

Supplemental Information

LAG-3 Inhibitory Receptor Expression Identifies

Immunosuppressive Natural Regulatory Plasma Cells

Andreia C. Lino, Van Duc Dang, Vicky Lampropoulou, Anna Welle, Jara Joedicke, Jelka Pohar, Quentin Simon, Jessie Thalmensi, Aurelia Baures, Vinciane Flühler, Imme Sakwa, Ulrik Stervbo, Stefanie Ries, Luc Jouneau, Pierre Boudinot, Takeshi Tsubata, Takahiro Adachi, Andreas Hutloff, Thomas Dörner, Ursula Zimmer-Strobl, Alex F. de Vos, Katja Dahlke, Gunnar Loh, Sarantis Korniotis, Christian Goosmann, Jean-Claude Weill, Claude-Agnès Reynaud, Stefan H.E. Kaufmann, Jörn Walter, and Simon Fillatreau

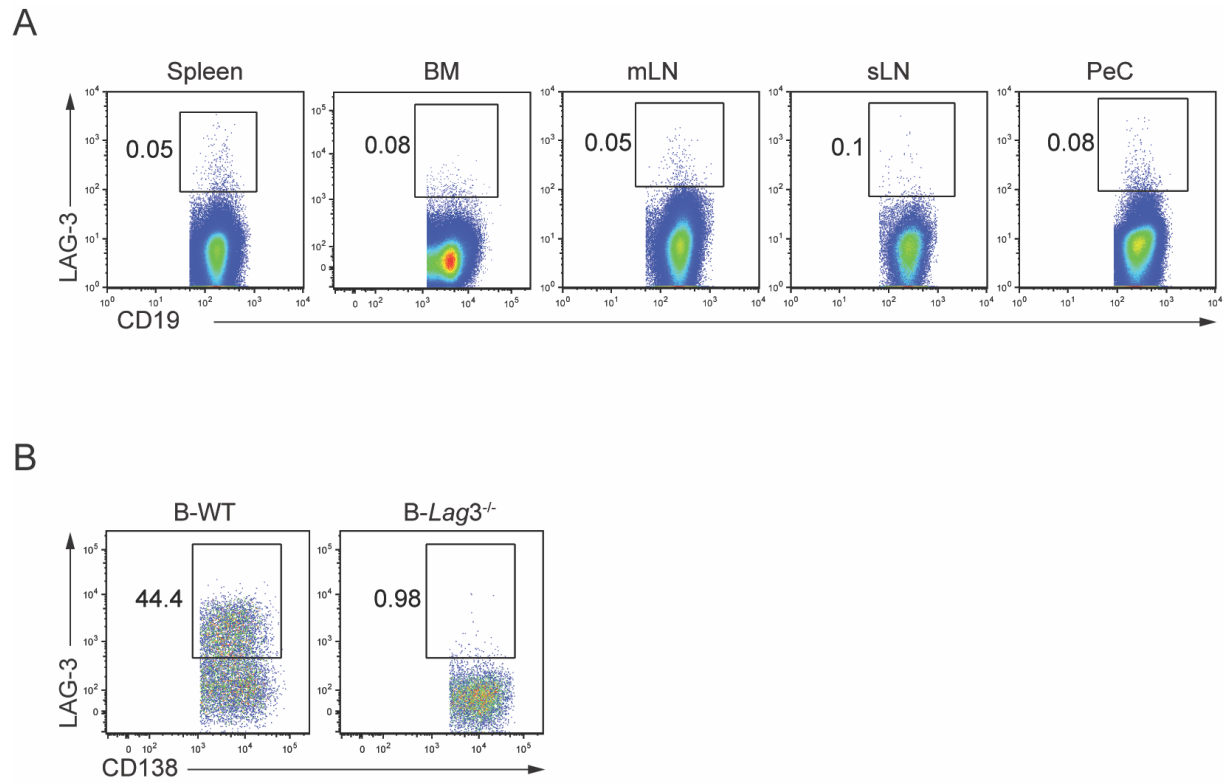
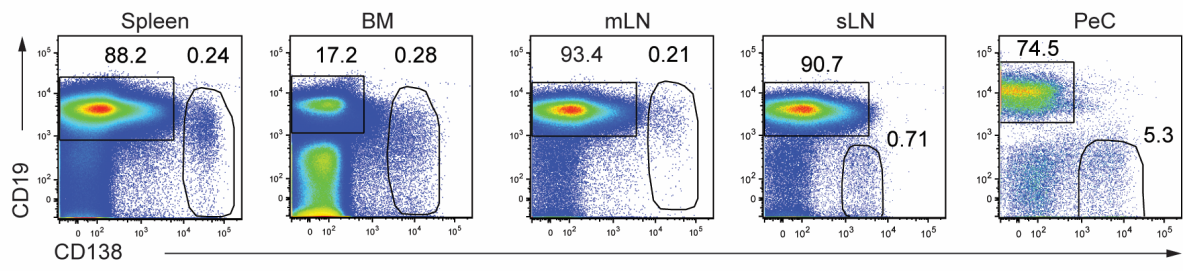


Figure S1: Expression of LAG-3 by B cells and plasmacytes in *Salmonella* infected mice, Related to Figure 1.

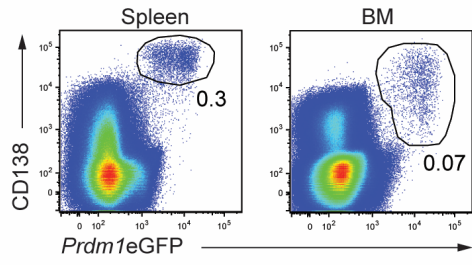
(A) Representative flow cytometry plots showing the expression of LAG-3 on CD19⁺CD138⁻ B cells from C57BL/6 mice in spleen, BM, mLN, sLN, and PeC on day 1 p.i. with *Salmonella* (SL7207, 10⁷ CFU).

(B) Representative flow cytometry plots showing the expression of LAG-3 on splenic CD138^{hi} cells of B-WT and B-*Lag3*^{-/-} mice on day 1 p.i. with *Salmonella* (SL7207, 10⁷ CFU).

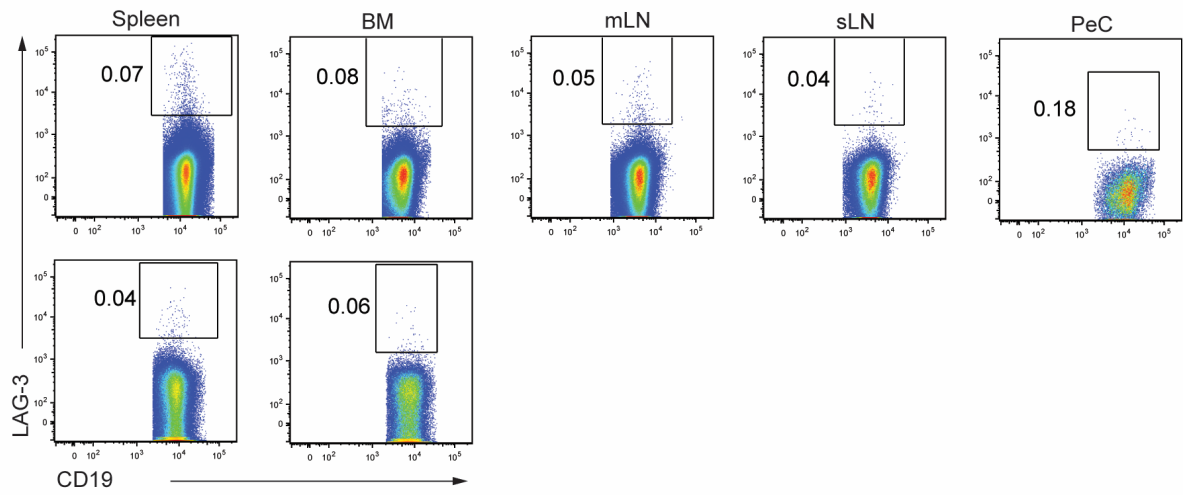
A



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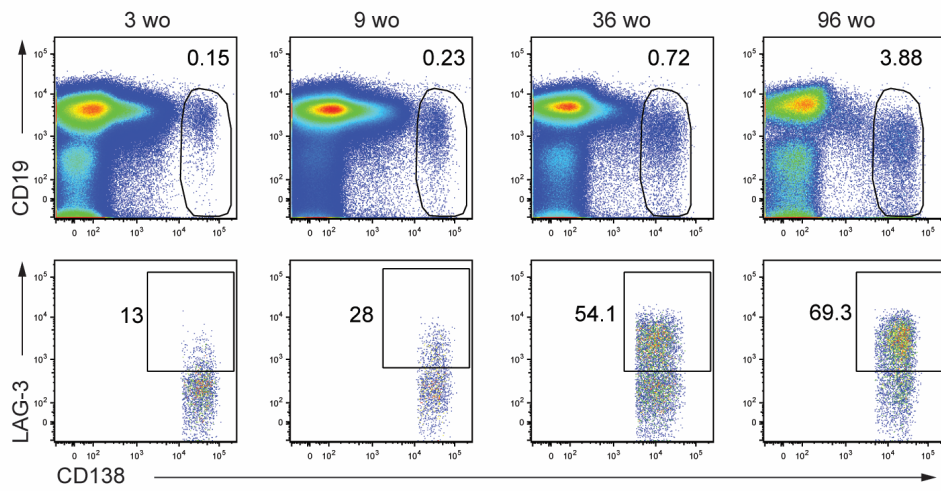


Figure S2: Presence of LAG-3-expressing plasma cells in naïve mice, Related to Figure 2.

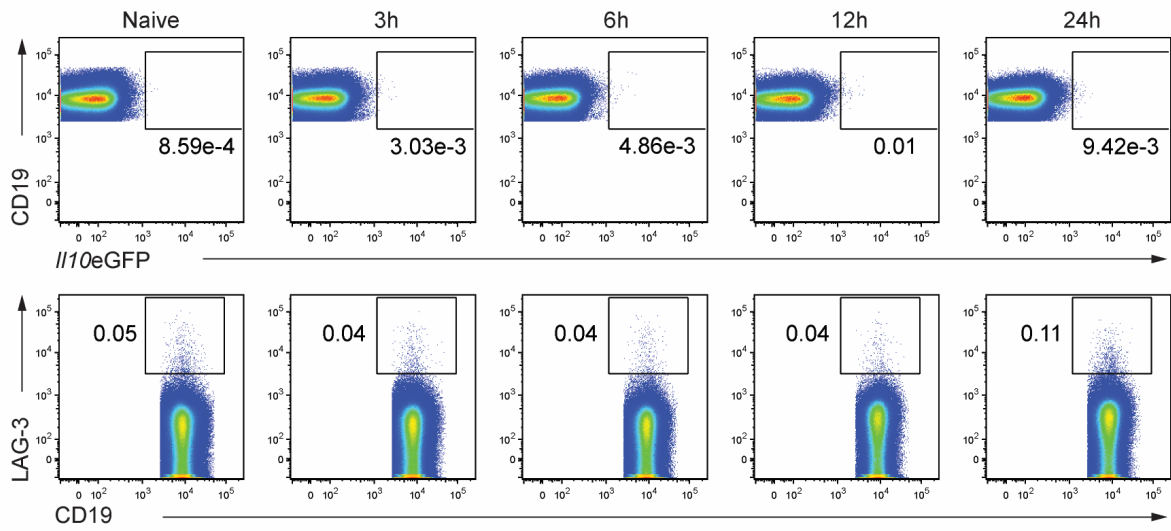
(A) Representative flow cytometry plots showing the expression of CD19 and CD138 on live CD4⁺CD8⁻ lymphocytes in the indicated tissues. The gates identify CD19⁺CD138⁻ B cells characterized in Figure S2C, and CD138^{+/hi} plasmocytes characterized in Figure 2A, for spleen, BM, mLN, sLN and PeC of naïve C57BL/6 mice.

(B) Representative flow cytometry plots showing the expression of CD138 and *Prdm1e*GFP on live CD4⁺CD8⁻ lymphocytes from spleen and BM of naïve *Prdm1e*GFP mice. The gates identify CD138^{+/hi}*Prdm1e*GFP⁺ plasmocytes characterized in Figure 2B.

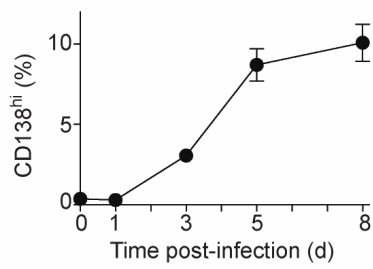
(C) Representative flow cytometry plots showing the expression of LAG-3 on CD19⁺CD138⁻ B cells in spleen, BM, mLN, sLN, and PeC from naïve C57BL/6 mice (top), as well as in spleen and BM from naïve *Lag3*^{-/-} mice (bottom).

(D) Representative flow cytometry plots showing the expression of CD19 and CD138 in live CD4⁺CD8⁻ lymphocytes (top) and the expression of LAG-3 and CD138 in live CD138^{hi} plasmocytes (bottom) from naïve C57BL/6 mice of indicated ages. The gates in top panels indicate CD138^{hi} cells analysed for the expression of LAG-3 and CD138 in bottom panels.

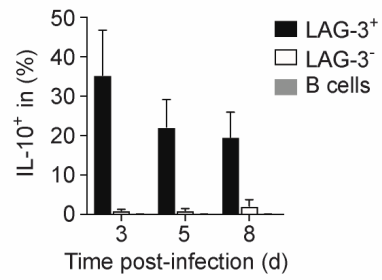
A



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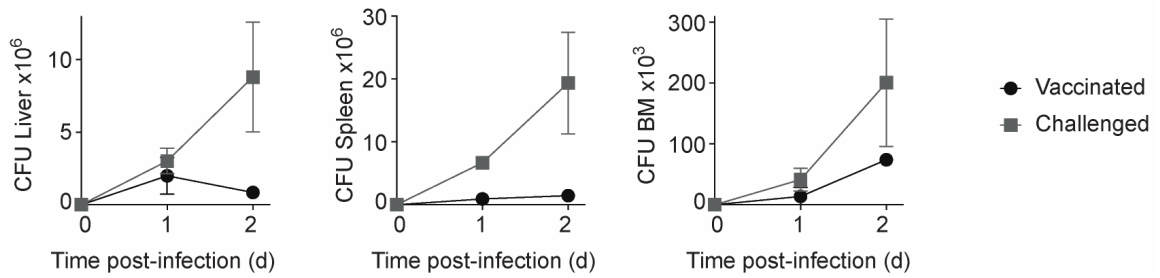


Figure S3: Induction of IL-10 expression in LAG-3⁺CD138^{hi} plasma cells during *Salmonella* infection, Related to Figure 3.

(A) *Il10eGFP* mice were challenged with *Salmonella* (SL7207, 10⁷ CFU), and spleens were analysed at indicated hours p.i. Representative flow cytometry plots showing the expression of *Il10eGFP* and CD19 (top), and the expression of LAG-3 and CD19 (bottom) on live CD19⁺CD138⁻ B cells.

(B) *Il10eGFP* mice were challenged with *Salmonella* (SL7207, 10⁷ CFU). Data show the frequency of CD138^{hi} plasmacytes in CD4⁻CD8⁻ live cells in spleen at indicated days p.i. Pool of two independent experiments with at least 6 mice per group.

(C) *Il10eGFP* mice were challenged with *Salmonella* (SL7207, 10⁷ CFU), and spleens were analyzed at indicated days p.i. Data show frequencies of *Il10eGFP*⁺ cells in CD19⁺CD138⁻ (B cells), LAG-3⁺CD138^{hi} (LAG-3⁺), and LAG-3⁻CD138^{hi} (LAG-3⁻) cells. Pool of two independent experiments with at least 6 mice per group.

(D) *Il10eGFP* mice were vaccinated (SL7207, 10⁶ CFU), and rechallenged 90 days later with *Salmonella* (SL7207, 10⁷ CFU) together with naive age-matched control *Il10eGFP* mice. CFU were determined in liver (left), spleen (middle), and BM (right) at indicated days p.i. Data are pooled from two independent experiments with at least n=6 per group.

Data show mean ± SEM.

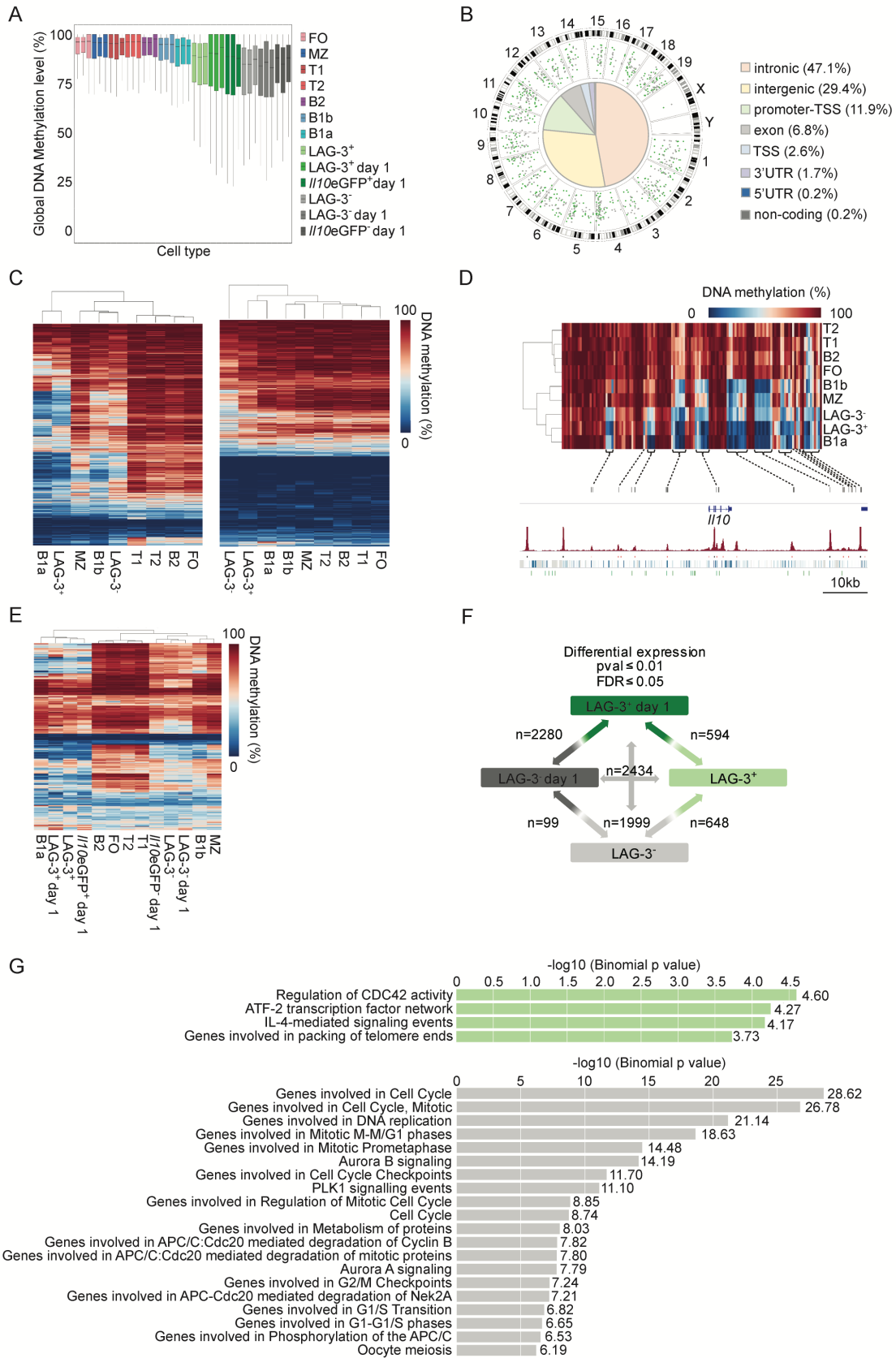


Figure S4. Analysis of DNA methylation and gene expression, Related to Figure 4

(A) Boxplot showing the genome-wide methylation percentage for the indicated B lineage cell subsets.

(B) Circular representation showing the distribution of the 469 DMR identified between LAG-3⁺CD138^{hi} cells and LAG-3⁻CD138^{hi} cells from naïve mice across the chromosomes and the genome. The methylation frequency for each DMR is indicated for LAG-3⁺CD138^{hi} cells (green dots) and LAG-3⁻CD138^{hi} cells (grey dots). The genomic positions of the DMR on the chromosomes are given by their angular value, and their methylation ratio are indicated by their height from the centre (radius). The pie chart shows the global distribution of the DMR within functional genomic regions.

(C) Heat-maps displaying the methylation percentage of each CpG found in the 469 DMR (see Figure 4B) that are hypo-methylated (left) or hyper-methylated (right) in LAG-3⁺CD138^{hi} cells compared to LAG-3⁻CD138^{hi} cells.

(D) Heat-map showing the methylation ratio for all covered CpG positions in the *Il10* locus for the indicated B lineage cell subsets, and additional genomic information, as in Figure 4D. In addition, the location of Egr2 motifs is indicated by green bars.

(E) Heat-map displaying the methylation ratio for the DMR distinguishing LAG-3⁺CD138^{hi} cells and LAG-3⁻CD138^{hi} cells from naïve mice for the indicated B lineage cell subsets.

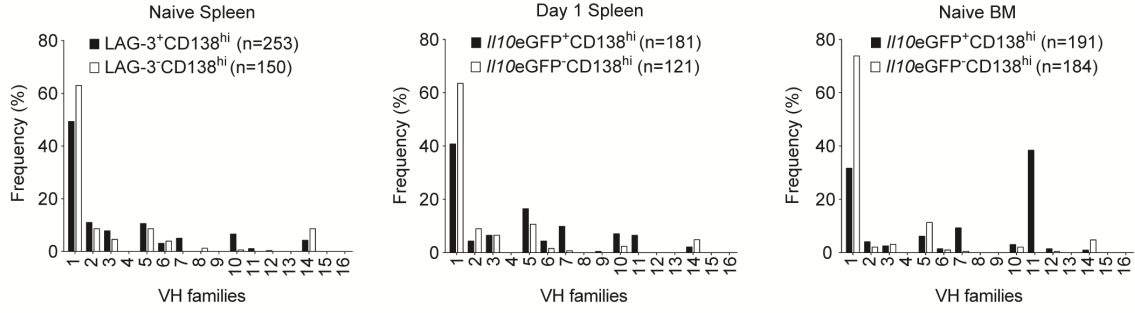
(F) Representation of the numbers of DEG (p-value ≤ 0.01 and FDR ≤ 0.05) for the indicated pairwise comparisons. The union of the ensembles of DEG contains 3631 elements.

(G) Gene set enrichment analysis for the DEG between LAG-3⁺CD138^{hi} cells and LAG-3⁻CD138^{hi} cells from naïve mice (MSigDB Pathway ontology). Upper and lower panels were obtained using the genes expressed at higher amounts in LAG-3⁺CD138^{hi} cells, and LAG-3⁻CD138^{hi} cells, respectively.

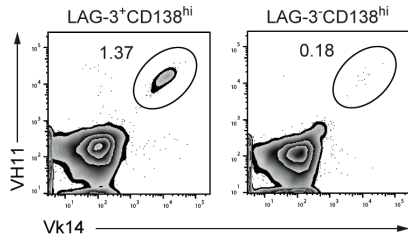
(B-E) Coverage weighted average methylation ratio from 3 replicates for each cell type are shown.

(A, E, F) All samples were from naïve mice except where indicated. (B, C, D, G) All samples were from naïve mice.

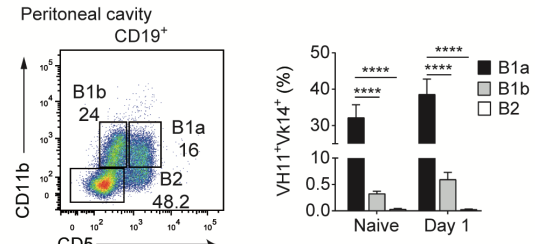
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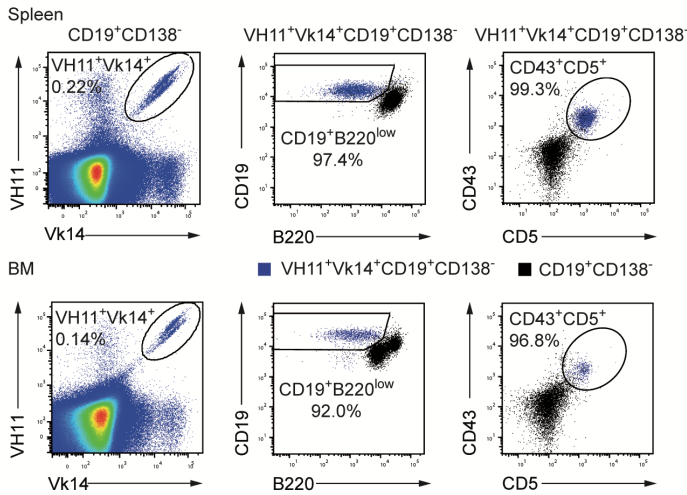
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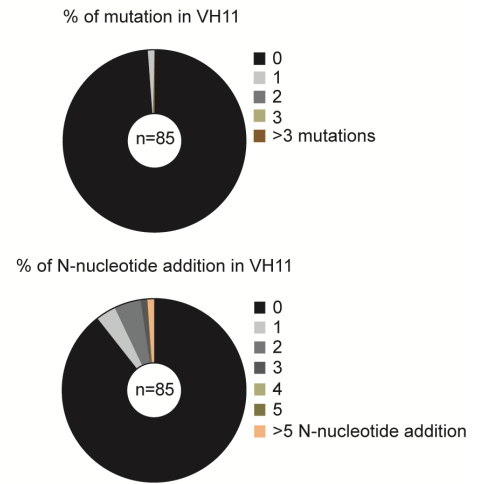
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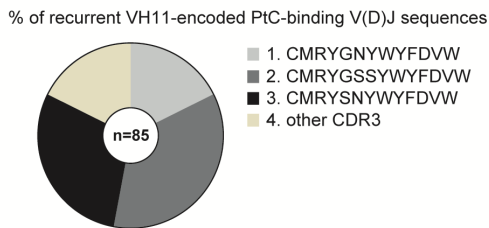
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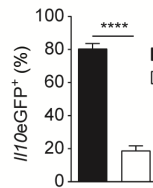
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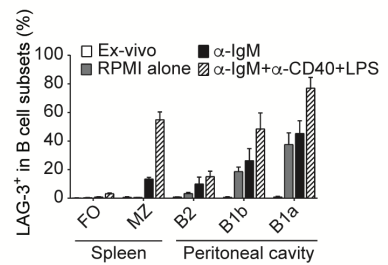
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I

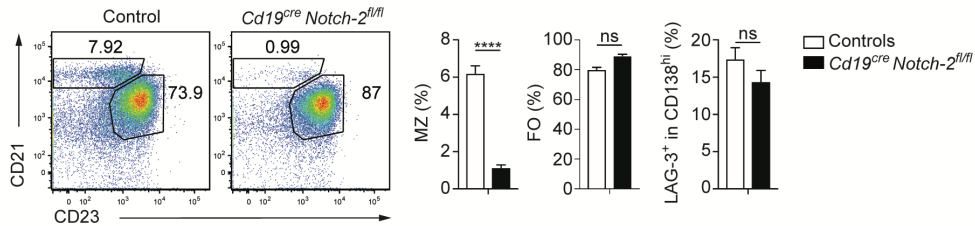


Figure S5: BCR repertoire and antigen recognition properties of LAG-3- and IL-10-expressing plasmocytes, Related to Figure 5.

- (A) Spleen LAG-3⁺CD138^{hi} (naïve C57BL/6 mice, n=253) and LAG-3⁺CD138^{hi} (naïve C57BL/6 mice, n=150), spleen *Il10*eGFP⁺CD138^{hi} (day 1, n=181) and *Il10*eGFP⁻CD138^{hi} (day 1, n=121), BM *Il10*eGFP⁺CD138^{hi} (naïve, n=191) and *Il10*eGFP⁻CD138^{hi} (naïve, n=184) plasmocytes were isolated from naïve and day 1-challenged mice (SL7207; 10⁷ CFU). Their *Igh* rearrangements were obtained by single cell PCR, and analysed using IMGT/V-Quest. Data show the IGHV family gene usage for the indicated populations.
- (B) Representative flow cytometry plots showing the expression of VH11 and Vk14.126 on the indicated spleen cell populations from naïve (LAG-3⁺CD138^{hi}, LAG-3⁻CD138^{hi}) *Il10*eGFP mice.
- (C) Representative flow cytometry plots showing the gating strategy to identify B1a (CD19⁺CD11b⁺CD5⁺), B1b (CD19⁺CD11b⁺CD5⁻) and B2 (CD19⁺CD11b⁻CD5⁻) B cell subsets in PeC (gated on live CD19⁺ B cells). The histogram indicates the frequency of VH11⁺Vk14.126⁺ among B1a, B1b and B2 subsets in naïve and day 1-challenged *Il10*eGFP mice (SL7207, 10⁷ CFU). Data are pooled from 2 independent experiments (n=6 per time point).
- (D) Representative flow cytometry plots showing the gating strategy to characterize VH11⁺Vk14.126⁺ B1a cells in spleen (upper) and BM (bottom). Left plots show the expression of VH11 and Vk14.126 in CD19⁺CD138⁻ B cells. Middle and right plots show the expression of CD19 and B220, as well as of CD43 and CD5, respectively, on VH11⁺Vk14.126⁺CD19⁺CD138⁻ B cells from the spleen and BM of naïve C57BL/6 mice (in blue). The expression of these markers on CD19⁺CD138⁻ B cells is indicated by the overlaid cells in black on the same plots. The percentages in the middle and right plots are the frequencies of CD19⁺CD138⁻VH11⁺Vk14.126⁺ having the expression of CD19 and B220, as well as of CD43 and CD5, falling within the gates. Data is representative of 2 independent experiments.
- (E) Frequency of IgH sequences having the indicated numbers of somatic mutations (top) or N-nucleotide additions (bottom) among all obtained VH11-positive sequences in spleen and BM from naïve and challenged mice.
- (F) Frequency of IgH CDR3 sequences associated with PtC binding among all obtained VH11-positive IgH sequences in spleen and BM from naïve and challenged mice.
- (G) Frequency of *Il10*eGFP⁺ cells within PtC- and non-PtC-reactive CD138^{hi} in BM from naïve *Il10*eGFP mice. Data are pooled from 4 independent experiments (n=12).
- (H) LAG-3 expression on B cell subsets *ex vivo*, and after overnight culture (RPMI, αIgM, αIgM+αCD40+LPS). Pools of 2-3 experiments.
- (I) Flow cytometry plots and quantification of spleen B cell subsets in naïve *Cd19-cre-Notch2^{fl/fl}* mice (n=7) and littermate controls (pooled *Cd19-cre* and *Notch2^{fl/fl}*, n=8). Pool of 2 experiments (n=6). Data show mean ± SEM. Results were compared using two-tailed unpaired t-test with Welch's correction in case of unequal variances (**** p<0.0001).

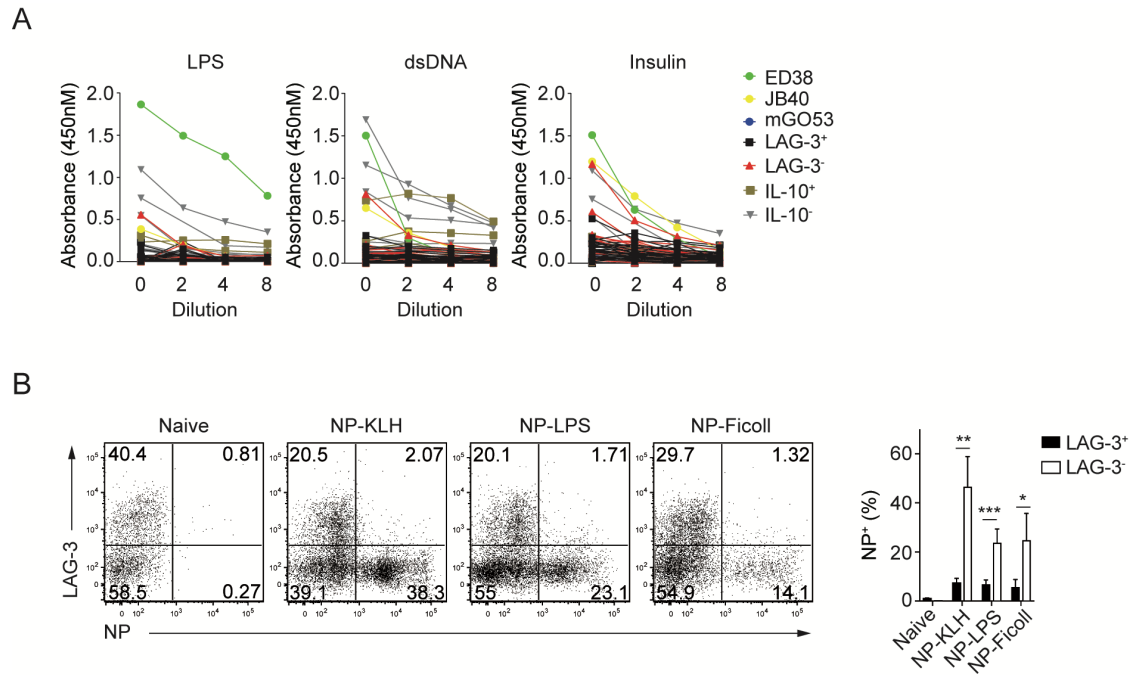
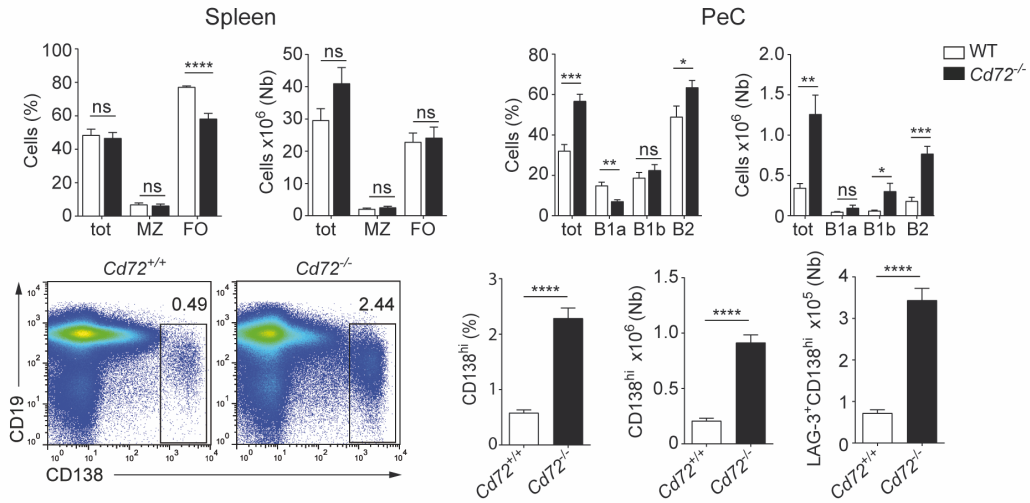


Figure S6: Reactivity of plasmocytes, Related to Figure 6.

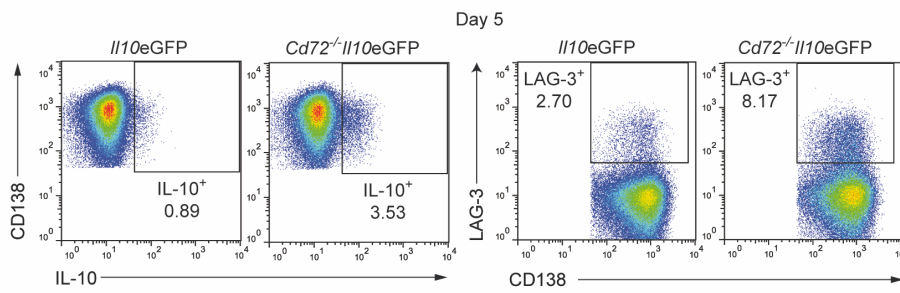
(A) Reactivity of recombinant immunoglobulin from spleen LAG-3⁺ (n=25) and LAG-3⁻ (n=19), and BM *Il10*eGFP⁺ (n=6) as well as *Il10*eGFP⁻ (n=14) plasmocytes for LPS, dsDNA and insulin by ELISA. Green, yellow and blue lines are high positive ED38, low positive JB40, and negative control mGO53 antibodies, respectively.

(B) Flow cytometry plots showing binding of NP-allophycocyanin and expression of LAG-3 on CD138^{hi} cells from spleen of naive and i.p. immunized mice (day 7 p.i.). Frequency of NP-binding cells within LAG-3⁺CD138^{hi} and LAG-3⁻CD138^{hi} spleen cells. Representative of 2 experiments (n=6). Data show mean \pm SEM (*p<0.05, **p<0.01; *** p<0.001)

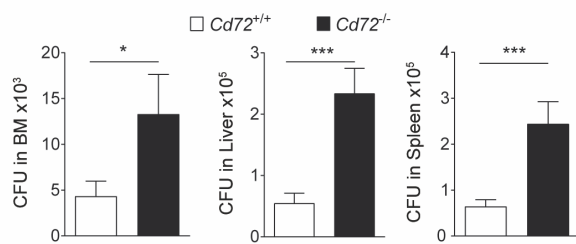
A



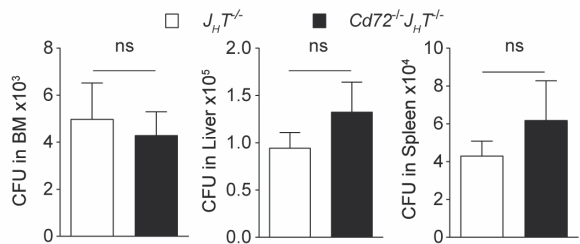
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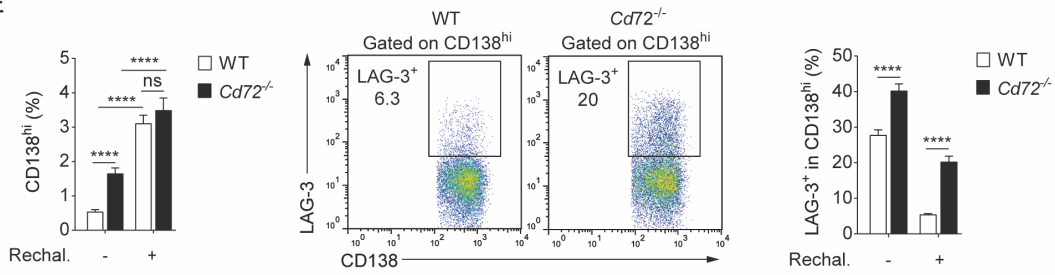
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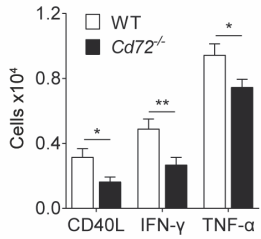
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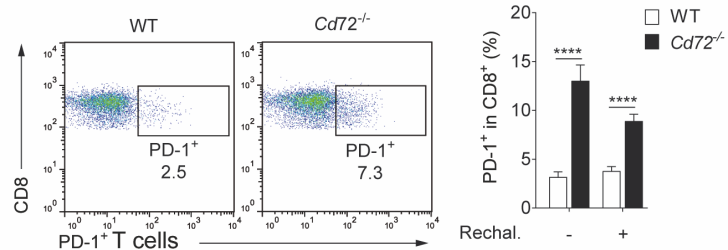


Figure S7: Host response of CD72-deficient mice during *Salmonella* infection, Related to Figure 7.

(A) Frequencies and numbers of total (tot) and indicated B cell subsets in spleen and PeC of naïve *Cd72*^{-/-} and control mice. Pool of 2 experiments (n=6) (top). Flow cytometry plots and quantifications for CD138^{hi} and LAG-3⁺CD138^{hi} cells in spleen of naïve *Cd72*^{-/-} and littermate control. Pool of 3 experiments (n=13) (bottom).

(B) *Il10eGFP* and *Cd72*^{-/-}*Il10eGFP* shown in Figure 7A. Representative flow cytometry plots showing the expression of *Il10eGFP* and LAG-3 by CD138^{hi} cells at day 5 p.i. Data are representative from 3 independent experiments (n=9-12 per group).

(C) CFU on day 3 p.i. (SL1344, 100 CFU) for *Cd72*^{-/-} and littermate controls. Pool of 5 experiments (n=22-25 per group)

(D) *Cd72*^{-/-}JHT (n=11) and JHT (n=10) mice were challenged with *Salmonella* (SL1344, 100 CFU). Data show CFU in BM, liver, and spleen on day 3 p.i. Data are pooled from 2 independent experiments.

(E) *Cd72*^{-/-} and WT mice shown in Figure 7E. Histogram (left) shows the frequency of CD138^{hi} cells in spleen before (day 90) and 5 days after re-challenge. Representative flow cytometry plots show the expression of LAG-3 in spleen CD138^{hi} cells on day 5 after re-challenge (middle). Histogram (right) shows the frequency of LAG-3⁺ cells in CD138^{hi} cells in spleen on day 90 and day 5 after rechallenge.

(F) *Cd72*^{-/-} and WT mice shown in Figure 7G. Numbers of CD40L-, IFN- γ -, and TNF- α -expressing *Salmonella*-reactive memory CD4⁺ T cells in BM on day 90 post-vaccination.

(G) *Cd72*^{-/-} and WT mice shown in Figure 7H. Representative flow cytometry plots (day 95) and quantifications showing the frequency of PD-1⁺ cell in spleen CD8⁺ T cells on days 90 and 95.

Data show mean \pm SEM. (n.s.p > 0.05, *p<0.05, **p<0.01; **** p<0.0001).

Table S1. Top 50 genes that are higher expressed in naïve CD138^{hi}CD19⁺LAG-3⁺ cells compared to naïve CD138^{hi}CD19⁺LAG-3⁻ cells. Related to Figure 4.

Pvalue based top 50 genes that are higher expressed in naïve CD138 ^{hi} CD19 ⁺ LAG-3 ⁺ compared to naïve CD138 ^{hi} CD19 ⁺ LAG-3 ⁻					
Rank	Gene symbol	logFC	PValue	FDR	Synonyms
1	Lag3	4.91	1.33E-07	0.001528	CD223 LAG-3 Ly66
2	Cpq	2.35	8.83E-07	0.002004	1190003P12Rik 2610034C17Rik Hls2 Lal-1 Pgcp
3	Vav3	1.96	1.16E-06	0.002004	A530094I06Rik AA986410 Idd18.1
4	Bcl2	1.67	1.4E-06	0.002004	AW986256 Bcl-2 C430015F12Rik D630044D05Rik D830018M01Rik
5	Zfp3611	2.05	1.71E-06	0.002178	AW742437 AW743212 Berg36 Brf1 D530020L18Rik ERF1 TIS11b cMG1
6	Egr1	1.95	2.04E-06	0.002343	A530045N19Rik ETR103 Egr-1 Krox-1 Krox-24 Krox24 NGF1-A NGF1-A NGFIA TIS8 Zenk Zfp-6 Zif268 egr
7	Inpp4a	1.89	3.49E-06	0.002981	107kDa 9630012D15 D130048C09Rik R74740
8	Kctd12	1.45	3.66E-06	0.002981	AU046135 AW538430 Pfet1 Pfetin
9	Prg2	1.84	4.22E-06	0.002981	EMBP MBP mMBP mMBP-1
10	Il4i1	2.70	4.59E-06	0.002981	Fig1 Fig1-ps LAAO LAO
11	Sema6d	1.91	4.65E-06	0.002981	1110067B02Rik AA409156 D330011G23 mKIAA1479
12	Otud1	1.90	5.44E-06	0.003125	4933428L19Rik
13	Bcl2l15	1.96	6.53E-06	0.003273	Bfk Gm566
14	Ppfibp2	1.45	6.68E-06	0.003273	Cclp1
15	Sirpa	1.32	7.47E-06	0.003273	A1835480 Bit CD172a P84 Ptpps1 SHP-1 SHPS-1 SIRP
16	Sell	2.59	7.49E-06	0.003273	A1528707 CD62L L-selectin LECAM-1 Lnhrl Ly-22 Ly-m22 Lyam-1 Lyam1
17	Jade2	1.31	8.61E-06	0.003273	1200017K05Rik AI480685 Phf15 mKIAA0239
18	Krt222	1.45	9.23E-06	0.003273	6330509G02Rik
19	AW112010	1.97	9.69E-06	0.003273	-
20	Klf4	1.94	9.72E-06	0.003273	EZF Gklf Zie
21	Rgcc	1.52	1.04E-05	0.003273	1190002H23Rik Rgc-32 Rgc32
22	Vegfa	2.01	1.06E-05	0.003273	Vegf Vpf
23	Bhlhe40	2.58	1.08E-05	0.003273	Bhlhb2 C130042M06Rik CR8 Clast5 Dec1 Shar p2 Stra13 Stra14
24	Ggh	2.00	1.18E-05	0.00346	gamma-GH
25	Oosp2	1.62	1.44E-05	0.003949	Gm99 Plac11 Tmem122
26	Ly6k	1.67	1.48E-05	0.003951	2410015A16Rik 3110035B01Rik mLy-6K
27	Cd200	1.65	1.77E-05	0.004608	Mox2 OX2
28	Serpina3f	1.45	2.06E-05	0.005062	2A1 BC049975
29	Plekha1	1.24	2.13E-05	0.005062	AA960558 C920009D07Rik TAPP1
30	Nfkbiz	1.12	2.22E-05	0.005062	AA408868 INAP Mail
31	Ackr3	1.92	2.25E-05	0.005062	AW541270 CXC-R7 CXCR-7 Cmkor1 Cxcr7 RDC-1 Rdc1
32	Rnf130	1.47	2.42E-05	0.005351	2510042A13Rik G1RZFP G1rp GOLIATH GP
33	Snn	1.14	2.87E-05	0.006151	2810407J07Rik AI848521 AW547286
34	Egr2	2.55	0.00003	0.006272	Egr-2 Krox-20 Krox20 NGF1-B Zfp-25 Zfp-6
35	Phlda1	1.47	3.24E-05	0.006652	DT1P1B11 TDAG51 Tdag
36	Il10	1.57	3.48E-05	0.006801	CSIF Il-10
37	Ephx1	1.93	3.76E-05	0.007191	EH Eph-1 Eph1 Ephx1 mEH
38	AB124611	1.49	5.07E-05	0.008396	Gm1706
39	Btla	1.34	0.000056	0.008941	A630002H24

40	Irf2bp2	1.15	5.77E-05	0.009071	E130305N23Rik IRF-2BP2
41	Clec2i	1.80	5.85E-05	0.00908	Clr-g Clrg Dcl1 LCL-1 OCILrP2
42	Pglyrp1	1.31	6.71E-05	0.009416	PGRP PGRP-S Pglyrp Tag7 Tasg7 Tnfsf3l
43	Abhd6	1.49	6.95E-05	0.009616	0610041D24Rik AA673485 AV065425
44	Srsf7	0.89	0.000073	0.009846	35kDa 9430065L19Rik 9G8 NX-96 Sfrs7
45	Egfl	3.38	7.36E-05	0.009846	Dffrx Fam Egf-1 Egfa
46	Ctss	1.32	0.000077	0.010022	Cats
47	Twsg1	1.26	7.88E-05	0.010022	1810013J15Rik 9030422N06Rik AW552143 D17Ert403e Tsg Twg
48	Zswim6	1.46	7.94E-05	0.010022	2900036G02Rik mKIAA1577
49	Gpr18	1.49	8.27E-05	0.010103	-
50	Rtp4	1.62	8.39E-05	0.010104	5830458K16Rik Ifrg28

Abbreviations

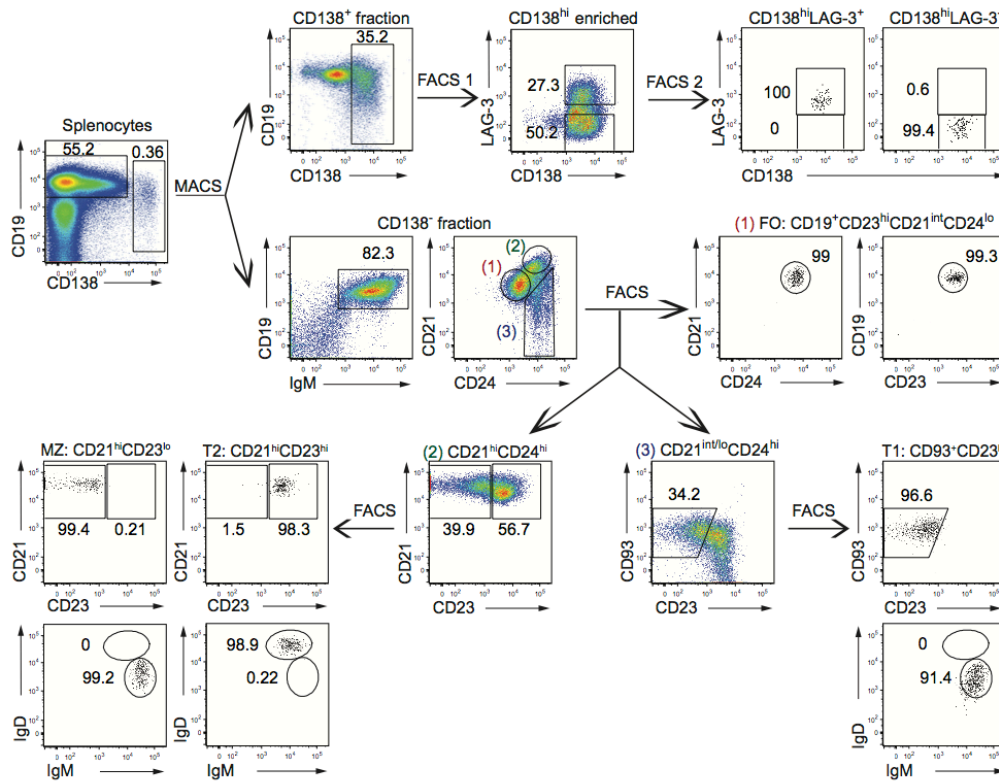
LogFC	Log2 of fold change
FDR	False Discovery Rate

Table S2. Top 50 genes that are higher expressed in naïve CD138^{hi}CD19⁺LAG-3⁻ cells compared to naïve CD138^{hi}CD19⁺LAG-3⁺ cells. Related to Figure 4.

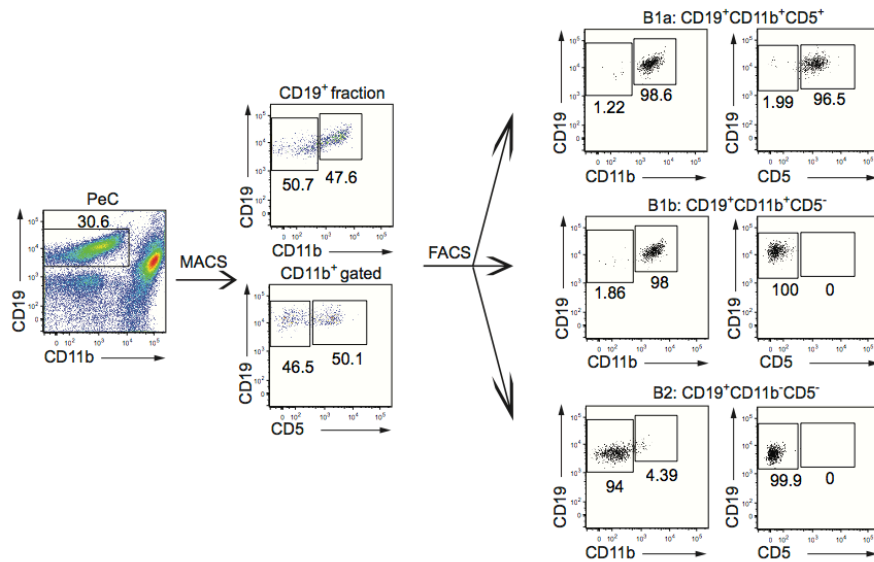
Pvalue based top 50 genes that are higher expressed in naïve CD138 ^{hi} CD19 ⁺ LAG-3 ⁻ compared to naïve CD138 ^{hi} CD19 ⁺ LAG-3 ⁺					
Rank	Gene symbol	logFC	PValue	FDR	Synonyms
1	Ighe	-4.86	5.86E-07	0.002004	NA
2	Ighg3	-2.79	7.65E-07	0.002004	NA
3	Cdk1	-1.76	9.26E-07	0.002004	Cdc2 Cdc2a p34<CDC2>
4	Mcm5	-1.81	1.25E-06	0.002004	AA617332 AI324988 AL033333 Cdc46 Mcnd5 P1-CDC46
5	Cdc45	-2.12	3.06E-06	0.002981	Cdc45l
6	Kpna2	-1.41	0.000004	0.002981	2410044B12Rik IPOA1 PTAC58 Rch1
7	Ada	-1.14	4.83E-06	0.002981	-
8	Ighg2c	-3.01	4.93E-06	0.002981	NA
9	Eno1	-1.34	5.79E-06	0.003164	0610008I15 AL022784 Eno-1 MBP-1
10	Ncapg	-1.84	8.08E-06	0.003273	5730507H05Rik Hcapg MFT.M05.13
11	Rrm2	-1.63	8.94E-06	0.003273	AA407299 R2
12	Kif11	-1.64	9.79E-06	0.003273	Eg5 Kif8 Kif11 Kns1
13	Slpi	-2.10	9.81E-06	0.003273	-
14	Ighg2b	-2.66	1.03E-05	0.003273	NA
15	Ccnb2	-1.69	1.04E-05	0.003273	CycB2
16	Nuf2	-2.30	1.26E-05	0.003614	2410003C07Rik AU044112 C85691 Cdc41 NUF2R
17	Cep55	-1.82	0.000014	0.003929	1200008O12Rik 2700032M20Rik
18	Spc24	-2.04	1.88E-05	0.004793	2410030K01Rik AV109292 Spbc24
19	Rfc4	-1.21	0.000021	0.005062	A1 AI894123 AU040575 RFC37
20	Nmral1	-1.27	2.18E-05	0.005062	1110025F24Rik AI256624
21	Ssr2	-1.57	2.89E-05	0.006151	1500032E05Rik AI315033 AU020133 TLAP TRAPB TRAPbeta
22	Prelid1	-1.00	3.35E-05	0.006744	2610524G07Rik Preli
23	Cdkn3	-1.82	3.49E-05	0.006801	2410006H10Rik KAP
24	Cdc20	-1.83	4.37E-05	0.008221	2310042N09Rik C87100 p55CDC
25	Tg	-1.33	4.74E-05	0.008396	Tgn cog
26	Selm	-1.17	4.78E-05	0.008396	1500040L08Rik A230103K18 Selm Sepm
27	Ighv1-63	-2.70	4.86E-05	0.008396	NA
28	Ddost	-1.15	4.97E-05	0.008396	-
29	Ahey	-1.19	5.06E-05	0.008396	AA987153 AL024110 CuBP SAHH
30	Igkv4-69	-1.91	0.000051	0.008396	NA
31	NA	-3.48	5.12E-05	0.008396	NA
32	Ighv1-75	-4.83	5.12E-05	0.008396	NA
33	Top2a	-1.38	5.61E-05	0.008941	Top-2
34	Ighv15-2	-6.47	6.09E-05	0.009245	NA
35	Tigit	-1.83	6.12E-05	0.009245	ENSMUSG00000071552 Vstm3
36	H2-Eb1	-0.87	6.34E-05	0.009363	Eb H-2Eb H2Eb Ia-4 Ia4
37	Spc25	-1.26	6.39E-05	0.009363	2600017H08Rik 2610205L13Rik Spbc25
38	Kif22	-1.89	6.44E-05	0.009363	AU021460 C81217 Kid Kif22a
39	Tcf19	-1.50	6.58E-05	0.009416	5730403J10Rik AW495861
40	Mad2l1	-1.14	6.72E-05	0.009416	AA673185 MAD2
41	Cxcr3	-2.33	7.37E-05	0.009846	Cd183 Cmkar3
42	Igkv3-10	-1.93	7.74E-05	0.010022	NA
43	Pbk	-2.26	7.84E-05	0.010022	2810434B10Rik AW538537 D14Ertd732e TOPK
44	Ighv1-47	-5.14	8.04E-05	0.010035	NA
45	Dnpep	-1.03	8.16E-05	0.010078	AA407814

46	Srm	-0.92	0.000088	0.010211	AA407669 SpdST SpdSy
47	Gstp2	-1.30	9.19E-05	0.010345	GSTpiA Gst-3 Gst3
48	Tmem97	-1.47	9.61E-05	0.010583	1810014L12Rik AI115531 AL022956 D11Bhm 182e
49	Shcbp1	-1.71	9.65E-05	0.010583	mPAL
50	Cenpe	-1.81	9.77E-05	0.010583	312kDa AU019344 BC049989 C530022J18 CE NP-E Kif10
Abbreviations					
LogFC		Log2 of fold change			
FDR		False Discovery Rate			

A



B



Methods S1: Strategy for the isolation of B lineage cell subsets, Related to STAR Methods

The isolation of spleen and PeC subsets are illustrated in panels A and B, respectively. For spleen, CD138⁺ cells were first enriched by autoMACS after incubation with anti-CD138-PE (clone 281-2, BD Pharmingen) followed by anti-PE microbeads (Miltenyi Biotec). The CD138⁺ fraction was then stained with anti-CD19, CD138, LAG-3, and CD11b/CD11c/CD3/PI. An additional enrichment of CD138^{hi} cells was performed by flow cytometry sort (FACS 1), and LAG-3⁺CD138^{hi}CD11b-CD11c-CD3-PI⁻ and LAG-3-CD138^{hi}CD11b-CD11c-CD3-PI⁻ cells were then isolated by a second flow cytometry sort (FACS 2). Plasmacytes were isolated on

day 1 p.i. with 10^7 CFU *Salmonella* (SL7207) using the same strategy, according to LAG-3⁺, LAG-3⁻, *I110eGFP*⁺ and *I110eGFP*⁻ phenotypes. FMO controls were used to identify the LAG-3⁺ and *I110eGFP*⁺ subsets. B cell subsets were isolated from the CD138⁻ fraction obtained after autoMACS. First, T cells were depleted from the CD138⁻ fraction by autoMACS. Follicular B cells (FO) (fraction 1 in the CD21 versus CD24 flow cytometry plot) were then isolated from this T cell-depleted CD138⁻ fraction by flow cytometry as CD19⁺CD23^{hi}CD21^{int}CD24^{lo}CD11b⁻CD11c⁻CD3⁻PI⁻ following staining with anti-CD19, CD21, CD23, CD24, CD11b/CD11c/CD3/PI. To obtain the other B cell subsets, the T cell-depleted CD138⁻ fraction was stained with anti-CD19, IgM, IgD, CD21, CD23, CD24, CD93, and CD11b/CD11c/CD3/PI. Then, the fractions (2) CD19⁺IgM⁺CD21^{hi}CD24^{hi}CD11b⁻CD11c⁻CD3⁻PI⁻ and (3) CD19⁺IgM⁺CD21^{lo/int}CD24^{hi}CD11b⁻CD11c⁻CD3⁻PI⁻ were enriched by flow cytometry. The marginal zone (MZ) CD24^{hi}CD21^{hi}CD23^{lo} and transitional 2 (T2) CD24^{hi}CD21^{hi}CD23^{hi} were then sorted from fraction (2), and the transitional 1 (T1) CD24^{hi}CD21^{lo/int}CD23^{lo}CD93⁺ from the fraction (3) by flow cytometry. For PeC, B cells were first purified by autoMACS via CD19-positive selection, then stained with anti-CD19, CD11b, CD5, CD11c, CD3, and PI. B1a: CD19⁺CD11b⁺CD5⁺CD11c⁻CD3⁻PI⁻; B1b: CD19⁺CD11b⁺CD5⁻CD11c⁻CD3⁻PI⁻ and B2: CD19⁺CD11b⁻CD5⁻CD11c⁻CD3⁻PI⁻ were then flow cytometry-sorted.