Supplementary Materials and Methods

Cells and Reagents

Heparinized blood was obtained from consenting patients with a confirmed diagnosis of CLL; collected with approval by the Mayo Clinic Jacksonville Institutional Review Board, in accordance with the Declaration of Helsinki. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient from CLL patients (n=36) as well as from healthy human donors (n=5) for cell death assays, serving as effector cells. CD19+ CLL cells were selected out by magnetic bead separation followed by CD5+ cell selection using Biotin conjugated anti human CD5 (EasySep™ Human CD19 Positive Selection kit andEasySep™ Release Human Biotin Positive Selection Kit, StemCell Technologies, Vancouver BC, Canada). In most experiments we additionally used purified CD19+/CD38^{hi} and CD19+/CD38^{lo} CLL patient cells. Briefly, CD19+/CD5+ B cells from CLL patients were sorted using automatic magnetic sorter (RoboSep™, Stemcell Technologies, MA, USA) followed by staining with APC conjugated anti-CD38 antibody and flow sorted using FACS Aria II (BD Biosciences, San Jose, CA, USA) according to manufacturer's instructions. Cells were then treated with trypsin-EDTA for 10 min and washed twice followed by culture in AIM-V serum-free media for $\geq$24h. CD38 expression in purified cells was again reassessed using a multi-epitope FITC conjugated anti-CD38 antibody (Cytognos CD38 multi-epitope-FITC antibody, Ref: CYT-38F2). Post-sorted purity was validated using an Attune NxT flow cytometer (Invitrogen, MA, USA). JVM13 (CD38+) and MEC1 (CD38-) cell lines were obtained from DSMZ and used in some experiments. All cells were cultured per conditions previously described.[1, 2] Ibrutinib and kuromanin were purchased from SelleckChem (Houston, TX, USA). Daratumumab was acquired through clinical sources.

CD38 and FcγRIII expression analysis

CD38 expression and mean fluorescence intensity (MFI) on CD19+/CD5+ CLL cells was determined using a PE conjugated anti-CD38 antibody on an Attune NxT flow cytometer (Invitrogen, MA, USA). Quantification of CD38 cell surface molecules was determined using BD Quantibrite™ beads (BD Biosciences, NJ, USA), followed by analysis on BD QuantiCALC™ software (BD Biosciences, NJ, USA) and with data expressed as number of antibodies bound per cells (sAbc). Expression of FcγRs on CD19+CD5+ primary CLL cells was determined by labelling with antibodies for FcγRI (CD64, Biolegends, CA, USA), FcγRII (CD32, Biolegends, CA, USA) and FcγRIII, (CD16, BD Pharmingen, MN, USA)]. After 20 min of incubation in dark, cells were analyzed on a flow cytometer (Attune NxT, Invitrogen, CA, USA), using appropriate isotype control. Data are represented as % expression of FcγRs.

Cell death assays

Antibody-dependent cell mediated cytotoxicity (ADCC)

ADCC was carried out as described by de Weers et al.[3] Target cells were labeled with Calcein-AM (1μM, 30 minutes, 37ºC in dark; ThermoFisher Scientific, Waltham, MA, USA); subsequently, washed thrice with PBS; plated in triplicate at 1×10^4cells/well in 96-well round bottom plates. Cells were pre-incubated (15 minutes, 37ºC in dark) with isotype control (IgG1-b12, 0.1μg/mL), vehicle control (DMSO, 0.001%), ibrutinib (1μM), daratumumab (0.1μg/mL) or combination of ibrutinib and daratumumab (ID). Daratumumab and ibrutinib doses for *in vitro/ex vivo* studies have been previously established.[4-6] Isotype IgG1-b12 antibody was added instead of mAb to determine spontaneous calcein release and Triton X-100 (1%) was used to determine the maximal calcein release. Thereafter, fresh human PBMCs were added at an Effector: Target (E:T) ratio of 50:1 (optimized in a previous report)[3] and cells were incubated for 6 hours at 37°C in dark. The plates were centrifuged, supernatant transferred into glass bottom black plates (ThermoFisher Scientific, Waltham, MA, USA) and fluorescence was measured in a Synergy spectrophotometer (Bio-Tek,

Winooski, VT, USA) (excitation laser: 488nm ± 10; band-pass filter: 520nm ± 20). The % of cellular cytotoxicity was calculated using the following formula:

$$\text{Specific lysis} = 100 \times \frac{\text{Experimental release (RFU)} - \text{Spontaneous release (RFU)}}{\text{Maximal release (RFU)} - \text{Spontaneous release (RFU)}}$$

Complement-dependent cytotoxicity (CDC)

CDC was determined in a manner similar to ADCC where Calcein-AM-labeled CLL cells were pre-incubated with IgG1-b12 (0.1µg/mL), DMSO (0.001%), ibrutinib (1µM), daratumumab (0.1µg/mL) or ID. 10% normal human serum was added and incubated with CLL cells for 1hr at 37°C. Cell viability and specific lysis was determined per the formula in the ADCC assay and as described by de Weers et al.[3]

Antibody-dependent cellular cytotoxicity (ADCP)

Macrophages (Mφ) were generated from peripheral blood monocytes, isolated from healthy human donors. Monocytes were cultured for 7 days in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 50 µg/mL / 50 U/mL penicillin/streptomycin, and granulocyte-macrophage colony-stimulating factor (10 ng/mL); the culture medium was renewed every 3 days. Macrophages were detached on day 7 with 0.1% trypsin-EDTA and characterized by flow cytometry (CD11b+; BD Biosciences, CA USA). Macrophages were seeded at 2×10^5 cells/well into tissue culture treated 24-well plates and allowed to adhere overnight at 37°C in dark. Phagocytosis (ADCP) was assessed with similar treatment as ADCC and CDC assays where Calcein-AM-labeled CLL cells were pre-incubated with IgG1-b12 (0.1µg/mL), DMSO (0.001%), ibrutinib (1µM), daratumumab (0.1µg/mL) or ID combination. After 6h of incubation, the non-phagocytosed target cells were collected. Macrophages were detached with 0.1% trypsin-EDTA, stained for CD11b-phycoerythrin expression and added to the nonphagocytosed target cells,. The

amount of doubly positive target cells (CD11b-phycoerythrin and calcein) was determined on an Attune NxT flow cytometer (Invitrogen, MA, USA).

Apoptosis assay

To measure % apoptosis, dual staining for Annexin V-FITC and propidium iodide (PI) or 7AAD in CLL cells treated for 24h with IgG1-b12 control Ab (0.1µg/mL), kuromanin (10uM), daratumumab (0.1 µg/mL), ibrutinib (1µM), ID or IK combination was performed as previously described.[7, 8] FcγR cross linking-dependent apoptosis was measured in primary CLL cells after blocking with or without pan-FcγR blocker (Human TruStain FcX, Biolegend, San Diego, CA) for 1h (±FcX) followed by treated with isotype IgG1 Ab (control), daratumumab (Dara, 0.1ug/mL) for 24h and subsequent staining with annexin-V and 7ADD; data were analysed using flow cytometric analysis. Comparative significance analyses between the groups (brackets) show p-values. $p<0.05$ was considered as statistically significant.

Determination of mitochondrial membrane potential

Mitochondrial membrane potential was measured using the The MitoProbe™ DiIC1(5) Assay Kit (Invitrogen, MA, USA). Cells (5×10^5) were incubated with kuromanin (10uM), daratumumab (0.1 µg/mL), ibrutinib (1µM) or ID for 24 h, followed by loading with with 250 ng/ml of $DiIC_1(5)$. Mitochondrial membrane potential was analyzed on an Attune NxT flow cytometer (Invitrogen, MA, USA).

Immunoblotting

Immunoblotting was performed using cell lysates as described after treatment.[9, 10] Briefly, protein-containing immunoblot were incubated overnight at 4˚C with antibodies against Lyn, Syk, BTK, PLCγ2, ERK1/2, AKT and their phosphorylated isoforms; as well as GAPDH (Cell Signaling Technology, MA, USA). After incubation with corresponding secondary antibody, the protein

bands were visualized using a chemiluminescent substrate and analysed by GE Amersham imager 600 (GE Healthcare Bio-Sciences, PA, USA). Densitometry analysis was performed using GelQuant software (BioChem Solutions, CA USA) and data was normalized to loading control (GAPDH).

In vivo disseminated-disease leukemia xenograft model

A partially humanized mouse model of CLL was established in a manner as previously reported by de Weers et al, Nijhof et al and Herman et al.[4, 11,12] All experiments were conducted in accordance with the Mayo Clinic Institutional Animal Care and Use Committee (IACUC) and institutional guidelines. Briefly, luciferase-labeled JVM13 B-leukemia cells (JVM13-Luc, 8×10^6 cells) were injected I.V. into the tail veins of NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice (The Jackson Laboratory). After 7 days, mice were randomized to receive either 1. vehicle (control, I.P.), 2. Effector cells only (healthy donor PBMCs, $8x10^6$ cells, I.V.), 3. Daratumumab alone on a weekly schedule (10 mg/kg, I.P.), 4. ibrutinib (6mg/kg, I.P.), 5. daratumumab + effector cells, 6. Ibrutinib + effectors, 7. Ibrutinib and daratumumab (ID) combination and 8. ID combination + effectors. This treatment schema is largely consistent with prior investigations of daratumumab.[4, 11,12] An initial saturating dose of daratumumab (20 mg/kg, I.P.) was administered to mice on day 8 after tumor implantation, thereafter followed by a 10 mg/kg dose given on days 11, 15, 20 and 26. In the daratumumab-receiving study arm, effector cells were given 1 day before the daratumumab injection. Tumor burden was quantified by bioluminescent signal intensity on an IVIS imaging machine and body weight was calculated on the same days also. Mice in the treatment groups that received effector cells were euthanized on day 30 to avoid any possibility of graft-versus-host disease−like symptoms, which could impact the results of the study.

Statistical analysis

ADCC, CDC and ADCP experiments were performed using primary CLL cells; plated in triplicate, and in some cases performed twice where sufficient number of cells were available. Results are expressed as mean ± SEM, mean ± SD or mean ± range (where applicable). Statistical analysis comparing results in cell death assays and protein expression were evaluated by non-parametric Mann–Whitney test or non-parametric Kruskal Wallis multiple comparison test (as applicable), using Graph Pad Prism software, version 5 (La Jolla, CA, USA); $p<0.05$ was considered as statistically significant. Spearman's test of correlation was used to determine association between CD38 expression and cell death; performed using R Statistical Software (version 3.1.1; R Foundation for Statistical Computing, Vienna, Austria). Overall survival was determined by Kaplan Meir analysis.

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Supplementary Figure Legends.

Supplemental Figure 1. Flow-sorting strategy used to enrich for CD19+CD5+/CD38^{hi} and CD19+CD5+/CD38^{lo} primary CLL cells.
CD19+CD5+ cells were first selected out from the peripheral blood mononuclear cells of patients with a confirmed diagnosis of CLL by magnetic bead selection and labelled with an anti-CD38 APC antibody. CD19+CD5+ cells were gated based on forward scatter /side scatter (upper panel, green box) followed by doublet discrimination (middle red outlined box) and dot plots for CD38 APC (lower panel, blue box) positive cells. After that, CD9+CD5+CD38 APC-labelled cells were gated similarly (all middle panel, respectively; red box outline). By using the same gating panel, post sort purity of CD38+ and CD38- cells were validated.

Supplemental Figure 2. Validation of CD38 epitope recovery following labeling and flow sorting of CLL cells with an anti-CD38 antibody.
A. Schema illustrating methodology implemented to determine recovery of the CD38 epitope to which daratumumab binds. Briefly, CD19+/CD5+ cells were selected for by magnetic bead separation from the PBMCs of patients with a confirmed diagnosis of CLL. One aliquot was untreated and a second aliquot treated with daratumumab (0.1ug/mL, 2hr). Subsequently, both aliquots were labeled with either anti-CD38 APC-conjugated Ab (**Panel B**) or a novel detection antibody that binds CD38 at 2 distinct epitopes (Cytognos CD38 multi-epitope-FITC antibody, Ref: CYT-38F2, **Panel C**). In the aliquot that was treated with daratumumab, it was clear that daratumumab binds to an epitope similar to an anti-CD38 APC Ab as ~0.04% of cells were detected as being CD38+ (**Panel D**). However, by using the multi-epitope anti-CD38 FITC Ab, we detected ~36% of CD38+ cells (vs. ~38% CD38+ cells in untreated counterparts) as shown in **Panel E**. Next, to determine whether trypsinization, washing and 24hr recovery of CLL cells would sufficiently wash off any detection Ab that competes with Daratumumab binding, we labeled untreated CD19+/CD5+ CLL cells with an anti-CD38 PE Ab; followed by treatment with trypsin-EDTA for 10 min and washing (twice) and culturing in AIM-V serum-free media for 24h. These cells were then labeled with either anti-CD38 APC or multiepitope anti-CD38 FITC and CD38+ cells analyzed for. Indeed, CLL cells under both conditions; using 2 distinct anti-CD38 Abs shows that our recovery process results in virtually identical CD38 expression and detection pre- and post-trypsinization (**compare Panel B and F**) with either an APC conjugated Ab or a multiepitope FITC conjugated Ab (**compare C and G**).

Supplemental Figure 3. CD38 expression and immune-effector mediated cytolysis by daratumumab in CLL cell lines, JVM13 and MEC1.
A. CD38 expression in JVM13 and MEC1 cells. Immune-mediated and direct anti-tumor effects of ibrutinib, daratumumab (Dara) or the Ibr + Dara combination were evaluated in CD38+ JVM13 and CD38- MEC1 CLL cell lines. **B.** MEC1 or JVM13 cells (targets) were labeled with Calcein-AM and cocultured with or without healthy donor PBMCs (effectors) at an E:T ratio of 50:1 for 4hr. Specific lysis from ADCC was calculated as described in the Materials & Methods section. Spontaneous release was determined using IgG1-b12 isotype antibody [0.1µg/mL]. **C.** Similarly, CDC was measured in Calcein-AM labeled MEC1 or JVM13 cells incubated with human serum (10%) from a healthy donor for 1hr and effect of ibrutinib, Dara or combination

treatment was determined. **D.** ADCP was examined in Calcein-AM labeled CLL cell lines treated with either Ibr, Dara or Ibr + Dara by incubating target tumor cells with human monocyte-derived macrophages (effectors) from a healthy donor at an E:T ratio of 2:1. Flow cytometry analysis to detect CD11b+ macrophage engulfment of calcein-labeled tumor cells was used to calculate %phagoctyosis.

Supplemental Figure 4. Scatterplots for where a correlation between cell death (CDC, ADCP, apoptosis) and CD38 expression was noted.

Supplemental Figure 5. Daratumumab induces apoptosis in JVM13 and MEC1 cells.
Apoptosis was measured in MEC1 or JVM13 cells treated with isotype IgG1 Ab (control), daratumumab (Dara, 0.1ug/mL), ibrutinib (Ibr, 1uM) or Ibr + Dara (ID) for 24h, followed by staining with annexin-V/propidium iodide (PI) and flow cytometry analysis. Comparative significance analyses between the groups (brackets) show p-values. $p \leq 0.05$ was considered statistically significant.

Supplemental Figure 6. FcγR expression in CD19+CD5+ CLL cells.
Surface expression (%) of three FcγR's namely FcγRI (CD64); FcγRII (CD32) and FcγRIII (CD16) were measured on primary CLL cells isolated from 5 patients by flow cytometry. Data are represented as % expression of different FcγR's. A representative CLL patients (Pt. 8) scatter plots for each of the three FcγR's is shown.

Supplemental Figure 7. Basal expression of B-cell receptor (BCR) signaling proteins is higher in CLL cells from patients with CD38+ disease.
Proteins associated with BCR signaling were examined by western blot and densitometry analysis. **A - F**. Phosphorylated (p-) and total protein levels for Lyn, Syk, BTK, PLCγ2 and ERK1/2 as well as AKT were probed for using anti-human Ab's against the aforementioned proteins in primary CD19+ CLL cells. A comparative analysis of baseline expression of BCR signaling proteins relative to GAPDH (in Non-IgM stimulated) CLL cells from clinically defined CD38+ patients (n=5) or CD38- patients (n=5) disease was performed. *$p \leq 0.05$, ** $p \leq 0.001$

Supplemental Figure 8. Comparative analysis of BCR signaling protein expression in CLL cells from CD38+ or CD38- disease after ex vivo treatment with daratumumab or ibrutinib + daratumumab.
Proteins associated with BCR signaling were examined by western blot and densitometry analysis. **A - F**. Phosphorylated (p-) and total protein levels for Lyn, Syk, BTK, PLCγ2 and ERK1/2 as well as AKT were probed for using anti-human Ab's against the aforementioned proteins in primary CD19+ CLL cells, which were treated (for 2hr) with 1uM Daratumumab (D) +/- 1uM ibrutinib (ID). First, baseline expression of BCR signaling proteins relative to GAPDH was determined, followed by a comparative analysis of the % change in these proteins in CLL cells from CD38+ patients (n=5) or CD38- patients (n=5) after treatment with D or ID compared to IgG1-treated control cells. *$p \leq 0.05$, ** $p \leq 0.001$

Supplemental Figure 9. Targeting CD38 with kuromanin or daratumumab downregulates expression of B-cell receptor (BCR) signaling proteins and its effects are augmented by

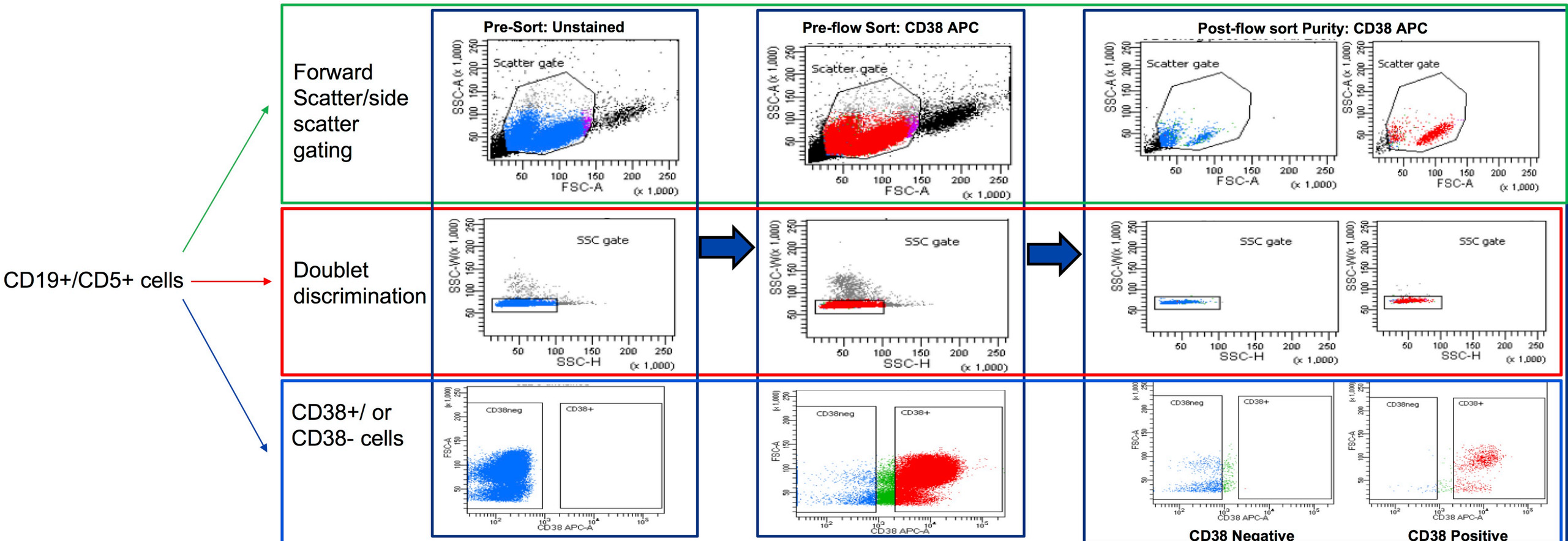
ibrutinib.
Proteins associated with BCR signaling were examined by western blot and densitometry analysis. **A - F**. Phosphorylated (p-) and total protein levels for Lyn, Syk, BTK, PLCγ2 and ERK1/2 as well as AKT were probed for using anti-human Ab's against the aforementioned proteins in primary CD19+ CLL cells, which were treated (for 2hr) with DMSO (control), ibrutinib (Ibr), kuromanin (Kuro) or the combination of the two agents. **G.** Representative western blots from a CD38+ and CD38- CLL Pt. are shown. **H.** Similarly, we performed western blot analysis in flow-sorted CD19+/CD38^{hi} and CD19+/CD38^{lo} cells from Pt. 19, treated with daratumumab (Dara) +/- Ibr and probed for pBTK, BTK, PLCγ2 and pPLCγ2 for 2hr. Comparative significance analyses between the groups (brackets) show p-values. $p \leq 0.05$ was considered as statistically significant.

Supplemental Figure 10. Ibrutinib does not decrease CD38 MFI in CD38- primary CLL cells.
CD19+ cells from CD38+ Pts. (1, 2, 9 and 11) and CD38- Pts. (3, 4, 5, 6 and 8) were treated ex vivo with ibrutinib (Ibr, 1μm) or DMSO for 24h. Cells were washed twice and labeled with anti-CD38 Ab, followed by flow cytometry analysis for determination of CD38 expression. % CD38+ cells pre-and pos-Ibr are shown **A.** and **B**. CD38 MFI in was also assessed in a similar manner pre-and post-Ibr exposure in **C.** CD38+ cells and **D.** CD38- CLL Pt. cells. While increase in MFI was noted in CD38+ and CD38- cells, the change was not statistically significant. Experiments were performed in triplicate.

Supplemental Figure 11. Daratumumab or kuromanin +/- ibrutinib induce apoptosis of CLL cells (analysis with secondary probe using 7AAD).
Apoptosis was measured in primary CLL cells (n=7 patients), treated with isotype IgG1 Ab (control), daratumumab (Dara, 0.1ug/mL), ibrutinib (Ibr, 1uM) or Ibr + Dara (ID) as well as kuromanin (10uM) +/- Ibr, for 24h, followed by staining with annexin-V/7ADD and flow cytometry analysis. $p \leq 0.05$ was considered as statistically significant.

Supplemental Figure 12. Targeting CD38 with daratumumab or kuromanin +/- ibrutinib disrupts mitochondrial membrane permeability in CLL cells.
Change in mitochondrial transmembrane permeability was examined in CLL cells from 30 patients treated with isotype IgG1 control Ab, daratumumab (Dara, 0.1ug/mL), ibrutinib (Ibr, 1uM) or Ibr + Dara (ID) as well as kuromanin (10uM) +/- Ibr, for 24h, using a membrane-potential-sensitive cyanine dye $DiIC_1(5)$, which accumulates in the mitochondria. A decrease in $DiIC_1(5)$ MFI represents an increase in mitochondrial transmembrane permeability and is associated with mitochondrial-mediated apoptosis. **A.** All CLL cells (n=30 patients) and **B.** Subset analysis in CLL cells from CD38+ and CD38- patients is shown. **C.** Histograms illustrating change in $\Delta\Psi m$ due to Dara from representative CD38+ CLL case (Pt. 9) and a CD38- case (Pt. 8). $DiIC_1(5)$ MFI amplitude with a left shift on the horizontal axis indicate lower MFI (increased membrane permeability and disruption in $\Delta\Psi m$). **D - G.** A similar analysis was separately performed in flow-sorted CD19+/CD38^{hi} and CD19+/CD38^{lo} CLL cells from 5 patients treated with the aforementioned agents. Results expressed as mean ± SEM. Comparative significance analyses between the groups (brackets) show p-values. $p \leq 0.05$ was considered as statistically significant.

Supplemental Figure 1.



Supplemental Figure 2

A. Workflow for Panels B– E

PBMCs from CLL Pt.

↓

CD19+/CD5+ selection

↙ ↘

CD19+/CD5+ CLL cells (Untreated) | CD19+/CD5+ CLL cells (Dara treated)

labeled with anti-CD38 APC

↓ | ↓

CD38 epitope analysis using: anti-CD38 APC *(Panel B)* or anti-CD38 multiepitope FITC *(Panel C)*

CD38 epitope analysis using: anti-CD38 APC (*Panel D*) or anti-CD38 multiepitope FITC (Pan*el E*)

A. Workflow for Panels F– G

PBMCs from CLL Pt.

↓

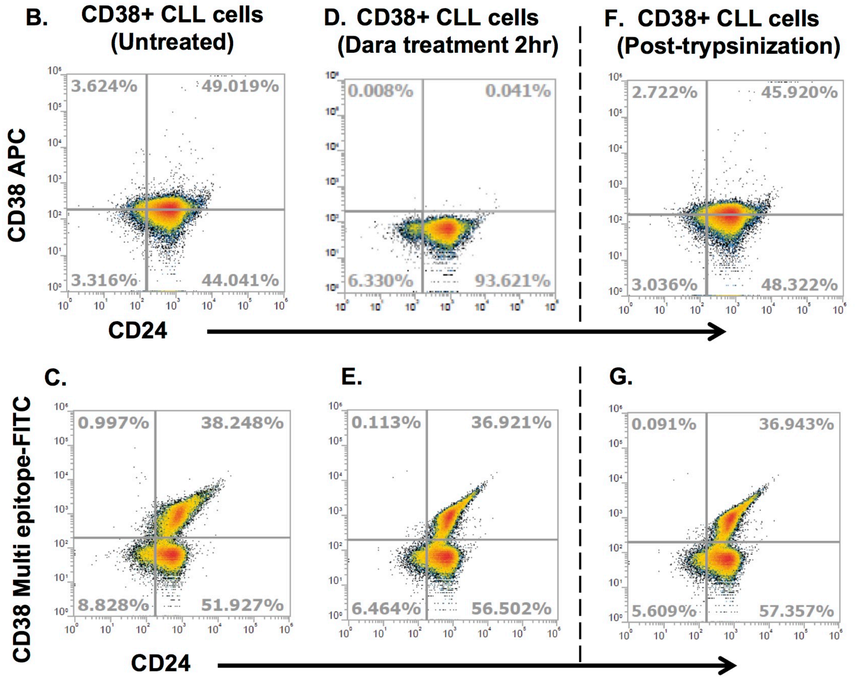
CD19+/CD5+ selection

↓

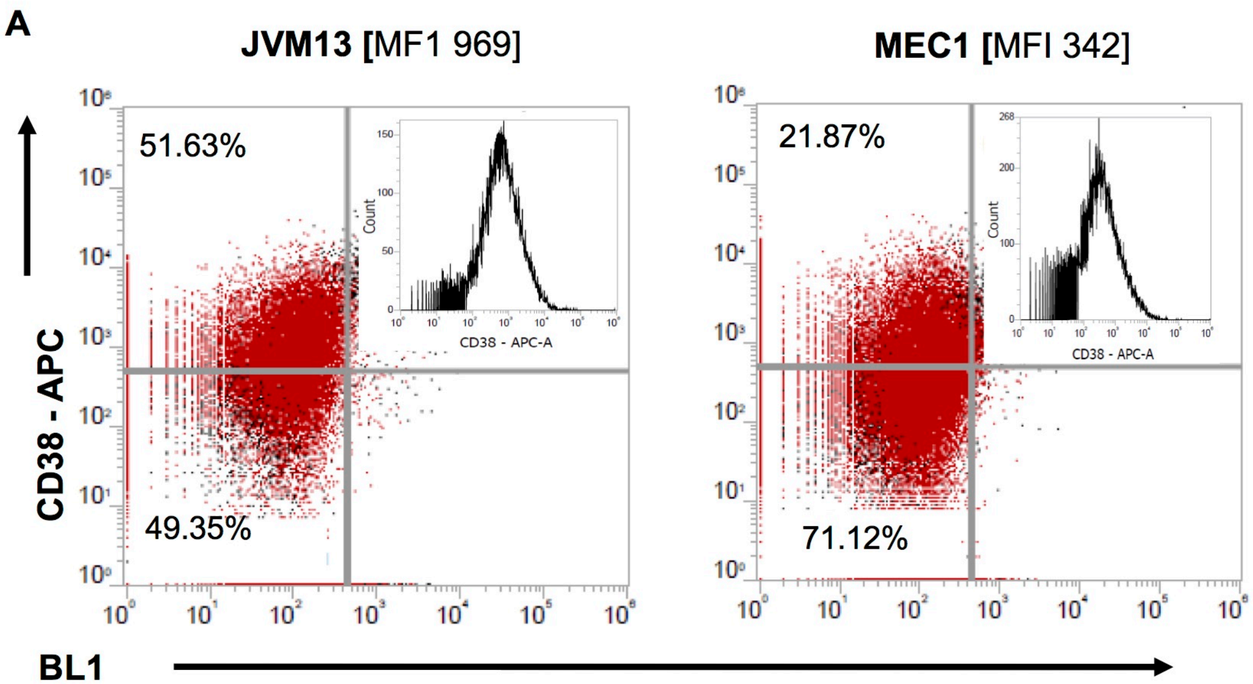
labeled with anti-CD38 PE (585±15nM)

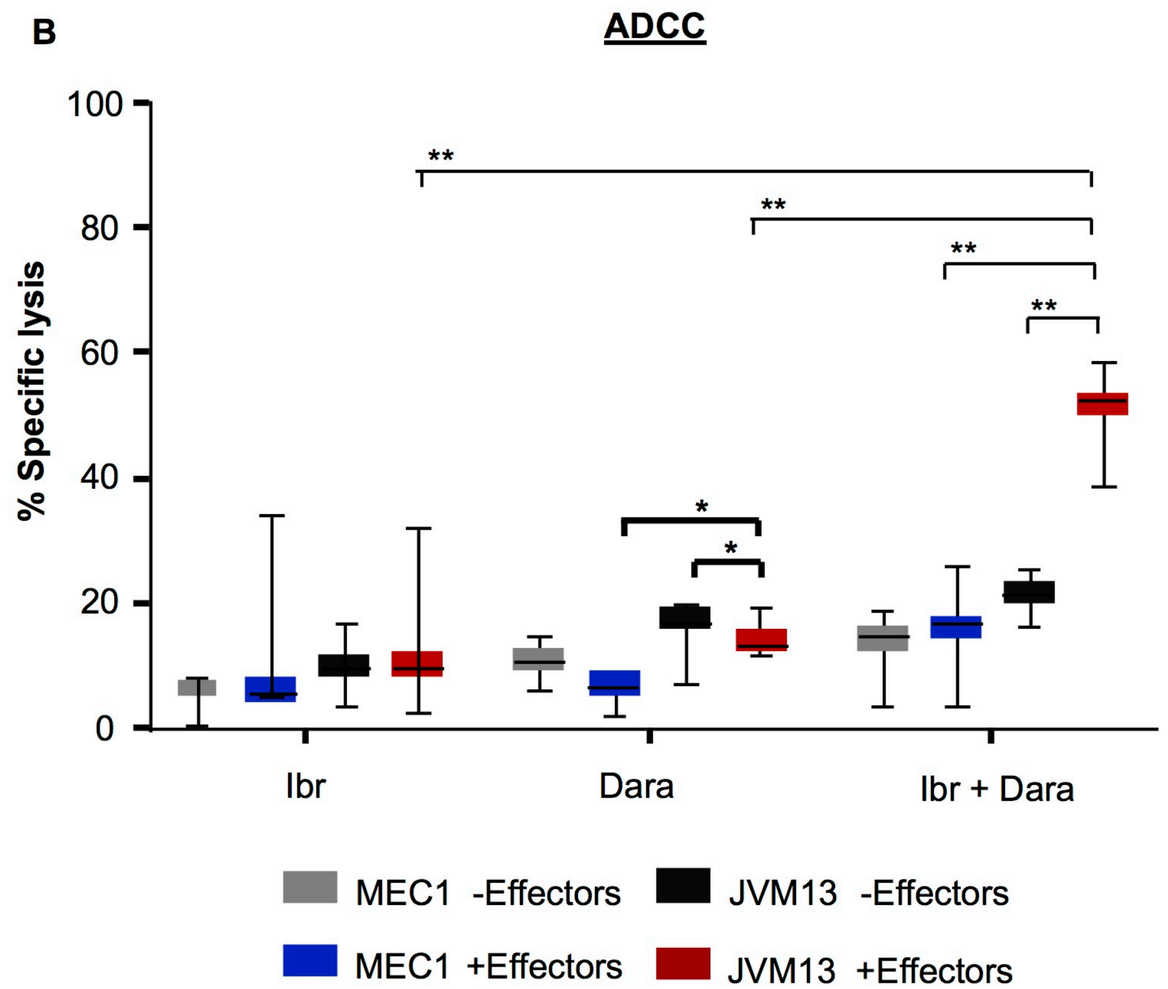
↓ *trypsinized, washed, 24h recovery*

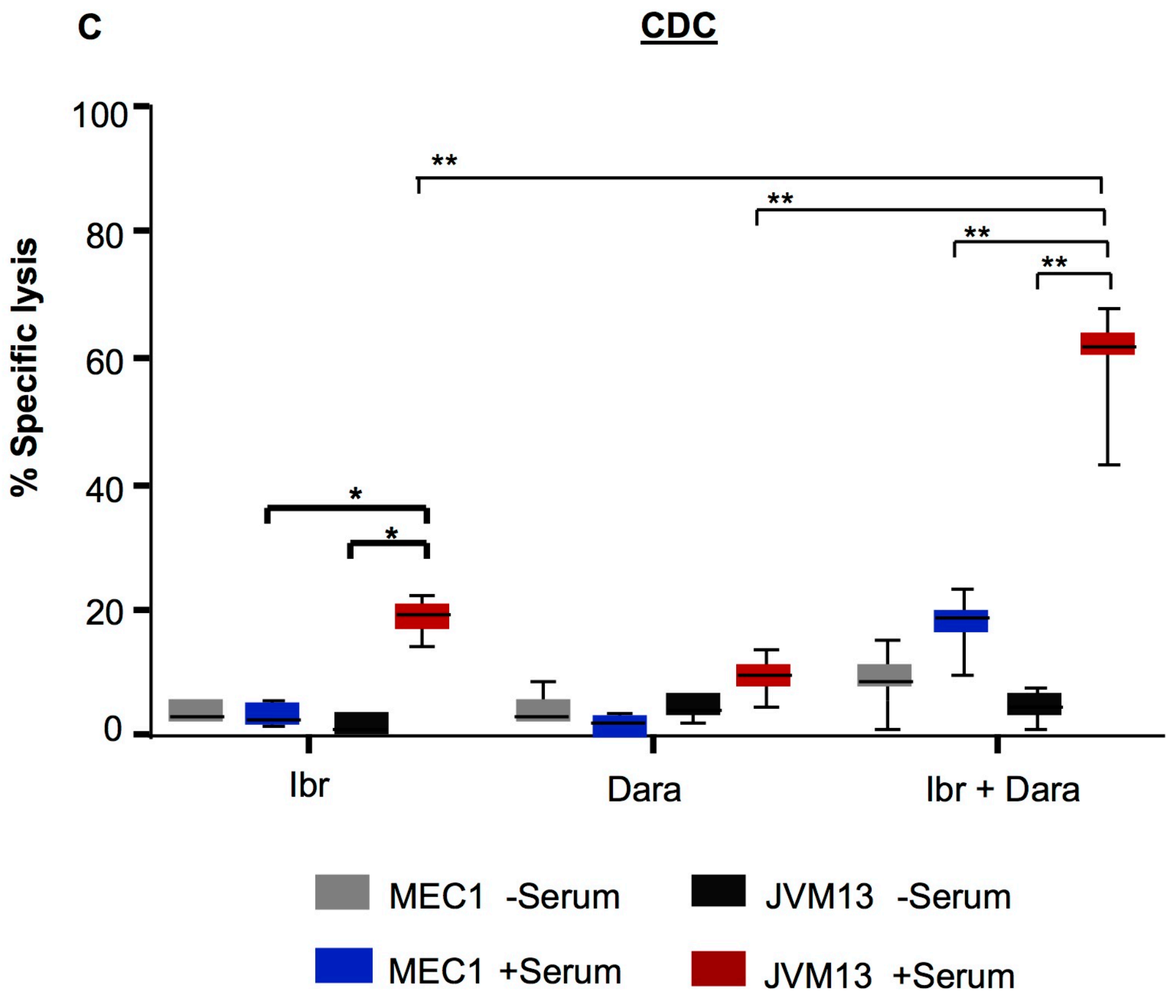
anti-CD38 APC (670±30) (*Panel F*) or anti-CD38 multiepitope FITC (*Panel G*)

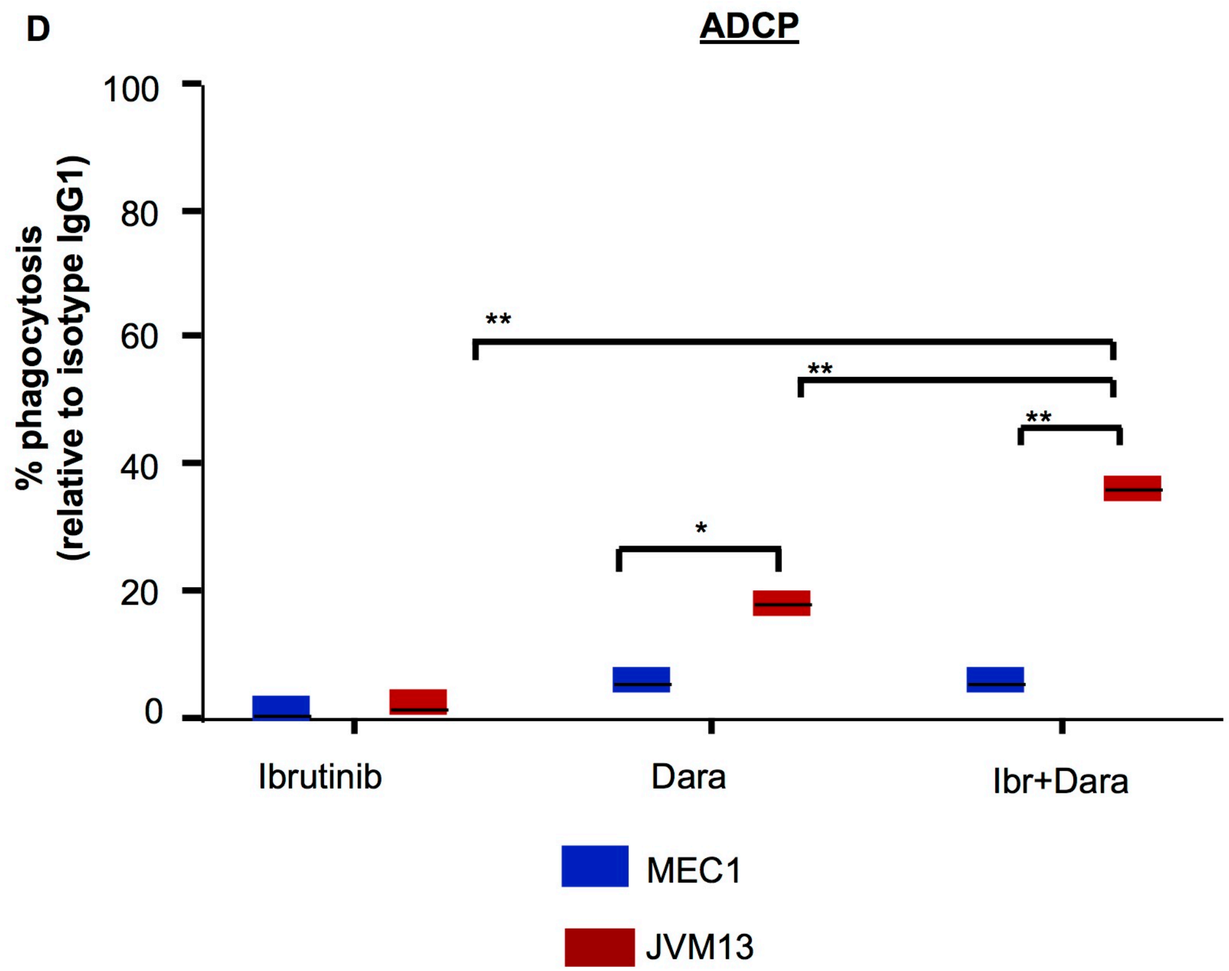


Supplemental Figure 3

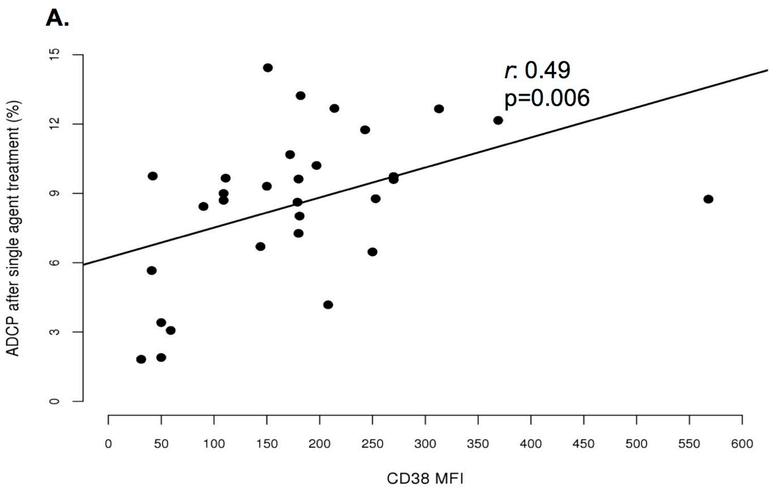


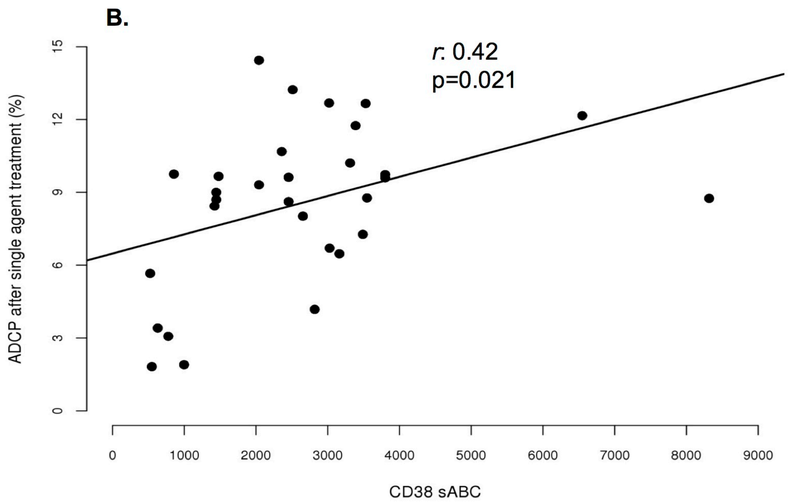


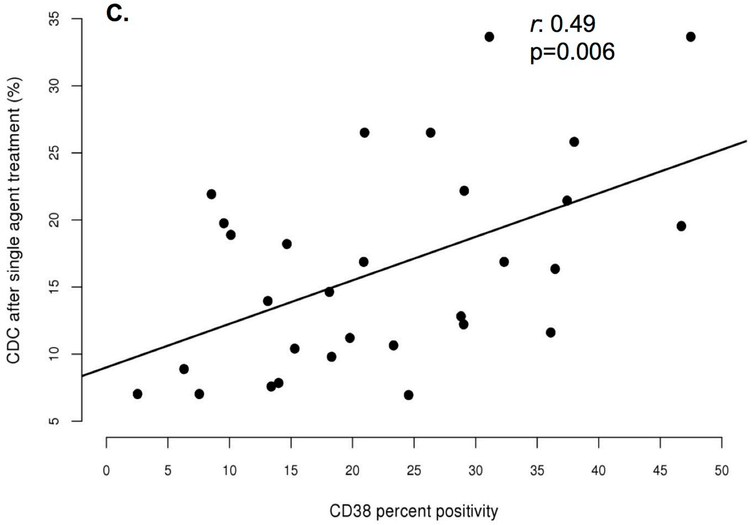


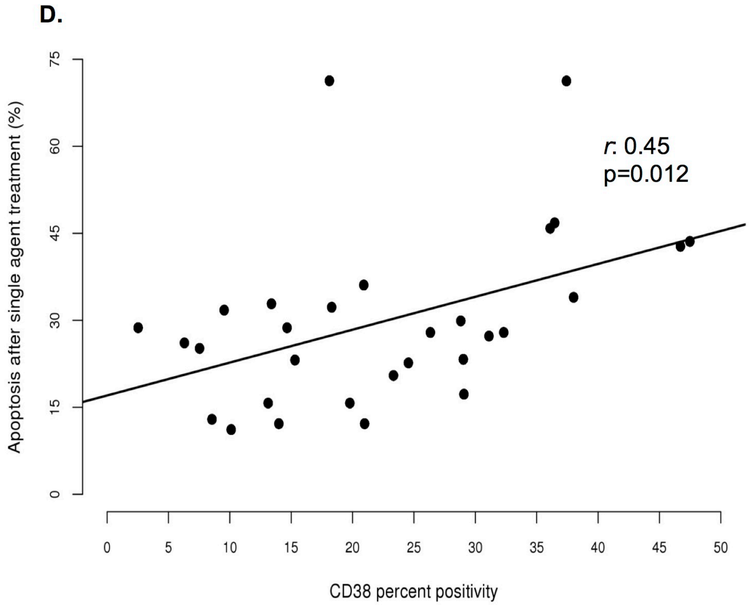


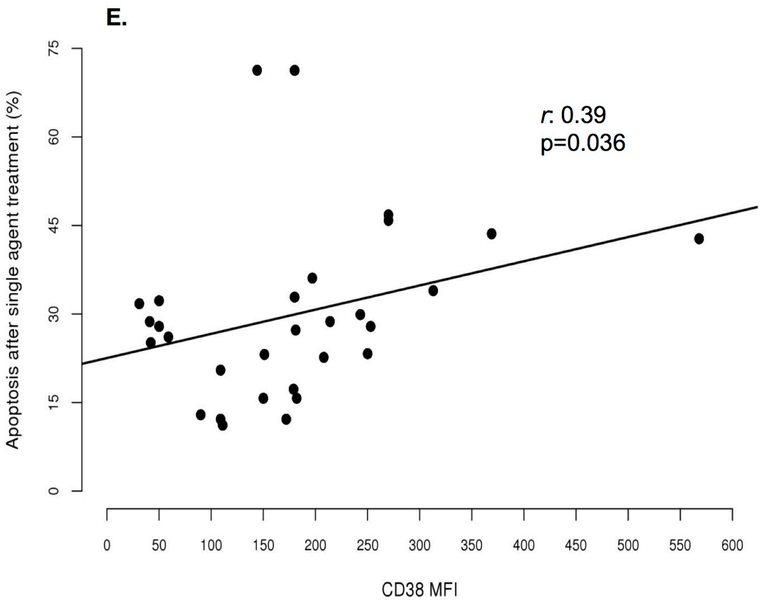
Supplemental Figure 4

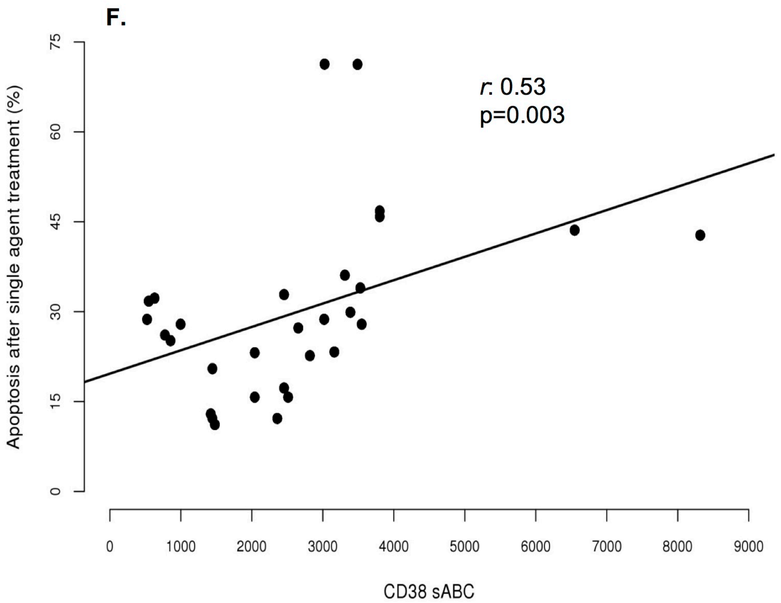




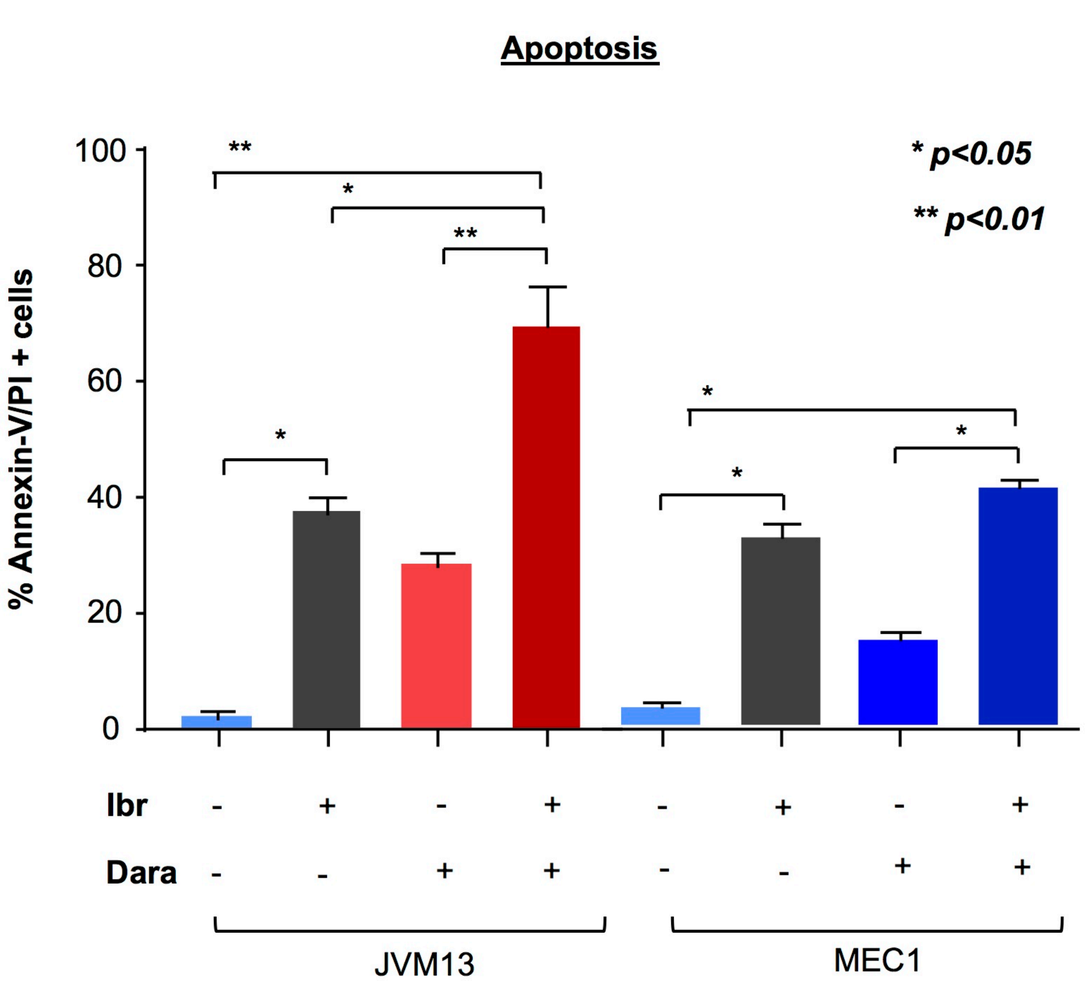


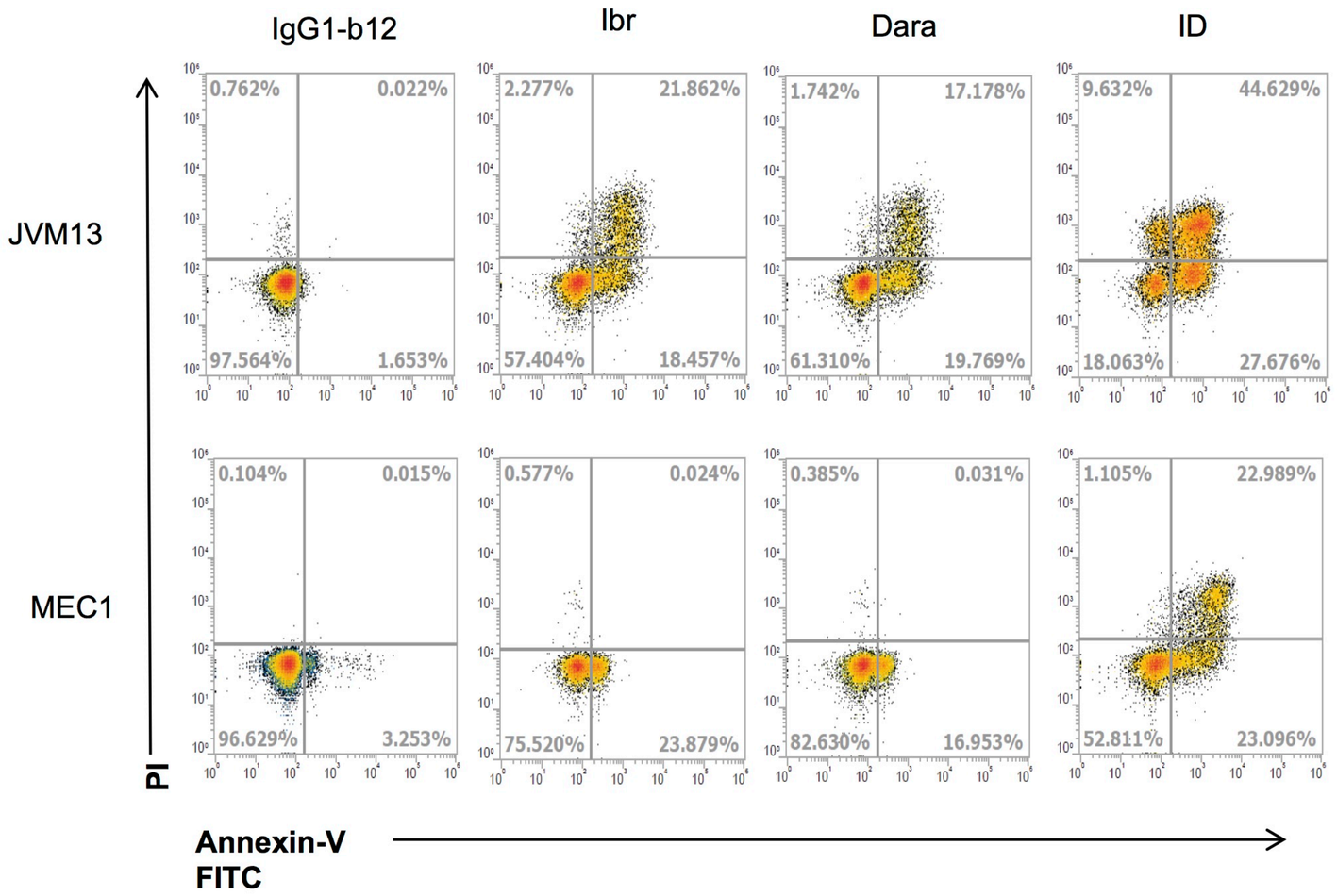




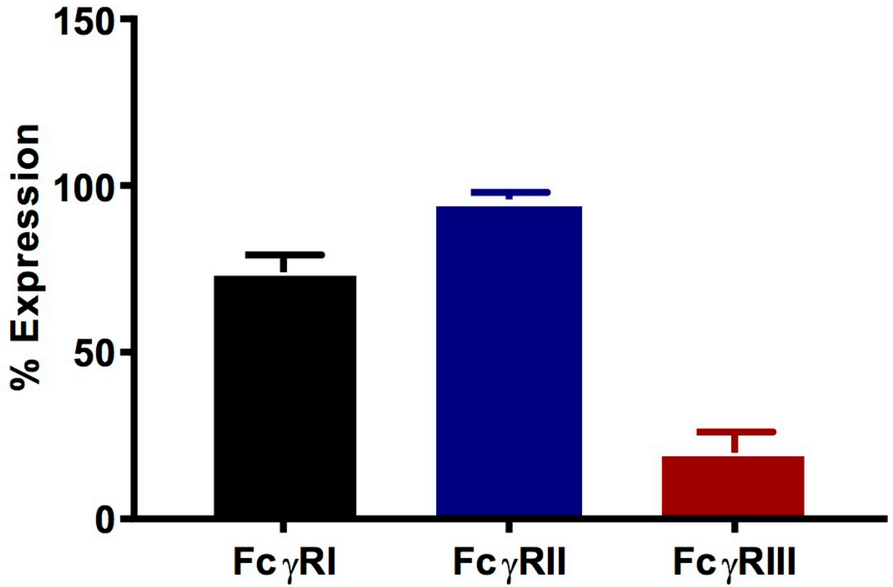


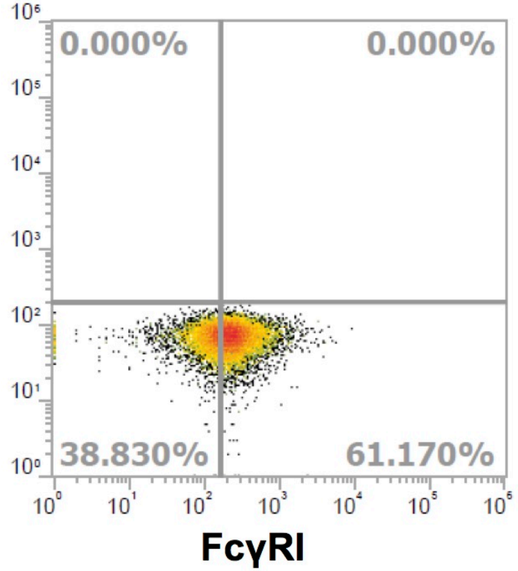
Supplemental Figure 5

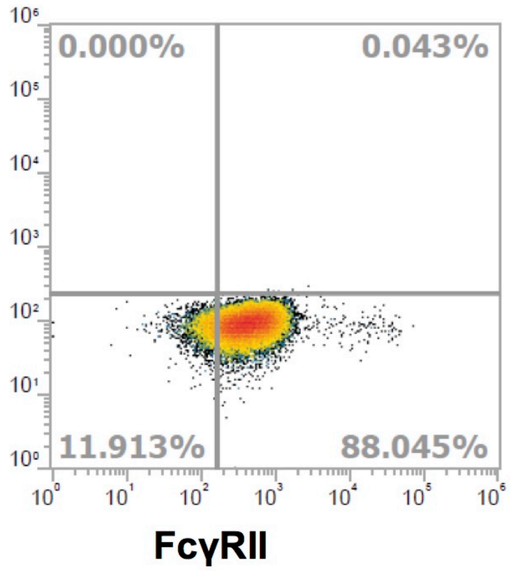


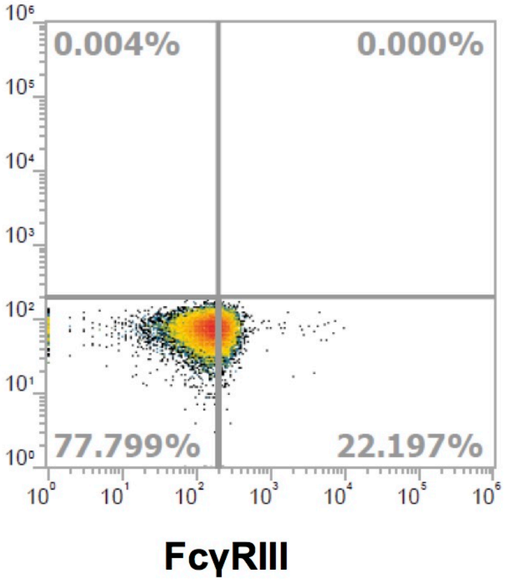


Supplemental Figure 6

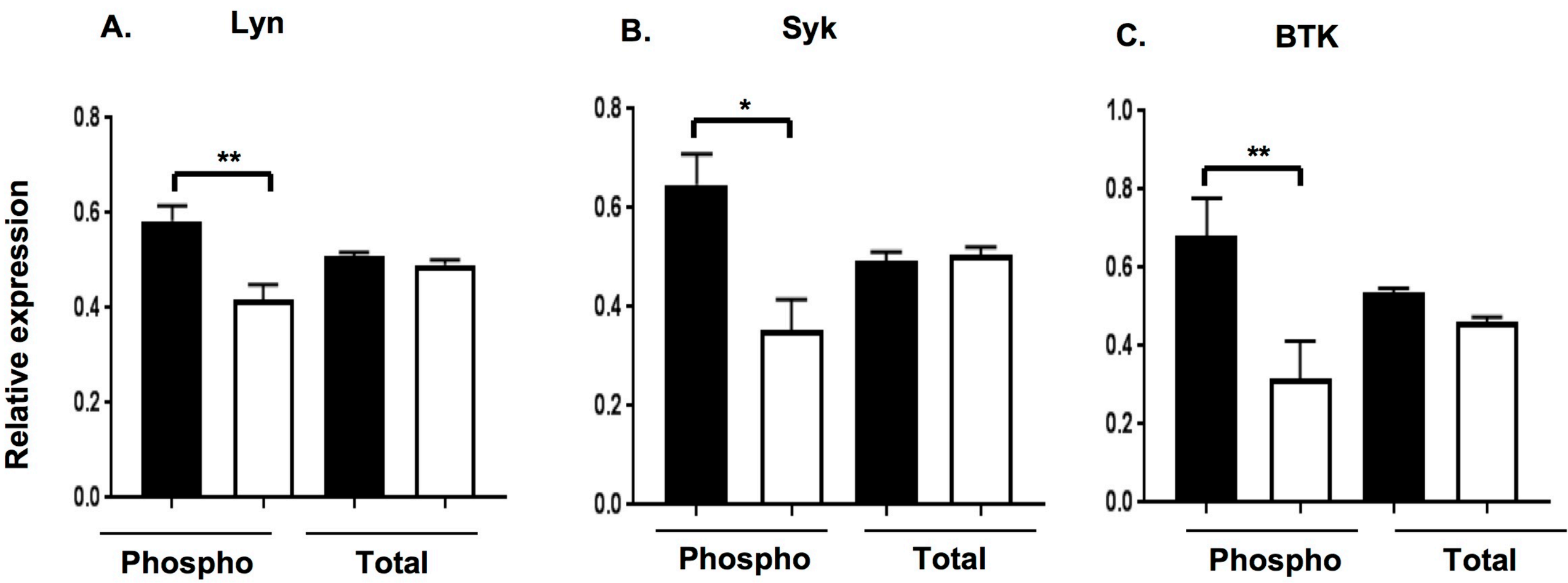




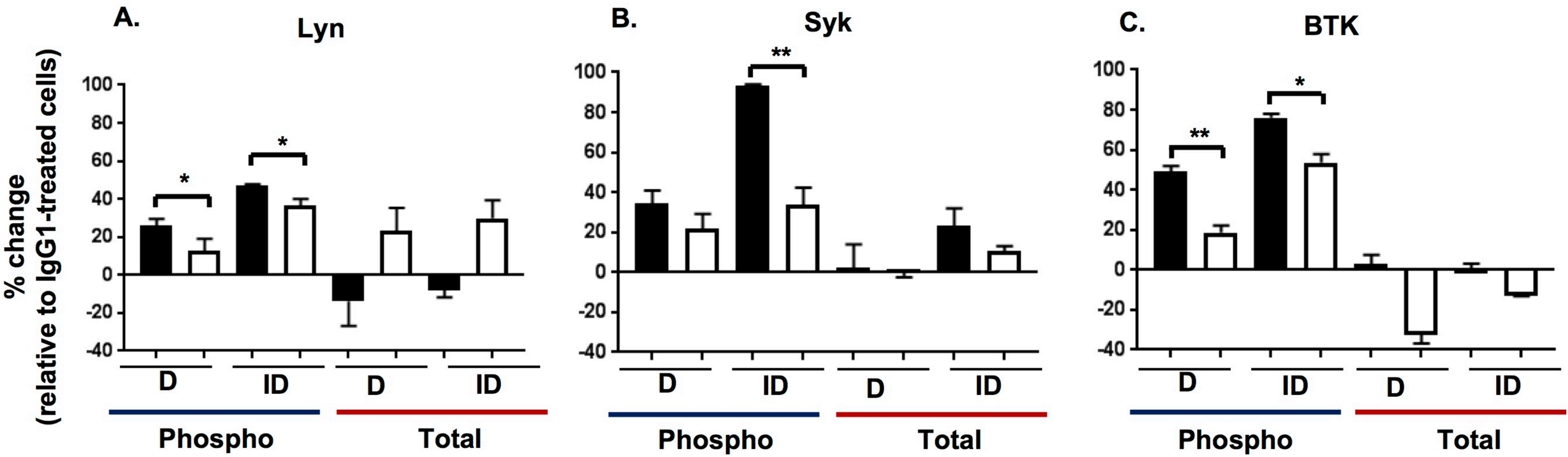


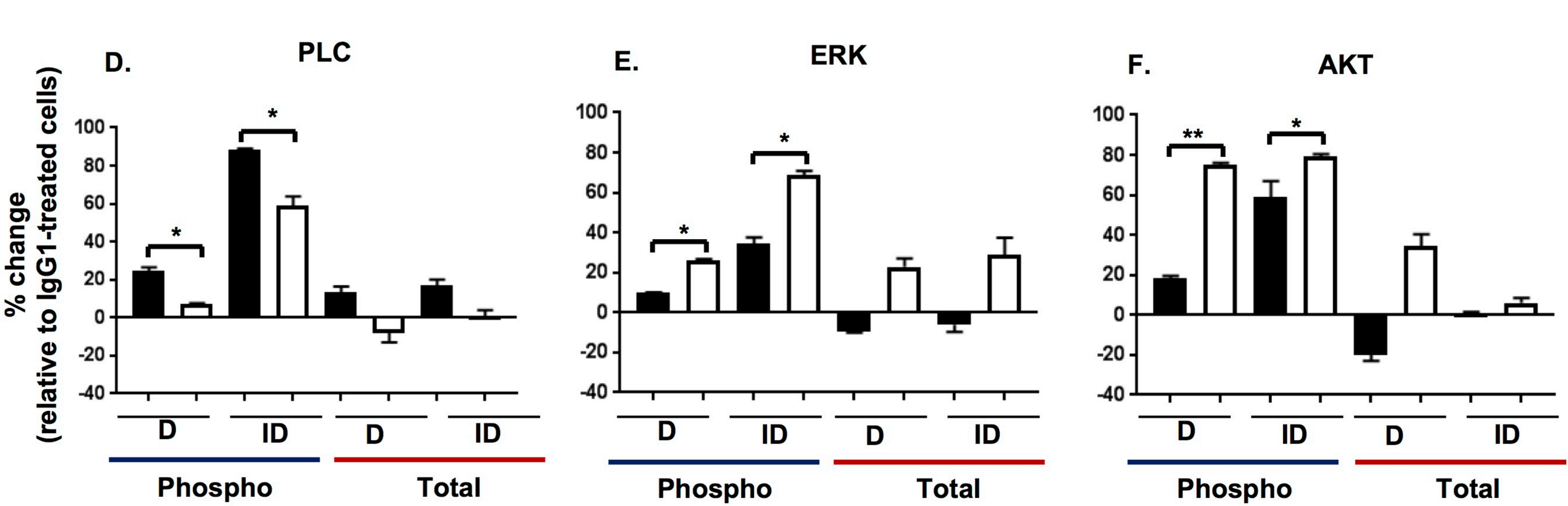


Supplemental Figure 7

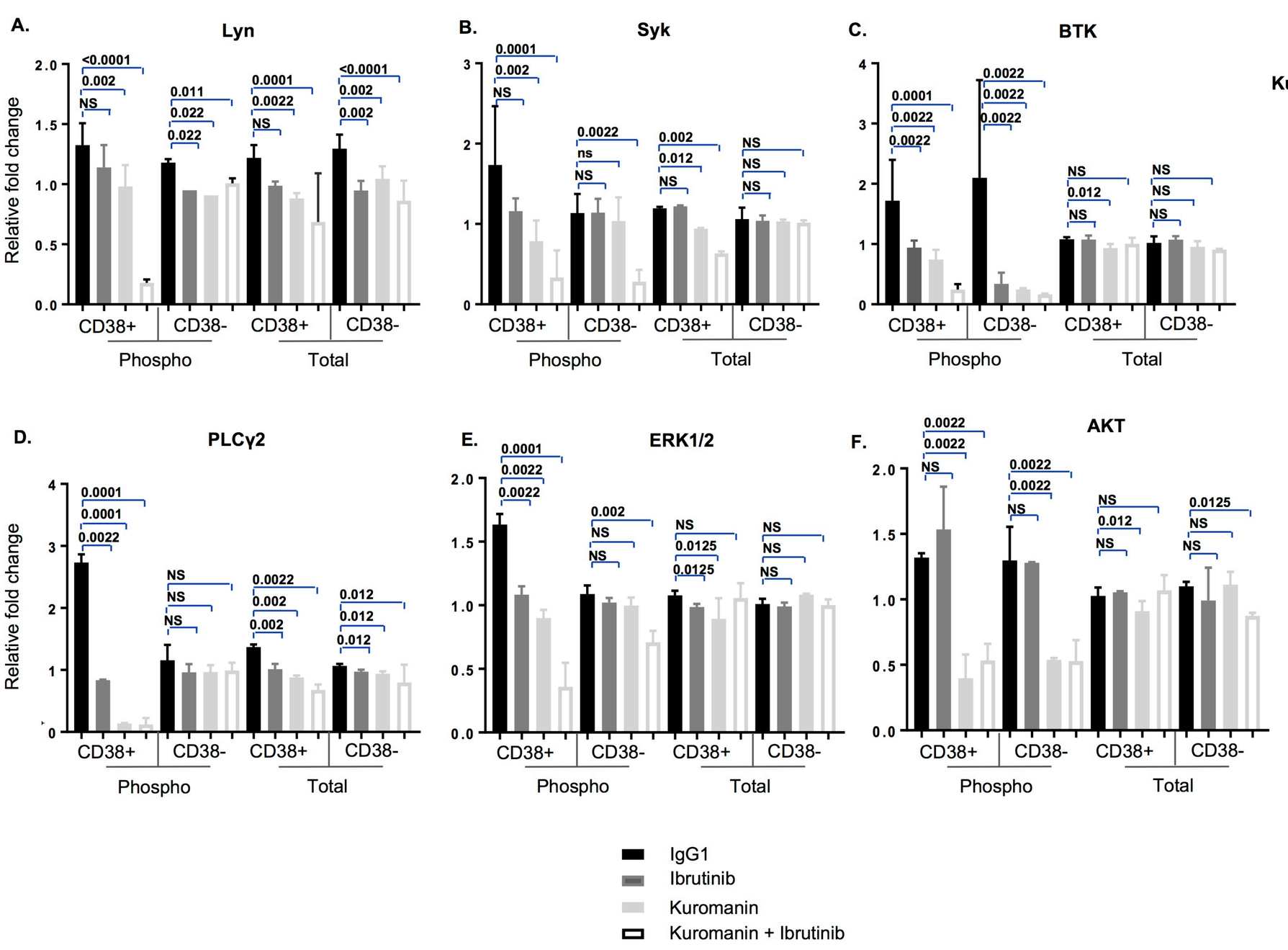


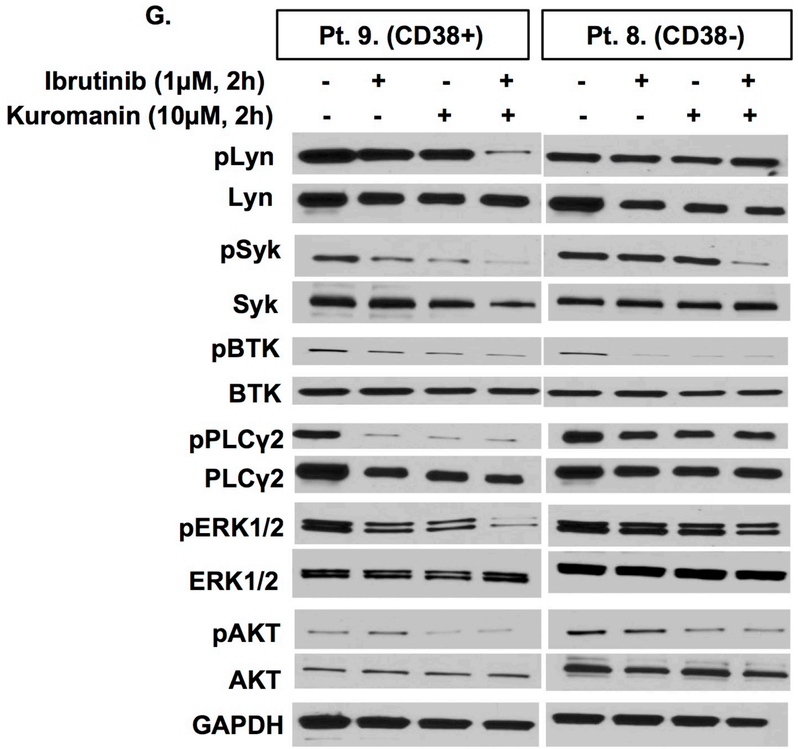
Supplemental Figure 8

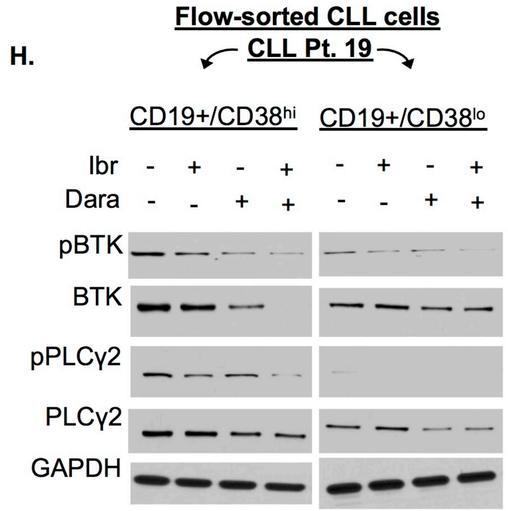




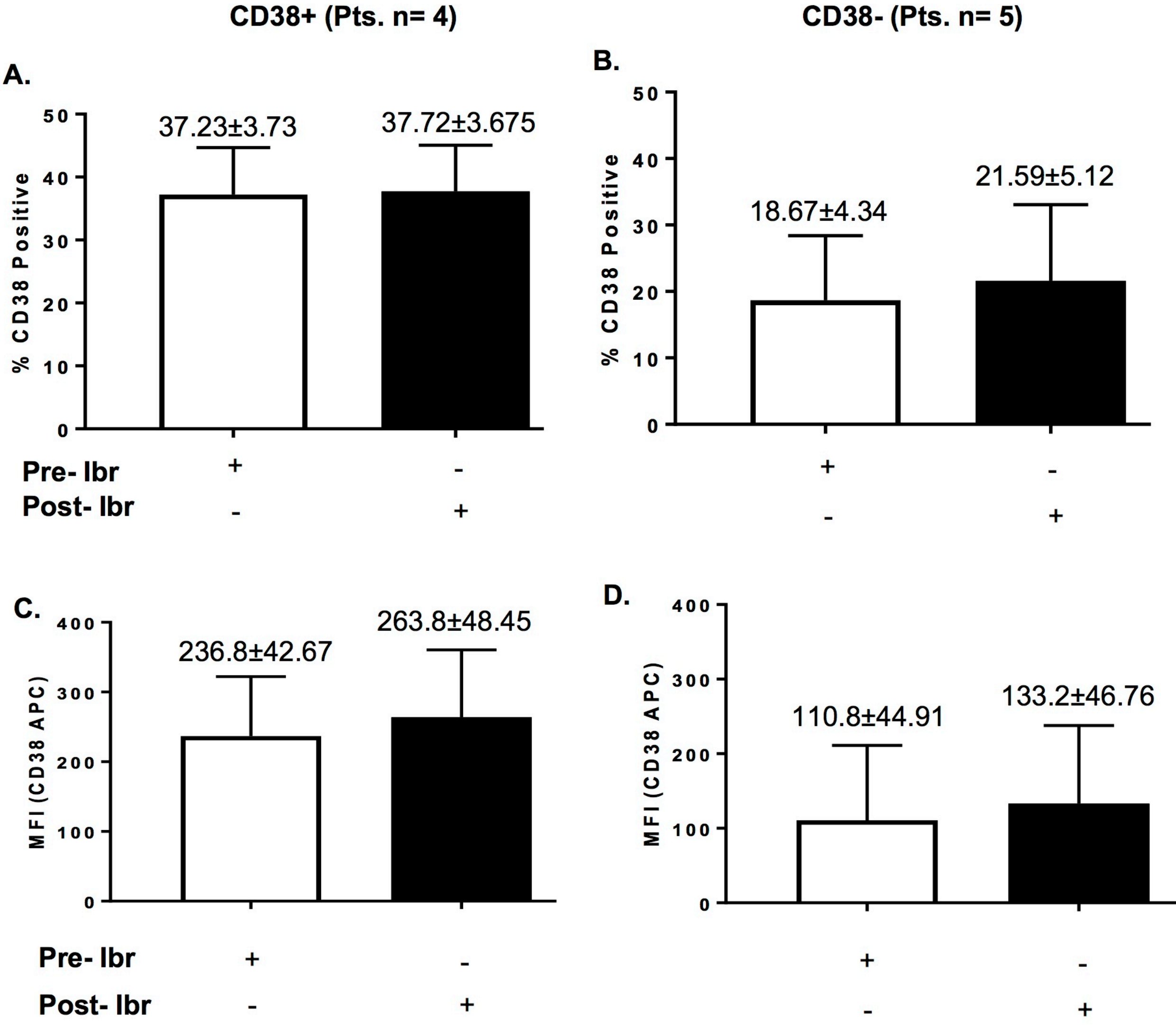
Supplemental Figure 9



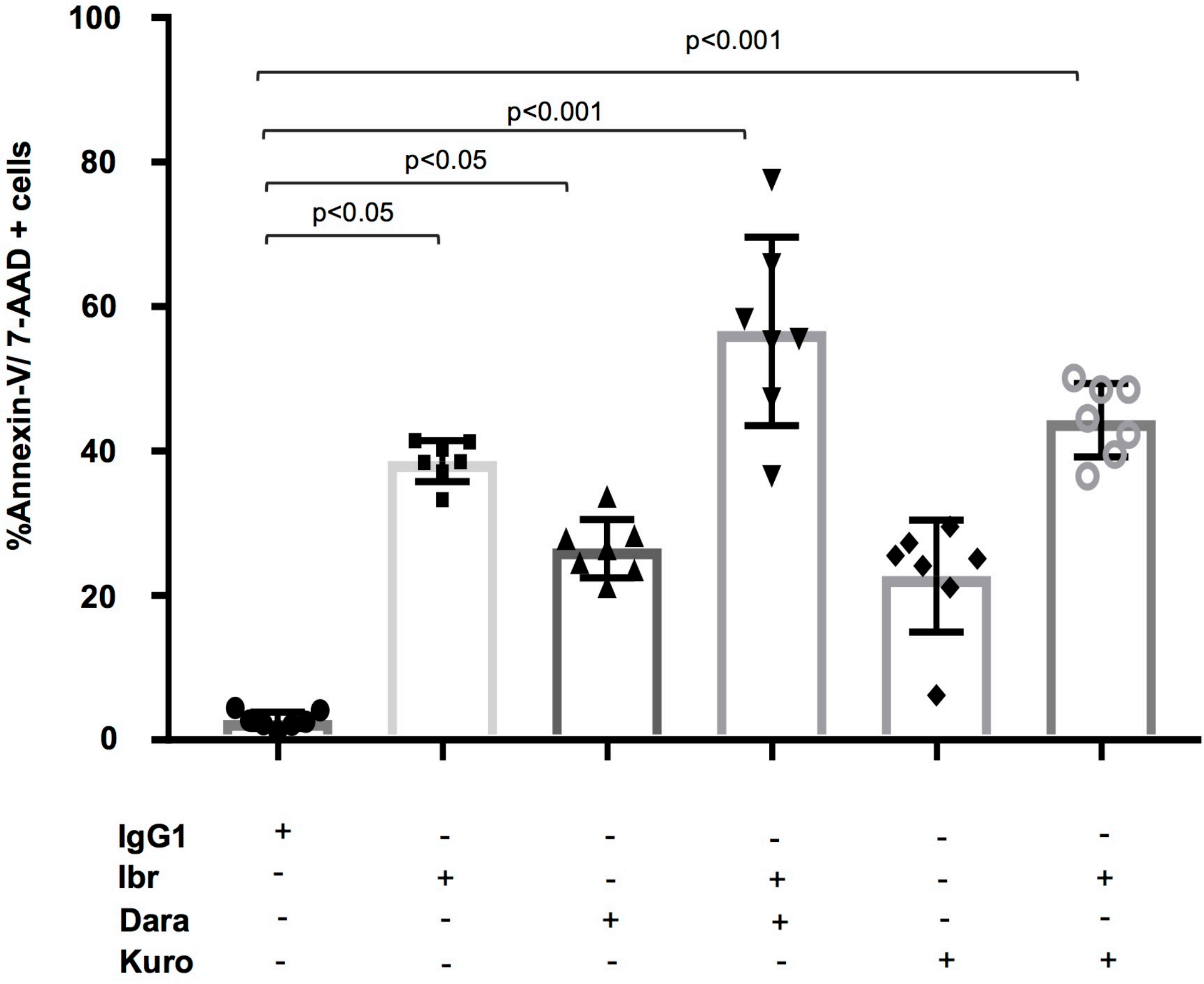




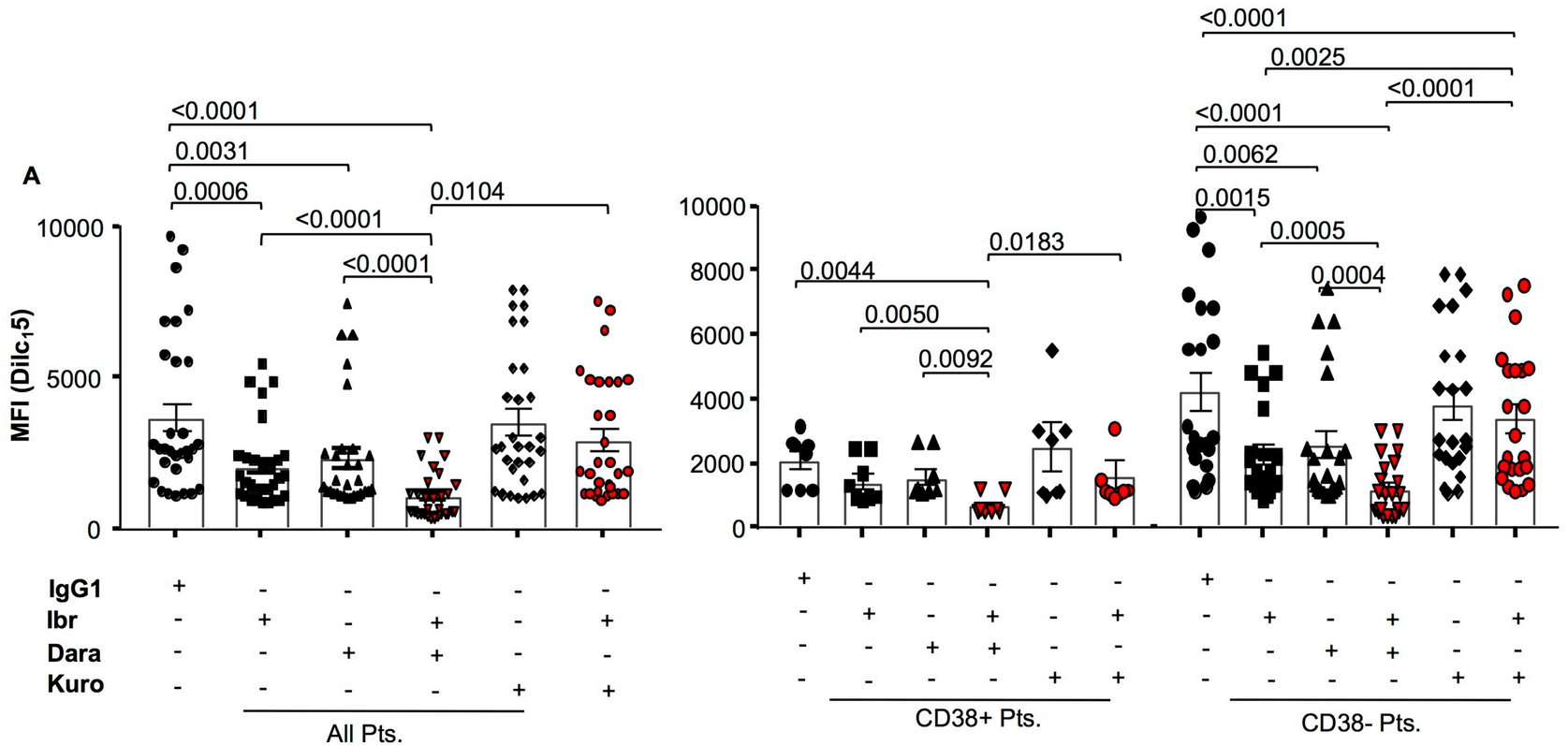
Supplemental Figure 10

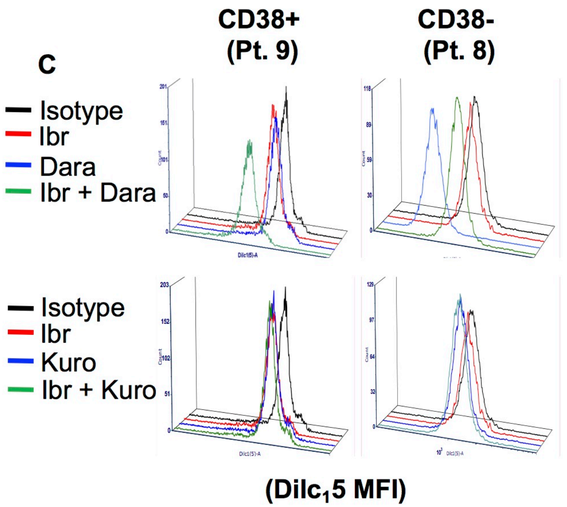


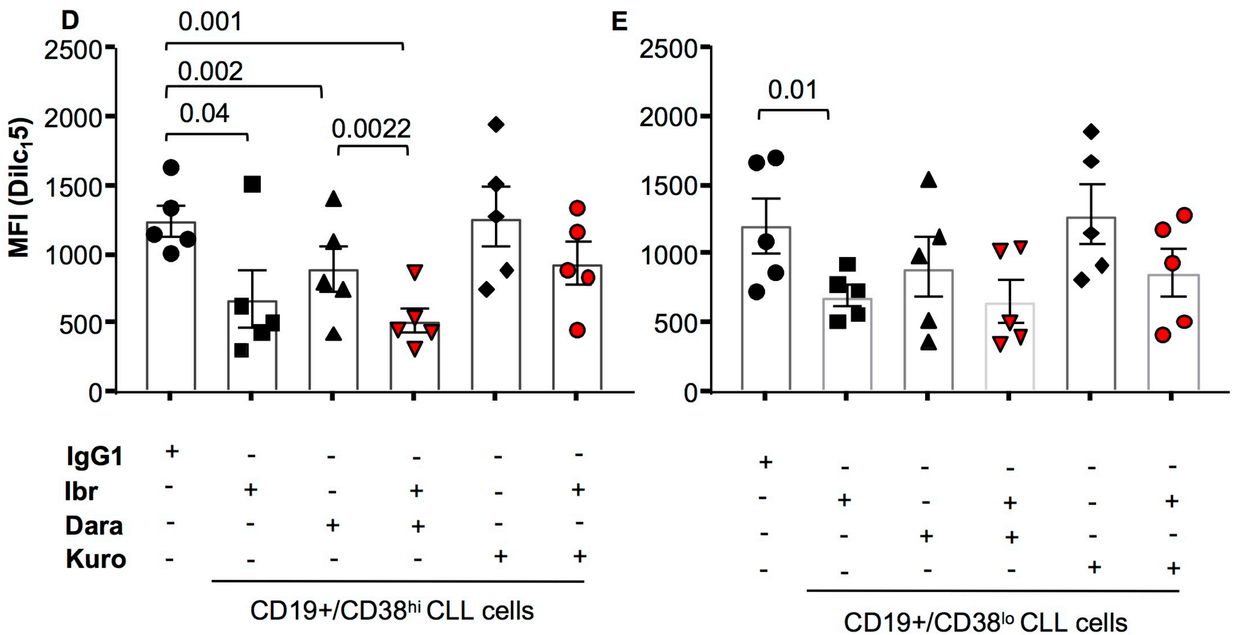
Supplemental Figure 11

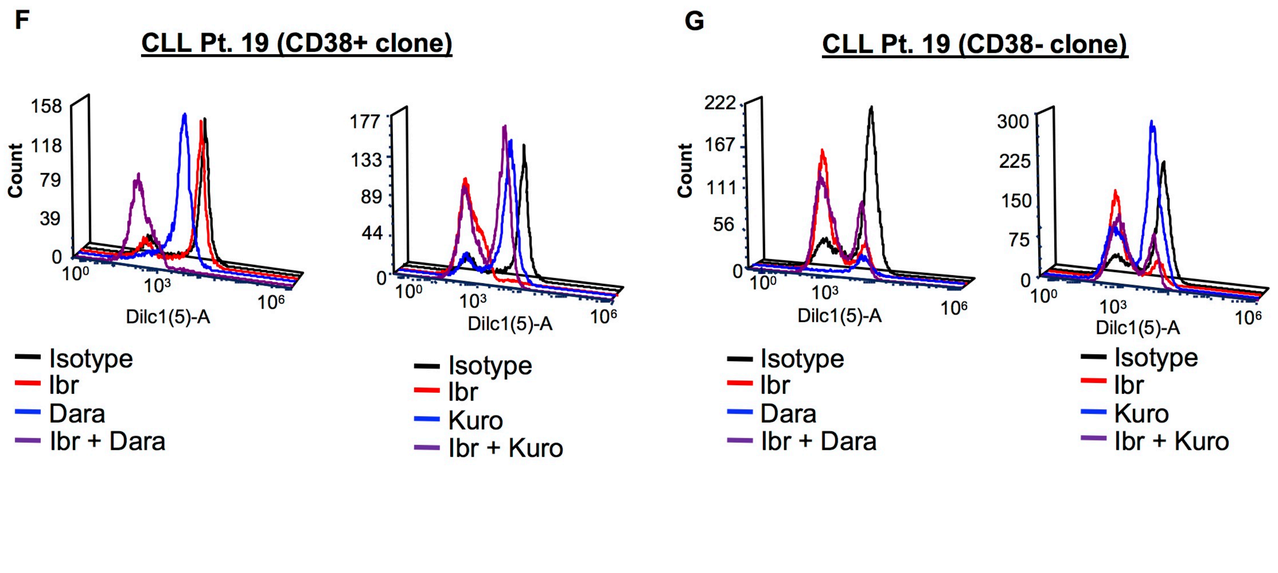


Supplemental Figure 12









Supplementary Tables.

Table S1. Clinical characteristics of CLL patients whose cells were used in the study.

Pt. #	Gender	Age	Rai Stage	Tx. status	IgVH	ZAP-70+	Del 17P+	Complex karyotype	Cytogenetics	CD38 (% positivity)	CD38 (MFI)	CD38 (sAbc)
1*	M	79	0	R/R	mutated	Yes	Yes	No	RB1 del	47.48	369	6548
2*	F	69	I	TN	un-mutated	Yes	No	No	trisomy 12, del 13q	31.11	181	2654
3	M	71	I	PT	N/A	N/A	No	Yes	IgH-BCL2 fusion, trisomy 12, del 13q	29.02	250	3162
4	M	90	0	PT	N/A	N/A	Yes	No	trisomy 12	26.34	50	997
5	M	67	I	PT	mutated	Yes	No	No	normal	20.91	197	3311
6	M	65	II	PT	mutated	N/A	No	No	del 13q	9.54	31	548
7	M	72	I	TN	un-mutated	Yes	No	No	trisomy 12	10.11	111	1479
8	M	58	0	TN	un-mutated	No	No	No	del 6q	7.54	42	855
9*	F	74	I	TN	un-mutated	N/A	No	No	N/A	38.01	313	3528
10	M	76	0	TN	mutated	No	No	No	trisomy 12	29.07	179	2454
11*	M	66	III	TN	un-mutated	Yes	No	No	del 13q	36.46	270	3801
12	M	53	I	PT	un-mutated	Yes	No	No	normal	28.81	243	3388
13	M	42	I	TN	un-mutated	Yes	Yes	Yes	del 13q, del 6q, del 11q	13.39	180	2454
14	M	59	I	TN	mutated	Yes	No	No	del 11q	18.11	144	3025
15	M	64	0	TN	mutated	Yes	No	No	del 13q	13.12	182	2511
16*	F	69	I	TN	un-mutated	No	Yes	Yes	del 11q, del 13q	46.71	568	8317
17*	M	71	IV	PT	un-mutated	Yes	No	No	del 13q	36.1	270	3801
18*	F	43	0	TN	un-mutated	Yes	No	No	del 13q	37.43	180	3489
19	M	65	III	R/R	N/A	Yes	Yes	Yes	del 13q, del 2q, del 11p	20.98	109	1445

20	M	64	II	TN	mutated	No	No	No	del 13q	24.55	208	2818
21	M	61	II	R/R	N/A	Yes	Yes	No	del 13q	2.53	41	524
22	F	78	0	TN	mutated	No	No	No	normal	6.3	59	776
23	F	58	0	TN	mutated	No	No	No	normal	18.3	50	630
24	F	50	IV	PT	N/A	Yes	No	No	del 13q	15.3	151	2041
25	M	71	II	PT	mutated	N/A	No	No	trisomy 12	23.33	109	1445
26*	M	68	IV	R/R	un-mutated	No	No	No	del 11q, del 13q	32.31	253	3548
27	M	69	IV	TN	mutated	No	No	No	normal	19.78	150	2041
28	M	80	0	R/R	N/A	No	No	Yes	del 11q, del 13q, IGH-BCL2 fusion	8.54	90	1422
29	M	60	I	TN	N/A	No	No	No	trisomy 12	13.99	172	2358
30	M	67	I	R/R	un-mutated	N/A	No	No	normal	14.66	214	3019
[a]31^{++}	M	66	0	TN	mutated	N/A	No	No	del 13q	15.98	137	2832
[a]32	M	66	I	R/R	un-mutated	Yes	No	No	del 13q	8.72	113	857
[a]33*	F	69	I	TN	un-mutated	Yes	No	No	del 13q	38.54	347	4142
[a]34*	F	50	I	TN	N/A	No	No	No	normal	45.95	401	5498
[a]35	F	74	0	R/R	un-mutated	N/A	No	No	normal	7.58	127	1011
[a]36	F	68	I	R/R	N/A	Yes	Yes	Yes	normal	10.54	132	954

NA, Not available

* Patients that were designated as CD38+ using the standardized clinical definition where >30% of CD19+/CD5+ CLL clones express CD38.

31^{++}, only used in experiments where CLL cells were flow-sorted for purifying CD19+/$CD38^{hi}$ and CD19+/$CD38^{lo}$ cell populations but were not used in other (non-flow sorted) experiments. And as such his data was not included in the overall correlation analysis or patient demographics summary in Supplementary Table 1.

[a.] Due to low tumor cell volume, these patients CLL cells were not used in cell death (ADCC, CDC, ADCP, apoptosis) assays. Cells were used in either immunoblot assays or experiments where FcγR-cross-linking-mediated cell death was assessed.

Table S2: Summary of Patient characteristics

Variant	Summary (N=36)
Median Age (years)	67 (range, 42, 90)
Gender (Male)	25 (76.7%)
Rai stage	
0	11 (30.5%)
I	15 (41.6%)
II	4 (11.1%)
III	2 (5.5%)
IV	4 (11.1%)
IgVH mutation	12 (33.3%)
Deletion 17p positive	7 (19.4%)
Complex karyotype	6 (16.6%)
ZAP-70 positive	17 (47.2%)

Information was unavailable for 9 patients regarding IgVH mutation status and for 8 patients regarding ZAP70 status.

* Notably, these data are from all 36 patients whose samples were used in at least 1 experiment in the study

Table S3: Correlations between CD38 measures and cell death measures

	Correlation with CD38 MFI		Correlation with CD38 sABC		Correlation with CD38 percent positivity	
Cell death measure	Spearman's *r*	P-value	Spearman's *r*	P-value	Spearman's *r*	P-value
Single agent treatment (%)						
ADCC	0.30	0.11	0.32	0.081	0.19	0.32
CDC	0.23	0.22	0.29	0.12	0.49	0.006
ADCP	0.49	0.006	0.41	0.021	0.11	0.57
Apoptosis	0.39	0.036	0.53	0.003	0.45	0.012
Combination treatment (%)						
ADCC	0.38	0.037	0.34	0.069	0.46	0.012
CDC	0.41	0.024	0.38	0.040	0.52	0.003
ADCP	0.62	<0.001	0.68	<0.001	0.37	0.044
Apoptosis	0.13	0.49	0.25	0.18	0.28	0.13

P-values result from Spearman's test of correlation. Samples from 30 patients (as listed in Table 1), were used in these analyses.

Table S4: Associations between patient characteristics and cell death measures after single agent daratumumab treatment

	Association with ADCC		Association with CDC		Association with ADCP		Association with Apoptosis	
Characteristic	Median cell death (%)	P-value	Median cell death (%)	P-value	Median cell death (%)	P-value	Median cell death (%)	P-value
Age		0.58		0.036		0.45		0.38
≤ 67 years (N=16)	16.5		13.4		9.7		29.3	
> 67 years (N=14)	10.3		19.2		8.8		26.7	
Gender		0.13		0.57		0.60		0.20
Male (N=23)	10.7		14.6		9.3		27.9	
Female (N=7)	25.5		19.5		8.0		32.3	
Rai stage		0.53		0.19		0.59		0.61
0-I (N=12)	14.3		17.6		9.2		28.3	
II-IV (N=10)	13.6		11.4		8.9		25.5	
IgVH mutation		0.46		0.46		0.38		0.37
No (N=12)	10.7		17.6		9.6		31.3	
Yes (N=9)	19.7		14.0		9.0		26.1	
Del17p		0.37		0.34		0.53		0.44
No (N=24)	11.4		14.3		9.2		26.7	
Yes (N=6)	20.3		23.0		8.7		30.8	
Complex karyotype		0.52		0.56		0.48		0.49
No (N=25)	12.1		14.6		9.3		27.9	
Yes (N=5)	15.1		19.5		8.7		23.3	
ZAP-70		0.93		0.25		0.11		0.095
No (N=10)	16.9		10.5		8.7		23.9	
Yes (N=14)	18.8		15.5		9.6		31.4	

P-values result from a Wilcoxon rank sum test. Numerical variables were dichotomized at the sample median for ease of presentation. Information was unavailable regarding IgVH mutation status (N=7) and ZAP-70 (N=6). Samples from 30 patients (as listed in Table 1), were used in these analyses.

Table S5: Associations between patient characteristics and cell death measures after combination treatment

	Association with ADCC		Association with CDC		Association with ADCP		Association with Apoptosis	
Characteristic	Median cell death (%)	P-value	Median cell death (%)	P-value	Median cell death (%)	P-value	Median cell death (%)	P-value
Age		0.093		0.55		0.26		0.55
≤ 67 years (N=16)	33.7		33.0		20.4		62.8	
> 67 years (N=14)	43.6		34.9		22.7		63.1	
Gender		0.096		0.30		0.50		0.28
Male (N=23)	34.0		30.5		21.0		60.4	
Female (N=7)	54.6		42.9		23.5		67.3	
Rai stage		0.98		0.88		0.14		0.43
0-I (N=12)	35.2		32.9		24.0		64.0	
II-IV (N=10)	42.7		35.4		16.8		59.5	
IgVH mutation		0.51		0.31		0.25		0.35
No (N=12)	36.3		35.8		24.0		65.7	
Yes (N=9)	32.5		34.5		17.3		61.8	
Del17p		0.021		0.036		0.90		0.59
No (N=24)	34.3		28.9		21.7		62.8	
Yes (N=6)	57.7		48.3		20.1		63.2	
Complex karyotype		0.17		0.42		0.45		0.084
No (N=25)	34.6		34.5		21.0		63.8	
Yes (N=5)	48.3		38.0		24.0		56.6	
ZAP-70		0.21		0.15		0.084		0.32
No (N=10)	34.7		26.8		17.0		60.3	
Yes (N=14)	45.0		36.2		24.1		64.0	

P-values result from a Wilcoxon rank sum test. Numerical variables were dichotomized at the sample median for ease of presentation. Information was unavailable regarding IgVH mutation status (N=7) and ZAP-70 (N=6). Samples from 30 patients (as listed in Table 1), were used in these analyses.

Table S6. % cell death in CLL patients whose cells were used across various immune-effector mediated and direct cell death assays.

				Single agent Daratumumab				Ibrutinib + Daratumumab (ID)			
Pt. #	**CD38 (% positivity)**	**CD38 (MFI)**	**CD38 (sAbc)**	**%ADCC**	**%CDC**	**%ADCP**	**Apoptosis**	**%ADCC (ID)**	**%CDC (ID)**	**%ADCP (ID)**	**Apoptosis (ID)**
1	47.48	369	6548	49.93	33.65	12.16	43.6	91.32	64.01	30.08	62.44
2	31.11	181	2654	5.86	33.65	8.02	27.28	76.32	51.22	23.19	68.73
3	29.02	250	3162	8.47	12.22	6.47	23.28	48.33	23.4	21.24	56.62
4	26.34	50	997	5.15	26.51	1.9	27.9	43.8	50.78	5.86	64.4
5	20.91	197	3311	19.71	16.88	10.21	36.08	33.98	35.47	24.18	64
6	9.54	31	548	6.26	19.76	1.82	31.75	14.73	45.32	11.16	60.33
7	10.11	111	1479	6.69	18.89	9.66	11.16	27.91	21.81	24.63	63.84
8	7.54	42	855	10.25	7.03	9.75	25.16	29.82	12.21	16.41	76.7
9	38.01	313	3528	30.16	25.82	12.66	33.96	67.46	45.32	34.78	67.26
10	29.07	179	2454	30.07	22.17	8.62	17.26	34.8	35.18	16.56	80.4
11	36.46	270	3801	26.51	16.35	9.73	46.8	50.78	42.91	14.23	81.9
12	28.81	243	3388	10.68	12.82	11.75	29.9	33.39	23.22	19.71	58.94
13	13.39	180	2454	10.74	7.59	9.62	32.85	31.78	37.98	23.97	63.91
14	18.11	144	3025	29.36	14.64	6.7	71.3	22.14	19.29	26.87	80.84
15	13.12	182	2511	17.81	13.96	13.23	15.73	32.52	36.83	25.17	56.52
16	46.71	568	8317	35.54	19.54	8.75	42.76	75.06	45.88	34.63	64.21
17	36.1	270	3801	5.52	11.61	9.6	45.86	72.69	24.56	37.83	77.97
18	37.43	180	3489	21.83	21.44	7.27	71.27	39.25	35.41	27.21	76.08
19	20.98	109	1445	15.14	26.51	8.7	12.18	60.58	50.78	16.18	35.21
20	24.55	208	2818	21.67	6.95	4.18	22.66	34.58	26.46	21.01	47.94

21	2.53	41	524	25.47	7.03	5.66	28.72	54.82	14.56	14.68	58.43
22	6.3	59	776	25.54	8.89	3.07	26.1	42.95	20.54	11.62	46.72
23	18.3	50	630	9.36	9.8	3.41	32.25	16.07	19.91	4.87	61.78
24	15.3	151	2041	31.48	10.41	14.44	23.15	54.58	42.91	23.49	81.79
25	23.33	109	1445	7.35	10.65	9	20.49	27.72	19.21	16.3	60.36
26	32.31	253	3548	12.05	16.88	8.77	27.9	19.3	36.2	22.18	42.19
27	19.78	150	2041	5.75	11.21	9.31	15.73	30.81	34.52	17.32	58.72
28	8.54	90	1422	25.91	21.92	8.436	12.93	43.45	22.09	24.23	20.14
29	13.99	172	2358	10.4	7.85	10.68	12.18	35.68	27.21	15.7	81.9
30	14.66	214	3019	8.68	18.21	12.68	28.72	20.02	30.54	24.08	57.88