Supplementary Information for:

Unmasking the suppressed immunopeptidome of EZH2 mutated diffuse large B-cell lymphomas through combination drug treatment

Short Title: Restoring the suppressed immunopeptidome of DLBCL

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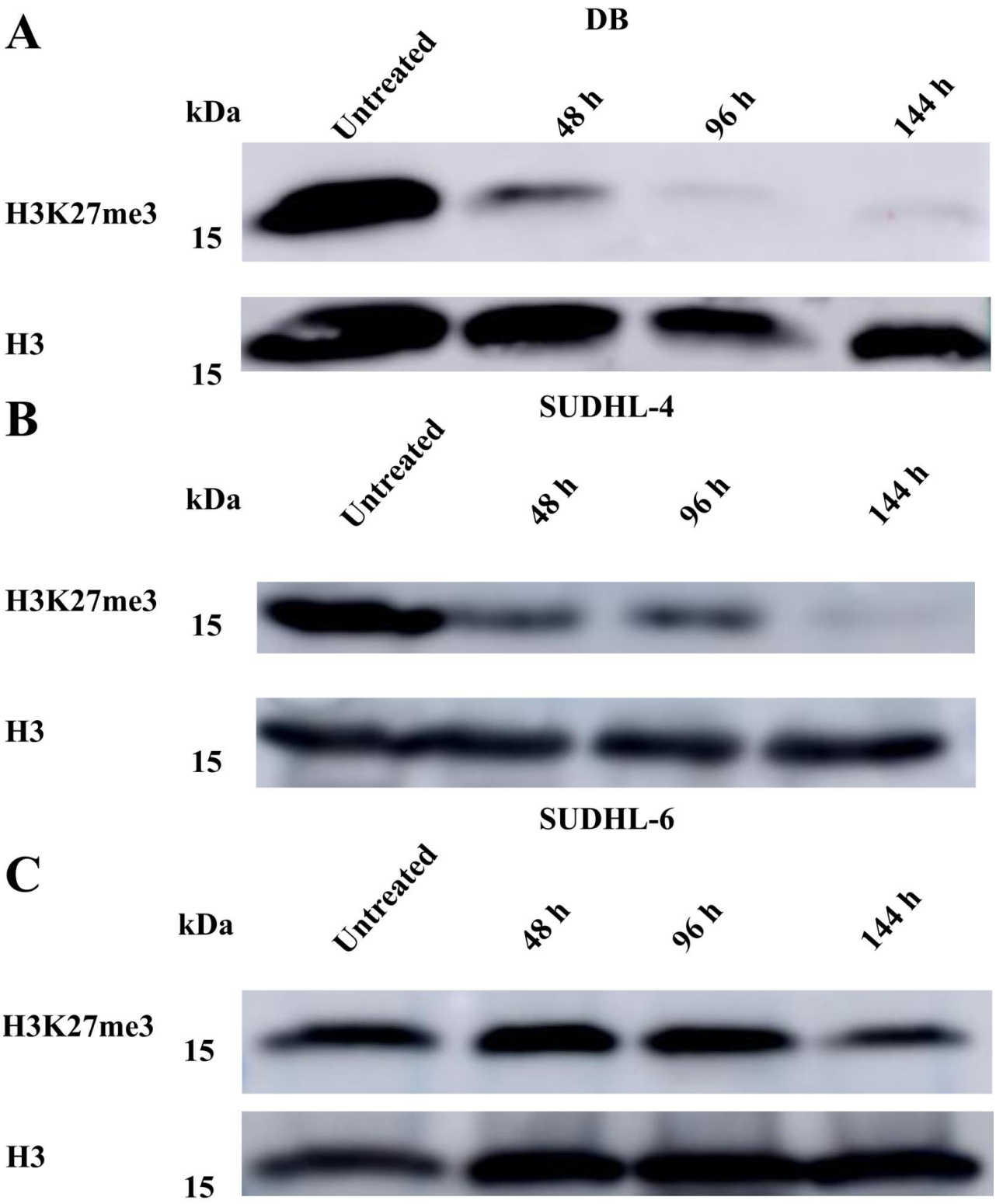
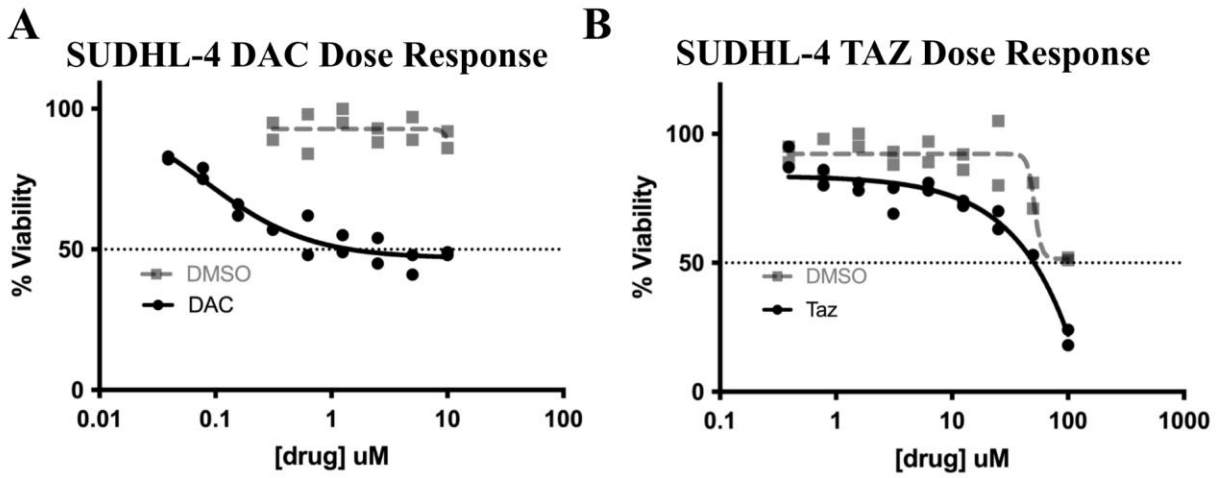
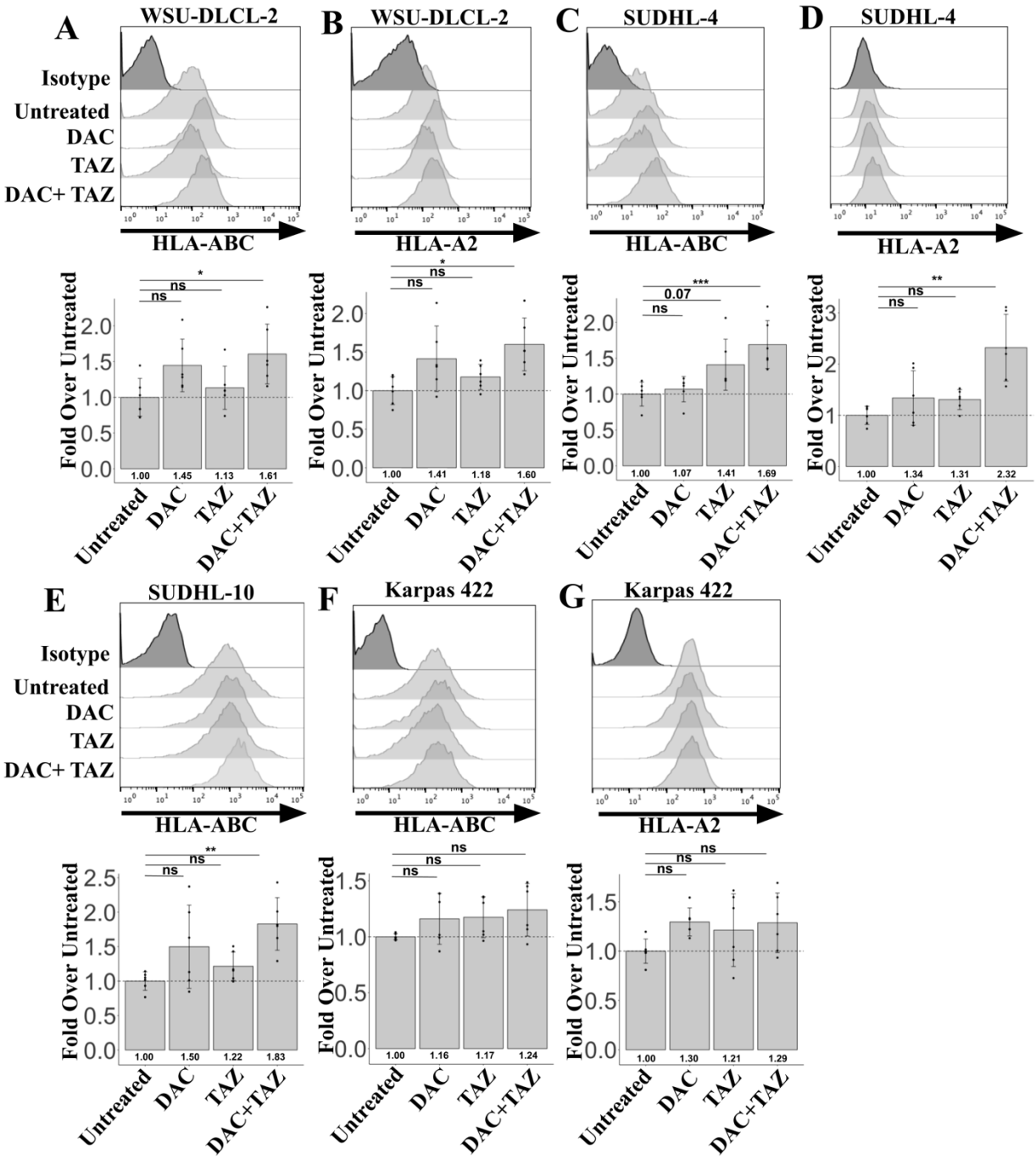


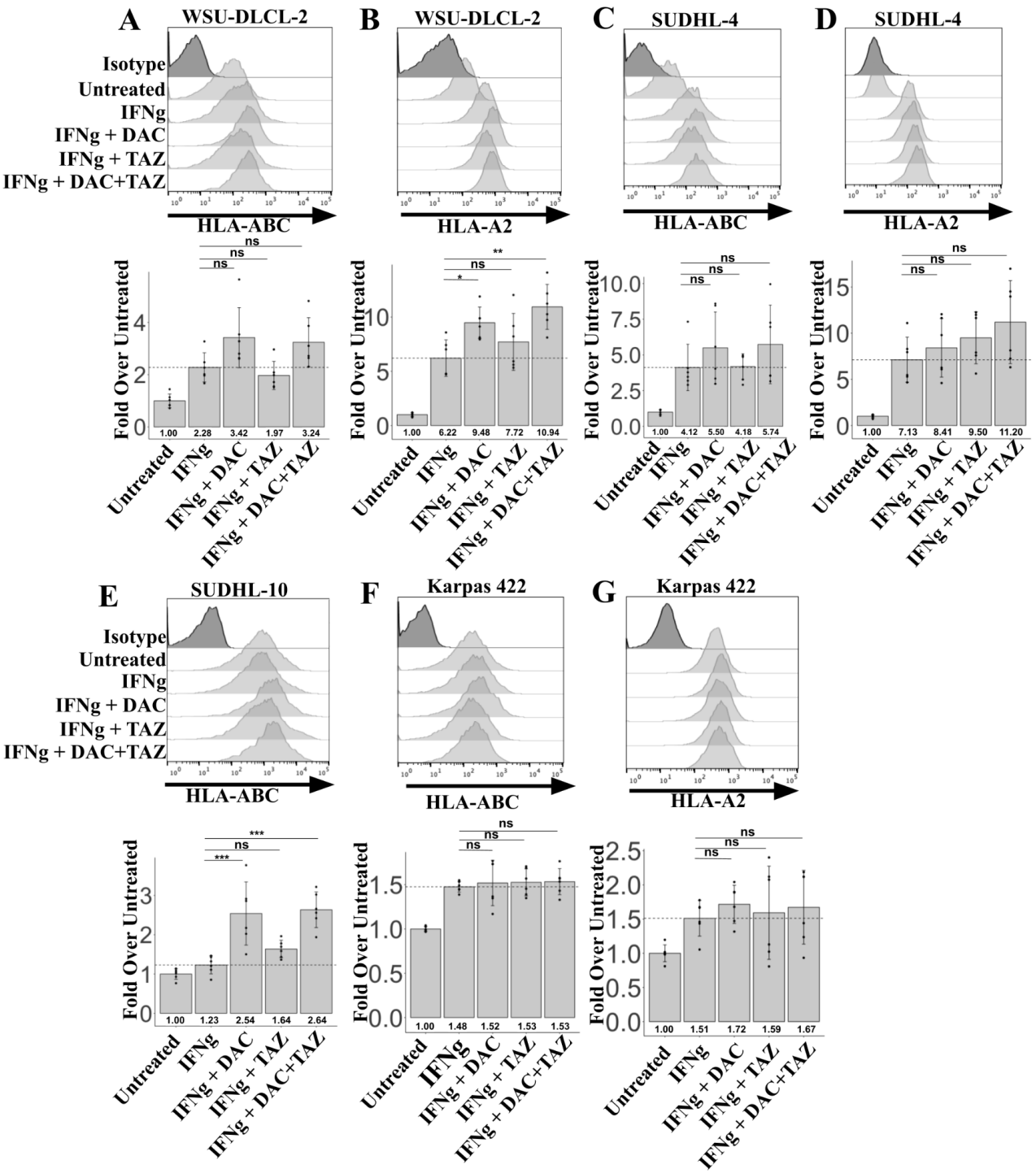
Supplemental Figure 1. Tazemetostat time course on H3K27me3 marks after treatment. (A) DB cells were treated with vehicle control for 144 hours or TAZ for 48, 96, or 144 hours, with drug and media being refreshed after each 48 hours. H3K27me3 levels were determined by western blot. Total H3 was used as a loading control. (B) SUDHL-4 cells treated as in A. (C) SUDHL-6 cells treated as in A.



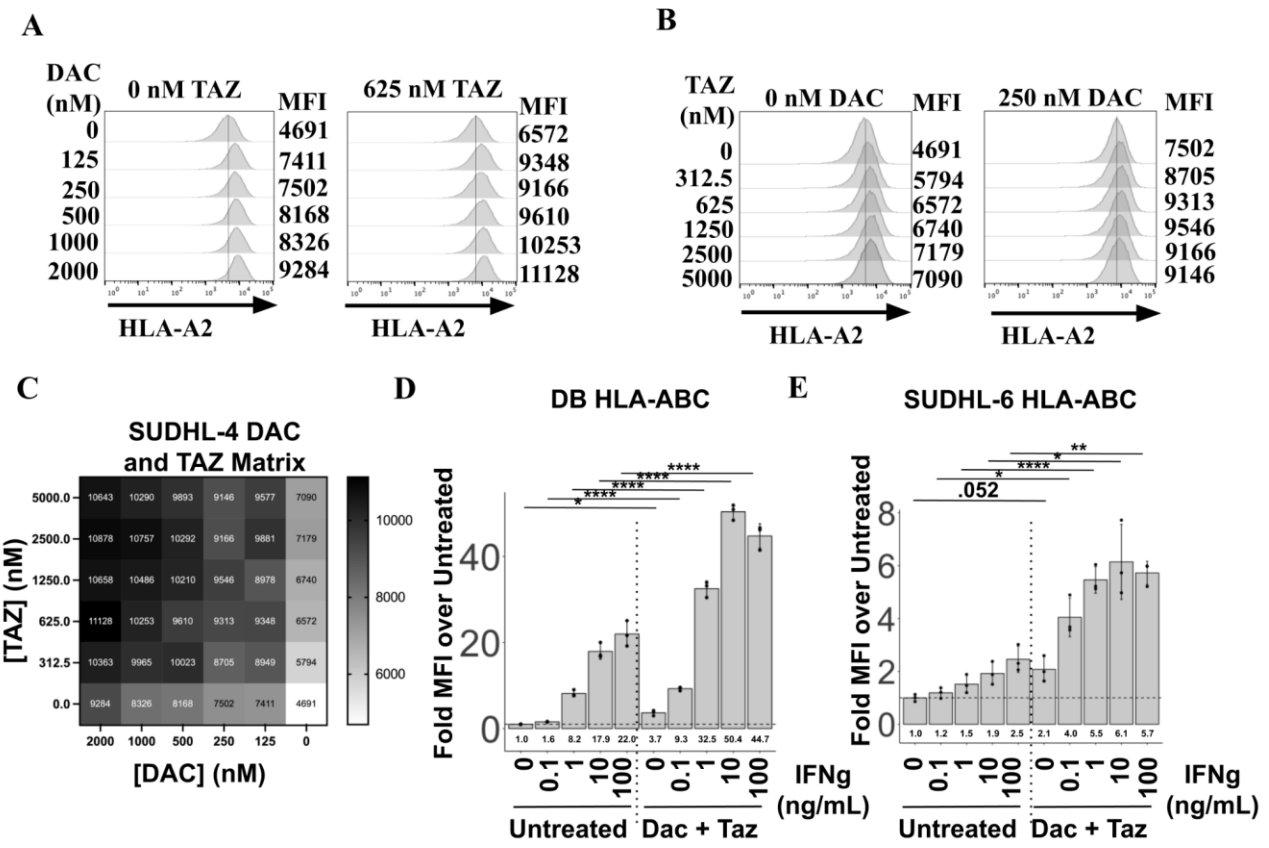
Supplemental Figure 2. Decitabine and tazemetostat dose responses on SUDHL-4 cells. (A) DAC from a 1mM stock in DMSO was diluted serially on SUDHL-4 cells for two days. Control DMSO was also added at equivalent concentrations as the DAC solution. (B) TAZ from a 10mM stock was diluted on SUDHL-4 cells as in (A). Each dot represents a technical replicate, N=2.



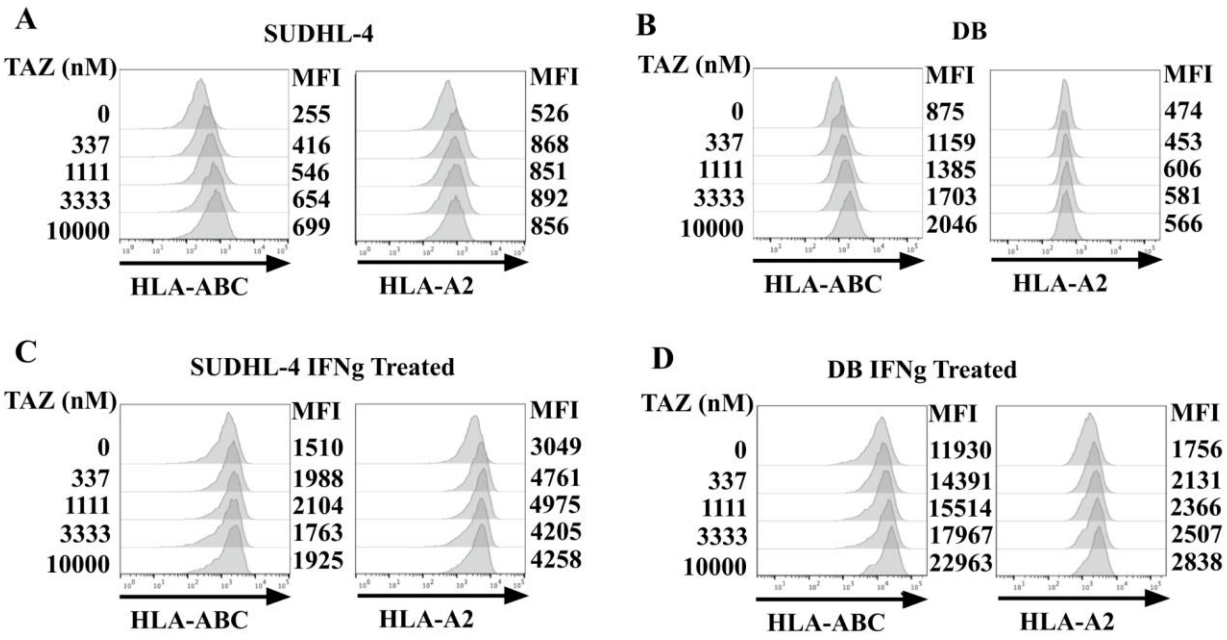
Supplemental Figure 3. Decitabine and tazemetostat upregulated HLA in WSU-DLCL2, SUDHL-4, SUDHL-10, but not Karpas 422 cell lines. (A-G) Cells were treated with indicated 125nM DAC, or 1uM TAZ. (A-B) WSU-DLCL2, (C-D) SUDHL-4, (E) SUDHL-10, and (F-G) Karpas 422 cells were assayed for HLA-ABC (A,C,E,F) and HLA-A-02 (B,D,G) expression by flow cytometry. ANOVA using untreated as control was performed, followed by a post-hoc tukey's test for individual experimental groups. Mean and standard deviation displayed for N=3 technical replicates per 2 biological replicates. * p<.05, ** p<.01, *** p<.001, **** p<.0001.



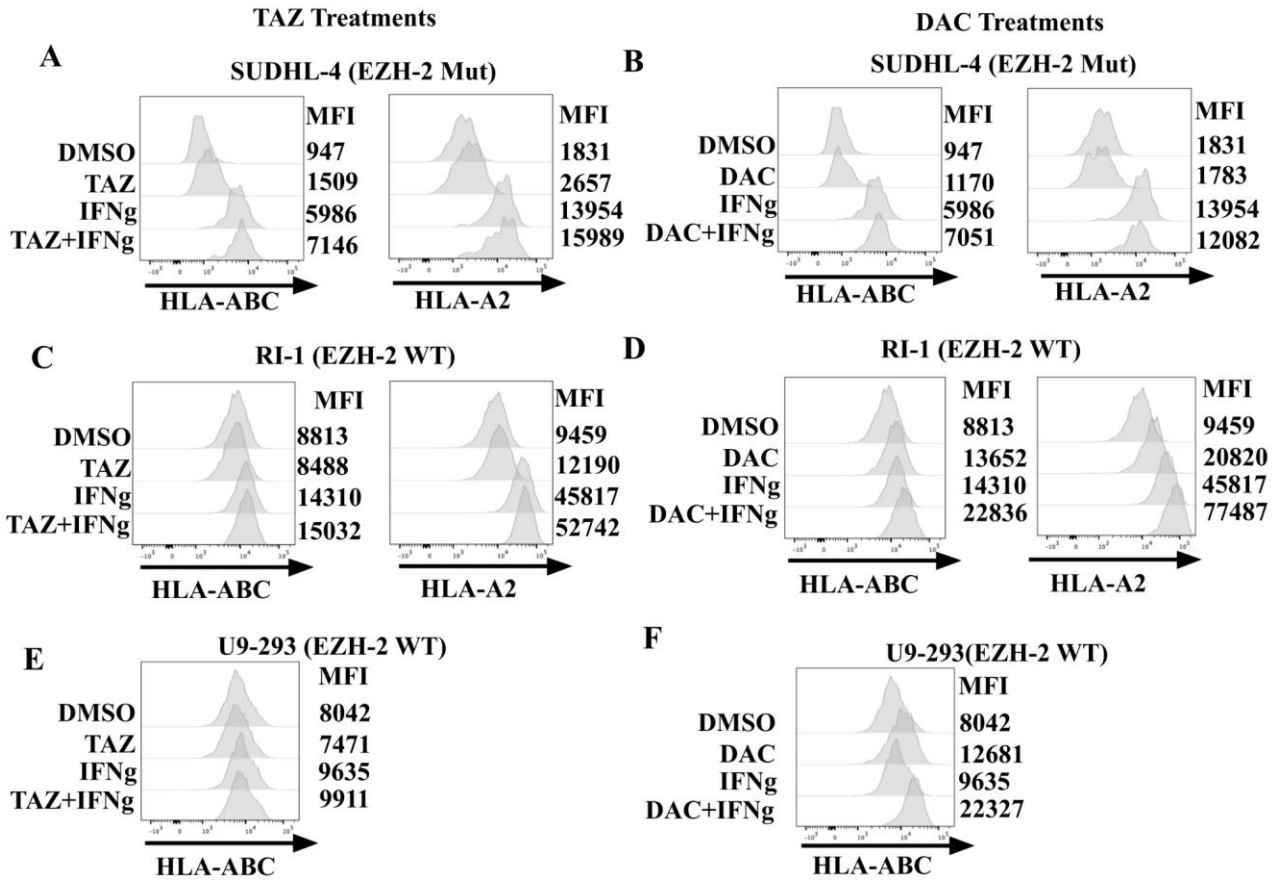
Supplemental Figure 4. Decitabine and tazemetostat on IFNg-mediated HLA upregulation in WSU-DLCL2, SUDHL-4, SUDHL-10, and Karpas 422 cell lines. (A-G) Cells were treated with indicated 125nM DAC, or 1uM TAZ alongside 10ng/mL IFNg. (A-B) WSU-DLCL2, (C-D) SUDHL-4, (E) SUDHL-10, and (F-G) Karpas 422 cells were assayed for HLA-ABC (A,C,E,F) and HLA-A-02 (B,D,G) expression by flow cytometry. ANOVA using IFNg as control was performed, followed by a post-hoc tukey's test for individual experimental groups. Mean and standard deviation displayed for N=3 technical replicates per 2 biological replicates. * p<.05, ** p<.01, *** p<.001, **** p<.0001.



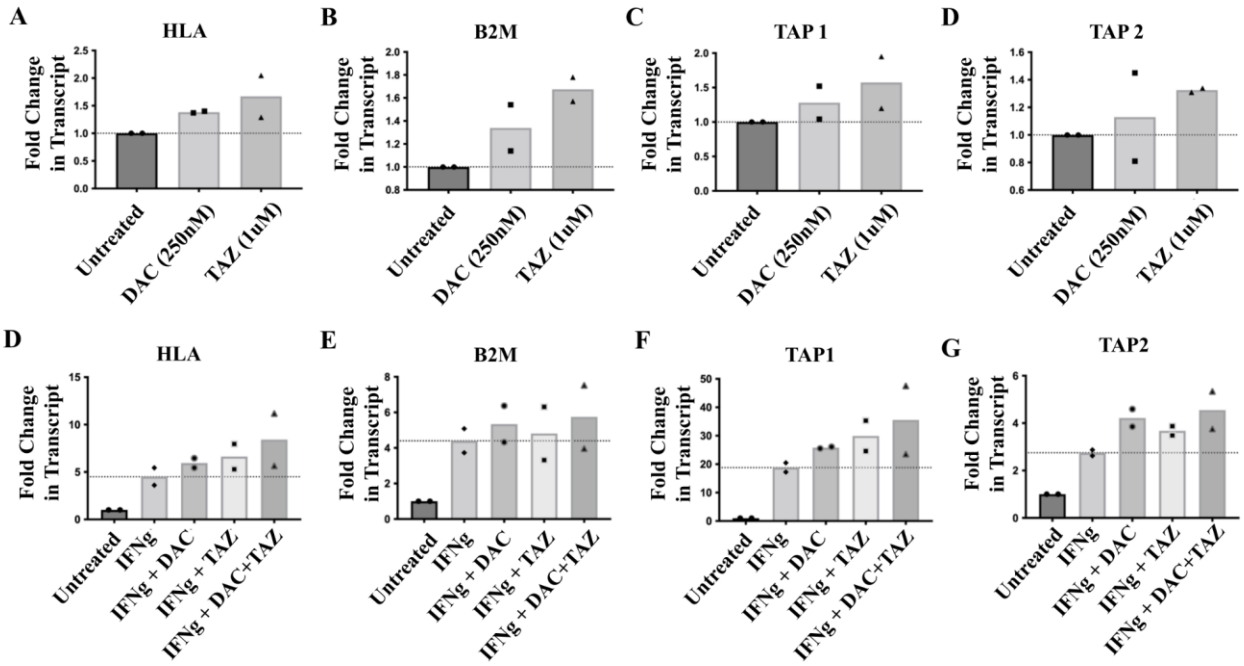
Supplemental Figure 5. Decitabine and tazemetostat both regulated HLA expression in DLBCL in a dose responsive manner. (A-B) SUDHL-4 cells were treated with 100 ng/mL Interferon-γ. (A) Dose responses of DAC were performed in the absence (left) or presence (right) of 625nM TAZ. (B) Dose responses of TAZ were performed in the absence (left) or presence (right) of 250nM DAC. (C) The full matrix of (A-B) is shown here with MFI values for HLA-A2. (D-E) A dose response of IFN-g was performed plus or minus DAC and TAZ on (D) DB or (E) SUDHL-6 cells. Mean and standard deviation displayed for N=3 technical replicates. A student's T test was performed between untreated and DAC + TAZ at each IFNg concentration. * p<.05, ** p<.01, *** p<.001, **** p<.0001.



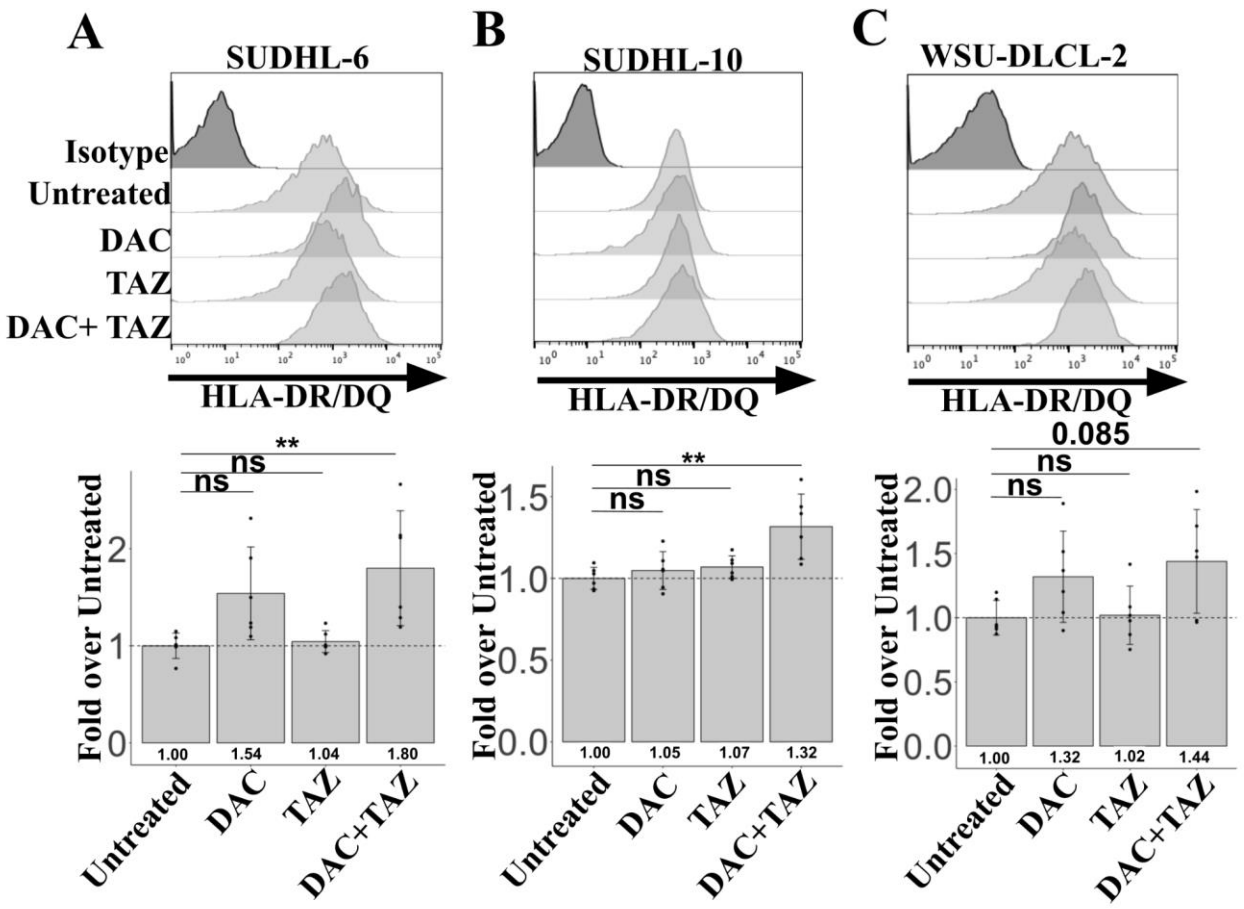
Supplemental Figure 6. Tazemetostat upregulated HLA expression on DLBCL in a dose dependent manner. (A) SUDHL-4 cells and (B) DB cells were treated with indicated concentrations of TAZ. Left panel demonstrates the HLA-ABC expression and the right panel shows the HLA-A2 expression. (C-D) Cells were treated as in (A-B) in addition to 100ng/mL IFNg.



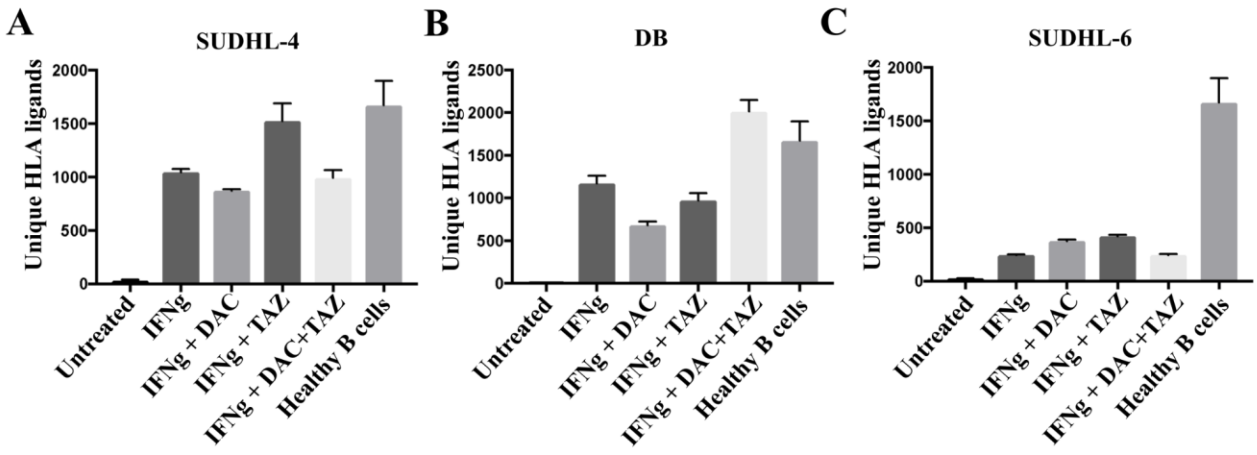
Supplemental Figure 7. Tazemetostat does not upregulate HLA in EZH2 WT DLBCL. (A) SUDHL-4 cells were treated with 1uM TAZ, 10ng/mL IFNg, or the combination of both. HLA-ABC and HLA-A2 surface expression was assayed by flow cytometry. Mean fluorescence intensity numbers are displayed. (B) SUDHL-4 cells were treated with 125nM DAC, 10ng/mL IFNg or the combination of both. Flow analysis displayed as in A. (C) RI-1 cells treated as in A. (D) RI-1 cells treated as in B. (E) HLA A2- U9-293 cells treated as in A. (F) HLA A2- U9-293 cells treated as in B.



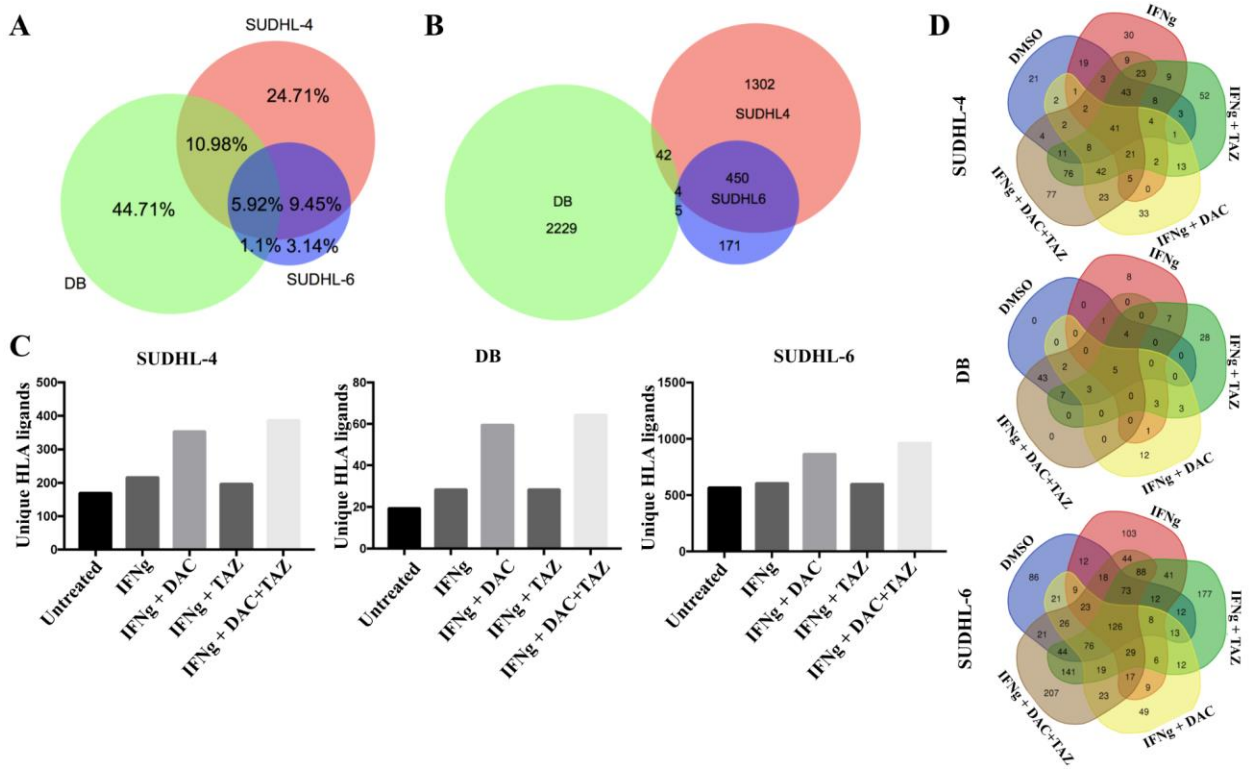
Supplemental Figure 8. SUDHL-4 cells showed modest increases in antigen presentation transcript. (A-D) SUDHL-4 cells were treated with either 250nM DAC, 1uM TAZ and transcript levels of (A) HLA-A, (B) beta-2-microglobulin, (C) TAP 1, and (D) TAP 2 were assessed. (E-F) Cells were treated as in (A-D) in addition to IFNg. A line denotes the level for IFNg treated alone. Mean and technical replicates displayed for 2 biological replicates.



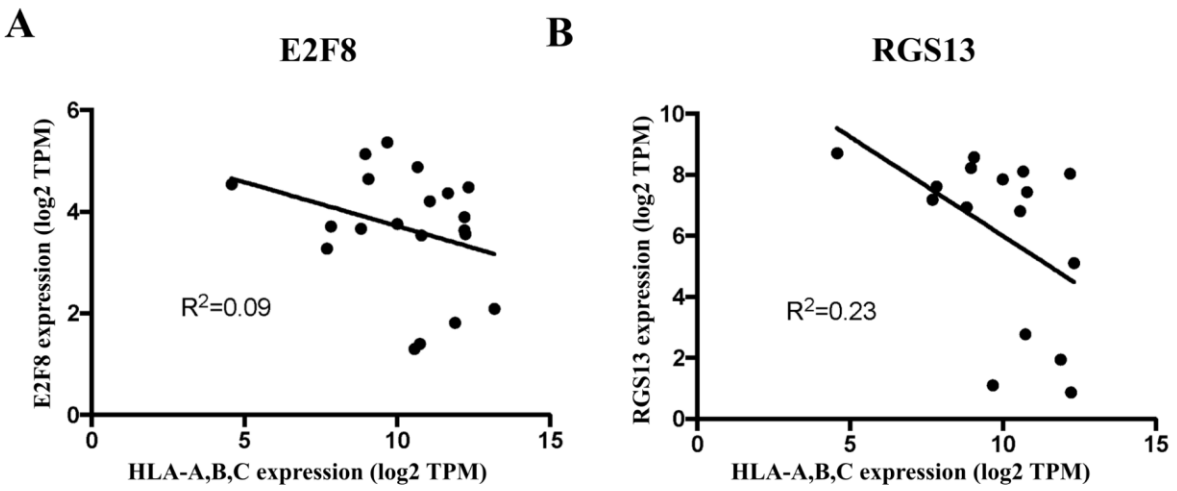
Supplemental Figure 9. Decitabine upregulated HLA Class II in DLBCL cell lines. (A-C) Cells were treated with indicated 125nM DAC, or 1uM TAZ for (A) SUDHL-6 (B) SUDHL-10 (C) WSU-DLCL-2 cell lines, and assayed for expression of HLA-DR/DQ. Mean and standard deviation displayed for N=3 technical replicates per 2 biological replicates. * p<.05, ** p<.01, *** p<.001, **** p<.0001.



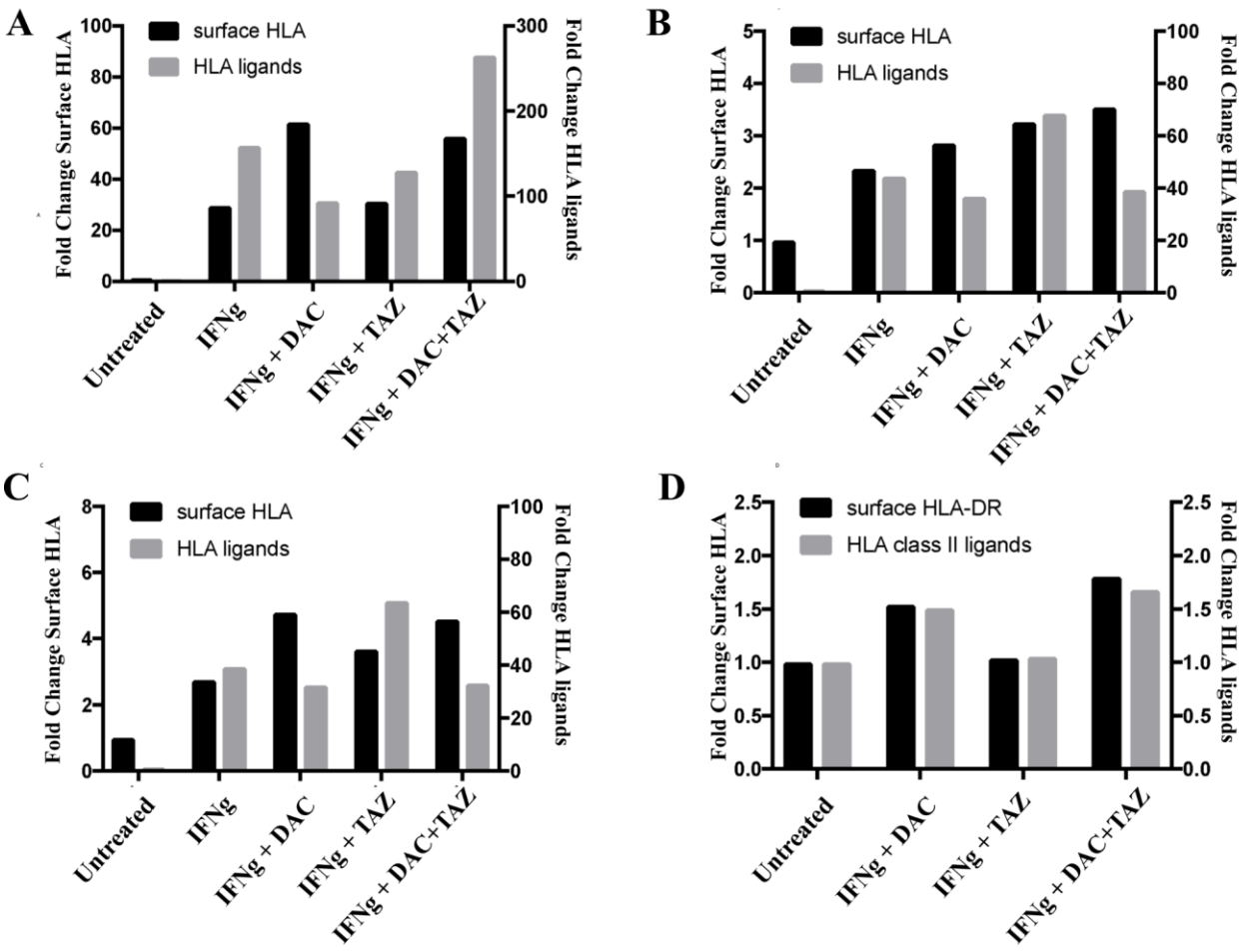
Supplemental Figure 10. Epigenetic drug treatment in combination with IFNg induced presentation of HLA ligands in DLBCL cell lines and healthy B cells. Cancer cells were treated with 125 nM DAC, 1uM TAZ and 100 ug/ml IFNg. B cells were isolated through CD19 MACS positive selection. Treatments were applied to (A) SUDHL-4, (B) DB and (C) SUDHL-6 cells. Results show unique HLA ligands identified by mass spectrometry after assignment by netMHCpan4.0. Results are shown for 2 biological replicates. Error bars indicate mean with range.



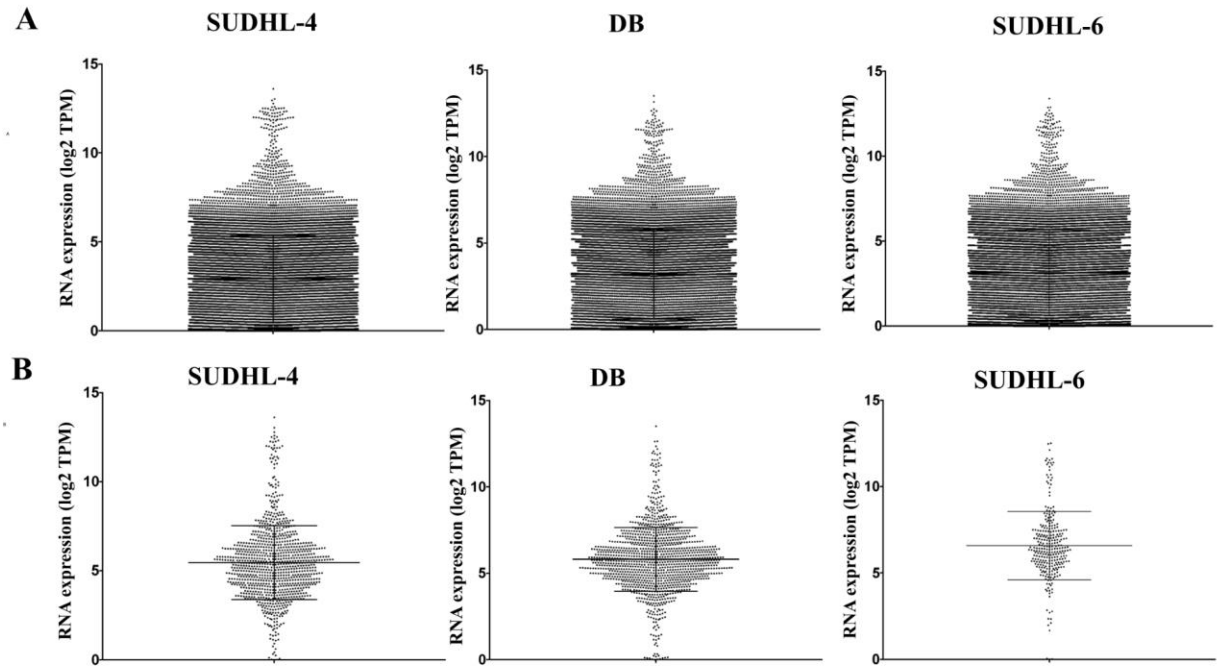
Supplemental Figure 11. HLA class I and HLA class II peptide characteristics. (A)Venn diagram of source proteins with percent overlap between conditions (B) Venn diagram of HLA class I peptides for DB, SUDHL-4 and SUDHL-6 cells. (C) Unique HLA class II numbers according to different treatment strategies. (D) Venn diagrams for the treatment strategies shown in (C).



Supplemental Figure 12. Correlation of HLA-A,B,C expression with E2F8 and RGS13 gene expression in DLBCL cell lines. Expression data were downloaded from the CCLE[48] and added for HLA-A, HLA-B and HLA-C. Then HLA-A,B,C expression was plotted against (A) E2F8 expression and (B) RGS13 expression. Transcripts with 0 reads were excluded. No significant correlations were observed.



Supplemental Figure 13. Data summary for surface HLA levels and the corresponding immunopeptidome. Data are presented as fold changes over untreated cell lines. Left y-axis depicts HLA surface levels by flow cytometry, right y-axis shows HLA ligands for (A) DB – HLA class I (B) SUDHL-4 – HLA class I (C) SUDHL-6 – HLA class I (C) SUDHL-6 – HLA class II.



Supplemental Figure 14. RNA expression levels of whole cell transcriptome and corresponding source proteins for HLA ligands. (A) RNA expression levels as published by the CCLE[48]for SUDHL-4 (left), DB (middle) and SUDHL-6 (right) cells. (B) RNA expression levels of genes which contributed to the described MHC class I immunopeptidome for SUDHL-4 (left), DB (middle) and SUDHL-6 (right) cells. Means with SD are depicted.