cells without DSB (−OHT) or after DSB induction (+OHT). Results are from two independent experiments. t, mean lifetime (nanosecond); sem, standard error of the mean; N, number of measurements; FRET % = (tD − tDA)/tD, with tD being lifetime of donor alone and tDA being lifetime of the donor in the presence of acceptor; p-value, variance analysis (threshold 5%).

**Reference**