SUPPLEMENTAL MATERIAL

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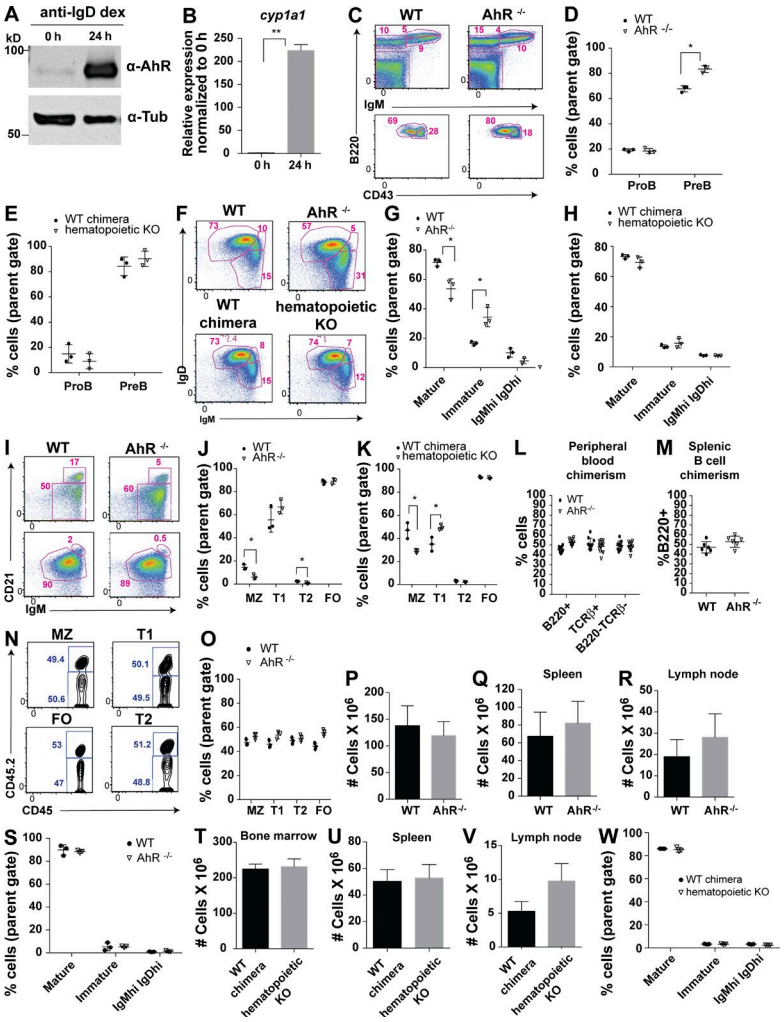

Figure S1. **AhR induction in B cells and role of AhR in B cell development.** (A) Western blotting on WT splenic B cell lysates either unstimulated (0 h) or 24 h stimulated with anti-IgD dextran showing AhR expression and tubulin (Tub) serving as the loading control. A representative blot from three experiments is shown. (B) Relative *cyp1a1* mRNA expression (normalized to β actin) in 24 h anti-IgD dextran–stimulated WT splenic B cells compared with unstimulated (0 h) cells. $n = 3$. Mean ± SD is shown. (C) Representative flow cytometry plot on live singlets from BM of WT and AhR$^{-/-}$ mice showing B220 versus IgM (top) and B220 versus CD43 (bottom; gated on B220^{lo} IgM^{-}). Pro–B cells were gated as B220^{lo} IgM-CD43^{+}, whereas pre–B cells were B220^{lo} IgM-CD43^{-}. (D) Frequency of pro–B cells (ProB) and pre–B cells (PreB) from a representative experiment with three mice per group (WT and AhR$^{-/-}$). Two experiments were performed. (E) Frequency of pro– and pre–B cells from WT chimera and hematopoietic KO (chimera) mice. $n = 3$. Mean ± SD is shown. (F) Representative flow cytometry plot on live B220^{+} splenocytes from WT and AhR$^{-/-}$ mice (top) and WT chimera and hematopoietic KO (bottom), showing IgM versus IgD, mature (IgD^{hi} IgM^{+}), immature (IgD^{lo} IgM^{+}), and IgM^{hi} IgD^{hi}. (G) Frequency of mature, immature, and IgM^{hi} IgD^{hi} B cells from a representative experiment with three mice per group (WT and AhR$^{-/-}$). Three experiments were performed. (H) Frequency of mature, immature, and IgM^{hi} IgD^{hi} B cells from WT chimera and hematopoietic KO (chimera). $n = 3$. Mean ± SD is shown. (I) Representative flow cytometry plot on live B220^{+}CD23^{-} (top) and B220^{+}CD23^{+} (bottom) splenocytes from WT and AhR$^{-/-}$ mice, showing CD21 versus IgM. MZ (B220^{+}CD23^{-} CD21^{hi} IgM^{hi}), T1 (B220^{+}CD23^{-} CD21^{lo} IgM^{+}), T2 (B220^{+}CD23^{+} CD21^{hi} IgM^{hi}), and follicular (FO; B220^{+}CD23^{+} CD21^{+} IgM^{+}) B cells are shown. (J) Frequency of MZ, T1, T2, and FO B cells from a representative experiment with three mice per group (WT and AhR$^{-/-}$). Two experiments were performed. (K) Frequency of MZ, T1, T2, and FO B cells from WT chimera and hematopoietic KO (chimera). $n = 3$. Mean ± SD is shown. (L) Chimerism of B, T, and non-B/T cells in peripheral blood of mixed BM chimeric mice 8 wk after reconstitution as assessed by flow cytometry using CD45.2 (AhR$^{-/-}$) and CD45.1 (WT) congenic markers. $n = 10$. Mean ± SD is shown. Four independent chimera sets were generated. (M) Splenic B cell chimerism in mixed BM chimeric mice as assessed by flow cytometry. $n = 6$. Mean ± SD is shown. (N) Representative flow cytometry plot showing contribution by WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the MZ, T1, T2, and FO splenic B cell subsets in mixed BM chimeras. (O) Quantification summary of contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the MZ, T1, T2, and FO splenic B cell subsets in mixed BM chimeras. $n = 3$. Mean ± SD is shown. (P–R) BM, spleen, and LN cellularity, respectively, in WT and AhR$^{-/-}$ mice. $n = 6$–9. Mean ± SD is shown. (S) Quantification summary of LN B cells (live singlet B220^{+}CD43^{-}) from WT and AhR$^{-/-}$ mice with IgD and IgM as markers of maturity. One representative experiment of two with three mice per group (mean ± SD) is shown. (T–V). BM, spleen, and LN cellularity, respectively, in WT chimera and hematopoietic KO chimera. $n = 3$. Mean ± SD is shown. (W) Quantification summary of LN B cells (live singlet B220^{+}CD43^{-}) from WT chimera and hematopoietic KO chimera with IgD and IgM as markers of maturity. $n = 3$. Mean ± SD is shown. *, $P < 0.05$; **, $P < 0.005$ (Student's *t* test).

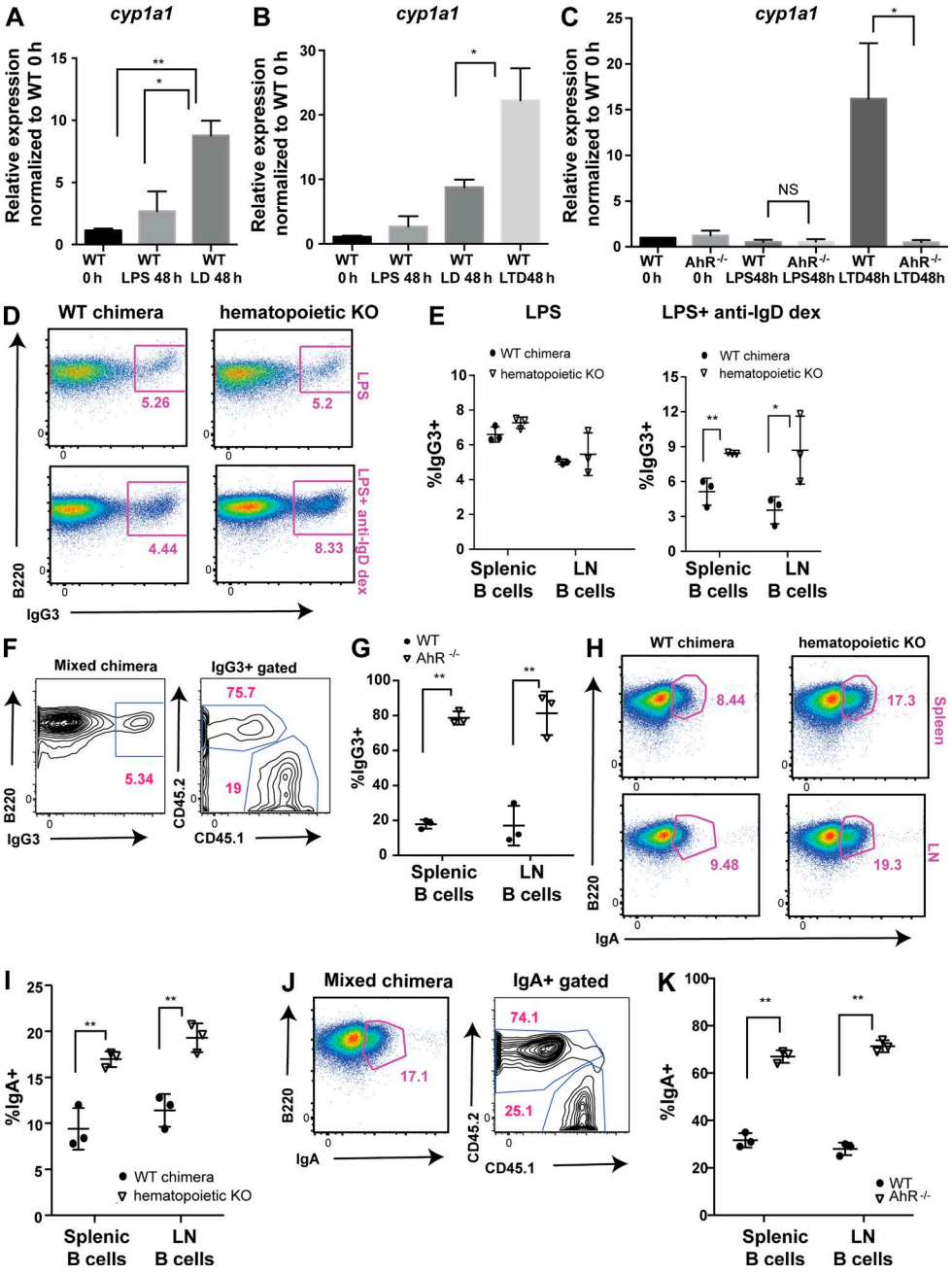

Figure S2. ***cyp1a1* expression and CSR in AhR single and mixed chimeras.** (A and B) Relative *cyp1a1* mRNA expression (normalized to β actin) under different stimulation conditions (48 h) compared with unstimulated (0 h) in WT splenic B cells. (C) Relative *cyp1a1* mRNA expression (normalized to β actin) in WT and AhR$^{-/-}$ splenic B cells as a function of time and stimulation. (D) Representative flow cytometry plot of WT chimera and hematopoietic KO (chimera) LN B cells stimulated for 96 h with LPS (top) and LPS + anti-IgD dextran (dex; bottom) for CSR to IgG3. (E) Quantification summary of cell-surface IgG3 expression at 96 h in splenic and LN B cells of WT chimera and hematopoietic KO chimera mice stimulated with LPS (left) and LPS + anti-IgD dextran (right). (F) Representative flow cytometry plot showing percent IgG3$^+$ splenic B cells at 96 h stimulation with LPS + anti-IgD dextran (left) and contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the IgG3$^+$ population (right) in mixed BM chimeras. (G) Quantification summary of relative contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the IgG3$^+$ population in splenic and LN B cells at 96 h after stimulation. (H) Representative flow cytometry plot of purified splenic (top) and LN (bottom) B cells from WT chimera and hematopoietic KO chimera stimulated for 96 h with LTD, showing cell-surface IgA expression as a measure of CSR. (I) Quantification summary of percent IgA$^+$ splenic and LN B cells from WT chimera and hematopoietic KO chimera stimulated for 96 h with LTD. (J) Representative flow cytometry plot showing percent IgA$^+$ LN B cells at 96 h stimulation with LTD (left) and contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the IgA$^+$ population (right) in mixed BM chimeras. (K) Quantification summary of relative contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) to the IgA$^+$ population in splenic and LN B cells at 96 h after stimulation. (A–C, E, G, I, and K) $n = 3$. Mean ± SD is shown. *, $P < 0.05$; **, $P < 0.005$ (Student's *t* test).

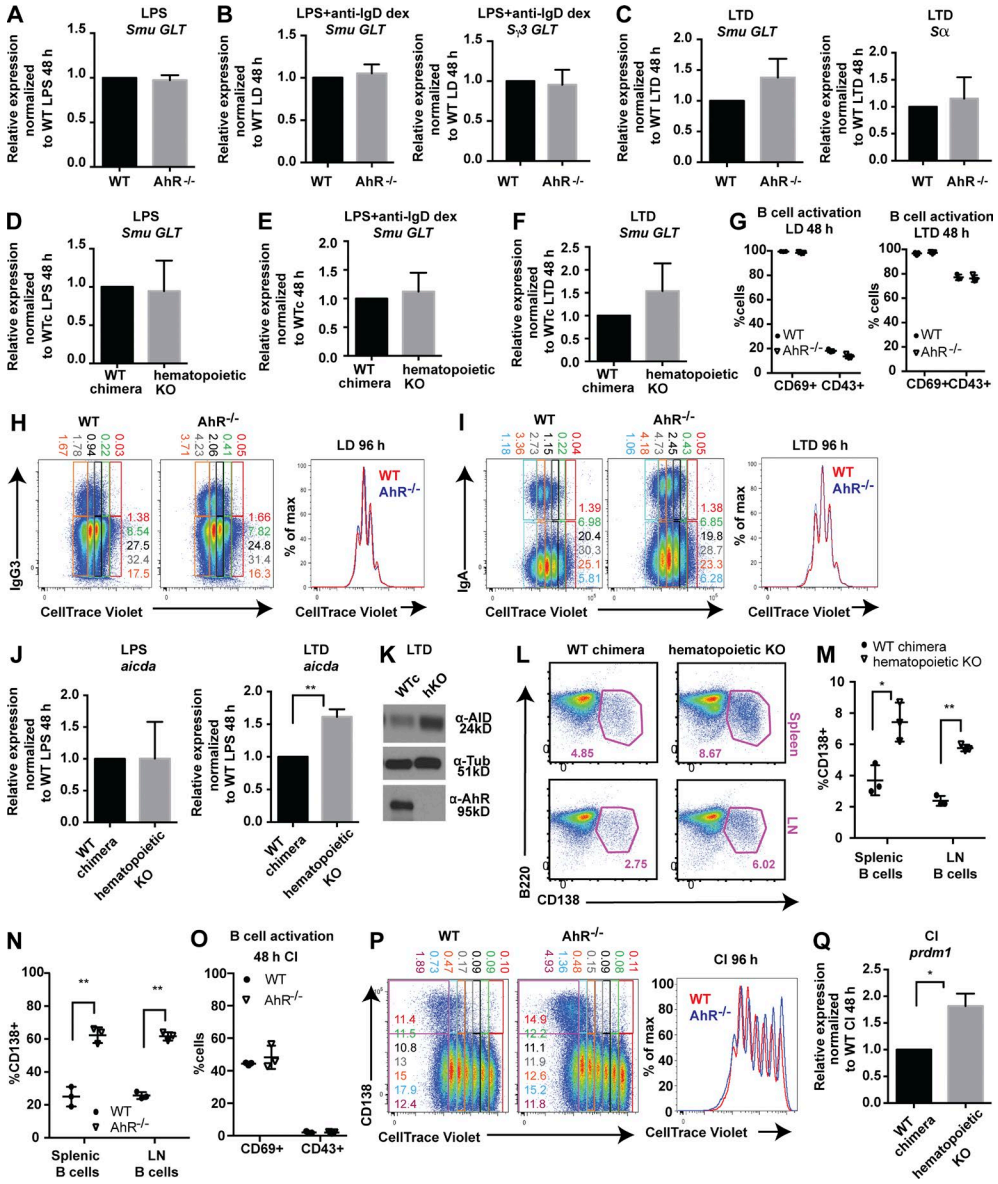

Figure S3. **Germline transcription, proliferation in AhR-deficient B cells, and AID and Blimp-1 expression in AhR single chimeras.** (A–C) Relative expression of Sμ, Sγ3, and Sα germline transcript (GLT) at 48 h of LPS, LPS + anti-IgD dextran (dex; LD), and LPS + anti-IgD dextran + TGFβ (LTD), respectively (normalized to β actin) in WT and $AhR^{-/-}$ splenic B cells. $n = 3$. Mean ± SD is shown. (D–F) Relative expression of Sμ germline transcript at 48 h of LPS, LD, and LTD (normalized to β actin) in WT chimera (WTc) and hematopoietic KO chimera. $n = 3$. Mean ± SD is shown. (G) B cell activation as measured by surface expression of CD69 and CD43 at 48 h after stimulation (LD [left] and LTD [right]) by flow cytometry. $n = 3$. Mean ± SD is shown. (H and I) Cell proliferation as measured by CTV dye dilution versus IgG3 (H) and IgA (I) isotype switching (dot plot; left) and histogram overlay of CTV dilution (right) in WT versus $AhR^{-/-}$ B cells at 96 h LD (H) or LTD (I) stimulation. Bins are color coded, and frequency of cells in each bin is color matched. A representative result from three mice is shown. (J) Relative mRNA expression of *aicda* (normalized to β actin) under LPS and LTD stimulation at 48 h in WT chimera and hematopoietic KO chimera splenic B cells. $n = 3$. Mean ± SD is shown. (K) Western blot showing AID, AhR, and tubulin (Tub; loading control) expression under LTD stimulation (72 h) of WT chimera and hematopoietic KO chimera (hKO) splenic B cells. A representative of two experiments is shown. (L) Representative flow cytometry plot of WT chimera and hematopoietic KO chimera splenic (top) and LN (bottom) B cells stimulated with anti-CD40 + IL-4 (CI) for 96 h, showing $CD138^+$ plasma cells. (M) Quantification summary of percent $CD138^+$ splenic and LN B cells from WT chimera and hematopoietic KO mice. $n = 3$. Mean ± SD is shown. (N) Quantification of percent contribution by WT (CD45.1) and $AhR^{-/-}$ (CD45.2) splenic and LN B cells to the $CD138^+$ population in mixed BM chimeras. $n = 3$. Mean ± SD is shown. (O) B cell activation as measured by surface expression of CD69 and CD43 at 48 h after CI stimulation. $n = 3$. Mean ± SD is shown. (P) Cell proliferation as measured by CTV dye dilution versus CD138 expression (dot plot; left) and histogram overlay of CTV dilution (right) in WT versus $AhR^{-/-}$ B cells at 96 h CI stimulation. Bins are color coded, and frequency of cells in each bin is color matched. A representative result from three mice is shown. (Q) Relative mRNA expression of *prdm1* (normalized to β actin) at 48 h CI stimulation between WT chimera and hematopoietic KO chimera splenic B cells. $n = 3$. Mean ± SD is shown. *, $P < 0.05$; **, $P < 0.005$ (Student's *t* test).

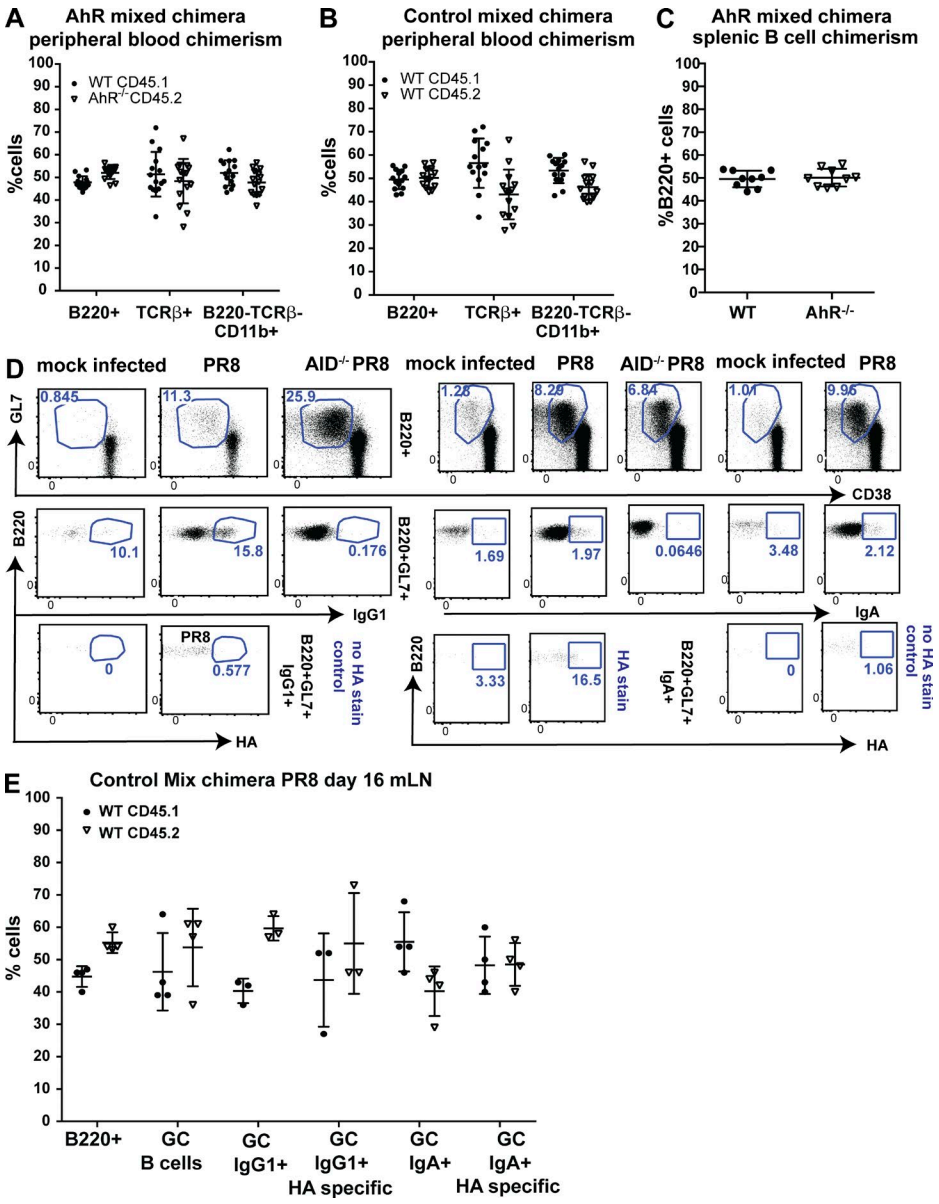

Figure S4. **Reconstitution of AhR mixed and control chimeras and HA stain specificity.** (A) Quantification summary of percent contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) cells to B, T, and myeloid cells in peripheral blood at 7 wk after reconstitution of AhR mixed chimeras. $n = 15$. Mean ± SD is shown. (B) Quantification summary of percent contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) cells to B, T, and myeloid cells in peripheral blood at 7 wk after reconstitution of WT control mixed chimeras. $n = 15$. Mean ± SD is shown. (C) Quantification summary of percent contribution of WT (CD45.1) and AhR$^{-/-}$ (CD45.2) cells to the splenic B cell pool. $n = 9$. Mean ± SD is shown. (D) Representative flow cytometry plot showing AhR mixed chimera (mock or PR8 infected) and PR8-infected AID KO mediastinal LN B cell responses; B220^{+} cells that are GL7^{+} and either IgG1 or IgA^{+} (specific for HA) are shown of the left and right, respectively. AID KO serves as the control for specificity of IgG1 (left) staining and IgA (right middle) and no HA stain control (similar to mock infected) for detection of HA-specific B cells (bottom left and bottom right). (E) Quantification summary of percent contribution of WT (CD45.1) and WT (CD45.2) cells of control mix chimeras to the B220^{+}, germinal center (GC)$^{+}$, GC IgG1^{+}, GC IgG1^{+} HA-specific, GC IgA^{+}, and GC IgA^{+} HA-specific compartment in the mediastinal LN at day 16 after infection. $n = 3$–4. Mean ± SD is shown (Student's t test).