YMTHE, Volume *31*

Supplemental information

Development of an mRNA-lipid nanoparticle vaccine against Lyme disease

Matthew Pine, Gunjan Arora, Thomas M. Hart, Emily Bettini, Brian T. Gaudette, Hiromi Muramatsu, István Tombácz, Taku Kambayashi, Ying K. Tam, Dustin Brisson, David Allman, Michela Locci, Drew Weissman, Erol Fikrig, and Norbert Pardi

Supplemental Information

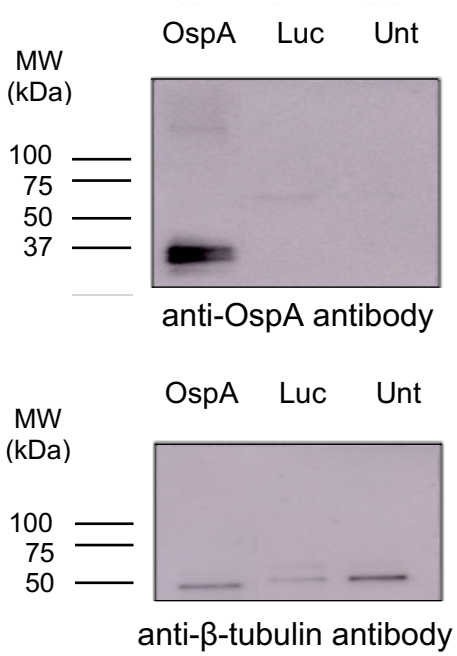


Figure S1. Protein production from OspA mRNA *in vitro*. Neuro 2-a cells were transfected with OspA- or Luc-encoding mRNAs. OspA protein expression in whole-cell lysate was detected by Western blot, utilizing untransfected cells (Unt) and Luc mRNA-transfected cells as negative controls. Membrane was stripped and re-probed with anti-beta tubulin antibody as a loading control. MW: molecular weight

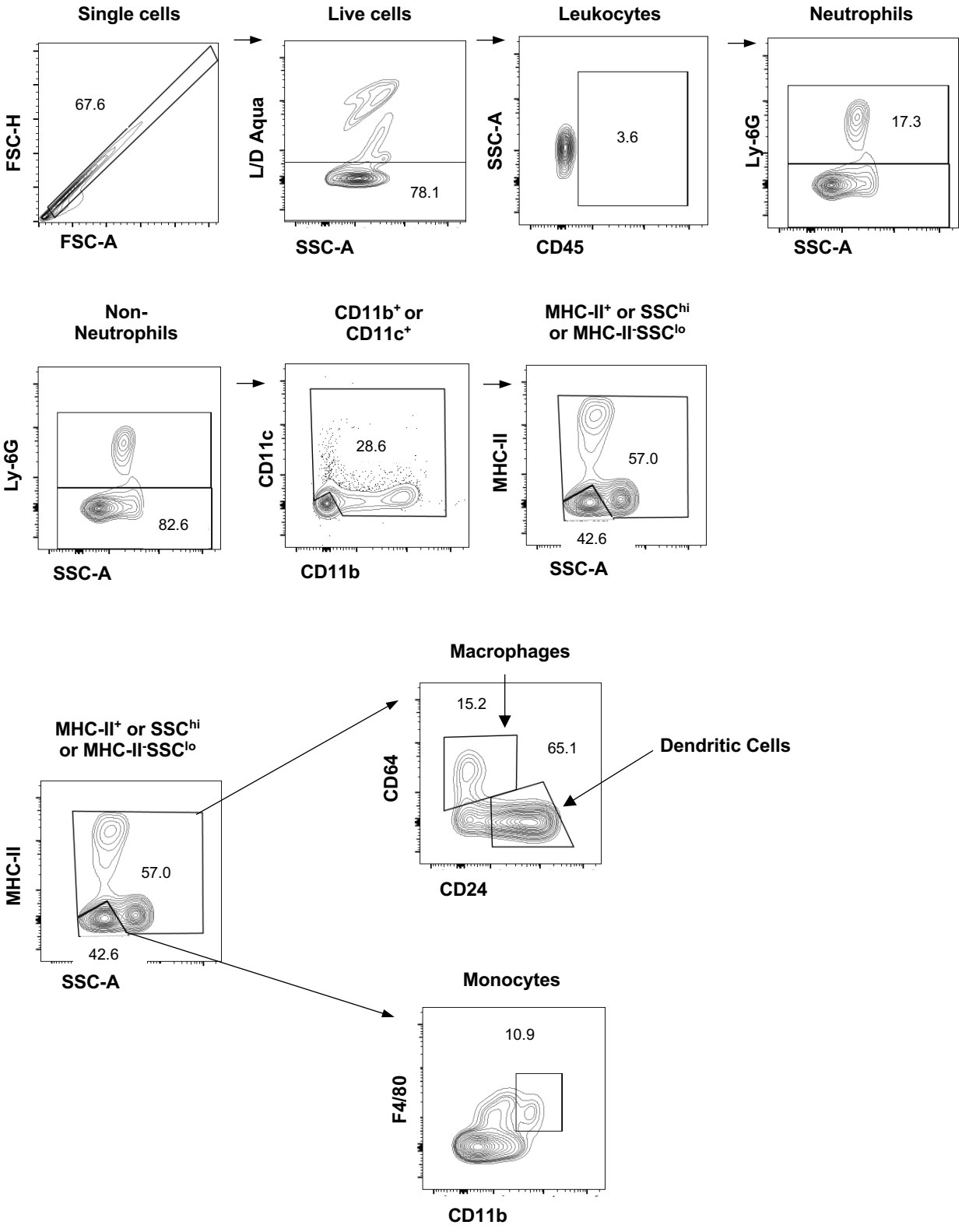


Figure S2. Flow cytometric gating strategy for the investigation of innate immune cell responses after OspA mRNA-LNP and rOspA + alum immunizations in mice. Representative flow cytometry plots for innate immune cell populations (neutrophils, macrophages, dendritic cells, monocytes).

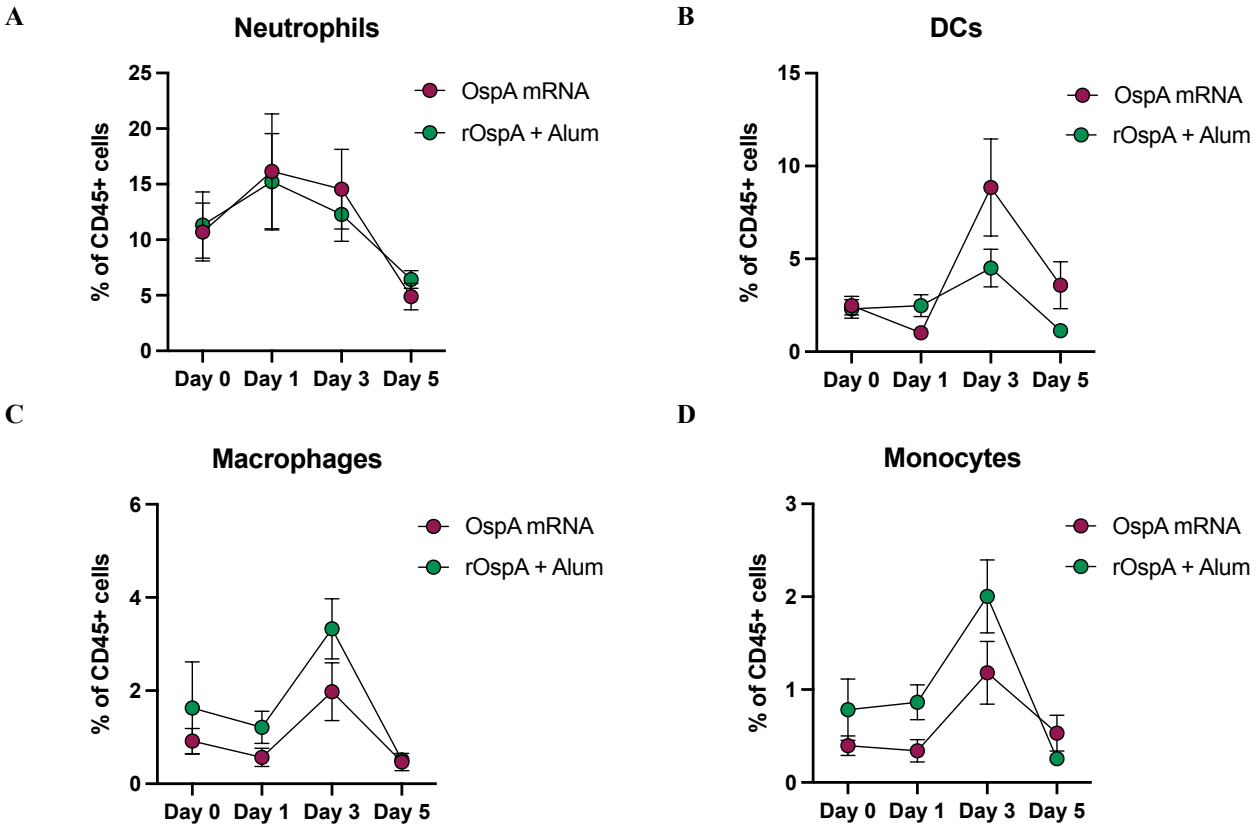


Figure S3. Assessing innate cell populations in injection site muscle of immunized mice. Mice were vaccinated intramuscularly with a single dose of 3 µg of OspA mRNA-LNP or 1 µg of rOspA + alum and innate immune cell responses were assessed 1, 3 and 5 days post injections. Non-injected mice were used as controls (day 0). Frequencies of (A) neutrophils ($CD45^+Ly\text{-}6G^+$) (B) dendritic cells ($CD45^+Ly\text{-}6G^-CD11b/CD11c^+MHCII^+/SSC^{hi}CD64^-CD24^+$) (C) macrophages ($CD45^+Ly\text{-}6G^-CD11b/CD11c^+MHCII^+/SSC^{hi}CD64^+CD24^-$) and (D) monocytes ($CD45^+Ly\text{-}6G^-CD11b/CD11c^+MHCII^-/SSC^{lo}CD11b^{hi}F4/80^+$) Data represent mean ± SEM (n = 5 mice per group). Data from one experiment is shown.

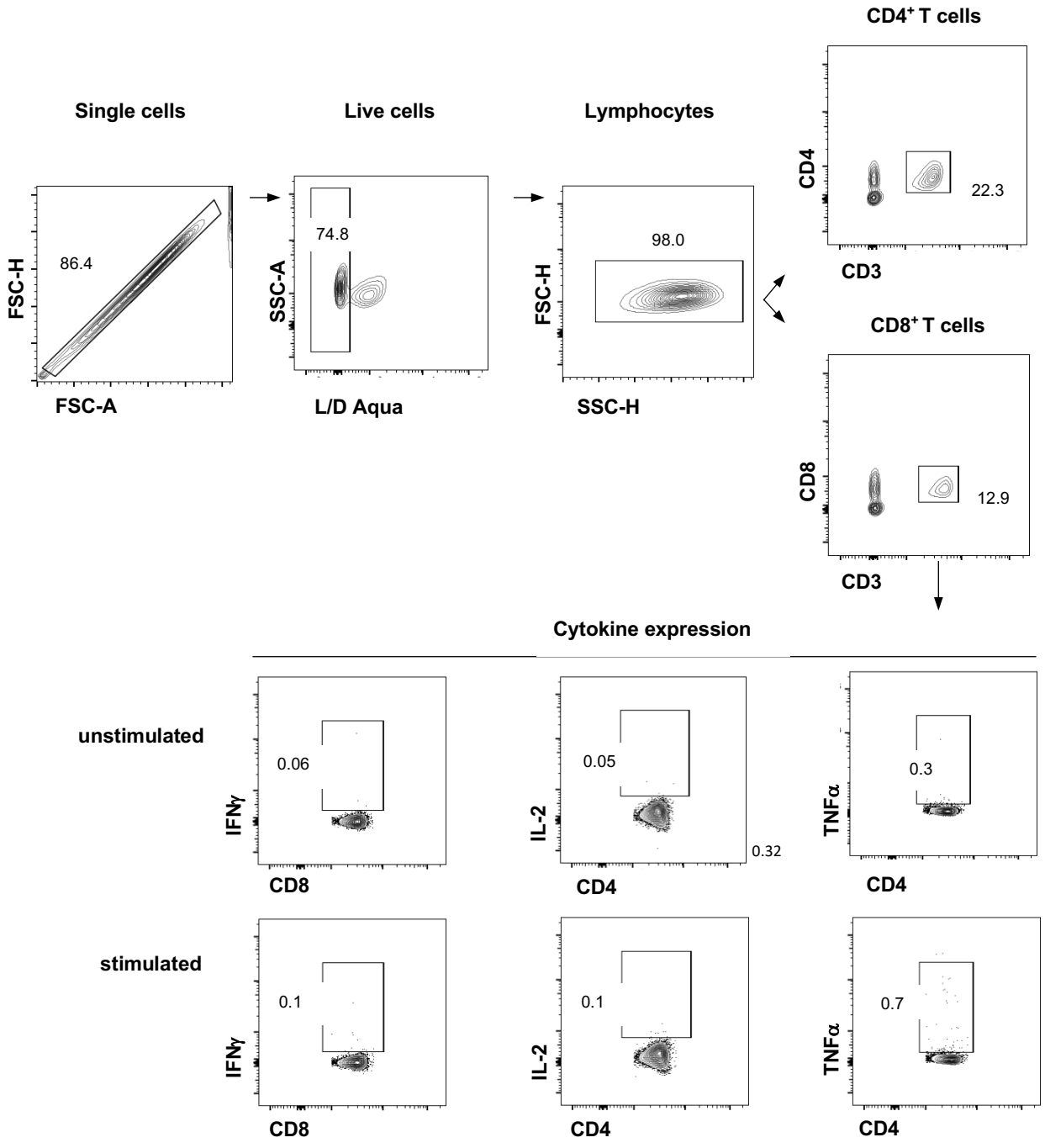
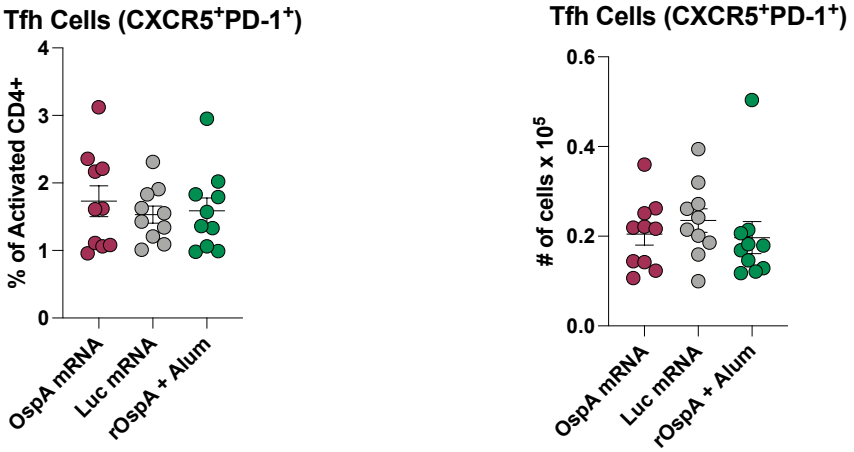


Figure S4. Flow cytometric gating strategy for the investigation of T cell responses in OspA mRNA-LNP-immunized mice. Representative flow cytometry plots for unstimulated and peptide-stimulated samples are shown.

A



B

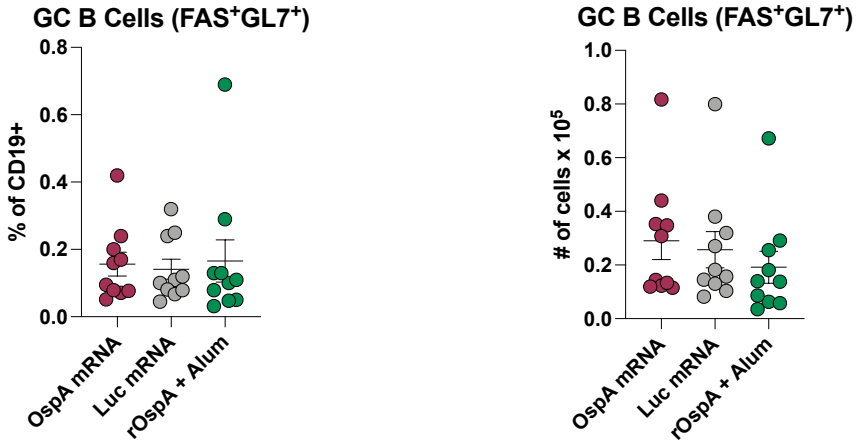
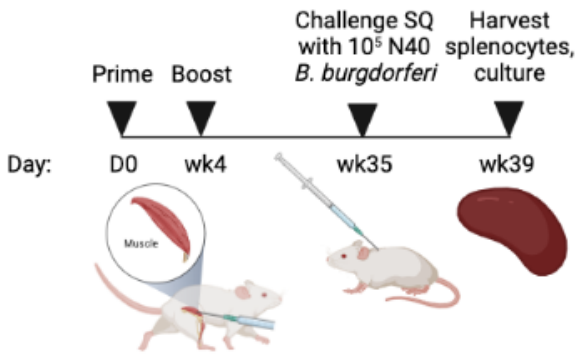


Figure S5. Assessing T follicular helper and germinal center B cells in spleens of immunized mice. (A) Tfh cell ($B220^{-}CD4^{+}CD62L^{-}PD\text{-}1^{+}CXCR5^{+}$) frequencies (left panel) and absolute numbers (right panel). (B) GC B cell ($CD19^{+}CD3^{-}FAS^{+}GL7^{+}$) frequencies (left panel) and absolute numbers (right panel). Each symbol represents one animal, and data represent mean ± SEM (n = 10 mice per group). Data from two independent experiments are shown.

A



B

Vaccine	Culture
OspA mRNA-LNP	0/5
Luc mRNA-LNP	4/4
rOspA + alum	0/5

Figure S6. Nucleoside-Modified OspA mRNA-LNP immunization protects mice from infection with *Borrelia burgdorferi* (A) Mice were vaccinated and boosted intramuscularly with OspA or Luc mRNA-LNPs or rOspA + alum as described in Figure 4. Thirty-five weeks after the prime dose, mice were challenged subcutaneously with 10^5 *Borrelia burgorderi* (strain N40) and then sacrificed 25 days later. Splenocytes were cultured for detection of *B. burgdorferi* infection. (B) Number of animals with detectable *B. burgdorferi* burden five days after spleen harvest. Data is obtained from one experiment.

Table S1. Antibodies used for innate cell flow cytometry

Stain	Fluorochrome	Clone	Vendor	Catalog #
CD11c	BV421	N418	BioLegend	117330
CD45	BV605	30-F11	BioLegend	103139
I-A/I-E	BV650	M5/114.15.2	BD Biosciences	563415
CD24	BV711	M1/69	BD Biosciences	563405
CD11b	APC-Cy7	M1/70	BD Biosciences	557657
Ly-6G	AF700	1A8	BD Biosciences	561236
CD64	PE-CF594	X54-5/7.1	BioLegend	139320
F4/80	PE	BM8	BioLegend	123109

Table S2. Antibodies used for T follicular helper (Tfh) cell flow cytometry.

Stain	Fluorochrome	Clone	Vendor	Catalog #
CXCR5	Biotin	SPRCL5	eBioscience	13-7185-82
Streptavidin	BV421	-	BioLegend	405225
B220	BV650	RA3-6B2	BioLegend	103241
CD4	PerCP-Cy5.5	RM4-5	BioLegend	100540
CD44	BV605	IM7	BioLegend	103047
CD62L	BUV395	MEL-14	BD	740218
PD-1	PE	RMP1-30	BioLegend	109104
Bcl6	AF647	K112-91	BD	624024
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S3. Antibodies used for germinal center B (GC B) cell flow cytometry.

Stain	Fluorochrome	Clone	Vendor	Catalog #
CD138	Biotin	281-2	BD Biosciences	553713
Streptavidin	BV650	-	BioLegend	405232
CD3e	BUV395	145-2c11	BD Biosciences	563565
CD19	BV605	6D5	BioLegend	115540
GL7	PerCP-Cy5.5	GL7	BioLegend	144610
FAS	PE	Jo2	BD Biosciences	554258
IgD	PE-Cy7	11-26c.2a	BioLegend	405720
OspA Tetramer 1	AF488	-	Labeled in-house	See methods
OspA Tetramer 2	AF647	-	Labeled in-house	See methods
RBD Tetramer	BV421	-	Labeled in-house	See methods
Live/Dead	eFluor 780	-	eBioscience	65-0865-14

Table S4. Antibodies used for memory B cell (MBC) and long-lived plasma (LLPC) cell flow cytometry.

Stain	Fluorochrome	Clone	Vendor	Catalog #
B220	BV421	RA3-6B2	BioLegend	103240
CD138	BB700	281-2	BD Biosciences	742124
CD38	AF700	90	Invitrogen	56-0381-82
IgD	APC-Cy7	11-26C.2A	BioLegend	405716
CD19	BV711	6D5	BioLegend	115555
GL7	PE	GL7	BioLegend	144608
FAS	PE-Cy7	Jo2	BD Biosciences	55763
CD4	PE-Cy5	H129.19	BD Biosciences	553654
CD8a	PE-Cy5	53-6.7	BD Biosciences	553034
Ter-119	PE-Cy5	TER119	BioLegend	116210
F4/80	PE-Cy5	BM8	Invitrogen	15-4801-82
OspA Tetramer 1	AF488	-	Labeled in-house	See methods
OspA Tetramer 2	AF657	-	Labeled in-house	See methods

Table S5. Antibodies used for long-lived plasma cell (LLPC) ELISpot assays.

Stain	Conjugate	Clone	Vendor	Catalog #
IgG1	biotin	polyclonal	Southern Biotech	1070-08
IgG2a	biotin	polyclonal	Southern Biotech	1080-08
IgG2b	biotin	polyclonal	Southern Biotech	1090-08
IgG3	biotin	polyclonal	Southern Biotech	1100-08
ExtrAvidin	alkaline phosphatase	-	Sigma-Aldrich	E2636

Table S6. Primers used for *Borrelia burgdorferi* detection.

Primer	**Sequence**
*mactin*_F	CATTGCTGACAGGATGCAGAAGG
*mactin*_R	TGCTGGAAGCTGGACAGTGAGG
*flaB*_F	ACAGCTGAAGAGCTTGGAATG
*flaB*_R	CTTGGTTTGCTCCAACATGAAC