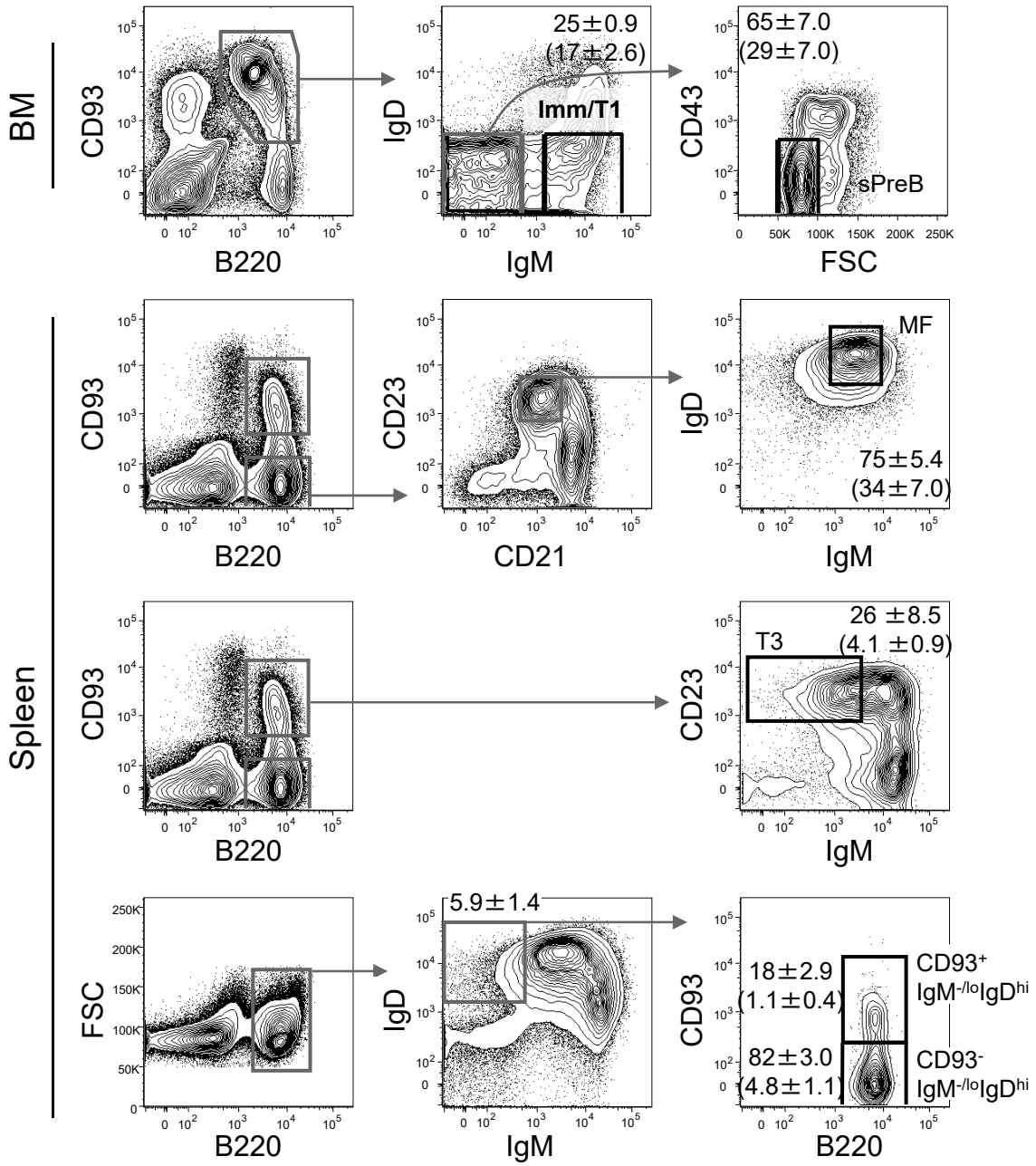

Figure S1. Gating strategy for the identification of B-cell subsets and cell sorting
Representative FACS diagrams for the determination/sorting of B-cell subsets are shown. Top row: $B220^{lo}CD93^{+}$ live leukocytes in the BM (left) were further analyzed for IgM and IgD expressions (middle), and $IgM^{+}IgD^{-}$ cells were defined as immature/transitional-1 (Imm/T1) B cells. $IgM^{-}IgD^{-}$ cells were further gated on $FSC^{lo}CD43^{-}$ cells to obtain small pre-B cells (right). Second row: mature follicular (MF) B cells were defined as $B220^{hi}CD93^{-}CD21^{dull}CD23^{+}IgM^{int}IgD^{hi}$ cells in spleen. Third row: transitional-3 (T3) B cells were defined as $B220^{low}CD93^{+}IgM^{-/lo}CD23^{+}$ cells in spleen. Bottom row: Anergic B cells were defined as $B220^{+}IgM^{-/lo}IgD^{hi}$ cells in spleen, and $CD93^{+}$ and $CD93^{-}$ anergic B cells were further dissociated by CD93 expressions (right). The percentage (mean ± S.D.; n = 5) of gated cells in each panel (in $B220^{+}$ cells) are shown. B cells were double-sorted to ensure purity. Doublets (by FSC-H/FSC-A) and dead cells (PI^{+}) were excluded from our analyses.

Figure S1. Nojima *et al.*

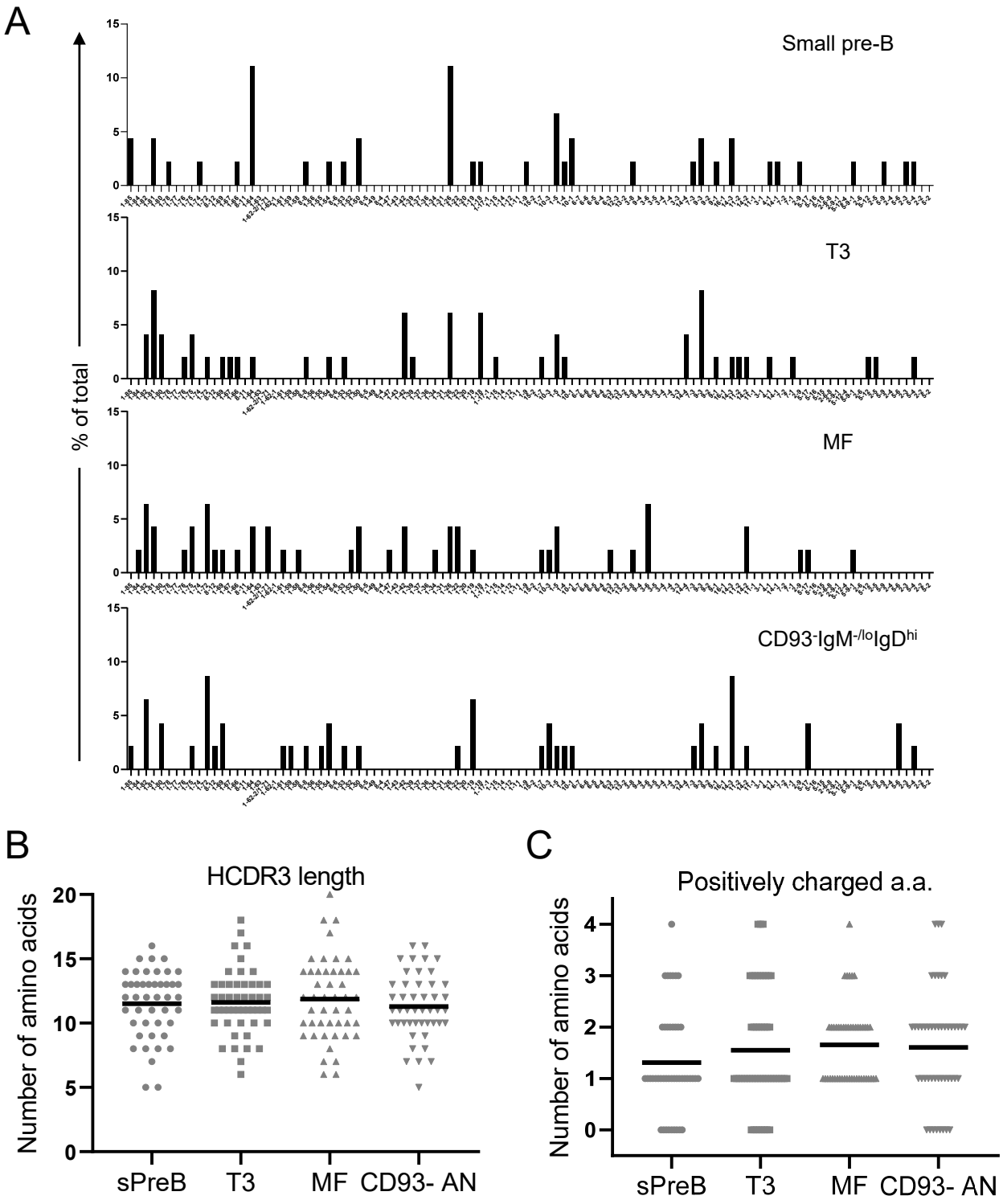

Figure S2. IgH gene features of B-cell subsets

After Nojima cultures, VDJ rearrangements were amplified from indicated B-cell subsets and V_H gene usage (A), HCDR3 amino acid length (B), and number of positively charged amino acids in HCDR3 were determined using IMGT/V-QUEST. Small pre-B (n = 45), T3 (n = 49), MF (n = 47) and CD93$^-$ IgM$^{-/lo}$IgD$^+$ B cells (n = 46). Due to sparse data limitation, no statistical assessment was provided for V_H distribution. (B and C) Each symbol represents an individual sample and horizontal bars indicate an average of samples. No statistical significance was obtained for HCDR3 length or positively charged amino acids (tested by Kruskal-Wallis test).

Figure S2. Nojima *et al.*

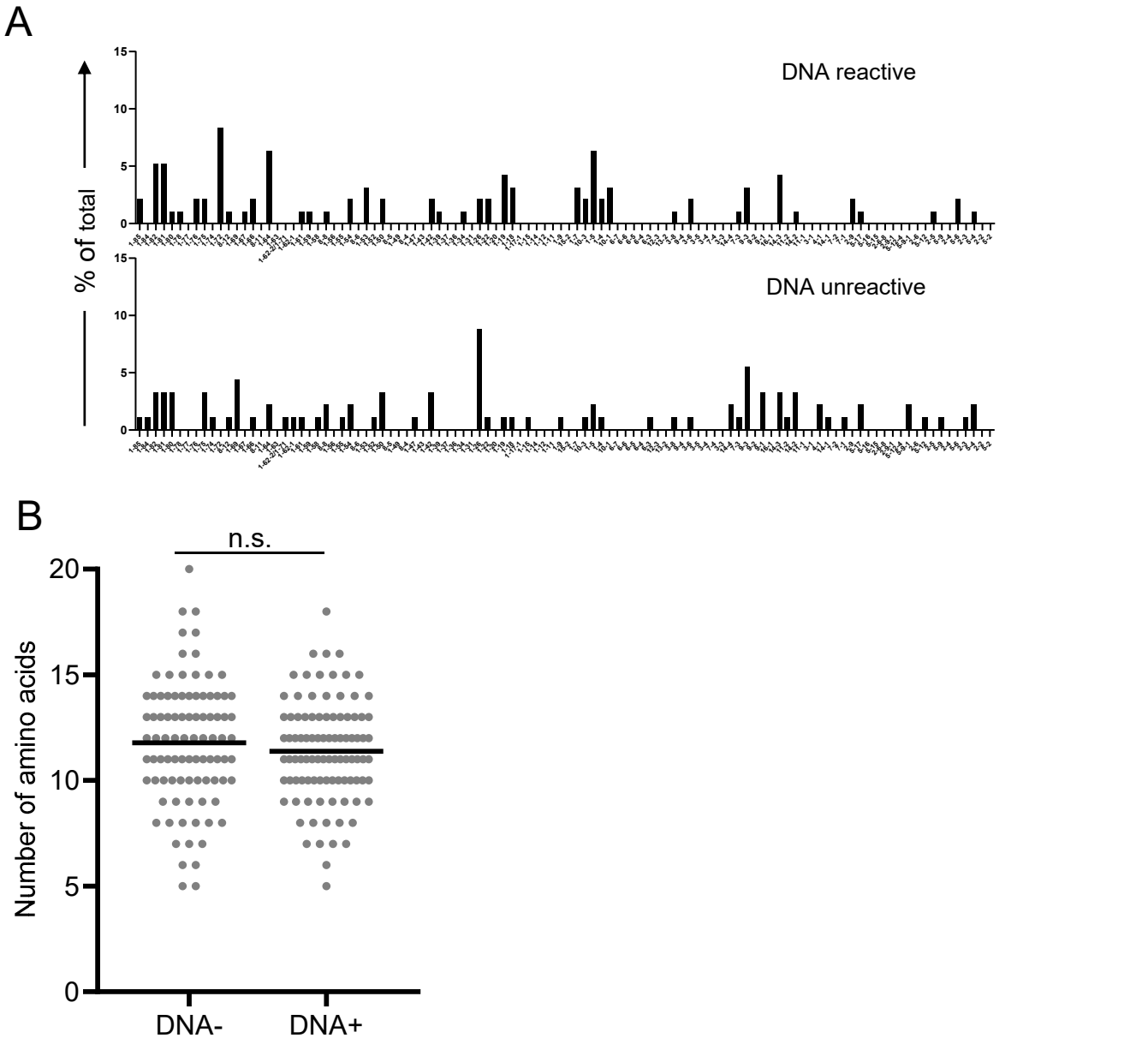

Figure S3. IgH gene features of DNA-binding and DNA-non-binding B cells
VDJ sequence samples described in Figure S2 were combined, and then separated based on DNA reactivity of their corresponding culture supernatant IgGs (Fig. 4A). V_H gene usage (A) and HCDR3 amino acid length (B) were compared between DNA reactive (n = 96) and DNA unreactive (n = 91) samples. Due to sparse data limitation, no statistical assessment was provided for V_H gene usage. (B) Each symbol represents an individual sample and horizontal bars indicate an average of samples. No statistical significance was obtained for HCDR3 length (tested by Mann-Whitney's *U* test).