Preclinical evaluation of the simultaneous inhibition of MCL-1 and BCL-2 with the combination of S63845 and venetoclax in multiple myeloma

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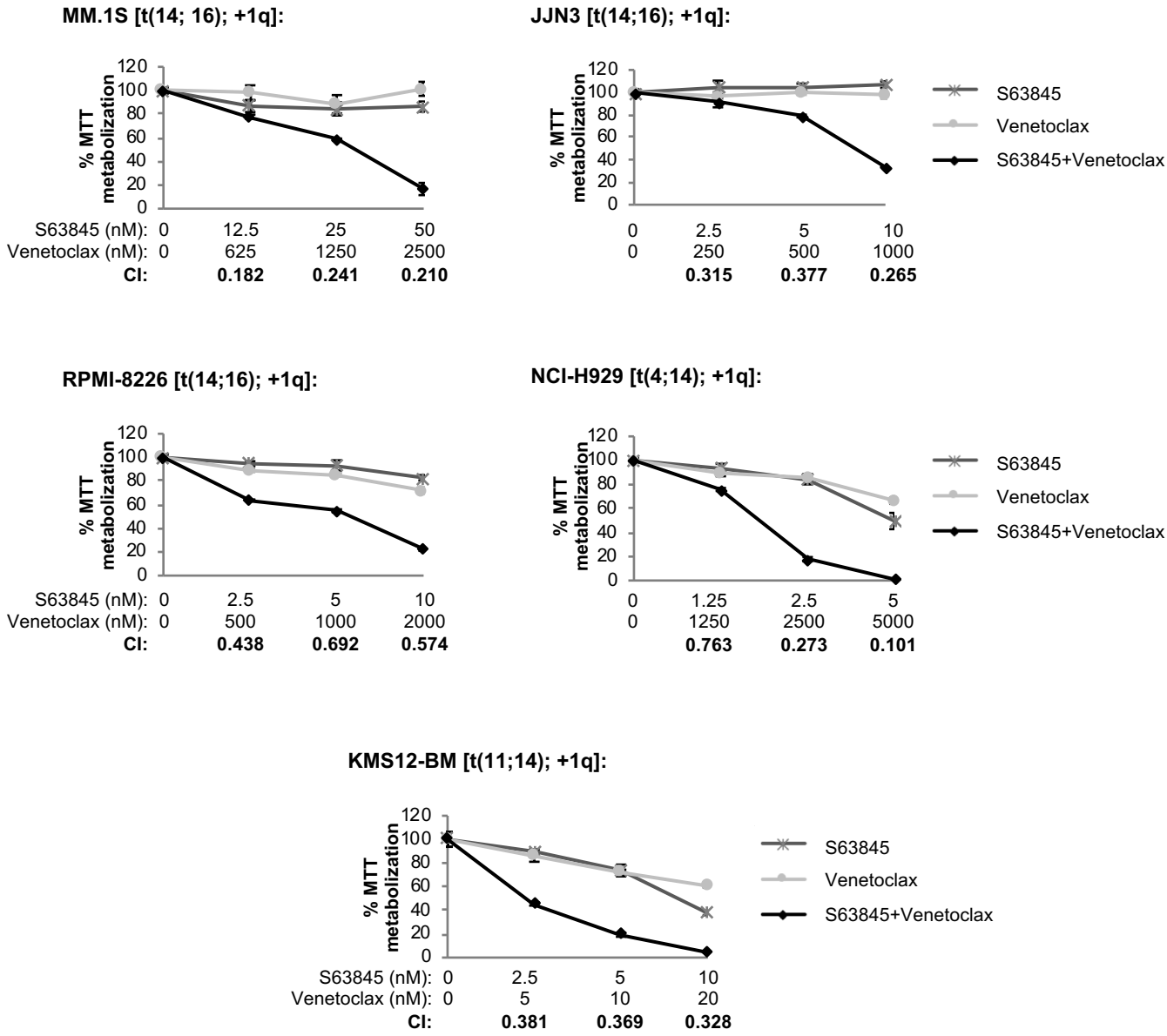
SUPPLEMENTARY FIGURE 1

A

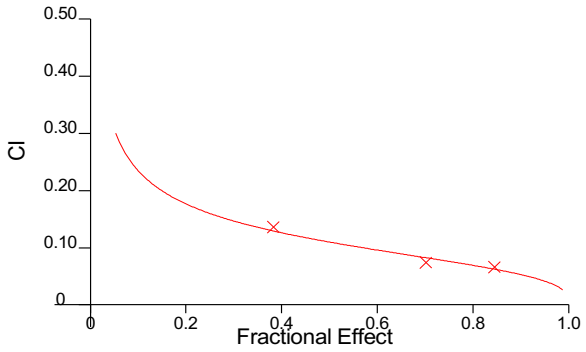


Supplementary Figure 1. S63845 + venetoclax combination has potent *in vitro* anti-myeloma activity. The indicated MM cell lines were incubated with increasing concentrations of S63845 or venetoclax for 48 hours maintaining the ratio of drug concentrations. Cell viability was analyzed by MTT assay. Average absorbance is shown relative to the percentage of the control. Data presented are means ± SD (n=3).

SUPPLEMENTARY FIGURE 2

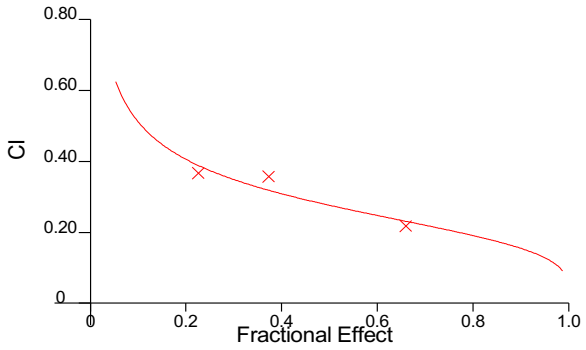
MM.1S:

S63845 (nM)	Venetoclax (nM)	Fa	CI
12.5	625	0.383	0.137
25	1250	0.701	0.075
50	2500	0.845	0.066



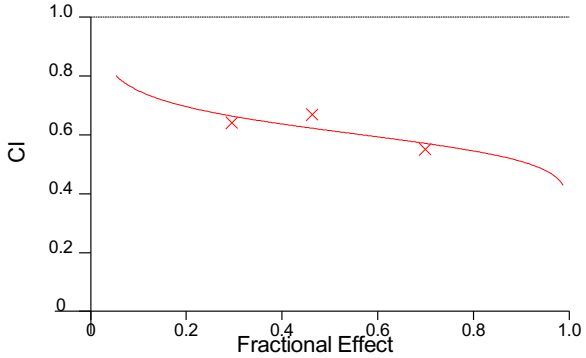
JJN3:

S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	250	0.226	0.368
5	500	0.373	0.358
10	1000	0.659	0.219



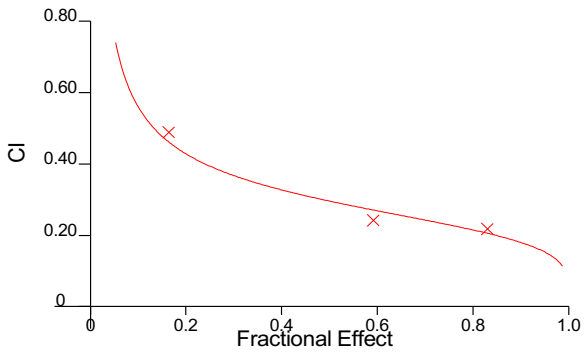
RPMI-8226:

S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	500	0.295	0.642
5	1000	0.463	0.671
10	2000	0.670	0.552



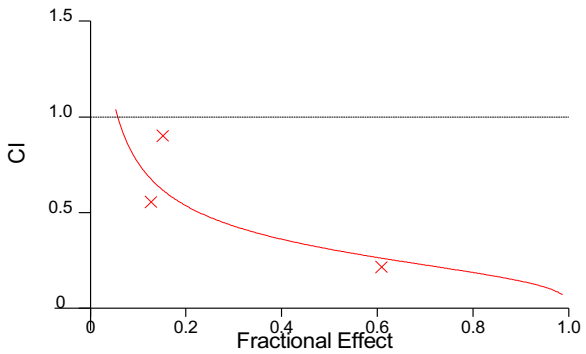
NCI-H929:

S63845 (nM)	Venetoclax (nM)	Fa	CI
0.625	625	0.163	0.489
1.25	1250	0.591	0.243
2.5	2500	0.830	0.219



KMS12-BM:

S63845 (nM)	Venetoclax (nM)	Fa	CI
1.25	2.5	0.127	0.558
2.5	5	0.152	0.903
5	10	0.609	0.218

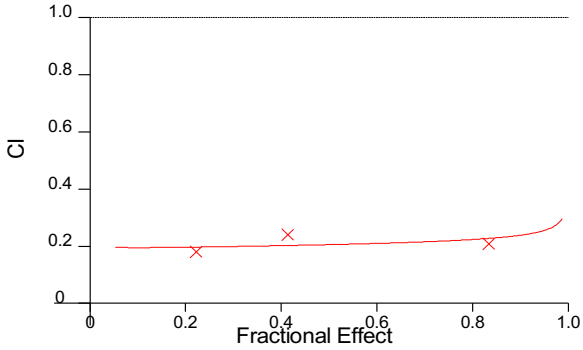


Supplementary Figure 2. The S63845 + venetoclax combination exhibits a strong synergism according to the apoptosis data obtained by flow cytometry. MM.1S, JJN3, RPMI-8226, NCI-H929 and KMS12-BM cells were exposed to increasing doses of S63845 or venetoclax alone or in combination for 48 hours maintaining a constant ratio of drug concentrations. Combination indices (CI) for each point combination were calculated with the Annexin V/PI apoptotic data assessed by flow cytometry and using the Calcusyn program (a CI of 1 indicates an additive effect, CIs < 1 a synergistic effect and CI > 1 antagonism). Since a constant drug ratio combination design was maintained for each cell line, an algebraic estimation of Fa (fraction of cells affected) – CI plot could be inferred with the Calcusyn software.

SUPPLEMENTARY FIGURE 3

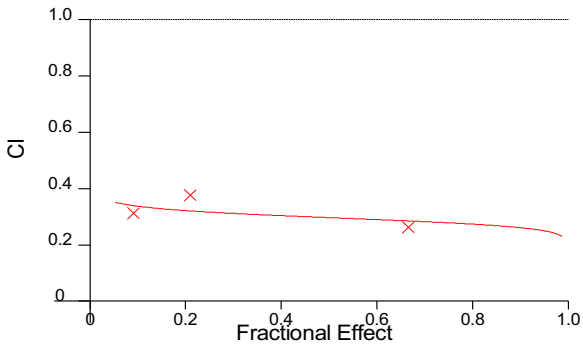
MM.1S:

S63845 (nM)	Venetoclax (nM)	Fa	CI
12.5	625	0.222	0.182
25	1250	0.414	0.241
50	2500	0.834	0.210



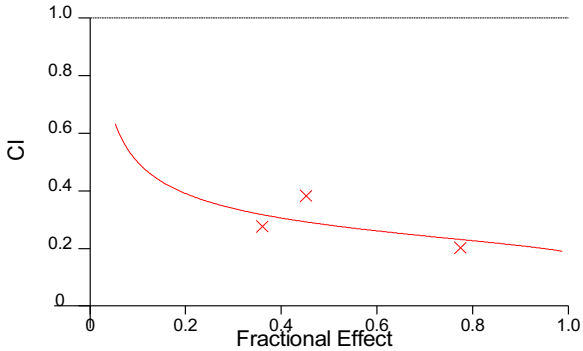
JJN3:

S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	250	0.091	0.315
5	500	0.210	0.377
10	1000	0.665	0.265



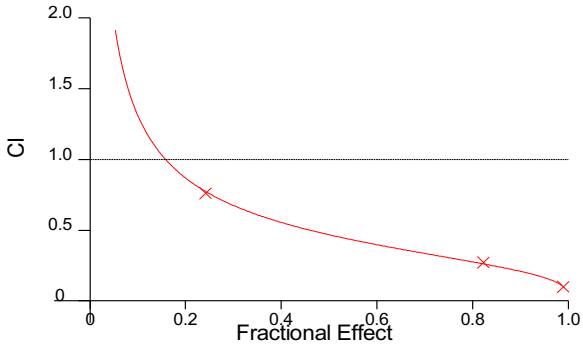
RPMI-8226:

S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	500	0.361	0.278
5	1000	0.452	0.383
10	2000	0.775	0.203



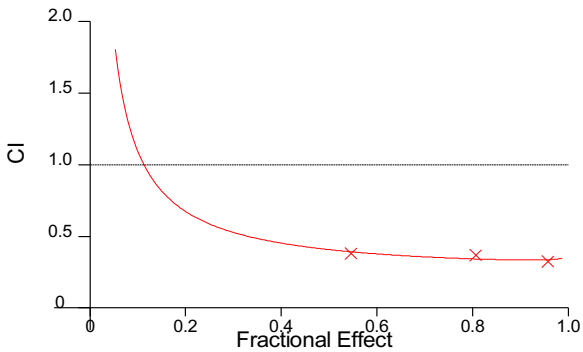
NCI-H929:

S63845 (nM)	Venetoclax (nM)	Fa	CI
1.25	1250	0.242	0.763
2.5	2500	0.823	0.273
5	5000	0.990	0.101



KMS12-BM:

S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	5	0.546	0.381
5	10	0.807	0.369
10	20	0.957	0.328

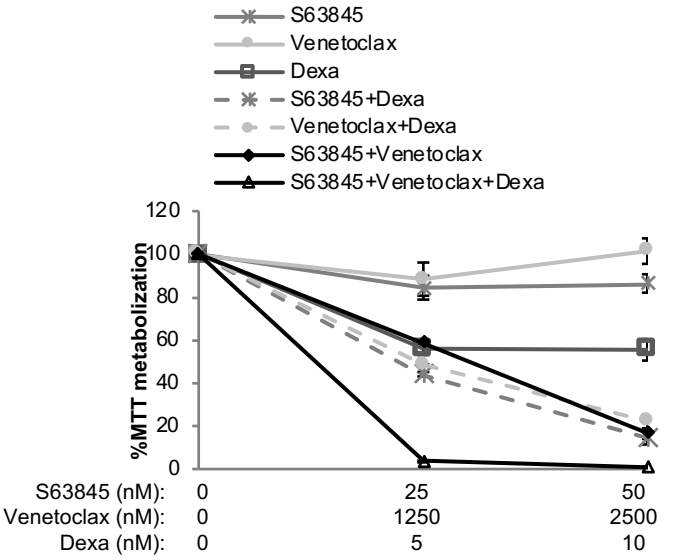


Supplementary Figure 3. The S63845 + venetoclax combination leads to strong synergism as indicated by viability data from MTT assays. The MM.1S, JJN3, RPMI-8226, NCI-H929 and KMS12-BM myeloma cell lines were exposed to increasing doses of S63845 or venetoclax or their combinations for 48 hours maintaining a constant ratio of drug concentrations. Combination indices (CI) for each point combination were calculated with the data obtained from the MTT assays with the Calcusyn program (a CI of 1 indicates an additive effect, CIs < 1 a synergistic effect and CI > 1 antagonism). Since a constant drug ratio combination design was maintained for each cell line, an algebraic estimation of a Fa (fraction of cells affected) – CI plot could be inferred with the Calcusyn software.

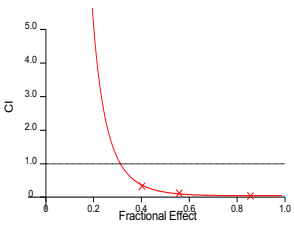
SUPPLEMENTARY FIGURE 4

A

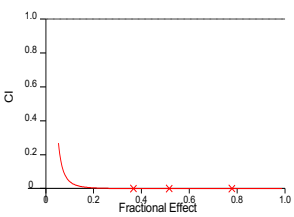
MM.1S:



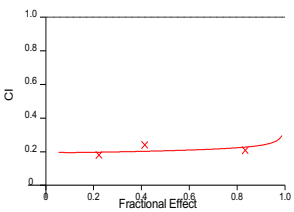
S63845 (nM)	Dexa (nM)	Fa	CI
12.5	2.5	0.403	0.331
25	5	0.559	0.128
50	10	0.856	0.048



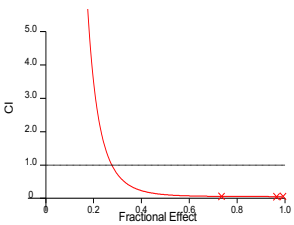
Venetoclax (nM)	Dexa (nM)	Fa	CI
625	2.5	0.367	0.556
1250	5	0.516	0.250
2500	10	0.778	0.181



S63845 (nM)	Venetoclax (nM)	Fa	CI
12.5	625	0.222	0.182
25	1250	0.414	0.241
50	2500	0.834	0.210

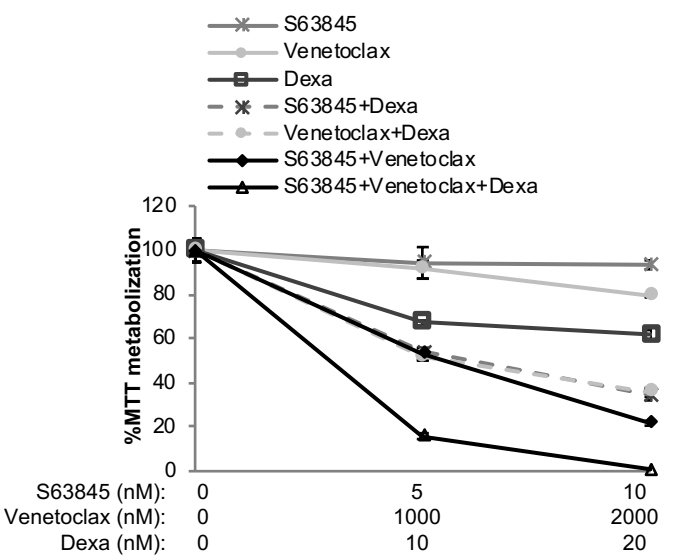


S63845 (nM)	Venetoclax (nM)	Dexa (nM)	Fa	CI
12.5	625	2.5	0.735	0.069
25	1250	5	0.965	0.054
50	2500	10	0.992	0.061

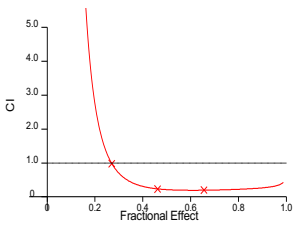


B

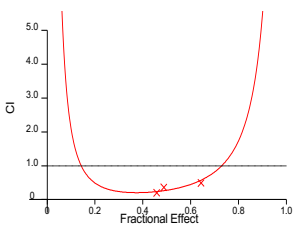
RPMI-8226:



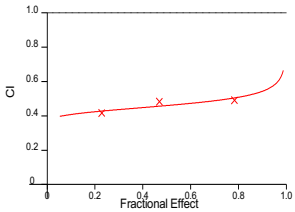
S63845 (nM)	Dexa (nM)	Fa	CI
2.5	5	0.270	0.983
5	10	0.461	0.239
10	20	0.655	0.203



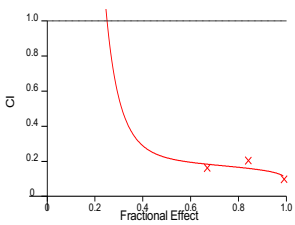
Venetoclax (nM)	Dexa (nM)	Fa	CI
500	5	0.459	0.207
1000	10	0.487	0.370
2000	20	0.644	0.494



S63845 (nM)	Venetoclax (nM)	Fa	CI
2.5	500	0.228	0.417
5	1000	0.469	0.485
10	2000	0.783	0.491

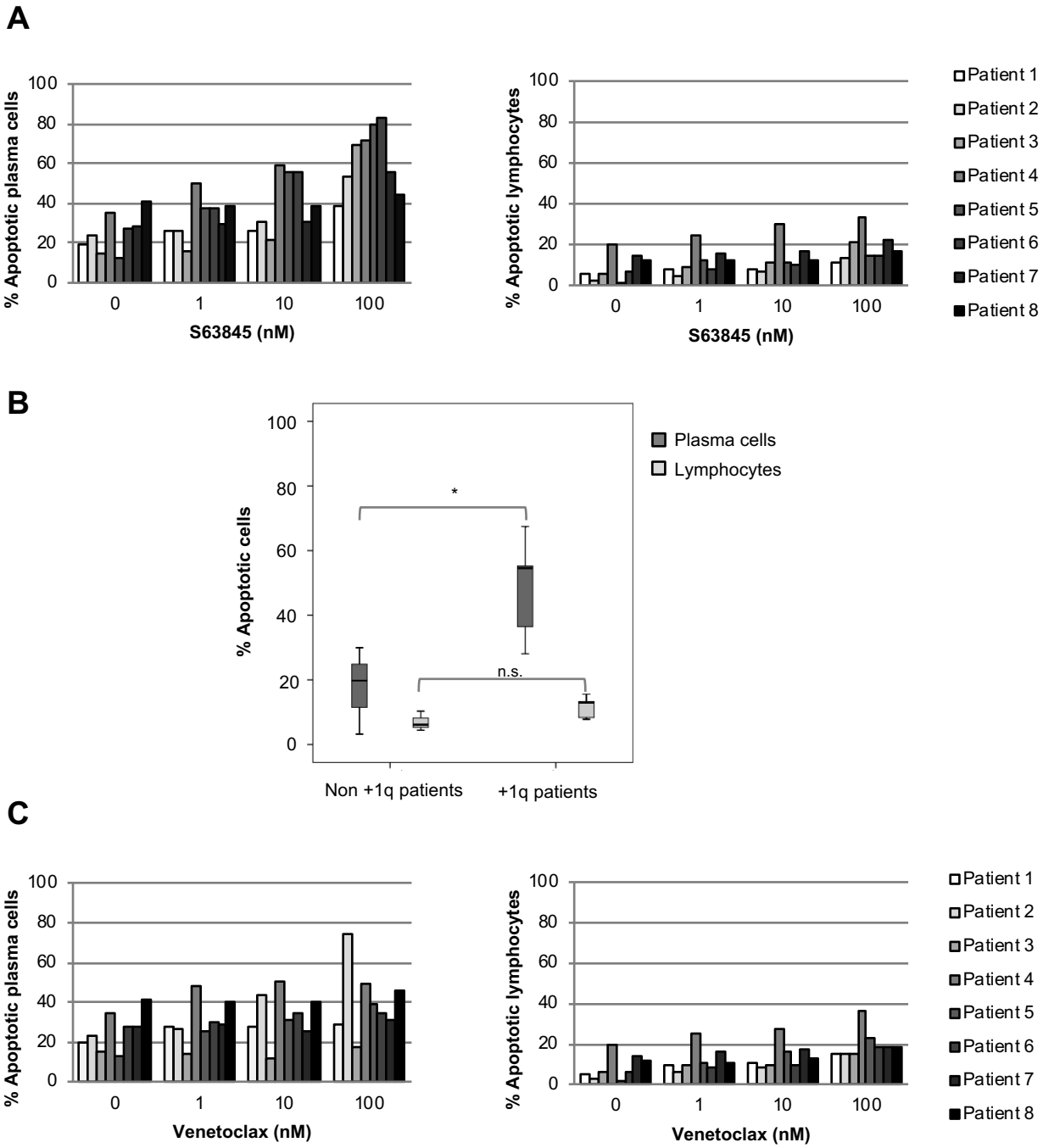


S63845 (nM)	Venetoclax (nM)	Dexa (nM)	Fa	CI
2.5	500	5	0.670	0.164
5	1000	10	0.841	0.205
10	2000	20	0.991	0.099



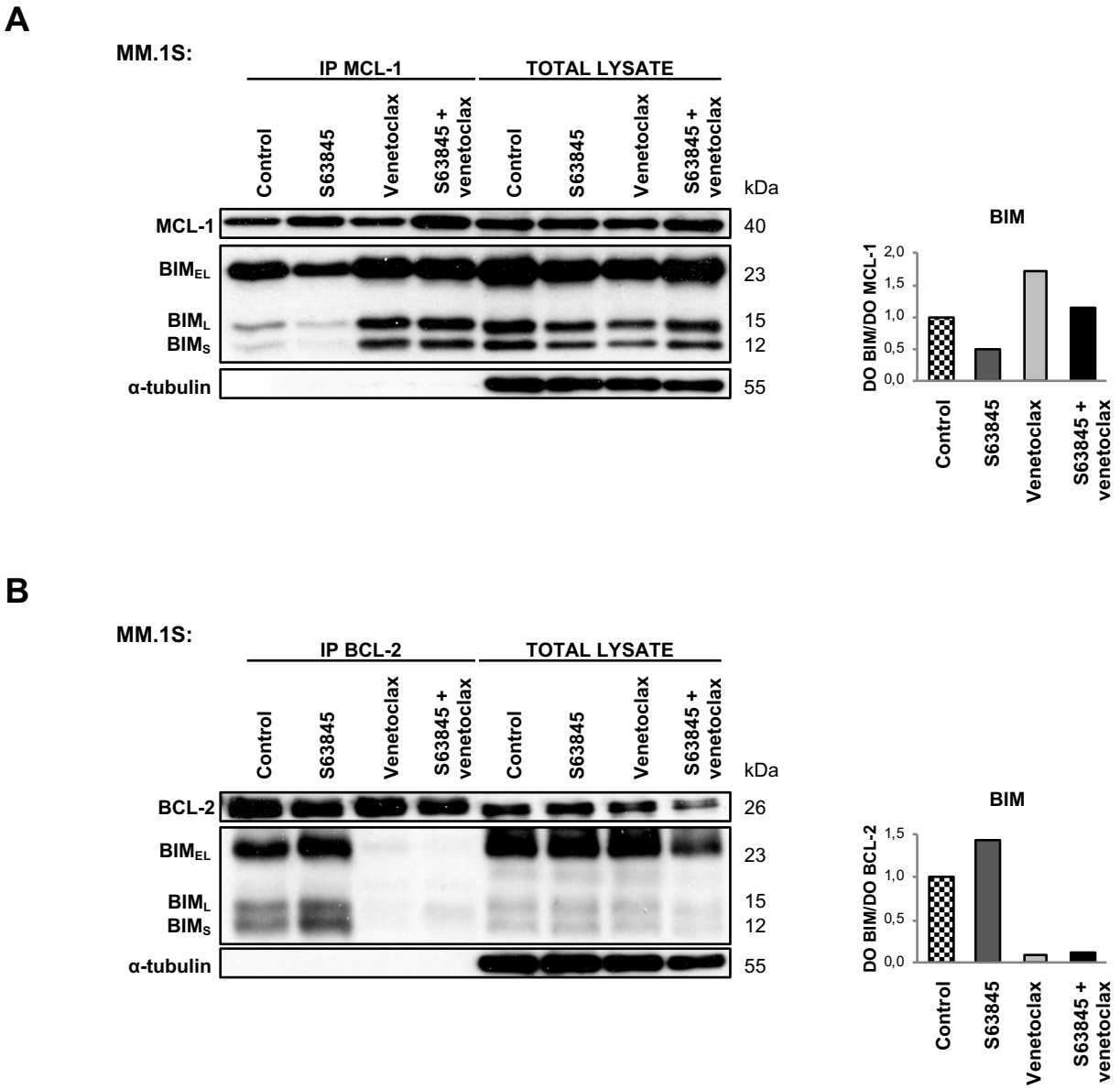
Supplementary Figure 4. The efficacy of the triple combination of S63845 + venetoclax + dexamethasone is superior to the double combination of S63845 + venetoclax. (A) MM.1S and (B) RPMI-8226 cell lines were exposed to increasing concentrations of S63845, venetoclax and dexamethasone and their combinations for 48 hours, maintaining a constant ratio drug combination design. Cell viability was analyzed by the MTT assay as shown in the graphs. Combination indices (CI) and inferred Fa-CI plots for the double and triple treatments were derived using the Calcusyn software.

SUPPLEMENTARY FIGURE 5



Supplementary Figure 5. S63845 and venetoclax show *ex vivo* anti-myeloma activity in monotherapy. (A) Bone marrow cells obtained from eight patients with MM were treated *ex vivo* with increasing doses of S63845 for 24 hours. Apoptosis induction was analyzed in myeloma plasma cells and normal lymphocytes by flow cytometry. (B) Percentage of apoptosis 24 hours after treatment with S63845 100nM was significantly higher (Student's t-test, $p < 0.05$) in plasma cells, but not in lymphocytes, from patients with +1q alterations (n=5) than in the rest of patients (n=3). (C) Apoptosis induction was also evaluated in myeloma plasma cells and normal lymphocytes from the same patients than in A treated with increasing doses of venetoclax for 24 hours.

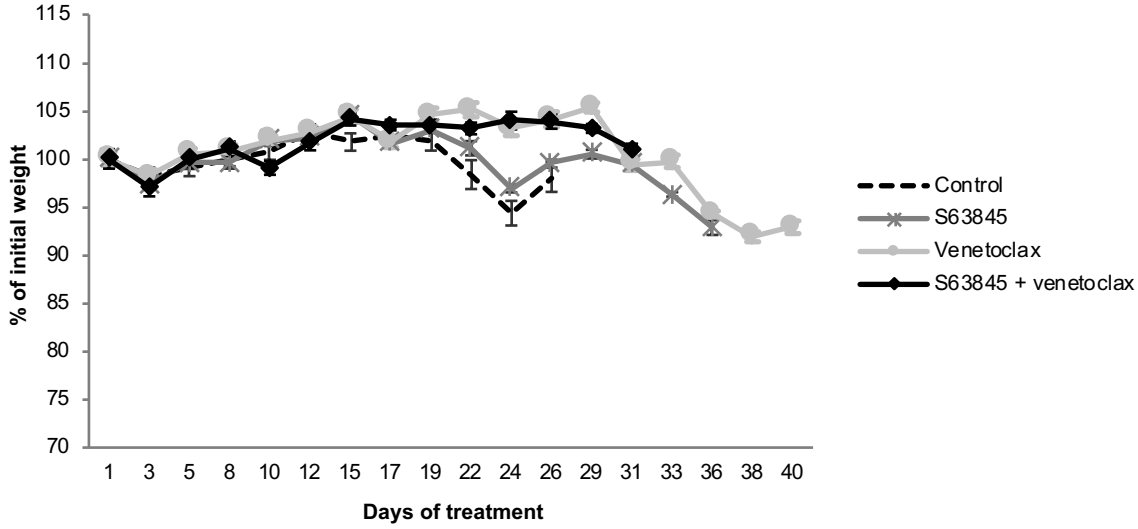
SUPPLEMENTARY FIGURE 6



Supplementary Figure 6. S63845 + venetoclax combination disrupts MCL-1/BIM and BCL-2/BIM complexes, and shifts MM cell dependence to the alternative anti-apoptotic protein. MM.1S cells were treated with S63845 12.5 nM and venetoclax 625 nM alone or in combination for 24 hours. Protein lysates were subjected to immunoprecipitation with an anti-MCL-1 (A) or an anti-BCL-2 (B) antibodies, and BIM bound to the anti-apoptotic proteins was then analyzed by immunoblotting. BIM levels were quantified by densitometry analysis of bands (using ImageJ software) normalized to those of MCL-1 or BCL-2, and depicted as bar diagrams. Whole cell lysates of each cell line are also shown.

SUPPLEMENTARY FIGURE 7

A



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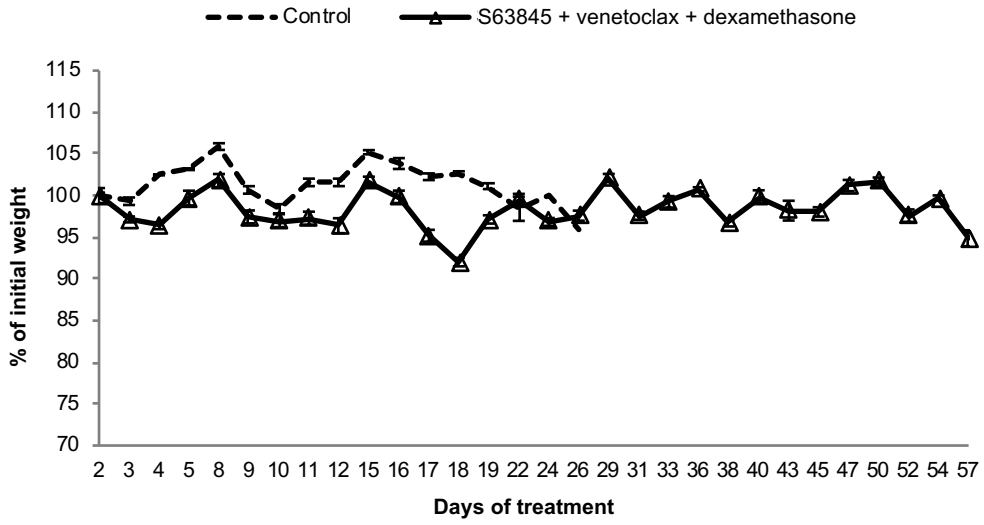

Supplementary Figure 7. The double combination of S63845 + venetoclax and the triple combination with dexamethasone did not reduce mouse body weight in a disseminated model of MM. (A) Percentages of mouse body weight variation of S63845, venetoclax and the double combination treatments during the study shown in Figure 3A-C. (B) Percentage of mouse body weight variation during the study with the triple combination of S63845 + venetoclax + dexamethasone shown in Figure 3D. Data represented are the mean ± SEM.