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Supplemental Methods

Acquisition of tissues from human organ donors

Human tissues were obtained from deceased organ donors at the time of organ acquisition for clinical transplantation as previously described, and through an approved research protocol and MTA with LiveOnNY, the organ procurement organization for the New York metropolitan area. The information on donors used in this study is presented in Supplemental Table 1. All donors were previously healthy, were free of cancer, and were serologically negative for Hepatitis B, C, and HIV. Isolation of tissues from organ donors does not qualify as “human subjects” research, as confirmed by the Columbia University IRB.

Flow cytometric analysis of cryopreserved samples

Cryopreserved cells of spleen, blood, bone marrow or intestinal tissue were thawed at 37°C and instantly passed through a 70µm cell strainer into RPMI medium containing 10% FCS. After centrifugation cells were resuspended in HBSS +Ca/+Mg, containing 45U/ml DNaseI (Sigma) and 2% FCS. The reaction was stopped by adding 4mM EDTA to the cells. 3-4x10^6 cells per 1.2ml-microtube were stained in a volume of 200µl HBSS -Ca/-Mg with 3% FCS and 2mM EDTA (referred to as staining buffer (SB)). All antibodies and staining reagents used in this study are listed in Supplemental Tables 2 and 3. For the experiments described in figures 1 and 2 all 33 spleen samples were stained with “Stain 1” (Supplemental Table 2). Fixable viability stain (FVS) 510 (Beckton Dickinson) or Ghost 510 (Tonbo Biosciences) were used in a separate step for 15

minutes at room temperature to exclude dead cells. The blood cells of 16 donors, bone marrow cells from 16 donors and intestinal cells of 11 donors were stained with the same 10-color flow panel, 2 additional blood and 6 intestinal tissue samples were stained with “Stain 2”. For latter flow panel FVS700 (Beckton Dickinson) was used to exclude dead cells. For all panels the antibody staining was performed for 40 min on ice in the dark. To detect biotinylated antibodies, after washing, an incubation with Streptavidin-BUV737 for 15 minutes on ice was performed. Cells were then fixed in 1% PFA for 30min before analysis on a BD LSRII. Data analysis was performed in FlowJo v.9 (Treestar). For the experiment described in supplemental figure 3 a total of 9x spleen, 8x blood and 7x intestinal tissue cells were stained with “Stain 4”. For this panel ZombieNIR was used to exclude dead cells. After fixation in 1% PFA cells were analysed on the Cytek Aurora and data analysis perfomed in FlowJo v.10 (Treestar).

Functional analysis of CD45RB/ CD69 subsets in spleen.

Cryopreserved cells were thawed as previously described and stained with CD19-APC-Vio770, CD27-BUV737, CD45RB-FITC and CD69-BV510 (Supplemental Table 3) for 40min on ice in the dark. For exclusion of dead cells 7-AAD was added to the samples right before FACS-sorting on an BD AriaII. Defined CD45RB/ CD69 subsets from $CD19^+$ $CD27^-$ (naïve) and $CD19^+$ $CD27^+$ (memory) B cells were individually sorted into 1ml RPMI 1640 (Corning), containing 10% heat-inactivated FBS (Gemini), 1x Penicillin/ Streptomycin, 10mM HEPES, 2mM L-Glutamin (all Gibco) and 0.05mM β-Mercaptoethanol (Sigma) (in future referred to as R10). After sorting centrifugation and counting of the cells was performed and equal cell numbers of the different CD45RB/

CD69 subsets seeded into 96-well plates in a volume of 100µl R10 each. Cells were stimulated with 1µg/ml ODN2006 (5'-tcgtcgttttgtcgttttgtcgtt-3') by adding 100µl 2x ODN2006 prepared in R10 and incubated at 37°C and 5% CO_2. Cells were harvested on day 5 and divided into flow cytometrical analysis and ELISpot assay. Cells were pipetted up and down in wells and transferred to a 1.2ml microtube, volume was measured and half of it transferred to a second 1.2ml microtube. After centrifugation one tube was resuspended in 200µl SB containing antibodies CD86-PE, CD25-PE-Dazzle594, CD38-BUV395, IgM-PE-Cy5, CD19-APC-Vio770 and CD27-BUV737 (Supplemental Table 3) for flow cytometric analysis. Staining was performed for 40min on ice in the dark. For live/ dead exclusion fixable viability stain FVS700 (BD) was used for 15min at room temperature. Cells were then fixed in 1% PFA for 30min before analysis on a BD LSRII. Data analysis was performed in FlowJo v.9 software (Treestar). The second tube was resuspended in R10 medium and used for ELISpot assay. Statistical analysis of functional data: In each case, we compared the 5 cell populations (naïve DN, CD45SP, 45/ 69 DP, 45/ 69 DN, CD69SP) in a pairwise fashion (i.e., a total of 10 comparisons). For each pairwise comparison, we first calculated a P value using a paired t-test. We then computed corresponding q values by adjusting the P values for multiple comparisons using a Bonferroni correction. Using a paired test accounts for different donor-specific baselines and evaluates whether there is a difference between the different cell populations after accounting for these differential baselines (i.e., looks at donor-specific relative changes).

ELISpot assay

After coating 4-HBX plates overnight at 4°C with 5µg/ml anti-human lambda and kappa (Southern Biotech) each in 1xPBS, they were washed three times with PBS/ 0,05% Tween20 followed by two times with distilled water. Wells were blocked with 200µl 1xPBS/ 5% heat-inactivated FBS for 2h at room temperature or overnight at 4°C. Afterwards they were washed twice with distilled water and 200µl R10 were added to each well. Plates were incubated at 37°C until plating cells. Cells were harvested as described above and 100µl cells per well added to row A for according sample. Duplicates for IgG and IgM were diluted in a 1:3 series by transferring 100µl to next row with multichannel pipet. Last row of each plate didn´t receive cells and is considered blank. After a short spin cells were incubated at 37°C and 5% CO_2 for 5h. After another washing step with PBS/ Tween and distilled water 50µl secondary antibody anti-human IgG-AP or anti-human IgM-AP (both Sigma) were added to each well. Plates were incubated overnight at 4°C. Another washing cycle was followed by developing the plates. Therefore BCIP in solution was mixed with 3% low-melt agarose and 40µl added per well. Plates were cooled down for 60min at room temperature and developed overnight at 4°C flipped upside down. Within 24h spots were counted and used for analysis.

RNA sequencing

Cryopreserved cells of spleen and ileum were thawed and stained with CD19-APC-Vio770, CD27-BV421, CD45RB-FITC and CD69-BV510 (Supplemental Table 3). For exclusion of dead cells 7-AAD was added to the samples right before FACS-sorting on

an BD AriaII. Defined CD45RB/ CD69 subsets from $CD19^+$ $CD27^-$ (naïve) and $CD19^+$ $CD27^+$ (memory) B cells were individually sorted into RNA-protect® Cell Reagent (Qiagen 76526), washed with PBS and resuspended in RLT-buffer (Qiagen) containing 1% β-ME. After running through a shredder-column (Qiagen QIAshredder 79656) samples were frozen as RLT lysates at -80°C until RNA was isolated using RNAeasy micro or mini kits (Qiagen). Libraries were prepared using the Illumina TruSeq Total RNA kit with ribosomal depletion or the SMART-Seq v4 Ultra Low Input RNA Kit for sequencing and Illumina Nextera XT DNA Library preparation kit following the manufacturer's protocol. Cluster generation and 75 bp Paired-read dual-indexed sequencing was performed on Illumina NextSeq 500 at the Health Sciences Sequencing Core in Pittsburgh or on a HiSeq 2500 sequencer at the Sulzberger Genome Center in NY. RNA-seq data uploaded to GEO (accession code GSE154583).

RNA sequencing analysis

Reads were mapped to the human reference genome build hg19 using STAR [46] (version 2.3.0e) with default parameters. FeatureCounts [47] was used to compute the number of reads mapped to each gene with options "-s 0 -t exon -g gene_name". Downstream statistical analysis was performed with R programming language. DESeq2 [48] was used for differential gene expression analysis, using batch and donor information as covariates to control confounding effects. Genes were considered as significantly differentially expressed between two groups if FDR≤0.05 and absolute value of log2 fold change >1. For heatmap visualization, gene read counts were first normalized with DESeq2 [48] for library depth, and then sequencing batch effect and donor difference

effect removed with function ComBat in the sva [49] package. Principal component analysis (PCA) was performed using function *prcomp* in R, with centering, scaling, and cor options on. Heatmaps were plotted with Z-score of log base 10 normalized read counts across samples as input with the *pheatmap* package.

For GSEA analysis in Supplemental Figure 4, plasmablast gene signatures are the top 200 genes ordered by log fold change from GenomicScape (http://www.genomicscape.com/microarray/nbtopc.php) and plasma cell signatures were from ref. [50]. Statistical analysis is described in the figure legend.

Supplemental references:

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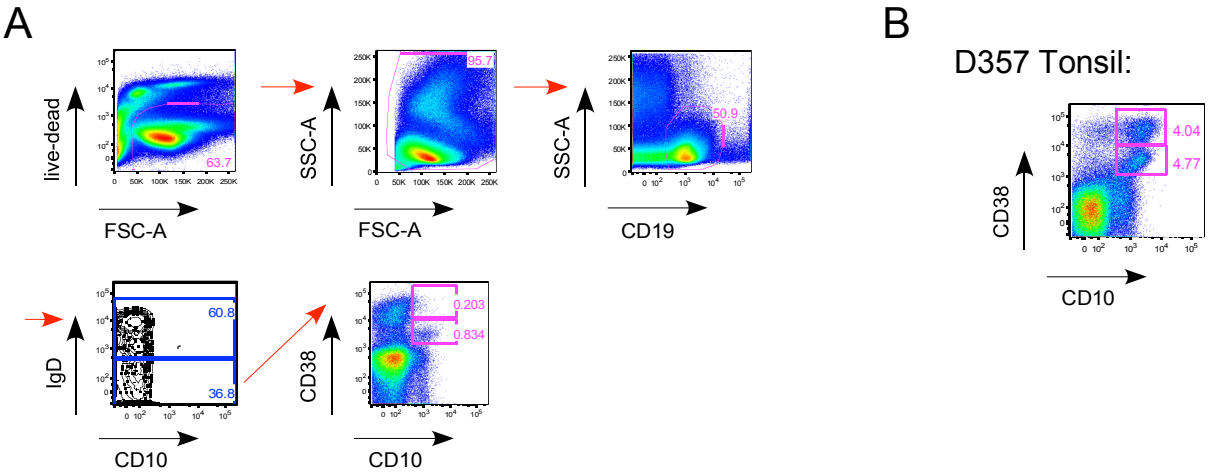
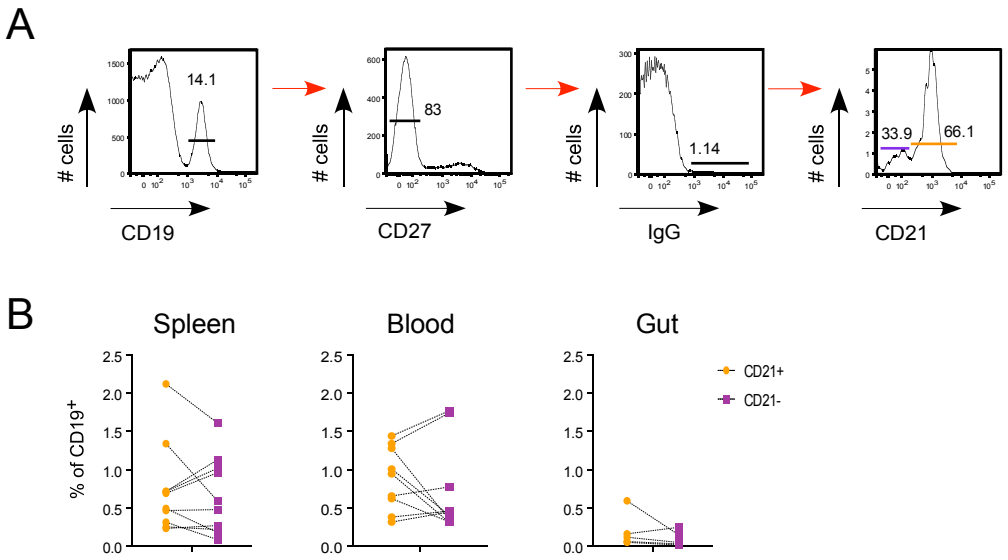
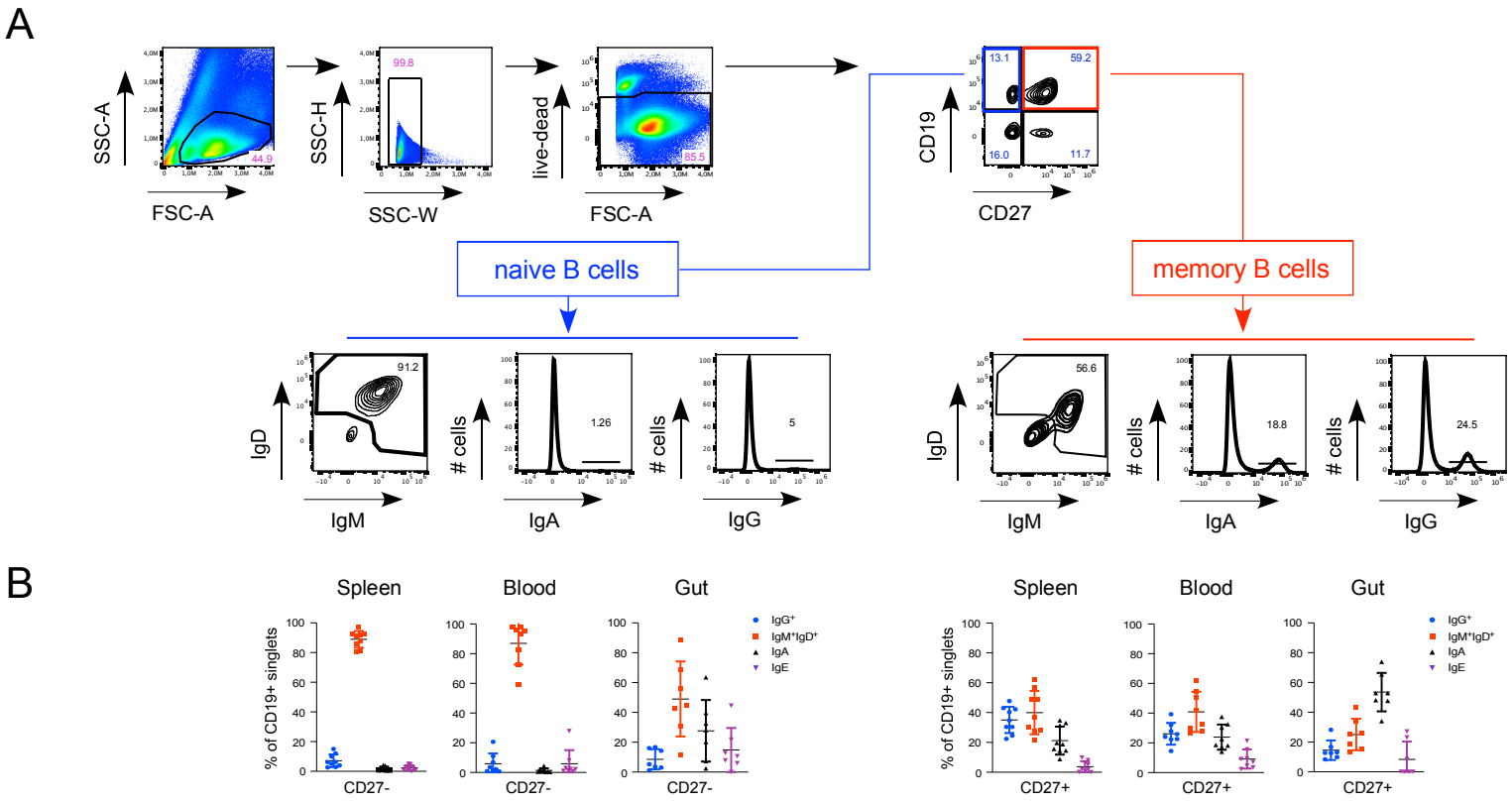
50. L. M. Valor, B. Rodríguez-Bayona, A. B. Ramos-Amaya, J. A. Brieva, and A. Campos-Caro. The transcriptional profiling of human in vivo-generated plasma cells identifies selective imbalances in monoclonal gammopathies. *PLoS ONE* **12** (8), e0183264 (2017).



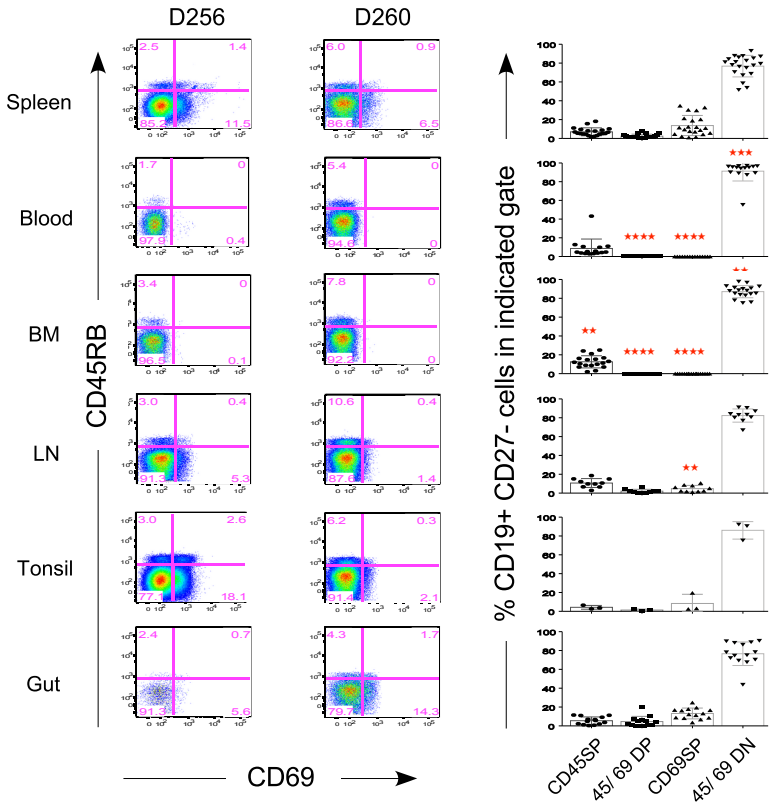
Supplemental Figure 1: Gating strategy for germinal center B cells in tissues. (A) Gating strategy modified from Milpied et al. used for flow cytometric analysis using the 10color-screening panel “Stain 1" for detection of germinal center B cells within splenocytes of D230. Arrows indicate subsequent gating of populations and numbers next to outlined areas indicate percent gated populations. Analyzed were live singlets which are $CD19^{+}$, IgD^{-}, $CD10^{+}$, and either $CD38^{int}$ (0.834%) or $CD38^{hi}$ (0.203%). (B) Gated as (A) from tonsil of donor D357 as positive control.



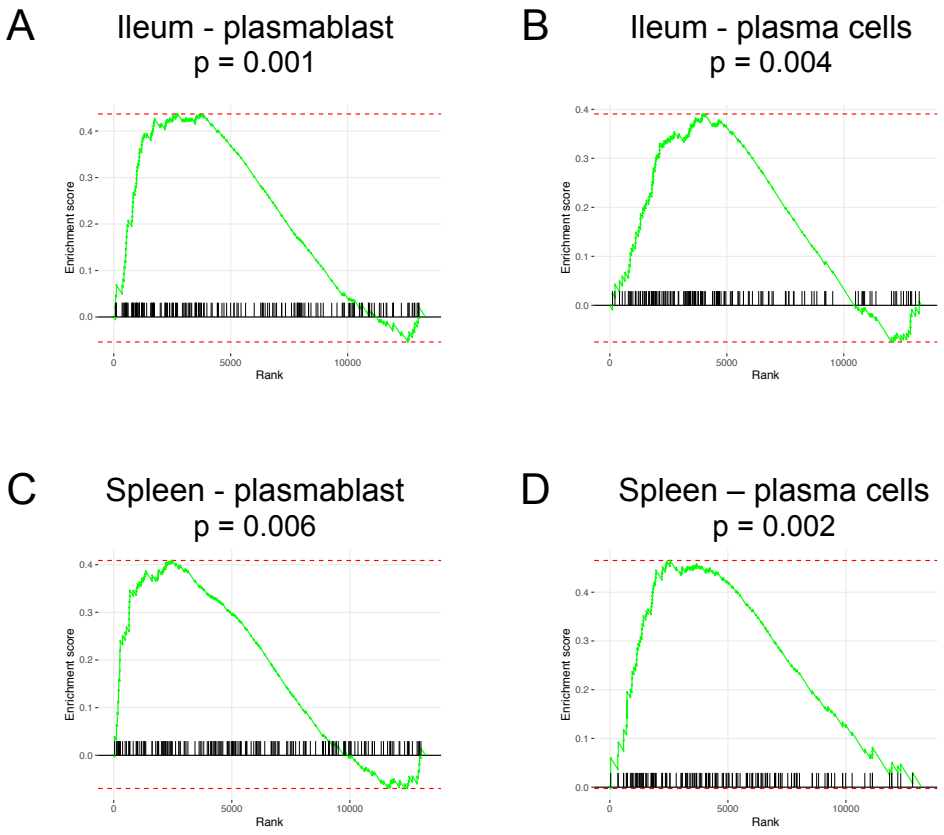
Supplemental Figure 2: Gating strategy for atypical MBC in tissues. (A) Gating strategy used for flow cytometric analysis of panel "Stain 2" (Supplemental Tables 2 & 3) for detection of atypical MBC in blood of D260 as example. Arrows indicate subsequent gating of populations and numbers next to outlined areas are percent gated populations. Analyzed were all live, single cells (see gating in Supplemental Figure 1) that are $CD19^+$, $CD27^-$, IgG^+, and either $CD21^-$ (33.9%; atypical MBC) or $CD21^+$ (66.1%). (B) Following the gating strategy in (A), a total of 10x spleen, 9x blood and 4x intestinal tissue samples were analyzed. The frequencies of $CD21^+$ (orange circles) and $CD21^-$ (purple squares) cells are shown as percentage of live $CD19^+$/ $CD27^-$/ IgG^+ with lines linking individual samples.



Supplemental Figure 3: Flow cytometric analysis of isotype distribution among NBC and MBC within tissues. (A) Gating strategy for flow cytometric detection of isotype distribution among splenic NBC (left, $CD27^-$) and MBC (right, $CD27^+$) of D215 using panel "Stain 4" (see supplemental table 2 & 3). Arrows indicate subsequent gating of populations and numbers next to outlined areas indicate percent gated populations. Analyzed were live singlets which are $CD19^+$, $CD27^-$ NBC (blue) or $CD27^+$ MBC (red, top panel) and either IgM^+IgD^+, IgA^+ or IgG^+ (lower panel). (B) Surface immunoglobulin isotype distribution of indicated human organ donor samples of NBC (left) and MBC (right). "Inferred" IgE frequencies of MBC across tissues are plotted as difference from 100% using the sum of measured IgM/IgD, IgG and IgA frequencies.



Supplemental Figure 4: Subsets defined by CD45RB and CD69 for CD19$^+$ CD27$^-$ B cells of different human tissue samples. Cryopreserved human cells of spleen, blood, bone marrow, lymph nodes, tonsil or intestinal tissue were thawed and stained as previously described. Results are a summary of flow panels “Stain1 “ and “Stain 2". Cells were aquired on a BD LSRII and live singlets were analyzed using FlowJo (Treestar). The left side of the graph shows the subsets defined by CD45RB and CD69 for CD19$^+$ CD27$^-$ (naive) B cells of the depicted tissues samples of D256 and D260 as dot plot diagrams. Numbers next to the outlined areas indicate the percent gated populations. The right side of the graph shows the percentages of CD19$^+$ CD27$^-$ cells in all four defined subsets (SP=single positive; DP=double positive; DN=double negative) discovered in different tissue samples. The results show the summary of 21 spleen, 15 blood, 18 bone marrow (BM), 10 lymph node (LN), 3 tonsil and 14 intestinal tissue (Gut) samples analyzed. Stars indicate significant differences of percentages found by using the unpaired two-tailed t-test for the CD45RB/ CD69 subsets in other tissue samples compared to the same subset in spleen (★★ p<0.005; ★★★ p<0.0005; ★★★★ p<0.0001).



Supplemental Figure 5: Gene set enrichment analysis (GSEA) of CD45RB/ CD69 subsets of spleen and gut. RNA sequencing of naive and MBC subsets of cells as in Figure 6. GSEA plots illustrating significant upregulation of plasmablast (**A**) and plasma cell (**B**) gene signatures in ileum DP MBC vs. splenic DN naive B cells. GSEA plots as in A and B showing significant upregulation of plasmablast (**C**) and plasma cell (**D**) gene signatures in splenic DP MBC compared to splenic DN naive B cells. In each plot, the x-axis shows the genes ranked by log fold change between the populations described above, and the y-axis shows the running enrichment score on the signature gene set with nominal p value indicated for 1000 permutations.

Supplemental Table 1. List of donors used in this study.

Donor ID	Age	Sex	Race	Sample	Data	1	2	3	4	5	6	S1	S2	S3	S4	S5
138	35	M	White	Spleen	Flow	X	X									
144	60	M	White	Spleen	Flow	X	X									
145	58	M	White	Spleen	Flow	X	X									
147	36	F	Black	Spleen	Flow	X	X									
149	55	M	White	Spleen	Flow	X	X									
153	46	M	Hispanic	Spleen	Flow	X	X		X						X	
154	54	M	Hispanic	Spleen	Flow	X	X									
159	57	F	White	Spleen, Blood, BM, Gut	Flow	X	X	X	X						X	
160	55	M	Black	Spleen, Blood, BM	Flow	X	X									
163	53	F	Black	Spleen	Flow	X	X									
164	58	F	Black	Spleen, Blood, BM, Gut	Flow	X	X	X	X						X	
168	56	F	Hispanic	Spleen, Blood, BM	Flow	X	X	X	X						X	
169	33	M	White	Spleen	Flow	X	X		X						X	
178	51	M	Black	Blood, BM	Flow		X									
181	46	M	Black	Spleen, Blood, BM	Flow	X	X	X	X						X	
182	46	M	Hispanic	Spleen, Blood, BM, Gut	Flow	X	X	X	X						X	
183	69	F	Black	Spleen	Flow	X	X									
185	57	M	White	Spleen	Flow	X	X		X						X	
186	35	M	White	Spleen, Blood, BM	Flow	X	X	X	X					X	X	
193	50	M	Hispanic	Spleen, Blood, BM	Flow, RNA-seq	X	X	X	X		X			X	X	X
197	70	F	Hispanic	Spleen, Blood, Lymph Node	Flow	X	X		X						X	
205	45	F	Hispanic	Spleen, Blood, BM, Lymph Node	Flow	X	X	X	X					X	X	
215	45	M	Hispanic	Spleen, Blood, BM, Gut	Flow	X	X	X	X				X	X	X	
217	49	M	Black	Spleen, Blood, BM	Flow	X	X		X						X	
226	66	F	White	Spleen, Blood, BM, Lymph Node, Gut	Flow	X	X		X					X	X	
228	69	M	White	Spleen, Blood, BM, Lymph Node, Gut	Flow	X	X		X				X		X	

Donor ID	Age	Sex	Race	Sample	Data	1	2	3	4	5	6	S1	S2	S3	S4	S5
230	52	F	Hispanic	Spleen, Blood, BM, Lymph Node, Gut	Flow	X	X		X			X		X	X	
243	67	F	White	Spleen, Blood, BM, Lymph Node	Flow	X	X	X	X						X	
246	73	M	White	Spleen, Blood, BM, Lymph Node	Flow	X	X	X	X					X	X	
256	70	M	Hispanic	Spleen, Blood, BM, Lymph Node, Gut, Tonsil	Flow, RNA-seq	X	X	X	X		X		X	X	X	X
260	34	M	Hispanic	Spleen, Blood, BM, Lymph Node, Gut, Tonsil	Flow, RNA-seq	X	X	X	X		X		X	X	X	X
269	61	M	White	Spleen, Blood, BM	Flow, ELISpot	X	X	X	X	X					X	
305	28	F	White	Spleen, Gut	Flow, RNA-seq	X	X		X		X				X	X
315	63	M	Hispanic	Spleen	Flow, ELISpot	X	X		X	X					X	
424	71	F	Black	Spleen	Flow, ELISpot					X						
443	47	F	Black	Spleen	Flow, ELISpot					X						
462	76	F	Asian	Spleen	Flow, ELISpot					X						
124	26	M	Black	Gut	Flow	X	X		X						X	
209	59	M	White	Tonsil	Flow				X					X	X	
214	30	M	White	Gut	Flow	X	X		X						X	
219	50	M	Hispanic	Gut	Flow				X						X	
221	29	M	Hispanic	Gut	Flow				X						X	
290	77	F	Black	Spleen, Blood, Gut	Flow	X	X		X				X	X	X	
300	56	M	Hispanic	Spleen, Blood, Gut	Flow	X	X		X					X	X	
328	52	M	Hispanic	Gut	Flow	X	X									
357	18	F	Black	Tonsil, Gut	Flow	X	X					X				
371	62	M	Black	Gut	Flow	X	X									
192	78	M	White	Spleen, Blood, Gut	Flow								X			
333	19	M	Unknown	Spleen, Blood	Flow								X			

Donor ID	Age	Sex	Race	Sample	Data	1	2	3	4	5	6	S1	S2	S3	S4	S5
365	28	F	Hispanic	Spleen, Blood	Flow								X			
388	49	M	Asian	Spleen, Blood, Gut	Flow								X			

This table shows the donor samples used for the separate figures 1-6 and supplementary figures 1-5 with ID, age in years, sex, M = male, F = female, race and a list of the different samples analyzed. Behind that is depicted the data generated, flow, RNA-sequencing = RNA-seq or ELISpot.

Supplemental Table 2. Antibody staining cocktails for flow cytometry.

Stain 1	Stain 2	Stain 3	Stain 4
CD19-APC-Vio770	CD19-APC-Vio770	CD19-APC-Vio770	CD19-APC-Vio770
CD27-BV421	CD27-BV421	CD27-BV421	CD27-BV421
IgD-PE-Cy7	IgD-PE-Cy7	IgD-PE-Cy7	IgD-PE-Cy7
CD10-PE-Cy5	IgM-PE-Cy5	IgM-APC	IgM-PE-Cy5
CD38-BUV395	CD21-PerCP-Cy5.5	CD21-PerCP-Cy5.5	CD21-PerCP-Cy5.5
CD138-APC	CD69-BV510	CD69-BV510	CD69-BV510
CD95-FITC	CD45RB-FITC	CD45RB-FITC	CD45RB-FITC
IgM-PE	IgA-PE	IgA-PE	IgA-PE
IgG-bio	IgG-bio	IgG-bio	IgG-bio
+ SA-BUV737	+ SA-BUV737	+ SA-BUV737	+ SA-BUV737
FVS/ Ghost 510	FVS 700	FVS 700	ZombieNIR

Listed are the antibodies used for the different flow cytometric staining panels.

Supplemental Table 3. List of antibodies used.

Antibody	Company	Catalog Number	Clone
CD19-APC-Vio770	Miltenyi	130-096-643	LT19
CD27-BV421	BioLegend	302824	O323
IgD-PE-Cy7	BD Pharmingen	561314	IA6-2
IgM-PE-Cy5	BD Pharmingen	551079	G20-127
CD38-BUV395	BD Horizon	563811	HB7
CD21-PerCP-Cy5.5	BioLegend	354908	Bu32
CD69-BV510	BioLegend	310936	FN50
CD45RB-FITC	Thermo Scientific	MA-1-19571	MEM-55
IgA-PE	Southern Biotech	2050-09	
IgG-bio	BD Pharmingen	555785	G18-145
Streptavidin-BUV737	BD Horizon	564293	
CD10-PE-Cy5	BD Pharmingen	555376	HI10a
IgM-APC	BD Pharmingen	551062	G20-127
CD138-APC	BioLegend	352308	DL-101
CD95-FITC	BioLegend	305606	DX2
IgM-PE	BD Pharmingen	555783	G20-127
CD27-BUV737	BD Horizon	564301	L128
CD86-PE	BioLegend	305406	IT2.2
CD25-PE-Dazzle594	BioLegend	356126	M-A251
anti-human-lambda	Southern Biotech	2071-01	
anti-human-kappa	Southern Biotech	2061-01	
anti-human-IgG-AP	Sigma-Aldrich	A3187	
anti-human-IgM-AP	Sigma-Aldrich	A3275	
Fixable Viability Stain 700	BD Horizon	564997	
Fixable Viability Stain 510	BD Horizon	564406	
Ghost Dye Violet 510	Tonbo	13-0870	
ZombieNIR	BioLegend	423105	

The table shows all used antibodies with fluorochrome, company name, catalog number and clone name.