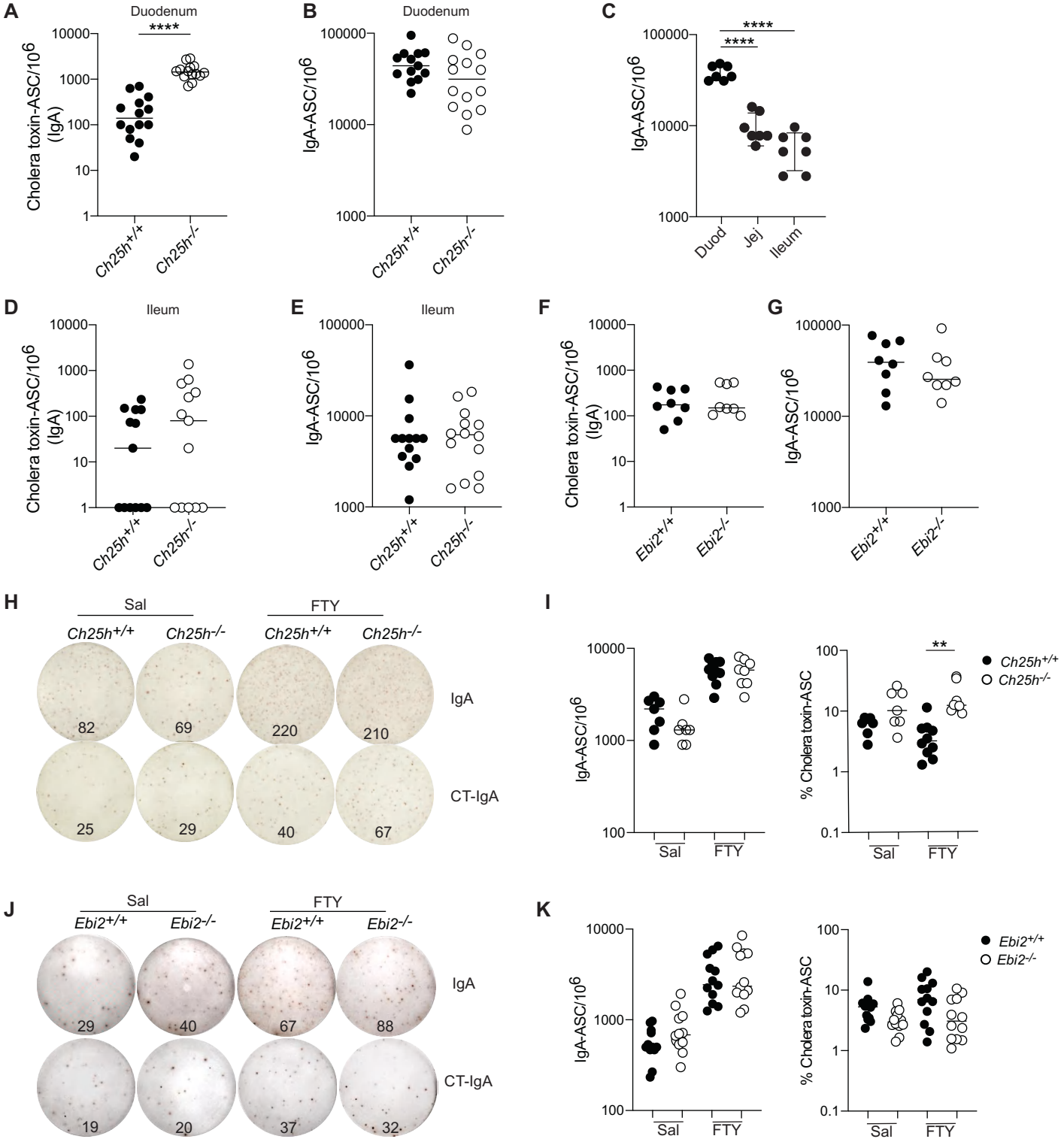

Figure S1. 25-HC controls antigen-specific IgA secretion in duodenum, but not in the ileum. Related to Figure 1.
(A) ELISPOT of cholera toxin-specific IgA and (B) total IgA secretion in duodenum of *Ch25h*$^{-/-}$ and littermate control mice. (C) Number of secreting IgA PCs in Duodenum, Jejunum and Ileum, analyzed by ELISPOT. (D) cholera toxin-specific IgA and (E) total IgA in the ileum of *Ch25h*$^{-/-}$ and littermate control mice, depicted by ELISPOT. (F,G) ELISPOT data showing IgA-ASC and total IgA in duodenum of *Ebi2*$^{-/-}$ and littermate control mice. (H) Image of representative ELISPOT of IgA and cholera toxin-specific IgA secreting cells in Peyer's patches of *Ch25h*$^{-/-}$ and littermate control mice orally immunized with cholera toxin for 3 weeks and treated for one week with FTY720 or saline by i.p. injection. (I) Graphs showing the plotted analysis of the total number of IgA secreting PCs and the frequency of the cholera toxin-specific IgA PCs, detected by ELISPOT assay depicted in (H). (J) Representative ELISPOT of Peyer's patch IgA and cholera toxin-specific IgA secreting cells from *Ebi2*$^{-/-}$ and littermate control mice treated for 3 weeks with cholera toxin by oral gavage and one week with FTY720 or saline by IP injection. (K) Total IgA and cholera toxin-specific IgA ASC in Peyer's patches of *Ebi2*$^{-/-}$ and littermate control mice. Graphs show data from 3≥ experiments. **p<0.01 (ANOVA multiple comparation).

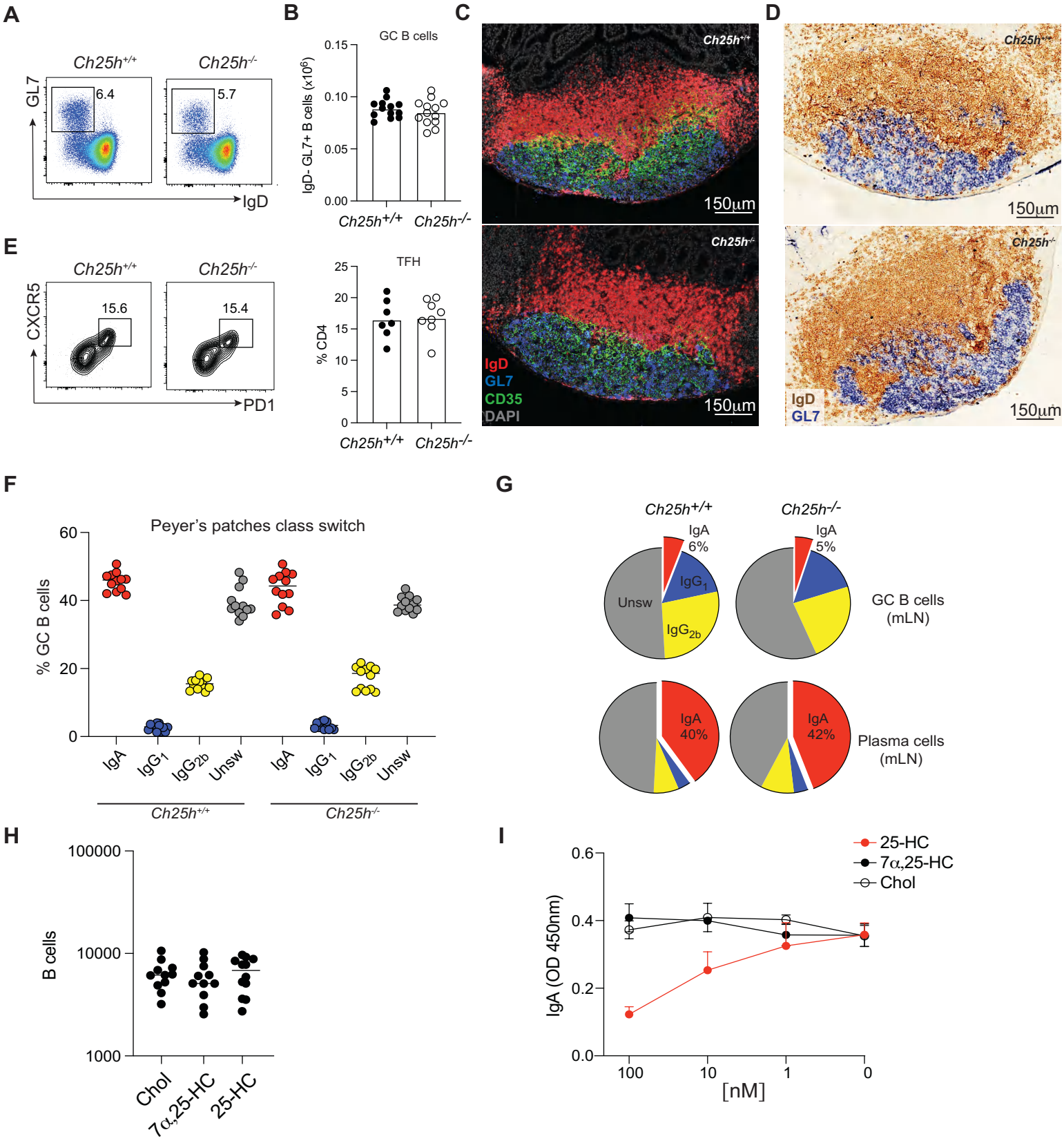

Figure S2. Ch25h deficiency does not impact germinal center or class switch recombination. Related to Figure 2.
(A) Representative flow cytometry plot of GC (B220+ IgD- GL7+) and (B) total number of GC B cells from Peyer's patches of *Ch25h*$^{-/-}$ and littermate control mice. (C) IF and (D) IHC of Peyer's patches from *Ch25h*$^{-/-}$ and littermate control mice stained with the indicated antibodies, 20X magnification. (E) Representative flow cytometry plot and compiled data for TFH cells (CD4+CXCR5+PD1+ cells) from *Ch25h-/-* and littermate control mice. (F) Compiled data of class switch recombination in GC B cells of Peyer's patches and (G) class switch recombination in GC B cells and PCs from mesenteric lymph nodes of the indicated genotype assessed by FACS. (H) Number of GC B cells quantified by flow cytometry after culture with NB21 cells and treatment with of cholesterol, 25-HC or 7α,25-HC. (I) Quantification of secreted IgA by ELISA from Peyer's patches GC B cells cultured with NB21 supernatant and anti-CD40 and stimulated with the indicated sterols for 3.5 days. Data were pooled from at least 3 independent experiments in (B,E, and F) or 2 independent experiments in (H and I). Statistics were measured by unpaired Student's t test (B,E) and two-way ANOVA (F,H, I).

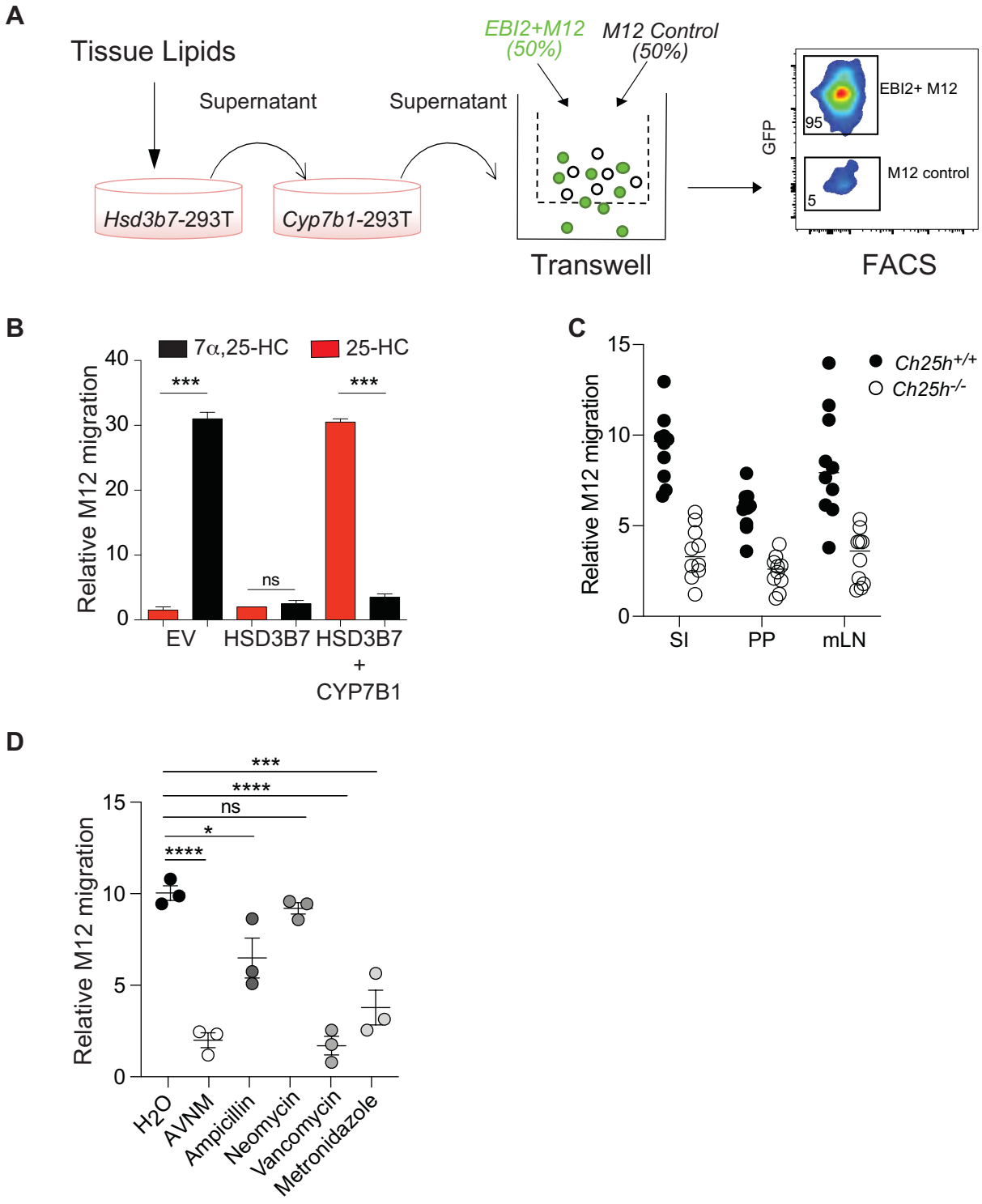

Figure S3. 25-HC quantification in Peyer's patches in steady state and after antibiotic treatment. Related to Figure 3.
(A) Schematic illustration of transwell migration assay with EBI2+ M12 for the quantification of 25-HC in intestinal SLO using transfected 293T cell. (B) Validation of the strategy in (A) using synthetic 25-HC and 7α,25-HC. Graph shows the relative migration of EBI2+ M12 cells from 3 experiments. (C) 25-HC quantification in the indicated tissue from Ch25h-/- and littermate control mice. (D) 25-HC quantification in Peyer's patches of mice treated for one week with the indicated antibiotics. Data are representative of at least 3 independent experiments (B and C) or 2 independent experiments in (D). Statistic was measured as *** $p<0.005$ (unpaired Student's t test (B,C)) and *$p<0.05$, **$p<0.01$,***$p<0.005$,****$p<0.001$ (two-way ANOVA (D).

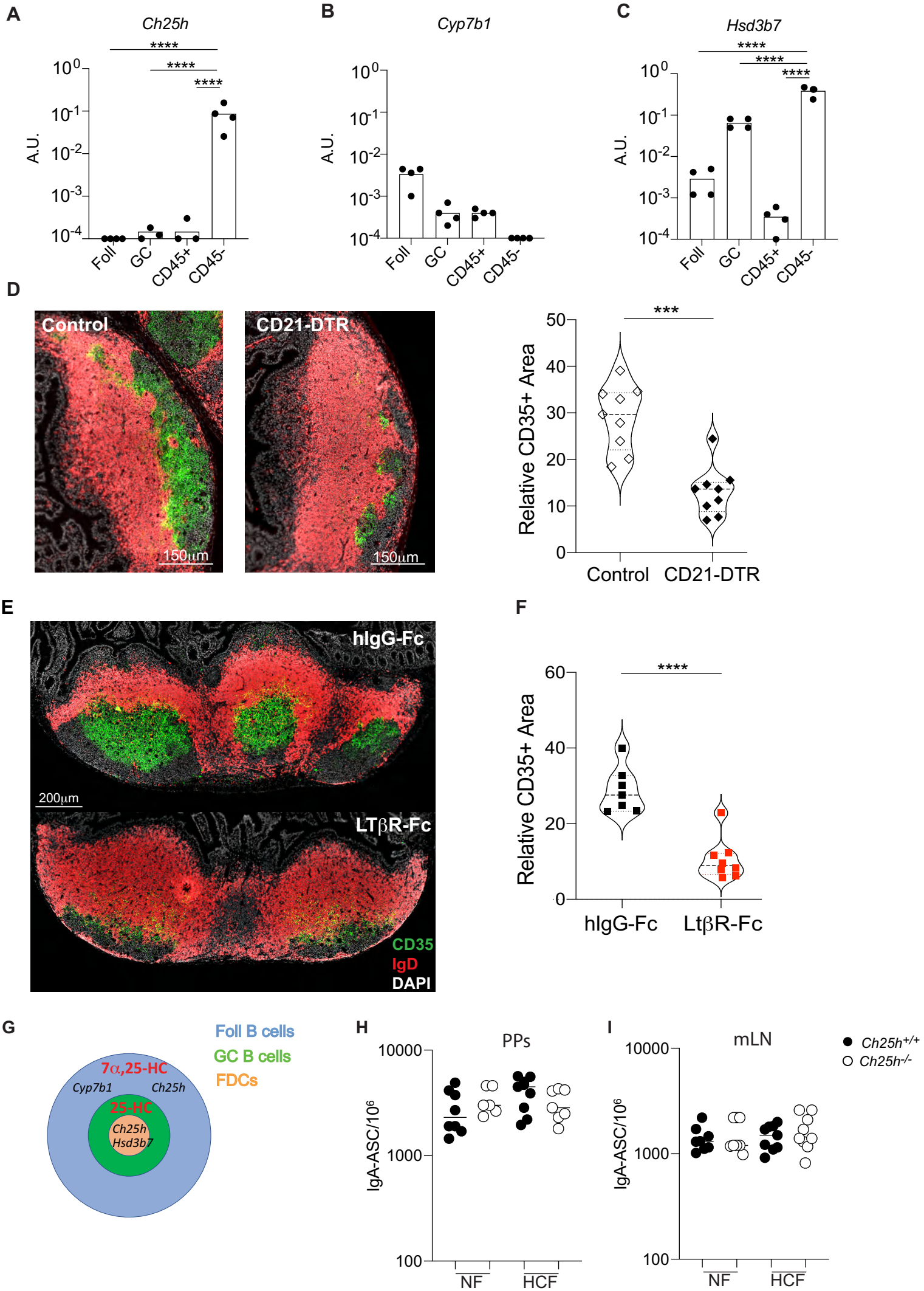
A
Ch25h
****
****
****
A.U.
Foll
GC
CD45+
CD45-
B
Cyp7b1
A.U.
Foll
GC
CD45+
CD45-
C
Hsd3b7
****
****
****
A.U.
Foll
GC
CD45+
CD45-
D
Control
CD21-DTR
150µm
150µm
***
Relative CD35+ Area
Control
CD21-DTR
E
hIgG-Fc
200µm
LTβR-Fc
CD35
IgD
DAPI
F
****
Relative CD35+ Area
hIgG-Fc
LtβR-Fc
G
Foll B cells
GC B cells
FDCs
7α,25-HC
Cyp7b1
Ch25h
25-HC
Ch25h
Hsd3b7
H
PPs
IgA-ASC/10⁶
NF
HCF
I
mLN
IgA-ASC/10⁶
NF
HCF
Ch25h+/+
Ch25h-/-

Figure S4. Non-hematopoietic expression of Ch25h and FDC depletion validation. Related to Figure 4.
(A) mRNA expression of *Ch25h*, (B)*Cyp7b1* and (C)*Hsd3b7* in sorted follicular and GC B cell, and in MACS-enriched $CD45^+$ and $CD45^-$ cells from Peyer's patches. (D) Immunofluorescence of Peyer's patches cryosection and compiled quantification of relative FDCs ($CD35^+$) area from BM chimera CD21-DTR and control mice, treated with 100 ng of diphtheria toxin for 16 hours. Sections were stained with the indicated antibodies, 20x magnification. (E) Immunofluorescence of Peyer's patches cryosection from C57/Bl6 mice treated with 100ng of LTβR-Fc or isotype control (hIgG-Fc) for 4 days. Sections were stained with the indicated antibodies, 20x magnification. (F) Quantification of relative FDCs ($CD35^+$) area. (G) Model for maintenance of 25-HC niche in germinal center. (H) ELISPOT of IgA in Peyer's patches and (I) mesenteric lymph nodes of $Ch25h^{-/-}$ and littermate control mice treated for 1 week with regular chow or 0.15% cholesterol supplement diet. ELISPOT data were pooled from at least 3 independent experiments. *** $p<0.005$, **** $p<0.001$ (unpaired Student's t test (D,F,H,I) and **** $p<0.001$ (two-way ANOVA (A,B,C)).

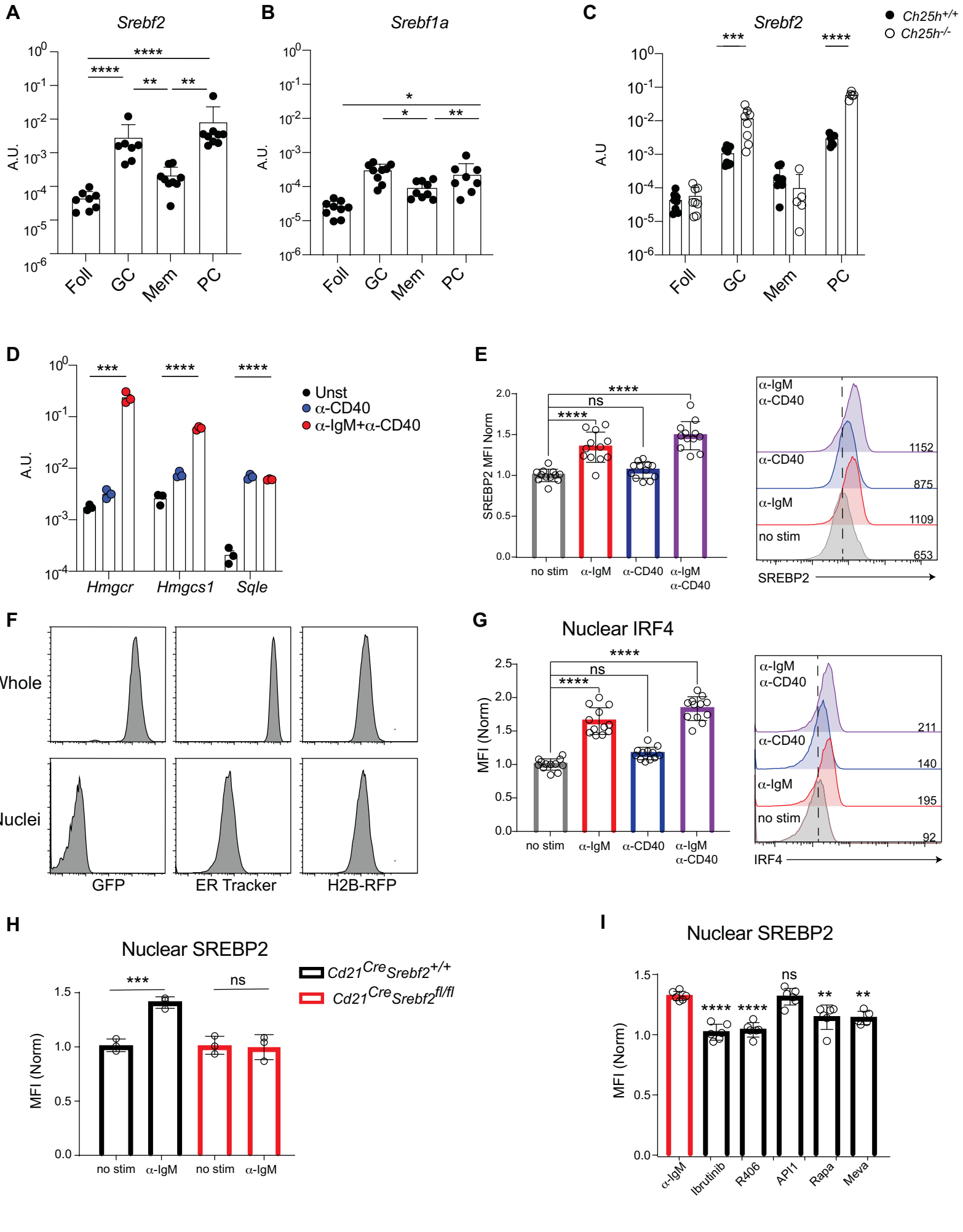
A
Srebf2
A.U.
Foll
GC
Mem
PC
B
Srebf1a
C
Srebf2
Ch25h+/+
Ch25h-/-
D
Unst
α-CD40
α-IgM+α-CD40
Hmgcr
Hmgcs1
Sqle
E
SREBP2 MFI Norm
no stim
α-IgM
α-CD40
α-IgM α-CD40
SREBP2
F
Whole
Nuclei
GFP
ER Tracker
H2B-RFP
G
Nuclear IRF4
MFI (Norm)
IRF4
H
Nuclear SREBP2
Cd21Cre Srebf2+/+
Cd21Cre Srebf2fl/fl
I
Nuclear SREBP2
Ibrutinib
R406
API1
Rapa
Meva

Figure S5. 25-HC reduces SREBP2 level and alters SREBP2-mediated transcription in vitro and in vivo. Related to Figure 5. Transcript level of *Srebf2* (A) and *Srebf1a* (B) in B cell subsets from mesenteric lymph nodes of wild-type mice and (C) from mesenteric lymph nodes of *Ch25h*$^{-/-}$ and littermate control mice measured by qPCR. (D) qPCR of mevalonate pathway and SREBP2 targets on B cells stimulated for 48hrs with a-CD40 or with a-CD40 with a-IgM. (E) Mean Fluorescence index (MFI) and representative histograms of total SREBP2 detected by flow cytometry in B cells stimulated for 16h with the indicated stimuli. (F) Whole cell and nuclei isolation stain of transduced WEHI for presence of cytoplasmic, ER and nuclear markers. (G) Mean Fluorescence index (MFI) of intranuclear IRF4 detected by flow cytometry in B cells stimulated for 16h with the indicated stimuli. (H) Mean Fluorescence index (MFI) of nuclear SREBP2 detected by flow cytometry in B cells isolate from *Cd2*$^{Cre/+}$*Srebf2*$^{flox/flox}$ and LTC stimulated or unstimulated for 16h with a-IgM. (I) MFI of SREBP2 nuclear fraction on B cells stimulated in vitro with the indicated inhibitors. Data were produced from at least experiment 2-3 independent experiments. *p<0.05, **p<0.01,***p<0.005, **** p<0.001 (two-way ANOVA).

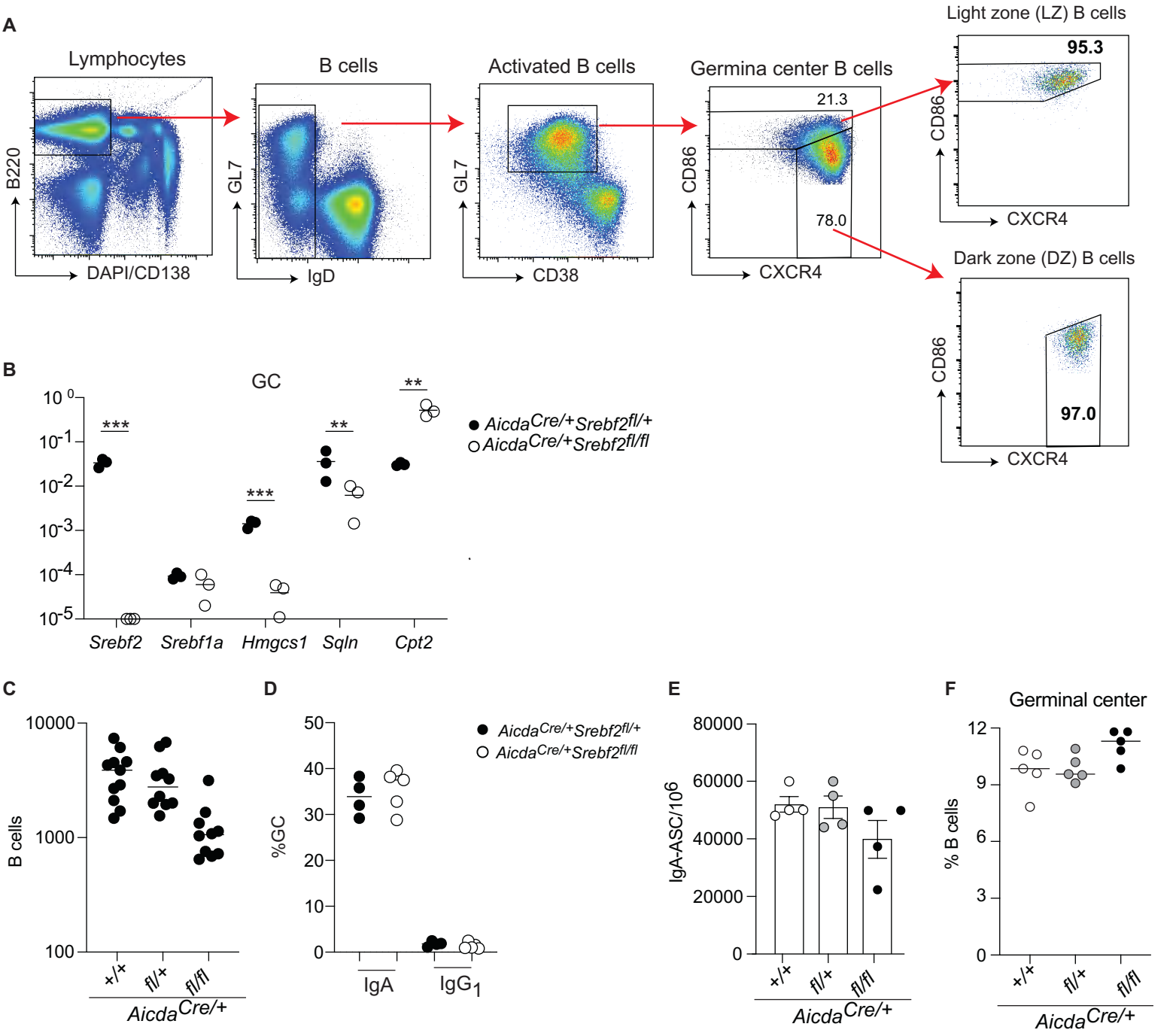

Figure S6. Sorting strategy for LZ and DZ GC B cells in Peyer's patches. Related to Figure 6.

(A) Flow cytometry sorting strategy and purity of CD86+CXCR4Lo (LZ) and CD86LoCXCR4+ (DZ) from Peyer's patches. (B) mRNA quantification of *Srebf2* and SREBP2 gene targets in sorted GC B cells from $Aicda^{Cre/+}Srebf2^{+/+}$ and $Aicda^{Cre/+}Srebf2^{flox/flox}$ mice. (C) Number of B cells from the indicated genotype recovered after 3.5 days of NB21 culture. (D) IgA and IgG1 class switch in GC B cells from the indicated genotype. (E) Quantification of IgA secreting PCs by ELISPOT in lamina propria of the mice of the indicated genotype. (F) Frequency of GC B cells detected by flow cytometry in Peyer's patches of the indicated mice. Data were plotted from 2 independent experiments in (B) or 3 independent experiments in (C,D,E,F). **p<0.01,***p<0.005, (unpaired t-test in (B) and two-way ANOVA in (C,D,E,F)).

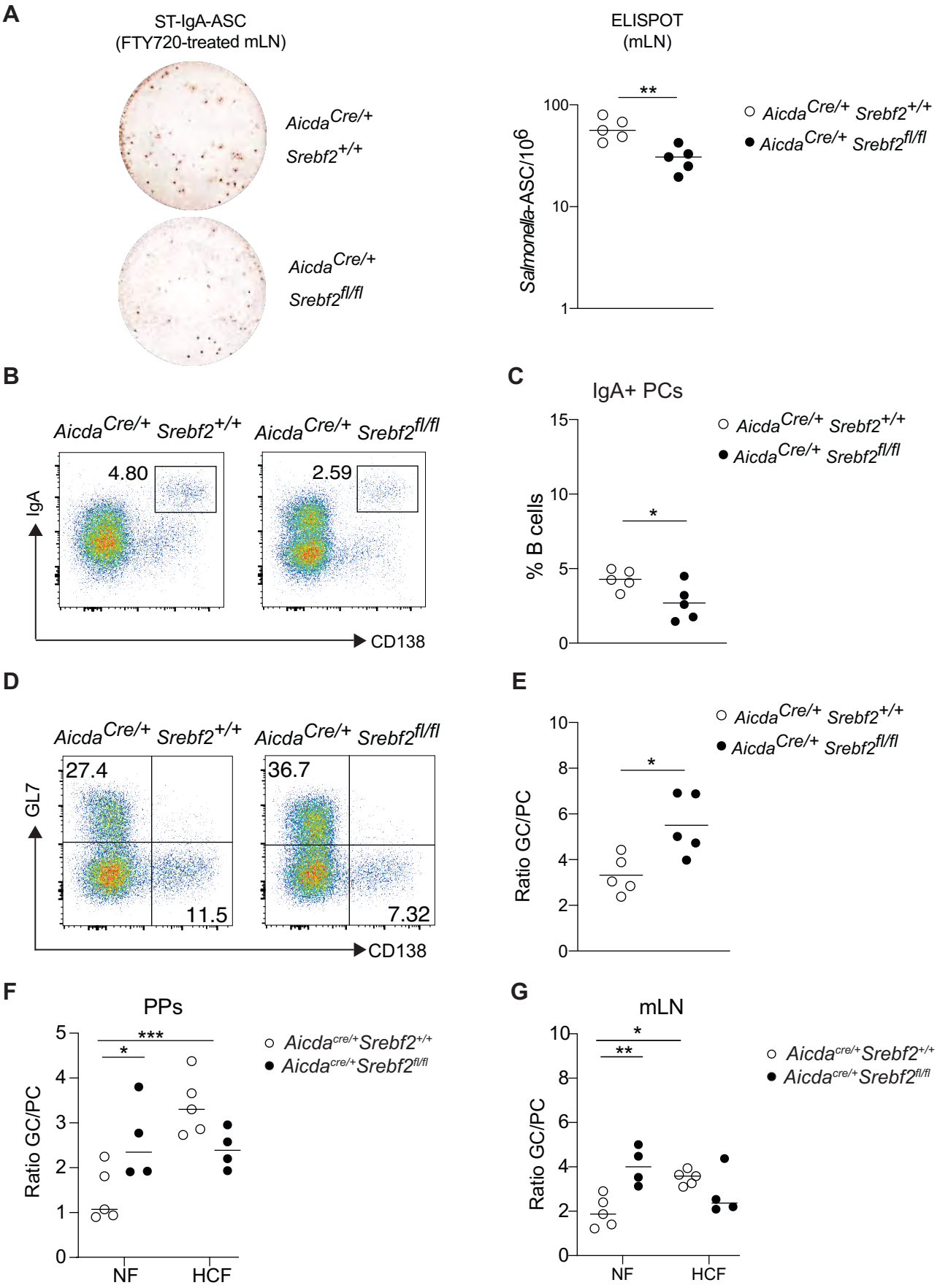

Figure S7. Salmonella-specific IgA response in mesenteric lymph nodes is controlled by GC B cell SREBP2 and it is sensitive to dietary cholesterol. Related to Figure 7.

(A) Representative ELISPOT and compiled data showing Salmonella-specific IgA secreting cells in mesenteric lymph nodes of *Aicda*$^{Cre/+}$*Srebf2*$^{+/+}$ and *Aicda*$^{Cre/+}$*Srebf2*$^{flox/flox}$ mice infected with Salmonella and treated with FTY720. (B) Representative flow cytometry and (C) frequency of IgA+ PCs from mesenteric lymph nodes of *Aicda*$^{Cre/+}$*Srebf2*$^{+/+}$ and *Aicda*$^{Cre/+}$*Srebf2*$^{flox/flox}$ mice infected with Salmonella and treated with FTY720 as in (A). (D) Representative flow cytometry of GC B cell (GL7+) and PCs (CD138+) in activated B cells from mesenteric lymph nodes of *Aicda*$^{Cre/+}$*Srebf2*$^{+/+}$ and *Aicda*$^{Cre/+}$*Srebf2*$^{flox/flox}$ mice infected with Salmonella and treated with FTY720 as in (A, B). (E) Ratio of GC B cell and PCs as in (D). (F) Ratio of GC B cells and PCs in activated B cells from Peyer's patches and (G) mesenteric lymph nodes of *Aicda*$^{Cre/+}$*Srebf2*$^{+/+}$ and *Aicda*$^{Cre/+}$*Srebf2*$^{flox/flox}$ mice treated for 1 week with HCF or NF and FTY720 upon infection. Each symbol represents one mouse from 2/3 independent experiments. *p<0.05, **p<0.01,***p<0.005 (two-way ANOVA).

Table S1: RT-qPCR primers list. Related to Figure 4.

Gene target	Fw	Rev	Source
Ch25h	5'-CTGCCTGCTGCTCTTCGACA-3'	5'-CCGACAGCCAGATGTTAATCA-3'	Yi et al., 2012
Cyp7b1	5'-TCAGGAAAGGCAAGATCTGCTGA-3'	5'-CCTGTTGACTGCAGGAAACTGTCA-3'	Yi et al., 2012
Hsd3b7	5'-ACCATCCACAAAGTCAACG-3'	5'-TCTTCATTGCCCCTGTAGA-3'	Yi et al., 2012
Srebp2	5'-CTTGACTTCCTTGCTGCA-3'	5'-GCGTGAGTGTGGGCGAATC-3'	Reboldi et al.,2016
Srebp1a	5'-TTGGCACCTGGGCTGCT-3'	5'-GCGCCATGGACGAGCTG-3'	Reboldi et al.,2016
HmgCoA reductase	5'-CTTGTGGAATGCCTTGTGATTG-3'	5'-AGCCGAAGCACATGAT-3'	Reboldi et al.,2016
HmgCoA sinthetase 1	5'-GGAAGCTTTGGGGACGTTA-3	5'-ACACTCCAACCCTCTTCCCT-3'	Reboldi et al.,2016
Sqalene	5'-CCAACTCAATGGGTCTGTTCCT-3'	5'-TGGCTTAGCAAAGTCTTCCAACT-3'	this paper
Ldlr	5'-GAAGTCGACACTGTACTGACCACC-3'	5'-CTCCTCATTCCCTCTGCCAGCCAT-3'	Reboldi et al.,2016
Fasn	5'-ACCCGAGGGATCTGGTGAA-3'	5'-CTGTCGTGTCAGTAGCCGAG-3'	Reboldi et al.,2016
Prdm1	5'-AACACGTGGTACAACCCAAAG-3'	5'-GGCTGCAGAGATGGATGTAG-3'	this paper
βActin	5'-ACAACGGCTCCGGCATGTGCA-3'	5'-GTGTGGTGCCAGATCTTCTCCA-3'	Liu et al.,2011