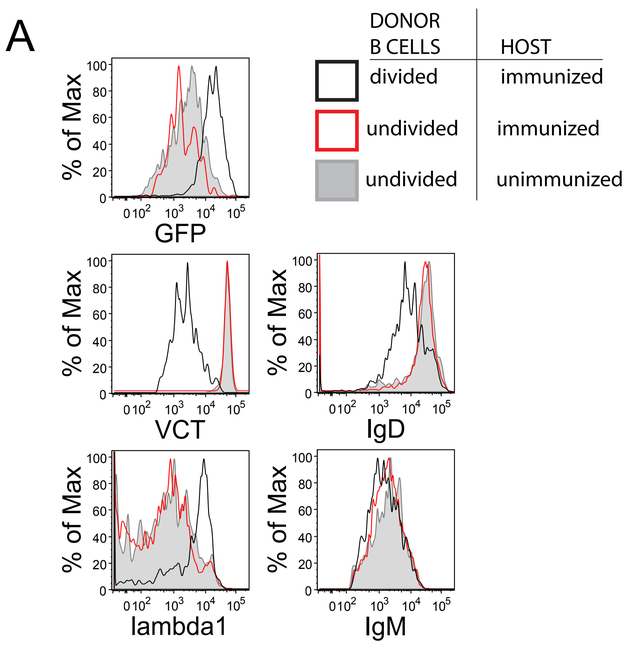
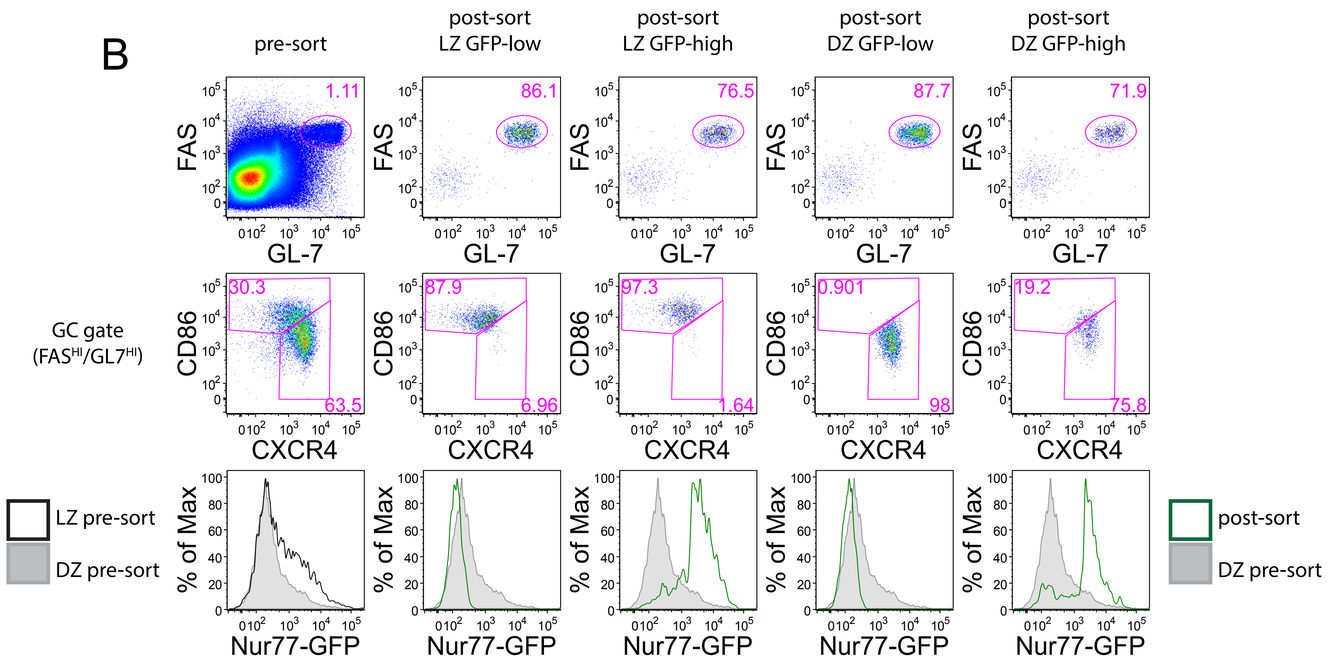
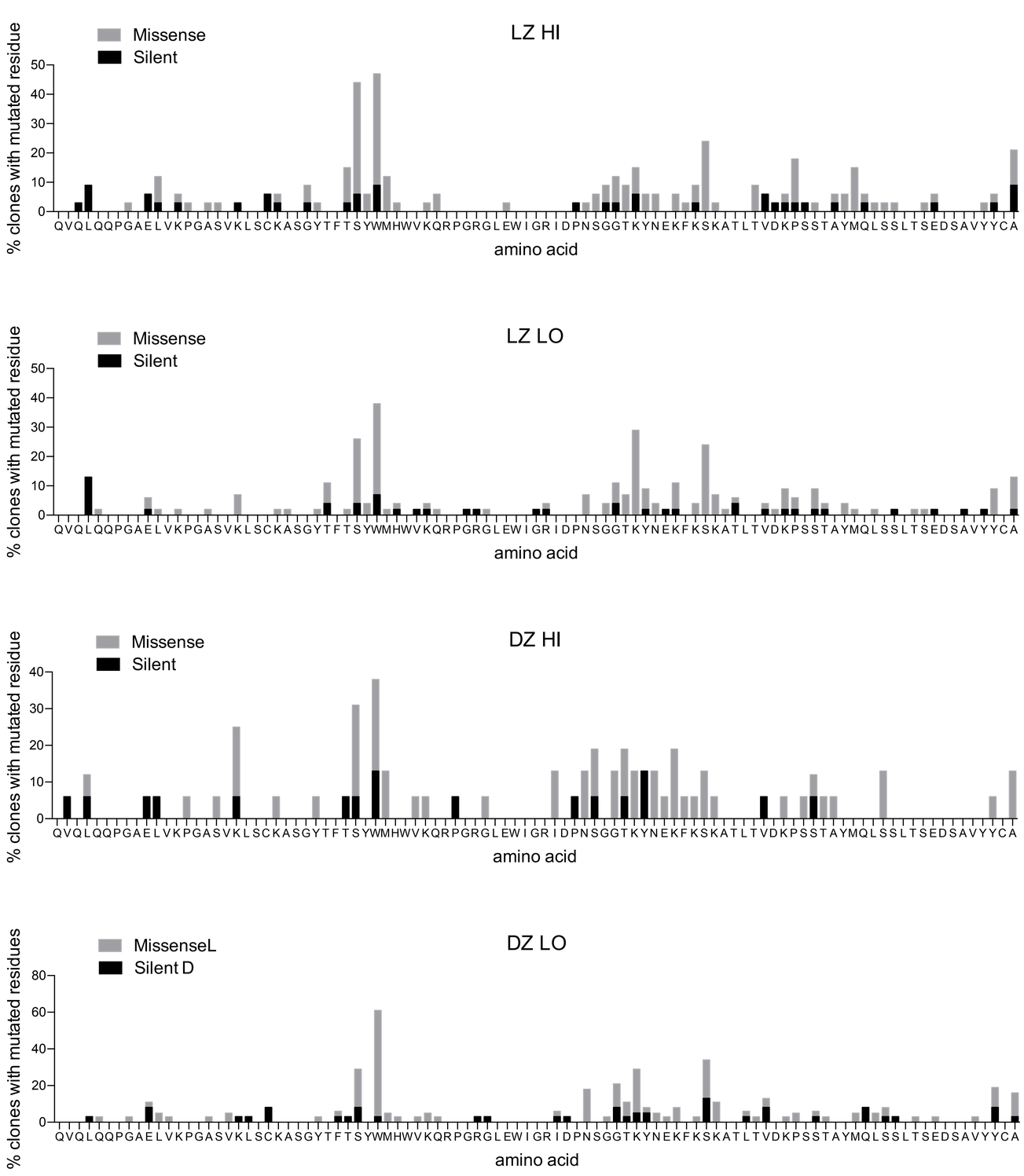
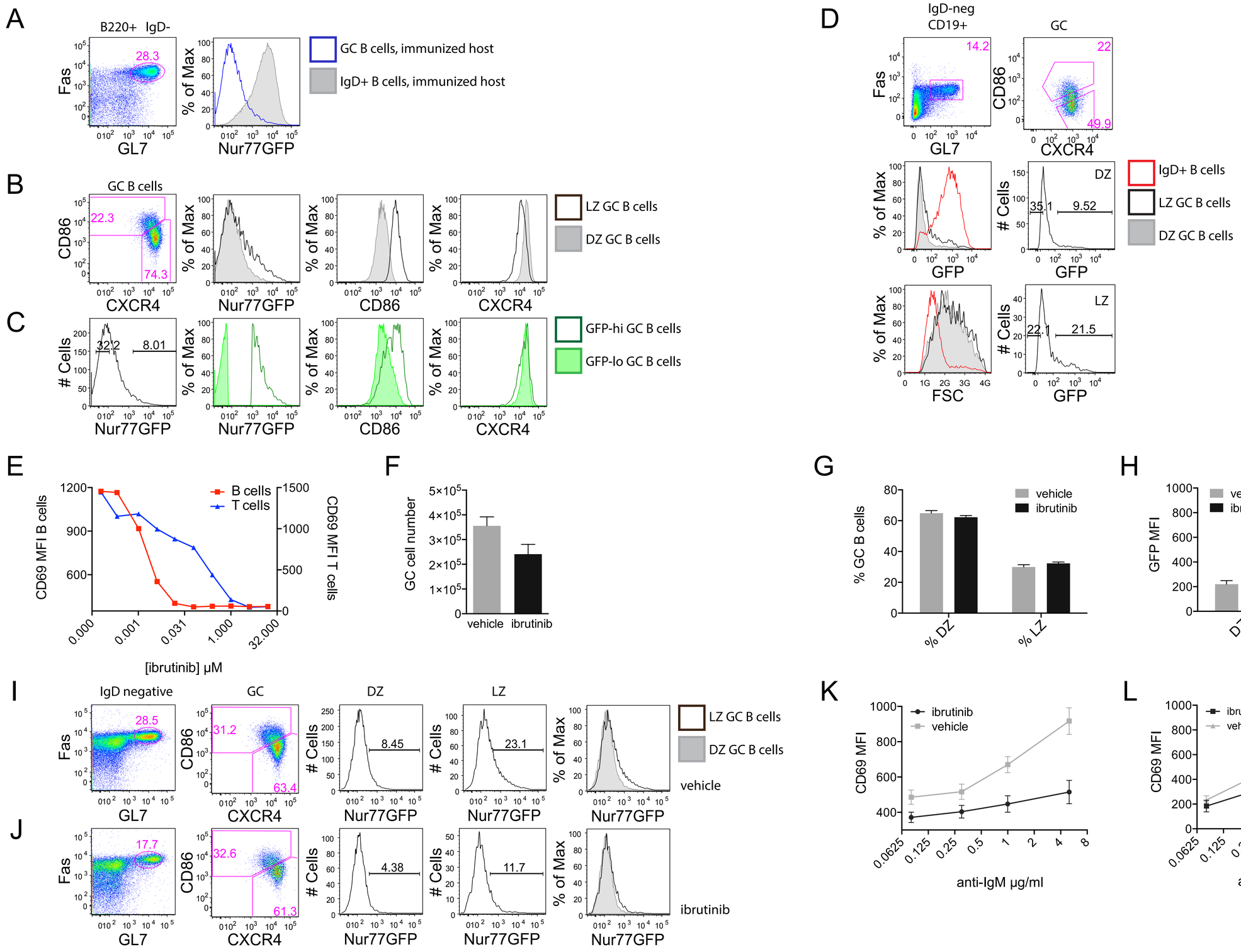






Supplemental Figure 1. Sorting and mutation analysis of NP-specific Vh186.2 heavy chain from immunized Nur77-eGFP reporter mice

A. Splenocytes from recipient mice described in Fig. 1A, B were harvested and stained for CD45.2, IgD, IgM, and λ1 expression. Donor (CD45.2)+ B cells were gated on the basis of Cell Trace Violet dilution to identify divided (black line) or undivided cells from immunized (red line) or unimmunized (gray histogram) hosts. Histograms represent overlayed expression of aforementioned markers in gated cell populations. Data are representative of 4 biological replicates.
B. Unrestricted repertoire reporter mice were stained, fixed and single-cell sorted 10 days after immunization with NP-KLH according to gates depicted in left-hand panels to obtain GC ($fas^{hi}GL7^{hi}$) LZ ($CD86^{hi}CXCR4^{lo}$) and DZ ($CD86^{lo}CXCR4^{hi}$) GFP-hi and GFP-lo samples. Post-sort purity was assessed by flow. Histograms in bottom panels represent GFP expression in individual post-sort samples overlayed onto a pre-sort DZ gate for reference. **C**. Vh186.2 heavy chains were isolated and sequenced as described in Figure 2D according to gating scheme depicted in Figure S1B. Graphs depict % clones with silent or missense mutations at each residue within Vh186.2 sequence.



Supplemental Figure 2. LCMV-infection of Nur77-eGFP reporter mice with and without ibrutinib treatment
A-C. Unrestricted repertoire C57/Bl6 reporter splenocytes were harvested and stained 15 days after LCMV infection. Plots and gating scheme is identical to that depicted in Figure 3A-C. Data are representative of at least 2 independent experiments with 2-3 mice per time point per experiment. **D.** Unrestricted repertoire reporter mice were stained, sorted into trizol 9 days after LCMV infection according to the gating scheme depicted to identify GFP-hi or GFP-lo LZ ($CD86^{hi}CXCR4^{lo}$) and DZ ($CD86^{lo}CXCR4^{hi}$) phenotype. Sort parameters are representative of 3 independent sorts. **E.** Reporter lymphocytes were treated and stained as in Figure 4A. Graph depicts CD69 MFI of CD4 T cells or B cells. Data are representative of 2 independent experiments. **F-J.** Reporter mice were treated with vehicle (top panels) or ibrutinib (bottom panels) on days 10, 11, and 12 following LCMV infection, and splenocytes were stained on day 13. Graphs represent absolute numbers of splenic GC B cells (F), and % LZ and DZ phenotype GC B cells (G), and GFP MFI within LZ and DZ phenotype GC B cells (H) from mice treated with ibrutinib (black) or vehicle (gray). **I, J.** Plots represent gating to identify GC ($IgD^{neg}Fas^{hi}GL7^{hi}$) and LZ/DZ subsets as in Fig. 1E. Histograms represent GFP expression in DZ or LZ GC B cells. GFP-high gates are drawn on the basis of GFP-negative mice. Data are representative 3 independent experiments, each composed of 2-3 biological replicates per treatment condition. **K, L.** Lymphocytes from inhibitor treated reporter mice harvested and treated as described in Figure 4D, E. Graph depicts CD69 MFI of B cells (K) or T cells (L) normalized to PMA-treated samples as in Figure 4D, E. Data plotted in H-L are the mean +/- SEM of 3 biological replicates.