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Supplemental Information

Teleost IgD^{+}IgM^{-} B Cells Mount Clonally Expanded and Mildly Mutated Intestinal IgD Responses in the Absence of Lymphoid Follicles

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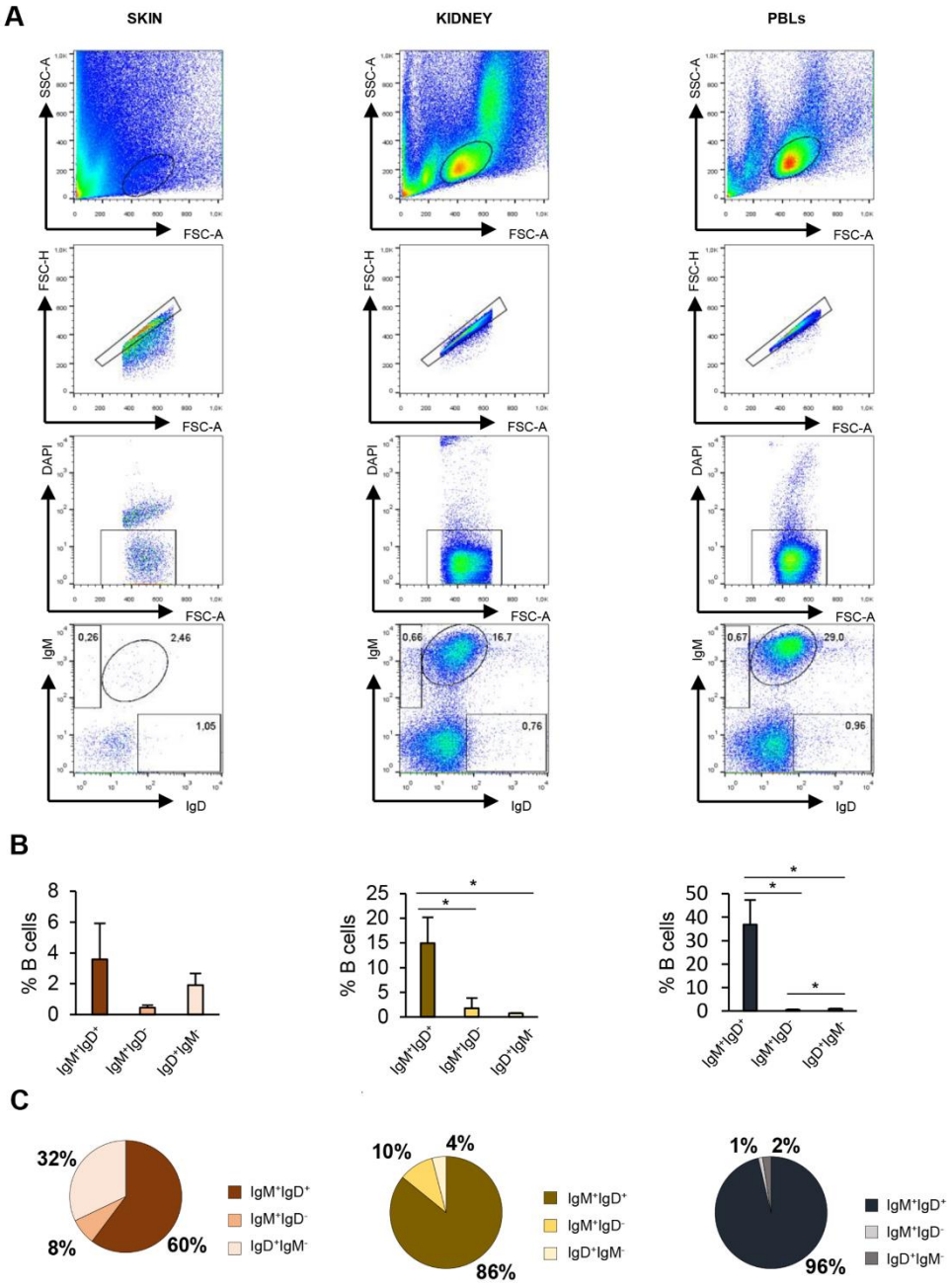

Figure S1. Characterization of rainbow trout B cell populations expressing surface IgM and IgD in skin, kidney and blood. Related to Figure 1. (A) Leukocytes from skin, kidney and blood of rainbow trout were isolated and labeled with specific monoclonal antibodies against trout IgM and IgD and analyzed by flow cytometry. Cells were gated as lymphoid on the basis of their FSC and SSC and percentages of IgM^+IgD^+, IgM^+IgD^-, IgD^+IgM^- determined on singlet and live (DAPI negative) cells. Density plots show a representative example for each tissue from one of six individuals. The percentages of live IgM^+IgD^+, IgM^+IgD^-, IgD^+IgM^- B cells among cells in the lymphocyte gate are shown. (B) Graphs show the mean percentages of IgM^+IgD^+, IgM^+IgD^- and IgD^+IgM^- B cells among total lymphoid cells in each tissue (mean + SD, $n = 6$). Asterisks denote statistically significant differences between subsets as indicated ($*P < 0.05$). (C) Relative percentages of the different B cells subsets among the total IgM^+ and IgD^+ cells were calculated and plotted as pie charts ($n = 6$).

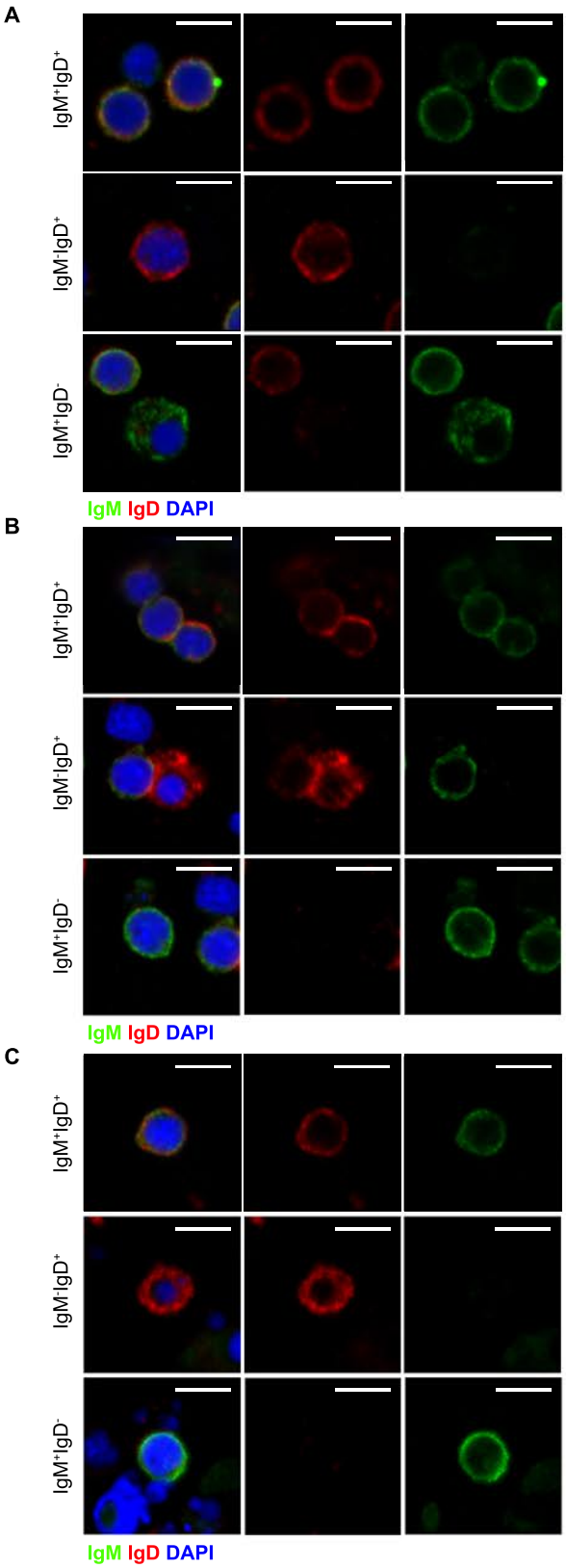

Figure S2. Higher magnification images showing the presence of immunoreactive IgD$^+$/IgM$^+$, IgD$^+$/IgM$^-$ and IgM$^+$/ IgD$^-$ B cells from spleen (A), gills (B) and gut (C). Related to Figure 1. Cells were fixed and labeled with trout anti-IgM (green, FITC) and anti-IgD (red, APC) antibodies and counterstained with DAPI (blue). Scale bars, 5 mm.

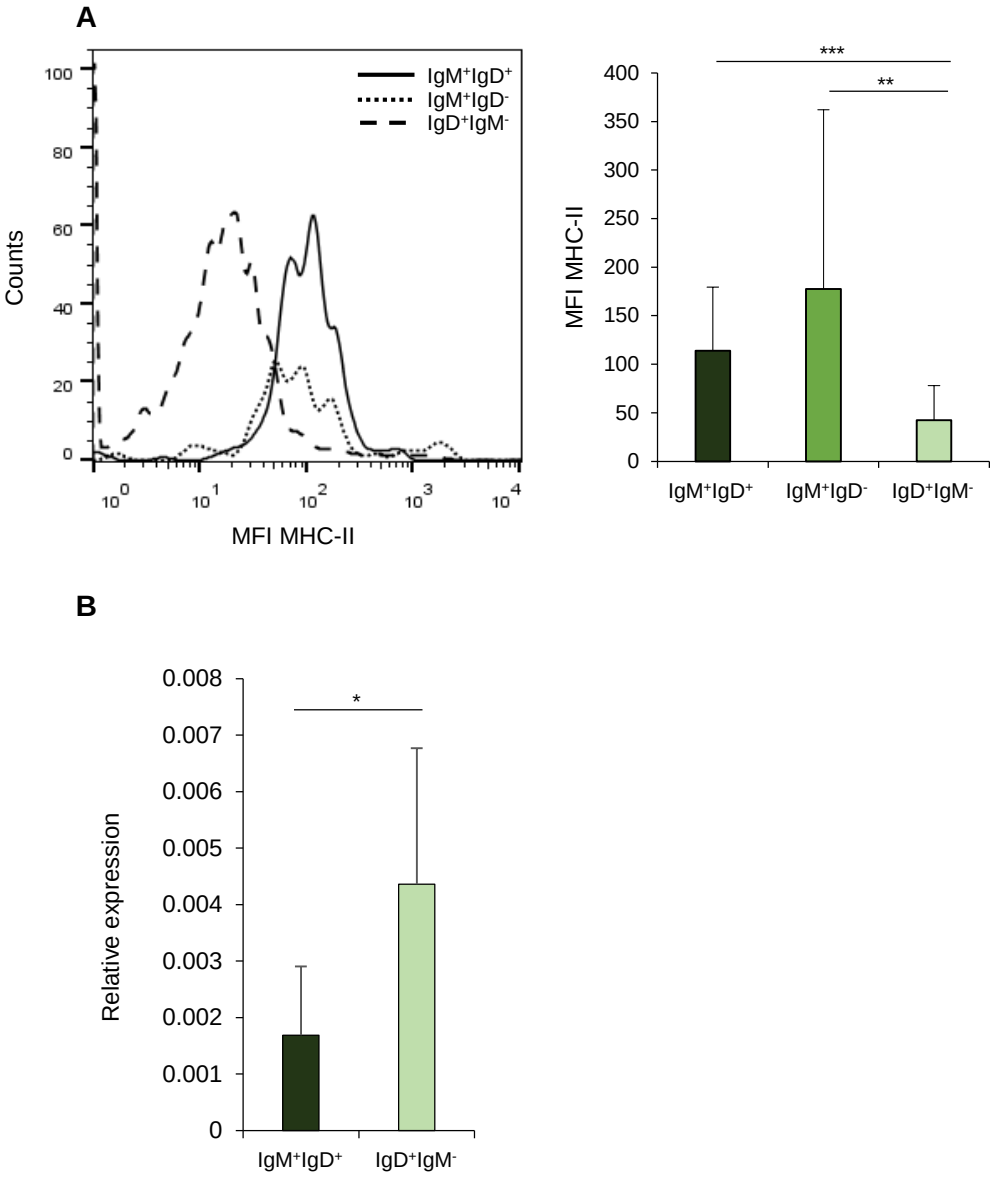

Figure S3. IgD$^+$/IgM$^-$ B cells display a plasmablast-like phenotype. Related to Figures 3 and 4. (A) Levels of surface MHC II of IgM$^+$IgD$^+$, IgM$^+$IgD$^-$, IgD$^+$IgM$^-$ B cell populations in rainbow trout gut. Leukocytes from rainbow trout gut were isolated and labeled with specific monoclonal antibodies against trout IgM, IgD and MHC II and analyzed by flow cytometry. Cells were gated as lymphoid on the basis of their FSC and SSC and IgM$^+$IgD$^+$, IgM$^+$IgD$^-$, IgD$^+$IgM$^-$ B cell subsets identified within singlet and live (DAPI negative) cells. Histograms showing MHC II expression levels in IgM$^+$IgD$^+$, IgM$^+$IgD$^-$, IgD$^+$IgM$^-$ B cells obtained in one representative fish are shown together with a quantification of MHC II MFI values presented as mean + SD (n = 13). (B) Rainbow trout blood leukocytes were labeled with specific monoclonal antibodies against trout IgM and IgD. IgM$^+$IgD$^+$ and IgD$^+$IgM$^-$ B cell subsets were then sorted by flow cytometry and levels of transcription of Blimp1 estimated by real time PCR. Quantification of Blimp1 average expression is shown (mean + SD, n = 5). Asterisks denote statistically significant differences between subsets as indicated (*P < 0.05, **P < 0.01 and ***P < 0.001).

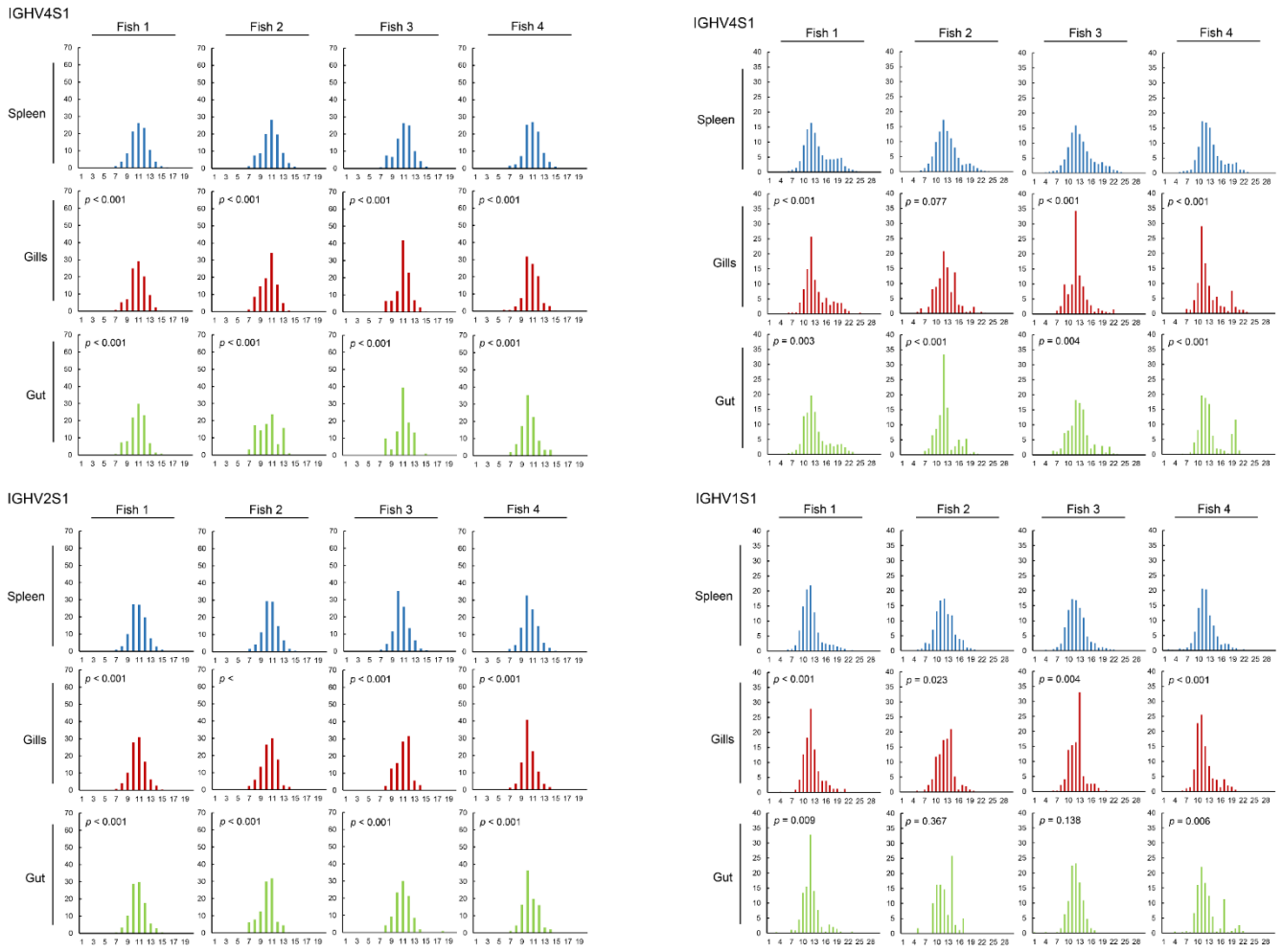

Figure S4. IgM and IgT CDR3 spectratyping in spleen, gills and gut. Related to Figure 3. CDR3 spectratyping of transcripts found in different organs for each fish analysed using sequences that corresponded to the two most frequent IgM VH families, namely IGHV4S1 and IGHV2S1 (A) and to the two most frequent IgT VH families, namely IGHV4S1 and IGHV1S1 (B). Statistical significance in CDR3 length distribution was calculated using the Kolmogorov–Smirnov test for each fish comparing mucosal organs to the spleen. Observed *P* values for each comparison are indicated in figure in the spectratyping representation from gills or gut.

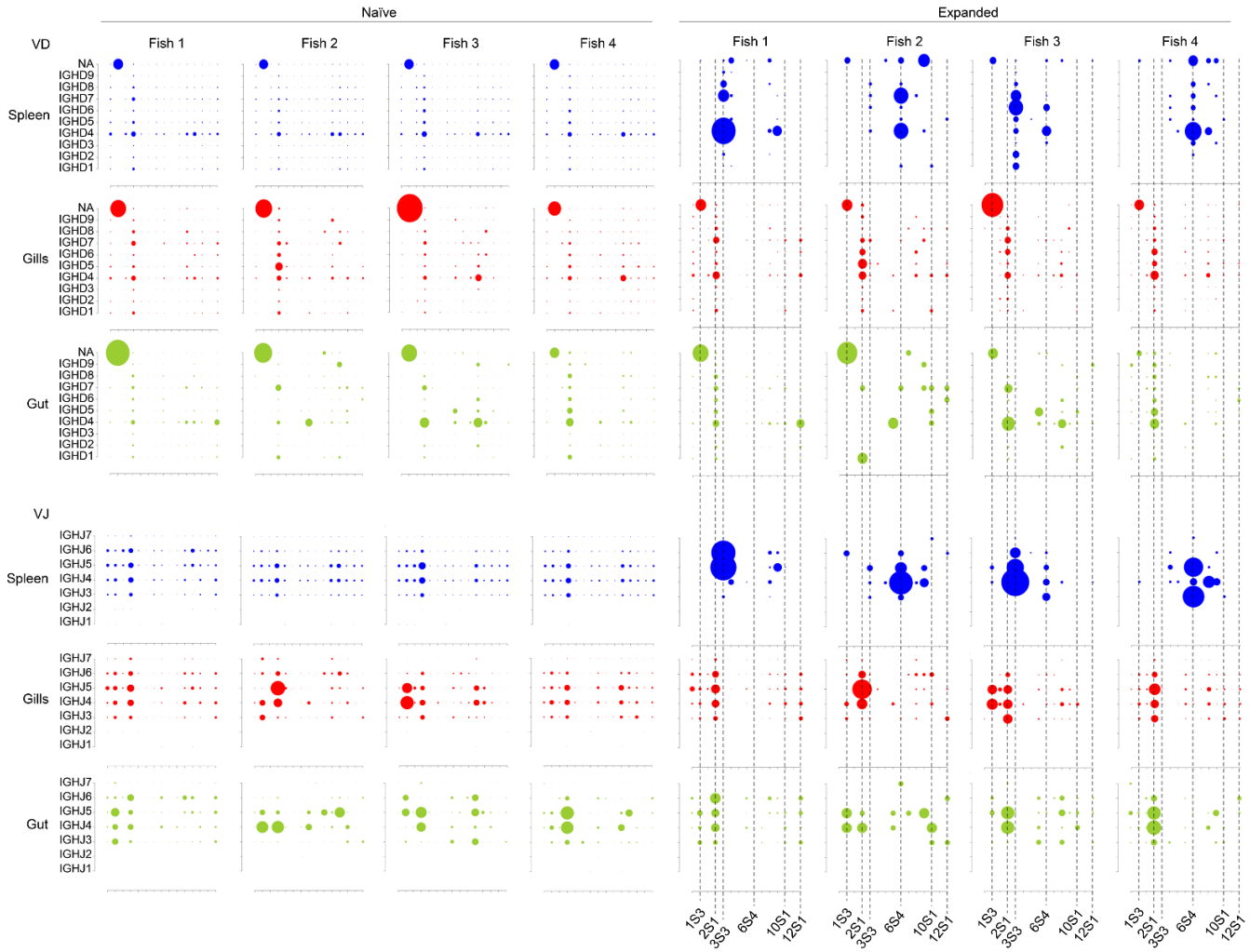

Figure S5. 2D Plot representation of V-D and V-J usage. Related to Figure 4. Figure showing the V-D and V-J combinations used in IgD isotype in naïve and expanded sequences from gills, gut and spleen for each donor fish analysed. Spots show the relative frequency for each specific V-D and V-J combination reaching at least 2.5% in a sample. Dotted lines highlight VH families showing differences in frequencies in expanded B cells regarding naïve B cells.