Graphical Abstract

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In Brief

SAP is required for proper T cell help in germinal centers (GCs). Biram et al. show that SAP-independent GCs are formed within Peyer's patches. These GCs host highly diversified clones that are subjected to mild selection forces, demonstrating that clonal diversification can be uncoupled from clonal selection in

Highlights

- Chronic germinal centers in Peyer's patches are formed in SAP-deficient mice
- SAP-independent germinal centers arise in response to influenza infection
- Few highly diversified clones dominate the SAP-independent germinal centers
- Germinal center B cells in SAP-deficient mice are subjected to mild selection forces

Cell Reports

B G De a I U . e m SAP-M a Se F e C G m a G e e Be ' Pa e

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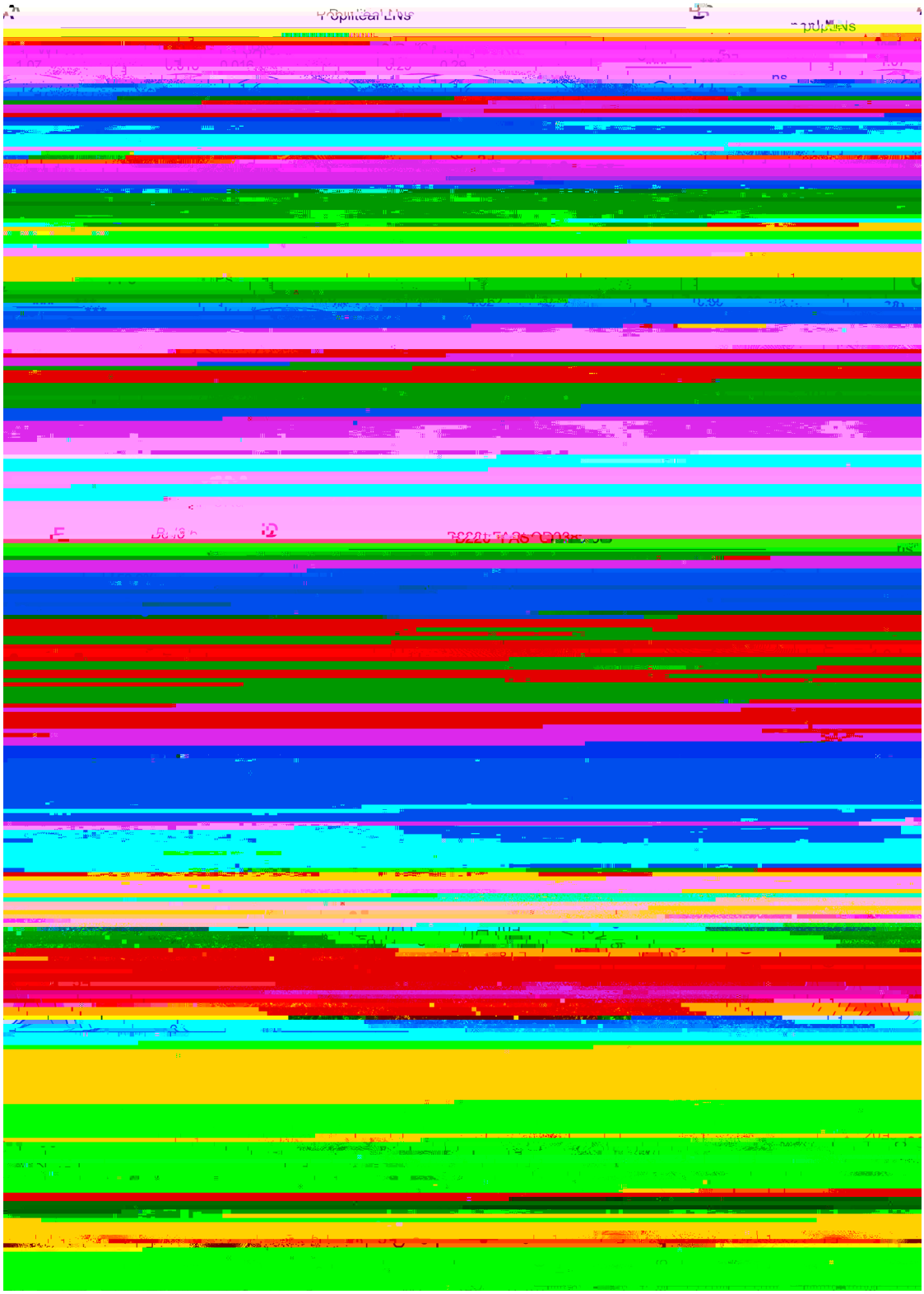
https://doi.org/10.1016/j.celrep.2020.01.032

Antibodies secreted within the intestinal tract provide protection from the invasion of microbes into the host

of a BCR, GC structures were detected in the gut-associated lymphoid tissues (GALTs) of LMP2A transgenic mice (Casola et al., 2004). Moreover, it was demonstrated that in transgenic

2B). In addition, no GC cells were detected in PPs derived from TCRα^{KO} mice; however, significant frequencies of GC cells were detected in the PPs of unimmunized SAP-deficient mice—3.4-fold lower than in WT (Figures 2A and 2C). In addition, GCs in the PPs of SAP-deficient mice showed normal distribution between the dark zone (DZ) and light zone (LZ) compartments (Figure 2D). Bcl6 is a key transcription factor that is essential for reprogramming activated B cells to differentiate into GC B cells (Basso and Dalla-Favera, 2012). B cells from WT and SAP-deficient mice expressed similar levels of mRNA transcripts and protein of Bcl6 (Figures 2E and 2F). These findings indicate that the GC structures observed in the PPs of SAP-deficient mice are bona fide GCs.

Many chronic GCs are found within PPs, and therefore it is difficult to discern whether these GCs are driven by specific gut-derived antigens. To examine whether SAP is required for pathogen-induced GC formation within other mucosal tissues, we infected WTte(P-.8(t4)-fe)19SAP(n)15.2(d)-417(W)0T8.6((RJ/Cs91Tf1.9851314TD(a)Tj/F41Tf6.3842TD[(A)--d(th)efi.6(am)ci.9(ue)e5.2(t)0(i7(W4t



F . e 2. GC B G . PP SAP-D e . M e E e T a Ma e

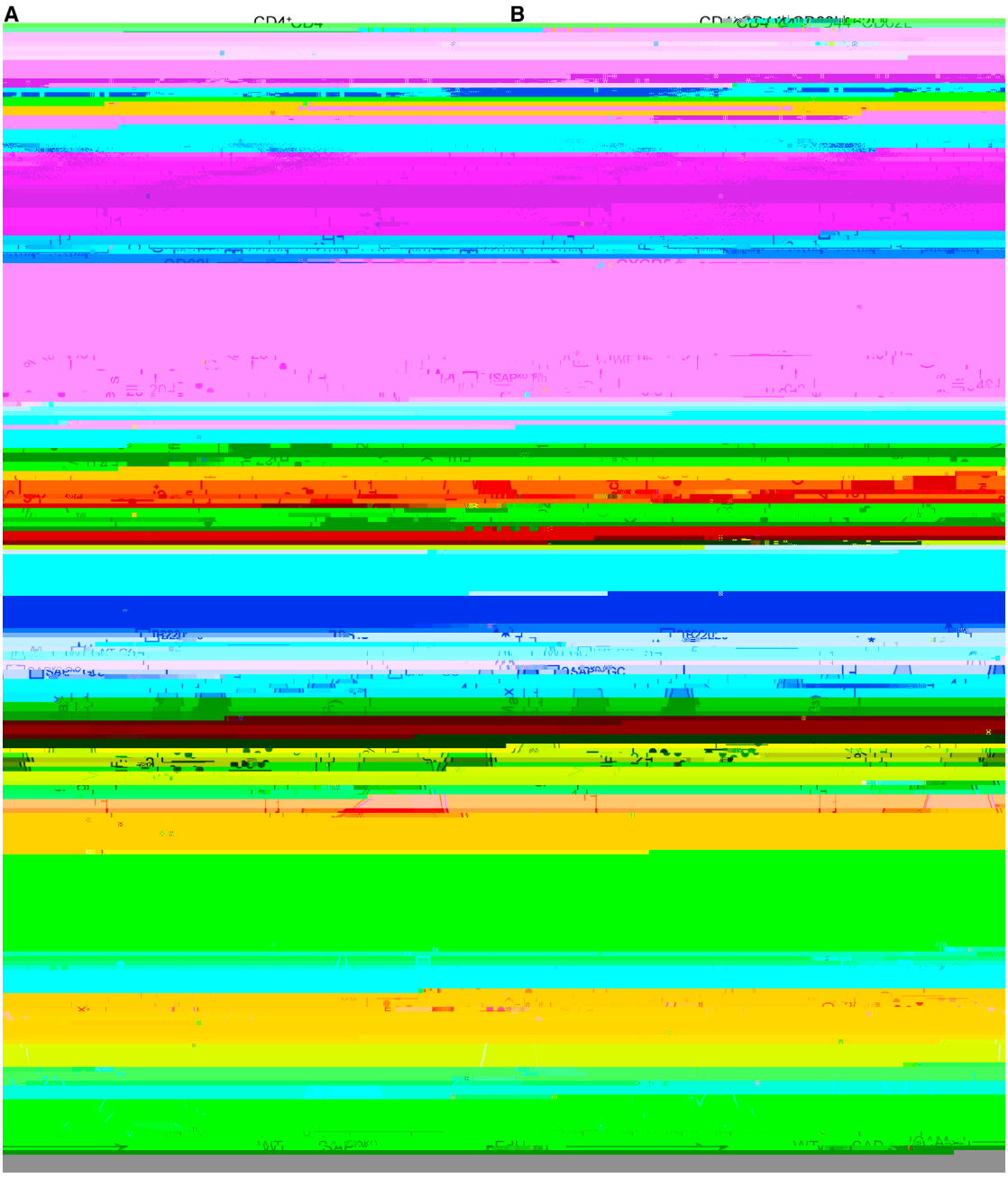


Figure 3. The Presence of TFH Cells in PP GC Is Maintained in SAP-Deficient Mice

(A) Representative flow cytometry plots showing activated Th cell ($CD44^{hi}$ $CD62L^{lo}$) frequencies in the PPs of WT and SAP^{KO} mice.

(B) Representative flow cytometry plots showing Tfh cells ($CXCR5^{+}$ $PD\text{-}1^{+}$) gated from the activated T cell population in the PPs of WT and SAP^{KO} mice.

(C and D) Graphs showing the frequencies of $CD4^{+}$ activated (C) and Tfh cells (D), as in (A) and (B), respectively.

(E) Representative histogram showing Bcl6 expression in WT and SAP^{KO} Tfh cells. Expression in naive T cells is shown as a negative control, and frequencies are summarized in the graph.

(F) Graph showing the ratio between GC B cells and Tfh cells in WT and SAP^{KO} PPs.

(G–I) Representative histogram and quantification of ICOSL (G), CD40 (H), and ICAM-1 (I) expression in PP GC B cells.

(J) Representative flow cytometry plots showing EdU uptake by WT and SAP^{KO} GC B cells, 2.5 h following EdU administration. Frequencies of EdU^{+} cells are summarized in the graph.

Data are pooled from two independent experiments, with three mice in each experiment. Each dot represents a single mouse; line represents the mean. *$p < 0.05$, ***$p < 0.0001$, two-tailed Student's t test. ns, not significant.

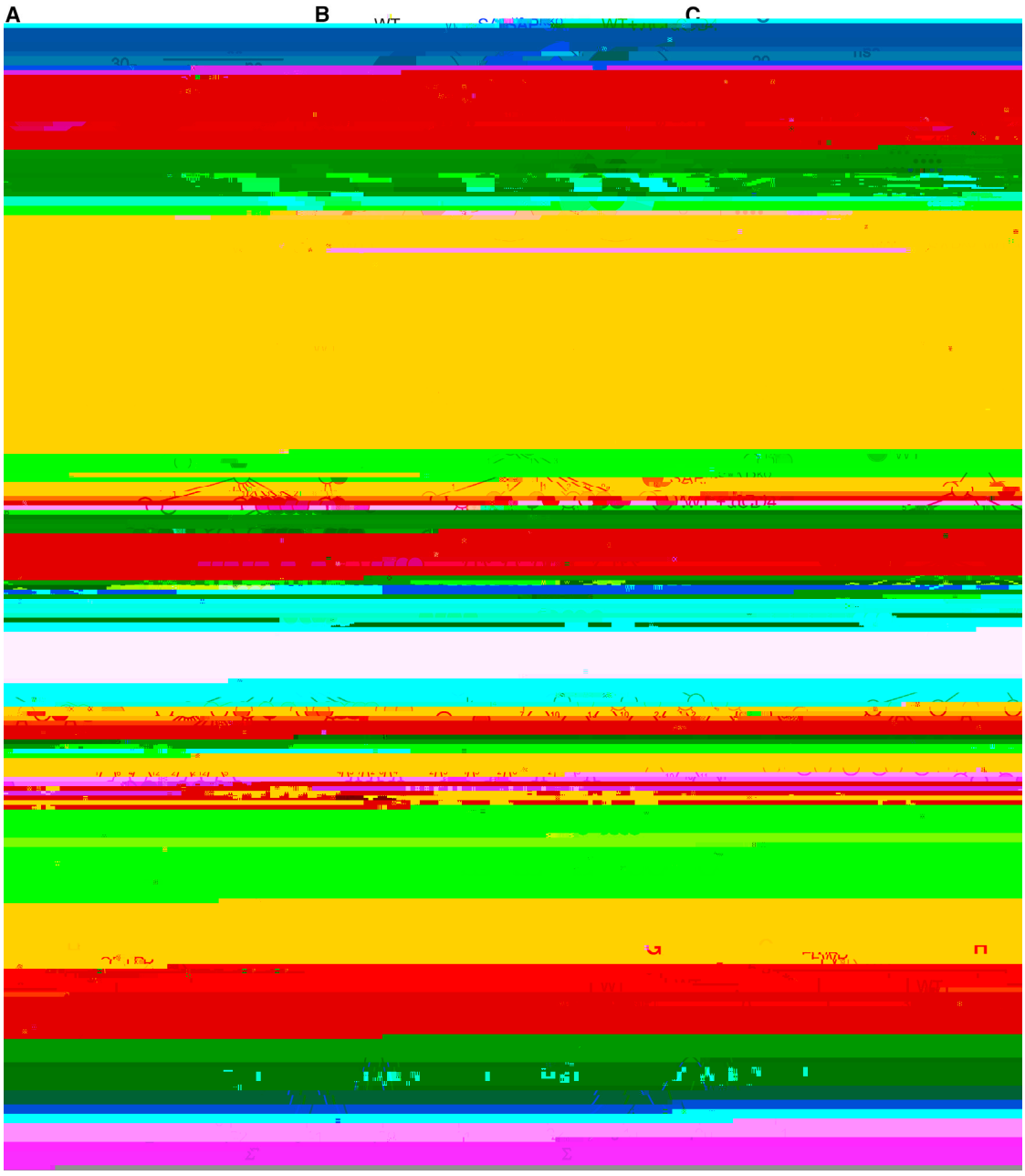


Figure 5. Clonal Diversity of Mouse PP GC

(A) The number of V-regions detected in IgA sequences of GC-derived ($GL7^+$ FAS^+ IgA^+) B cells recovered from a single PP derived from either WT, SAP^{KO}, or WT mice treated with αCD4-depleting antibody for 14 days (WT + αCD4).

whereas non-synonymous mutations in the DNA, which result in changes in the nucleic acid code, may affect Ig affinity (Yaari et al., 2012). Hence, affinity-based clonal selection is characterized by an increase in the ratio of replacement versus silent mutations. We found that B cells in SAP-deficient mice showed a lower ratio of synonymous to non-synonymous mutations, both in the CDR and FWR sequences, compared to WT mice (Figures 5G and 5H). Furthermore, mice that were treated with anti-CD4 antibody for 2 weeks also showed a reduction in selection strength, but to a lesser extent than that observed in SAP-deficient mice (Figures 5G and 5H). Similar differences were observed when clones bearing the same V genes were compared between WT and SAP-deficient mice (Figure S6). Since the BASELINe analysis compares each Ig sequence to its corresponding germline sequence, independent of clonal expansion, the difference in clonal divergence did not affect the results of this analysis. We conclude that selection forces within chronic GCs of the PP depend on SAP, whereas clonal diversification can take place in the absence of SAP-dependent T cell help.

Positive selection of B cells by T cells in GCs depends on the upregulation of Myc and its downstream genes (Calado et al., 2012; Dominguez-Sola et al., 2012). To understand why positive selection is perturbed in the absence of SAP in PPs, we sorted GC B cells from WT and SAP-deficient mice and examined their transcriptome by bulk RNA sequencing analysis. We found that GC B cells derived from SAP-deficient mice show altered gene expression profiles (Figure S5F). Gene

conclude that SAP controls PC formation in the gut at least partially through regulation of the GC size.

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		

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Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Ziv Shulman (ziv.shulman@weizmann.ac.il). This study did not generate new unique reagents.

Zeiss LSM 880 upright microscope fitted with Coherent Chameleon Vision laser was used for whole lymph node scan imaging experiments. Images were acquired with a femtosecond-pulsed two-photon laser tuned to 940 nm. The microscope was fitted with a

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Students' t tests or one-way ANOVA with bonferroni posttest were used for statistical analysis and indicated in figure legends. Unless otherwise indicated, the data in figures were displayed as the mean ± SEM and n represents number of mice analyzed. p values are