Figure Legends

Figure S1. Effect of panobinostat (LBH589) and everolimus (RAD001) depending on the dose in HL cell lines. (A) Cells were incubated with diluent control, LBH589 (0.02-1μM), RAD001 (0.1μM) or the combination of LBH589 and RAD001 and after 24 hours, cell viability was determined by an MTS assay and the dose-effect curves were determined using the Calcusin software. Data represents the mean of three independent experiments performed in triplicate. (B) Combination index values for LBH589 plus RAD001 in HL cells lines after 24hours. Values <1 indicate synergy.

Figure S2. Effects of panobinostat (LBH589) and everolimus (RAD001) combination on apoptosis in HL cell lines. HL cell lines were incubated for 48 hours with diluent control, LBH589 (0.02-0.1 μM), RAD001 (0.1 μM) or the combination of LBH589 and RAD001 and, intracellular protein levels were examined by Western blot. LBH589 induced cleavages of caspase 9, and PARP and, decreased XIAP level in all cell lines, whereas RAD001 had no effect on these proteins.

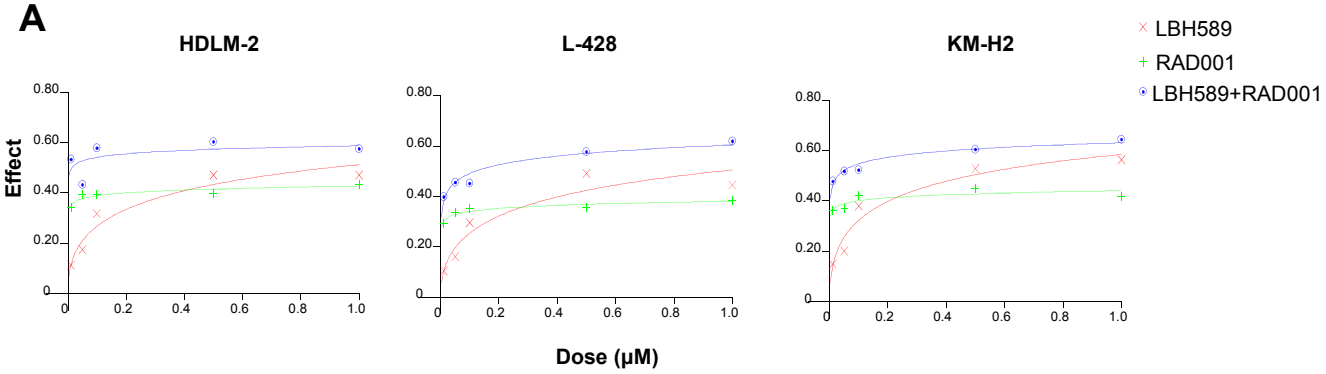
Figure S3. Effects of panobinostat (LBH589) and everolimus (RAD001) combination on cytokine secretion in HL cell lines. Cells were incubated with diluent control or LBH589 (0.02-0.1μM), RAD001 (0.1μM) or the combination of LBH589 and RAD001 and after 48 hours, supernatants were examined for cytokine levels using a multiplex assay. (A) Heatmap of the cytokine concentration data transformed in log2 for all 3 HL cell lines. The data is normalized and presented in reference to the mean value of all points (5 pg/ml). Thus, red color indicates values > 5 pg/ml, and green color represent values < 5 pg/ml. As shown, all cell lines produced low concentrations of IL10, EGF, Eotaxin, IL1-beta, IL4, IL2, and interferon gamma. The blue, green and red bars on top of the figure denote different cell lines. (B) Effect of drug treatment on the level of 30 cytokines in the HL cell lines. Data presented in reference to base line concentration (DSMO-treated) of each cytokine, which is shown in black color. Red color indicates an incres in cytokine level above baseline, and green color indicates a decrease in cytokine level below the base line. The colored bars on top of each panel indicate different treatment conditions.

Figure S4. Effects of higher doses of panobinostat (LBH589) and everolimus (RAD001) combination on STATs activation in HL cell lines. Cells were incubated with diluent control or LBH589 (0.5 μM), RAD001 (0.5 μM) or the combination of LBH589 and RAD001 and after 48 hours, cell lysates were prepared and resolved by SDS-PAGE, transferred to nitrocellulose membrane and probed with anti-phospho-STAT3, anti-STAT3, anti-phospho-STAT5, anti-STAT5, anti-phospho-STAT6 and anti-STAT6 antibodies. Higher concentrations of LBH589 was more potent than lower concentrations (Figure 5B) in inhibiting STAT3 phosphorylation Figure S5.

Figure 5. Effects of panobinostat (LBH589) on STATs activation in 2 other HL cell lines, L-540 and HD-MyZ. (A) Cells were incubated with diluent control or LBH589 (0.1μM), RAD001 (0.1μM) or the combination of LBH589 and RAD001 and after 12, 24 and 48 hours, cell lysates were prepared and resolved by SDS-PAGE, transferred to nitrocellulose membrane and probed with anti-phospho-STAT3 (Tyr 705), anti-STAT3, anti-phospho-STAT5 (Tyr 694), anti-STAT5,

anti-phospho-STAT6 (Tyr 641), anti-STAT6 and anti-beta actin antibodies. (B) Cells were incubated with diluent control or LBH589 (0.1μM), and after 48 hours, supernatants were examined for TNF-alpha levels.

A



B

LBH589 (μM)	RAD001 (μM)	Combination index		
		HDLM-2	L-428	KM-H2
0.01	0.01	0.01	0.03	0.03
0.05	0.05	0.14	0.08	0.09
0.1	0.1	0.06	0.16	0.1
0.5	0.5	0.22	0.27	0.42
1	1	0.57	0.38	0.58

Fig. S1

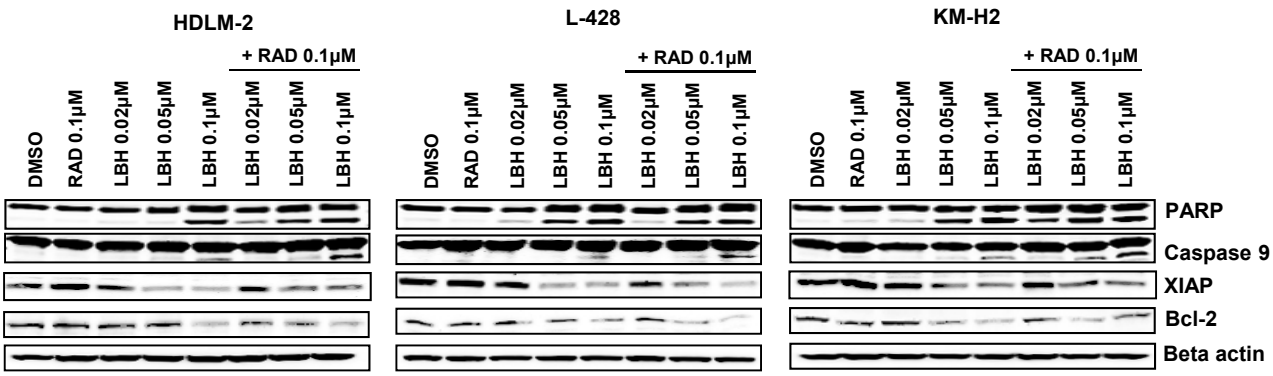
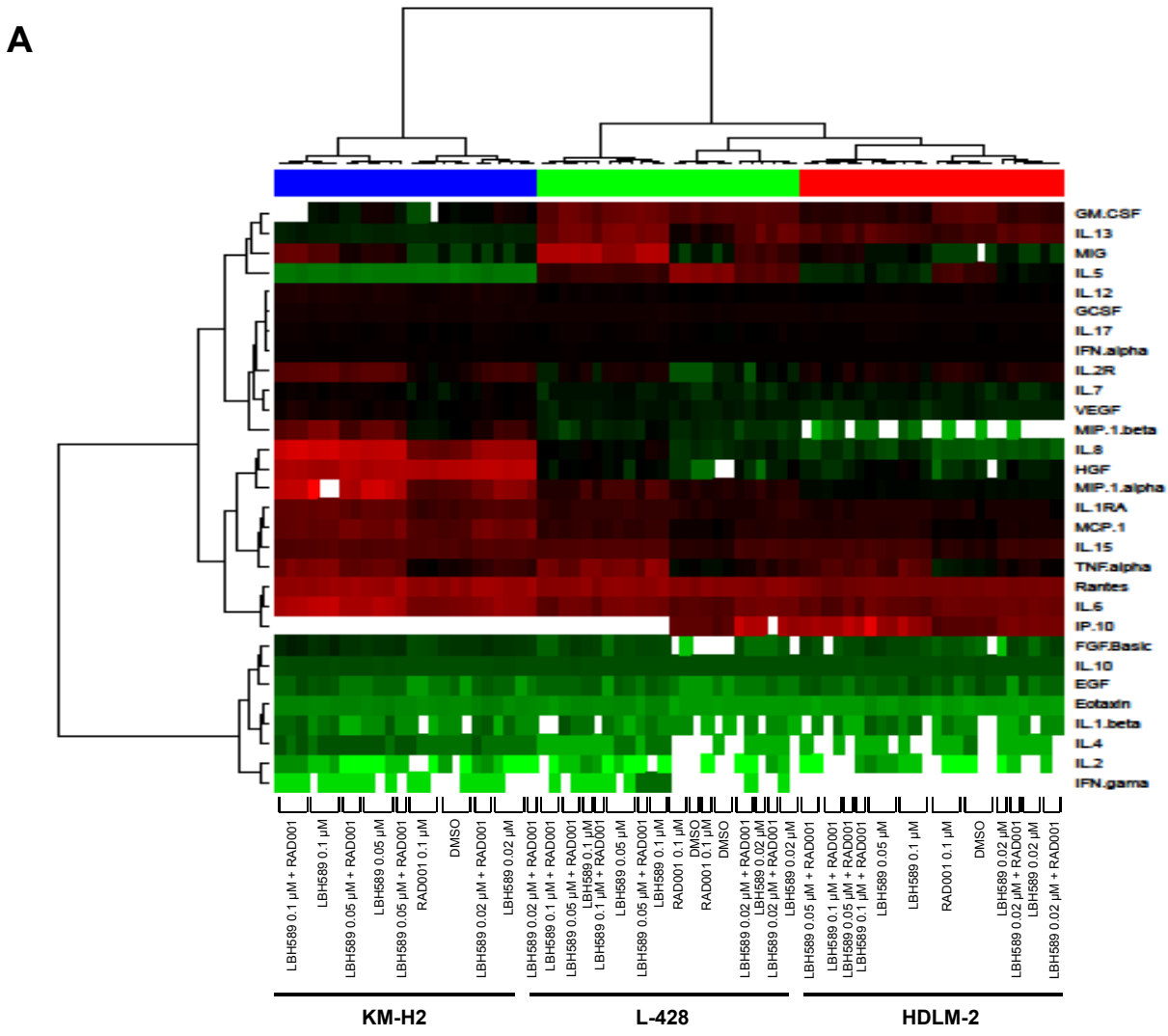

HDLM-2
L-428
KM-H2
+ RAD 0.1μM
DMSO
RAD 0.1μM
LBH 0.02μM
LBH 0.05μM
LBH 0.1μM
PARP
Caspase 9
XIAP
Bcl-2
Beta actin

Fig. S2


A
GM.CSF
IL.13
MIG
IL.5
IL.12
GCSF
IL.17
IFN.alpha
IL.2R
IL.7
VEGF
MIP.1.beta
IL.8
HGF
MIP.1.alpha
IL.1RA
MCP.1
IL.15
TNF.alpha
Rantes
IL.6
IP.10
FGF.Basic
IL.10
EGF
Eotaxin
IL.1.beta
IL.4
IL.2
IFN.gama
LBH589 0.1 µM + RAD001
LBH589 0.1 µM
LBH589 0.05 µM + RAD001
LBH589 0.05 µM
LBH589 0.05 µM + RAD001
RAD001 0.1 µM
DMSO
LBH589 0.02 µM + RAD001
LBH589 0.02 µM
LBH589 0.02 µM + RAD001
LBH589 0.1 µM + RAD001
LBH589 0.1 µM + RAD001
LBH589 0.05 µM
LBH589 0.05 µM + RAD001
LBH589 0.1 µM
RAD001 0.1 µM
DMSO
DMSO
LBH589 0.02 µM + RAD001
LBH589 0.02 µM
LBH589 0.02 µM + RAD001
LBH589 0.02 µM
LBH589 0.05 µM + RAD001
LBH589 0.1 µM + RAD001
LBH589 0.05 µM + RAD001
LBH589 0.1 µM + RAD001
LBH589 0.05 µM
LBH589 0.1 µM
RAD001 0.1 µM
DMSO
LBH589 0.02 µM
LBH589 0.02 µM + RAD001
LBH589 0.02 µM
LBH589 0.02 µM + RAD001
KM-H2
L-428
HDLM-2

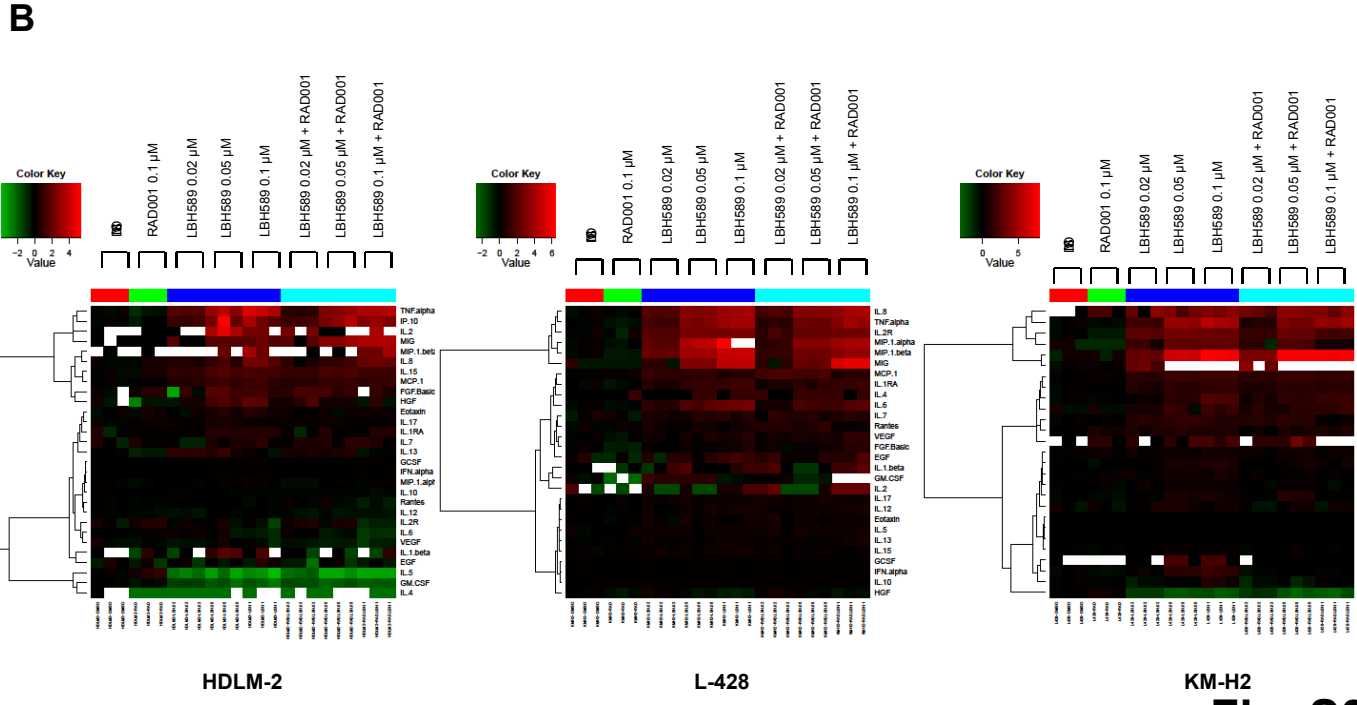

B
Color Key
Value
RAD001 0.1 µM
LBH589 0.02 µM
LBH589 0.05 µM
LBH589 0.1 µM
LBH589 0.02 µM + RAD001
LBH589 0.05 µM + RAD001
LBH589 0.1 µM + RAD001
HDLM-2
L-428
KM-H2

Fig. S3

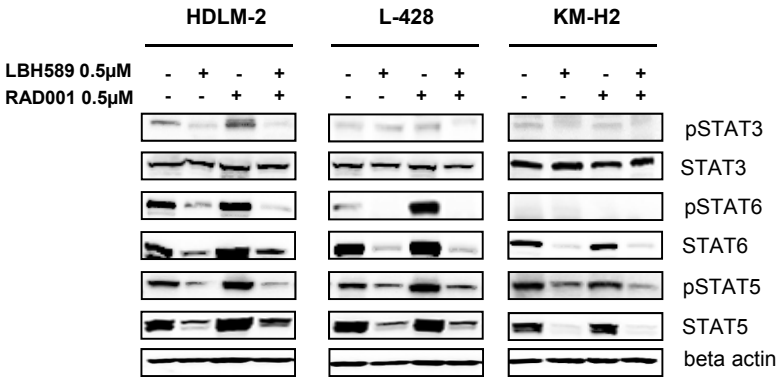
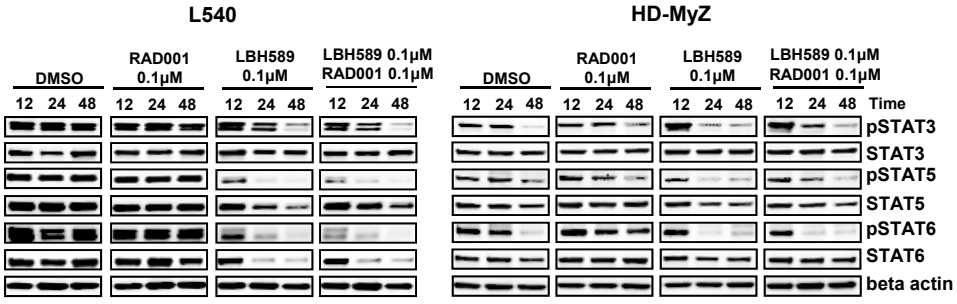

HDLM-2
L-428
KM-H2
LBH589 0.5μM
RAD001 0.5μM
pSTAT3
STAT3
pSTAT6
STAT6
pSTAT5
STAT5
beta actin

Fig. S4

A


L540
HD-MyZ
DMSO
RAD001 0.1μM
LBH589 0.1μM
LBH589 0.1μM RAD001 0.1μM
12 24 48
Time
pSTAT3
STAT3
pSTAT5
STAT5
pSTAT6
STAT6
beta actin

B

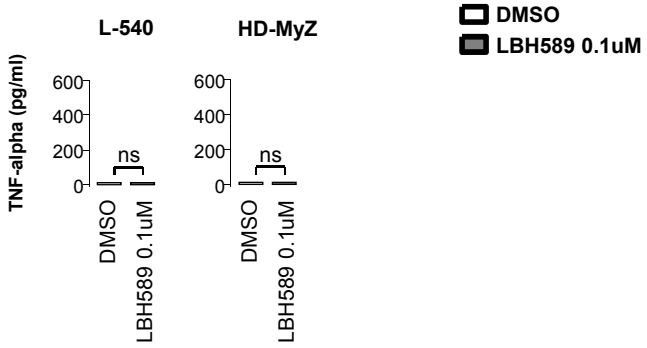

DMSO
LBH589 0.1uM
L-540
HD-MyZ
TNF-alpha (pg/ml)
600
400
200
0
ns
DMSO
LBH589 0.1uM

Fig. S5