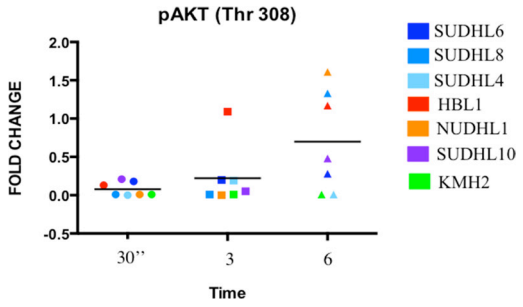
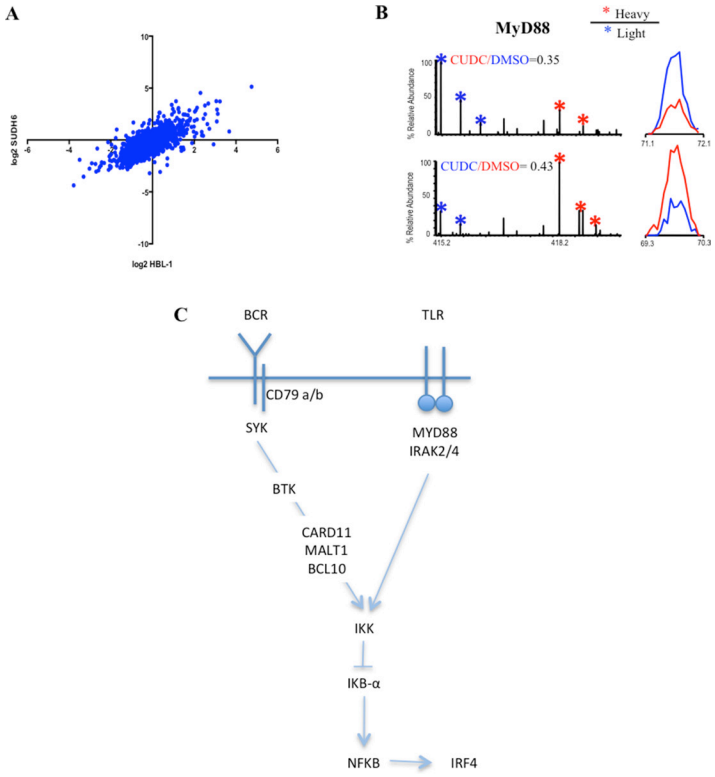
Dual inhibition of histone deacetylases and phosphoinositide 3-kinase enhances therapeutic activity against B cell lymphoma

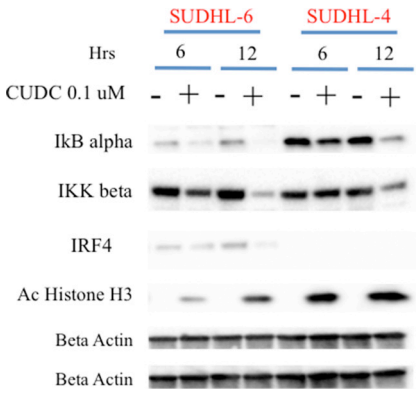
Supplementary Materials



Supplementary Figure 1: Scatterplot summarizing the results of phospho AKT (Thr 308) assessed by luminex multiplex assay, showing significant decrease in phosphorylation levels at 30 minutes in our cell line panel (*n* = 7) after treatment with CUDC-907 0.1 µM for 24 hours. However, AKT phosphorylation progressively increased in most cells in time dependent manner.



Supplementary Figure 2: (**A**) Scatterplot of quantitative proteomics data showing protein changes in HBL-1 (x axis) and SUDHL-6 cells (y axis) in a log2 scale. Average log2 fold change of forward and reverse experiments of HBL-1 and SUDHL-6 cells were used to generate the scatterplot. (**B**) Representative MS spectra from MyD88 are shown with forward (top panel) and reverse (lower panel) experiments after treatment with CUDC-907 0.1 µM for 24 hours in HBL-1. The selected ion current chromatograms for the (M+2H)2+ ions from tryptic peptide LLELLTK for the forward and reverse experiments are shown to the right of the associated spectrum. The light isoform (m/z = 415.3) is shown in blue and heavy isoform (m/z = 418.3) is shown in red. The integrated area under the curve was used to calculate the ratio of the two isoforms. (**C**) Schematic representation of the B-cell receptor (BCR) and Toll like receptor (TLR) pathways, which converge to activate NF-kB.



Supplementary Figure 3: Western blot demonstrating that CUDC-907 (0.1 µM for 6 and 12 hours) inhibits NF-kB activation by increasing IkB alpha levels and decreasing IKK beta levels in two representative GCB DLBCL cell lines (SUDHL-6 and SUDHL-4). The changes were associated with a decrease in IRF4 levels.

Supplementary Table 1: Table summarizing the CUDC-907 IC50 dose at 72 hours for all the lymphoma cell lines used

Cell line	CUDC-907 IC50 (µM) 72Hrs
HDLM2	0.6843
KMH2	0.2023
SUDHL-4	0.1224
L-428	0.0919
BJAB	0.0498
HBL-1	0.0394
DB	0.0355
NUDHL-1	0.0262
SUDHL-10	0.0254
RAMOS	0.0243
RAJI	0.0218
Ri-1	0.0182
U-2932	0.0153
CA-46	0.0135
SUDHL-6	0.0119
OCI-LY-19	0.0089
SUDHL-8	0.0087
TMD-8	0.0070
OCI-LY-10	0.0047
U-2973	0.0042

Supplementary Table 2: Table summarizing the panobinostat and BKM-120 IC50 dose at 48 hours for DLBCL cells

Cell line	Panobinostat IC50 (µM) 48 Hrs	BKM-120 IC50 (µM) 48 Hrs
HBL-1	0.033	0.65
TMD-8	0.085	0.9
U-2932	0.62	1.2
SUDHL-8	0.12	0.5
SUDHL-6	0.05	0.8
SUDHL-4	0.025	1.5
OCI-LY-19	0.01	0.1
NUDHL-1	0.007	0.5
SUDHL-10	0.05	0.8
U-2973	0.40	0.4

Supplementary Table 3: Proteins that are commonly down or up-regulated following treatment of HBL-1 and SUDHL-6 cells with 0.1 µM CUDC-907 for 24 hours. See_Supplementary_Table 3

Supplementary Table 4: Table summarizing the cell lines used including cell of origin and source

Cell line	Cell of Origin	Source
Diffuse Large B cell Lymphoma		
SUDHL-4	GCB	DSMZ
SUDHL-6	GCB	DSMZ
OCI-LY-19	GCB	DSMZ
SUDHL-8	GCB	ATCC
BJAB	GCB	MDCC (Davis lab)
U-2932	ABC	DSMZ
OCI-LY-3	ABC	DSMZ
Ri-1	ABC	DSMZ
HBL-1	ABC	MDCC (Davis lab)
TMD-8	ABC	MDCC (Davis lab)
OCI-LY-10	ABC	WCMC (Cesarman lab)
NUDHL-1	DH	DSMZ
SUDHL-10	DH	ATCC
Hodgkin Lymphoma		
HDLM2	-	DSMZ
KMH-2	-	DSMZ
L-428	-	DSMZ
Large Cell Lymphoma B-Lymphoblast		
DB	-	ATCC
Burkitt Lymphoma		
RAJI	-	ATCC
RAMOS	-	ATCC
CA-46	-	ATCC