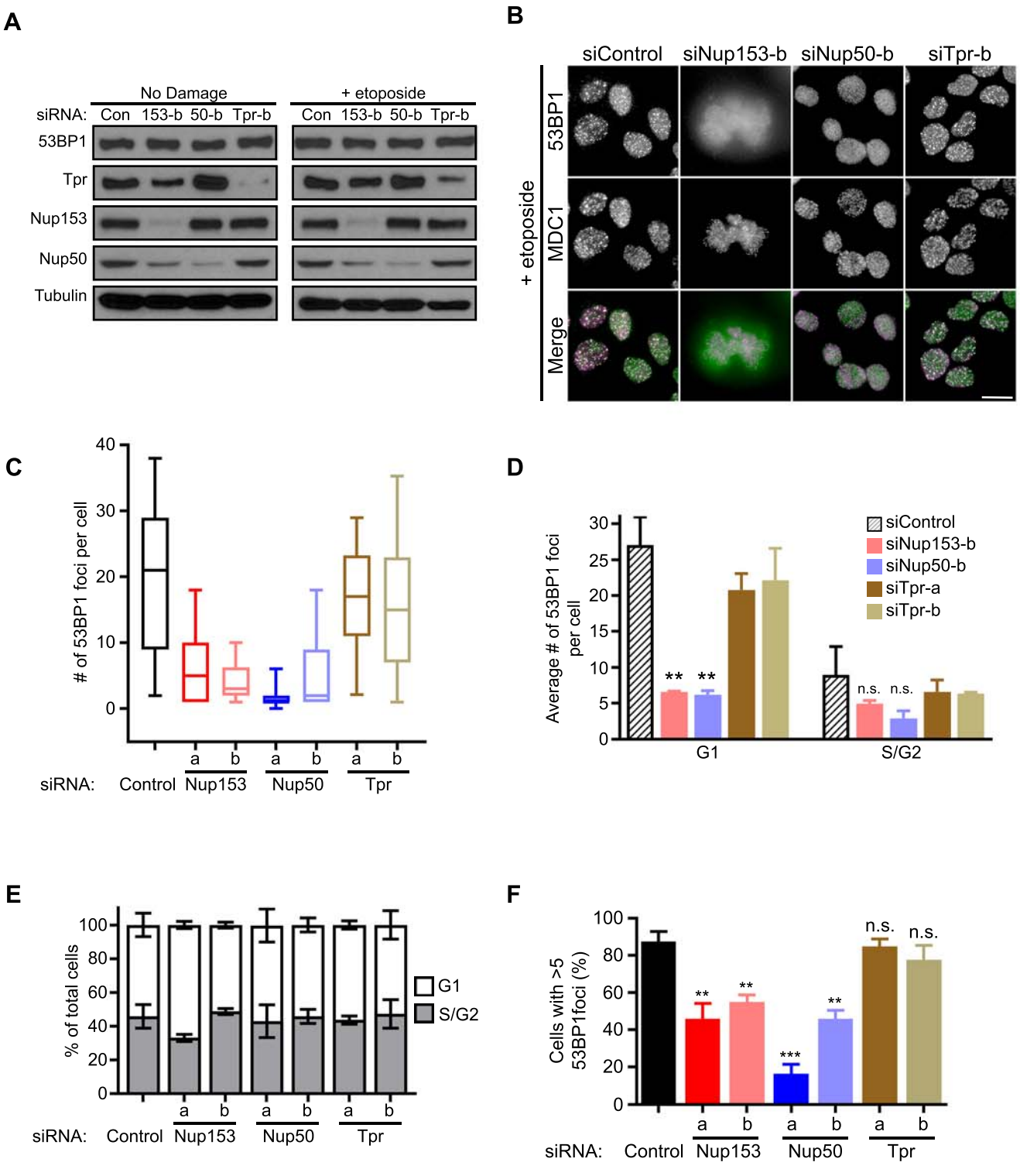
Mackay, et al., Supplemental Figure S1



Supplemental Figure S1. Independent siRNA oligos confirm a requirement for Nup153 and Nup50 in intranuclear targeting of 53P1 following DNA damage. (A) Western blot analysis confirmed knockdown of the indicated proteins in the absence or presence of DNA damage induced by etoposide. Note that total cellular levels of 53BP1 are not affected after depletion of Nup153 or Nup50. (B) U2OS cells were transfected with either control or gene-specific siRNAs, targeting distinct mRNA sequences from those used throughout the manuscript. 48 hours later, cells were treated with 20μM etoposide for 30 minutes, followed by a 90 minute recovery in fresh medium, and analyzed for the formation of nuclear 53BP1 foci (green). DNA damage foci were detected using antibodies for MDC1 (magenta) or γ-H2AX (not shown). Scale bar, 20μm. (C) Quantification of the number of 53BP1 foci per cell upon induction of DNA damage after treatment with the indicated siRNAs. Data shown are the combined results from analysis of >300 nuclei from 3 independent experiments. Boxplots represent the 25th, median, and 75th percentile of values from the indicated treatments. Whiskers represent the 10th and 90th percentiles. (D) Quantification of 53BP1 foci in cyclin A-negative (G1) and cyclin A-positive (S/G2) cells upon induction of DNA damage after treatment with the indicated siRNAs. Error bars represent the mean and standard deviation from 3 independent experiments where >100 cells were scored. Hatched bars for siControl samples indicate data from Fig. 1D, which are included here for comparison. (E) Quantification of cyclin A-negative (G1) and cyclin A-positive (S/G2) cells upon induction of DNA damage after treatment with the indicated siRNAs. Error bars represent the mean and standard deviation from 3 independent experiments where >500 cells per treatment were analyzed. (F) Quantification of the number of cells with >5 53BP1 nuclear foci upon induction of DNA damage after treatment with the indicated siRNAs. Error bars represent the mean and standard deviation from 3 independent experiments where 300-500 cells were scored. $^{**}p<0.002$; $^{***}p<0.0001$; n.s., not significant; compared to control siRNA (Student's t-test).

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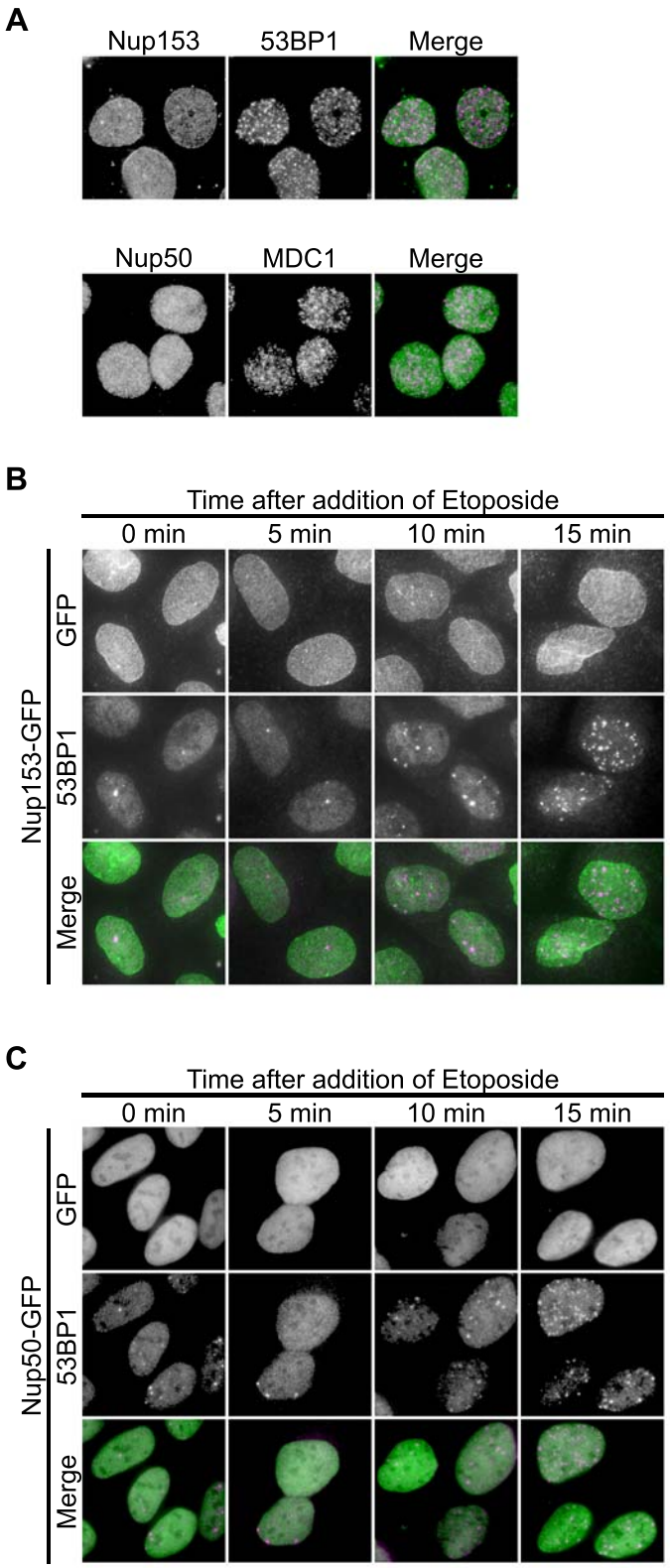
Supplemental Figure S2



Supplemental Figure S2. Nup153 and Nup50 do not target to sites of DNA damage, even at very early stages of the DNA damage response. (A) U2OS cells were treated with 20μM etoposide for 30 minutes and analyzed for either Nup153 (green) and 53BP1 (magenta), or Nup50 (green) and MDC1 (magenta), as indicated. Note that although both Nup153 and Nup50 localize to the nuclear rim and nucleoplasm, they do not concentrate at DNA damage foci. (B and C) U2OS cells stably expressing either Nup153-GFP (B) or Nup50-GFP (C) were treated with etoposide and harvested every 5 minutes for 30 minutes. Recombinant proteins were detected using a GFP-specific antibody (green), while damage foci were detected with 53BP1 (magenta).

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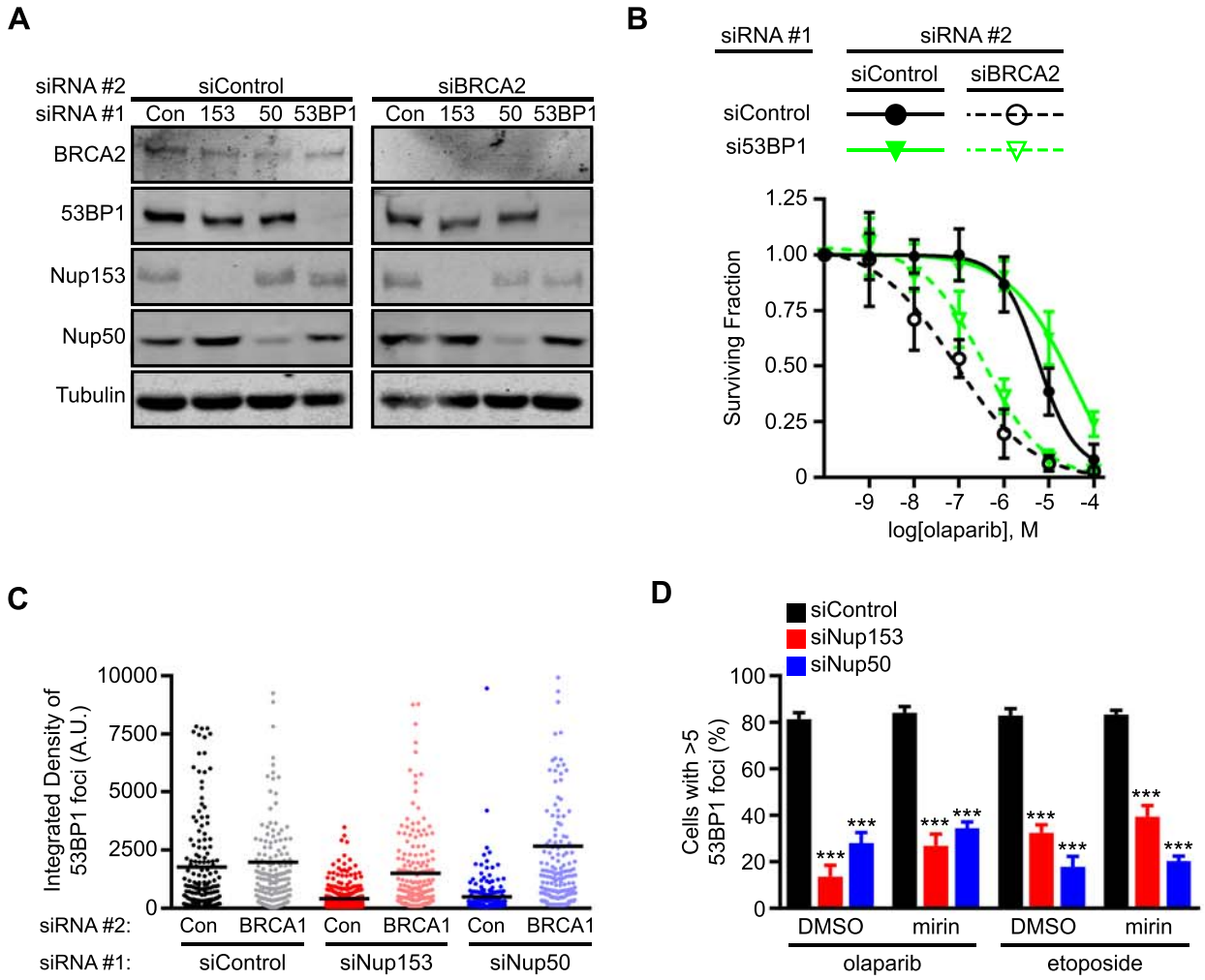
Supplemental Figure S3



Supplemental Figure S3. (A) BRCA2 depletion results in PARPi sensitivity that is not counteracted by low levels of 53BP1. U2OS cells were treated according to the experimental timeline described in Fig. 6A. Following both siRNA treatments, cells were incubated with 100μM olaparib and harvested 24 hours later for western blot analysis. (B) Quantification of cell viability at the indicated olaparib concentrations following codepletion of 53BP1 and BRCA2. Control samples, with and without BRCA2 depletion, are shown for comparison. Error bars represent the mean and standard deviation of 3 independent experiments, and curves were fitted using GraphPad Prism. (C) Quantification of the integrated density (mean intensity x area) of 53BP1 foci after treatment with the indicated siRNAs and incubation with etoposide. Each point represents an individual 53BP1 focus, while the black bars represent the mean integrated density of >100 foci per treatment. (D) U2OS cells were treated as in Fig. 8, with the modification that 16 hours before analysis, cells were incubated with either DMSO or 30μM mirin. Mirin (or DMSO) was also present during incubation with etoposide. Graph represents the quantification of the number of cells with >5 53BP1 nuclear foci upon induction of DNA damage after treatment with the indicated siRNAs. Error bars represent the mean and standard deviation from 3 independent experiments where 300-500 cells were scored. ***$p<0.0001$, compared to control siRNA within each group (Student's t-test). (E) BARD1 is effectively depleted under our experimental conditions. U2OS cells were treated according to the experimental timeline described in Fig. 7A. Following both siRNA treatments, cells were incubated with either DMSO (no damage) or olaparib for 24 hours, harvested and subjected to western blot analysis.