Synergistic interaction between HDAC and MCL-1 inhibitors through downregulation of BCL-X_L in multiple myeloma

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Figure legends

Supplement figure 1.

Concurrent MCL-1 and HDAC inhibition synergistically kills MM cells *in vitro.* Cell viability of indicated human MM cell lines 96h after treatment with S63845 (A)-(H) and venetoclax (I)-(N) alone or in combination with either panobinostat (A),(C),(E),(G),(I),(K),(M) or ricolinostat (B),(D),(F),(H),(J),(L),(N). Results are presented relative to 0.1% DMSO control. Combination index (CI) values of < 0.8, 0.8–1.2, and >1.2 were interpreted as synergistic, additive, and antagonistic drug activity, respectively. (O)-(P) Assessment of cleaved caspase 3 and cleaved PARP was performed via flow cytometry 48 or 72 hours post treatment induction, respectively. (Q)-(R) BAK activation was determined by staining with antibodies against its active form. Graphs show the mean +/- standard deviation of the mean (SDM) of triplicate experiments. Differences between groups were calculated with one-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction, where **** denotes P <0.0001, ** denotes P<0.001 and * denotes P <0.05

Supplement figure 2.

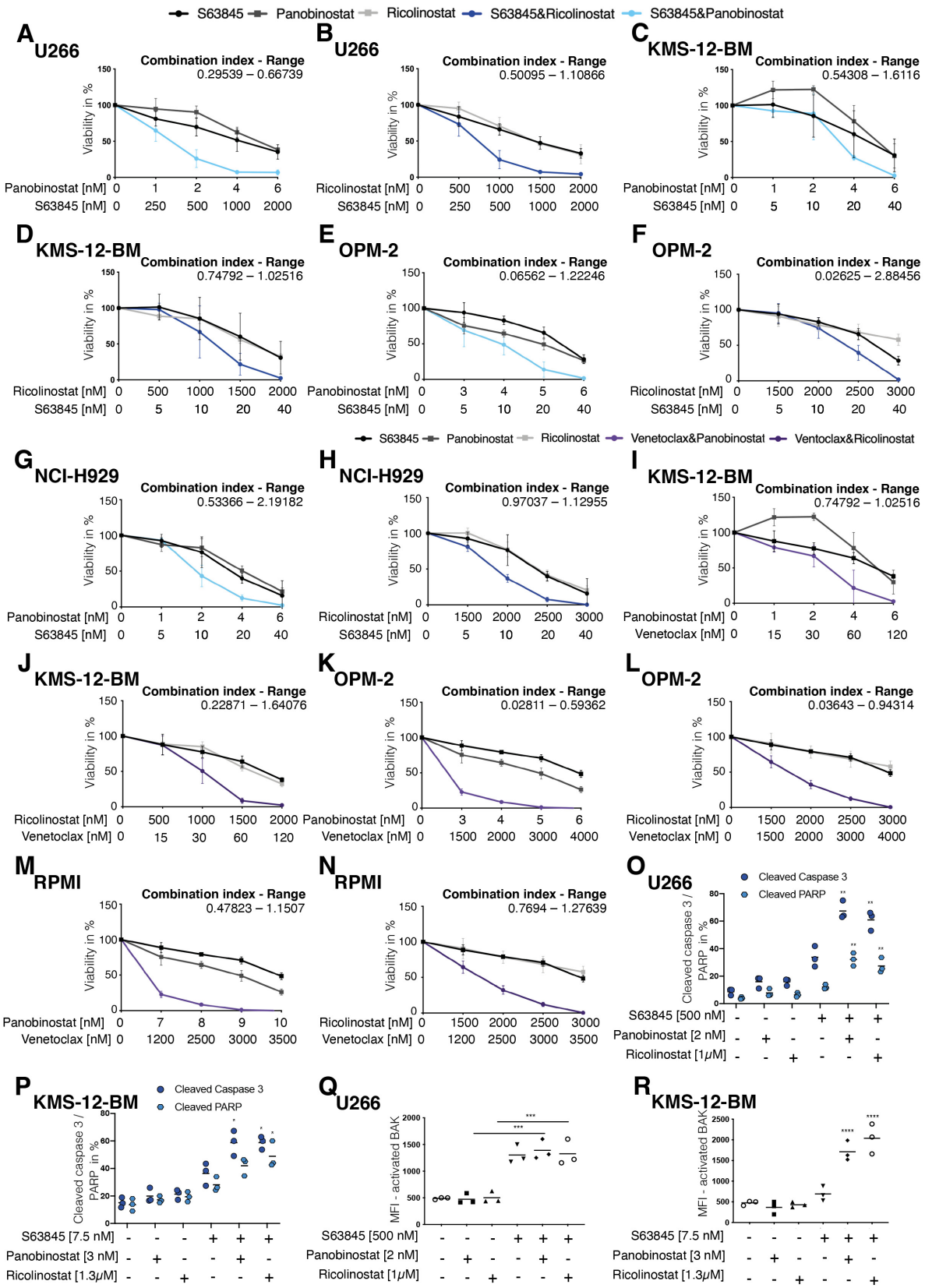
S63845 in combination with HDACi downregulates BCL-X_L and BCL-X_L overexpression reduces apoptosis by sequestering BIM and BAK. (A-B) Immunoblot analysis of the indicated Bcl-2 family members was performed 24 h post treatment initiation. One representative experiment of three independent biological replicates is shown. (C-E) Cell cycle analysis was performed 24h after treatment initiation with either single-agent or combination treatment of S63845 and HDACi. Differences between groups were calculated with three-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction. (FG) MM cells transduced with pcW57.1 EGFP (left panel) or pcW57.1 BCL-X_L (right panel) were treated for 24h with 0.5 μg/ml doxycycline to induce protein overexpression and afterwards exposed to the indicated treatments. Apoptotic cells were assessed 24 hours post treatment induction. Results indicate the mean +/- SDM of three independent experiments. Differences between groups were calculated with one-way ANOVA, corrected for multiple comparison with Bonferroni-Holm correction, where **** denotes P <0.0001, ** denotes

P<0.001 and * denotes *P* <0.05 (H–I) Co-immunoprecipitation experiments in U266 cells transduced with either pcW57.1-EGFP (right panels) or pcW57.1-BCL-X_L (left panels) were performed after 24h pretreatment with 0.5μg/ml doxycycline to induce protein overexpression and subsequent drug exposure for 24 hours.

Supplement figure 3.

Ricolinostat promotes the activity of S63845 independent of HDAC6 inhibition. (A–D) Co-immunoprecipitation experiments in MM cells transduced with either pcW57.1-EGFP (right panels) or pcW57.1-BCL-X_L (left panels) were performed after 24 h pretreatment with 0.5 μg/ml doxycycline to induce protein overexpression and subsequent drug exposure for 24 hours. (E) KMS-12-BM and (F) U266 cells were either treated with panobinostat or ricolinostat for 24h, then whole-cell lysates were blotted for the indicated proteins. (G) Single cell clones of U266 cells harboring a Tet-on miR-E vector expressing either a shRNA targeting Renilla (control) or two different shRNAs targeting HDAC6 were exposed to 0.3 μg/ml doxycycline for 48 h and whole-cell lysates were blotted for the indicated proteins. Western blots are representative for three independent experiments. (H-J) U266 cells were pretreated with doxycycline for 48 h and viability was assessed after an additional 48 h treatment with increasing concentrations of S63845 as indicated in the figure. Results show the mean +/- SD of three independent experiments performed in triplicates.

Supplement figure 1.



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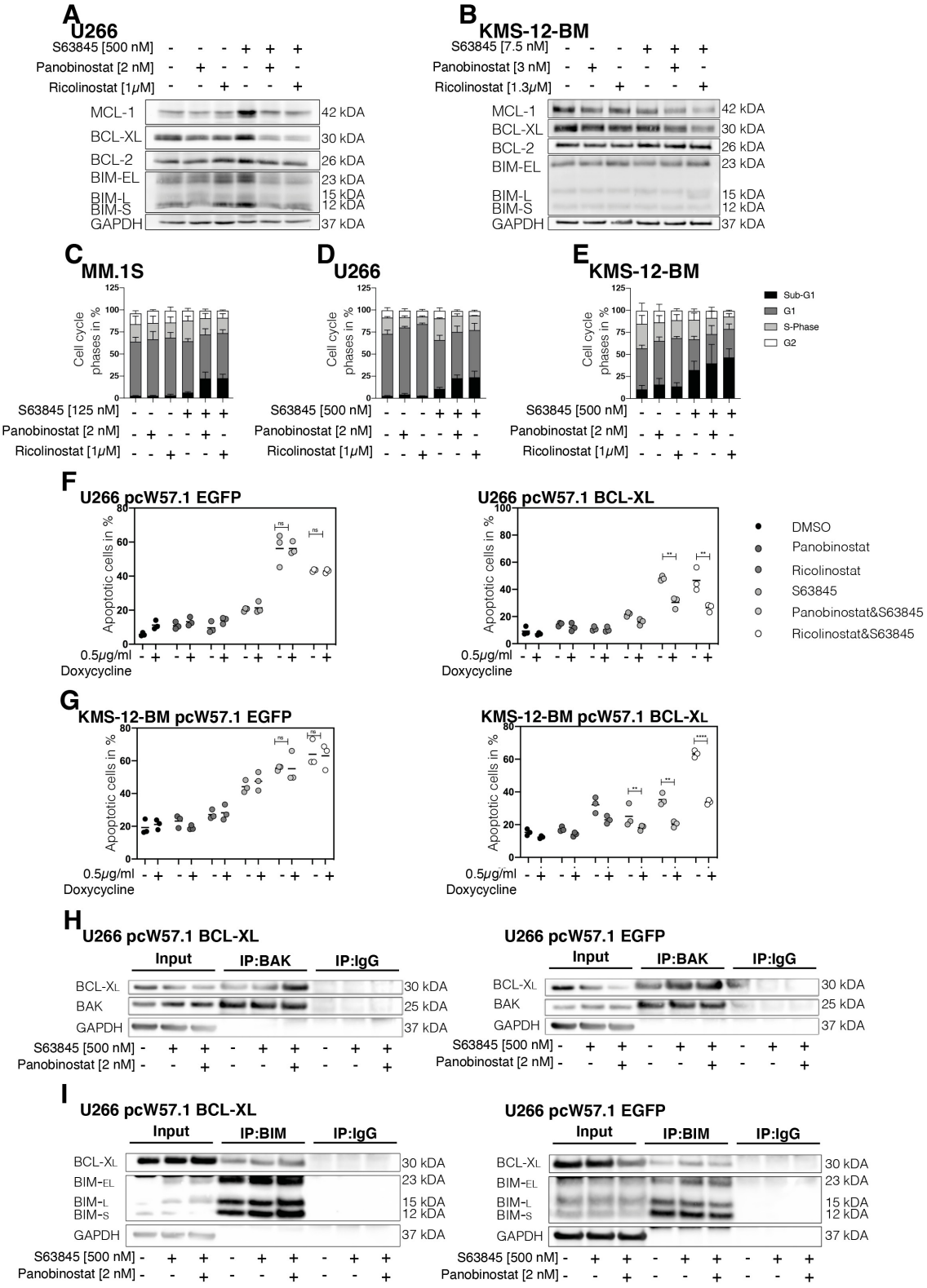

Supplement figure 3.