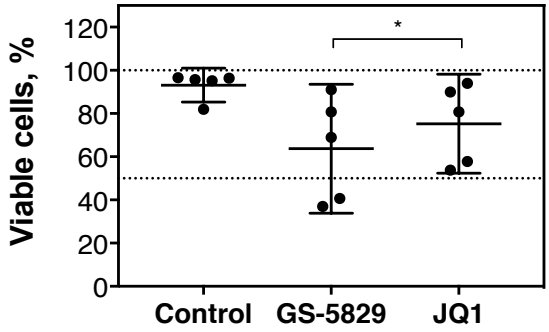
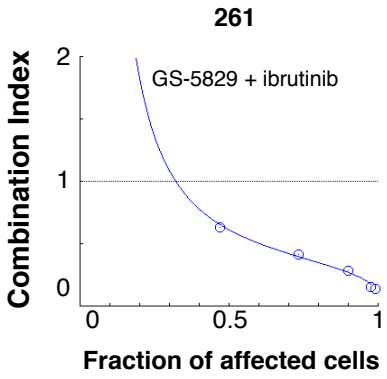
Supplementary Information



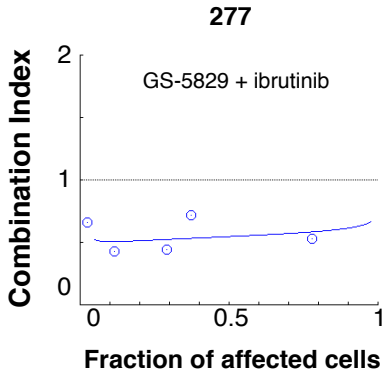
Supplementary Figure 1. GS-5829 is a more potent inducer of apoptosis in primary CLL cells than JQ1. Primary CLL cells co-cultured with NLCs were treated with 400 nM GS-5829 or JQ1 for 120 hours. The mean with 95% CI of viable CLL cells percentage by DiOC6/PI staining as measured in 5 samples. Paired t-test.

A



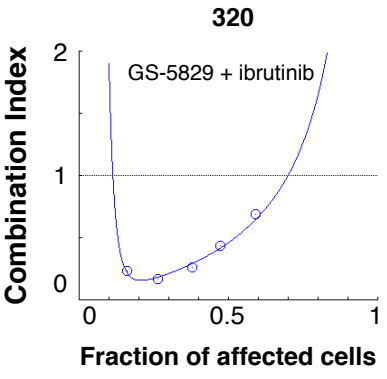
GS-5829, nM	Ibrutinib, nM	*CI*
50	125	0.637
100	250	0.413
200	500	0.286
400	1000	0.156
800	2000	0.139

B



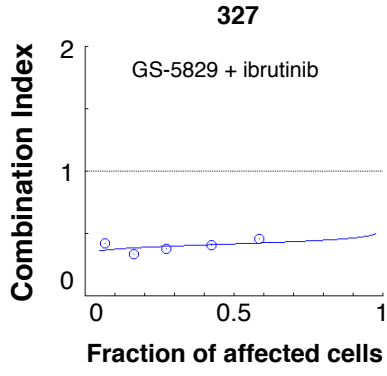
GS-5829, nM	Ibrutinib, nM	*CI*
50	125	0.662
100	250	0.435
200	500	0.445
400	1000	0.723
800	2000	0.531

C



GS-5829, nM	Ibrutinib, nM	*CI*
50	125	0.239
100	250	0.174
200	500	0.263
400	1000	0.435
800	2000	0.690

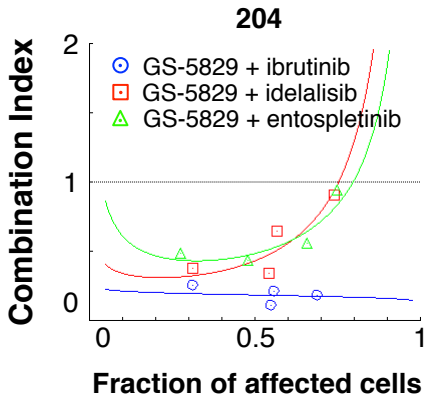
D



GS-5829, nM	Ibrutinib, nM	*CI*
50	125	0.421
100	250	0.340
200	500	0.378
400	1000	0.410
800	2000	0.458

Supplementary Figure 2. GS-5829 and ibrutinib synergistically induce apoptosis in primary CLL cells. (A-D) Fa-*CI* plots generated using data from 4 samples demonstrate that GS-5829 and ibrutinib synergistically induce apoptosis in primary CLL cells co-cultured with NLCs.

A

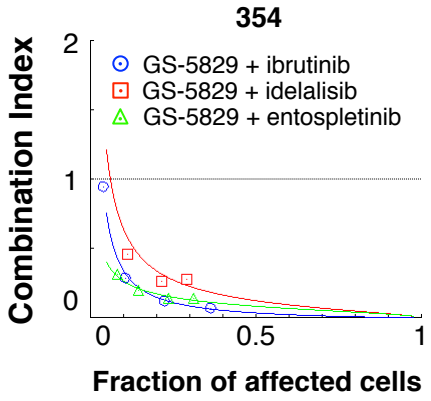


GS-5829, nM	Ibrutinib, nM	*CI*
100	250	0.256
200	500	0.115
400	1000	0.217
800	2000	0.187

GS-5829, nM	Idelalisib, nM	*CI*
100	250	0.381
200	500	0.341
400	1000	0.646
800	2000	0.912

GS-5829, nM	Entospletinib, nM	*CI*
100	250	0.486
200	500	0.437
400	1000	0.565
800	2000	0.948

B

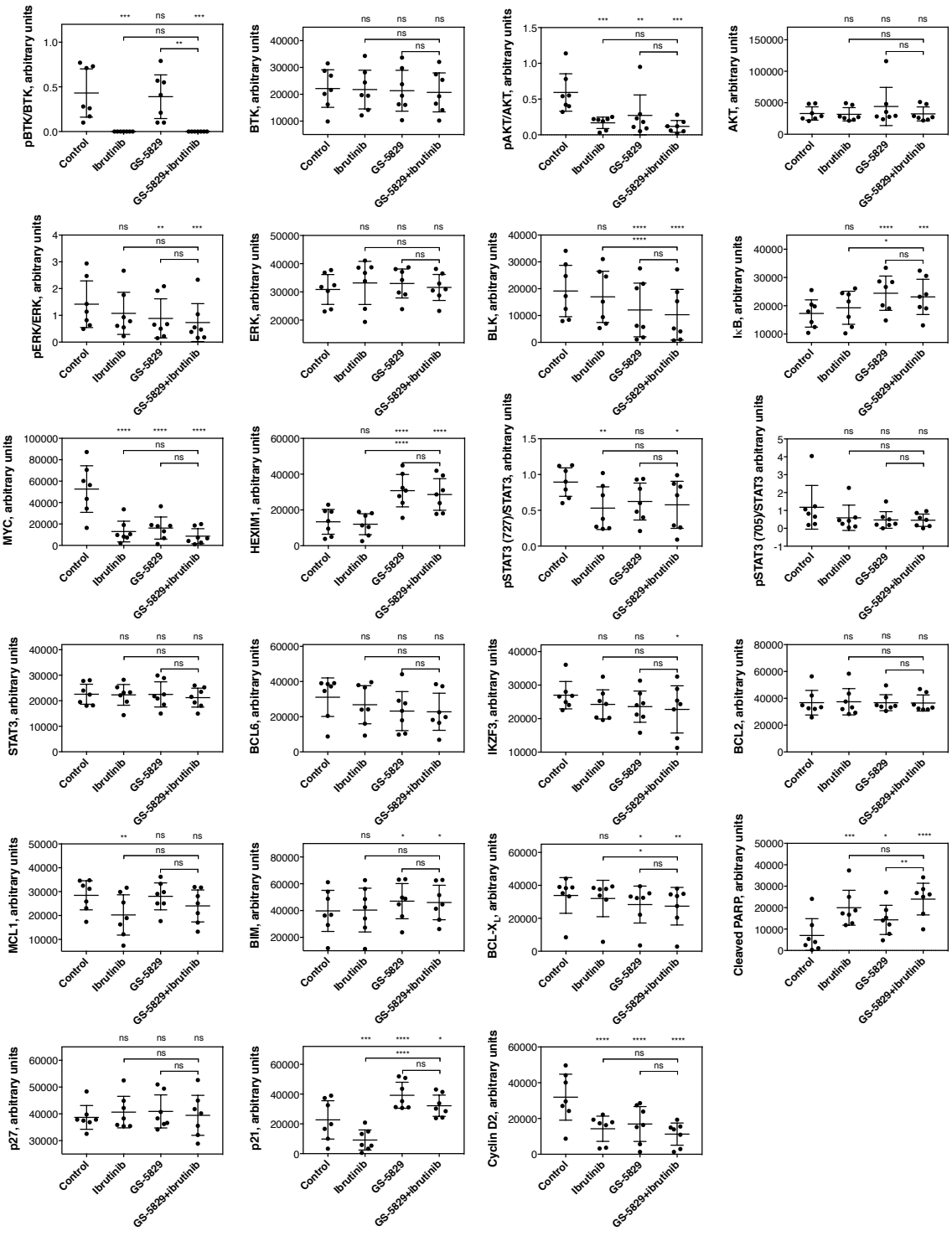


GS-5829, nM	Ibrutinib, nM	*CI*
100	250	0.943
200	500	0.289
400	1000	0.119
800	2000	0.069

GS-5829, nM	Idelalisib, nM	*CI*
100	250	2.075
200	500	0.463
400	1000	0.265
800	2000	0.282

GS-5829, nM	Entospletinib, nM	*CI*
100	250	0.318
200	500	0.201
400	1000	0.139
800	2000	0.139

Supplementary Figure 3. GS-5829 and the BCR signaling inhibitors demonstrate synergistic activity in a model of CLL microenvironment. (A-B) Fa-*CI* plots generated using data from 2 samples demonstrate that GS-5829 and the BCR signaling inhibitors (ibrutinib, idelalisib, entospletinib) synergistically induce apoptosis in primary CLL cells co-cultured with NLCs.



Supplementary Figure 4. GS-5829 changes the levels of multiple proteins in CLL. The protein content of CLL cells after 24 hours of treatment with GS-5829, ibrutinib or both was analyzed by Western blotting. The results were quantified by densitometry and normalized to β-actin level in each sample. The mean with 95% CI of normalized intensity as measured in 7 samples. Repeated measures one-way ANOVA with Sidak's post test.