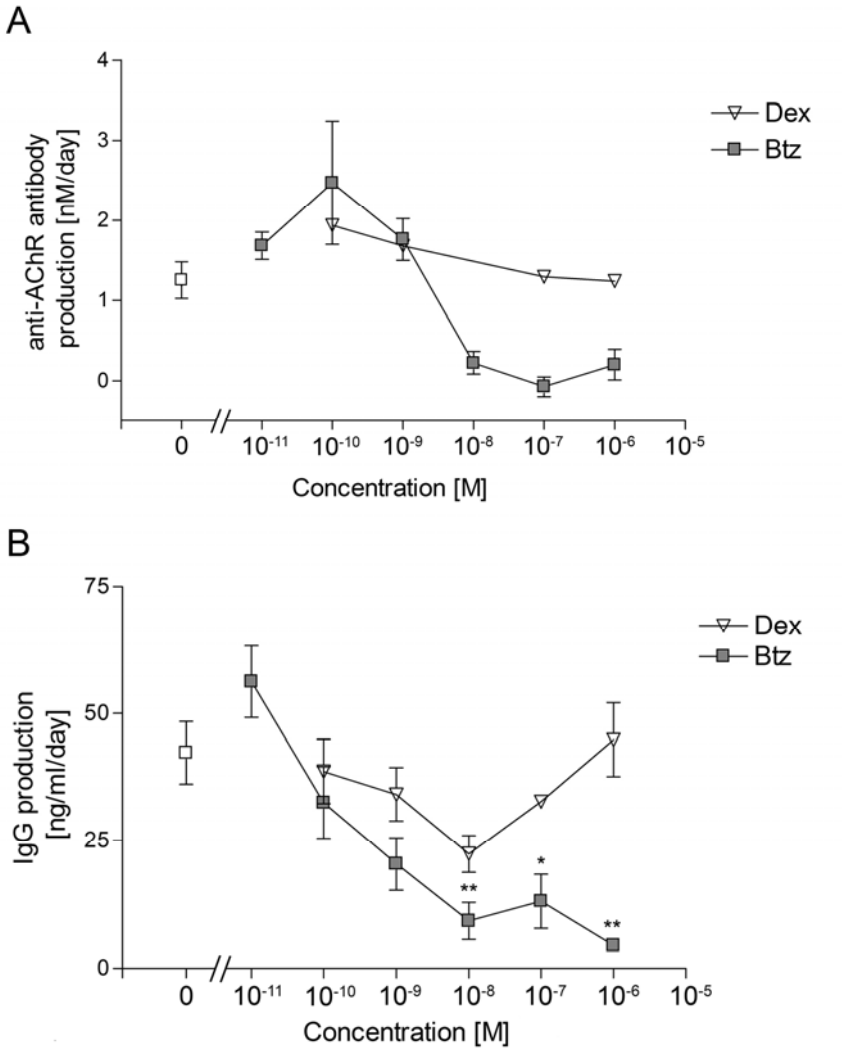
Supplemental Table S1

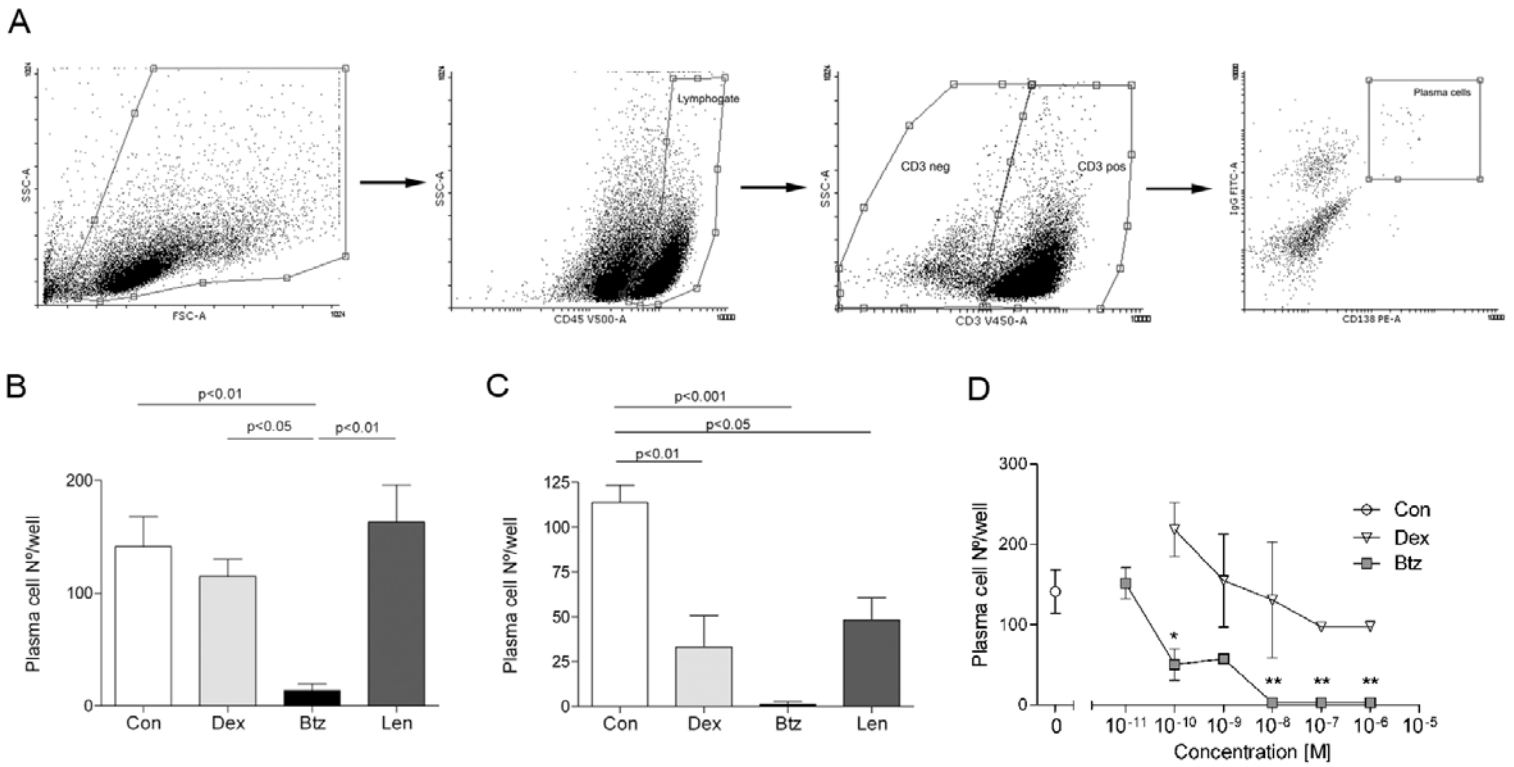
Total number of events counted for each of the subpopulations analyzed by flow cytometry.

Patient	Viable cells (Nº Events)	$CD3^+$ cells (Nº Events)	$CD19^+$ cells (Nº Events)
MG-1	1,312	859	115
MG-2	2,143	1,573	168
MG-3	1,443	805	126
MG-4	4,183	1,831	116
MG-5	25,517	16,894	916
MG-6	3,953	*N/A*	*N/A*

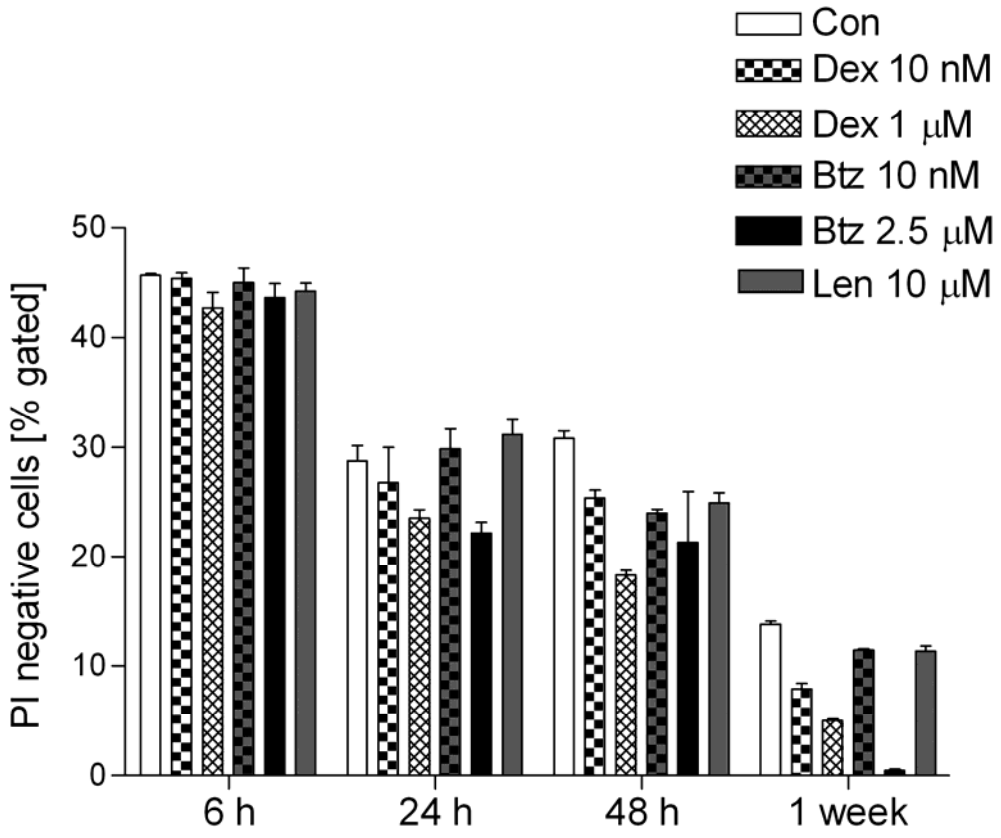
N/A: not analyzed



Supplemental Figure 1. Drug effects on fresh thymic cell cultures. Thymic cells from patient MG-10 were dispersed mechanically and enzymatically immediately after thymectomy and cultured for 14 days. Bortezomib and dexamethasone were added at different concentrations on days 7 and 11, and cells were collected on day 14. Both AChR Ab (A) and IgG (B) production were reduced by bortezomib at concentrations ≥1x 10^{-8}M, while dexamethasone had no significant effects on either. Each point represents the average of at least two samples, and error bars correspond to the SEM. One-way ANOVA and Bonferroni post-hoc testing were used for statistical analysis. * $p<0.05$; ** $p<0.01$, compared with the untreated cultures (white square).



Supplemental Figure 2. Cytofluorometric analysis of drug effects on CD138^{+} cells. Thymic cells were cultured for 14 days and drugs were added on days 7 and 11. Cells were collected and labeled for FACS analysis on day 14. **A.** Gating strategy for selection of the plasma cell population. We gated on CD45^{+}, CD3^{-} and IgG^{high}/CD138^{+} cells and excluded debris and doublets (not shown). Approximately 150,000 events per sample were recorded. Samples exposed to bortezomib showed a very significant reduction in absolute plasma cell numbers when enumerated under the microscope after cytocentrifugation (**B**), and this largely correlated with the drug effects observed by quantifying them by FACS (**C**). Dexamethasone and lenalidomide also reduced their absolute numbers significantly (**C**), but to a lesser extent than bortezomib. (**D**) A broader range of concentrations was tested for both bortezomib and dexamethasone; only bortezomib induced a significant depletion of plasma cell numbers (1x 10^{-6}M - 1x 10^{-8}M and 1x 10^{-10}M). One-way ANOVA was used for statistical comparison between conditions, each bar or point represents the average of 3 replicates and error bars correspond to the SEM. We used cells from patient MG-5 because of higher CD138^{+} frequencies/ absolute numbers.



Supplemental Figure 3. General toxicity of the experimental drugs on thymic cells. Thymic cells from patient MG-9 were cultured for 1 week in the presence of different concentrations of the experimental drugs. Cell viability was evaluated at 6 h, 24 h, 48 h and 1 week by measuring propidium iodide (PI) incorporation by flow cytometry; samples were gated in the FSC-SSC dot-plot to exclude membrane debris. Cell viability decreased with time in all the conditions and was lower after 1 week exposure to dexamethasone (10 nM and 1 μM) and bortezomib 2.5 μM than to bortezomib (10 nM) or lenalidomide (10 μM). Each bar represents the average of 3 replicates and error bars the SEM.