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Supplemental information

Impact of supraphysiologic MDM2 expression on chromatin networks and therapeutic responses in sarcoma

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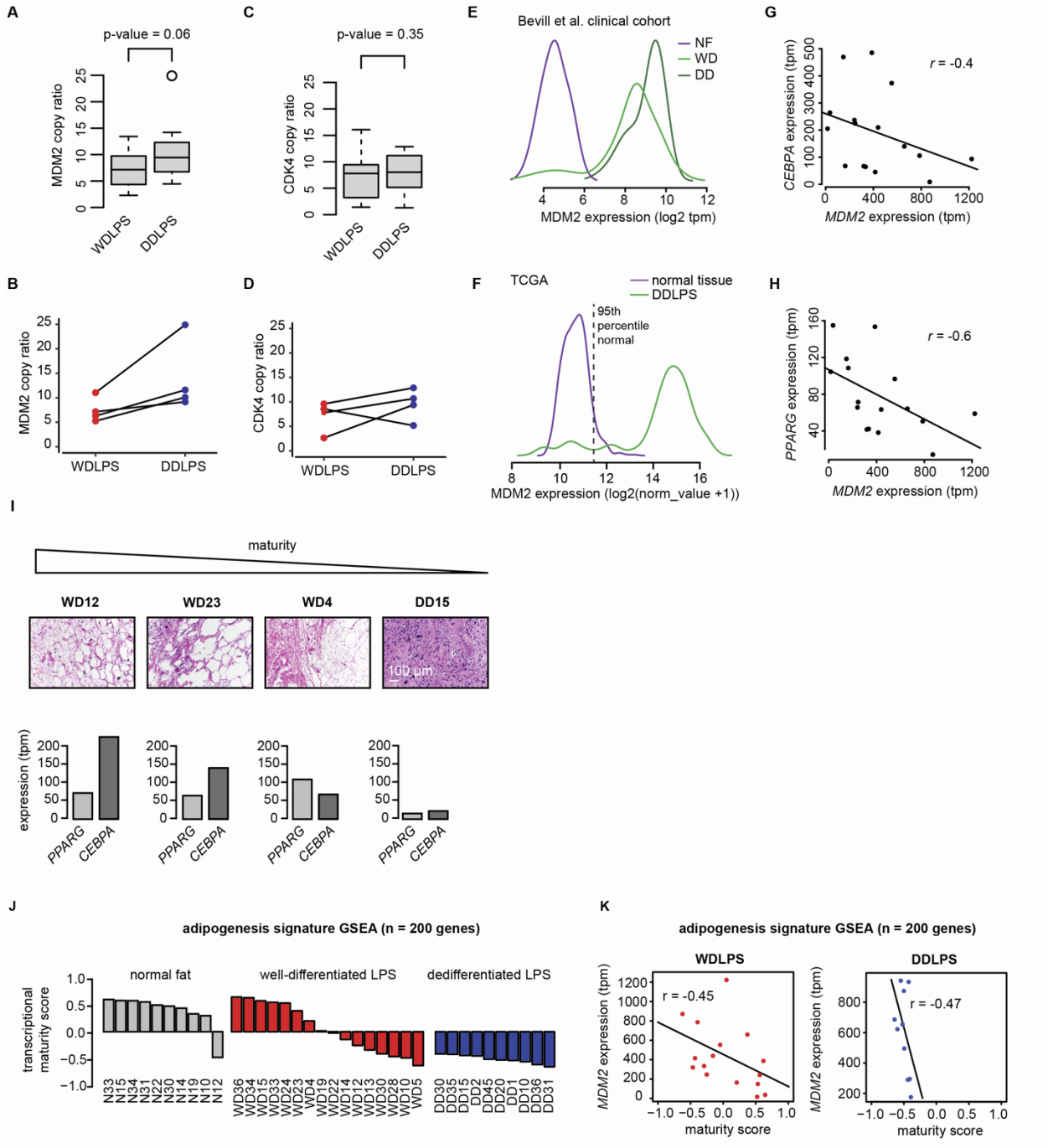

Figure S1 | MDM2 copy number and expression correlate with dedifferentiated liposarcoma states related to Figure 1. (A) Boxplot representing copy number ratio of *MDM2* in WD (*n* = 11) and DD (*n* = 10) tumors, one sided t-test. **(B)** *MDM2* copy number ratio of paired WD/DD tumors (*n* = 4). Lines connect samples derived from the same tumor, one sided, paired t-test p-value = 0.04. **(C)** Boxplot representing copy number ratio of *CDK4* in WD (*n* = 11) and DD (*n* = 10) tumors, one sided t-test. **(D)** *CDK4* copy number ratio of paired WD/DD tumors (*n* = 4). Lines connect samples derived from the same tumor, one sided, paired t-test p-value = 0.17. **(E)** Histograms showing log 2 tpm values for MDM2 in normal fat, WDLPS and DDLPS samples in our clinical cohort. **(F)** Histograms showing normalized log2 MDM2 expression in

normal tissues (*n* = 737) and DDLPS (*n* = 59) in the TCGA. Dashed line represents the top 5% of normal tissue expression values. **(G)** Scatter plots show correlation between *MDM2* expression (x-axis) and *CEBPA* (y-axis) for WDLPS tumors (*n* = 16), *r* = spearman correlation. **(H)** Scatter plots show correlation between *MDM2* expression (x-axis) and *PPARG* (y-axis) for WDLPS tumors (*n* = 16), *r* = spearman correlation. **(I)** Hematoxylin and eosin staining for representative well-differentiated (WD) and dedifferentiated (DD) tumors, ranked by maturity score (top) and showing expression of master adipocytic regulators PPARG and CEBPA (bottom). **(J)** Waterfall plots show maturity scores of normal fat and primary tumors using the GSEA adipogenesis signature. **(K)** Scatter plots show correlation between *MDM2* expression (y-axis) and maturity score using GSEA adipogenesis gene signature (x-axis) for WDLPS and DDLPS tumors.

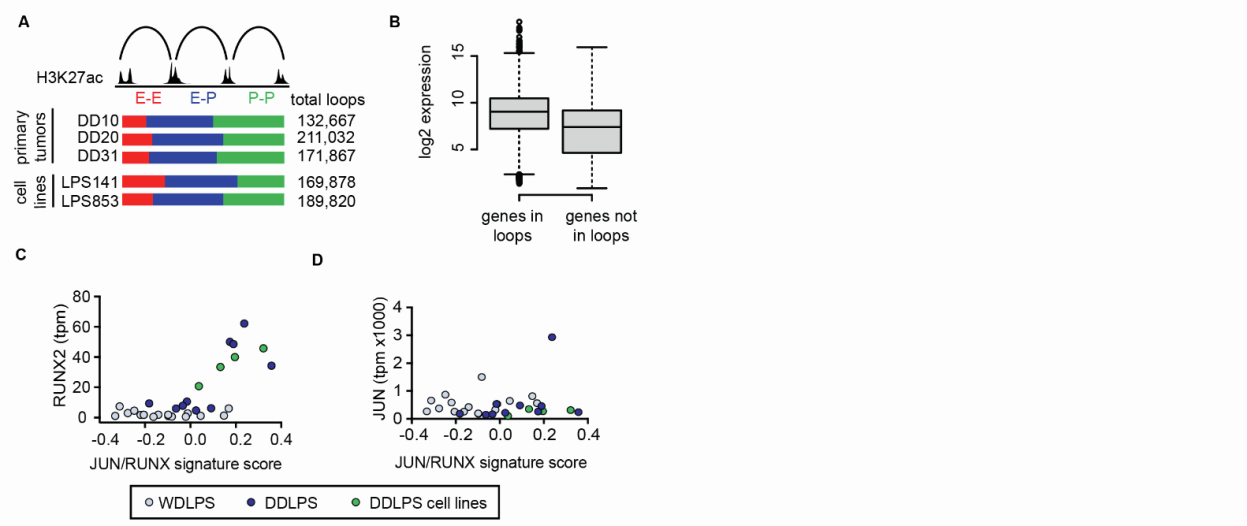

Figure S2 | HiChIP defines transcriptional circuits in liposarcoma tumors and cell lines related to Figure 2. (A) Summary of E-E (red), E-P (blue), and P-P (green) contact loops called from HiChIP data in three DDLPS tumors (DD10, DD20, and DD31) and two DDLPS cell lines (LPS141 and LPS853). Line width of loops in the top schematic indicates relative contribution to the total dataset. **(B)** Boxplot depicting expression of all genes engaged in HiChIP loops compared to all other expressed genes not in loops in LPS141 cell line, two tailed t-test p-value < 2.2e-16. **(C)** Scatter plot of *RUNX2* expression (y-axis) and RUNX2/JUN target genes expression score (x-axis) across WD (*n* = 16) and DD tumors (*n* = 10) and DD cell lines (*n* = 4). **(D)** Scatter plot of *JUN* expression (y-axis) and RUNX2/JUN target genes expression score (x-axis) across WD (*n* = 16) and DD (*n* = 10) tumors and DD cell lines (*n* = 4).

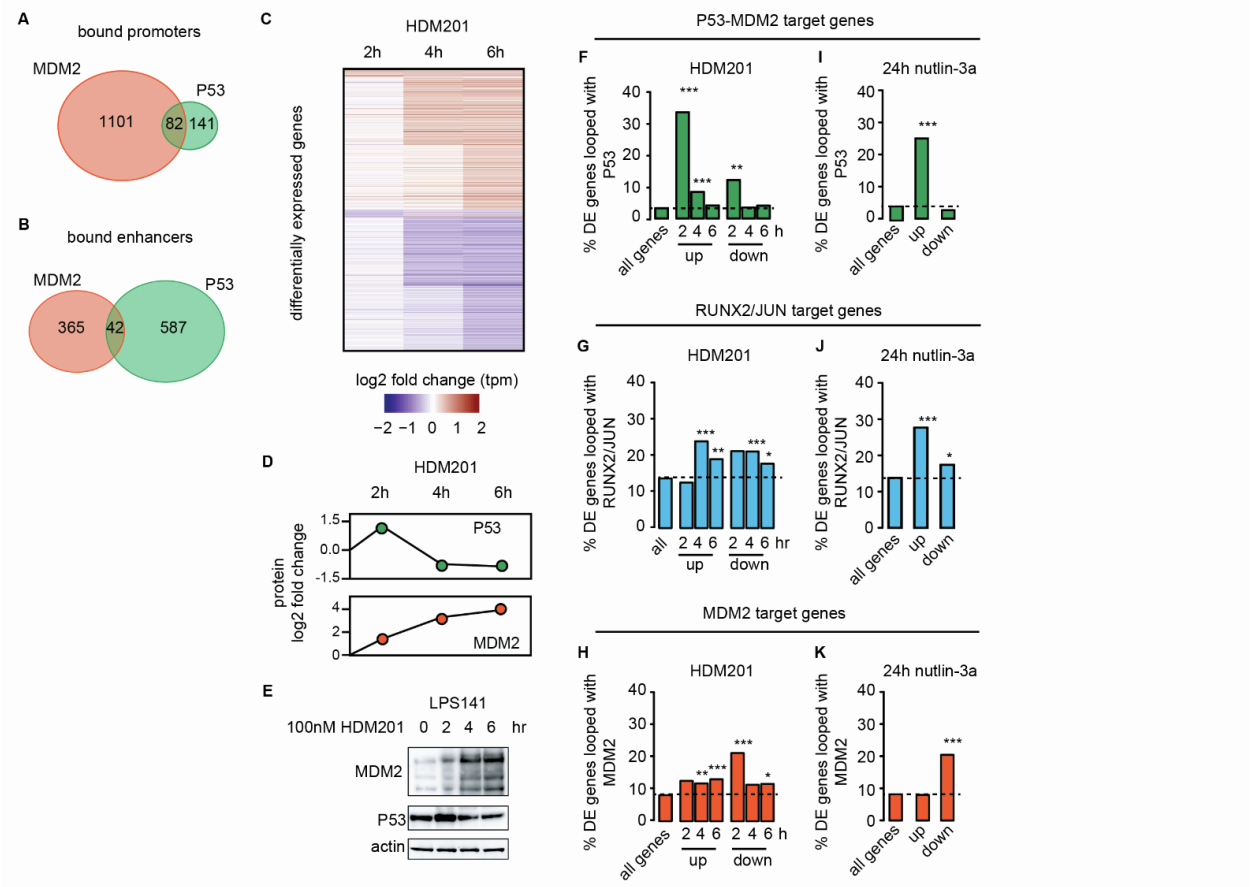

Figure S3 | HDM201 defines the impact of MDM2 on transcriptional circuits in LPS141 cells related to Figure 3. (A-B) Venn diagrams showing overlap between MDM2 and P53 binding sites at **(A)** promoters and **(B)** enhancers. **(C)** Heatmap of differentially expressed genes in response to a time course (2h, 4h, 6h) of HDM201 treatment (100nM) in LPS141 cells. Expression levels were quantified from 2 biological replicates. **(D)** MDM2 and P53 protein levels plotted across a time course (2h, 4h, 6h) of HDM201 treatment (100nM) in LPS141 cells. **(E)** Western blot used for protein level quantification in panel B. **(F-K)** Bar plots depicting percent of genes differentially expressed at either 2h, 4h, or 6h treatment with HDM201 (100nM) or 24h treatment with Nutlin-3a (1μM) that are engaged in HiChIP loops bound by F and I) P53, G and J) RUNX2/JUN, or H and K) MDM2. Significant enrichment of up/down-regulated TF-bound genes was determined using a two-sided Fisher exact test by comparing to all expressed genes bound by a given transcription factor. Significant p-values for each comparison are F) 2h up: < 2.2e-16 , 4h up: 3.3e-13 , 2h down: 1.1e-4 G) 4h up: 2.8e-13, 6h up: 9.5e-05, 4h down: 3.4e-07, 6h down: 3.7e-03 H) 4h up: 2.3e-04, 6h up: 2.6e-06, 2h down: 1.5e-4, 6h down: 3.6e-03 I) 24h up: 3.3e-18 J) 24h up: 0.04 K) 24h down: 1.0e-08.

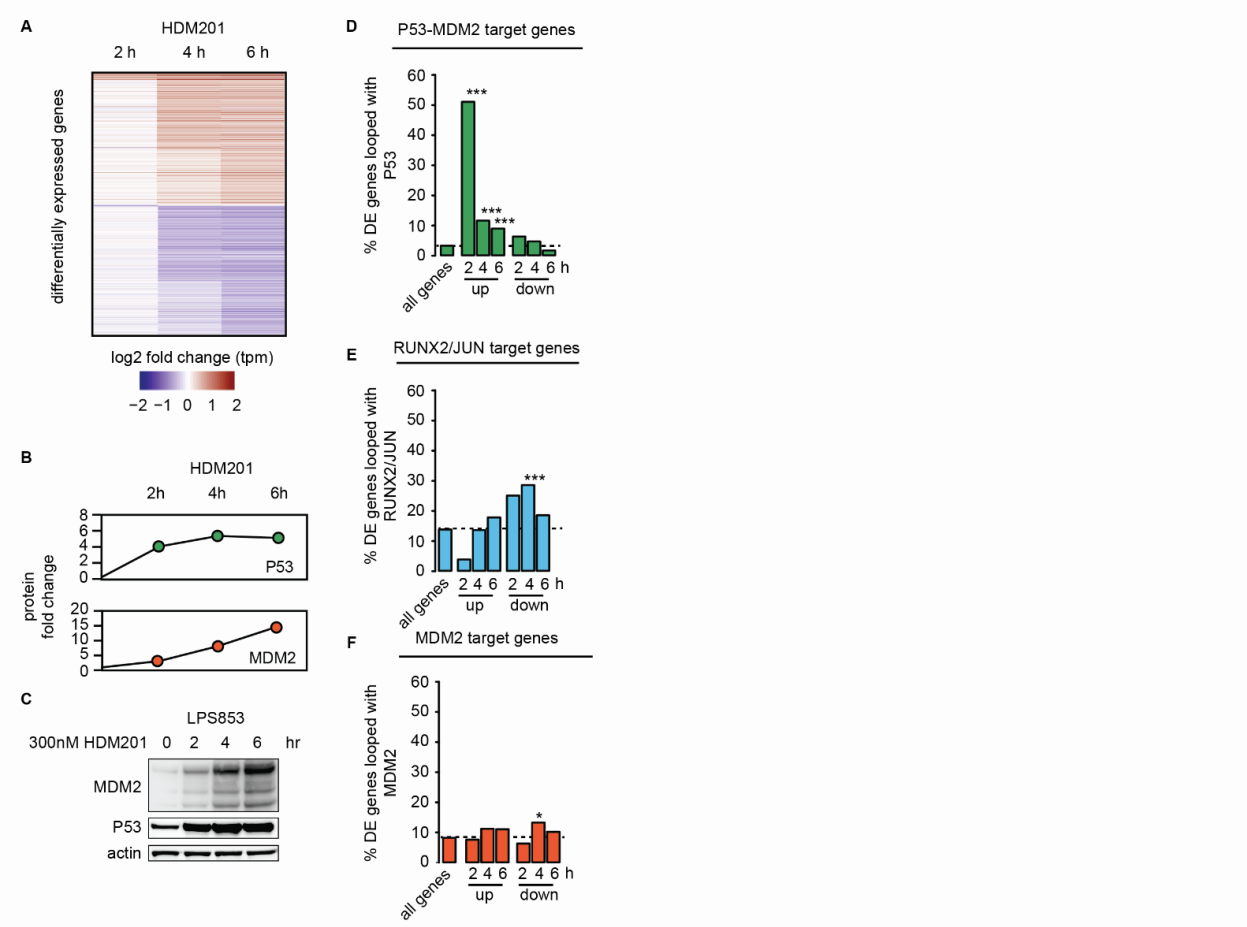

Figure S4 | HDM201 defines the impact of MDM2 on transcriptional circuits in LPS853 cells related to Figure 3. (A) Heatmap of differentially expressed genes in response to a time course (2h, 4h, 6h) of HDM201 treatment (300nM) in LPS853 cells. Expression levels were quantified from 2 biological replicates. **(B)** MDM2 and P53 protein levels plotted across a time course (2h, 4h, 6h) of HDM201 treatment (300nM) in LPS853 cells. **(C)** Western blot used for protein level quantification in panel B. **(D-F)** Bar plots depicting percent of genes differentially expressed at either 2h, 4h, or 6h treatment with HDM201 (300nM) that are engaged in HiChIP loops bound by D) P53, E) RUNX2/JUN, or F) MDM2. Significant enrichment of up/down-regulated TF-bound genes was determined using a two-sided Fisher exact test by comparing to the background of all expressed genes bound by a given transcription factor. Significant p-values for each comparison are D) 2h up: 2.6e-21 , 4h up: 2.1e-16 , 6h up: 4.6e-08, 4h down: 0.03 E) 6h up: 0.01, 4h down: 9.4e-16, 6h down: 0.01 F) 2h up: 9e-03 , 4h up: 0.02, 4h down: 9.4e-06.

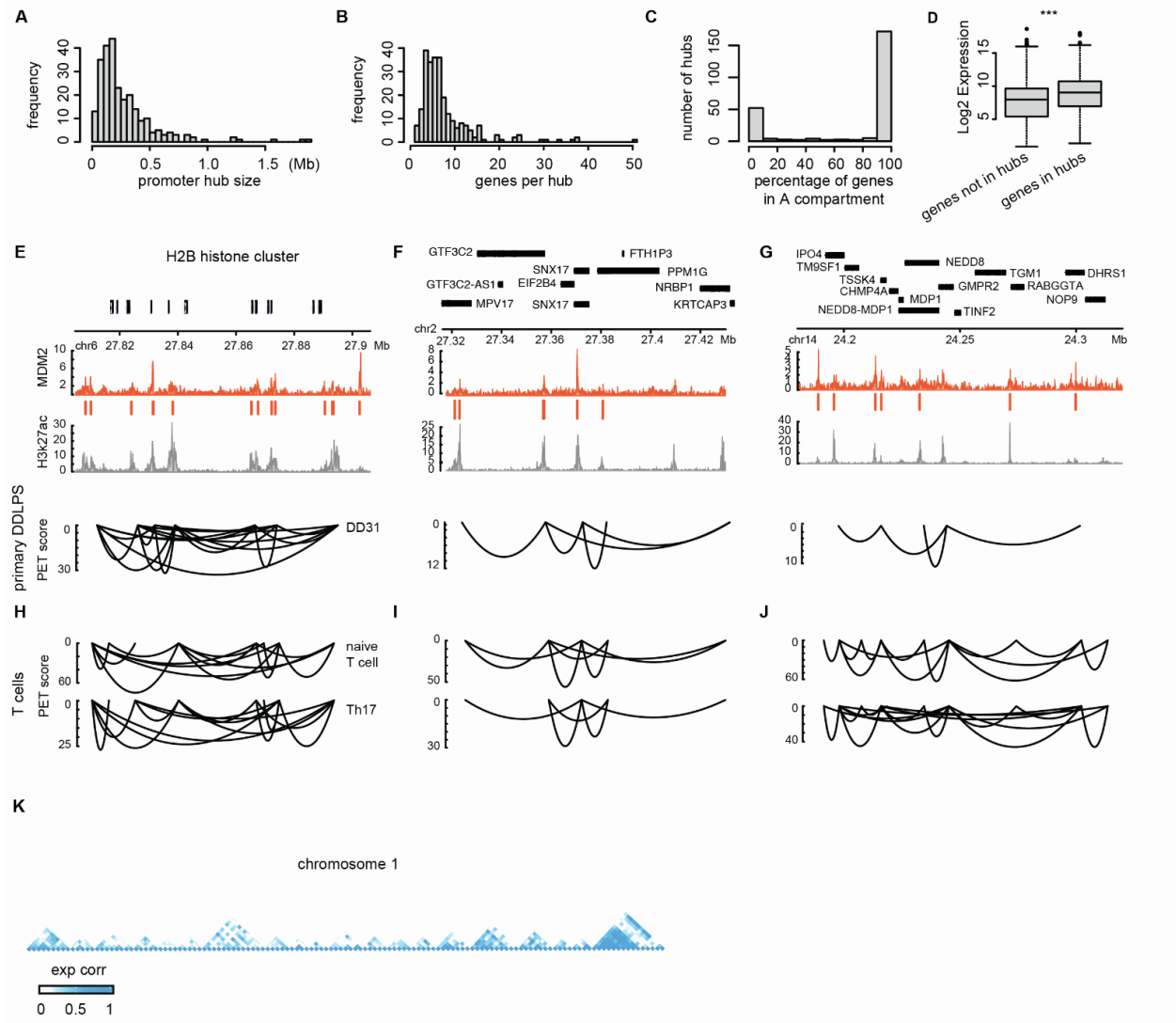

Figure S5 | Multivalent promoter hubs in HiChIP datasets related to Figure 4. (A) Histogram depicting linear size (Mb) of promoter hubs (*n* = 258). **(B)** Histogram depicting number of genes contained within each promoter hub (*n* = 258). **(C)** Histogram depicting the percentage of genes in each promoter hub (*n* = 258) that are contained within the euchromatic A compartment. **(D)** Boxplot comparing gene expression of genes in hubs with expression of genes not in hubs. Two-sided t-test p-value< 2.2e-16. **(E-G)** Genomic tracks for three genomic regions with representative promoter hubs. Top tracks represent MDM2 binding intensity and called peaks (orange), and H3K27ac intensity (grey) in LPS141 cells. Bottom track depicts HiChIP promoter-promoter loops in a dedifferentiated tumor (DD31). Loop height is proportional to the paired end tag (PET) score. Genes are shown above. **(H-J)** HiChIP promoter-promoter loops in two T-cell populations (naive T cell and Th17) shown for the same promoter hubs as above. **(K)** Heatmap depicting gene expression correlation across all promoter-promoter hubs contained on Chromosome 1 (*n* = 60). Each triangle represents a given hub and heat in blue represents the degree to which genes are coordinately expressed across TCGA datasets.

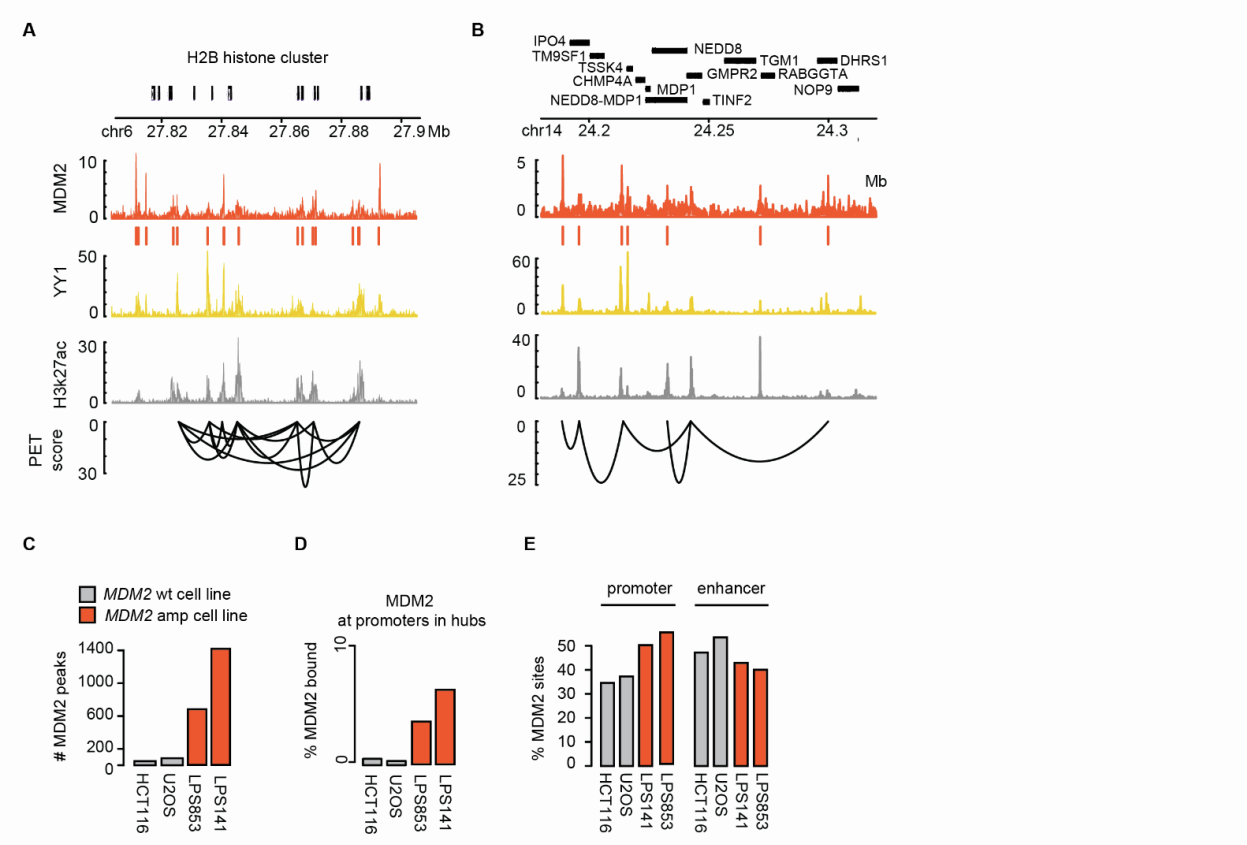

Figure S6 | Recruitment of supraphysiologic MDM2 to promoter hubs related to Figure 4. **(A-B)** Genomic tracks for representative promoter hubs in the LPS853 cell line. Tracks represent, from top to bottom, MDM2 binding intensity (orange), YY1 binding intensity (yellow) and H3K27ac intensity (gray), and paired end tag (PET) scores for promoter-promoter loops in H3K27ac HiChIP. **(C)** Bar plot of total MDM2 ChIP-seq peaks defined in MDM2 wildtype cell lines in gray (HCT116 and U2OS) or MDM2 amplified cell lines in orange (LPS141 and LPS853). **(D)** Bar plot shows percent of hub promoters bound by MDM2 in wildtype cell lines in gray (HCT116 and U2OS) or MDM2 amplified cell lines in orange (LPS141 and LPS853). **(E)** Bar plot shows the proportion of MDM2 binding sites that coincide with promoters or putative enhancers. MDM2 wildtype cell lines are in gray (HCT116 and U2OS) and MDM2 amplified cell lines are in orange (LPS141 and LPS853).

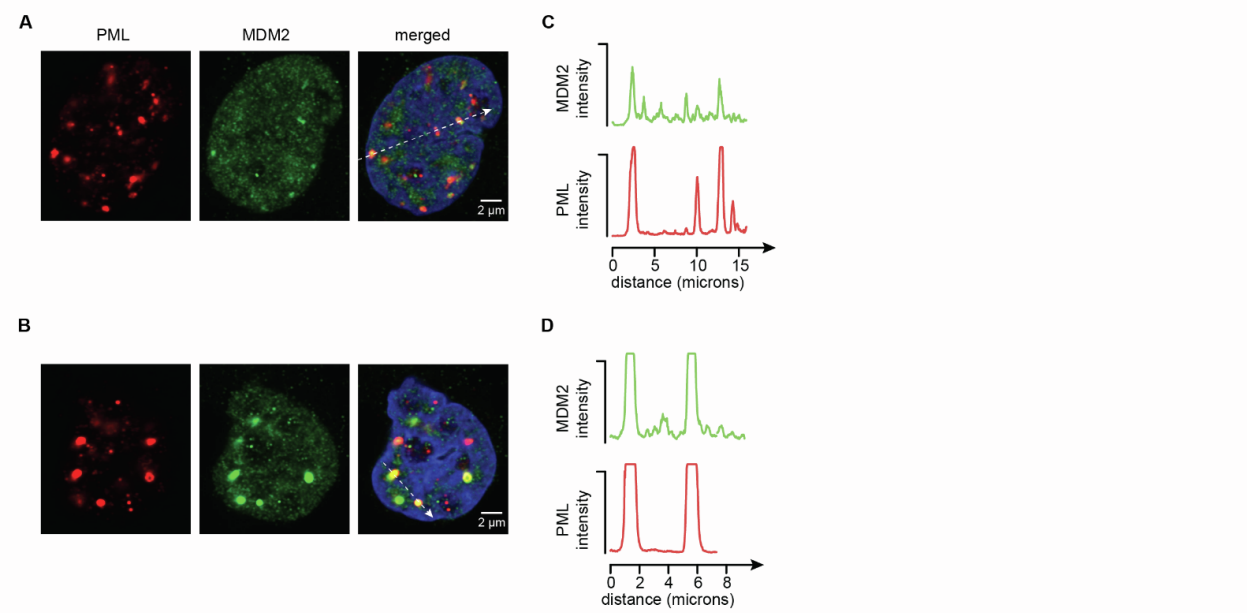

Figure S7 | MDM2 colocalizes in nuclear foci with PML related to Figure 5. (A-B) Representative 63x confocal immunofluorescence images of MDM2 (green), PML (red), and DAPI (blue) in the LPS853 cell line. **(C-D)** Quantification of MDM2 and PML intensity profiles across white dashed line shown in panel a and b respectively.

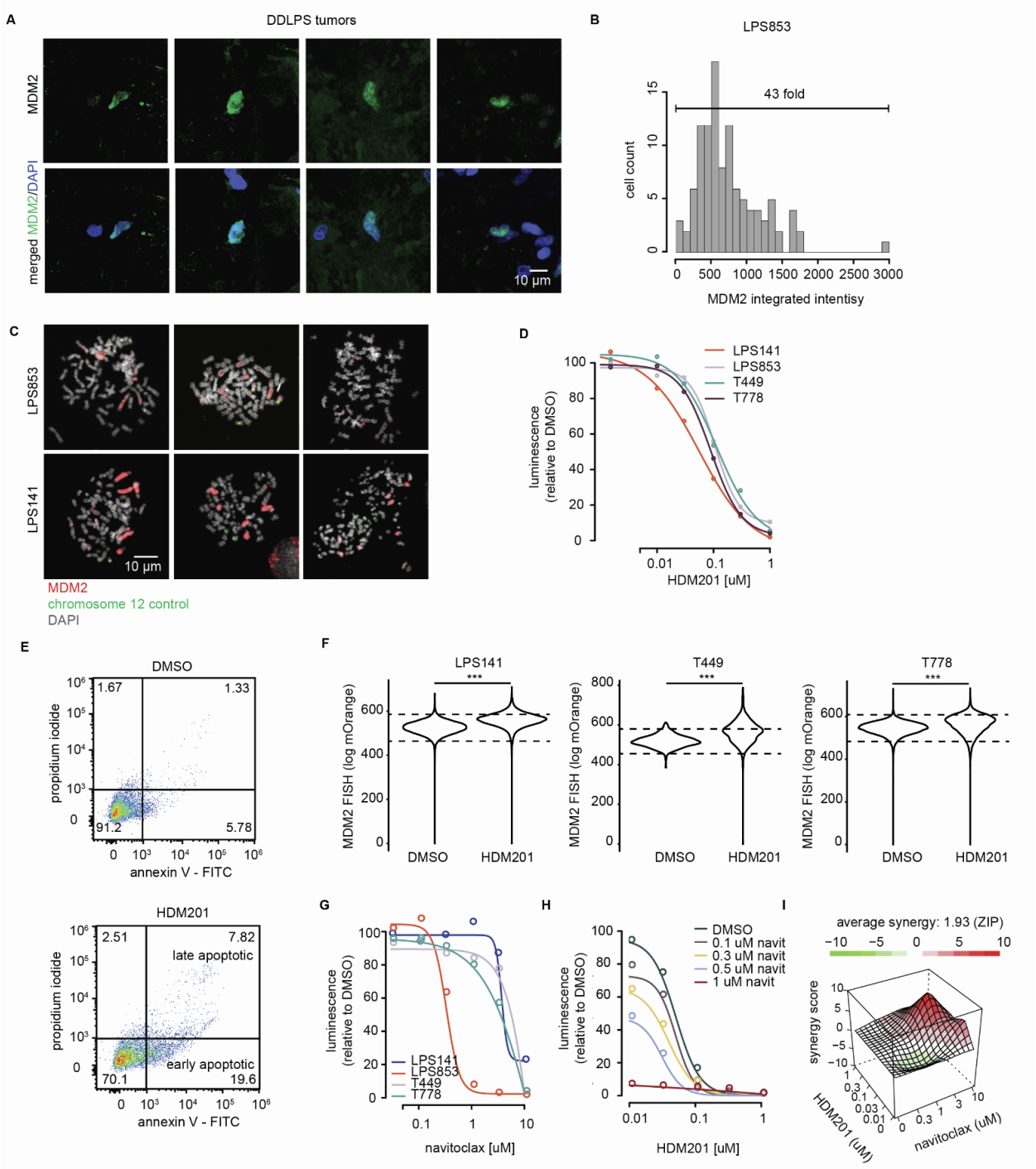

Figure S8 | Therapeutic vulnerabilities of liposarcomas and their association with MDM2 levels related to Figure 6. (A) Representative 25x confocal images of MDM2 immunofluorescence (green) in primary DDLPS tumors, Nuclei are marked with DAPI (blue). **(B)** Histogram of MDM2 integrated intensity across a population of untreated LPS853 cells. n = 109. **(C)** Representative 40x images of MDM2 FISH (red) or control chromosome 12 FISH (green) on metaphase spreads of LPS141 and LPS853 cells. Chromosomes are stained with DAPI (gray). **(D)** Dose response curves showing cell titer glo measurements of ATP luminescence (y axis) across increasing doses of HDM201 (x axis) in liposarcoma cell lines treated for 96 hours. (*n* =

6). **(E)** Flow cytometry scatter plot of LPS853 cells stained with propidium iodide (PI) (y-axis) and annexin V (x-axis) following treatment with DMSO (left) or 300nM HDM201 (right) for 24 hours. Gating indicates PI and annexin V positive cells with percent cells in each gate indicated in red. **(F)** Violin plots show MDM2 FISH distribution for LPS141, T449 and T778 cells following 3 days treatment with DMSO (control) or 500nM HDM201. (*n* = ≥ 20,000 cells per condition). Dashed lines represent the top 5% and bottom 5% of the DMSO control. Comparisons of HDM201 to DMSO control for each cell line displayed two tailed p-values <2.2e-16. **(G)** Dose response curves showing cell titer glo measurements of ATP luminescence (y axis) across increasing doses of Navitoclax (x axis) in liposarcoma cell lines treated for 96 hours. (*n* = 6). **(H)** Dose response curves across a constant dose range of HDM201 (x axis) in the LPS853 cell line treated for 96 hours. Each curve represents cell titer glo for HDM201 alone or with addition of Navitoclax at increasing doses. (*n* = 4). **(I)** 3D drug interaction landscapes using the Zero Interaction Potency (ZIP) model for data in panel G. Scores are plotted for each dose combination (right). Positive scores representing dose combinations that yield higher growth inhibition than either single agent alone.

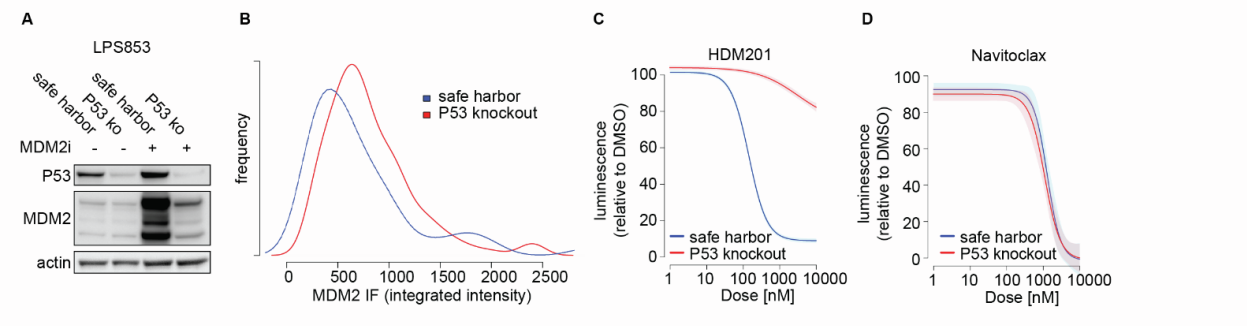

Figure S9 | Generation of a P53 knockout LPS853 cell line related to Figure 6. (A) Western blot showing P53 and MDM2 protein in LPS853 cells treated with safe harbor or P53 knockout small guide RNAs. Cells were treated 24h with DMSO (control) or 300nM HDM201. **(B)** Histogram of MDM2 integrated intensity across safe harbor or P53 knockout LPS853 cells. (*n* = 171). **(C-D)** Dose response curves showing cell titer glo measurements of ATP luminescence (y axis) across increasing doses of **(C)** HDM201 or **(D)** Navitoclax (x axis) in safe harbor or P53 knockout LPS853 cells treated for 96 hours. (*n* = 6).