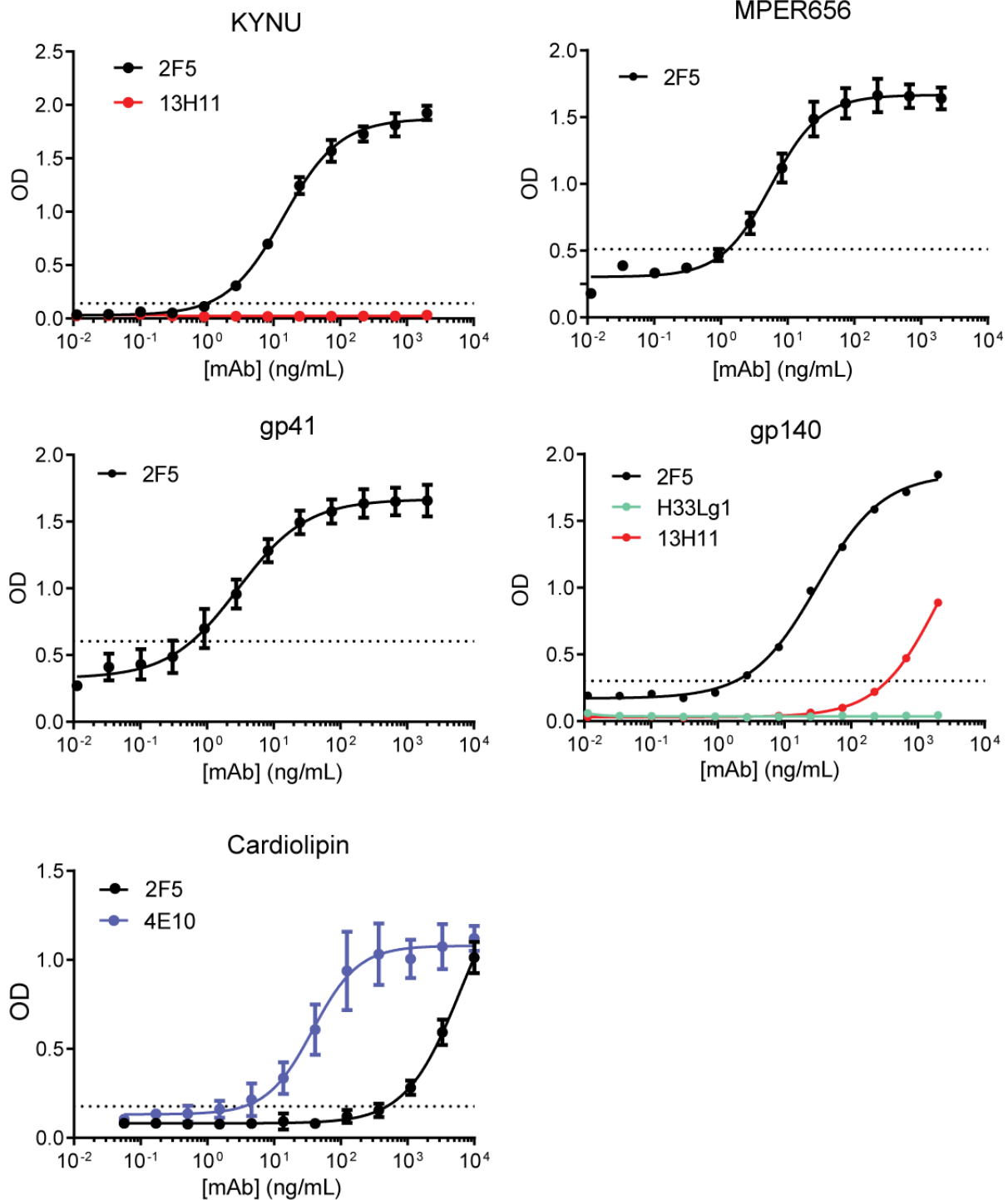


**Supplemental Figure 1. Gating strategy for sorting single B cells from pre- and post-tolerance compartments.** *A*, Small pre-B (B220<sup>int</sup>CD93<sup>+</sup>IgM<sup>lo</sup>IgD<sup>-</sup>CD43<sup>-</sup>FSC<sup>lo</sup>) and immature B (B220<sup>int</sup>CD93<sup>+</sup>IgM<sup>lo</sup>IgD<sup>-</sup>CD43<sup>-</sup>CD23<sup>-</sup>) cells were sorted from BM. *B*, Anergic (B220<sup>+</sup>IgM<sup>-</sup>IgD<sup>+</sup>) and MF (B220<sup>hi</sup>CD93<sup>-</sup>IgM<sup>int</sup>IgD<sup>hi</sup>CD23<sup>+</sup>CD21<sup>lo</sup>) B cells were sorted from splenocytes. In the second column from the left, non-B cells (B220<sup>-</sup>) from parent gate R1 are superimposed as red cells on the plot of total B cells (blue, from parent gate R2) as a reference for gating IgM<sup>-</sup> cells.



**Supplemental Figure 2. Representative standard curves for ELISA.** Serial 3-fold dilutions of monoclonal antibodies were applied to antigen-coated plates as positive and negative controls for specific antigen binding. 13H11 is a low-avidity gp140-specific Ab. H33Lg1 is specific for 4-hydroxy-3-nitrophenyl (NP) acetyl. 4E10 is bispecific for gp41 MPER and anionic lipids. Dashed lines indicate the cutoff for antigen binding, set at 6 standard deviations above the mean signal from supernatants from B-cell-negative wells.