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Supplemental information

Shift in MSL1 alternative polyadenylation in response to DNA damage protects cancer cells from chemotherapeutic agent-induced apoptosis

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A

Gene Ontology, 135 proximal APA shift genes

Biological Process

Gene Ontology Terms	FDR
No statistically significant results	*N/A*

Molecular Function

Gene Ontology Terms	FDR
No statistically significant results	*N/A*

Cellular Compartment

Gene Ontology Terms	FDR
transcription elongation factor complex (GO:0008023)	3.39E-02

Gene Ontology, 124 distal APA shift genes

Biological Process

Gene Ontology Terms	FDR
No statistically significant results	*N/A*

Molecular Function

Gene Ontology Terms	FDR
No statistically significant results	*N/A*

Cellular Compartment

Gene Ontology Terms	FDR
protein-containing complex (GO:0032991)	1.48E-02
nuclear lumen (GO:0031981)	3.11E-02

B

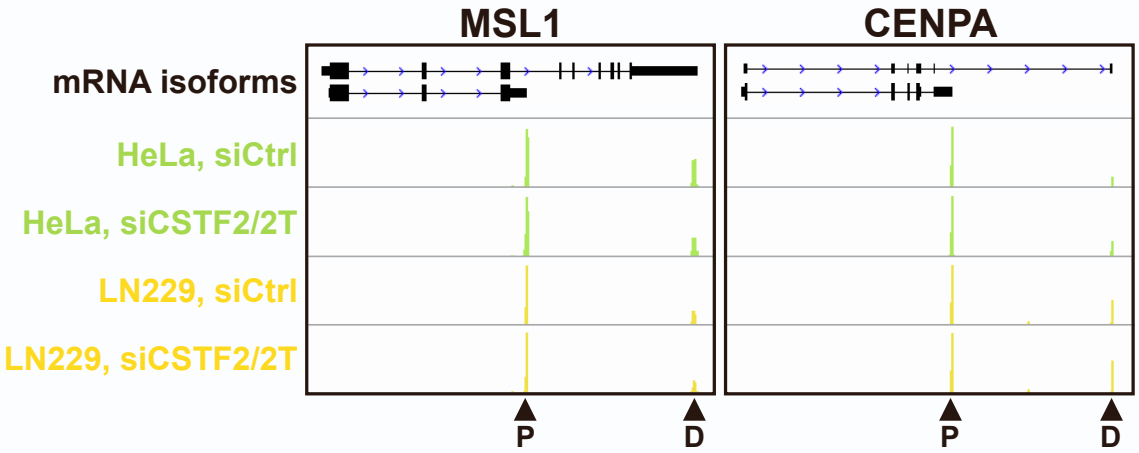

Figure S1. Gene Ontology analysis of genes with DOXO-induced APA shifts, related to Figure 1. (**A**) Tables showing results of GO analysis from the 135 genes with DOXO-induced proximal APA shift (left) and from the 124 genes with DOXO-induced distal APA shift (right). FDR: false-discovery rate. (**B**) Diagrams showing GENCODE annotations and PAPERCLIP results (merged from both replicates) for MSL1 (left) and CENPA (right). Each track of PAPERCLIP results is individually scaled. Arrowheads denote poly(A) sites identified by PAPERCLIP. P: Proximal. D: Distal. Data from (Hwang et al. 2016).

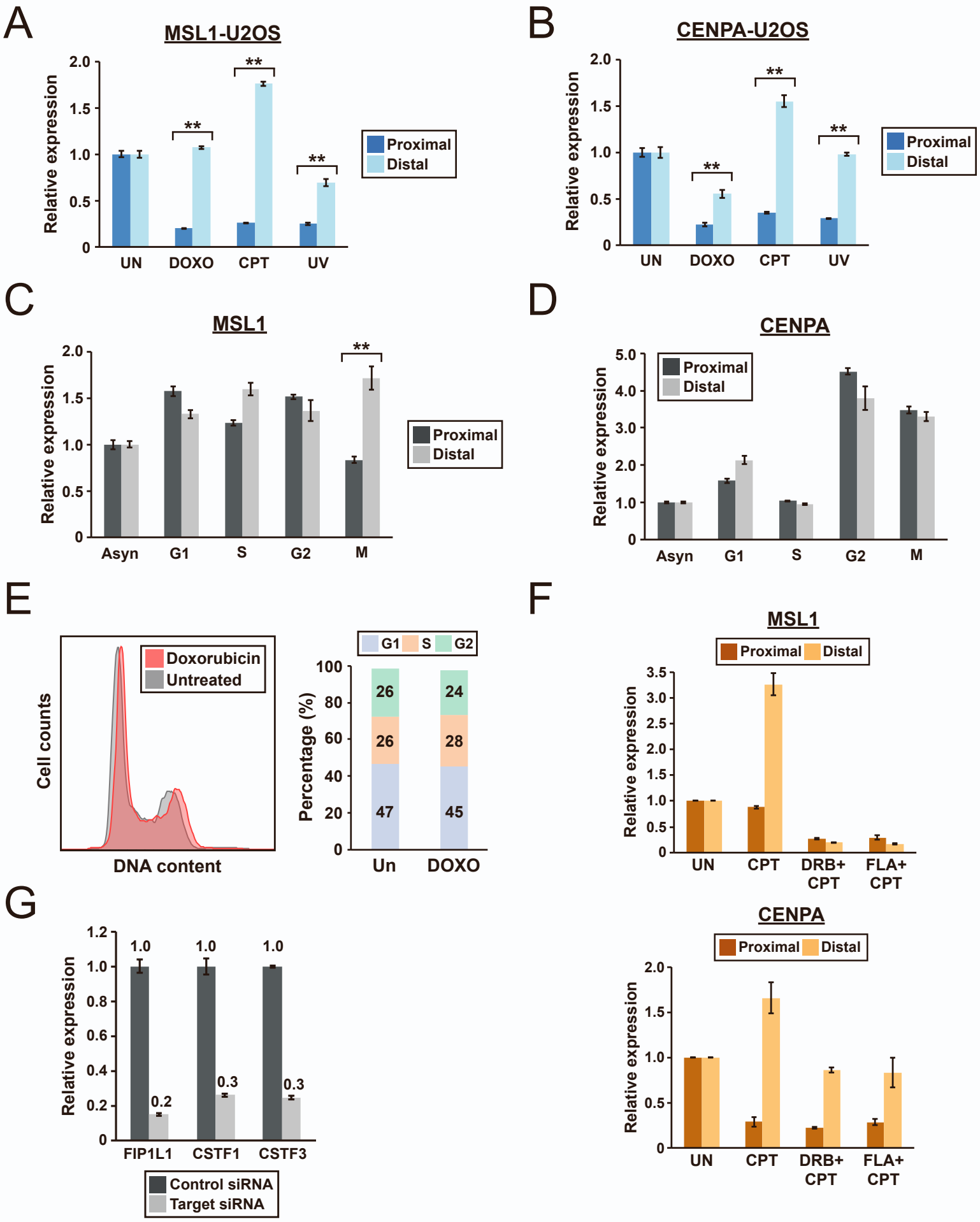

Figure S2. Distal APA shifts of MSL1 and CENPA are observed in U2OS cells with DNA damage but they are not caused by alterations in the cell cycle profile, related to Figure 2.

(**A and B**) Bar graphs showing quantitation of MSL1 and CENPA mRNA isoforms by qRT-PCR in U2OS cells with different treatments. UN: untreated; DOXO: doxorubicin, 1 μg/mL; CPT: camptothecin, 5 μM; UV: ultraviolet light, 30 J/m^2. (**C and D**) Bar graphs showing quantitation of MSL1 and CENPA mRNA isoforms by qRT-PCR in synchronized HeLa cells in different phases of the cell cycle. Asyn: Asynchronous. (**E**) (Left) A histogram showing cell-cycle profiles from HeLa cells with and without 8-hour doxorubicin treatment. (Right) A bar graph showing the percentages of cells in G1/S/G2 phases of the cell cycle from the same experiment. (**F**) Bar graphs showing quantitation of MSL1 (top) and CENPA (bottom) mRNA isoforms by qRT-PCR in HeLa cells with different treatments for 8 hours (n=2). UN: untreated; CPT: camptothecin, 5 μM; DRB+CPT: 100 μM DRB plus 5 μM CPT; FLA+CPT: 1 μM Flavopiridol plus 5 μM CPT. (**G**) A bar graph showing quantitation of FIP1L1, CSTF1 and CSTF3 mRNA expression by qRT-PCR in HeLa cells transfected with a control siRNA or the corresponding target siRNAs. In all panels, error bars indicate SEM. Statistical significance of the distal-to-proximal isoform ratio is determined by two-tailed t-test. **: $p<0.01$.

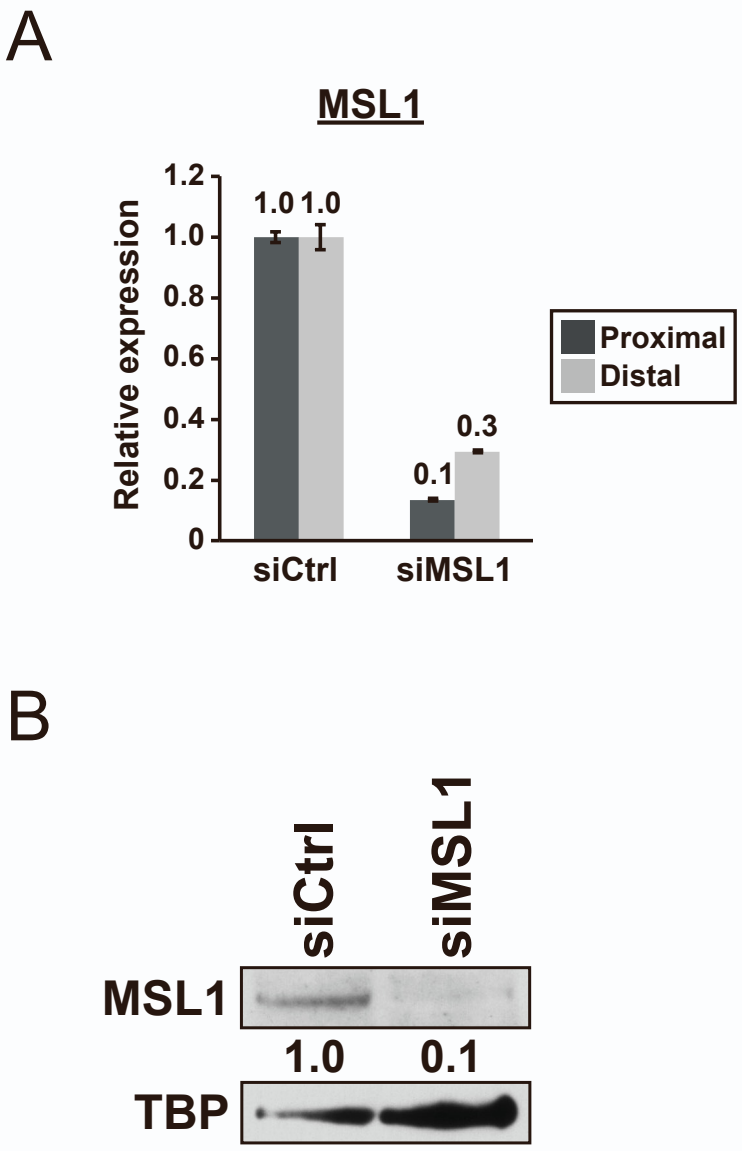

Figure S3. Characterization of the MSL1 siRNA, related to Figure 3. (**A**) A bar graph showing quantitation of MSL1 mRNA isoforms by qRT-PCR in LN229 cells transfected with a control siRNA (siCtrl) or the MSL1 siRNA (siMSL1). (**B**) Immunoblots showing quantitation of the full-length MSL1 protein in the nuclear lysates of HeLa cells transfected with a control siRNA (siCtrl) or the MSL1 siRNA (siMSL1). TBP serves as the loading control.

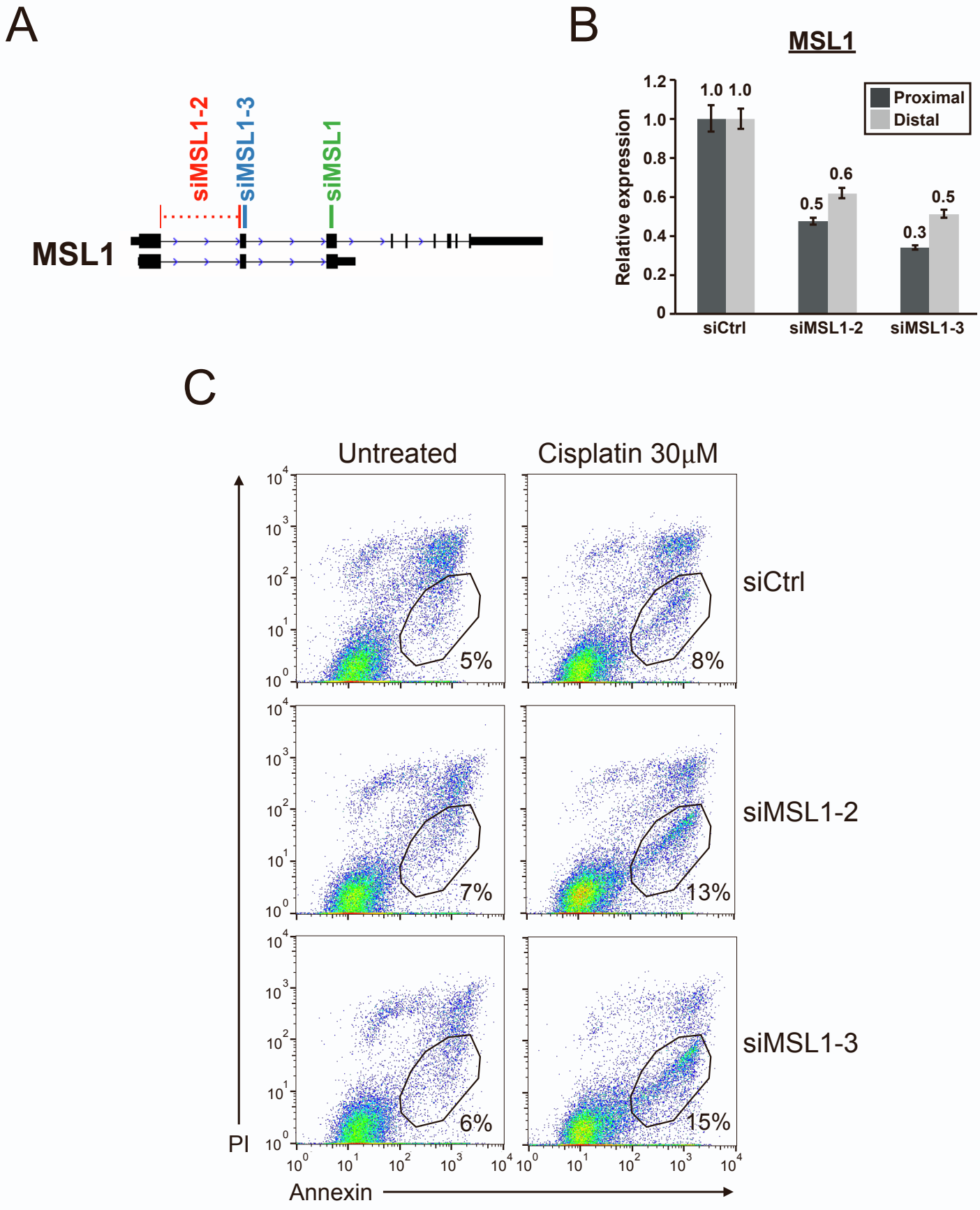

Figure S4. Enhanced apoptosis in HCT116 cells is observed with two additional MSL1 siRNAs, related to Figure 4. (**A**) Illustrations showing locations of the target sequences from 3 different MSL1 siRNAs (siMSL1, siMSL1-2, siMSL1-3) in both MSL1 mRNA isoforms. All 3 siRNAs target both MSL1 mRNA isoforms. (**B**) A bar graph showing quantitation of MSL1 mRNA isoforms by qRT-PCR in HeLa cells transfected separately with siMSL1-2, siMSL1-3, or a control siRNA (siCtrl). Error bars indicate SEM. (**C**) Representative flow cytometry results from the apoptosis assay in HCT116 cells transfected with either siMSL1-2, siMSL1-3, or a control siRNA (siCtrl). The numbers indicate the percentage of apoptotic cells (the circled [PI-low, Annexin-high] population). PI, propidum iodide.

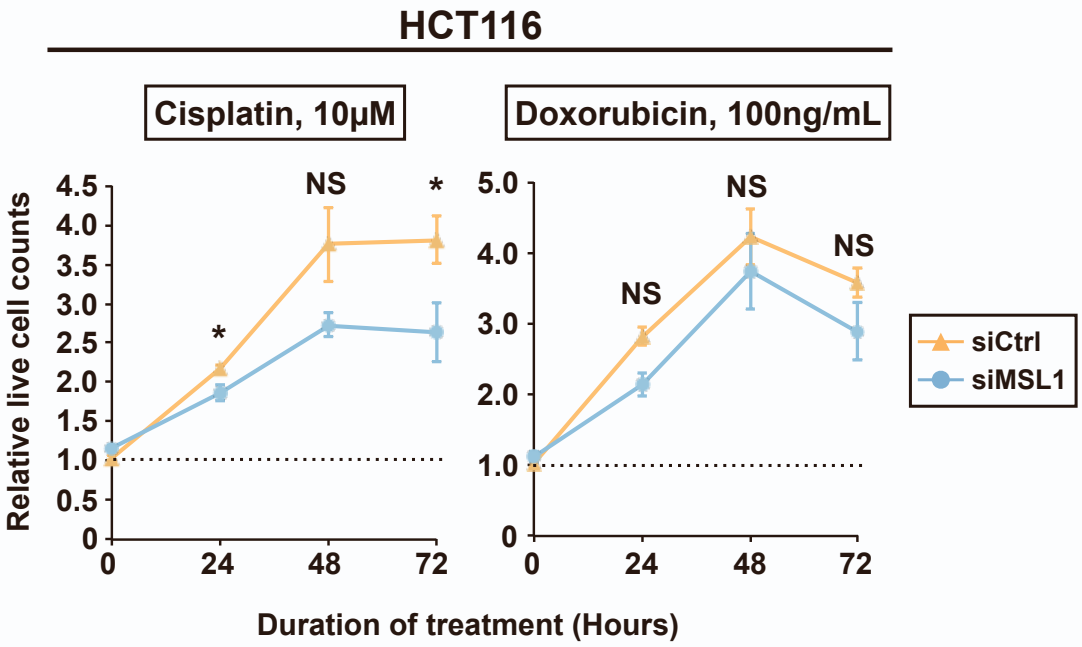

Figure S5. MSL1 knockdown amplifies the cytotoxicity of chemotherapeutic agents in HCT116 cells, related to Figure 5. Line graphs showing the relative numbers of live HCT116 cells in the presence of chemotherapeutic agents (left panel: 10 μM cisplatin; right panel: 100 ng/mL doxorubicin) from the control (siCtrl) and MSL1-knockdown (siMSL1) groups from three independent experiments (n=3). The number of untreated cells at the onset of treatment is set to 1, which is indicated by a dashed line. NS: not significant; *: $p<0.05$.

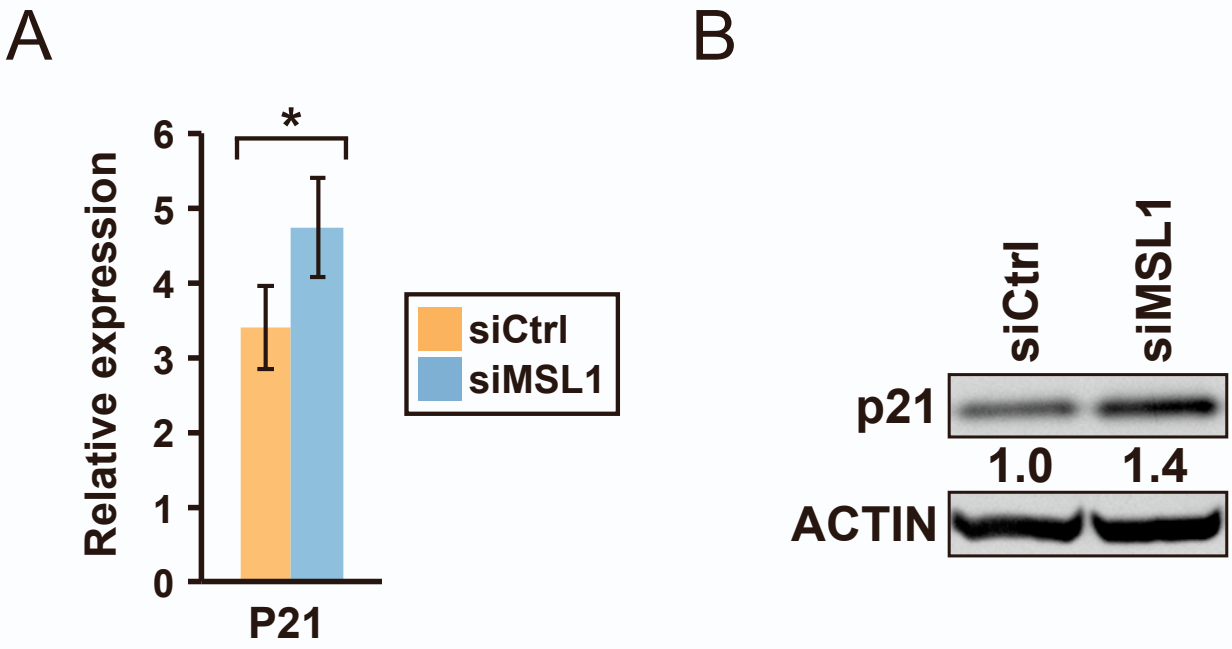

Figure S6. Knockdown of MSL1 increases p21 expression in cisplatin-treated HCT116 cells, related to Figure 6. (**A**) A bar graph showing the expression of p21 after 8hrs of 40 μM cisplatin treatment in control and MSL1-knockdown HCT116 cells, as measured by qRT-PCR from three biological replicates (n=3). Expression in the untreated cells (not shown) is set to 1 for each gene. *: $p<0.05$. Error bars indicate SEM and statistical significance is determined by one-tailed t-test. (**B**) Representative immunoblots from two independent experiments showing an increased expression of p21 in MSL1-knockdown HCT116 cells after 8hrs of 40 μM cisplatin treatment. Actin serves as the loading control.

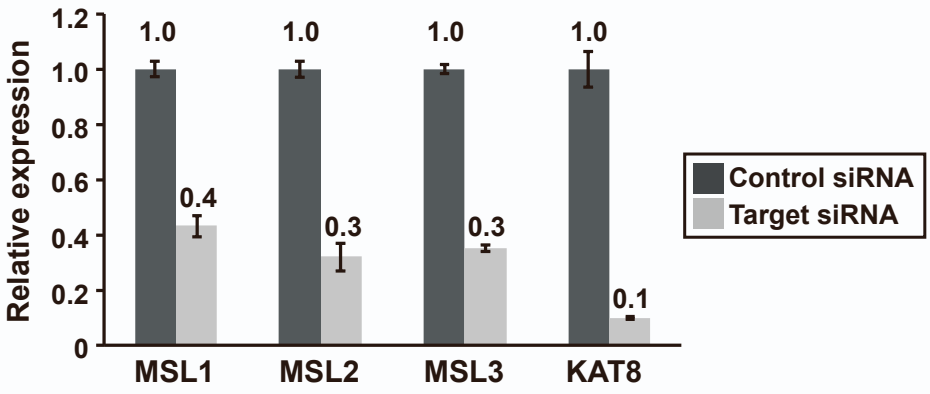

Figure S7. Knockdown of MSL components in HCT116 cells by siRNA transfection, related to Figure 7. A bar graph showing quantitation of MSL1, MSL2, MSL3 and KAT8 mRNA expression by qRT-PCR in HCT116 cells transfected with a control siRNA or the corresponding target siRNAs. Error bars indicate SEM.