Proteasome inhibitors block Ikaros degradation by lenalidomide in multiple myeloma

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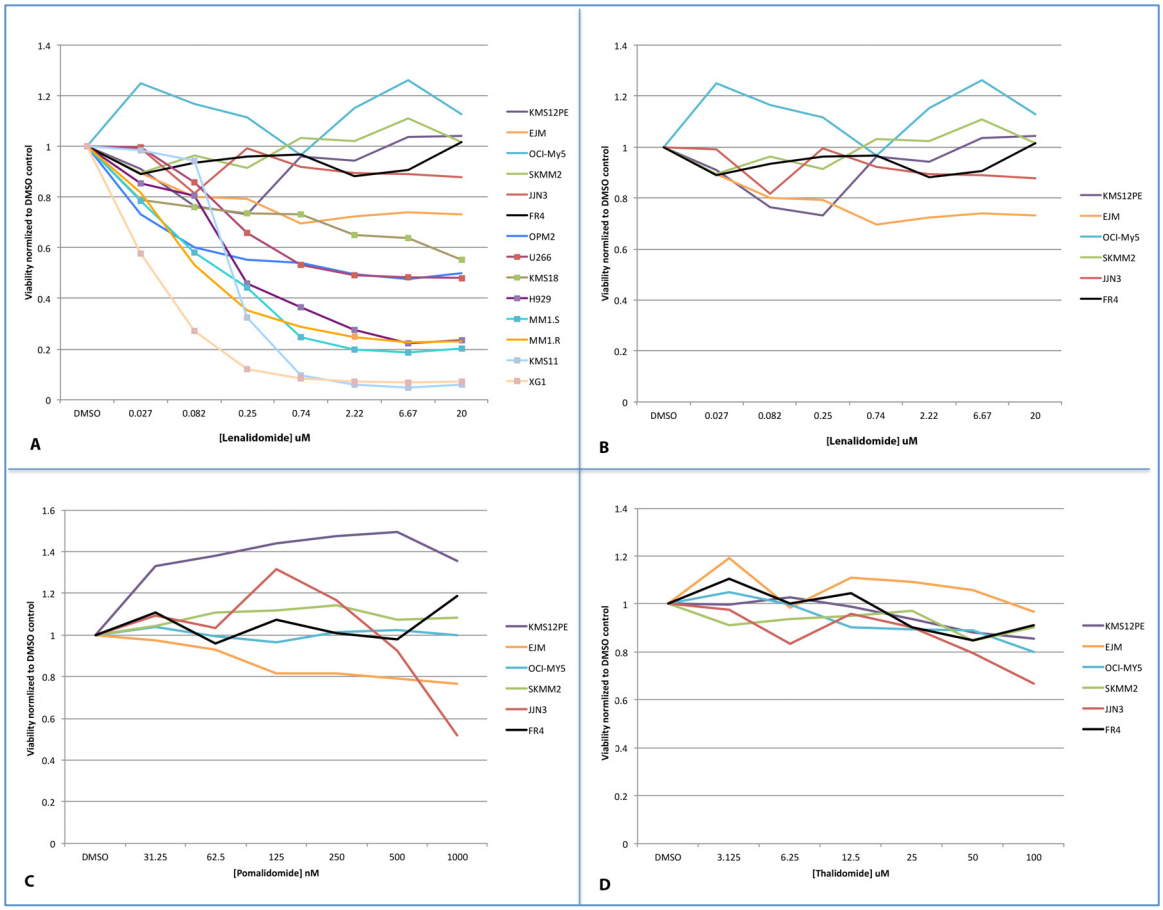


Figure 1 Supp.:

MTT assay of response of MM cell-lines to LEN (A). 14 myeloma cell-lines were treated with indicated doses of LEN and cell viability was measured at day 6 after treatment by MTT. Data were normalized to DMSO control. Figure (B) shows LEN resistant cell-lines, (C) and (D) display MTT results obtained under same condition using THAL and POM respectively.

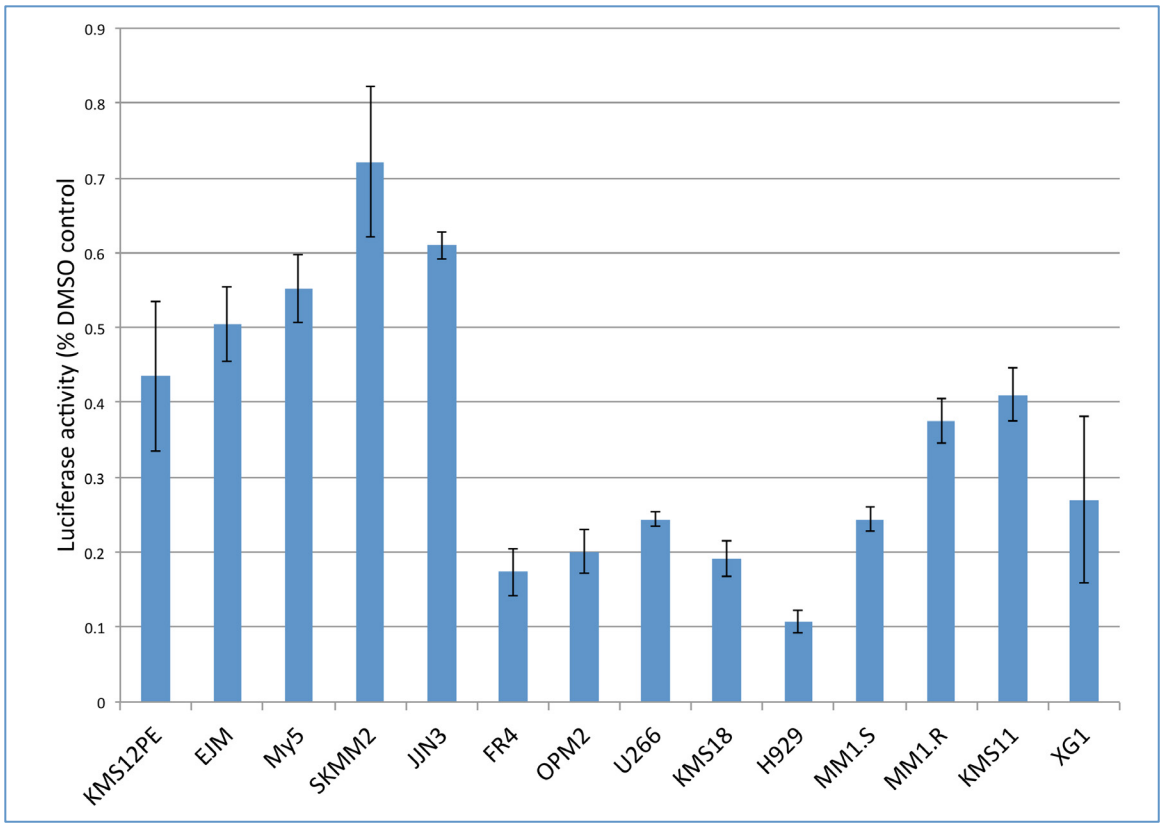
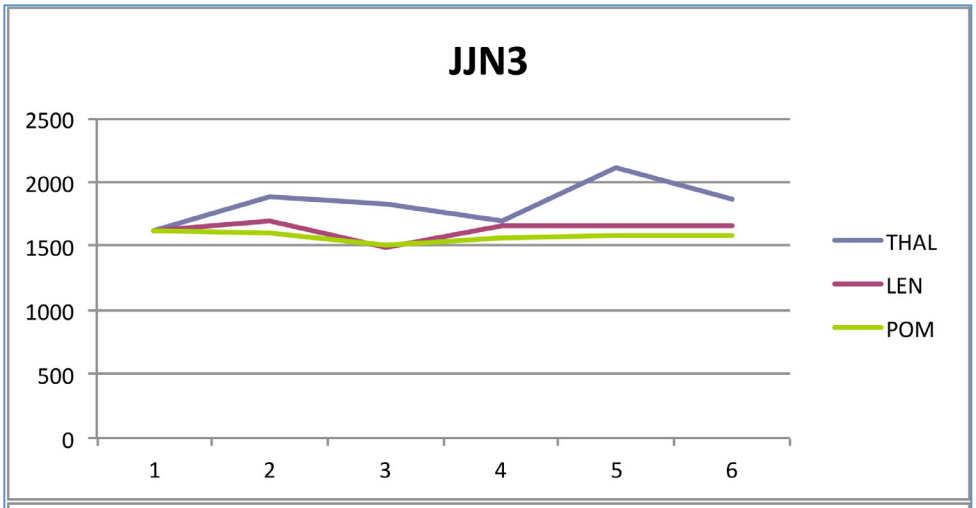
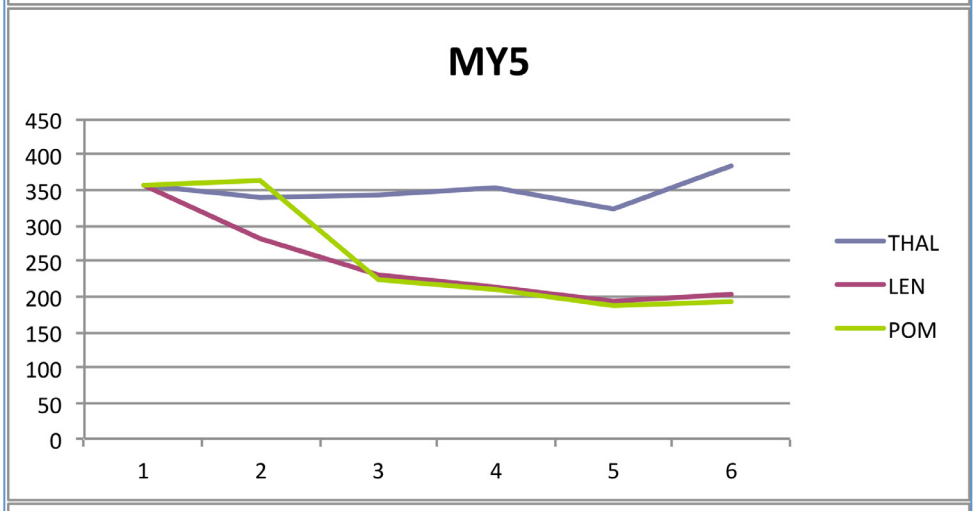
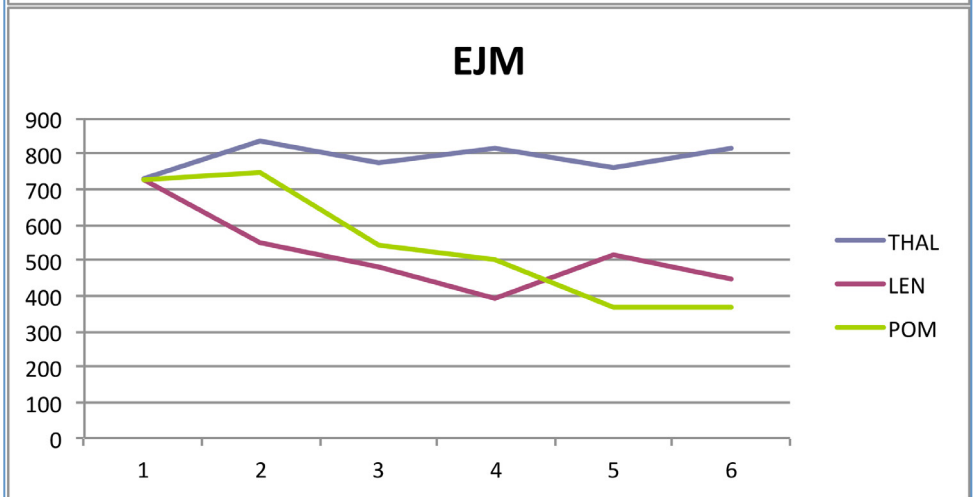


Figure 2 Supp.:

This figure displays Ikaros degradation efficiency after 24 hour of drug treatment. It correlates with response of multiple myeloma cells to LEN therapy using 24 hours drug treatment. 14 myeloma cell-lines with different sensitivity to IMiDs were infected with adenovirus expressing IKZF1-luciferase fusion proteins, after 24 hours, cells were treated with vehicle (DMSO) or LEN for 24 hours. Cells were harvested, lysed and luciferase activities were measured. Data generated from each cell-line were normalized to vehicle treated control.







	1	2	3	4	5	6
Thalidomide(uM)	DMSO	1.95	7.8	31.3	125	500
Lenalidomide(uM)	DMSO	0.0625	0.25	1	4	16
Pomalidomide(uM	DMSO	0.0078	0.0313	0.125	0.5	2

Figure 3 Supp.: IKZF1 degradation in the LEN resistant cell-lines JJN3, EJM and MY5. No reduction is seen in JJN3 to either THAL, LEN or POM whereas MY5 and EJM show IKZF1 reduction to LEN and POM treatment.

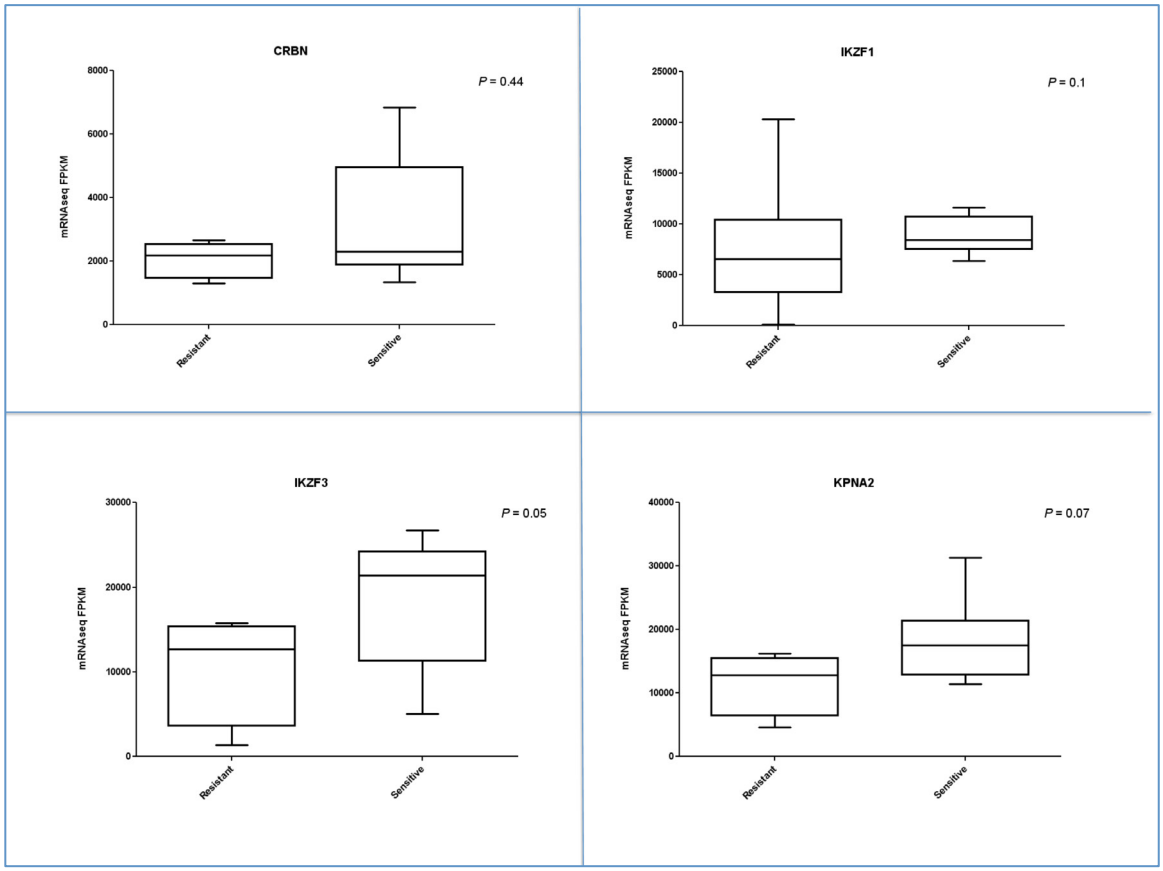


Figure 4 Supp.: RNAseq analysis indicated that CRBN, IKZF1/3 and KPNA2 gene expression tend to be more expressed in the sensitive vs. the resistant/intermediate sensitive cell-lines.

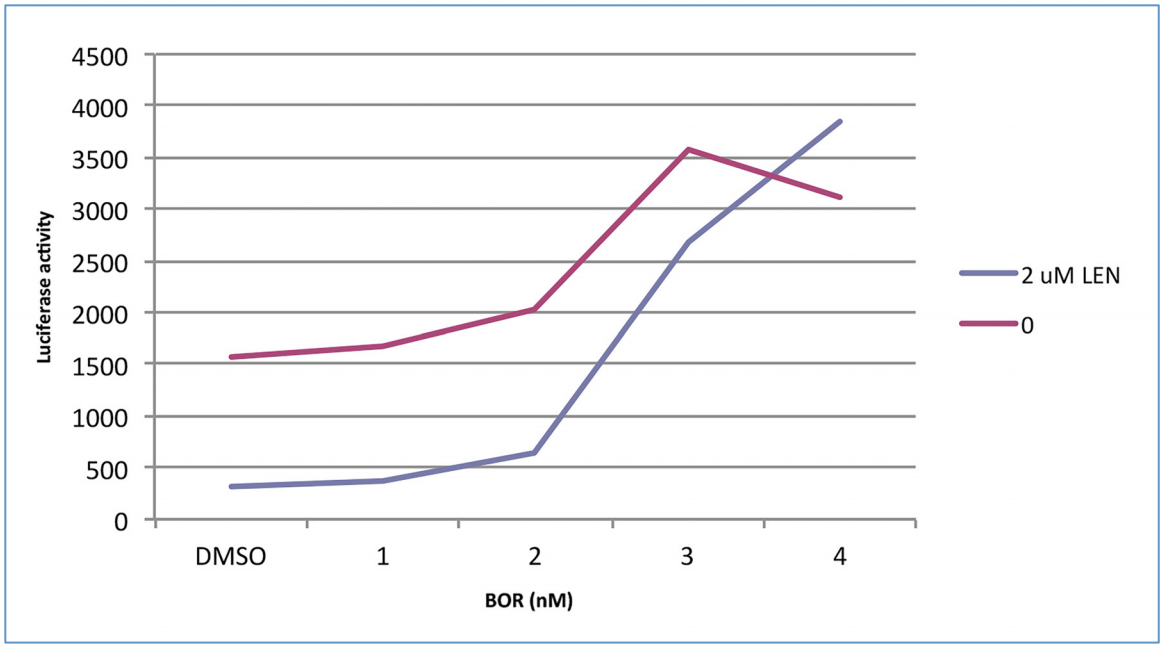
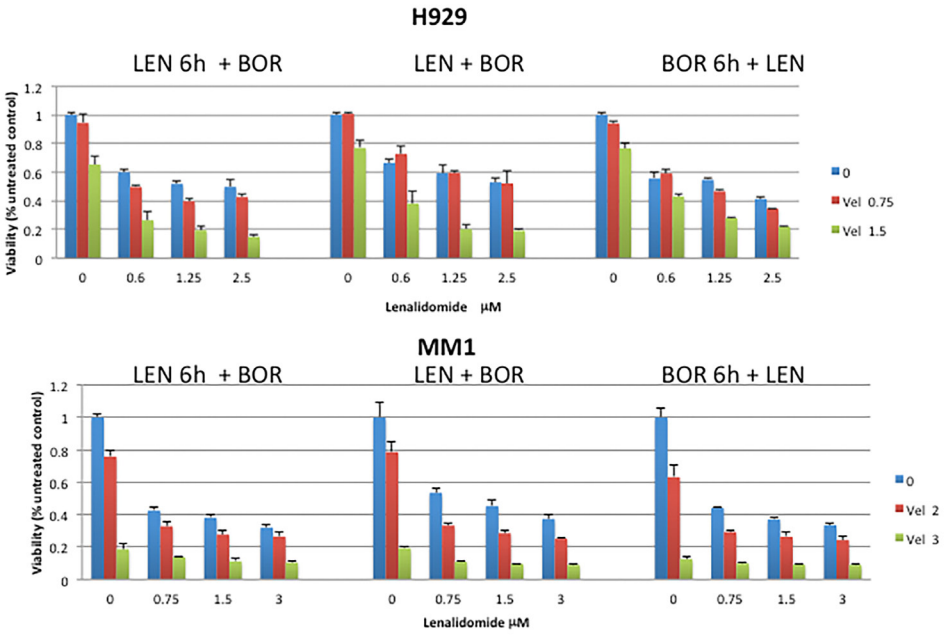


Figure 5 Supp.: Bortezomib abrogates LEN induced IKZF1Luc abrogation in H929 (exposure for 24 hours).



Len + Vel

CI For experimental values

Vel (Len+Vel) (nM)	Len (Len + Vel) (uM)	Fa	CI
0.75	0.6	0.272476	**2.668**
0.75	1.25	0.404997	**1.496**
0.75	2.5	0.478854	**1.427**
1.25	0.6	0.354946	**1.664**
1.25	1.25	0.498842	**1.21**
1.25	2.5	0.599147	**1.09**
1.5	0.6	0.61885	**1.033**
1.5	1.25	0.797847	**0.958**
1.5	2.5	0.808531	**0.964**

6 Hour Len + Vel

CI For experimental values

Vel (6H Len + Vel) (nM)	Len (6H Len + Vel) (uM)	Fa	CI
0.75	0.6	0.50325	**0.657**
0.75	1.25	0.602767	**0.477**
0.75	2.5	0.572642	**0.78**
1.25	0.6	0.580765	**0.672**
1.25	1.25	0.695043	**0.527**
1.25	2.5	0.725121	**0.51**
1.5	0.6	0.734776	**0.563**
1.5	1.25	0.805793	**0.489**
1.5	2.5	0.851281	**0.439**

6 Hour Vel + Len

CI For experimental values

Vel (Len + 6H Vel) (nM)	Len (Len + 6H Vel) (uM)	Fa	CI
0.75	0.6	0.408746	**1.675**
0.75	1.25	0.533742	**1.145**
0.75	2.5	0.654098	**0.797**
1.25	0.6	0.569682	**0.962**
1.25	1.25	0.720647	**0.705**
1.25	2.5	0.780409	**0.647**
1.5	0.6	0.7757	**0.702**
1.5	1.25	0.852013	**0.607**
1.5	2.5	0.887262	**0.559**

MM1S

CI For experimental values

Len + Vel

Vel (Len + Vel) (nM)	Len (Len + Vel) (uM)	Fa	CI
0.5	0.75	0.516523	**0.806**
0.5	1.5	0.586762	**0.865**
0.5	3	0.646781	**0.982**
0.75	0.75	0.508637	**0.931**
0.75	1.5	0.574052	**1.027**
0.75	3	0.651191	**1.026**
1.5	0.75	0.532033	**1.047**
1.5	1.5	0.5989	**1.107**
1.5	3	0.647041	**1.288**
2	0.75	0.667272	**0.781**
2	1.5	0.714479	**0.812**
2	3	0.747886	**0.888**
3	0.75	0.891373	**0.811**
3	1.5	0.904238	**0.805**
3	3	0.910619	**0.809**

CI For experimental values

6 Hour Len + Vel

Vel (Len +6H Vel) (nM)	Len (Len + 6H Vel) (uM)	Fa	CI
0.5	0.75	0.552288	**1.374**
0.5	1.5	0.624183	**1.126**
0.5	3	0.659235	**1.352**
0.75	0.75	0.607369	**0.879**
0.75	1.5	0.669144	**0.785**
0.75	3	0.680898	**1.135**
1	0.75	0.583175	**1.201**
1	1.5	0.642948	**1.108**
1	3	0.667879	**1.404**
1.5	0.75	0.579591	**1.447**
1.5	1.5	0.670514	**1.051**
1.5	3	0.686907	**1.331**
2	0.75	0.707358	**0.845**
2	1.5	0.736981	**0.853**
2	3	0.757221	**0.914**
3	0.75	0.901645	**0.694**
3	1.5	0.908813	**0.678**
3	3	0.910025	**0.678**

CI For experimental values

6 Hour Vel + Len

Vel (6H Len + Vel) (nM)	Len (6H Len + Vel) (uM)	Fa	CI
0.5	0.75	0.527277	**1.787**
0.5	1.5	0.616458	**1.229**
0.5	3	0.643965	**1.63**
0.75	0.75	0.583669	**1.087**
0.75	1.5	0.618238	**1.305**
0.75	3	0.673137	**1.253**
2	0.75	0.601126	**1.433**
2	1.5	0.662743	**1.337**
2	3	0.678879	**1.662**
3	0.75	0.833065	**1.066**
3	1.5	0.863443	**1.037**
3	3	0.873378	**1.035**

CI > 1 ; antagonism
CI = 1 ; additive
CI < 1 ; synergy

Figure 6 Supp.: (A) MTT of combined LEN and BOR treatment demonstrates synergistic toxic effects in both H929 and MM1.S cell-line. (B) Calcusyn analysis of combined LEN and BOR treatment in H929 and 8226 cell-line (table). Highest synergy is observed when LEN is administered 6 hours before BOR treatment.

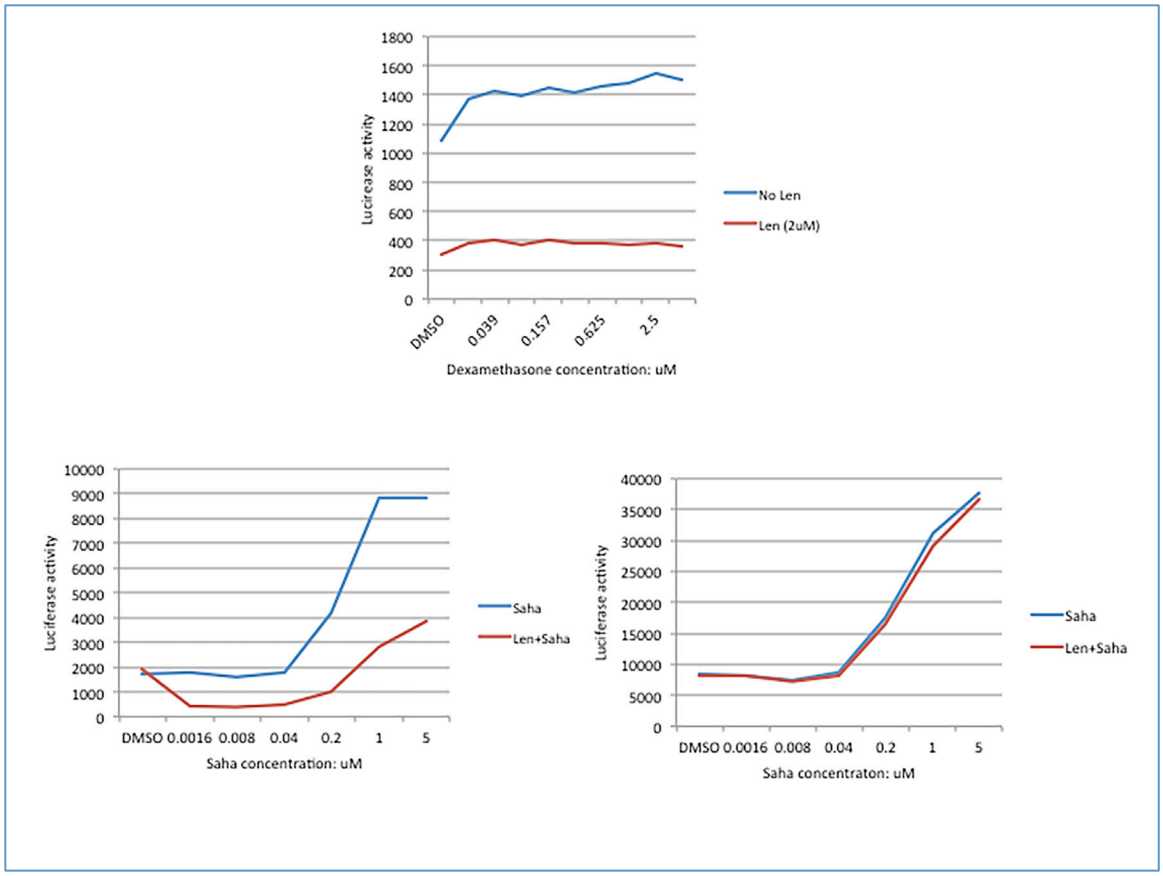


Figure 7 Supp.: No significant influence on LEN-induced IKZF1 degradation by either LEN in combination with DEX or the pan-HDAC inhibitor SAHA. However, SAHA was shown to enhance both luciferase control and IKZF1-luciferase accumulation.