SUPPLEMENTAL MATERIAL

Schickel et al., https://doi.org/10.1084/jem.20160201

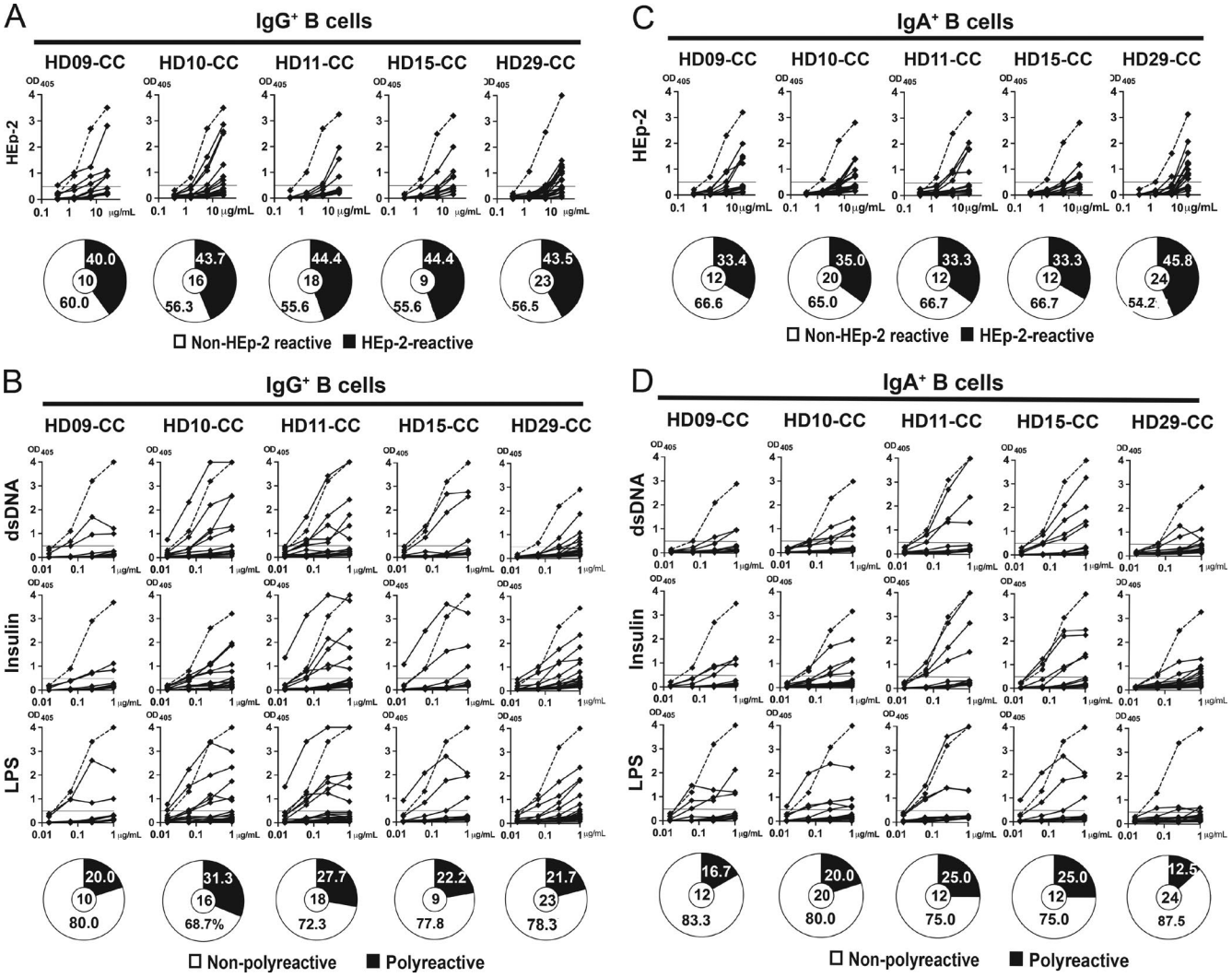

Figure S1. **Frequencies of HEp2-reactive and polyreactive IgG$^+$ and IgA$^+$ B cells from HDs that do not carry the *1858T PTPN22* risk allele.** (A–D) Recombinant antibodies expressed by CD19$^+$CD27$^+$IgG$^+$ B cells (A and B) and CD19$^+$CD27$^+$IgA$^+$ B cells (C and D) from five HDs that do not carry the *1858T PTPN22* risk allele were tested by ELISA for reactivity against HEp-2 cell lysate (A and C) or polyreactivity defined by binding to three antigens (dsDNA, insulin, and LPS; B and D). Continuous lines show binding for each cloned recombinant antibody. Dotted lines show the ED38-positive control. Horizontal lines show cutoff OD_{405nm} for positive reactivity. For each individual, the frequency of reactive and nonreactive B cells are summarized in pie charts, with the number of antibodies tested shown in the center.

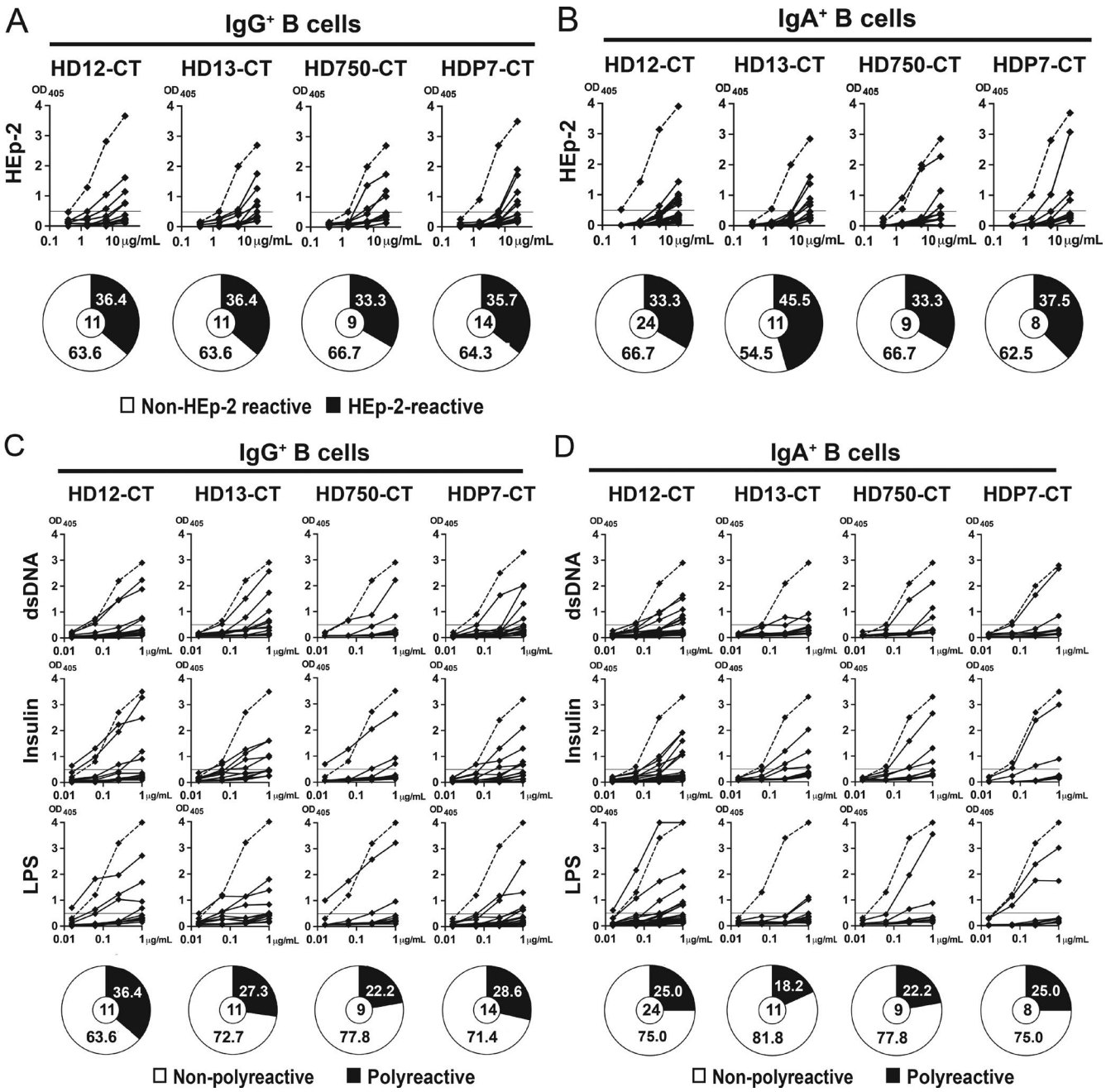

Figure S2. **Frequencies of HEp2-reactive and polyreactive IgG$^+$ and IgA$^+$ B cells from *1858T PTPN22* healthy carriers.** (A–D) Recombinant antibodies expressed by CD19$^+$CD27$^+$IgG$^+$ B cells (A and C) and CD19$^+$CD27$^+$IgA$^+$ B cells (B and D) from four HDs that carry the *1858T PTPN22* risk allele were tested by ELISA for reactivity against the HEp-2 cell lysate (A and B) or polyreactivity defined by binding to three antigens (dsDNA, insulin, and LPS; C and D). Continuous lines show binding for each cloned recombinant antibody. Dotted lines show the ED38-positive control. Horizontal lines show cutoff OD_{405nm} for positive reactivity. For each individual, the frequency of reactive and nonreactive B cells is summarized in pie charts, with the number of antibodies tested shown in the center.

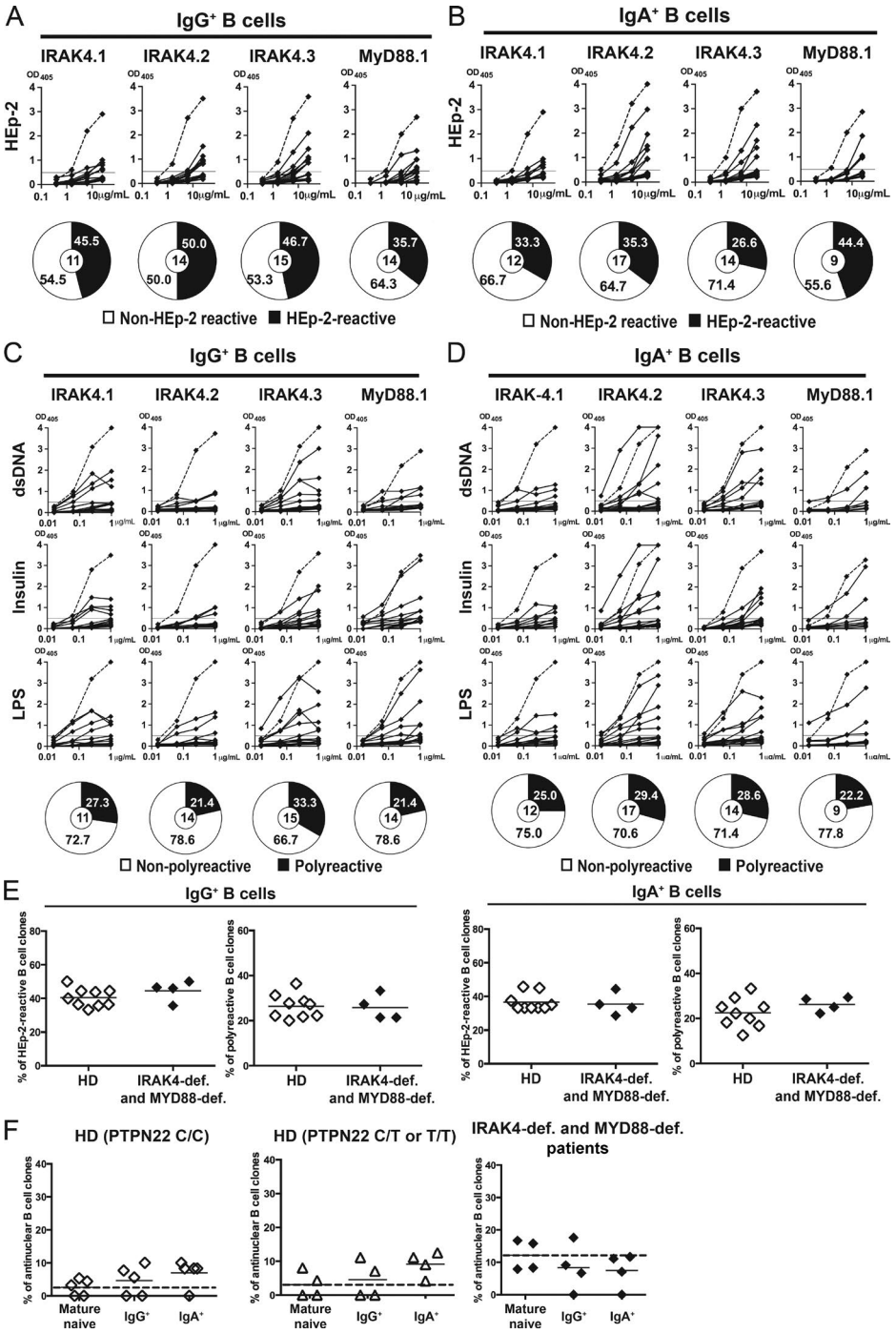

Figure S3. **Frequencies of HEp2-reactive and polyreactive IgG$^+$ and IgA$^+$ B cells from IRAK4- and MYD88-deficient patients.** (A–D) Recombinant antibodies expressed by CD19$^+$CD27$^+$IgG$^+$ B cells (A and C) and CD19$^+$CD27$^+$IgA$^+$ B cells (B and D) from three IRAK4-deficient patients and one MYD88-deficient patient were tested by ELISA for reactivity against the HEp-2 cell lysate (A and B) or polyreactivity defined by binding to three antigens (dsDNA, insulin, and LPS; C and D). Continuous lines show binding for each cloned recombinant antibody. Dotted lines show the ED38-positive control. Horizontal lines show cutoff OD_{405nm} for positive reactivity. For each individual, the frequency of reactive and nonreactive B cells is summarized in pie charts, with the number of antibodies tested shown in the center. (E) The frequencies of HEp-2–reactive and polyreactive clones between pooled HDs (five PTPN22 C/C and four PTPN22 C/T) and IRAK4- and MYD88-deficient patients in the IgG$^+$ (left) and IgA$^+$ (right) B cell compartments are compared. (F) Antinuclear frequencies are compared between the mature naive, IgG$^+$, and IgA$^+$ B cells from five HDs without (left) or four with the *1858T PTPN22* risk allele (middle) and in three IRAK4-deficient patients and one MYD88-deficient patient (right). Each diamond represents an individual, horizontal bars show means, and dashed lines show mean frequencies in the respective mature naive B cells. def., deficient.

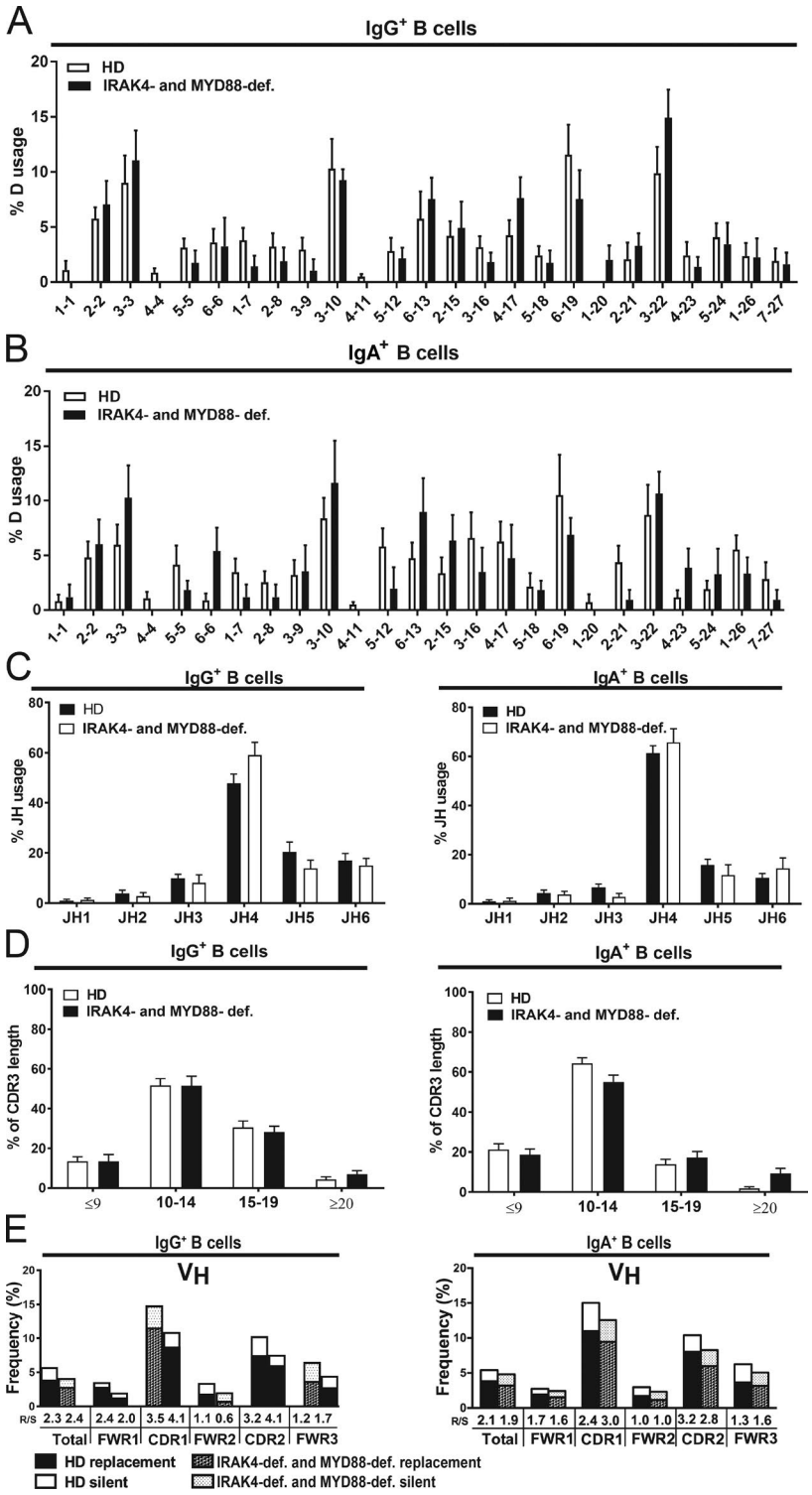

Figure S4. **D and JH gene segment usage and IgHCDR3 length of memory B cells from HDs and IRAK4- and MYD88-deficient patients.** (A–D) DH gene usage frequencies in IgG$^+$ (A) and IgA$^+$ (B) B cells are represented for 14 HDs and 5 IRAK4- and 1 MYD88-deficient patient, and sequence analyses were performed on 354 IgG$^+$ and 295 IgA$^+$ single B cells from HDs and 119 IgG$^+$ and 94 IgA$^+$ B cells from IRAK4- and MYD88-deficient patients. JH gene frequencies are represented in C, and IgH CDR3 lengths are shown in D. (E) Frequency of mutations in *VH*, *Vκ*, and *Vλ* genes from IgG$^+$ (left) and IgA$^+$ (right) B cells calculated from the number of replacement and silent nucleotide exchanges per base pair in FWRs and CDRs. The replacement versus silent (R/S) mutation ratio for each region is indicated. Error bars represent SEM. def., deficient.

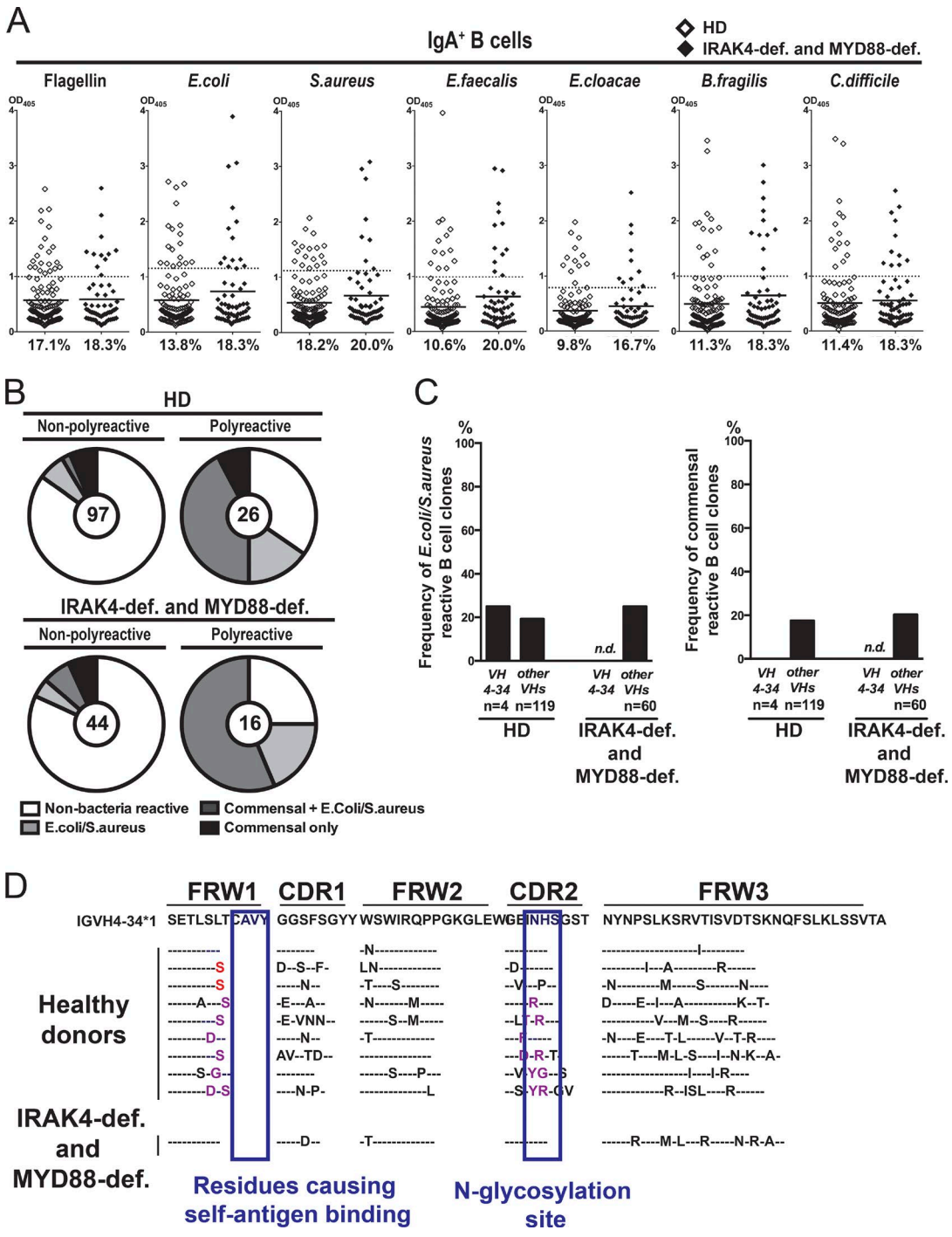

Figure S5. **Antibacteria reactivity of IRAK4- and MYD88-deficient IgA⁺ B cells.** (A) Recombinant antibodies cloned from 123 single IgA⁺ B cells isolated from HDs and from 60 single IgA⁺ B cells from IRAK4- and MYD88-deficient patients were tested by ELISA for reactivity against flagellin, *E. coli*, *S. aureus*, *E. faecalis*, *E. cloacae*, *B. fragilis*, and *C. difficile*. Each diamond represents a single antibody. For each bacteria strain, frequency of the reactive clone is indicated. Antibodies above the dotted line were considered as reactive against the bacteria strains. (B) Pie charts representing bacteria reactivity among the nonpolyreactive and polyreactive IgA⁺ B cells clones in HDs and IRAK4- and MYD88-deficient IgA⁺ memory B cells. (C) Frequencies of *E. coli*– or *S. aureus*–reactive (left) and commensal bacteria–reactive (*E. faecalis*, *E. cloacae*, *B. fragilis*, or *C. difficile* reactive) clones (right) among the *VH4-34*–encoded antibodies versus other *VH*s in IgA⁺ B cells from HDs and IRAK4- and MYD88-deficient patients. (D) VH4-34 amino acid sequence alignment for IgA⁺ memory B cells from HDs and IRAK4- and MYD88-deficient patients. The germline *IGHV4-34*01* amino acid sequence is shown at the top. Identity to germline is denoted by a dash, and substituted residues are in black. In blue are the germline AVY and NHS sequences in FWR1 and CDR2. Mutations in AVY only are shown in red, and mutations in clones mutated for both AVY and NHS are represented in purple. def., deficient.