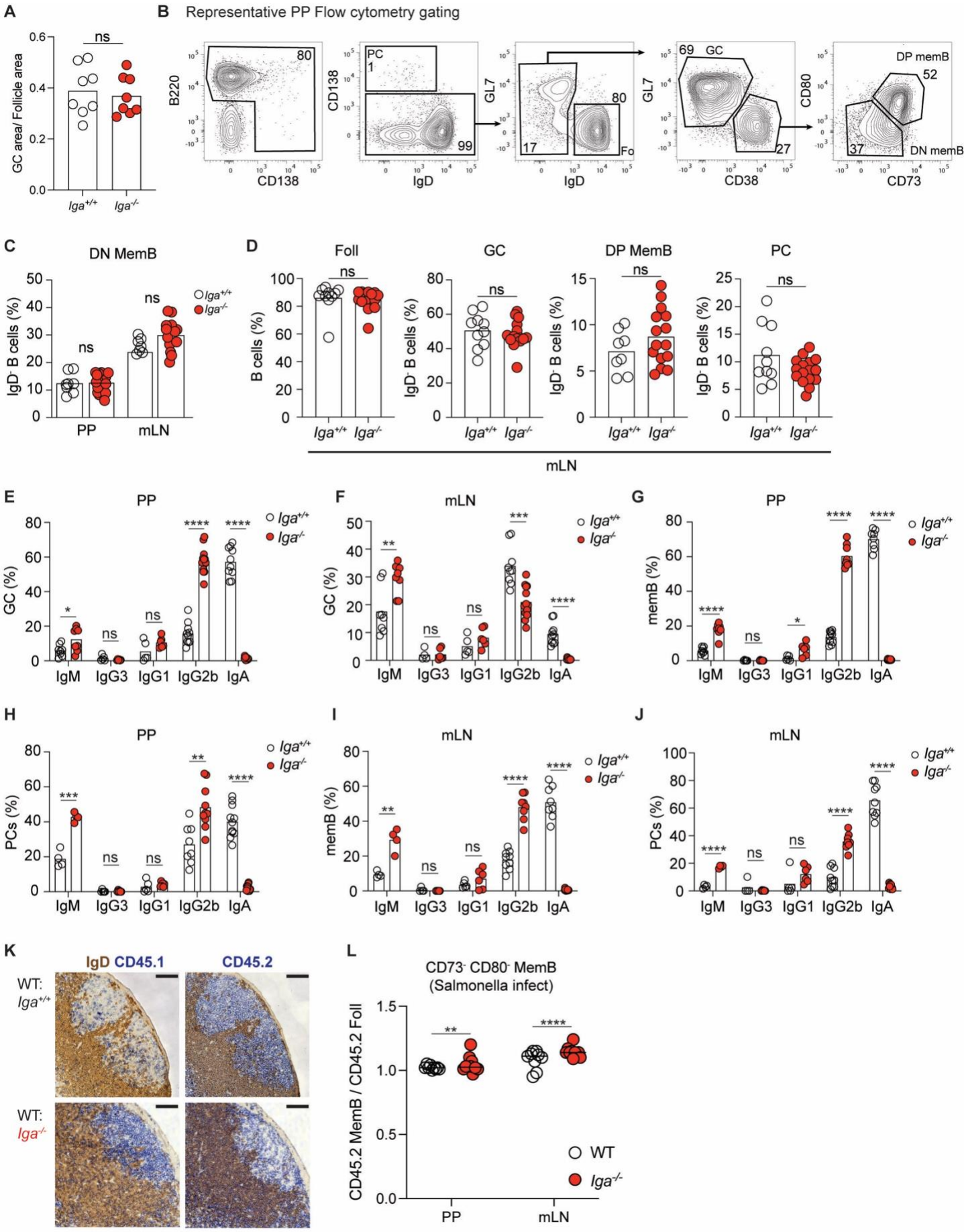
Supplemental Material



Supplemental Figure 1

Figure S1. Characterization of mucosal B cell compartments in *Iga*$^{-/-}$ mice, Related to Figure 1

(A) IHC ratio of GC area to follicle area of *Iga*$^{+/+}$ and *Iga*$^{-/-}$ PPs.

(B) Representative flow cytometry of PPs gated previously on live singlets.

(C) CD73^{-} CD80^{-} (DN) MemB as percentage of activated (IgD^{-}) B cells in *Iga*$^{+/+}$ and *Iga*$^{-/-}$ mice.

(D) mLN Foll B cells as percentage of B cells. GC, MemB, and PCs as percentage of activated B cells from *Iga*$^{+/+}$ and *Iga*$^{-/-}$ mice.

(E, F) Isotypes of GC B cells in PPs (E) and mLN (F).

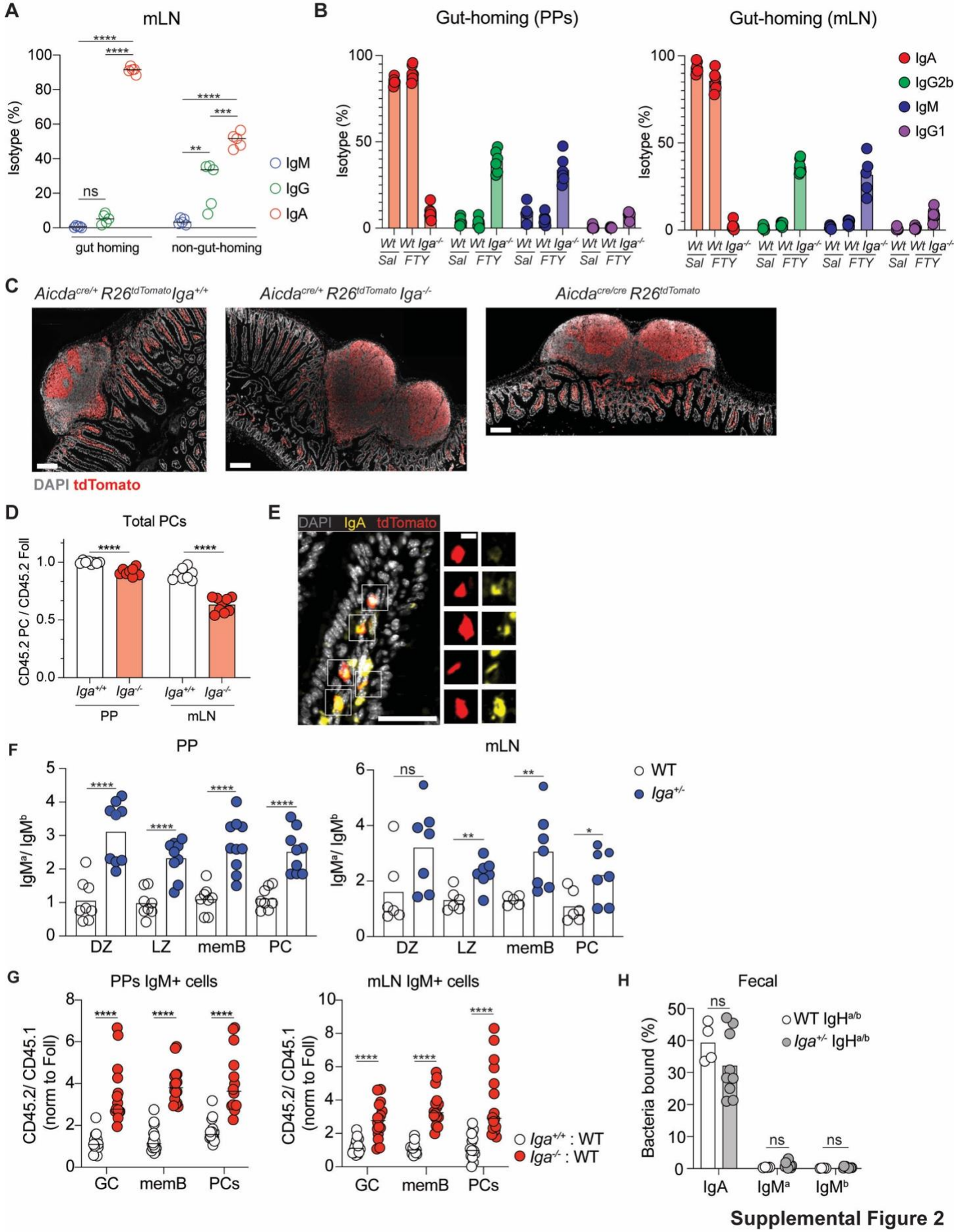
(G, H) Isotypes of MemB cells(G) and PCs(H) from PPs.

(I, J) Isotypes of MemB cells(I) and PCs (J) from mLN.

(K) Representative serial IHC sections of mixed BM chimeras stained with IgD (brown) and CD45.1 or CD45.2 (blue). Scale bars, 100 μm.

(L) Ratio of frequency of CD45.2 DN MemB cells to CD45.2 Foll B cells in PP and mLN of Salmonella infected mixed BM chimeras.

Data from at least 3 independent experiments with 1-3 mice per group (A-L). ns=not significant, *p < 0.005, **p < 0.001,***p < 0.0005, ****p < 0.0001; Unpaired two-tailed Student's t-test.



Supplemental Figure 2

Figure S2. Impact of IgA deficiency on mucosal B cell differentiation in a competitive setting, Related to Figures 2 and 3.

(A) Isotypes of gut-homing ($\alpha4\beta7^+$ $CCR9^+$) and non-gut-homing PCs from mLN of WT mice treated with FTY-720 for seven days.

(B) Isotypes of gut-homing and non-gut-homing PCs from PPs and mLN of WT and *Iga*$^{-/-}$ mice treated with FTY-720 for 7 days.

(C) Representative immunofluorescence of PP and small intestinal villi of *Aidca*$^{cre/+}$ $R26^{tdTomato}$ *Iga*$^{+/+}$, *Aidca*$^{cre/+}$ $R26^{tdTomato}$ *Iga*$^{-/-}$, *Aidca*$^{cre/cre}$ $R26^{tdTomato}$ mice stained with DAPI (gray) and tdTomato (red). Scale bars, 200 μm.

(D) Ratio of frequency of CD45.2 PCs to CD45.2 Foll B cells in PP and mLN of mixed BM chimeras.

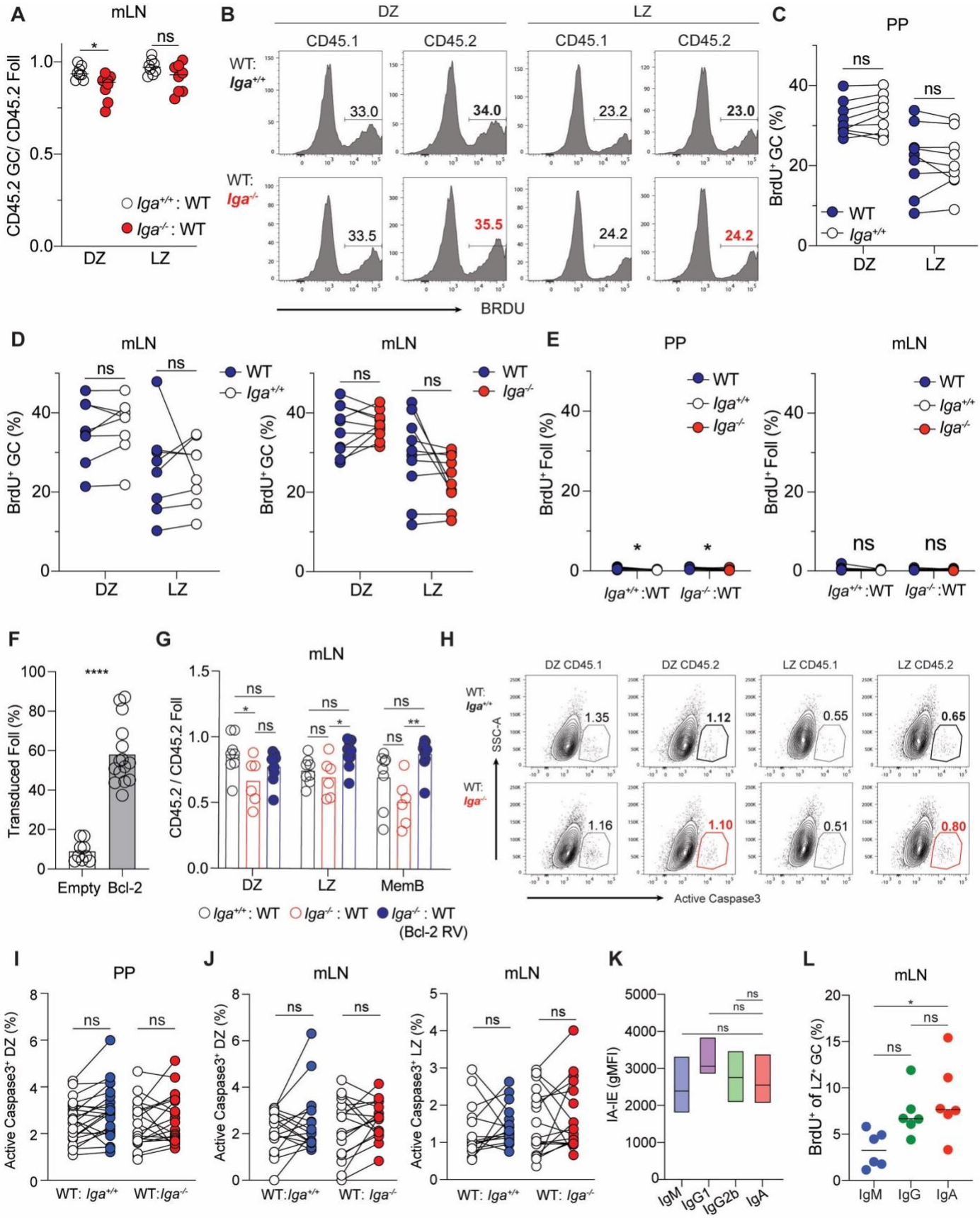
(E) Representative immunofluorescence of small intestinal villi stained for DAPI (gray), tdTomato (red), and IgA (yellow), in WT: *Aidca*^{cre} $R26^{tdTomato}$ *Iga*$^{+/+}$ mBM chimera. Scale bars, 50 μm. Inserts of IgA^+ $tdTomato^+$ cells within villi, scale bars, 10 μm.

(F) Ratio of frequency of IgM^a to IgM^b in indicated B cell populations from PP and mLN of WT and *Iga*$^{+/-}$ mice.

(G) Ratio of frequency of IgM^+ CD45.2 B cells to IgM^+ CD45.1 for indicated B cell populations of PP and mLN of mixed BM chimeras. Normalized to Foll CD45.2/ CD45.1 ratio.

(H) Percentage of endogenously coated fecal bacteria from WT and *Iga*$^{+/-}$ mice with indicated antibodies.

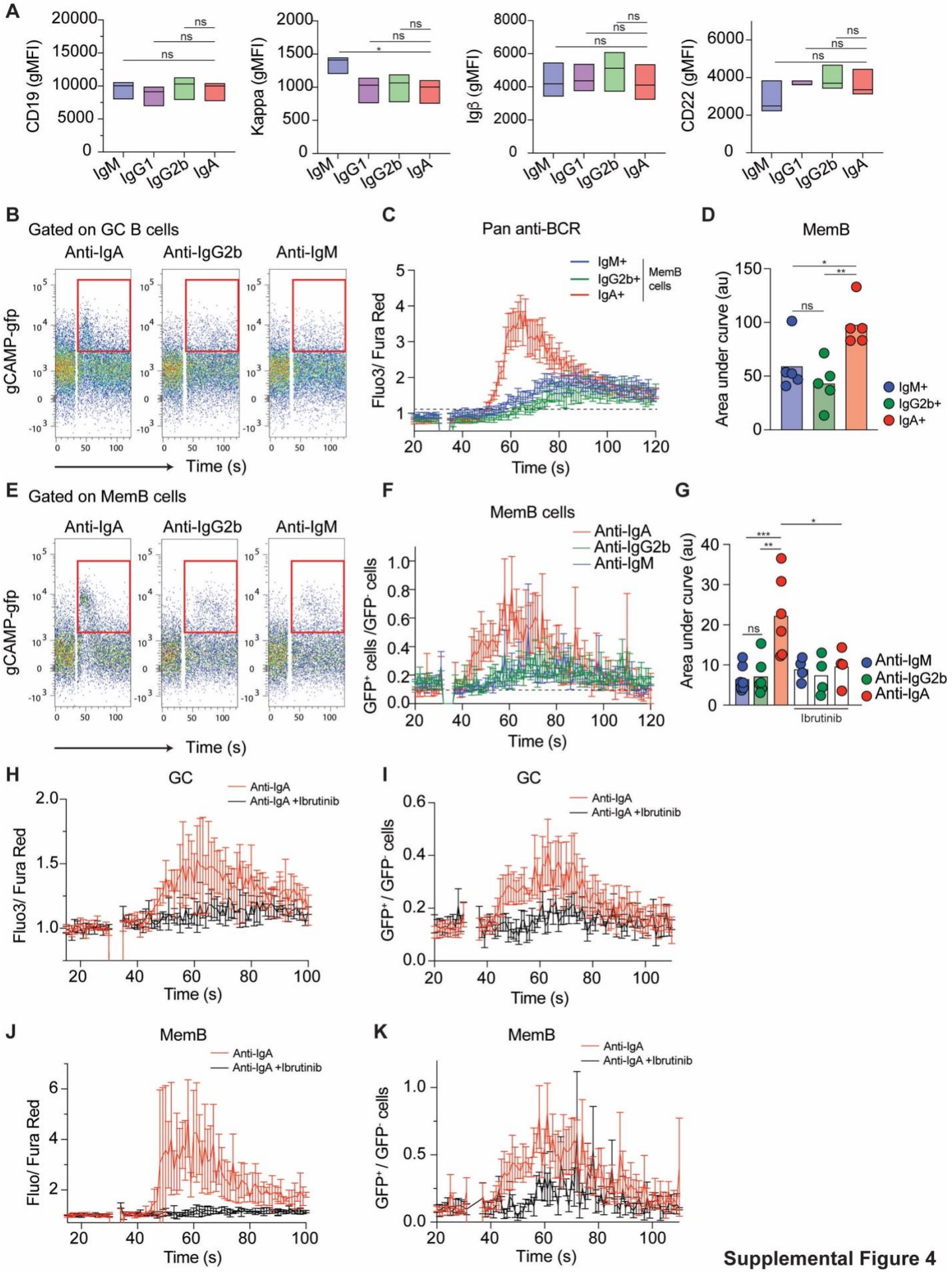
Compiled data from 2 independent experiments with 2-3 mice per group (A, B). Representative data from at least 3 independent experiments with at least 3 mice per group(D, G). Compiled data from 4 independent experiments with 2-4 mice per group (F). ns=not significant, *$p < 0.005$, **$p < 0.001$,***$p < 0.0005$, ****$p < 0.0001$. Unpaired two-tailed Student's t-test in d, f, g, h. One way ANOVA in a, b.



Supplemental Figure 3

Figure S3. Role of IgA BCR on light zone (LZ) and dark zone (DZ) GC selection, Related to Figure 4
(A) Ratio of frequency of CD45.2 DZ or LZ GC B cells to CD45.2 Foll B cells in mLN of mixed BM chimeras.
(B) Representative histograms of BrdU staining in PP DZ and LZ GC B cells of mixed BM chimeras.
(C) Percentage of $BrdU^+$ DZ or LZ GC B cells in PPs of WT: *Iga*$^{+/+}$ mixed BM chimeras after 3.5 hour BrdU pulse.
(D) Percentage of $BrdU^+$ DZ and LZ GC B cells from mLN of indicated mixed BM chimeras.
(E) Percentage of $BrdU^+$ Foll B cells from PP or mLN of indicated mixed BM chimeras.
(F) Percentage of transduced (empty vector or *Bcl2*) Foll B cells from retroviral BM chimeras.
(G) Ratio of frequency of specified CD45.2 B cells to CD45.2 Foll B cells in mLN of Bcl2 retroviral mixed BM chimeras.
(H) Representative active caspase 3^+ staining in DZ and LZ GC B cells of mixed BM chimeras.
(I) Percentage of active caspase3^+ DZ GC B cells from PPs of mixed BM chimeras.
(J) Percentage of active caspase3^+ DZ and LZ GC B cells from mLN of mixed BM chimeras.
(K) surface IA-IE gMFI on WT PP GC B cells for indicated isotypes.
(L) Percentage of $BrdU^+$ LZ GC B cells in mLN for indicated isotypes after 30 minute BrdU pulse. (IgG are defined as IgM-IgA-).

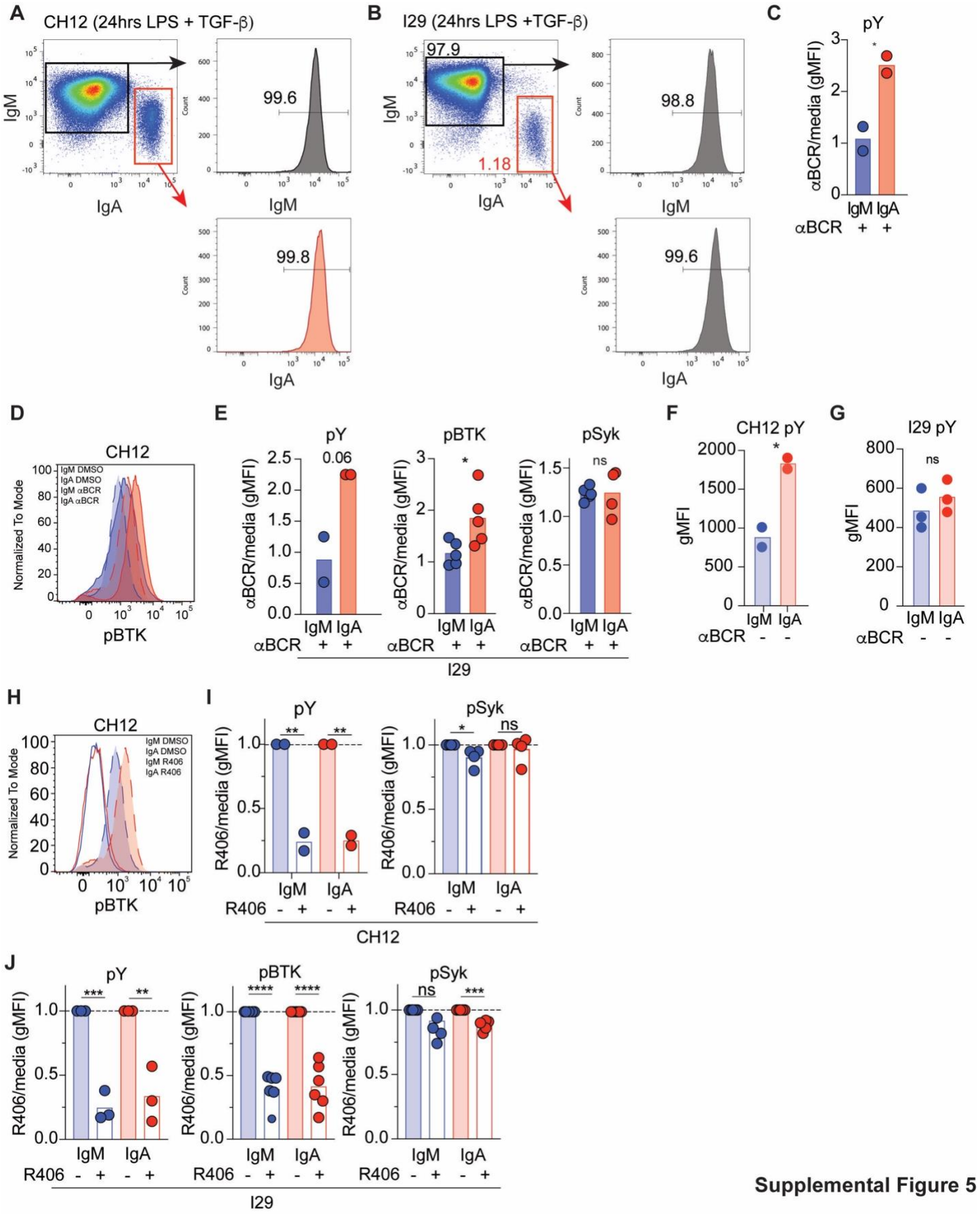
Data from at least 3 independent experiments with 2-3 mice per group (A, C-E, J, K). Data from 2 experiments with at least 3 mice per group (F, G, L). Unpaired two-tailed Student's t-test was used in a, c, d, e, h, i. One-way ANOVA was used in f, j, k. ns=not significant, *p<0.005, **p<0.001, ****p<0.0001.



Supplemental Figure 4

Figure S4. Measuring isotype dependent BCR signaling pathways ex vivo, Related to Figure 5.
(A) PP GC surface gMFI for indicated BCR components on specified isotypes**.**
(B) Representative flow cytometry of *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/gCAMP}$ PP GC B cells stimulated with indicated anti-BCR.
(C) Compiled calcium traces for IgM, IgG2b, and IgA MemB cells (defined as isotype negative populations) stimulated with pan anti-BCR.
(D) AUC of individual calcium traces calculated between 50 and 120 seconds for (C).
(E) Representative flow cytometry of *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/gCAMP}$ PP MemB cells stimulated with indicated anti-BCR.
(F) Compiled calcium traces from *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/gCAMP}$ PP MemB cells stimulated with indicated anti-BCR isotypes. Shown as ratio of frequency of gCAMP-GFP^{+} to gCAMP-GFP^{-} cells per second.
(G) AUC of individual calcium traces calculated between 50 and 100 seconds for (G). Indicated samples were pre-treated with ibrutinib prior to stimulation.
(H, I) are compiled calcium traces for *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/+}$ (H) or *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/gCAMP}$ (I) PP GC stimulated with anti-IgA. Indicated samples were pre-treated with ibrutinib prior to stimulation.
(J, K) are compiled calcium traces for *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/+}$ (J) or *Aicda*$^{cre/+}$ Rosa26$^{tdTomato/gCAMP}$ (K) PP MemB cells stimulated with anti-IgA. Indicated samples were pre-treated with ibrutinib prior to stimulation.

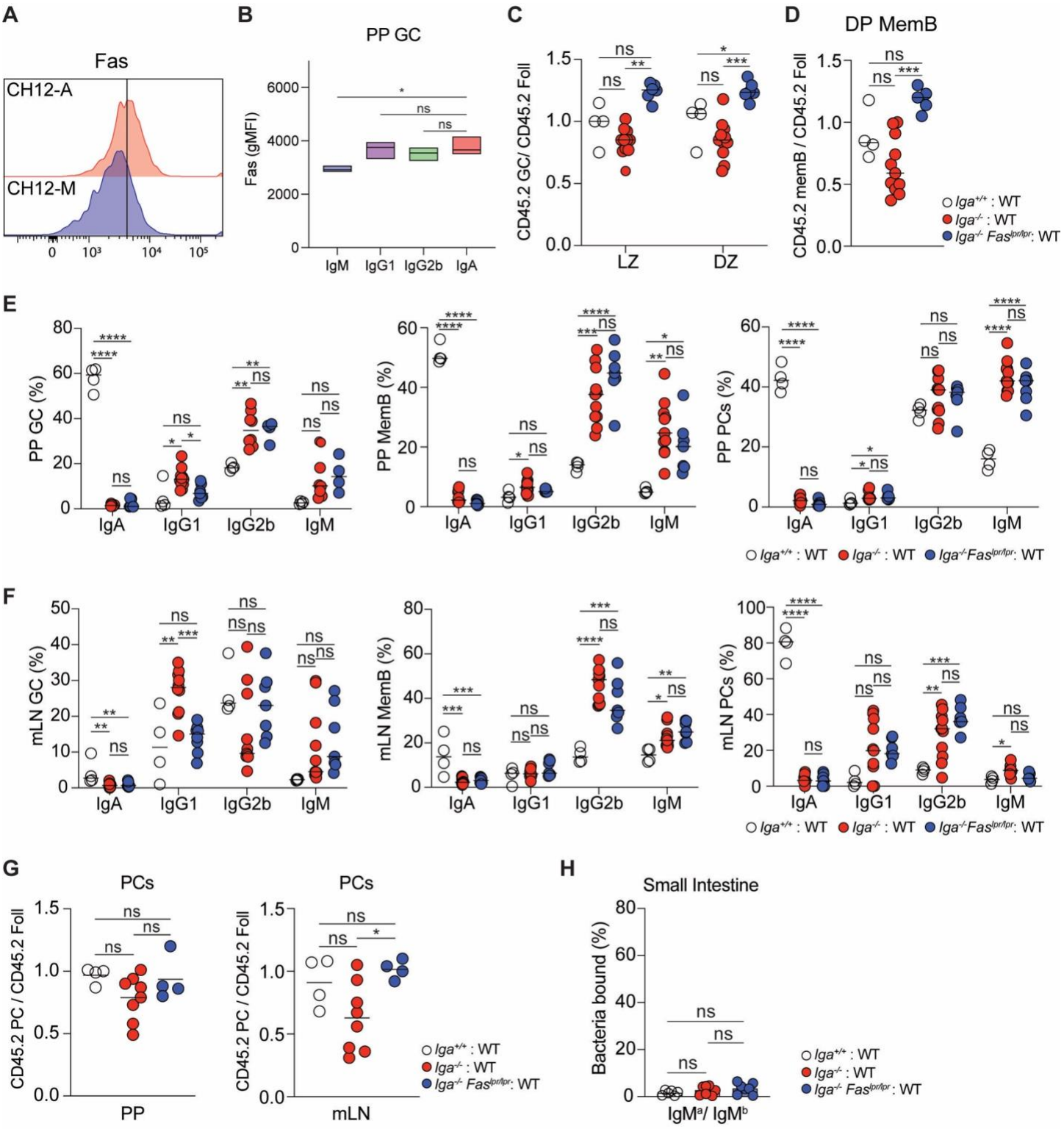
Data from at least 3 independent experiments with at least 2 mice per group (A). Data from 4 independent experiments (C, D, F, G). ns=not significant, *p<0.005, **p<0.001,***p<0.0005. One-way ANOVA in a, d, g.



Supplemental Figure 5

Figure S5. Measuring isotype dependent BCR signaling pathways in vitro, Related to Figure 5.
(A) Representative flow cytometry of CH12 cell line after in vitro class switch to IgA. CH12 expressing surface IgM or IgA were sorted to produce uniform cell lines expressing IgM or IgA BCR.
(B) Representative flow cytometry of I29 cell line in vitro class switch to IgA. I29 expressing surface IgM or IgA were sorted to produce uniform cell lines expressing IgM or IgA BCR.
(C) pY gMFI normalized to untreated(media) in CH12 cells after anti-BCR stimulation.
(D) Representative pBTK histogram in CH12 cells after anti-BCR stimulation.
(E) pY, pBTK, pSyk gMFI normalized to untreated in I29 cells after anti-BCR stimulation.
(F) pY gMFI in unstimulated CH12 cells.
(G) pY gMFI in unstimulated I29 cells.
(H) Representative histogram of pBTK after incubation with R406 in CH12 cells.
(I) pY and pSyk gMFI normalized to untreated in CH12 cells after incubation with R406. Compiled data from at least 3 independent experiments.
(J) pY, pBTK, and pSyk gMFI normalized to untreated in I29 cells after incubation with R406.

Compiled data from 2 independent experiments (C, F). Data from at least 3 independent experiments (E, G, J). ns=not significant, *$p < 0.005$, **$p < 0.001$, ***$p < 0.0005$, ****$p < 0.0001$. Unpaired two-tailed Student's t-test in c, e, f, g, I, j.



Supplemental Figure 6

Figure S6. Interplay between IgA and Fas during mucosal GC reaction and B cell differentiation, Related to Figure 6.

(A) Representative histograms of surface Fas on CH12 cells.
(B) Surface Fas gMFI on PP GC B cells for indicated isotypes.
(C) Ratio of frequency of CD45.2 LZ or DZ B cells to CD45.2 Foll B cells in mLN of mixed BM chimeras.
(D) Ratio of frequency of CD45.2 DP MemB cells to CD45.2 Foll B cells in mLN of mixed BM chimeras.
(E, F) Isotypes of PP(E) and mLN(F) for indicated B cells from mixed BM chimeras.
(G) Ratio of frequency of CD45.2 PCs to CD45.2 Foll from PPs and mLN of mixed BM chimeras.
(H) Percentage of endogenously coated small intestinal bacteria with IgM^a or IgM^b antibodies from mixed BM chimeras made in μMT hosts. Percentage shown as IgM^a/IgM^b ratio.

Compiled data from at least 3 independent experiments with at least 2 mice per group (B). Compiled data from 3 independent experiments with 1-3 mice per group (C-G). Compiled data from 3 independent experiments with 2-3 mice per group (H). ns=not significant, *$p < 0.005$, **$p < 0.001$, ***$p < 0.0005$, ****$p < 0.0001$; One-way ANOVA in B-H.