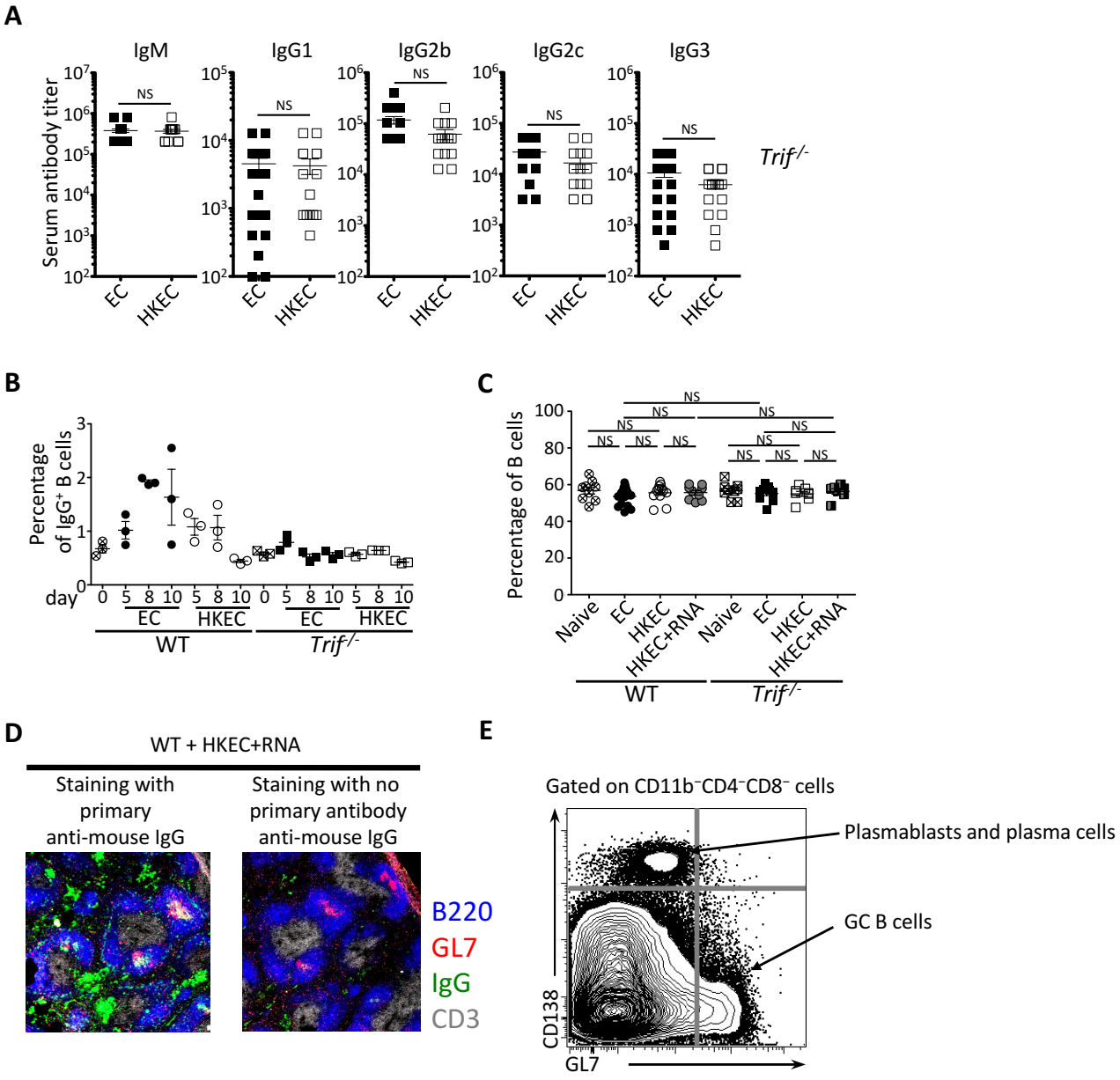

Figure S1 (related to Figure 1).
Analysis of total splenic B cell populations.
(**A**) Day 25 serum titers of class-specific anti-E. coli *(*EC*)* antibodies from *Trif*$^{-/-}$ mice vaccinated intraperitoneally with 5x10^7 live EC or heat-killed *EC* (HKEC). (**B**) Percentage of IgG$^+$ B cells in the spleens of either naïve WT and *Trif*$^{-/-}$ mice (day 0) or at days 5, 8 and 10 post-vaccination of WT and *Trif*$^{-/-}$ mice with 5x10^7 live EC or HKEC. (**C**) Percentage of total B cells in the spleens of naïve WT and *Trif*$^{-/-}$ mice or at day 7 post vaccination with EC, HKEC or HKEC+RNA(30 µg). Each symbol represents an individual mouse. (**D**) Immunofluorescence micrographs at 4X magnification of spleen sections from WT mice vaccinated with 5x10^7 HKEC+RNA(30 µg) at day 7. Staining was performed in the presence (left panel) or absence (right panel) of primary antibody specific for anti-mouse IgG (IgG1+IgG2a+IgG2b+IgG3). Scale bar = 300 µm. (**E**) Representative flow cytometry dot-plot showing CD138 and GL7 expression on CD11b$^-$ and TCRβ$^-$ splenic cells to exclude myeloid cells and T cells, respectively, from a WT mouse at day 7 post vaccination with 5x10^7 live EC. NS, not significant ($P > 0.05$); (two-tailed unpaired *t* test). Data in (A) and (B) are means±s.e.m.

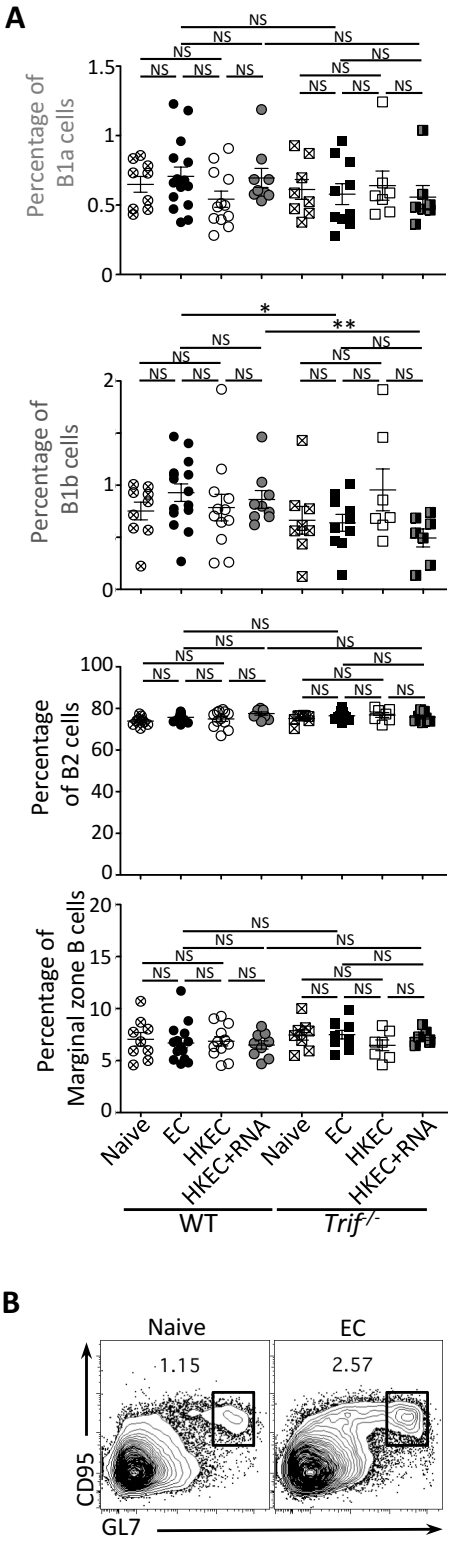

Figure S2 (related to Figure 2).

The different subsets of B cells in the spleen and B cell differentiation to GC B cell.

(**A**) Percentage of total B1a, B1b, B2 or MZ B cell populations in the spleens of WT and *Trif*$^{-/-}$ mice 7 days after injection of the vaccines indicated on the X-axis. Each symbol represents an individual mouse. NS, not significant ($P > 0.05$); (two-tailed unpaired *t* test). Data are means±s.e.m. (**B**) Representative flow cytometry analyses of CD95 and GL7 upregulation on CD19^{+} B cells 7 days after vaccination with live E. coli (EC). Numbers adjacent to outlined areas indicate percent of cells in gates.

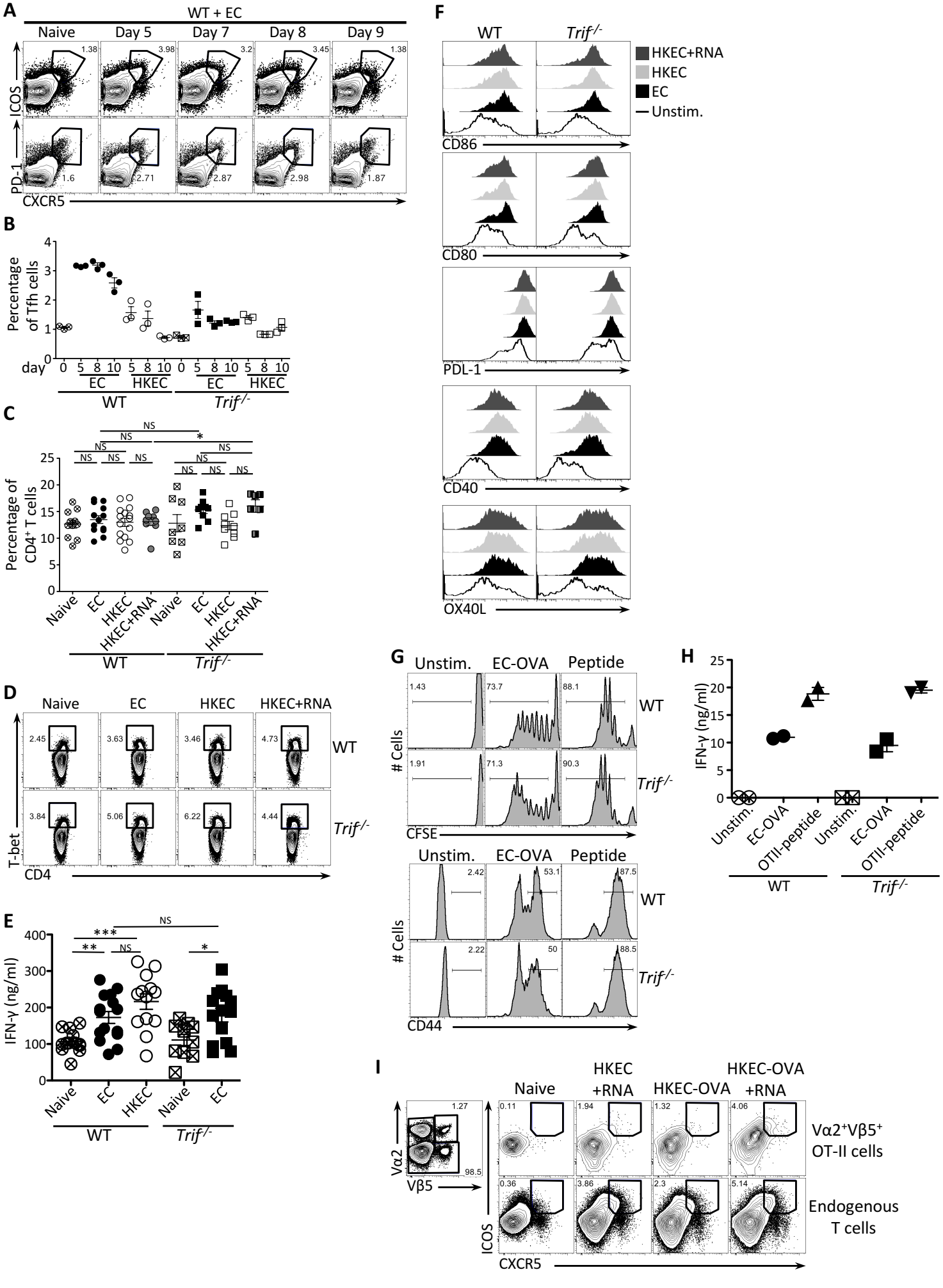
A
WT + EC
Naive
Day 5
Day 7
Day 8
Day 9
ICOS
PD-1
CXCR5
B
Percentage of Tfh cells
day
EC
HKEC
WT
Trif-/-
C
Percentage of CD4+ T cells
NS
Naive
EC
HKEC
HKEC+RNA
D
T-bet
CD4
E
IFN-γ (ng/ml)
F
WT
Trif-/-
HKEC+RNA
HKEC
EC
Unstim.
CD86
CD80
PDL-1
CD40
OX40L
G
Unstim.
EC-OVA
Peptide
Cells
CFSE
CD44
H
IFN-γ (ng/ml)
OTII-peptide
I
HKEC +RNA
HKEC-OVA
Vα2
Vβ5
ICOS
CXCR5
Vα2+Vβ5+ OT-II cells
Endogenous T cells

Figure S3 (related to Figure 3).
Further characterization of the $CD4^+$ T cell response after vaccination.
(**A**) Flow cytometry analyses of CXCR5 and ICOS double-positive cells (gated on $CD4^+CD3^+$ cells) and CXCR5 and PD-1 in the spleens of naïve WT mice or at days 5, 7, 8 and 9 post vaccination of WT mice with $5x10^7$ live E. coli (EC). (**B**) Percentage of CXCR5 and ICOS double-positive cells in the spleens of WT and *Trif*$^{-/-}$ mice that were either naïve or at days 5, 8 or 10 post vaccination with $5x10^7$ live EC, heat-killed E. coli (HKEC) at day 5, 8 or 10. (**C**) Percentage of $CD4^+CD3^+$ cells in the spleens of WT and *Trif*$^{-/-}$ mice that were naïve or at 5 days after vaccination. (**D**) Flow cytometry of T-bet expression in $CD4^+$ T cells in the spleens of naïve or vaccinated WT and *Trif*$^{-/-}$ mice at day 5. (**E**) Interferon-γ detection by ELISA after two days of re-stimulation on anti-CD3 coated plates of splenic cells from WT mice that had been vaccinated intraperitoneally 7 days earlier with EC or HKEC and *Trif*$^{-/-}$ mice that had been vaccinated with EC 7 days earlier. (**F**) Flow cytometry analyses of the upregulation of co-stimulatory molecules by bone marrow-derived dendritic cells from WT or *Trif*$^{-/-}$ mice stimulated or not with live EC, HKEC or HKEC+RNA(30 μg) after 24 hours (bacteria:cell ratio=20). (**G**) Naïve OT-II $CD4^+$ T cell proliferation (upper panel) and upregulation of CD44 (lower panel) after 5 days of culture with splenic $CD11c^+$ cells from WT or *Trif*$^{-/-}$ that were either unstimulated or stimulated with live recombinant OVA-expressing E. coli (EC-OVA) at a MOI 20 or with 2μM OT-II control peptide. (**H**) Detection by ELISA of IFN-γ production by OT-II T cells isolated at day 7 from WT mice that were naïve or vaccinated as in (**E**) and subjected to re-stimulation on anti-CD3 coated plates for 48 hours. (**I**) Flow cytometry analyses of the upregulation of surface CXCR5 and ICOS expression by adoptively transferred OT-II T cells (Vα2 and Vβ5 double positive, left panel) or endogenous $CD4^+$ T cells (double negative or Vα2 or Vβ5 single positive, left panel) in the spleens of naïve WT mice or at day 7 post vaccination of WT mice with HKEC+RNA, HKEC-OVA or HKEC-OVA+RNA(30 μg). NS, not significant ($P > 0.05$); *, $P<0.05$, **, $P\leq0.01$ and ***, $P\leq0.001$ (two-tailed unpaired *t* test). Numbers adjacent to outlined areas indicate percent of cells in gates.

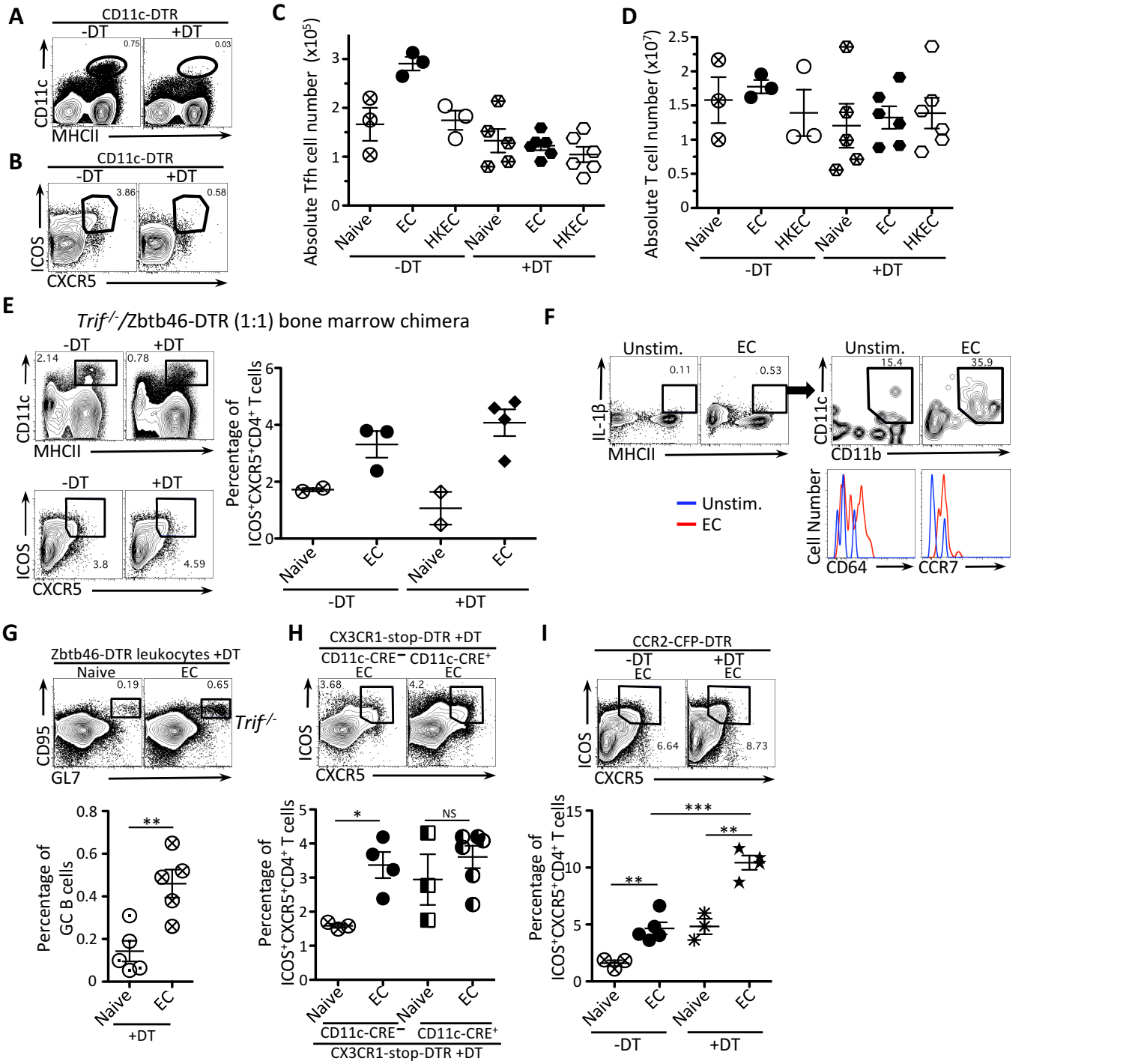
A
CD11c-DTR
-DT
+DT
0.75
0.03
CD11c
MHCII
B
CD11c-DTR
-DT
+DT
3.86
0.58
ICOS
CXCR5
C
Absolute Tfh cell number (x10^5)
Naive
EC
HKEC
-DT
+DT
D
Absolute T cell number (x10^7)
E
Trif-/-/Zbtb46-DTR (1:1) bone marrow chimera
2.14
0.78
3.8
4.59
Percentage of ICOS+CXCR5+CD4+ T cells
F
Unstim.
EC
0.11
0.53
IL-1β
MHCII
15.4
35.9
CD11c
CD11b
Cell Number
CD64
CCR7
G
Zbtb46-DTR leukocytes +DT
0.19
0.65
Trif-/-
CD95
GL7
Percentage of GC B cells
H
CX3CR1-stop-DTR +DT
CD11c-CRE− CD11c-CRE+
3.68
4.2
NS
CD11c-CRE−
CD11c-CRE+
CX3CR1-stop-DTR +DT
I
CCR2-CFP-DTR
6.64
8.73

Figure S4 (related to Figure 4).

Assessment of the antigen presenting cells driving Tfh cell differentiation.

(**A**) Flow cytometry analyses of $CD11c^{+}MHCII^{+}$ dendritic cells in the spleens of naïve chimeric mice that were generated by lethal irradiation of recipient WT mice reconstituted with bone marrow cells from CD11c-DTR mice. Eight weeks after reconstitution, mice were injected with PBS or diphtheria toxin (DT) and DC depletion was assessed 2 days later. (**B)** Flow cytometry analyses of Tfh cells (gated first on $CD4^{+}CD3^{+}$ cells followed by gating on double positive ICOS and CXCR5 T cells) in the spleens of CD11c-DTR chimeric mice 5 days after vaccination with live E. coli (EC) upon PBS or DT treatment. (**C**) Absolute numbers of Tfh cells in the spleens of CD11c-DTR chimeric mice 5 days after vaccination with live EC upon PBS or DT treatment (each symbol represents an individual mouse). (**D**) Absolute numbers of $CD4^{+}CD3^{+}$ T cells in the spleens of CD11c-DTR chimeric mice 5 days after vaccination with live EC upon after PBS or DT treatment (Each symbol represents an individual mouse). (**E**) Flow cytometry analyses of $CD11c^{+}$MHC-II^{+} dendritic cells in the spleens of naïve chimeric mice that were generated by lethal irradiation of recipient WT mice reconstituted with a mixture of bone marrow cells from Zbtb46-DTR and *Trif*$^{-/-}$ mice (ratio 1:1) . 8 weeks after reconstitution, mice were injected with PBS or diphtheria toxin (DT) and classical DC depletion was assessed 2 days later (upper left panel). Flow cytometry analyses of Tfh cells (gated first on $CD4^{+}CD3^{+}$ cells followed by gating on double positive ICOS and CXCR5 T cells) in the spleens of Zbtb46-DTR/*Trif*$^{-/-}$ chimeric mice 5 days after vaccination with live EC upon PBS or DT treatment (lower left panel). Percentage of Tfh cells in the spleens of Zbtb46-DTR/*Trif*$^{-/-}$ chimeric mice 5 days after vaccination with live EC upon PBS or DT treatment (each symbol represents an individual mouse, right panel). (**F**) Flow cytometry analysis of MHC-II^{+} cells producing IL-1β (intracellular staining) from *in vitro* culture of leukocytes from Zbtb46-DTR mice stimulated or not for 4hrs with EC in presence of DT and Brefeldin A (Upper left panel). Flow cytometry analysis of IL-$1\beta^{+}$MHC-II^{+} cells based on CD11b and CD11c expression (Upper right panel). Histogram overlay comparing the expression of CD64 and CCR7 comparing stimulated (red) or not (blue) $CD11b^{+}CD11c^{+}$ cells (lower panel). (**G**) Flow cytometry analysis (upper panel) and percentage (lower panel) of germinal center B cells obtained *in vivo* 7 days after vaccination with EC of *Trif*$^{-/-}$ mice that were adoptively transferred with Zbtb46-DTR leukocytes in the presence DT. (**H**) Flow cytometry analysis (upper panel) and percentage (lower panel) of $ICOS^{+}CXCR5^{+}$ Tfh cells in the spleens of CX3CR1-stop-DTR (either CD11c-CRE^{-} or CD11c-CRE^{+}) 5 days after vaccination with live EC after DT treatment (each symbol represents an individual mouse). (**I**) Flow cytometry (upper panel) and percentage (lower panel) of Tfh cells in the spleens of CCR2-CFP-DTR mice 5 days after vaccination with live EC upon PBS or DT treatment (each symbol represents an individual mouse).

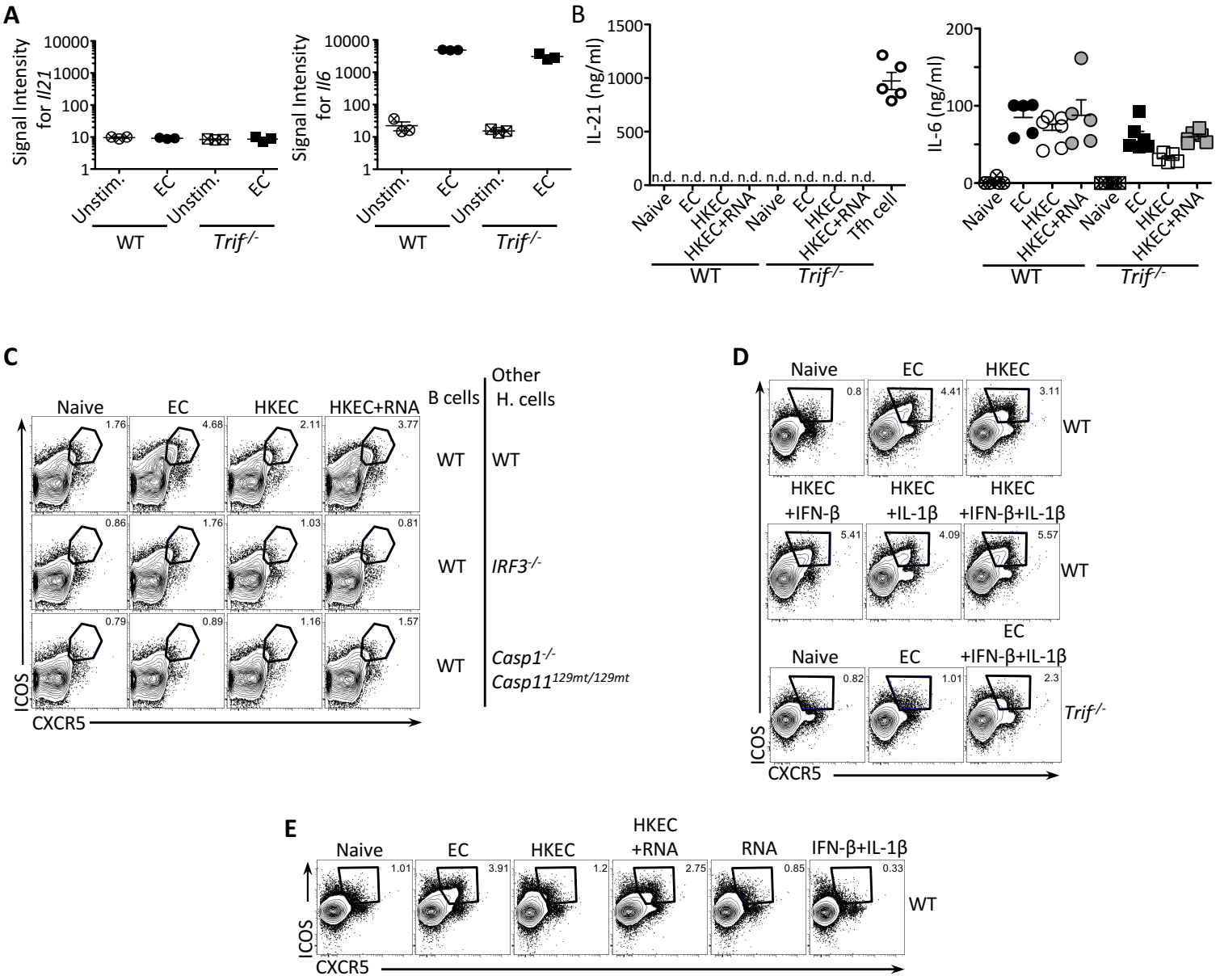

Figure S5 (related to Figure 5).

Assessment of cytokines in Tfh cell differentiation.

(**A**) Gene microarray analyses of WT and *Trif*$^{-/-}$ bone marrow macrophages untreated or treated with live *E. coli* for 6 hours (three biological replicates). Data were re-analyzed for *Il-6* and *Il-21* genes from our previous published observation in PMID: 21602824, Sander *et al. Nature* 2011 (Affymetrix Microarray data are available through the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE27960). (**B**) Detection by ELISA of IL-21 and IL-6 in the supernatants of cultured BMDCs from WT or *Trif*$^{-/-}$ mice 24 hours after stimulation with live EC, HKEC or HKEC supplemented with 10 μg/ml of RNA. The Tfh control condition used was supernatant of sorted CXCR5^{+} ICOS^{+} CD4 T cells re-stimulated for 2 days on anti-CD3 coated plates. n.d. not detected. (**C**)Flow cytometry analyses of Tfh cells at day 7 in the spleens of the indicated naïve or vaccinated chimeric mice generated similarly to the mice in Figure 4A. Lethally irradiated mice received a mixture of B cell-deficient BM (μMT, 80%) and B cell-sufficient BM (20%). BM from B cell sufficient WT mice were mixted with BM from μMT mice on either WT, *Irf3*$^{-/-}$ or *Casp1*$^{-/-}$*Casp*$^{129mt/129mt}$ background. (**D**) Flow cytometry analyses of ICOS^{+}CXCR5^{+}CD4^{+} Tfh cells in the spleens of WT or *Trif*$^{-/-}$ mice vaccinated with 5x10^{7} live EC, HKEC or HKEC supplemented with intravenous injection of 50U IFN-β, 1 μg IL-1β or both 20 hours after injection of the bacteria. Each symbol represents an individual mouse. (**E**) Detection of CXCR5^{+} ICOS^{+} CD4 T cells in WT mice vaccinated with 5x10^{7} live EC, HKEC or HKEC supplemented with 30 μg of RNA or after a single intraperitoneal injection of 30 μg of RNA alone or intravenous injection of 50U IFN-β and 1 μg IL-1β. Numbers adjacent to outlined areas indicate percent of cells in gates. Numbers adjacent to outlined areas indicate percent of cells in gates. NS, not significant ($P > 0.05$); *, P<0.05; **, P≤0.01 and ***, P≤0.001 (two-tailed unpaired *t* test). Numbers adjacent to outlined areas indicate percent of cells in gates.

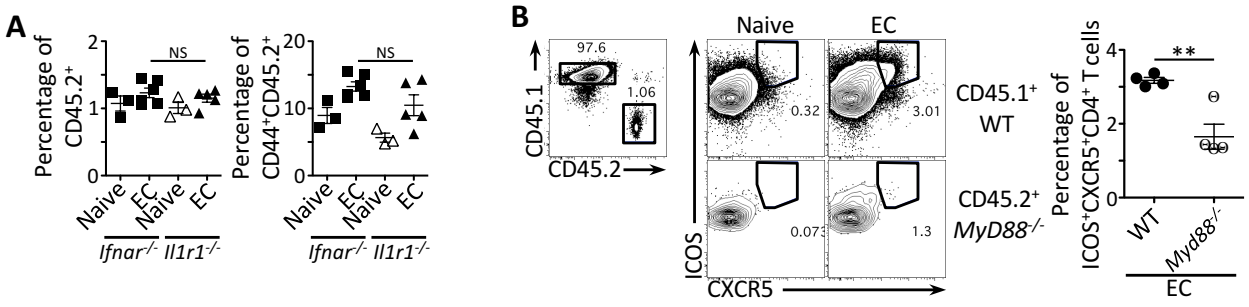

Figure S6 (related to Figure 6).

T cell intrinsic effect of IL-1β and IFN-β cytokines

(**A**) Percentage of CD45.2$^+$CD4$^+$ T cells observed in CD45.1 mice that were adoptively transferred with CD45.2$^+$ *Ifnar* $^{-/-}$ or *Il1r1*$^{-/-}$ CD4$^+$ T cells before and after vaccination with live E. coli (EC) (left panel). Percentage of activated CD44$^+$ T cells among the CD45.2$^+$ *Ifnar* $^{-/-}$ or *Il1r1*$^{-/-}$ CD4$^+$ T cells adoptively transferred into CD45.1 mice vaccinated or not with live EC (right panel). (**B**) Pre-gated on CD4 T cells, detection of adoptively transferred *MyD88*$^{-/-}$ CD45.2$^+$ CD4 T cells (leftmost panel) and Tfh differentiation of endogenous (CD45.1$^+$) or adoptively transferred CD4 T cells. NS, not significant ($P > 0.05$); *, P<0.05; **, P≤0.01 and ***, P≤0.001 (two-tailed unpaired *t* test). Numbers adjacent to outlined areas indicate percent of cells in gates.

		Total B cells			Total B2 cells			Total B1a cells			Total B1b cells			Total MZ B cells		
Mouse Cohort	**Number (n)**	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve
WT Naïve	8	**6.07E+07**	1.08E+07	1.00	**4.39E+07**	3.07E+06	1.00	**3.98E+05**	5.69E+04	1.00	**4.51E+05**	7.47E+04	1.00	**3.89E+06**	2.01E+05	1.00
WT *EC*	15	**6.99E+07**	2.18E+07	**1.15**	**5.32E+07**	4.21E+06	**1.21**	**5.18E+05**	6.25E+04	**1.30**	**6.86E+05**	1.03E+05	**1.52**	**4.64E+06**	3.86E+05	**1.19**
WT HK*EC*	12	**6.46E+07**	1.64E+07	**1.06**	**4.87E+07**	4.01E+06	**1.11**	**3.24E+05**	4.56E+04	**0.81**	**4.81E+05**	8.90E+04	**1.07**	**4.51E+06**	4.54E+05	**1.16**
WT HK*EC* +RNA	9	**5.18E+07**	6.44E+06	**0.85**	**4.06E+07**	1.79E+06	**0.93**	**4.37E+05**	8.22E+04	**1.10**	**4.56E+05**	6.27E+04	**1.01**	**3.31E+06**	1.31E+05	**0.85**
Trif$^{-/-}$ Naïve	8	**5.80E+07**	1.45E+07	1.00	**4.37E+07**	4.09E+06	1.00	**3.47E+05**	3.85E+04	1.00	**4.23E+05**	1.22E+05	1.00	**4.31E+06**	4.52E+05	1.00
Trif$^{-/-}$ *EC*	10	**6.32E+07**	2.60E+07	**1.09**	**4.86E+07**	6.60E+06	**1.11**	**3.64E+05**	5.56E+04	**1.05**	**4.44E+05**	9.85E+04	**1.05**	**4.66E+06**	6.12E+05	**1.08**
Trif$^{-/-}$ HK*EC*	7	**6.02E+07**	1.08E+07	**1.04**	**4.63E+07**	3.25E+06	**1.06**	**3.86E+05**	6.40E+04	**1.11**	**5.97E+05**	1.54E+05	**1.41**	**3.68E+06**	3.95E+05	**0.85**
Trif$^{-/-}$ HK*EC* +RNA	7	**4.84E+07**	9.50E+06	**0.83**	**3.58E+07**	2.18E+06	**0.82**	**2.76E+05**	5.21E+04	**0.80**	**2.47E+05**	5.55E+04	**0.58**	**3.95E+06**	2.76E+05	**0.92**

Supplementary Table 1 (Related to Figure 2)

Cell counts of total splenic B cell populations before or 7 days after the indicated vaccination.

		Total IgG$^+$ B cells			IgG$^+$ B2 cells			IgG$^+$ B1a cells			IgG$^+$ B1b cells			IgG$^+$ MZ B cells		
Mouse Cohort	**Number (n)**	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve	Absolute number	± s.em.	Fold increase over naïve
WT Naïve	8	**5.56E+05**	8.99E+04	1.00	**2.12E+05**	4.44E+04	1.00	**9.01E+03**	2.00E+03	1.00	**1.63E+04**	4.18E+03	1.00	**1.28E+04**	1.94E+03	1.00
WT *EC*	15	**1.61E+06**	1.75E+05	**2.89**	**5.43E+05**	8.54E+04	**2.57**	**3.91E+04**	7.58E+03	**4.35**	**8.47E+04**	2.12E+04	**5.20**	**3.46E+04**	4.14E+03	**2.71**
WT HK*EC*	12	**9.92E+05**	1.21E+05	**1.79**	**3.18E+05**	4.06E+04	**1.50**	**1.83E+04**	3.79E+03	**2.03**	**4.11E+04**	1.10E+04	**2.53**	**1.87E+04**	1.16E+03	**1.47**
WT HK*EC* +RNA	9	**1.24E+06**	1.40E+05	**2.23**	**6.12E+05**	2.60E+04	**2.89**	**3.72E+04**	7.81E+03	**4.13**	**7.75E+04**	2.54E+04	**4.76**	**4.86E+04**	8.95E+03	**3.80**
Trif$^{-/-}$ Naïve	8	**7.13E+05**	1.08E+05	1.00	**2.82E+05**	4.18E+04	1.00	**1.36E+04**	1.68E+03	1.00	**2.18E+04**	5.96E+03	1.00	**2.09E+04**	5.01E+03	1.00
Trif$^{-/-}$ *EC*	10	**7.87E+05**	1.01E+05	**1.10**	**3.21E+05**	4.23E+04	**1.14**	**1.87E+04**	3.97E+03	**1.37**	**2.77E+04**	7.39E+03	**1.27**	**2.84E+04**	5.47E+03	**1.36**
Trif$^{-/-}$ HK*EC*	7	**6.84E+05**	8.18E+04	**0.96**	**2.93E+05**	2.68E+04	**1.04**	**1.41E+04**	2.30E+03	**1.04**	**2.56E+04**	7.63E+03	**1.17**	**1.89E+04**	3.11E+03	**0.90**
Trif$^{-/-}$ HK*EC* +RNA	7	**6.53E+05**	7.76E+04	**0.92**	**2.28E+05**	3.12E+04	**0.81**	**1.38E+04**	2.86E+03	**1.02**	**1.39E+04**	7.28E+03	**0.64**	**2.67E+04**	6.10E+03	**1.28**

Supplementary Table 2 (Related to Figure 2)

Cell counts of IgG class-switched splenic B cells for each subset before or 7 days after the indicated vaccination.