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A dual function for the chromatin organizer Special A-T rich Binding Protein 1 in B-lineage cells

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1 **A dual function for the chromatin organizer Special A-T rich**
2 **Binding Protein 1 in B-lineage cells**

3

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16 **Keywords:** B cells, nuclear factor, ambivalent, somatic hypermutation

17

18

19 **Abstract**

20 SATB1 (Special A-T rich Binding protein 1) is a cell type specific factor that regulates the genetic
21 network in developing T cells and neurons. In T cells, SATB1 is required for lineage commitment,
22 VDJ recombination, development and maturation. Considering that its expression varies during B
23 cell differentiation, the involvement of SATB1 needed to be clarified in this lineage. Using a KO
24 mouse model deleting SATB1 from the pro-B cell stage, we examined the consequences of SATB1
25 deletion in naive and activated B cell subsets. Our model indicates firstly, unlike its essential
26 function in T cells, that SATB1 is dispensable for B cell development and the establishment of a
27 broad IgH repertoire. Second, we show that SATB1 exhibits an ambivalent function in mature B
28 cells, acting sequentially as a positive and negative regulator of Ig gene transcription in naive and
29 activated cells, respectively. Third, our study indicates that the negative regulatory function of
30 SATB1 in B cells extends to the germinal center response in which this factor limits somatic
31 hypermutation of Ig genes.

32

33 Introduction

34 The transcription factor Special A-T rich binding protein 1 (SATB1) is a factor able to bind to Matrix
35 Attachment Regions (MAR) in the nucleus ¹. This MAR-binding protein (MAR-BP) is indispensable
36 for T lymphocyte development ²⁻⁴ through its implication in properly organizing nuclear architecture,
37 especially chromatin folding ⁵⁻⁷. Murine SATB1, sharing more than 98% identity with its human
38 homologue, can multimerize through an ubiquitin-like domain ⁸ and interacts with chromatin through
39 its CUT-like and homeodomains respectively involved in DNA binding affinity and specificity ^{9,10}.
40 One historic feature of SATB1 is its ability to tie nuclear matrix proteins ^{11,12}. Another essential
41 feature, as a transcription factor, is its capacity to bind DNA for gene regulation. Strikingly, such
42 interactions were found to occur in nucleosome-dense regions, preferentially at AT-rich sequences
43 in the nucleosomal core ¹³, a feature that strongly supports its candidacy as a pioneer factor. Given
44 its multivalent potency as a nuclear matrix scaffolding factor, a genome organizer involved in
45 chromatin looping and a transcription factor, the exact mechanism linking this MAR-BP to gene
46 regulation remains puzzling. The large body of literature related to SATB1 in T cells suggest that
47 this factor is capable of flexible functions; either binding to euchromatin ^{5,6} or nucleosome-dense
48 regions ¹³, depending on the cell type and development stage assessed. Long distance interactions
49 between promoters and enhancers can be promoted by SATB1 homotetramerization ⁸. A negative
50 regulatory function for SATB1 was attributed to its capacity to recruit chromatin modifiers such as
51 Histone deacetylase 1 (HDAC1) ¹⁴. Indeed, SATB1 is subject to posttranslational modification:
52 phosphorylation of this MAR-BP modifies interactions with chromatin corepressor and coactivator
53 complexes, leading to a switch in its transcriptional activity ^{15,16}; SATB1 acetylation disrupts its
54 interaction with C-terminal binding protein 1 (CtBP1) ¹⁷. Moreover, regulation of *Satb1* gene is by
55 itself tightly regulated in T cells by interchanging promoter usage, resulting in fine tuning of protein
56 expression ^{18,19}. These multiple levels of regulation suggest the need for meticulous control of
57 SATB1 expression.

58 SATB1 deletion models in the mouse led to major alterations in neuronal, hematopoietic and
59 immune systems ^{2,20} and pointed out broad and critical functions for this protein in mouse
60 development. SATB1 is a major player in early hematopoiesis since it promotes hematopoietic
61 stem cell self-renewal (HSC) ²¹. Based on its relative expression level in differentiating HSC ²²,
62 SATB1 favors the lymphoid lineage ²³. In erythrocyte and myeloid cells, this protein also modulates
63 epigenetic marks by association with CBP (CREB binding protein) to control β -globin and NADPH
64 oxydase gene expression ²⁴⁻²⁶. There is also growing evidence showing that SATB1 is involved in
65 cancer and auto-immune diseases ^{6,7,27,28} highlighting the necessity to decipher its regulation in
66 immune cells.

67 Due to thymus alteration in *Satb1* KO mice ², SATB1's functions were largely studied in T-cells.
68 Initially described as a repressor factor ^{12,29}, SATB1 proved to be also a transcriptional activator

69 ^{6,30}. As an example of ambivalence, SATB1 could repress *c-myc* in resting T-cells while stimulating
70 its expression in activated T cells ³. It is now established that this MAR BP is a major regulator of
71 thymocyte development throughout all differentiation stages from progenitors to regulatory subsets
72 ^{28,31,32}. One notable role of SATB1 in T cells is its activator function for *Rag* gene expression in
73 order to promote VDJ recombination and shape the T cell receptor repertoire ^{33,34}. In this context,
74 SATB1 has been shown to bridge *Rag1* and *Rag2* gene promoters and the distant antisilencer
75 element (ASE) of this locus³³.

76 By contrast to the vast knowledge on SATB1 fine tuning and regulatory functions in T lymphocytes,
77 little is known on the potency of SATB1 to regulate B cell development, beyond HSC fate decision
78 ^{22,23}. Initial studies reported a discrete defect on B cell numbers in *Satb1* KO mice ². Taking
79 advantage of its elegant fluorescent reporter model, Yokota group's described fluctuations in *Satb1*
80 expression throughout B cell development with higher expression in naive B cell subsets. In this
81 study authors proposed that SATB1 is involved in BCR-mediated B cell survival ³⁵. Many aspects
82 of the literature point to a potential function for SATB1 during B cell development. Given its
83 regulatory function for *Rag* gene expression in T cells ³³, the potential implication of SATB1 in V(D)J
84 recombination of Ig genes in B cells needs to be examined. Moreover, since SATB1 was discovered
85 by its ability to bind the *MAR_{E μ}* region of the *IgH* locus ¹, we could postulate a role for SATB1 in Ig
86 heavy chain expression.

87 In this study, we examined SATB1 implication in B-lineage cell development and depicted its critical
88 function on immunoglobulin production using a conditional KO mouse model allowing deletion of
89 this factor in B-lineage cells. Although we found SATB1 nonessential for B cell development, we
90 showed that this factor displays an ambivalent function in late developing B cell subsets: acting
91 sequentially as a positive and then negative regulator of Ig gene transcription. The negative
92 regulatory function of SATB1 extends to the germinal center reaction in which this factor limits Ig
93 gene somatic hypermutation.

94

95 **Materials and Methods**

96
97 **Mice:** *Satb1*^{tm1a/wt} mice come from the Mouse Clinical Institute (MCI) (IR00004167/ P4167). First
98 crossings with 129S4/SvJae-*Gt(ROSA)26Sor*^{tm2(FLP*)sor}/J mice (The Jackson Laboratory) removed
99 reporter and marker cassette placed between *Frt* sites. Second crossings with B6.C(CG)-
100 *Cd79a*^{tm1(cre)Reth}/EhobJ mice (The Jackson Laboratory) deleted *Satb1-exon4* due to CRE
101 recombinase expression in B-lineage cells ³⁶. *Aicda*^{-/-} homozygous mice (kindly provided by Pr. T.
102 Honjo) were used to prepare control samples devoid of SHM, as reported in ³⁷. All experiments,
103 excepted 3C-HTGTS, were performed on two-month-old mice. Primers used for genotyping are
104 listed in *Supplementary Table S1*. All animal strains were bred and maintained in SPF conditions
105 at 21–23°C with a 12-h light/dark cycle. Procedures were reviewed and approved by the Ministère
106 de l'Enseignement Supérieur, de la Recherche et de l'Innovation APAFIS#16639-
107 2018090612249522v2.

108 **Western Blot:** B cells from spleen were sorted with EASYSEP MOUSE B cell isolation kit (Stem
109 Cell Technologies) and lysed using RIPA buffer (Santa Cruz) completed with protease inhibitor
110 (Orthovanadate, PMSF, anti-protease cocktail). Proteins were quantified with Pierce BCA Protein
111 Assay kit (Thermo Scientific) and denatured 5 minutes at 95°C. TGX Stain-free FastCast 12%
112 acrylamide gels (Bio-Rad Laboratories) were used to separate proteins that were transferred on
113 Transblot Turbo polyvinylidene fluoride membranes (Bio-Rad laboratories) with Transblot Turbo
114 Transfer System. After blocking incubation with Phosphate Buffer Saline (PBS)- 5% skim milk,
115 SATB1 primary antibody was incubated overnight at 4°C. Membrane was incubated with secondary
116 antibody with PBS-3% milk. Proteins were detected and quantified with PIERCE ECL Western
117 Blotting Substrate (Thermo Scientific) and ChemiDoc Touch Imaging System coupled to Image Lab
118 J6.0 software (Bio-Rad Laboratories). Antibodies and concentrations used are in *Supplementary*
119 *Table S2*. Unprocessed raw data are provided in *Supplementary Fig. S1A*)

120 **Enzyme-linked ImmunoSorbent Assay (ELISA):** Sera were collected from the blood of two-
121 month-old mice. Supernatants were obtained after sorting B cells from spleens and *in vitro* LPS-
122 activation during 4 days. Plates were coated overnight with 1µg/ml of primary antibody Goat Anti-
123 Mouse Unlabeled IgM, IgG3, IgG1 or IgA (Southern Biotech) diluted in sodium carbonate buffer.
124 Plates were blocked with PBS-3% bovine serum albumin 1 hour at 37°C. Supernatant or diluted
125 sera were incubated 2 hours at 37°C then secondary antibody conjugated with alkaline
126 phosphatase (Southern Biotech) was incubated 1 hour at 37°C. Enzymatic reaction was performed
127 by adding SigmaFast™ p-nitrophenyl phosphate tablet. DO were measured at 405 nm on Thermo
128 scientific MULTISKAN FC. Antibodies and concentrations used are in *Supplementary Table S2*.

129 **Flow Cytometry:** To analyze B cell populations, spleen and bone marrow were collected, crushed
130 and filtered through Clearline Streater 40 μ m (Fisherbrand); lymphocyte numbers were quantified
131 in a Cell-Dyn Emerald counter (Abbott). Cell suspensions were labeled in FACS Buffer (PBS buffer
132 (Eurobio), 10% BSA (Stem Cell Technologies) 2mM EDTA) containing antibodies for 20 minutes
133 at 4°C and analyzed on BD LSRFortessa SORP flow cytometer (BD Bioscience). For intracellular
134 staining, cells were first stained for surface markers. After washing, cells were treated with the
135 fixation/permeabilization kit Intraprep (Beckman Coulter, A07803), following the manufacturer's
136 instructions. Mean Fluorescence Intensity values were normalized to WT values; antibodies used
137 are provided in *Supplementary Table S2*. Gating strategies to evaluate proportions and numbers
138 of B cell subsets are provided in *Supplementary Fig. S1*.

139 **RNA extraction:** Pre-B cells, splenic resting B cells, *in vitro*-activated cells with LPS for 2 or 4
140 days, plasma cells and GC B cells were lysed with TRIZOL Reagent (Ambion Life Technologies)
141 and RNA extraction was done following recommendations of DirectZol RNA microprep kit
142 (ZymoResearch).

143 **RT-qPCR:** 1 μ g RNA from B cell subset samples was treated with DNase I Amplification Grade
144 (Invitrogen) and reverse transcription was done following the High Capacity cDNA Reverse
145 Transcription kit protocol (Applied Biosystem). Quantitative PCR was performed on 20ng cDNA
146 using either SensiFAST Probe Hi-ROX or SensiFAST SYBR Hi-ROX kits (BioLine) on a Quant
147 Studio III (Applied BioSystem). *IgH* and *Igk* primary transcripts were quantified as previously
148 described³⁸. *Satb1* transcripts were measured by TaqMan assays with Mm.PT.58.13287891 (IDT),
149 *Rag 1* and *Rag 2* transcripts with Mm01270936_m1 and Mm00501300_m1 (ThermoFisher),
150 respectively. Transcript quantification was carried out with normalization to *Hprt*
151 (Mm.PT.58.32092191 - IDT) for resting and *in vitro*-stimulated cells, and to *B220*
152 (Mm.PT.58.32086646 - IDT) for GC B cells. Specific primers designed to amplify membrane and
153 secreted *Ig μ* transcripts, as well as *S μ* transcripts are listed in *Supplementary Table S1*.

154 **Repertoire:** Pre-B cells from bone marrow were enriched using mouse CD25 MicroBead Kit
155 (Miltenyi Biotec). Library preparation was adapted from methods previously described^{39,40}.
156 Transcripts were amplified by 5'RACE PCR using reverse primer hybridizing within μ gene and Cap
157 race primer carrying Unique Molecular Identifiers (UMIs). ProtoScript® II (New England Biolabs,
158 Ipswich, MA) was used for reverse transcription and amplicons were obtained using Phusion® High
159 Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) according to the manufacturer's
160 instructions. (Primers are listed in *Supplementary Table S1*). Illumina adapter and tag sequences
161 were added by primer extension⁴². Resulting libraries were sequenced on Illumina MiSeq
162 sequencing system using MiSeq Reagent kit V3 600 cycles. Paired reads were merged as

163 previously described⁴² and UMIs were treated with MIGEC software. Repertoire analysis was done
164 using IMGT/HIGHV-QUEST online tool (<http://imgt.org/>)⁴³.

165 ***In vitro Stimulation:*** Splenic B cells isolated with EASYSEP MOUSE B cell isolation kit (Stem Cell
166 Technologies) were cultured at 1×10^6 cells/ mL in RPMI 1640 medium (Eurobio) supplemented with
167 10% fetal bovine serum (Dutscher), 2mM Glutamine (Eurobio), 1% Eagle's Non Essential Amino
168 Acids (Eurobio), 50U/ml of penicillin-streptomycin (Gibco), 1mM sodium pyruvate (Eurobio), 129
169 μ M 2- β mercaptoethanol (Sigma-Aldrich) in the presence of 1 μ g/ml Lipopolysaccharide (LPS-
170 Invivogen). An aliquot of cells was collected for analysis and sample preparation at day 2 or 4 after
171 stimulation. Same isolation protocol and cell culture reagents were used for Anti-CD40 + IL4 *in vitro*
172 stimulation by replacing LPS with 5 μ g/ml of anti-CD40 antibody (R&D Systems, listed in
173 *Supplementary Table S2*).and 20ng/mL recombinant mouse IL4 (Peprotech). An aliquot of cells
174 was collected for analysis and sample preparation at day 3 and 4 after stimulation.

175 ***In vitro Ethynil-DeoxyUridine (EdU) Incorporation:*** Splenic B cells were cultured as described
176 above and EdU was added 48 hours later and incorporated for 24 hours. Cells were then processed
177 by following recommendations of Click-it EdU Alexa Fluor 488 Flow Cytometry Assay Kit (Thermo
178 Fisher Scientific). EdU incorporation was evaluated by flow cytometry on BD LSRFortessa SORP
179 flow cytometer (BD Bioscience)

180 ***NP-CGG immunization:*** Nitrophenylacetyl-Chicken Gamma Globulin (NP-CGG) immunization
181 was realized by injecting 100 μ g NP-CGG (N-5055C-5 Biosearch Technologies) precipitated with
182 complete for the first intraperitoneal injection (day 0) and incomplete Freund's adjuvant for the
183 second one at day 12. Pre-immune sera were collected before day 0 and immunized sera were
184 collected at days 8 and 17. Mice were sacrificed at day 17 and splenic GC B cells were sorted to
185 extract DNA for SHM quantification by HTS.

186 ***Cell sorting:*** Plasma cells from spleen or plasmablasts from *in vitro* stimulation were sorted using
187 B220 and CD138 surface markers on BD FACS ARIA III (BD Bioscience). GC B cells were sorted
188 using B220 and GL7 cell surface markers (*Supplementary Table S2*).

189 ***Somatic Hypermutation Analysis:*** SHM analysis was performed on B220⁺/GL7⁺ GG B cells
190 sorted from Peyer's patches or immunized spleens from *wt*, *Satb1* cKO and *Aicda* KO mice. 5'*S μ* ,
191 3'*J_H4* and 3'*J_k5* intronic regions were amplified from 10,000 cells with specific primers (listed in
192 *Supplementary Table S1*) using Phusion High-Fidelity DNA Polymerase (New England Biolabs)
193 and according to the following program: denaturation (98°C 10s), hybridization (69°C 30s) and
194 amplification (72°C 1mn) during 38 cycles. Libraries were constructed with Ion Xpress Plus gDNA

195 Fragment Library kit (Cat. no. 4471269, Life Technologies) and sequenced on the Ion-Proton
196 System S5. SHM frequencies were determined using Raw Data analyzed with DeMinEr tool ³⁷

197 **RNA-Sequencing:** RNA-Seq analysis was performed either on splenic resting B cells sorted with
198 Easysep Mouse B cell isolation kit (Stem Cell Technologies), *in vitro*-activated B cells with LPS
199 (bulk culture, at day 2) and *in vitro* LPS-differentiated plasmablasts (sorted at day 4). Sample quality
200 controls and library preparation were performed at the GeT-Santé facility (Inserm, Toulouse,
201 France, get.genotoul.fr). i/ Quality control: RNA concentration and purity were determined using a
202 ND-2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). RNA integrity was
203 checked with a Fragment Analyzer (Agilent Technologies, Santa Clara, USA), using the RNA
204 Standard Sensitivity Kit. 260/280 purity ratios were all ≥ 1.8 , and integrity indices were of good
205 quality (8.3-10 RIN and > 1.7 28S/18S ratios). ii/ Library preparation (GeT-Santé): RNA-seq paired-
206 end libraries were prepared according to Illumina's protocol with some adjustments, using the
207 TruSeq Stranded Total RNA Gold library prep Kit (Illumina, San Diego, USA). Briefly, between 934-
208 1000 ng total RNA were first ribo-zero depleted using Illumina Ribo-Zero probes. Then, remaining
209 RNA were fragmented during 2 min and retrotranscribed to generate double stranded cDNA.
210 Compatible adaptors were ligated, allowing the barcoding of the samples with unique dual indices.
211 Libraries were amplified by 12 PCR cycles of PCR and an additional final purification step resulted
212 in 280-700 bp fragments. Libraries quality was assessed using the HS NGS kit on the Fragment
213 Analyzer (Agilent Technologies, Santa Clara, USA). iii/ Library quantification (GeT-PlaGe):
214 quantification and sequencing were performed at the GeT-PlaGe core facility (INRAE, Toulouse,
215 France). Libraries were quantified by qPCR using the KAPA Library Quantification Kit (Roche,
216 Basel, Switzerland) to obtain an accurate quantification. iiiii/ Sequencing (GeT-PlaGe): libraries
217 were equimolarly pooled and RNA sequencing was then performed on one S4 lane of the Illumina
218 NovaSeq 6000 instrument (Illumina, San Diego, USA), using the NovaSeq 6000 S4 v1.5 Reagent
219 Kit (300 cycles), and a paired-end 2 x 150 pb strategy.

220 **RNA-Sequencing Analysis:** Paired-end reads were mapped on GRCm38 mouse genome that
221 was previously indexed with "Mus_musculus.GRCm38.dna.primary_assembly.fa" and
222 "Mus_musculus.GRCm38.102.chr_patch_hapl_scaff.gtf" files from ENSEMBL release 102. Index
223 and mapping steps were both made with STAR v2.6.0c ⁴⁴. Then featureCounts v2.0.1 ⁴⁵ was used
224 to count reads by gene. An R script named template_script_DESeq2_CL.r of SARTools ⁴⁶ was run
225 first with all count data in order to retrieve a PCA and check if biological variability was the main
226 source of variance in the data. Then, the same script was run for each desired differential analysis
227 with count data from defined reference and interest conditions. Differentially regulated genes with
228 an adjusted p value of 0.05 and a foldchange ≤ -1.5 or ≥ 1.5 were selected for downstream analysis.
229 Gene SYMBOLs were converted to ENTREZIDs with the bitr function of the R ClusterProfiler

230 package ⁴⁷. The resulting ENTREZIDs and their associated log2foldchange were then used to
231 calculate enriched biological pathway profiles of different gene clusters (Down in cKO , Up in cKO
232 , Down in WT and Up in WT) using the CompareCluster function of ClusterProfiler ⁴⁷ with p value
233 and q value thresholds set to 0.01 and 0.05 respectively. The resulting enriched functional profiles
234 were filtered through a Gene Ontology list consisting of the hierarchical children of the following
235 Biological pathway terms: B cell costimulation, B cell selection, humoral immune response,
236 immunoglobulin production, memory B cell differentiation, regulation of the apoptotic process of
237 mature B cells, SHM (*Supplementary Table S3*). Terms that were enriched in all down-regulated
238 gene clusters or all up-regulated clusters were discarded.

239 **3C-HTGTS:** 3C-HTGTS was performed as previously described⁴⁸. Briefly, 10 million of resting and
240 LPS-stimulated B cells (at day 3) were crosslinked with 2% formaldehyde 10% FCS PBS for 10min
241 at RT under rotation. Crosslinking is stopped by adding glycine at 0.1M. Then, cells were lysed in
242 50mM Tris, 150mM NaCl, 5mM EDTA, 0.5%NP-40,1% TX-100 supplemented with protease
243 inhibitor (ROCHE #11873580001). Nuclei were resuspended in 0,3% SDS for 1h at 37°C at 900rpm
244 and then neutralized with Triton TX-100 for 1h. DNA restriction was performed using CviQ1
245 (Thermo Fisher ER0211) in B buffer (Thermo Fisher #BB5) overnight at 37 °C, before heat
246 inactivation for 25 min at 65 °C. Overnight ligation was performed at 16°C at 300rpm. Next, DNA
247 was treated by proteinase K and RNase and cleaned by phenol/chloroform. After 3C step, the LAM-
248 HTGTS protocol was performed ⁴⁹. Briefly, 3C DNA was sonicated using the Bioruptor (Diagenode;
249 two pulses at low intensity for 20s), and 10 µg was used for the LAM-HTGTS step. A S_μ bait was
250 used for primer elongation. These single-stranded DNA fragments were incubated with streptavidin
251 beads (Dynabeads C1 streptavidin beads; Invitrogen) overnight at RT and washed with BW buffer
252 (1M NaCl, 5mM Tris-HCl pH7.4, 0,5mM EDTA pH 8.0). Universal I7 adaptors were ligated before
253 the nested PCR performed with S_μ nested and universal I7 reverse primers (all primers are listed
254 in *Supplementary Table S1*). After the Tagged PCR with I7 and I5 Illumina primer, PCR products
255 were cleaned using PCR clean-up kit (Macherey-Nagel REF#740609) and validated after migration
256 on BioAnalyser (Agilent). 3C-HTGTS libraries were sequenced 300pb paired-end MiSeq V3 with
257 20% PhiX.

258 **Analysis of 3C-HTGTS:** Sequencing reads were aligned to the mm10 genome and processed as
259 previously described⁴⁹. Each 3C-HTGTS library plotted for comparison was normalized by
260 randomly selecting a number of junctions equal to the total number of junctions present in the
261 smallest library in the comparison set.

262 **Data availability:** Raw data from RNA-seq, SHM, Rep-Seq and 3C-HTGTS have been deposited
263 in the European Nucleotide Archive database under access number PRJEB52320.

264 **Results**

265

266 ***Conditional deletion of Satb1 in murine B lineage cells***

267 Since depletion of *Satb1* in ES cells and mouse embryos showed multiple dysfunctions and did not
268 allow animals to survive beyond 1 month of age ², we evaluated SATB1 contribution to B cell
269 development in a conditional knock out model inducing SATB1 depletion in B-lineage cells from
270 the pro-B cell stage. In our model, initially derived from *Satb1^{tm1a}* allele, *Satb1* conditional allele
271 (*Satb1^{flx}*) contains exon 4 flanked with two *LoxP* sites that permit its specific deletion when coupled
272 to one allele carrying a CRE recombinase insertion into *Cd79a* gene (*Cd79a^{cre}*) ³⁶ (Fig. 1A). Mice
273 carrying the homozygous SATB1 deletion in B-lineage cells (*Satb1^{flx/flx} Cd79a^{cre/+}*, referred as cKO
274 conditional Knock Out) were compared to heterozygous littermates (*Satb1^{flx/+} Cd79a^{cre/+}*, referred
275 as cHet: conditional Heterozygous) and to *wt* littermates carrying an identical conditional allele but
276 devoid of CRE expression (*Satb1^{flx/+} Cd79a^{+/+}*, referred as WT). Western blot experiments
277 performed on sorted splenic B cells from these three genotypes confirmed that *Satb1*-exon4
278 deletion induced a complete protein depletion (Fig. 1B, unprocessed raw data are provided in
279 *Supplementary Fig. S1*).

280

281 ***SATB1 depletion in B lineage cells allows normal B cell development and IgH repertoire***

282 By questioning ImmGen database ⁵⁰, we examined *Satb1* expression in developing B-lineage cells
283 and noted that all subtypes of B-lineage cells expressed *Satb1* transcripts (*Supplementary Fig.*
284 *S2A*). Common lymphoid progenitor and pre-pro B cells, also mentioned as Hardy fraction A, both
285 displayed higher SATB1 expression. This was not surprising given that SATB1 has been reported
286 to favor lymphocyte lineage differentiation from hematopoietic stem cells ²³. While a consistent drop
287 in *Satb1* transcription was observed at pro- and pre-B stages, a second wave of *Satb1* gene
288 expression occurred in transitional and mature resting B cells present in splenic follicles or the
289 marginal zone as well as in splenic memory B cells. *Satb1* gene expression further decreased in
290 antigen-activated cells such as germinal center centrocytes to reach its minimal level in proliferating
291 cell subsets such as centroblasts and plasmablasts (*Supplementary Fig. S2A*). In B cells from WT
292 mice, we experimentally confirmed a significant decrease in *Satb1* transcripts by RT-qPCR assays
293 when comparing naive and GC B cells sorted from Peyer's patches (Fig. 1C). The same findings,
294 corroborating expression profiles described in the ImmGen database, were very recently
295 documented in a mouse model carrying a Tomato-reporter transgene knocked into a *Satb1* allele
296 ³⁵. This converging evidence for variable expression of *Satb1*, according to B cell subsets,
297 supposes a narrow regulation and suggests an accurate function of this factor in the B cell lineage.

298 To determine SATB1 contribution throughout B cell development and maturation, we analyzed B
299 cell populations from WT, cHet and cKO mice by flow cytometry (*Supplementary Fig. S1*). Absolute
300 numbers of B-lineage cell subsets from bone marrow (Fig. 1D left and middle) and spleen (Fig. 1D
301 right) were not affected by *Satb1* gene inactivation suggesting, despite its contribution to
302 lymphocyte lineage initiation²², that SATB1 was not required for B cell development. Our findings
303 that early B cell development was not impaired in our cKO mice excluded any function for SATB1
304 in B-lineage choice maintenance. This data completed studies from Kanakura and Steidl groups
305^{21,22} that previously hypothesized that SATB1 function was restricted to stem cell renewal and fate
306 hematopoietic lineages.

307 Since SATB1-binding to the ASE region is known to modulate *Rag* gene expression in T cells and
308 incidentally impact TCR rearrangements³³, the consistent expression of this factor in early
309 committed and developing B cells could confer a similar function on Ig gene rearrangements. We
310 investigated this point by first analyzing *Rag* gene expression by RT-qPCR and found that sorted
311 bone marrow pre-B cells from WT and cKO animals expressed similar transcript levels
312 (*Supplementary Fig. S2B*). The striking different impact of SATB1 deletion on *Rag* expression in B
313 and T cells could be indirectly explored by comparing DNA accessibility in the region close to *Rag*
314 genes regions from WT B- and T-lineage cell precursors. Such a detailed study was provided by
315 the consortium of the Immunological Genome Project that performed ATAC-seq in B and T cell
316 precursors⁵¹. This study indicates that, while the DNA region encompassing the SATB1-binding
317 ASE regulatory element was accessible in T-lineage precursor cells, this was not the case in bone
318 marrow cell subsets of the B cell lineage (a comparison focused on *Rag* gene loci was provided in
319 *Supplementary Fig. S2C*). To note, B-lineage precursors display other accessible regulatory
320 regions such as the *Erag* element located closely to *Rag* genes regions. It is then reasonable to
321 suppose that ASE region is not active in the B cell lineage, this could potentially explain normal
322 *Rag* gene transcription in SATB1-deficient B cell precursors. Given that SATB1 acts as a chromatin
323 loop organizer in T cells^{5,34,52-54}, we suspected a potential effect of its deletion on *IgH V* region
324 accessibility in developing B cells. To assess this point we examined VDJ recombination diversity
325 by Repertoire-Sequencing experiments on RNA samples (RepSeq) extracted from pre-B cell-
326 enriched bone marrow fractions. Our data displayed an equivalent broad distribution of each
327 rearranged and expressed V gene (Fig. 1E and *Supplementary Fig. S2D*) in WT and cKO mice. A
328 similar representation of V_H family usage indicated that SATB1 deletion did not hamper
329 mechanisms leading to a diversified *IgH VDJ* repertoire in developing B cells. VDJ junction analysis
330 in pre-immune repertoire of WT and *Satb1* cKO models revealed comparable lengths of CDR3
331 regions as well as rather comparable distribution of P and N nucleotides (*Supplementary Fig. S2E*)
332 suggesting that *IgH V* region assembly and end-joining occurs normally in the absence of STAB1.
333 Altogether, our results indicate that SATB1 is dispensable to establish *IgH* repertoire and does not

334 affect *Rag* expression or influence *IgH* locus accessibility. Given that SATB1 deletion did not impair
335 VDJ rearrangements and B cell development, further studies could then be performed on peripheral
336 B cell subsets in this model without any bias. In line with this statement, we determined whether
337 SATB1 deletion could impact Ig isotype production and secretion in the mouse. Sera from WT,
338 cHet and cKO animals were collected at two months of age and IgM, IgG1, IgG3 and IgA levels
339 were quantified by ELISA assays. Homozygous and heterozygous KO mice displayed serum Ig
340 levels comparable to WT for each isotype (Fig. 1F), suggesting that SATB1 does not influence
341 global antibody production.

342

343 ***SATB1* depletion decreases *IgH* transcription in resting B cells**

344 Despite normal bone-marrow B cell development and Ig secretion in cKO mice, we however
345 suspected that SATB1 depletion might influence *IgH* locus expression, given its capacity to bind
346 MAR sequences *in vitro*⁵⁵ and to modulate MAR-containing reporter genes²⁹. We first examined
347 surface IgM expression levels, as a component of B cell receptors, on immature and mature B cell
348 subsets from bone marrow and spleen compartments in our model. By measuring IgM mean
349 fluorescence intensity (MFI) by flow cytometry, *Satb1* cKO mice displayed consistent and significant
350 decreased IgM surface expression when compared to WT, on bone marrow immature and
351 recirculating B cells (Fig. 2A) and on splenic transitional, marginal zone and follicular B cell
352 populations (Fig. 2B). The same significant decrease in IgM BCR expression, also observed on
353 naive B cells sorted from Peyer's patches (Fig. 2C), confirmed a specific function for SATB1 as a
354 positive regulator of BCR expression in resting B cells. This role for SATB1 in BCR surface
355 expression could explain its function as a contributing factor to B cell survival as described recently
356 by Ozawa et al³⁵. Indeed, the knock-in reporter mouse model used in their study leads to
357 inactivation of one *Satb1* allele and it is likely that such a deletion induces intermediate surface IgM
358 expression level similar to the one we observed in our heterozygous mice (*Supplementary* Fig. S3
359 A). It is reasonable to suppose that reduced BCR expression on resting B cells reduces ability of
360 these cells to respond to anti-IgM mediated *in vitro*-stimulation. One fair interpretation of the survival
361 defect described by Ozawa et al³⁵ could be simply the consequence of reduced BCR expression
362 in the knock-in model.

363 The decreased BCR expression observed in SATB1-deficient B lymphocytes raised the question
364 of a potential Ig gene transcription defect in our model. Quantification of *IgH* primary transcription
365 by RT-qPCR in resting splenic B cells showed a significant 2 fold-decrease of transcripts running
366 through the *J_H4* intron in cKO mice when compared to WT (Fig. 2D). We also evaluated the
367 respective proportion of membrane and secreted *Igμ* transcripts (*Supplementary* Fig. S3B), our
368 data showed that SATB1 deletion does not impair alternative splicing of the *Igμ* chain transcript.
369 Although not statistically significant, a similar downward trend was also observed for *Igκ*

370 transcription in mutants (Fig. 2D). It is established that MAR sequences flanking both sides of the
371 *IgH cEμ* intronic enhancer are able to bind SATB1⁵⁶. Whereas binding of equivalent regions in the
372 *Igκ* locus has never been shown, it has to be noted that only one upstream MAR is associated with
373 the *iEκ* enhancer. The presence of either one or two MAR sequences surrounding these intronic
374 enhancers could potentially explain differences observed for *IgH* and *Igκ* transcription in our *Satb1*
375 KO model. It is tempting to speculate that, through its DNA binding in proximity to intronic
376 enhancers, SATB1 could physiologically potentiate their transcriptional effect in resting B cells.
377 Although, this hypothesis is unlikely since IgM BCR expression was never compromised in resting
378 B cell populations of mice devoid of either *Eμ*, *iEκ* full regions or their associated-MARs⁵⁷⁻⁶⁰. In
379 contrast, the literature reports that BCR expression on resting B cells was decreased upon deletion
380 of components of the 3'regulatory regions of both *IgH*⁶¹ and *Igκ*⁶² loci, arguing for the importance
381 of close contact between 3'enhancers and respective promoters of rearranged V exons. In resting
382 B cells, such loops have been reported within the *IgH* locus in many studies⁶³. In line with a
383 proposed function for SATB1 as a promoter-enhancer loop regulator⁵⁴, a rationale hypothesis could
384 be that this MAR binding protein participates in physical interactions between rearranged *V_H* exons
385 and the 3'*RR* and consequently enhances Ig chain transcription in resting B cells. To evaluate such
386 chromatin loop interactions, we performed 3C-HTGTS experiments using a bait within the *Sμ*
387 region⁴⁸ of resting B cells from *wt* and mutant mice. Our data showed that loop interactions between
388 *Eμ* and *V_H* regions still occur at frequencies that do not significantly differ in resting B cells from
389 both genotypes (*Supplementary Fig. S4A*); this suggests that SATB1 is not required to form
390 chromatin loops in naive and LPS-activated B cells.

391 In line with the broad chromatin organization function proposed for SATB1 in T cells, we then
392 performed total RNA seq analysis of both WT and SATB1-deficient B cell subsets. Resulting
393 datasets were submitted to principal component analysis to validate reproducibility between
394 samples (*Supplementary Fig. S5A*). When comparing datasets obtained from resting splenic B
395 cells, our analysis only disclosed 65 genes displaying significant changes in expression: 53 were
396 overexpressed and only 12 were downregulated ($\log_2 FC > \pm 1.5$; Fig. 2E and *Supplementary Table*
397 *S4*). Indeed, our data supports the hypothesis of an ambivalent function for SATB1, displaying both
398 positive and negative regulatory actions for gene expression in resting B cells. Our study indicates
399 that SATB1 depletion does not induce drastic transcriptional changes in resting B cells. In contrast
400 to the T lineage^{7,27,64}, our data suggest that the intrinsic function of SATB1 in B cells may be
401 relatively focused. In this cell type, SATB1 would have a distinctly different role from that of a major
402 genome organizer.

403

404 ***SATB1 depletion increases IgH transcription and Ig synthesis in activated B cells***

405 We sought to evaluate effect of SATB1 depletion on B cell activation in response to mitogenic and
406 antigenic stimuli. We first performed *in vitro* stimulation of splenic B cells from both genotypes with
407 LPS and carried out RNA seq in a time-course manner on resting B cells (sorted at day 0), activated
408 B cells (bulk culture at day 2) and plasmablasts (sorted at day 4). Once sample reproducibility
409 validated (*Supplementary Fig. S5A*), we compared transcriptional programs induced by transition
410 from “resting to activated” and “activated to plasmablast” cell stages. Venn diagrams mainly
411 indicate that both B cell activation and differentiation programs induced by LPS were not drastically
412 impaired upon SATB1 depletion. Indeed, following *in vitro* culture, a vast majority of transcripts
413 displayed a common regulation profile in WT and SATB1-deficient models: *i.e.* 5460 transcripts
414 during the “resting to activated” transition and 2758 during the “activated to plasmablast” transition
415 (*Supplementary Fig. S5B*). Nevertheless, we submitted differentially expressed genes from both
416 transition programs, to a gene ontology analysis for biological processes relevant to B cell activation
417 (see list in *Supplementary Table S3*). During the “resting to activated” transition, SATB1 depletion
418 decreased expression of a few genes involved in humoral immune responses mediated by
419 circulating immunoglobulins (Fig. 3A, red dots) including *Lta*, a gene recently described as a SATB1
420 target in T cells⁵⁴, as well as *Tgfb1* encoding TGFβ, the cytokine involved in regulation of isotype
421 switching to IgA. Strikingly, the analysis specified that SATB1 depletion increased *Tcf3* expression
422 in activated B cells (Fig. 3A, blue dot), while its gene expression, encoding the E2A transcription
423 factor, was unchanged in hematopoietic stem cells devoid of SATB1⁶⁵. During the “activated to
424 plasmablast” transition, SATB1 depletion downregulates *Cd40* and few genes involved in Ig class
425 switching pathway (Fig. 3B, red dots); this effect on *Cd40* targeting was expected since already
426 observed upon SATB1 deletion in hematopoietic stem cells and T cells^{54,65}.

427 In parallel, in such *in vitro* stimulation assays, B cells were tested for intrinsic abilities to proliferate,
428 to undergo LPS-driven class switch recombination and to differentiate into plasmablasts. When
429 measured by EDU incorporation, the ability of SATB1-deficient B cells to proliferate was similar to
430 that of WT cells (*Supplementary Fig. S6A*). This suggested that, while decreasing IgM BCR
431 expression on naive cells, SATB1-deficiency is dispensable for cell cycle entry of splenic B cells
432 upon activation of TLR pathways. The same normal proliferation capacity in response to TLR or
433 CD40 triggering was also recently reported for B cells devoid of the entire *Satb1* gene region³⁵. In
434 our model, normal *in vitro* proliferation observed in B cells devoid of SATB1 was consistent to the
435 normal proportion of spontaneous GC B cells in Peyer’s patches of KO animals (Fig. 3C), or the
436 equivalent proportions of splenic GC centroblasts and centrocytes obtained after NP-CGG
437 immunization (*Supplementary Fig. S6B*). More strikingly, in clear contrast with the decreased *IgH*
438 expression in resting B cells, such *in vivo*-activated cells express higher BCR levels as evidenced
439 by IgM surface labelling (Fig. 3D). This highlights that the ambivalent function for SATB1 clearly
440 applies to Ig gene expression in developing mature B cells.

441 Class switching to IgG3 was examined by surface labelling of *in vitro*-activated splenic B cells by
442 LPS at day 4. Data showed equivalent numbers of IgG3-expressing cells in both WT and SATB1
443 KO models (*Supplementary Fig. S6C*) indicating that SATB1 is not required for IgG3 class
444 switching. RNA seq datasets from LPS- activated cells at day 2 also provided relevant portrayals
445 of germline transcription landscape in the *IgH* locus of WT and SATB1 deficient models
446 (*Supplementary Fig. S6D*). When examining germline transcription within the *I μ* -donor and *I γ 3*- or
447 *I γ 2b*-acceptor regions, both WT and SATB1-deficient models displayed identical profiles
448 suggesting that, in agreement with normal CSR to IgG3 observed at day 4, SATB1 depletion does
449 not impede LPS-induced germline transcription occurring prior to CSR.

450 When measured in the same *in vitro* assays, the intrinsic ability of splenic B cells to differentiate
451 into plasmablasts (CD138⁺) upon LPS activation was also similar in WT and our SATB1 KO model
452 (*Fig. 3E*). In agreement with *in vitro* data, NP-CGG immunization of mice induced generation of
453 plasma cells in normal proportions in the absence of SATB1 (*Fig. 3F*). Altogether, these data
454 suggest that SATB1 is dispensable for efficient differentiation into antibody secreting cells.

455 Although, once differentiated *in vitro* into plasmablasts, SATB1 deficient cells displayed higher
456 levels of intracellular IgM when analyzed by flow cytometry (*Fig. 3G*). This increase in Ig production
457 by plasmablasts was correlated with a significant increase of both *IgH* and *Igk* primary transcripts
458 (respectively 1.4 and 1.7) in LPS-activated cells from SATB1 deficient animals (*Fig. 3H*).

459 The effect of SATB1 deletion on global transcription of both IgH and IgL chains was also confirmed
460 by comparing RNA seq data from splenic B cells activated by LPS at day 2 (*Fig.3J*) or from sorted
461 plasmablasts at day 4 (*Fig.3K*). In the first case, among the 64 genes found upregulated in SATB1-
462 deficient models (\log_2 FC>+/-1.5), 20 of them corresponded to *IgVH* chain products and 23 others
463 were identified as *IgV* κ L chain products (*Fig.3J* and *Supplementary Table S5*). When comparing
464 plasmablasts, *IgV* H chain and *IgV* λ L chain products were strongly increased upon SATB1
465 depletion (*Fig. 3K* and *Supplementary Table S6*). Our data indicate that, beyond its modest effect
466 on B cell activation programs induced by LPS *in vitro*, the absence of SATB1 induces a more
467 pronounced transcription of Ig genes. Moreover, STAB1 is dispensable for proliferation, CSR and
468 differentiation into plasma cells.

469 As a parallel to LPS stimulation, we performed *in vitro* stimulation with anti-CD40+IL4 in our model
470 to evaluate, in another activation context, cell differentiation and Ig intracellular content in the
471 absence of SATB1. Our data show, as in LPS-stimulation, that SATB1-deficient B cells differentiate
472 efficiently into plasmablasts in response to anti-CD40+IL4 cocktail (*Supplementary Fig S6E left*). To
473 note, such plasmablasts devoid of SATB1 also contains high level of intracellular IgM, although
474 upregulation was not, in this case, increased significantly (*Supplementary Fig S6E right*). However,
475 our data unveil a switch in the regulatory function of SATB1 for Ig chain transcription, from positive
476 in naive to negative in activated B cells. This striking effect persists until the terminal stage since

477 SATB1-deficient plasma cells display a high content of Ig chains. However, SATB1-depletion does
478 not allow plasma cells to produce more Ig since our deficient mice display broadly normal levels of
479 serum antibody isotypes.

480

481 ***SATB1 depletion increases somatic hypermutation***

482 Since our group recently reported a critical function of *cEμ*-associated MARs for SHM of the *IgH*
483 locus in mouse models ⁶⁶, and according to historic studies in the *Igκ* locus ⁵⁹, it was questionable
484 whether MAR binding proteins, such as SATB1, could be involved in targeting somatic mutations
485 of Ig genes. We first evaluated the ability of SATB1 deficient B cells to support somatic mutations
486 by analyzing global SHM within intronic regions, not subject to antigen selection, located
487 immediately downstream from V exons of both *IgH* and *Igκ* loci and also upstream from *IgH Sμ*
488 regions in spontaneous GC B cells from Peyer's patches. As a possible regulation mechanism,
489 transcription taking place in the corresponding regions targeted by AID was also quantified in this
490 subset. Second, to more accurately evaluate the ability of SATB1-deficient B cells to undergo SHM
491 in response to specific antigen, we immunized mice with NP-CGG ⁶⁷. By analogy to B cells devoid
492 of *MARs_{Eμ}* regions, in which SHM machinery gains access more frequently to the region
493 downstream from *cEμ* (upstream from *Sμ*) ⁶⁸, we carefully quantified SHM in this same region (Fig
494 4A, *scheme*). In GC B cells sorted from Peyer's patches, while *Sμ* transcription was not significantly
495 raised (Fig. 4A, *left bar graph*, $p=0.3$), we found a significant increase of mutations in the absence
496 of SATB1 (1.88 mut/Kb) compared to WT (1.44 mut/Kb) (Fig. 4A, *middle bar graph*, $p=0.02$ and
497 *Supplementary Table S7A*). A similar and significant increase of SHM in this region was also
498 observed in splenic GC B cells sorted after NP-CGG immunization: while WT cells barely reached
499 0.22 mut/Kb, SATB1-deficient cells underwent two fold more mutations reaching 0.41 mut/Kb (Fig.
500 4A, *right bar graph*, $p=0.03$ and *Supplementary Table S7B*). This common feature shared by
501 *MARs_{Eμ}* KO- and *Satb1* KO-B cells suggests that SATB1 could be involved in limiting access of the
502 SHM machinery to donor *Sμ* regions in cells undergoing SHM. When transcription and SHM were
503 quantified downstream from the rearranged *IgH* variable regions (Fig 4B, *scheme*) in GC B cells
504 sorted from Peyer's patches, homozygous *Satb1* deletion increased transcription (Fig. 4A, *left bar*
505 *graph*, $p=0.3$) downstream from *J_{H4}* and raised, although not significantly, SHM frequencies (Fig.
506 4B, *middle bar graph*, $p=0.05$ and *Supplementary Table S7C*). These findings were clearly
507 confirmed upon NP-CGG immunization. Since B cell response to NP-CGG challenge is
508 preferentially dominated by mutated clones expressing the V_H186.2 segment ⁶⁷, quantification of
509 base substitutions in the *J_{H4}* intron downstream from the V_H186.2-rearranged exons is considered
510 as a reliable hallmark of antigen-induced SHM. Indeed, in SATB1-deficient GC B cells from
511 immunized mice, NP-CGG-induced mutations were significantly increased within the *J_{H4}* intron:
512 mutant cells displayed 5.06 mut/Kb while WT cells only reached 3.28 mut/Kb (Fig. 4B, *right bar*

513 *graph*, $p=0.02$ and *Supplementary Table S7D*). When, in the intronic region downstream from *Jk5*
514 of the *Igk* locus (Fig 4C, scheme), global transcription was significantly increased in mutant
515 samples, (Fig 4C, *left bar graph*, $p=0.03$), both spontaneous and NP-CGG-induced GC B cells
516 displayed an increased trend of mutation frequencies upon SATB1 depletion (Fig. 4C, *middle and*
517 *right bar graphs* and *Supplementary Table S7E and F*). Since B cell responses to DNP hapten
518 preferentially involve Ig composed of Ig λ 1 light chains ⁶⁹, it is likely that changes in mutation
519 frequency within the *Igk* light chain loci, in this case not significant, probably underestimated any
520 potential SHM increase induced by SATB1 depletion. While our Gene Ontology enrichment
521 analysis detects the “Somatic diversification of Igs” as a differentially regulated pathway during the
522 “resting to activated” transition, expression of major actors of mismatch repair (MMR) and base
523 excision repair (BER) pathways such as *Ung*, *Msh2*, *Msh6*, *Pms2* and *Polq* were similarly
524 upregulated in both wt and cKO samples (Fig. 3A). Beyond global pathways analysis, it was also
525 necessary to verify in detail whether SATB1 depletion could affect, through its transcription factor
526 function, expression of any potential factors, including AID, involved in SHM. Indeed, when
527 extracted from RNA-seq datasets, expression of 16 genes involved in SHM was unchanged upon
528 SATB1 deletion in both LPS-activated B cells as well as LPS-induced plasmablasts
529 (*Supplementary Fig. S7A*). Within Ig gene regions submitted to SHM analysis, substitution
530 frequencies calculated at each base displayed a globally increased pattern (although not
531 significant) that did not offer any hypothesis regarding the origin of the changes (*Supplementary*
532 *Fig. S7B*). It is well established that AID targeting for SHM is coupled to transcription initiated at V
533 promoters ⁷⁰. Given the significant increase of primary transcription occurring at *IgH* and *Igk* in GC
534 B cells devoid of SATB1 (Fig. 4B and 4C), one straightforward hypothesis to explain the global
535 SHM increase in our model could be an overall increase in AID targeting of Ig genes. Interestingly,
536 in line with this hypothesis, we recently highlighted increased AID deamination coupled to increased
537 transcription in our mouse model devoid of *MARs_{E μ}* regions when bred into a DNA repair-deficient
538 background ⁶⁸. Surprisingly, while genomic deletion of *MAR_{E μ}* or *MAR_{E μ}* regions in the mouse
539 decreased SHM ^{59,68}, suppression of SATB1 led to the opposite effect in the regions downstream
540 from the rearranged variable segments of *IgH* and *Igk* loci. This finding also plays in favor of a
541 protective function for SATB1 against mutations. In this case, since the regions involved are located
542 upstream from *E μ* and *E μ* , one could propose that the potential protective effect of SATB1 could
543 take place in resting B cells in which SHM is not expected to happen. Moreover, the discrepancy
544 between the effect of *MARs_{E μ}* deletion and SATB1-depletion on targeted mutations upstream from
545 intronic enhancers suggest that other interacting factors, beyond SATB1, participate in the complex
546 regulation of *cis*-acting MARs. While we recently proposed that *MARs_{E μ}* favor error-prone repair in
547 its upstream regions in order to optimize SHM ⁶⁸, our current study suggest that SATB1 does not
548 participate in the unfaithful repair processes associated with SHM.

549 Taken together, our data suggest that SATB1-deletion increases SHM of Ig genes through a
550 transcription-coupled mechanism that probably favors AID targeting.

551

552 **Discussion**

553 The fact that SATB1 plays a major role as a “genome organizer” in hematopoietic- and T-lineage
554 cells ^{7,28,30} suggested that it is also important in B-lineage cells, a cell type that also undergoes fine
555 developmental regulation of its expression ³⁵. By using a conditional deletion model in B cells, our
556 study fills in gaps concerning the function of SATB1 in this lymphocyte lineage. In contrast to its
557 function in early-developing T cells, SATB1 is dispensable for the establishment of the Ig gene
558 repertoire and overall early B cell development in the bone marrow; this result is consistent with
559 the pioneer SATB1-null mouse model in which B cell populations could be observed in the spleen².
560 When looking at regions involved in *Rag* genes regulation, accessibility data suggests that, unlike
561 its critical function in T cell precursors, ASE region is not active in B-lineage cells. This difference
562 suggests that *Rag* genes regulation process evolved separately in B and T lineages, at least with
563 regard to the regulatory function of SATB1. However, our model reveals that SATB1 is involved in
564 the control of Ig gene transcription in mature B cells. Although previously unknown in the B lineage,
565 our findings once again point to a dual function for SATB1 depending on the activation stage. We
566 show that SATB1 promotes Ig gene transcription in resting B cells, while in activated B cells, it acts
567 as a repressor. In contrast to the T cell lineage where SATB1 is considered a major regulatory
568 factor of the enhancer/super-enhancer network ^{53,54}, SATB1 deletion does not induce major
569 disruptions in the B cell transcriptome. Our invalidation model shows that only a reduced number
570 of genes expressed during B differentiation are impacted by SATB1 deletion. In agreement with
571 the repressive function of this factor in activated B cells, our study also shows that Ig genes are the
572 predominant targets of SATB1 in activated B cells. Our 3C-HTGTS data indicate that SATB1 is not
573 required to establish chromatin loops between *E μ* and *V_H* regions in resting and activated B cells.
574 A rationale hypothesis could be that the effect of SATB1 on *IgH* transcription is mainly due to its
575 function as a transcription factor rather than a genome organizer. Given the critical effect of SATB1
576 depletion on Ig gene transcription, it would certainly be interesting to evaluate SATB1 physiological
577 binding to regulatory regions in Ig gene loci in resting and activated B cells.

578 By clarifying that SATB1 is an essential activator of Ig chain transcription, and consequently BCR
579 expression, in resting B cells, our study extends recent work published by Yokota's team ³⁵ and
580 proposes a rational explanation for the survival function of SATB1 at this stage. These two studies
581 also point out the importance of SATB1 expression levels which could, as demonstrated in HSCs
582 ^{22,65}, fine-tune some critical genes or regulatory pathways in lymphocytes. Beyond the physiological
583 decrease in *Satb1* expression between naive and activated B stages, it is possible that this gene

584 undergoes splicing modifications in the B cell lineage as it is proposed in developing T cells by the
585 Spillianakis' group ⁷¹. The stability of SATB1 protein, according to its transcript variants, might also
586 be critical for its function in mature B cell subsets, as already proposed in T-lineage cells by the
587 Galande's group¹⁹. Interestingly, the dual function of SATB1 also observed in the B lineage,
588 switching from activator to repressor of transcription in activated B cells, is reminiscent of the
589 molecular switch observed upon phosphorylation and acetylation of this factor ¹⁶.

590 A surprising observation from our study is that SATB1 deletion increases somatic hypermutation of
591 the *IgH* locus, extending into the *Sμ* region. It is very likely that the *Igκ* locus is similarly influenced.
592 A direct impact of SATB1 on key factors involved in SHM, such as AID and proteins involved in
593 BER and MMR pathways, can be excluded since normal induction of their transcripts were
594 observed in the absence of SATB1. In this respect, SATB1 could play a protective role against
595 SHM in resting cells. This finding should be read in conjunction with our recent study of the MARs
596 regions associated with the *Eμ* enhancer of the *IgH* locus ⁶⁸, known to bind SATB1 ¹. The SATB1-
597 induced increase in SHM could logically be correlated with the observed increase in primary
598 transcription of the variable regions of Ig H and L chains. However, it is not excluded that SATB1
599 modifies the accessibility of these regions to AID or its cofactors. Since it is proposed that SATB1
600 stabilizes the unpaired DNA regions against unwinding ¹³, such an action of SATB1 taking place in
601 the *MARs-Eμ* region could contribute to the protection of its surrounding regions against unwanted
602 SHM. In line with the putative protective function of SATB1, it has been shown that this protein acts
603 as an accessory factor of BER through its interaction with Oxo-guanine-glycosylase 1 (OGG1) ⁷²,
604 a DNA glycosylase that usually promotes error-free repair and that is not involved in Ig gene SHM
605 ⁷³.

606 Further study of these modifications of SATB1 in B cells will be necessary to clarify the origin of its
607 versatility. It has recently been proposed that SATB1 isoforms are subject to phase separation in
608 T cells ⁷¹. Consistent with its localization to PML nuclear bodies ^{74,75}, this mode of regulation
609 deserves to be explored in the context of the B cell nucleus. Moreover, since chromatin loop
610 extrusion mechanisms are now proven to be critical for Ig gene regulation ⁷⁶, it might be relevant to
611 evaluate a potential implication of SATB1 in this process.

612

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614

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630 EP and SLN conceived and supervised the study. MT and OM developed the experimental model.
631 JP performed the bio-informatic analysis. MT, CB, EP and SLN wrote the manuscript.

632 **Competing Interest Statement:** The authors declare no competing financial interests.

633

634 **References**

635

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- 845

846 **Legend to Figures**

847

848 **Figure 1.**

849 **A)** Conditional deletion of *Satb1*-exon 4 by *Cd79a^{cre/+}* recombinase in B cells. Exon 4, cre site, frt
850 site, conditional allele (*Satb1^{flx}*) and Wild type allele (*Satb1⁺*) are indicated. **B)** SATB1 protein
851 quantification by Western Blot in splenic B cells from WT (*Satb1^{flx/+}*), conditional heterozygous
852 (*Satb1^{flx/+} Cd79a^{cre/+}*) and conditional homozygous (*Satb1^{flx/flx} Cd79a^{cre/+}*) mice. **C)** *Satb1* transcript
853 quantification by RT-qPCR (normalized to *Hprt*) in Peyer's patch naive and GC B cells sorted from
854 WT mice (n=8) from 3 independent experiments. **D)** Bar graphs displaying absolute numbers of B
855 cell subsets determined by flow cytometry from bone marrow (per femur, n=7-10) and spleen
856 (n=11-15) from 4-5 independent experiments. **E)** Pie chart representation of *IgHV* family gene
857 usage, quantified by RACE-RepSeq, expressed by pre-B cell-enriched bone marrow populations
858 in WT (n=5) and cKO mice (n=6) from 2 independent experiments. **F)** Immunoglobulin serum
859 isotype levels quantified by ELISA in 8 to 10 week-old WT and cKO mice (n=8-15) from 4
860 independent experiments. Error bars represent SEM; p-value was determined with two tailed Mann
861 Whitney test; non-significant (ns) and significant differences are indicated (***) p<0.001).

862

863 **Figure 2.**

864 **A)** Comparison of surface IgM fluorescence intensity, evaluated by flow cytometry, on immature
865 and recirculating bone marrow B cell subsets from two-month-old WT and homozygous cKO
866 animals. One representative histogram is shown (left), bar graph (right) displays normalized surface
867 IgM MFI in WT and cKO animals (n=7-10 from 4 independent experiments). **B)** Comparison of
868 surface IgM fluorescence intensity, evaluated by flow cytometry, on Follicular (FO), Marginal zone
869 (MZ), Transitional (Tr.1, Tr.2, Tr.3) splenic B cell subsets from two-month-old WT and homozygous
870 cKO animals. One representative histogram is shown (left), bar graph (right) displays normalized
871 surface IgM MFI in WT and cKO animals (n=13-14 from 5 independent experiments). **C)**
872 Comparison of surface IgM fluorescence intensity, evaluated by flow cytometry, on naive B cells
873 sorted from Peyer's Patches from two-month-old WT and homozygous cKO animals. One
874 representative histogram is shown (left), bar graph (right) display normalized surface IgM MFI in
875 WT and cKO animals (n=10-11 from 3 independent experiments). **D)** *IgH* and *Igk* primary
876 transcripts quantified by RT-qPCR (normalized to *Hprt*) in splenic resting B cells sorted from WT
877 and cKO mice (n= 6-10 from 5 independent experiments). **E)** Volcano plot from RNA-seq indicating
878 differentially expressed transcripts when comparing WT and cKO mice (n=2) resting B cells.
879 Transcripts located within Ig gene loci are indicated. Error bars represent SEM; p-value was
880 determined by two tailed Mann Whitney test; non-significant (ns) and significant differences are
881 indicated (* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001).

882

883 **Figure 3.**

884 **A)** Visual representation of Gene Ontology enrichment analysis of genes significantly associated
885 with biological pathways upon B cell transition from resting (day 0) to *in vitro*-activated (day 2)
886 subsets in WT and cKO mice. Datasets from RNA-seq experiments were used for the analysis. **B)**
887 Same representation as above for B cell transition from *in vitro*-activated (day 2) to plasmablast
888 (day 4) subsets. **C)** Germinal center (GC) B cell subsets evaluated by flow cytometry in Peyer's
889 patches of WT and cKO animals (n=12-18 from 4 independent experiments). **D)** Comparison of
890 surface IgM fluorescence intensity evaluated by flow cytometry, on Peyer's patch GC B cells from
891 two-month-old WT and homozygous cKO animals. One representative histogram is shown (left),
892 bar graph (right) displays normalized surface IgM MFI in WT and cKO animals (n=10-11 from 3
893 independent experiments) **E)** Proportion of plasmablasts evaluated by flow cytometry, obtained
894 following *in vitro* LPS activation (day 4) of splenic B cells from WT and cKO animals (n=13-18 from
895 6 independent experiments) (left). **F)** Proportion of plasma cells in the spleen evaluated by flow
896 cytometry following NP-CGG immunization of WT and cKO animals (n=6-7 from 1 experiment). **G)**
897 Comparison of intracellular IgM fluorescence intensity evaluated by flow cytometry, in *in vitro*-
898 differentiated plasmablasts (day4) from WT and homozygous cKO animals. One representative
899 histogram is shown (left), bar graph (right) displays normalized intracellular IgM MFI in WT and
900 cKO animals (n=9-10 from 4 independent experiments). **H)** *IgH* and *Igk* primary transcripts
901 quantified by RT-qPCR (normalized to *Hprt*) in *in vitro* LPS-activated B-lineage cells (day 4) of WT
902 and cKO animals (n=9-13 from 5 independent experiments). **J)** Volcano plots from RNA-seq
903 indicating differentially expressed transcripts when comparing WT and cKO mice (n=2) in *in vitro*
904 LPS-activated B cells (day 2). Transcripts located within Ig gene V regions are indicated. **K)** Same
905 analysis as above in *in vitro* LPS-differentiated plasmablasts (day 4). Error bars represent SEM; p-
906 value was determined by two tailed Mann Whitney test; non-significant (ns) and significant
907 differences were indicated (* p<0.05; ** p<0.01).

908

909 **Figure 4.**

910 Comparison of primary transcription and somatic hypermutation in WT and cKO animals, taking
911 place in various regions of Ig genes targeted by AID (left schemas), in spontaneous GC B cells
912 sorted from Peyer's patches (n=7-10 from 4 independent experiments, left and middle bar graphs).
913 Somatic hypermutation in splenic GC B cells sorted after NP-CGG immunization (n=7-8 from 4
914 independent experiments, right bar graphs). Data were obtained by NGS (Ion proton) combined
915 with DeMinEr filtering, an accurate dedicated method to quantify AID-induced substitutions in B-
916 lineage cells (50). **A)** Transcription and SHM upstream from *IgH* *Sμ* region. **B)** Transcription and
917 SHM within the *IgH* intron downstream from *VJ558* (consensus) to *J_{H4}*-rearranged exons (in
918 spontaneous GC B cells) or in the same region downstream from *V186.2* to *J_{H4}*-rearranged exons
919 (in NP-CGG-induced GC B cells). **C)** Transcription and SHM within the *Igk* intron downstream from

920 all-rearranged *Jk5* segments. Error bars represent SEM; p-value was determined by two tailed
921 Mann Whitney test; non-significant (ns) and significant differences were indicated (* $p < 0.05$).
922

Fig. 1

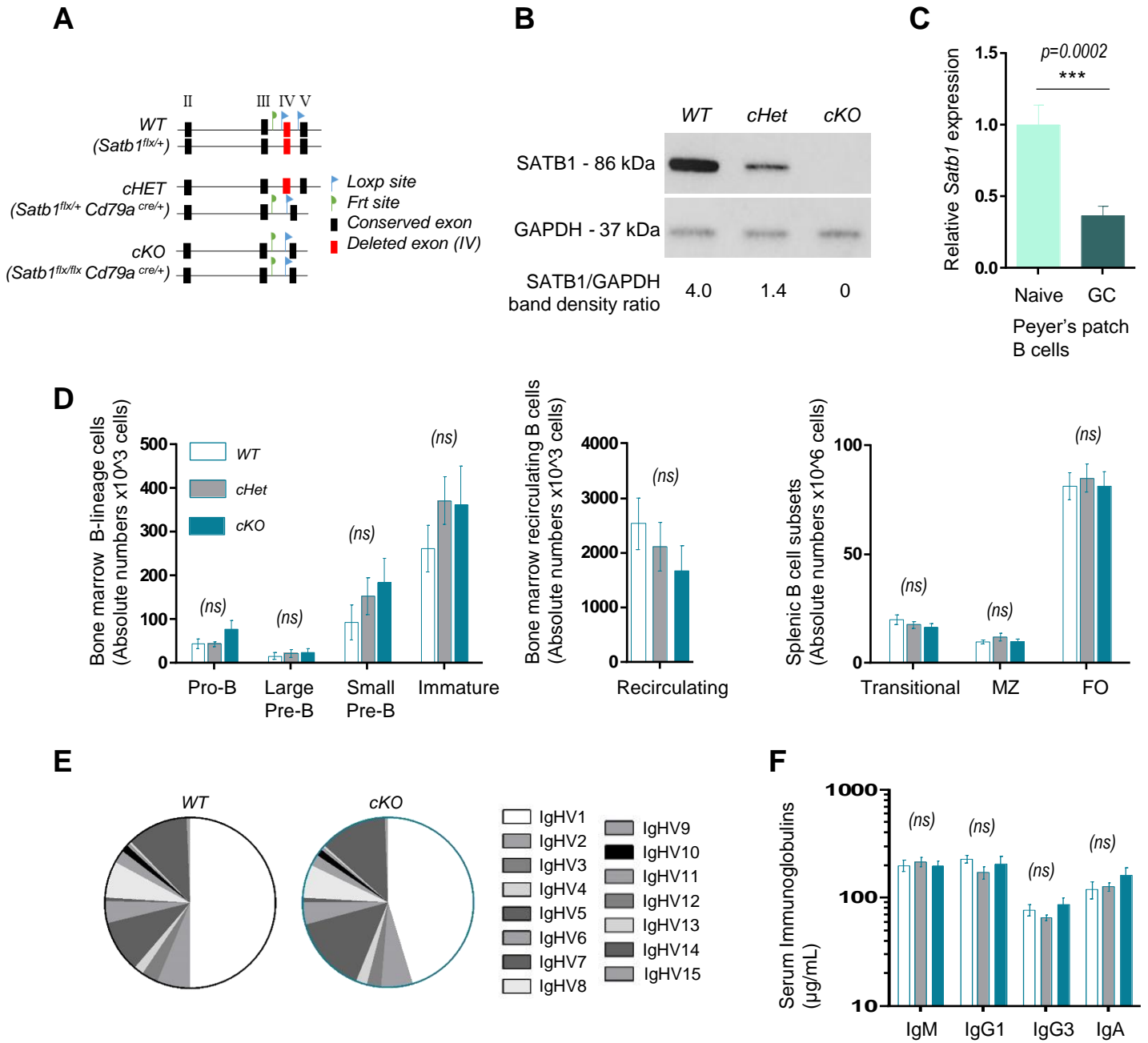
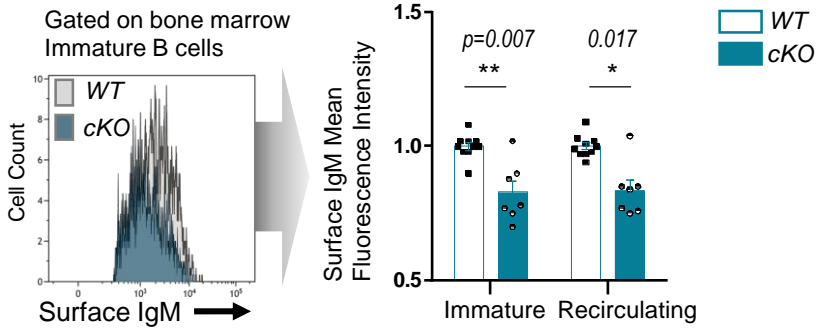
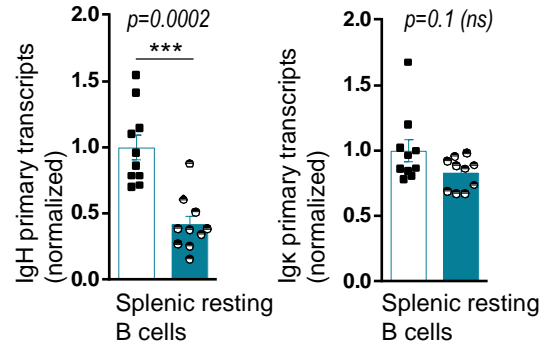


Fig. 2

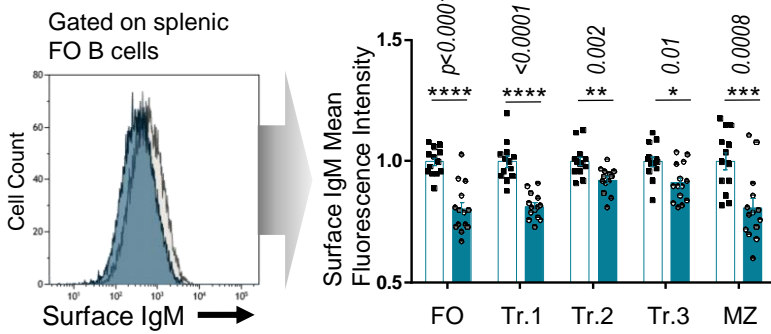
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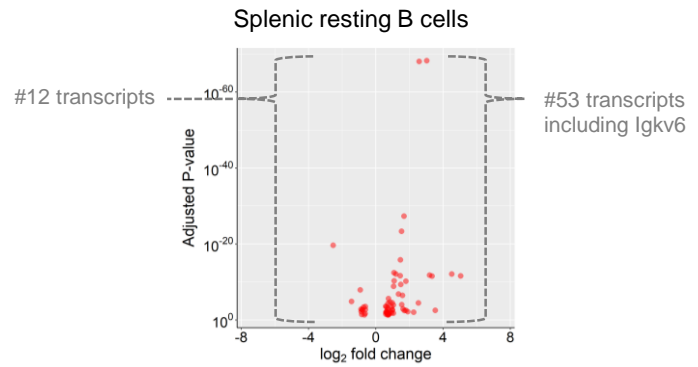
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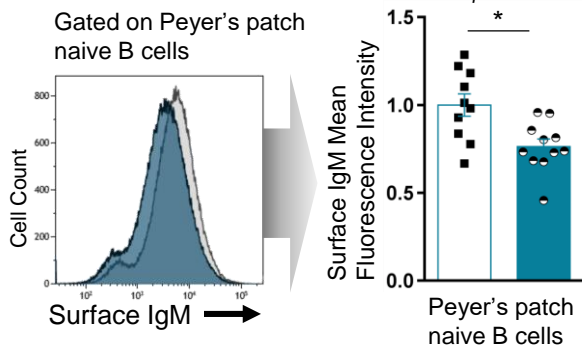


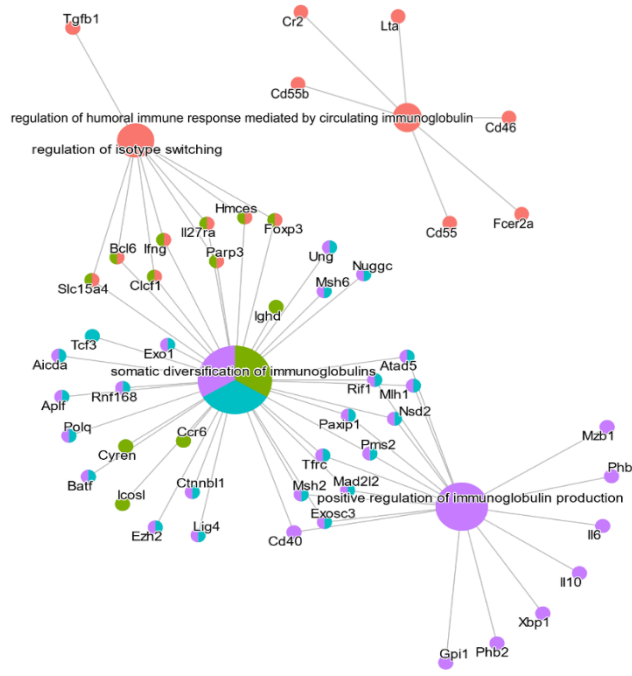
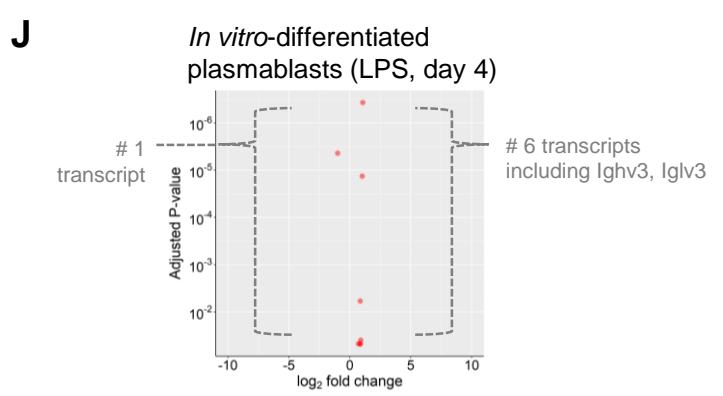
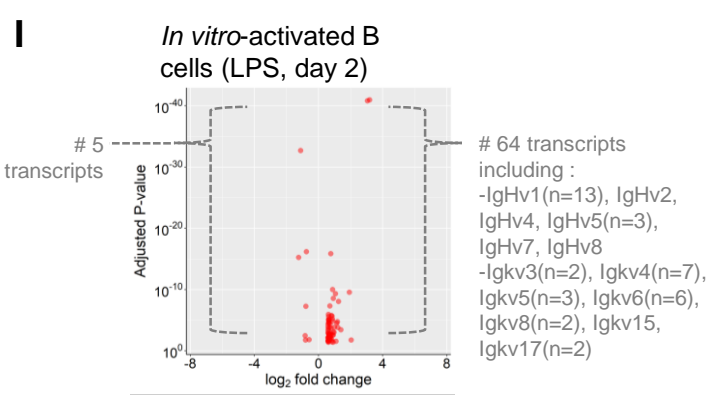
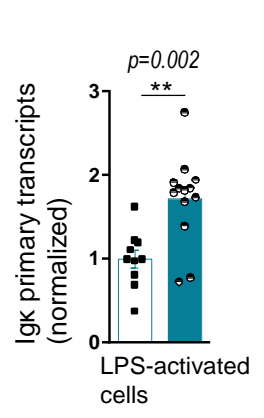
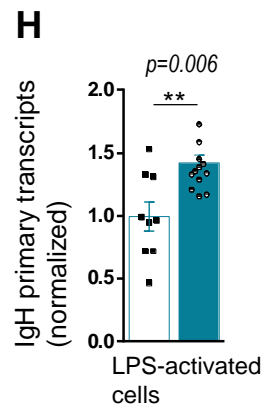
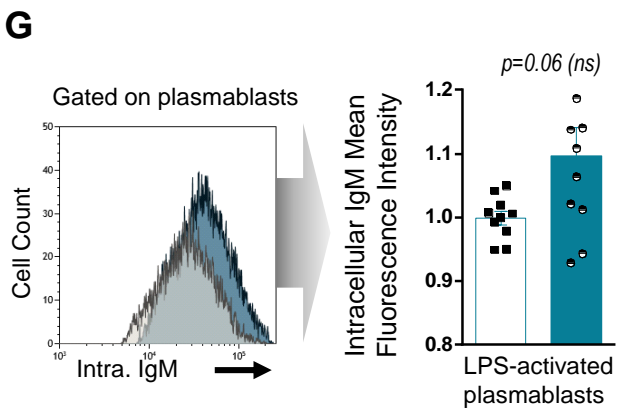
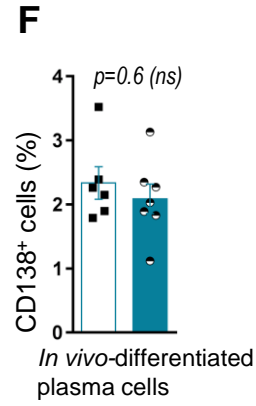
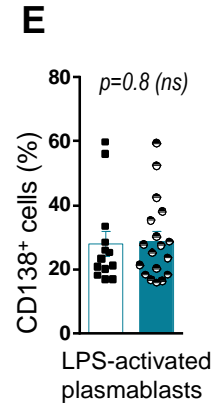
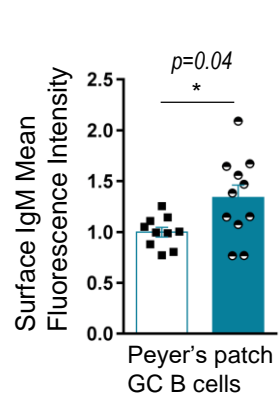
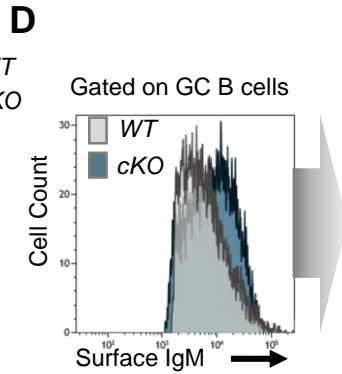
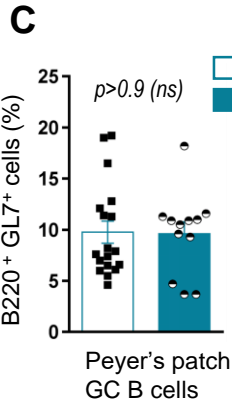
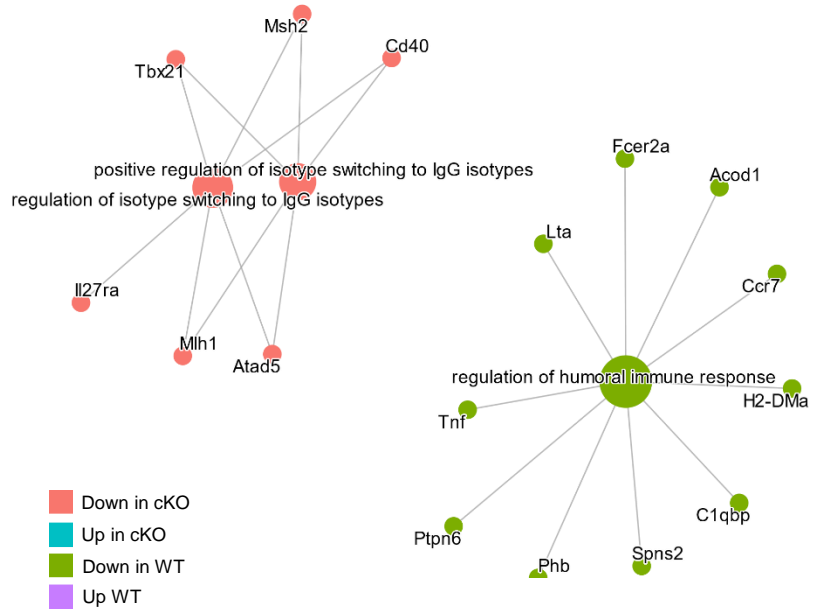
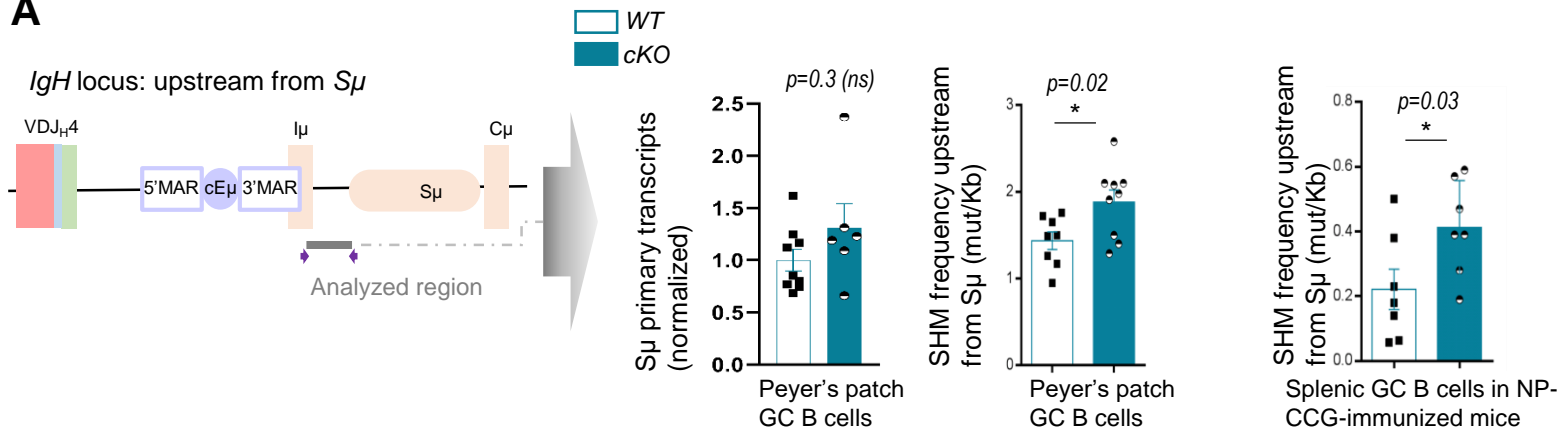
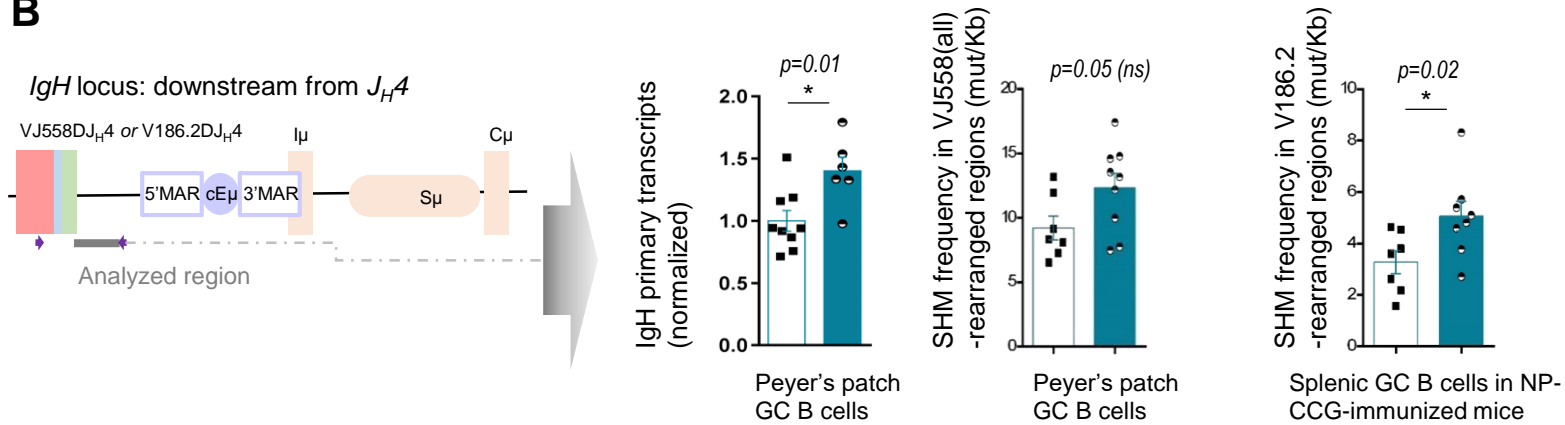
Fig. 3**A** Resting (day 0) to *in vitro*-activated (day 2) transition**B** *In vitro*-activated (day 2) to plasmablast (day 4) transition

Fig. 4

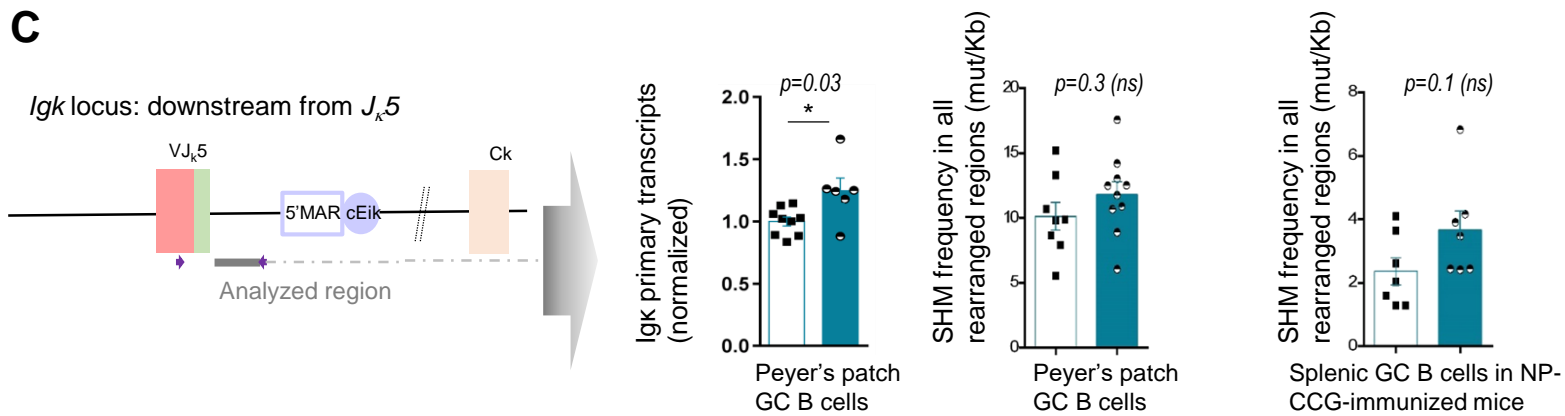
A



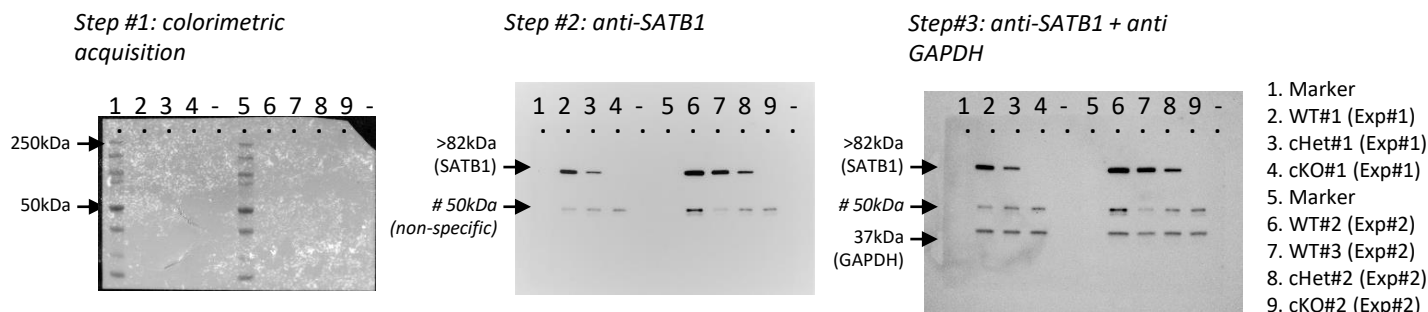
B



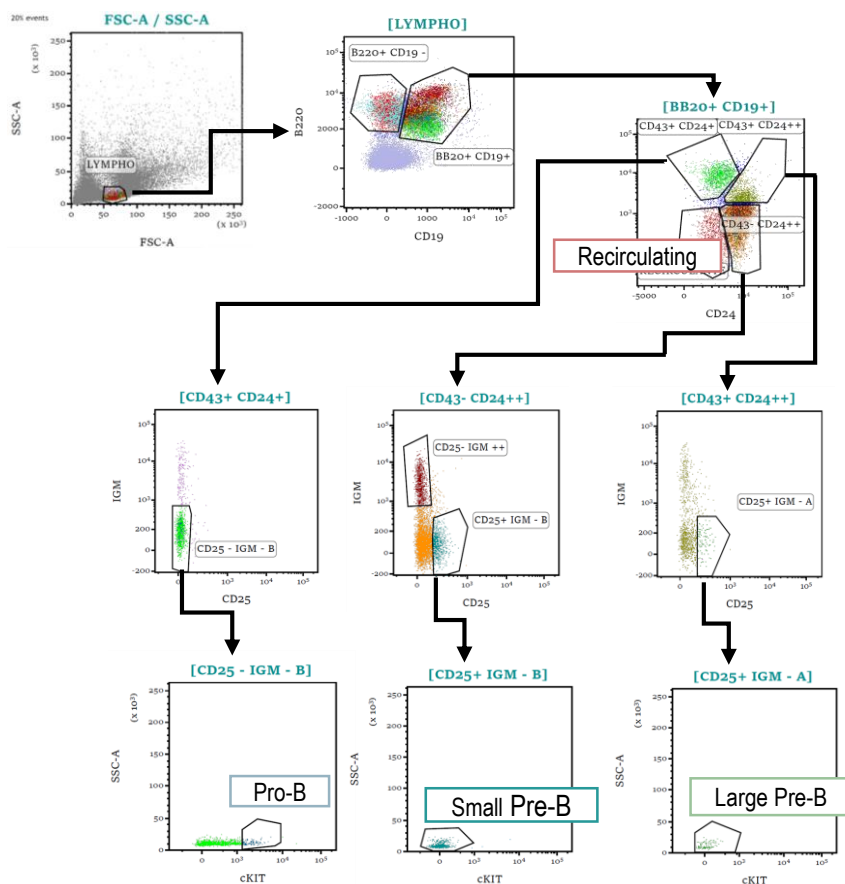
C



A



B



C

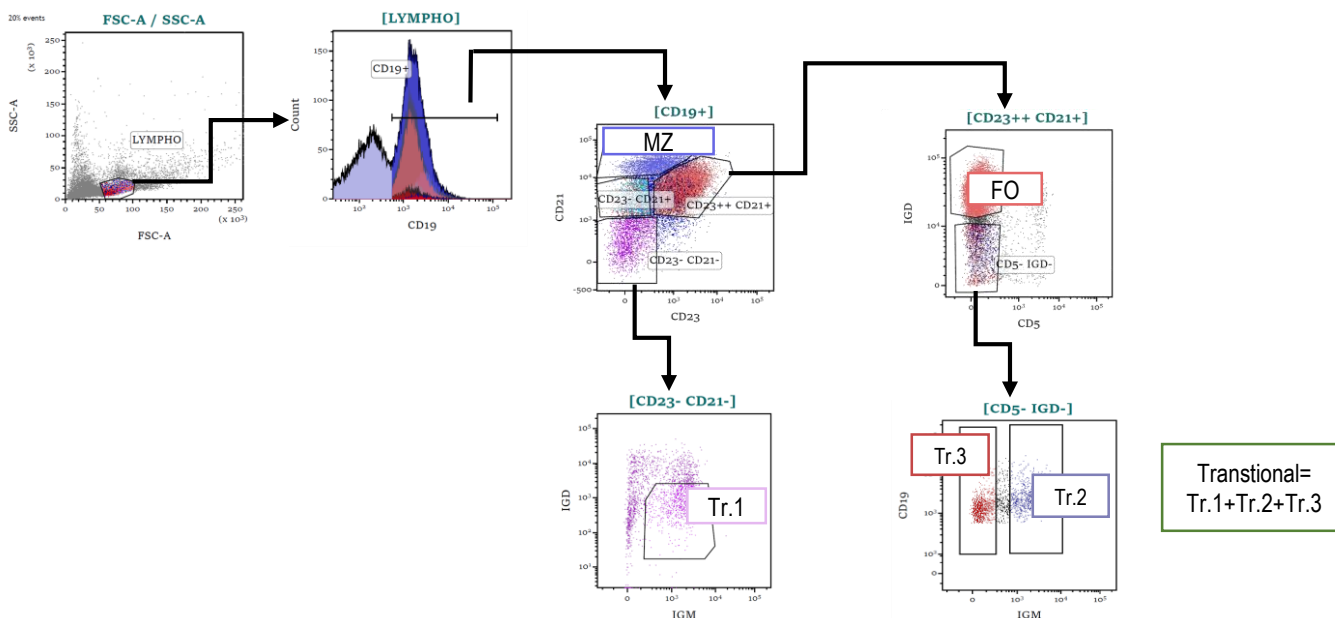


Fig. S1. (A) Unprocessed original images of Western blots quantifying SATB1 protein expression in sorted splenic B cells from WT, cKO heterozygous and cKO homozygous mice. **(B)** Gating Strategy used to analyse bone marrow mouse B cell subsets in Fig.1D. **(C)** Same as (B) for B cell subsets from the spleen.

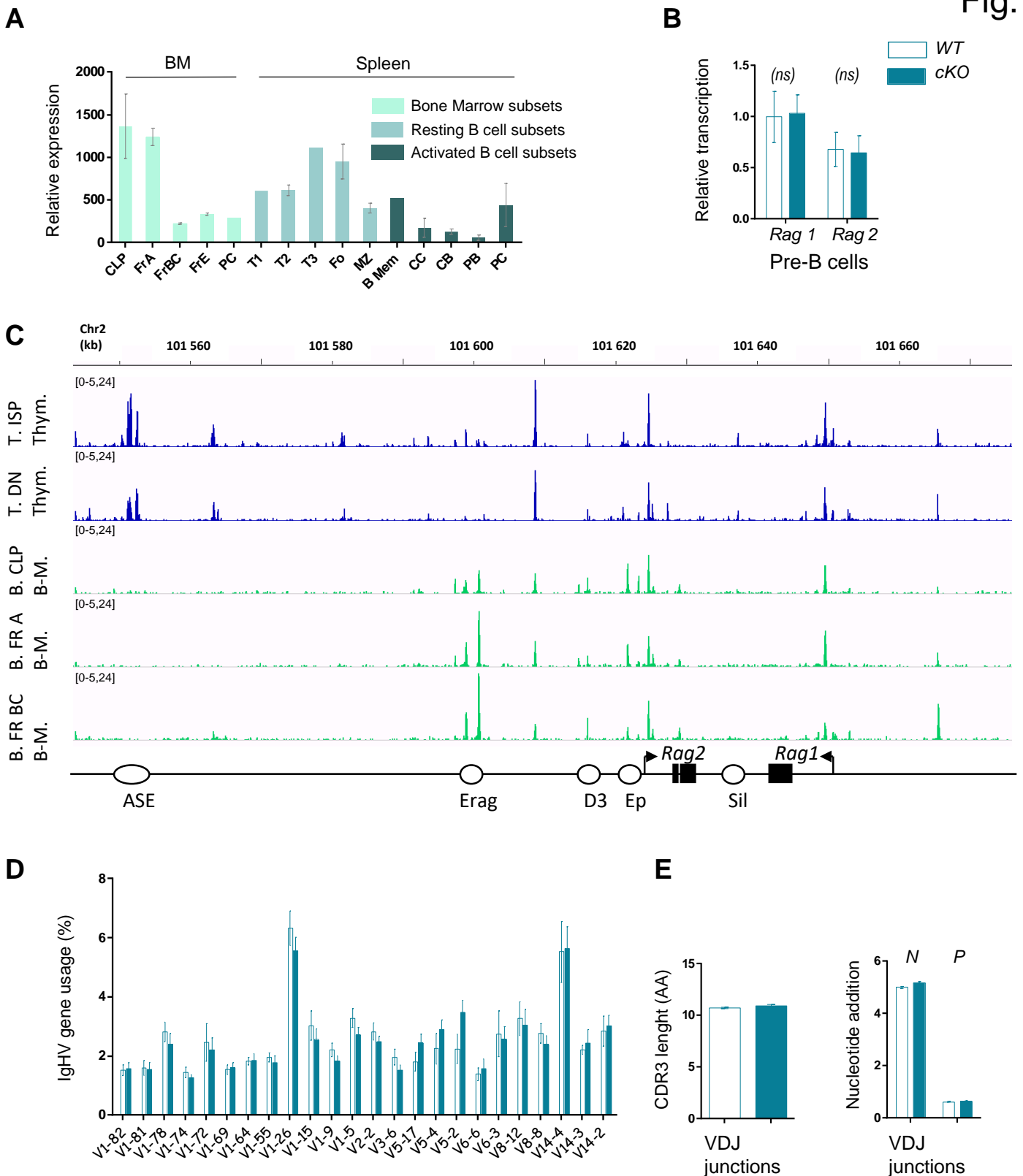


Figure S2. **(A)** Representation of SATB1 relative expression in murine *wt* B cell populations with Gene Skyline data browser (ImmGen ULI RNASeq data group⁴⁶). Populations include Common Lymphoid progenitor, Hardy fractions A, B, C, E and mature peripheral B cell subsets. **(B)** Transcription of *Rag1* and *Rag2* genes quantified in bone marrow sorted pre-B cells from *wt* ($n=6$) and *Satb1* *cKO* ($n=5$) mice. Error bars represent SEM; p-value was determined with two tailed Mann Whitney test. **(C)** Comparison of *Rag* gene loci accessibility by ATAC-seq in thymocytes (intermediate single-positive and double positive populations, upper lanes in blue) and bone-marrow developing B cells (common lymphoid progenitor, fractions A and BC, lower lanes in green) performed in *wt* mice, published data assembled by the ImmGen consortium^{46, 47}, relevant regulatory elements are indicated. **(D)** Comparison of *IgHV* gene relative proportion, quantified by RACE-RepSeq, expressed by pre-B cell-enriched bone marrow populations in *wt* ($n=5$) and *cKO* mice ($n=6$). **(E)** Comparison of CDR3 length (left panel), N and P-nucleotide addition (right panel) during VDJ recombination for *wt* ($n=5$) and *cKO* mice ($n=6$). Statistical analysis was not performed given the minor differences observed.

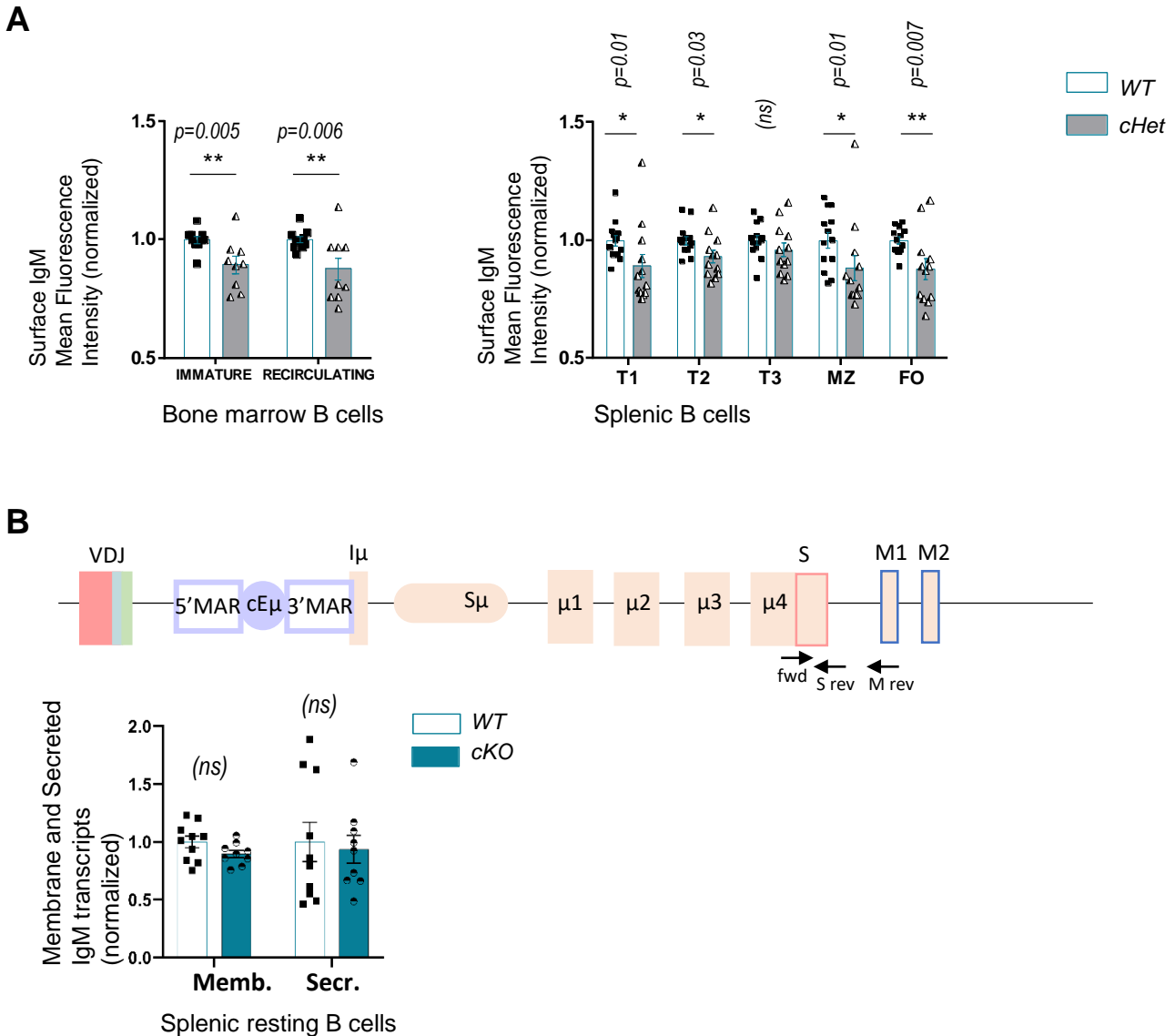


Fig. S3. (A) Mean Fluorescence Intensity measured by FACS analysis (normalized on WT samples) of IgM expressed at the surface of B cell subsets of 2-month-old WT and cKO heterozygous mice. Left panel includes data from bone marrow IgM expressing B cell subsets (n=7-9). Right panel includes data from B cell subset from the spleen (n=12-13). **(B)** Primer location to quantify Membrane and Secreted IgM transcripts on the mouse IgH locus (top, not to scale), RT-qPCR quantification (Normalized to *Hprt*) in splenic resting B cells of WT (n=10) and cKO (n=9) animals. Error bars represent SEM; p-value was determined by two tailed Mann Whitney test; non-significant (ns) and significant differences are indicated (* p<0.05; ** p<0.01).

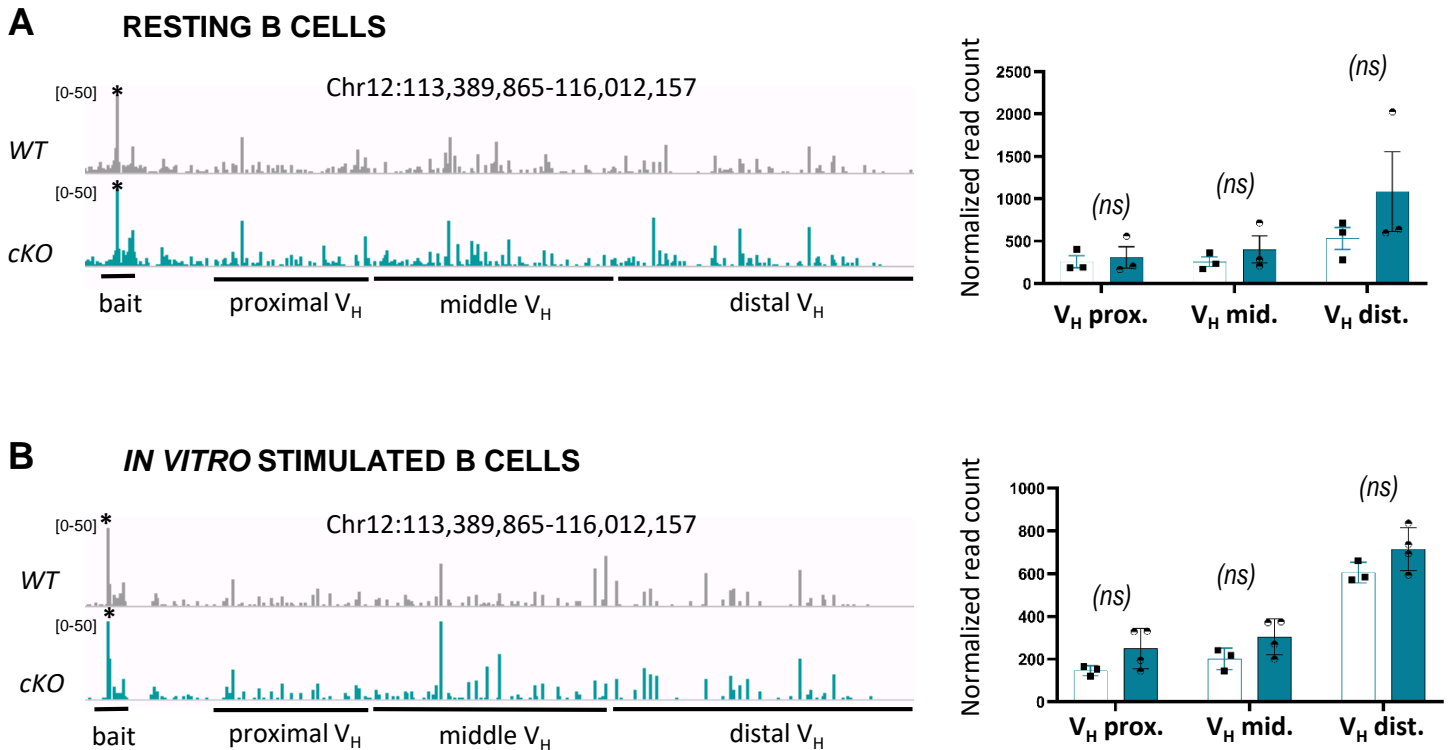


Fig. S4. (A) Chromatin loops in resting B cells from spleen ($n=3$ of each genotype). Intrachromosomal interactions at IgH locus measured by 3C HTGTS between the bait located close to $E\mu$ enhancer ($I\mu S\mu$ region) and the V_H family regions in B cells from WT and cKO mice (> 2-month-old). Left diagram: representative bedgraph of each genotype (IGV browser), showing physical interaction between bait (identified by an asterisk) and the various V_H segments (proximal, middle and distal) positioned on the germline locus. Right diagram display normalized coverage reads mapping with respective V_H family regions from 3 to 4 sample of each genotype.. **(B)** Same as (A) in LPS-activated B cells for 3 days ($n=3-4$ of each genotype). Error bars represent SEM; p-value was determined by two tailed Mann Whitney test; non-significant (ns) differences are indicated.

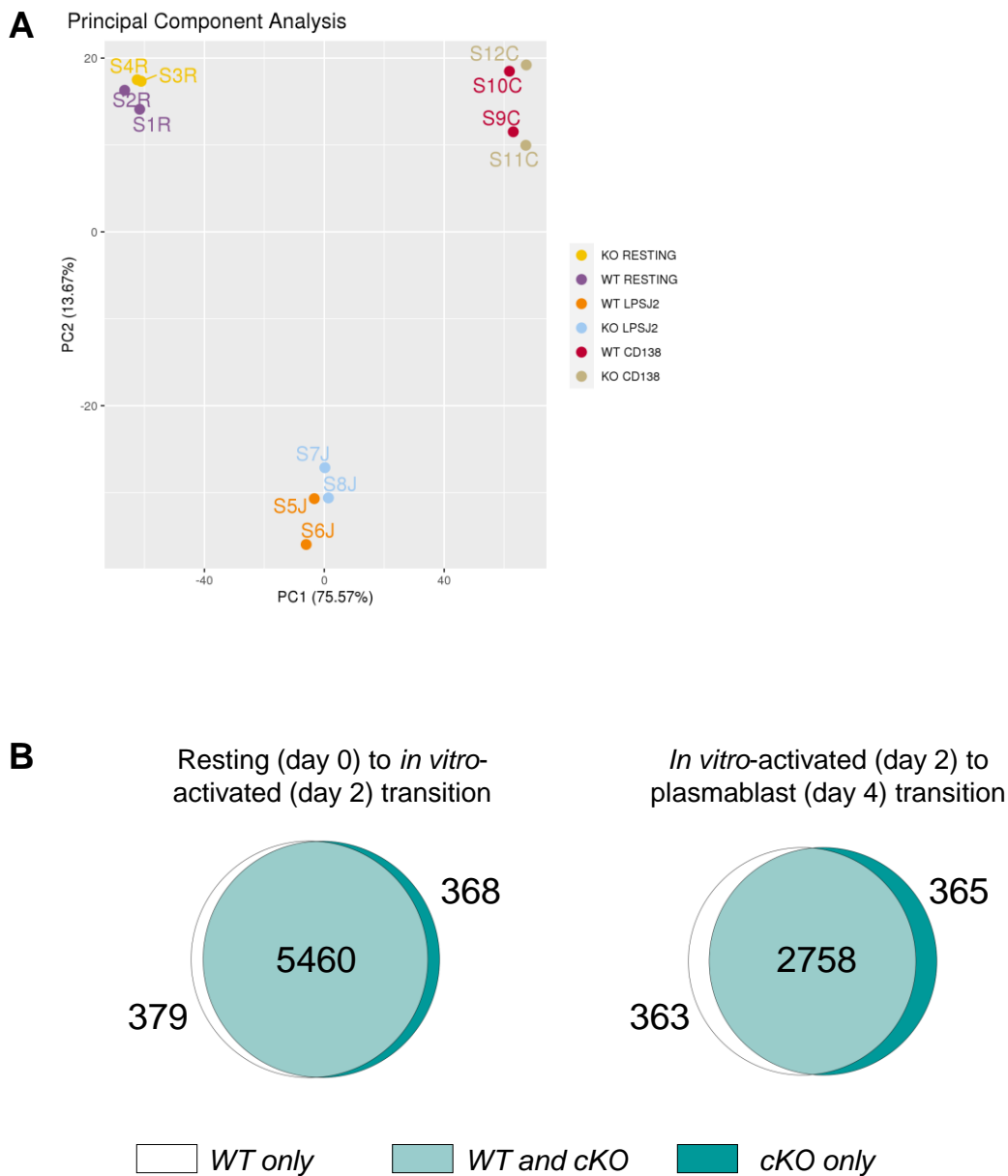


Fig. S5. (A) Principal Component Analysis of RNA sequencing of resting (R), LPS stimulated (J) or plasmablast (C) cells from WT and cKO mice (n=2). **(B)** Venn diagrams from RNA-seq displaying numbers and proportions of differentially expressed transcripts of B-lineage cells from WT and cKO mice (n=2) during resting to *in vitro*-activated (day 2) transition (left) and during *in vitro*-activated to plasmablast (day 4) transition (right)

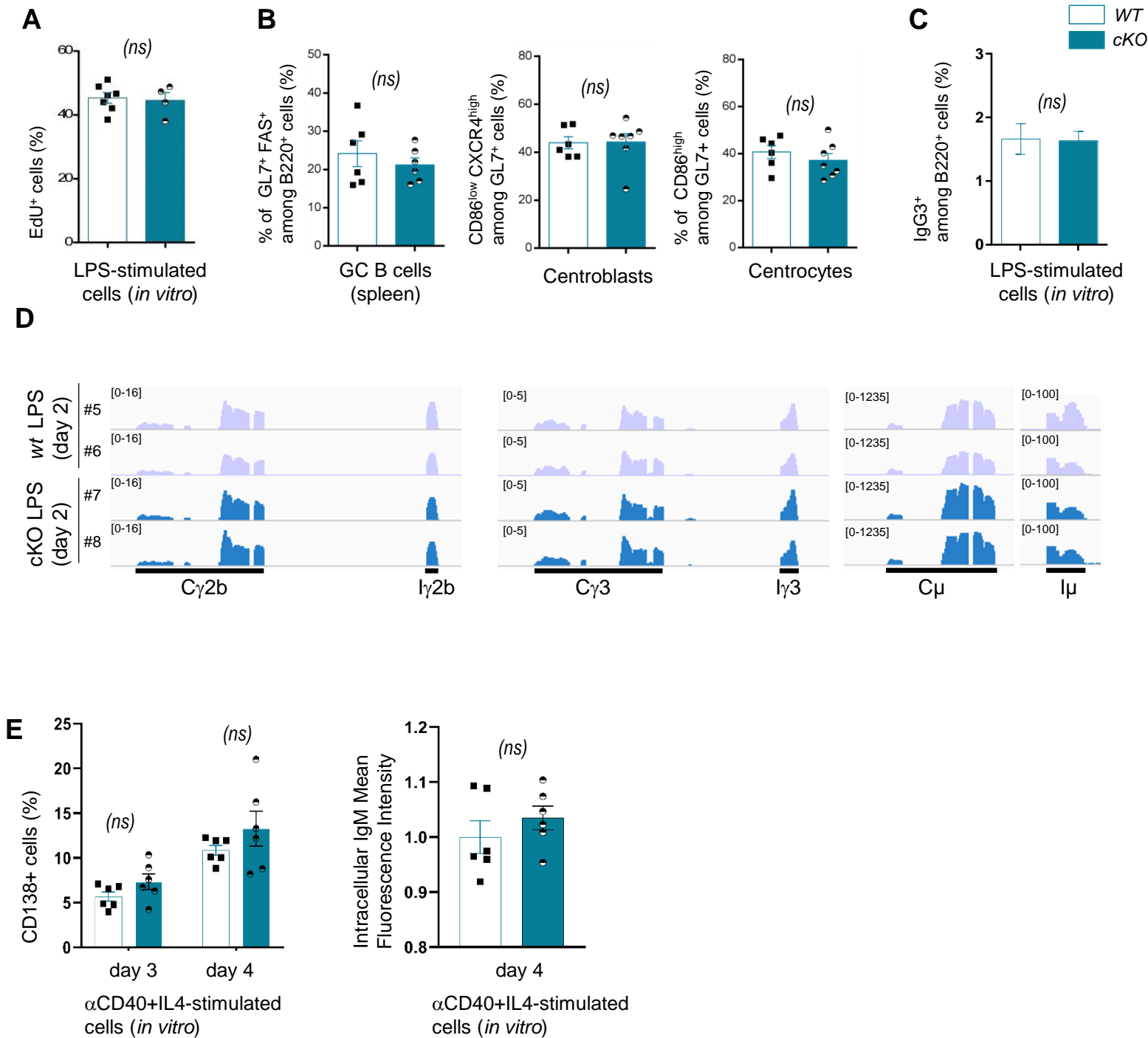


Fig. S6. (A) Percentage of EdU-incorporating splenic B cells from WT and cKO mice during *in vitro* LPS activation (n=4-7). (B) Detailed analysis of germinal center (GC) populations obtained following NP-CGG immunization of WT (n=6) and cKO (n=7) animals: percentage of GC B cells (B220⁺GL7⁺, left) among splenic B cells, percentage of centroblasts (CD86^{Low}CXCR4^{High}, upper right) & centrocytes (CD86^{High}, lower right) within the initial GC B cell subset. (C) IgG3 switched cells obtained after *in vitro* activation of splenic B cells with LPS at day 4. (D) Visual representation of relevant *IgH* constant gene transcripts induced in *in vitro* LPS-activated B cells (day 2) from WT and cKO mice (n=2). Bedgraph files from RNAseq analysis were aligned on mouse GRCm38/mm10 assembly using IGV browser. (E) Proportion of plasmablasts evaluated by flow cytometry, obtained following *in vitro* α CD40+IL4 activation (day 3 and day 4) of splenic B cells from WT and cKO animals (left); Comparison of intracellular IgM fluorescence intensity evaluated by flow cytometry, in *in vitro*-differentiated plasmablasts (α CD40+IL4, day4) from WT and homozygous cKO animals (n=6). Error bars represent SEM; p-value was determined by two tailed Mann Whitney test; non-significant (ns) differences are indicated.

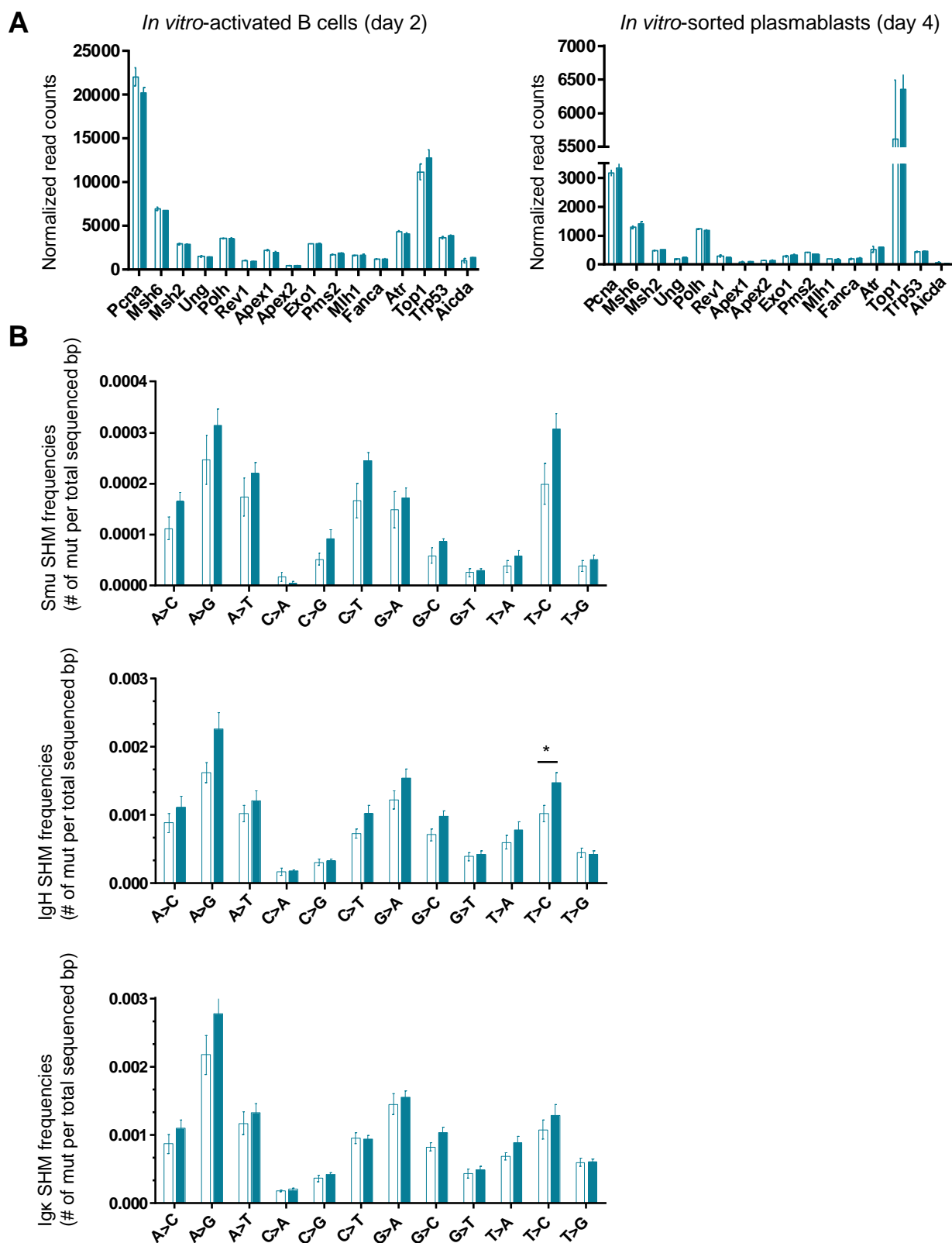


Fig. S7. (A) Relative expression of genes involved in SHM and SHM-associated DNA repair pathways in *in vitro* activated B cell- (day 2) or enriched-plasma cell- (day 4) subsets of WT and cKO mice evaluated by RNA-seq (n=2). **(B)** Base substitution patterns, reported in frequencies for each base, in GC B cells sorted from Peyer's patches of WT and cKO mice. SHM taking place within the region upstream from *IgH S μ* (top), downstream from rearranged-*J_H4* exons (middle) and downstream from rearranged-*Jk5* exons (bottom) (n=7-10). Data were obtained by NGS (Ion proton) combined to DeMinEr filtering. Error bars represent SEM; p-value was determined with two tailed Mann Whitney test, only significant differences were indicated (* p<0.05).

Supplementary Table S1: summary table of genotyping primers

Primer name	Sequence	Gene
EF	5'-TTTGCTCATGTGGAATGTCGAGGTA-3'	Satb1
KR	5'-GGGCAAGAACATAAAGTGACCCTC-3'C	Satb1
EF2	5'-AATAATCTGCTCCACTGCTCCACTGAGGACCCAC-3'	Satb1
ER3	5'-CCCTATTGCAGTGGGAATCAGCAT-3'	Satb1
MB1 CRE Wild Type Forward	5'-CTCTTTACCTTCCAAGCACTGA-3'	Mb1
MB1 CRE COMMON	5'-ACTGAGGCAGGAGGATTGG-3'	Mb1
MB1 CRE MUTANT Forward	5-'CATTTTCGAGGGAGCTTCA-3'	Mb1

Supplementary Table S2: antibodies used for Western blot, ELISA and flow cytometry

	Antibody	Clone	Dilution	Sources
WESTERN BLOT	SATB1	EPR3951	1/1000	ABCAM AB109122
	GAPDH	AF5718	1/5000	RD SYSTEMS
	Goat anti-rabbit	AB_2632593	1/5000	SOUTHERNBIOTECH
ELISA	Unlabeled Goat Anti Mouse IgM	1020-01	1/500	SOUTHERNBIOTECH
	Unlabeled Goat Anti Mouse IgG1	1070-01	1/500	SOUTHERNBIOTECH
	Unlabeled Goat Anti Mouse IgG3	1100-01	3/1000	SOUTHERNBIOTECH
	Unlabeled Goat Anti Mouse IgA	1040-01	1/250	SOUTHERNBIOTECH
	Goat Anti Mouse IgM-AP	1021-04	1/1000	SOUTHERNBIOTECH
	Goat Anti Mouse IgG1-AP	1070-04	1/1000	SOUTHERNBIOTECH
	Goat Anti Mouse IgG3-AP	1100-04	1/1000	SOUTHERNBIOTECH
	Goat Anti Mouse IgA-AP	1040-04	1/1000	SOUTHERNBIOTECH
Flow cytometry BONE MARROW B CELLS	cKIT BV421	2B8	1/100	BECTON DICKINSON 562609
	B220 BV510	RA3-6B2	1/100	BIOLEGEN D 103248
	CD25 APC	PC61	1/100	BECTON DICKINSON 561048
	CD24 FITC	M2/169	1/100	BECTON DICKINSON 553261
	CD43 PE	S7	1/100	BECTON DICKINSON PHARM 553271
	IGM PC7	II /41	1/100	EBIOSCIENCE 25-5790-82
	CD19 APCH7	1D3	1/100	BECTON DICKINSON PHARM 560143
Flow cytometry SPLENIC B CELLS	CD5 FITC	53-7.3	1/100	BECTON DICKINSON 5530211
	CD19 APCH7	1D3	1/100	BECTON DICKINSON PHARM 560143
	CD23 PC7	B3B4	1/100	BIOLEGEN D 101614
	CD21 PE	7G6	1/100	BECTON DICKINSON PHARM 552957
	IGM APC	II /41	1/100	EBIOSCIENCE 17-2590-82
	IGD BV421	11-26C-2A	1/100	BIOLEGEN D 405725
Flow cytometry PEYER 'S PATCHES CELLS	B220 APC	RA3-6B2	1/100	BIOLEGEN D 103212
	GL7 FITC	GL7	1/100	BECTON DICKINSON 553666
Flow cytometry CENTROCYTE / CENTROBLAST	B220 BV510	RA3-6B2	1/100	BIOLEGEN D 103248
	CD38 FITC	90	1/100	BECTON DICKINSON 558813
	FAS BV421	Jo2	1/100	BECTON DICKINSON 562633
	CXCR4 PE	2B11/CXCR4	1/50	BECTON DICKINSON PHARM 551966
	CD86 APC	GL1	1/50	BIOLEGEN D 105011
Flow Cytometry IN VITRO STIMULATED CELLS	B220 BV421	RA3-6B2	1/100	BECTON DICKINSON 562922
	IGM PC7	II/41	1/100	EBIOSCIENCE 25-5790-82
	IgG3 FITC	R40-82	1/100	BECTON DICKINSON 553403
	CD138 APC	281-2	1/100	BECTON DICKINSON 558626

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Supplementary Table S6: List of differentially expressed genes in in vitro-differentiated plasmablasts (day 4)

Genes upregulated in plasmablasts (Day4)						
MGI SYMBOL	WT	cKO	FoldChange	log2FoldChange	pvalue	padj
Igfv3	18404	38273	2.079	1.056	1.79921733414964e-12	2.31613247425083e-8
Rps7-ps3	1038	2096	2.02	1.015	4.144740942877341e-9	1.3338812539415e-5
Ighv3-8	6327	11801	1.865	0.899	2.16216554785762e-5	0.0397622244251016
Reep5	3030	5507	1.817	0.861	3.29776904916066e-5	0.0471690899664946
Glb1	1322	2165	1.638	0.712	3.02476519318689e-5	0.0471690899664946
Atf5	412	745	1.805	0.852	3.71673509239989e-5	0.0478455308444638

Supplementary Table S7: Total number of mutations, total number of bp analyzed and mutation frequencies within intron 5' to S μ (A, B); 3' to J_H4 (C, D) and 3' to J_k5 (E, F) in GC B cells of wt and Satb1 cKO. For each region tested for SHM, data were obtained from spontaneous GC B cells sorted from Peyer's patches (n=6-9 individual mice) or splenic GC B cells sorted upon NP-CGG-immunization (n=7-8 individual mice).

S7A

Intron 5' to Smu Peyer's patches GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8692	167 611	143 504 465	1,17	8690	151 915	100 956 853	1,50
8649	45 504	48 022 564	0,95	8645	54 050	38 428 176	1,40
8753	143 998	96 361 943	1,50	8756	311 660	148 421 088	2,10
8755	182 738	122 821 454	1,49	9601	144 996	73 409 771	1,98
8644	109 695	63 612 833	1,72	9604	163 545	78 032 498	2,10
7128	250 366	152 000 328	1,65	9877	162 887	85 164 912	1,91
7129	176 860	139 941 043	1,26	9880	113 784	87 909 626	1,29
9881	152 289	86 749 376	1,76	7111	485 120	187 778 619	2,58
				7112	252 555	121 402 038	2,08
Total	1 229 061	853 014 006	1,44	Total	1 840 512	921 503 581	1,88

S7B

Intron 5' to Smu NP CGG Splenic GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8139	19 700	111 850 026	0,18	6854	26 279	142 403 067	0,19
6858	48 673	211 877 661	0,23	6857	56 795	146 983 925	0,39
6859	8 202	129 181 296	0,06	6864	30 662	52 155 066	0,59
6863	7 520	132 861 196	0,06	6687	106 971	229 058 581	0,47
6692	25 891	179 825 690	0,14	6691	101 092	256 700 586	0,39
6693	109 022	217 449 208	0,50	6791	33 527	118 595 323	0,28
2544	67 300	178 614 933	0,38	2537	112 115	197 775 331	0,57
Total	286 308	1 161 660 010	0,22	Total	467 441	1 143 671 879	0,41

S7C

Intron 3' to J _H 4 Peyer's patches GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8692	57 561	7 906 004	7,28	8690	621 327	82 879 451	7,50
8649	654 842	100 297 965	6,53	8645	328 574	32 909 115	9,98
8753	4 064 096	339 723 680	11,96	8756	106 623	13 743 216	7,76
8755	218 499	26 244 087	8,33	9601	1 718 084	140 896 971	12,19
8644	136 754	10 382 456	13,17	9604	1 866 361	127 867 165	14,60
8646	475 950	58 858 803	8,09	9605	2 112 619	142 861 789	14,79
9881	195 215	21 355 525	9,14	9661	2 550 716	146 382 941	17,42
				9877	757 522	57 471 525	13,18
				9880	1 614 250	119 121 342	13,55
Total	5 802 917	564 768 520	9,21	Total	10 061 826	745 012 173	12,33

S7D

Intron 3' to J _H 4 NP CCG Splenic GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8139	152 171	39 872 610	3,81	8133	137 111	28 459 587	4,82
6858	84 367	38 653 923	2,18	6854	252 934	92 925 446	2,72
6859	125 264	78 908 567	1,59	6857	141 510	37 358 940	3,79
6863	153 921	58 613 945	2,62	6864	335 616	40 332 921	8,32
6692	317 757	68 343 790	4,65	6687	777 955	152 063 704	5,12
6693	270 476	59 540 608	4,55	6691	551 228	96 233 795	5,73
2544	151 910	41 648 331	3,64	6791	249 260	54 111 722	4,61
				2537	176 658	32 694 946	5,40
Total	1 255 866	385 581 774	3,29	Total	2 622 272	534 181 061	5,06
Total	1 255 866	385 581 774	3,29	Total	2 622 272	534 181 061	5,06

S7E

Intron 3' to Jk5 Peyer's patches GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8692	64 352	8 109 925	7,94	8690	977 250	109 513 034	8,92
8649	187 594	18 973 865	9,89	8645	133 351	11 622 636	11,47
8753	41 647	2 737 783	15,21	8756	1 092 197	76 790 583	14,22
8755	2 497 183	288 443 955	8,66	9601	1 097 124	100 505 774	10,91
8644	400 715	30 094 611	13,32	9604	1 941 371	110 394 569	17,59
9881	958 245	97 655 675	9,81	9605	779 895	62 212 383	12,54
7128	1 571 163	146 409 208	10,73	9877	820 899	65 692 001	12,50
7129	540 065	97 174 751	5,56	9880	2 136 606	163 662 781	13,05
				7111	963 266	90 045 963	10,70
				7112	1 256 410	223 536 225	6,07
Total	6 260 964	689 599 773	10,14	Total	6 842 087	536 730 980	11,80

S7F

Intron 3' to Jk5 NP CCG Splenic GC							
Satb1 ^{flx/+}				Satb1 ^{flx/flx} Cd79a ^{cre/+}			
N°	number of mutations	total number of pb analyzed	Frequency (mutation/Kb)	N°	number of mutation	total number of pb analyzed	Frequency (mutation/Kb)
8139	222 655	85 014 498	2,62	6854	171 154	69 778 869	2,45
6858	72 960	56 300 498	1,30	6857	271 677	110 895 960	2,45
6859	2 008	1 253 443	1,60	6864	280 371	67 161 110	4,16
6863	121 103	93 266 492	1,30	6687	371 949	94 961 964	3,92
6692	196 340	96 679 417	2,03	6691	249 263	71 580 665	3,48
6693	323 874	88 558 220	3,66	6791	247 551	102 051 591	2,43
2544	731 196	178 302 885	4,10	2537	622 428	91 007 705	6,84
Total	1 670 136	599 375 453	2,37	Total	2 214 393	607 437 864	3,68