

Supplemental Figures

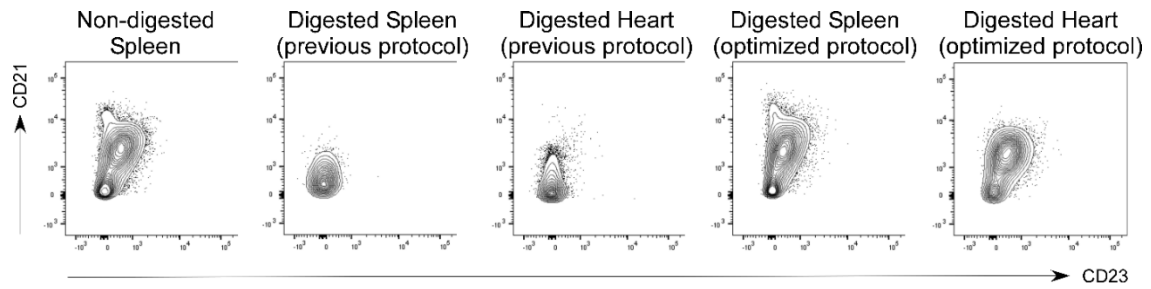


Figure S1: Optimization of tissue digestion protocol to prevent the cleavage of CD21 and CD23. In non-digested spleens, three populations of B cells are distinguished based on the expression of CD21 and CD23: $CD21^{high}CD23^{-}$, $CD21^{+}CD23^{+}$, and $CD21^{-}CD23^{-}$. Digestion with Collagenase using our previously published protocol (1) reduced staining for CD21 and CD23 in B lymphocytes isolated from the spleen and the heart. The use of purified Collagenase (optimized protocol) resulted in the preservation of these antigens in the spleen (positive control) and highlighted expression of CD21 and CD23 in myocardial B cells.

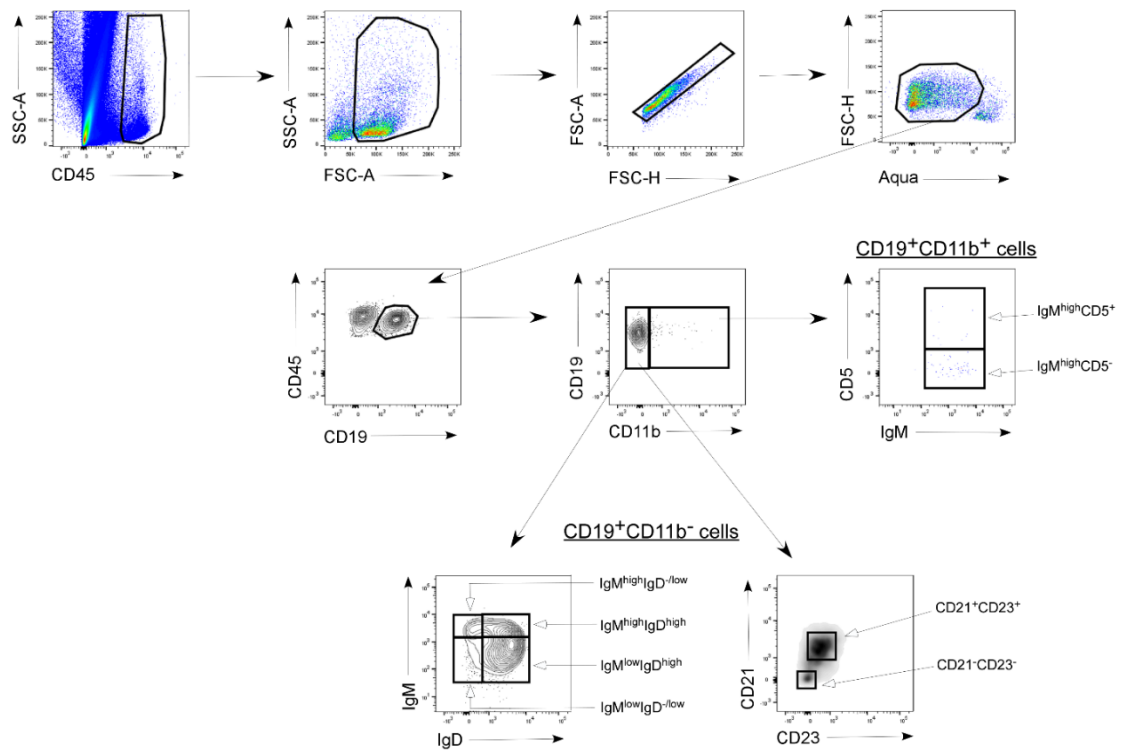


Figure S2: Gating strategy for the flow cytometric analysis of myocardial-associated B cells. B cells were gated as CD45⁺Aqua⁻CD19⁺. Most B cells were CD11b⁻ and further analyzed based on the expression of IgM, IgD, CD21, and CD23. CD11b⁺ B cells were analyzed based on the expression of CD5 and IgM.

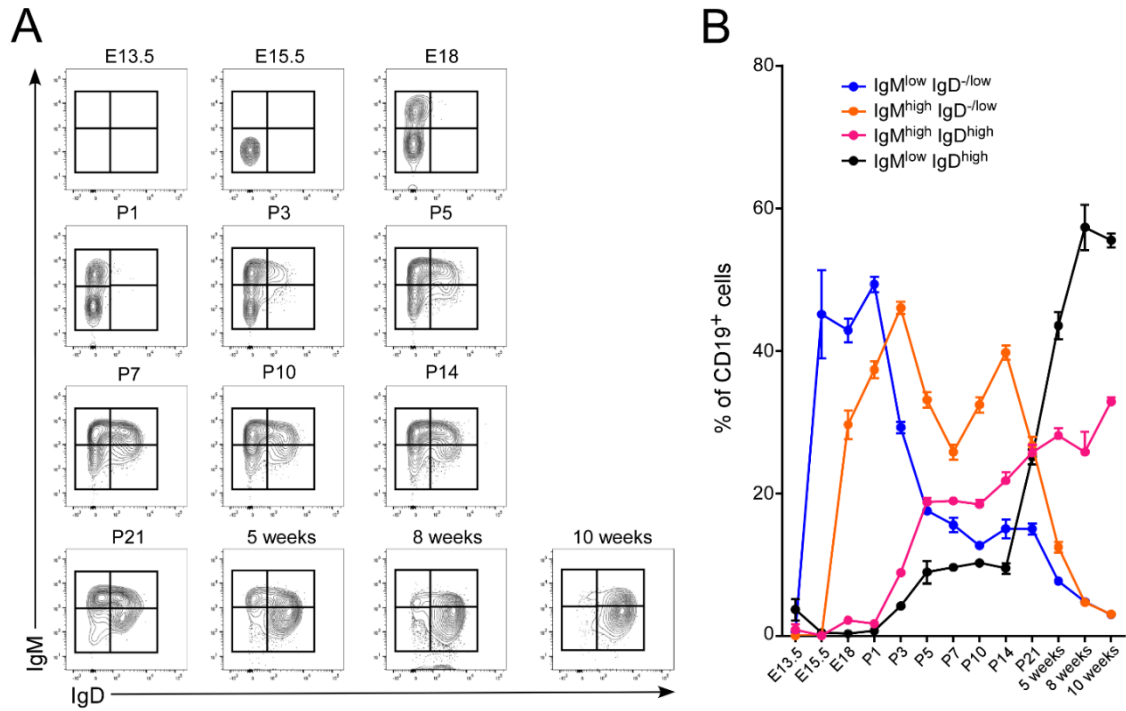


Figure S3: Flow cytometric profile of CD19⁺CD11b⁻ B cells showing the expression of IgM and IgD from embryonic to adult life. **A)** Representative flow charts showing the expression of IgM and IgD in CD19⁺CD11b⁻ B cells from embryonic to adult life in the heart. A population of IgM^{low}IgD^{-/low} cells first appeared in the heart at E15.5. IgM^{high}IgD^{low} cells were seen in the heart at E18. In the early post-natal life, the prevalence of IgM^{high}IgD^{high} cells increased, and the prevalence of IgM^{low}IgD^{-/low} decreased. In the adult heart, the majority of CD19⁺CD11b⁻ B cells were IgM^{low}IgD^{high}. **B)** Group-graph showing the dynamic changes in B cell composition from embryonic to adult life in the heart. n=4-7 samples/condition. From E13.5 to P7, 3-6 embryonic and neonatal hearts were pooled together in the same tube to constitute n=1.

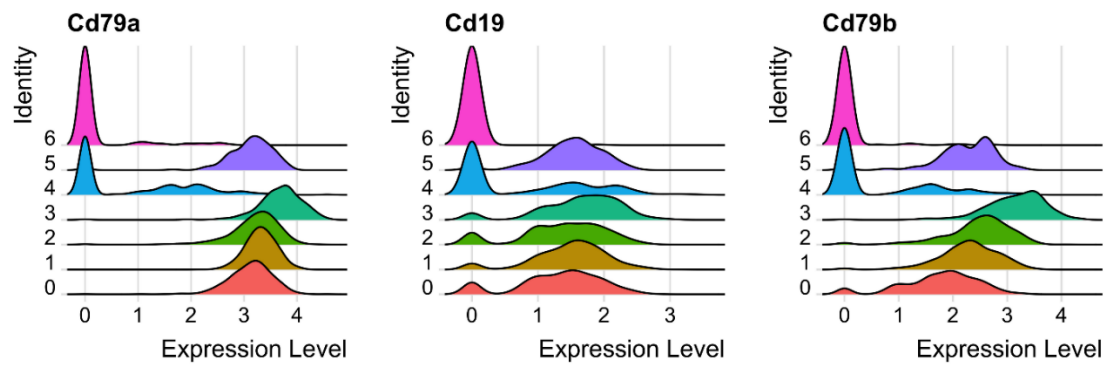


Figure S4: Expression of B cell specific markers in CD45⁺Aqua⁺CD19⁺ B cell clusters. B cells were sorted from blood, heart, liver, and lung. We performed unsupervised clustering of single-cell transcription data and identified 7 clusters (0 to 6). For the subsequent analysis, only cells that were positive for B cell specific markers (Cd79a, Cd19, and Cd79b) were used (cluster 6 and negative cells within cluster 4 were excluded).

Supplemental Table 1: Comparative analysis of B cell subsets ratio in the heart from embryonic day 13.5 (E13.5) to adulthood (10 weeks old). Data were analyzed by 2-way ANOVA followed by Tukey's post-hoc test to correct for multiple comparisons.

	<i>p</i> value			
	IgM ^{high} CD5 ⁺	IgM ^{high} CD5 ⁻	CD21 ⁺ CD23 ⁺	CD21 ⁻ CD23 ⁻
E13.5 vs. E15.5	0.9752	0.8299	n.a.	0.0504
E13.5 vs. E18	0.9914	0.8333	0.0848	<0.0001
E13.5 vs. P1	0.8916	0.5475	0.0021	<0.0001
E13.5 vs. P3	0.9900	0.6271	0.0026	<0.0001
E13.5 vs. P5	0.8948	0.7611	0.1667	<0.0001
E13.5 vs. P7	0.3486	0.8406	<0.0001	<0.0001
E13.5 vs. P10	0.5651	0.7948	0.0007	<0.0001
E13.5 vs. P14	>0.9999	0.6695	<0.0001	<0.0001
E13.5 vs. 3 weeks	0.9131	0.4794	0.0003	<0.0001
E13.5 vs. 5 weeks	0.9192	0.5455	<0.0001	0.0127
E13.5 vs. 8 weeks	0.8627	0.4267	<0.0001	0.3981
E13.5 vs. 10 weeks	0.8737	0.4279	0.0006	0.7456
E15.5 vs. E18	0.6658	0.9999	0.0848	0.1389
E15.5 vs. P1	0.5287	0.9998	0.0021	0.0537
E15.5 vs. P3	0.6609	>0.9999	0.0026	0.0779
E15.5 vs. P5	>0.9999	>0.9999	0.1667	0.9043
E15.5 vs. P7	0.9962	0.9996	<0.0001	>0.9999
E15.5 vs. P10	>0.9999	>0.9999	0.0007	>0.9999
E15.5 vs. P14	0.8475	>0.9999	<0.0001	0.8772
E15.5 vs. 3 weeks	0.5464	0.9936	0.0003	>0.9999
E15.5 vs. 5 weeks	0.5510	0.9998	<0.0001	0.1415
E15.5 vs. 8 weeks	0.5078	0.9647	<0.0001	0.0799
E15.5 vs. 10 weeks	0.5154	0.9666	0.0006	0.0715
E18 vs. P1	0.2096	0.0308	0.9994	0.0020
E18 vs. P3	>0.9999	0.1487	0.0699	0.0377
E18 vs. P5	<0.0001	0.9646	0.2216	0.0010
E18 vs. P7	<0.0001	>0.9999	<0.0001	<0.0001
E18 vs. P10	0.0037	0.9999	0.0005	<0.0001
E18 vs. P14	0.0836	0.3359	<0.0001	<0.0001
E18 vs. 3 weeks	0.3260	0.0080	0.0002	<0.0001
E18 vs. 5 weeks	0.4651	0.0287	<0.0001	<0.0001
E18 vs. 8 weeks	0.1208	0.0042	<0.0001	<0.0001
E18 vs. 10 weeks	0.1496	0.0029	0.0006	<0.0001
P1 vs. P3	0.0010	0.3758	0.0187	0.0098
P1 vs. P5	<0.0001	0.0046	0.2113	0.0002
P1 vs. P7	0.0001	0.0004	<0.0001	<0.0001
P1 vs. P10	0.0047	0.0017	0.0008	<0.0001
P1 vs. P14	<0.0001	0.0837	<0.0001	<0.0001
P1 vs. 3 weeks	0.8287	0.5443	0.0003	<0.0001
P1 vs. 5 weeks	0.9965	>0.9999	<0.0001	<0.0001
P1 vs. 8 weeks	0.7971	0.0345	<0.0001	<0.0001
P1 vs. 10 weeks	0.9932	0.1039	0.0007	<0.0001
P3 vs. P5	<0.0001	0.0106	0.3162	0.0007
P3 vs. P7	0.0002	0.0003	<0.0001	<0.0001
P3 vs. P10	0.0075	0.0035	0.0008	<0.0001
P3 vs. P14	0.0023	0.9242	<0.0001	<0.0001

P3 vs. 3 weeks	0.0032	0.0143	0.0003	<0.0001
P3 vs. 5 weeks	0.0770	0.3779	<0.0001	<0.0001
P3 vs. 8 weeks	0.0003	0.0002	<0.0001	<0.0001
P3 vs. 10 weeks	0.0015	0.0096	0.0007	<0.0001
P5 vs. P7	0.0110	0.1332	0.9999	0.0832
P5 vs. P10	0.4133	0.9244	0.9267	0.1048
P5 vs. P14	<0.0001	0.1181	0.2949	>0.9999
P5 vs. 3 weeks	<0.0001	0.0004	0.0035	0.0439
P5 vs. 5 weeks	<0.0001	0.0030	<0.0001	<0.0001
P5 vs. 8 weeks	<0.0001	<0.0001	<0.0001	<0.0001
P5 vs. 10 weeks	<0.0001	0.0019	<0.0001	0.0001
P7 vs. P10	0.8852	0.7541	0.0232	>0.9999
P7 vs. P14	0.0002	0.0022	0.0008	0.0307
P7 vs. 3 weeks	0.0001	<0.0001	0.0008	>0.9999
P7 vs. 5 weeks	<0.0001	0.0002	<0.0001	<0.0001
P7 vs. 8 weeks	<0.0001	<0.0001	<0.0001	<0.0001
P7 vs. 10 weeks	<0.0001	0.0004	0.0009	<0.0001
P10 vs. P14	0.0158	0.0288	0.0941	0.0311
P10 vs. 3 weeks	0.0051	0.0002	0.0006	0.9985
P10 vs. 5 weeks	0.0036	0.0011	<0.0001	<0.0001
P10 vs. 8 weeks	0.0042	<0.0001	<0.0001	<0.0001
P10 vs. 10 weeks	0.0041	0.0008	0.0004	<0.0001
P14 vs. 3 weeks	0.0001	0.0029	0.0016	0.0034
P14 vs. 5 weeks	0.0001	0.0802	<0.0001	<0.0001
P14 vs. 8 weeks	<0.0001	<0.0001	<0.0001	<0.0001
P14 vs. 10 weeks	<0.0001	0.0032	0.0002	<0.0001
3 weeks vs. 5 weeks	>0.9999	0.6136	<0.0001	<0.0001
3 weeks vs. 8 weeks	0.1508	0.4818	<0.0001	<0.0001
3 weeks vs. 10 weeks	0.5533	0.7636	0.0001	<0.0001
5 weeks vs. 8 weeks	0.7906	0.0348	0.0016	0.0192
5 weeks vs. 10 weeks	0.9368	0.1127	0.0738	0.0075
8 weeks vs. 10 weeks	>0.9999	>0.9999	0.9670	0.6045

*n.a. = not applicable: Mean Difference = 0.

Supplemental Table 2: List of the unique upregulated genes in CD21⁻CD23⁻, CD21⁺CD23⁺ and CD11b⁺ B cell subsets assessed by seruat.

CD21 ⁻ CD23 ⁻					
genes	p value	Avg logFC	pct.1	pct.2	P value adjusted
<i>Iglc1</i>	1.30E-214	1.397333	0.849	0.517	4.03E-210
<i>Vpreb3</i>	1.97E-94	1.15496	0.619	0.425	6.11E-90
<i>Ighm</i>	0	1.041216	0.974	0.951	0
<i>Ly6d</i>	1.06E-187	0.982473	0.932	0.834	3.28E-183
<i>Ms4a1</i>	9.02E-209	0.982102	0.906	0.784	2.80E-204
<i>Spib</i>	3.74E-169	0.925162	0.716	0.383	1.16E-164
<i>Cd79b</i>	7.85E-243	0.882916	0.94	0.876	2.44E-238
<i>Tagln2</i>	1.85E-84	0.862385	0.611	0.387	5.75E-80
<i>Rilpl2</i>	5.72E-133	0.843463	0.623	0.316	1.78E-128
<i>Iglc2</i>	9.12E-44	0.794945	0.773	0.751	2.83E-39
<i>Pafah1b3</i>	3.19E-137	0.788188	0.583	0.279	9.89E-133
<i>Iglc3</i>	1.79E-83	0.769403	0.828	0.694	5.57E-79
<i>Cd24a</i>	7.09E-105	0.74746	0.71	0.475	2.20E-100
<i>Cd2</i>	3.12E-118	0.734293	0.775	0.552	9.70E-114
<i>Myadm</i>	8.74E-143	0.719357	0.537	0.213	2.71E-138
<i>Hspa8</i>	5.42E-176	0.709709	0.935	0.822	1.68E-171
<i>Arl5c</i>	1.24E-72	0.706192	0.499	0.283	3.86E-68
<i>Ucp2</i>	1.13E-136	0.696751	0.804	0.575	3.51E-132
<i>Igkc</i>	3.64E-63	0.690625	0.953	0.961	1.13E-58
<i>Ptma</i>	4.46E-195	0.689157	0.961	0.905	1.39E-190
CD21 ⁺ CD23 ⁺					
genes	p value	Avg logFC	pct.1	pct.2	P value adjusted
<i>Fcer2a</i>	4.35E-227	0.838628	0.653	0.253	1.35E-222
<i>Ighd</i>	1.45E-188	0.667697	0.827	0.619	4.50E-184
<i>Neurl3</i>	6.94E-130	0.600094	0.58	0.284	2.15E-125
<i>Satb1</i>	1.91E-145	0.571445	0.778	0.506	5.94E-141
<i>Mef2c</i>	6.60E-135	0.491009	0.934	0.842	2.05E-130
<i>Fchsd2</i>	2.67E-80	0.457044	0.706	0.545	8.30E-76
<i>Gpr183</i>	2.72E-52	0.43904	0.563	0.407	8.45E-48
<i>Stap1</i>	3.11E-68	0.428216	0.724	0.578	9.64E-64
<i>Dmxl1</i>	2.49E-54	0.417994	0.667	0.533	7.73E-50
<i>Pgap1</i>	1.52E-47	0.400051	0.535	0.393	4.73E-43
<i>Lamb3</i>	5.12E-44	0.384832	0.459	0.312	1.59E-39
<i>Smad7</i>	8.91E-45	0.378965	0.514	0.365	2.77E-40
<i>Stat4</i>	5.21E-40	0.357963	0.414	0.275	1.62E-35
<i>Sell</i>	5.37E-51	0.352693	0.617	0.485	1.67E-46
<i>Zfp318</i>	7.23E-32	0.301359	0.435	0.308	2.25E-27
<i>Cr2</i>	4.70E-30	0.2908	0.324	0.201	1.46E-25
<i>H2-Ab1</i>	1.04E-68	0.276937	0.972	0.876	3.23E-64

<i>Vps37b</i>	4.93E-19	0.271257	0.601	0.543	1.53E-14
<i>Pxdc1</i>	3.54E-42	0.271227	0.289	0.137	1.10E-37
<i>H2-Aa</i>	4.10E-55	0.269677	0.957	0.86	1.27E-50
CD11b ⁺					
genes	p value	Avg logFC	pct.1	pct.2	P value adjusted
<i>Ly6a</i>	6.11E-166	1.063561	0.774	0.377	1.90E-161
<i>Itgb1</i>	5.09E-228	0.754993	0.621	0.142	1.58E-223
<i>S100a4</i>	5.35E-295	0.701682	0.393	0.031	1.66E-290
<i>Odc1</i>	1.05E-62	0.521991	0.626	0.321	3.25E-58
<i>Traf1</i>	1.06E-125	0.509379	0.563	0.172	3.28E-121
<i>Tyrobp</i>	6.82E-83	0.484692	0.509	0.185	2.12E-78
<i>Zbtb20</i>	3.47E-58	0.48393	0.632	0.329	1.08E-53
<i>Ms4a6c</i>	2.44E-138	0.475518	0.381	0.076	7.59E-134
<i>Gimap4</i>	4.05E-67	0.42572	0.746	0.416	1.26E-62
<i>Rplp1</i>	1.81E-57	0.424575	0.92	0.895	5.62E-53
<i>Sp140</i>	6.91E-61	0.409878	0.785	0.479	2.15E-56
<i>Cybb</i>	4.25E-62	0.402891	0.768	0.432	1.32E-57
<i>Id2</i>	1.14E-91	0.384671	0.387	0.11	3.53E-87
<i>H2-Q7</i>	9.53E-39	0.378988	0.752	0.497	2.96E-34
<i>Cd9</i>	9.98E-193	0.373671	0.442	0.073	3.10E-188
<i>Ptpn18</i>	1.45E-46	0.371551	0.799	0.641	4.50E-42
<i>Fxyd5</i>	9.39E-89	0.366216	0.67	0.286	2.92E-84
<i>Cd44</i>	1.71E-86	0.343201	0.553	0.204	5.30E-82
<i>Ctla4</i>	6.36E-134	0.336224	0.283	0.042	1.97E-129
<i>Socs3</i>	4.62E-59	0.334449	0.361	0.129	1.43E-54

Supplemental Table 3: Comparative analysis of B cell subsets ratio in the spleen from embryonic day 18 (E18) to adulthood (10 weeks old). Data were analyzed by 2-way ANOVA followed by Tukey's post-hoc test to correct for multiple comparisons.

	<i>p</i> value				
	IgM ^{high} CD5 ⁺	IgM ^{high} CD5 ⁻	CD21 ⁺ CD23 ⁺	CD21 ⁻ CD23 ⁻	CD21 ^{high} CD23 ⁻
E18 vs. P1	0.1978	0.0011	0.9877	0.2007	n.a.
E18 vs. P3	0.0120	0.0702	0.0452	>0.9999	n.a.
E18 vs. P5	<0.0001	0.7884	<0.0001	<0.0001	0.6491
E18 vs. P7	0.0167	0.7222	<0.0001	0.0101	0.1352
E18 vs. P10	0.0019	0.9942	0.0008	<0.0001	0.0355
E18 vs. P14	0.0003	0.9965	<0.0001	<0.0001	0.0733
E18 vs. 3 weeks	>0.9999	0.0871	0.0004	<0.0001	0.0049
E18 vs. 5 weeks	>0.9999	0.9479	<0.0001	<0.0001	0.0036
E18 vs. 8 weeks	0.8241	>0.9999	<0.0001	<0.0001	0.0035
E18 vs. 10 weeks	0.4782	>0.9999	<0.0001	<0.0001	0.0064
P1 vs. P3	0.0136	0.9794	0.0690	0.4794	n.a.
P1 vs. P5	0.0002	0.0032	<0.0001	<0.0001	0.6491
P1 vs. P7	0.0188	0.0068	<0.0001	0.0159	0.1352
P1 vs. P10	0.0023	0.0050	0.0010	<0.0001	0.0355
P1 vs. P14	0.0004	0.0013	<0.0001	0.0002	0.0733
P1 vs. 3 weeks	0.0300	0.0011	0.0005	0.0003	0.0049
P1 vs. 5 weeks	0.0003	0.0031	<0.0001	<0.0001	0.0036
P1 vs. 8 weeks	0.2533	0.0010	<0.0001	<0.0001	0.0035
P1 vs. 10 weeks	0.2941	0.0099	<0.0001	<0.0001	0.0064
P3 vs. P5	<0.0001	0.0559	0.0007	0.0014	0.6491
P3 vs. P7	0.0207	0.1232	<0.0001	0.0066	0.1352
P3 vs. P10	0.0046	0.0924	0.0008	0.0001	0.0355
P3 vs. P14	0.8614	0.0860	<0.0001	0.0005	0.0733
P3 vs. 3 weeks	0.0195	0.0353	0.0004	0.0003	0.0049
P3 vs. 5 weeks	0.0315	0.0991	<0.0001	<0.0001	0.0036
P3 vs. 8 weeks	0.0108	0.0698	<0.0001	<0.0001	0.0035
P3 vs. 10 weeks	0.0133	0.0766	<0.0001	<0.0001	0.0064
P5 vs. P7	0.2758	0.2584	0.0007	0.0737	0.4820
P5 vs. P10	0.1105	0.1536	0.0141	0.0381	0.0442
P5 vs. P14	<0.0001	0.4148	<0.0001	0.8193	0.0922
P5 vs. 3 weeks	<0.0001	0.3698	0.0016	0.4448	0.0040
P5 vs. 5 weeks	0.0002	0.1093	<0.0001	<0.0001	0.0037
P5 vs. 8 weeks	0.0001	0.7957	<0.0001	<0.0001	0.0035
P5 vs. 10 weeks	0.0002	0.5694	<0.0001	<0.0001	0.0065
P7 vs. P10	0.0467	0.9129	0.4812	0.2930	0.1039
P7 vs. P14	0.0253	0.9841	0.0019	0.0992	0.1485
P7 vs. 3 weeks	0.0193	0.0813	0.0058	0.1356	0.0064
P7 vs. 5 weeks	0.0203	0.9905	<0.0001	0.0287	0.0039
P7 vs. 8 weeks	0.0186	0.7910	<0.0001	0.0228	0.0036
P7 vs. 10 weeks	0.0189	0.5104	<0.0001	0.0179	0.0066
P10 vs. P14	0.0076	>0.9999	0.0409	0.4011	0.9538
P10 vs. 3 weeks	0.0027	0.0123	0.0052	0.8328	0.0340
P10 vs. 5 weeks	0.0031	>0.9999	<0.0001	<0.0001	0.0045
P10 vs. 8 weeks	0.0024	0.9985	<0.0001	<0.0001	0.0038
P10 vs. 10 weeks	0.0024	0.7074	<0.0001	<0.0001	0.0072
P14 vs. 3 weeks	0.0002	0.0553	0.1046	0.9983	0.4474

P14 vs. 5 weeks	0.0007	>0.9999	<0.0001	<0.0001	0.0046
P14 vs. 8 weeks	<0.0001	0.9990	<0.0001	<0.0001	0.0039
P14 vs. 10 weeks	0.0002	0.9090	<0.0001	<0.0001	0.0073
3 weeks vs. 5 weeks	>0.9999	0.0081	<0.0001	<0.0001	0.0060
3 weeks vs. 8 weeks	0.3757	0.1255	<0.0001	<0.0001	0.0044
3 weeks vs. 10 weeks	0.0849	0.0457	<0.0001	<0.0001	0.0084
5 weeks vs. 8 weeks	0.1140	0.9768	0.0170	0.0004	0.0829
5 weeks vs. 10 weeks	0.0023	0.5268	<0.0001	<0.0001	0.3702
8 weeks vs. 10 weeks	0.9641	>0.9999	0.0456	0.0007	0.9797

*n.a. – not applicable: Mean Difference = 0.

Supplemental Table 4: Comparative analysis of B cell subsets ratio in the heart. blood. spleen. lung. and liver from P1 to 5 weeks of age. Data were analyzed by 2-way ANOVA followed by Tukey's post-hoc test to correct for multiple comparisons.

	<i>p</i> value				
CD5⁺IgM^{high}	P1	P10	P14	P21	5 weeks
Heart vs. Blood	0.9952	0.8659	0.1571	0.5161	0.8766
Heart vs. Lung	0.3451	<0.0001	<0.0001	>0.9999	0.1441
Heart vs. Liver	0.9414	<0.0001	<0.0001	0.9938	0.9996
Heart vs. Spleen	0.9913	0.9997	0.0005	0.741	0.1741
Blood vs. Lung	0.581	<0.0001	<0.0001	0.5319	0.0123
Blood vs. Liver	0.7815	<0.0001	0.1657	0.7588	0.8041
Blood vs. Spleen	>0.9999	0.768	0.4492	0.0576	0.0161
Lung vs. Liver	0.0744	<0.0001	<0.0001	0.9915	0.2792
Lung vs. Spleen	0.623	<0.0001	<0.0001	0.8117	>0.9999
Liver vs. Spleen	0.7446	<0.0001	0.9722	0.4815	0.3228
CD5⁺IgM^{high}	P1	P10	P14	P21	5 weeks
Heart vs. Blood	0.0310	>0.9999	0.9998	0.2612	0.9991
Heart vs. Lung	0.4652	0.9082	0.0243	0.9889	<0.0001
Heart vs. Liver	0.3838	0.0028	0.0030	0.3645	0.1479
Heart vs. Spleen	<0.0001	0.5473	0.9769	0.3228	0.0074
Blood vs. Lung	0.6900	0.9480	0.0244	0.1297	<0.0001
Blood vs. Liver	<0.0001	0.0018	0.0105	0.9981	0.0867
Blood vs. Spleen	0.0139	0.6328	0.9501	0.0022	0.0160
Lung vs. Liver	0.0073	0.0001	<0.0001	0.1872	<0.0001
Lung vs. Spleen	0.0002	0.9643	0.1082	0.6842	0.4597
Liver vs. Spleen	<0.0001	<0.0001	0.0004	0.0029	<0.0001
CD21⁺CD23⁺	P1	P10	P14	P21	5 weeks
Heart vs. Blood	0.9995	0.1196	0.6867	0.0001	0.7120
Heart vs. Lung	0.9336	0.4350	0.6495	0.7056	0.0913
Heart vs. Liver	0.9978	<0.0001	0.0001	0.1036	0.0383
Heart vs. Spleen	0.9778	0.6524	0.9991	0.0121	0.1285
Blood vs. Lung	0.8545	0.9536	>0.9999	0.0158	0.0023
Blood vs. Liver	>0.9999	0.0429	0.0265	0.1514	0.4464
Blood vs. Spleen	0.9321	0.8295	0.8199	<0.0001	0.0038
Lung vs. Liver	0.8050	0.0053	0.0148	0.8257	<0.0001
Lung vs. Spleen	0.9996	0.9970	0.7964	0.0003	0.9999
Liver vs. Spleen	0.8984	0.0017	0.0003	<0.0001	<0.0001
CD21⁻CD23⁻	P1	P10	P14	P21	5 weeks
Heart vs. Blood	<0.0001	0.6507	0.9999	<0.0001	0.9699
Heart vs. Lung	<0.0001	0.0036	0.0069	0.9988	0.7935
Heart vs. Liver	0.0943	<0.0001	<0.0001	<0.0001	0.7570
Heart vs. Spleen	<0.0001	0.1109	0.0013	0.9994	0.9646
Blood vs. Lung	<0.0001	<0.0001	0.0080	<0.0001	0.9877
Blood vs. Liver	<0.0001	<0.0001	<0.0001	0.1720	0.3888
Blood vs. Spleen	<0.0001	0.8140	0.0048	0.0001	0.6987
Lung vs. Liver	<0.0001	<0.0001	<0.0001	<0.0001	0.1706
Lung vs. Spleen	0.7596	<0.0001	<0.0001	0.9889	0.3894
Liver vs. Spleen	<0.0001	<0.0001	<0.0001	<0.0001	0.9793

Supplemental Table 5: List of genes differentially expressed in the heart. blood. liver and lung associated B cells (FDR<0.05). Genes were filtered by Seruat as logFC<-0.25 and logFC>0.25.

Tissue	Gene	P value	Avg logFC	pct.1	pct.2	FDR
Heart	Tsc22d3	1.80E-39	0.51096	0.917	0.887	5.60E-35
Heart	H3f3b	2.32E-32	0.279229	0.965	0.962	7.22E-28
Heart	Ltb	1.03E-31	-0.4771	0.553	0.758	3.21E-27
Heart	Rsrp1	1.85E-28	0.265148	0.95	0.92	5.73E-24
Heart	Sertad1	1.34E-27	0.37262	0.761	0.61	4.16E-23
Heart	Ppp1r15a	2.84E-21	0.309911	0.723	0.552	8.83E-17
Heart	Hspa5	1.22E-19	0.260398	0.907	0.853	3.79E-15
Heart	Fam107b	1.64E-19	0.278719	0.893	0.89	5.09E-15
Heart	Hvcn1	9.35E-19	0.383688	0.738	0.623	2.90E-14
Heart	Ccr7	9.85E-17	-0.29137	0.84	0.922	3.06E-12
Heart	Dusp5	8.50E-12	0.388447	0.444	0.316	2.64E-07
Heart	Slfn2	2.45E-11	-0.26319	0.467	0.615	7.62E-07
Heart	Txnip	3.84E-11	0.267175	0.82	0.756	1.19E-06
Heart	Plk2	4.57E-09	0.296815	0.285	0.181	1.42E-04
Heart	Zfp36l2	5.35E-08	0.277817	0.756	0.718	0.00166
Blood	H3f3b	8.86E-96	-0.4975	0.96	0.963	2.75E-91
Blood	Slfn2	8.75E-77	0.614287	0.808	0.499	2.72E-72
Blood	Nr4a1	1.07E-75	-0.84732	0.667	0.852	3.31E-71
Blood	Junb	1.12E-75	-0.57947	0.952	0.967	3.47E-71
Blood	Hspa8	5.92E-68	-0.46816	0.942	0.952	1.84E-63
Blood	Eif1	4.55E-62	-0.31926	0.959	0.96	1.41E-57
Blood	Ltb	2.22E-61	0.559167	0.865	0.658	6.91E-57
Blood	Cd79b	2.51E-58	0.349156	0.969	0.922	7.79E-54
Blood	Hspa5	1.17E-55	-0.47158	0.781	0.896	3.63E-51
Blood	Vps37b	7.50E-55	-0.66371	0.608	0.804	2.33E-50
Blood	Rsrp1	1.16E-52	-0.40427	0.888	0.94	3.62E-48
Blood	Fos	1.73E-50	-0.75049	0.095	0.377	5.39E-46
Blood	Ftl1	9.72E-50	-0.38472	0.943	0.944	3.02E-45
Blood	Crem	1.28E-48	-0.55081	0.259	0.544	3.97E-44
Blood	Ubc	3.78E-48	-0.37127	0.926	0.936	1.17E-43
Blood	Ppp1r15a	4.48E-47	-0.52638	0.399	0.66	1.39E-42
Blood	Tob2	6.16E-47	-0.5052	0.471	0.709	1.91E-42
Blood	Sertad1	1.29E-44	-0.49682	0.481	0.702	3.99E-40
Blood	Dusp1	7.40E-44	-0.64697	0.253	0.518	2.30E-39
Blood	Actg1	1.16E-36	-0.38401	0.907	0.939	3.61E-32
Blood	Srgn	1.35E-35	-0.3406	0.932	0.939	4.19E-31
Blood	Pim1	5.44E-34	-0.45582	0.557	0.73	1.69E-29
Blood	Rel	5.07E-32	-0.43411	0.631	0.787	1.58E-27
Blood	Hsp90ab1	1.73E-31	-0.32178	0.933	0.947	5.36E-27
Blood	Kdm6b	2.37E-30	-0.3896	0.298	0.528	7.37E-26
Blood	Dnaja1	3.54E-30	-0.38298	0.578	0.745	1.10E-25
Blood	Il16	3.93E-30	0.334229	0.523	0.313	1.22E-25

Blood	Cebpb	4.77E-30	-0.42578	0.197	0.419	1.48E-25
Blood	Myc	9.11E-30	-0.52867	0.052	0.237	2.83E-25
Blood	Klf4	9.93E-30	-0.3727	0.081	0.276	3.09E-25
Blood	Tsc22d3	1.01E-29	-0.46513	0.875	0.9	3.14E-25
Blood	Hsp90aa1	1.80E-29	-0.39176	0.586	0.743	5.59E-25
Blood	Cytip	7.60E-29	-0.31311	0.892	0.928	2.36E-24
Blood	Arhgap45	9.25E-29	0.263477	0.939	0.853	2.87E-24
Blood	Litaf	2.07E-28	-0.43192	0.392	0.588	6.42E-24
Blood	Fosb	2.26E-27	-0.32846	0.054	0.228	7.02E-23
Blood	Nfkbid	3.24E-27	-0.39184	0.364	0.571	1.01E-22
Blood	Dusp5	1.85E-26	-0.45738	0.199	0.398	5.76E-22
Blood	Dynll1	4.45E-26	-0.33004	0.735	0.837	1.38E-21
Blood	Fam107b	5.85E-25	-0.31526	0.855	0.904	1.82E-20
Blood	Tnfrsf13c	3.20E-24	0.285198	0.621	0.43	9.94E-20
Blood	Gem	5.71E-24	-0.38636	0.223	0.416	1.77E-19
Blood	Iglc3	7.73E-24	0.26969	0.828	0.685	2.40E-19
Blood	Ifngr2	2.71E-23	0.284059	0.375	0.202	8.42E-19
Blood	Ifrd1	3.77E-23	-0.30587	0.27	0.465	1.17E-18
Blood	Plaur	3.97E-23	-0.41226	0.676	0.76	1.23E-18
Blood	Plk2	4.28E-23	-0.46589	0.084	0.248	1.33E-18
Blood	Ier2	1.92E-20	-0.27193	0.865	0.898	5.97E-16
Blood	Per1	1.48E-19	-0.27087	0.243	0.422	4.58E-15
Blood	Tob1	1.17E-18	-0.30751	0.292	0.464	3.63E-14
Blood	Clk1	1.48E-18	-0.25864	0.56	0.717	4.58E-14
Blood	Got1	1.51E-18	-0.33578	0.275	0.439	4.69E-14
Blood	Ppp1r18	1.90E-18	0.26068	0.714	0.588	5.91E-14
Blood	Wsb1	3.26E-18	-0.27466	0.452	0.614	1.01E-13
Blood	Cd86	2.45E-17	-0.28552	0.175	0.332	7.61E-13
Blood	Tra2a	3.70E-17	-0.29084	0.335	0.497	1.15E-12
Blood	Sik1	1.95E-16	-0.27574	0.237	0.39	6.05E-12
Blood	Gimap1	1.97E-16	0.252142	0.652	0.515	6.12E-12
Blood	Rilpl2	3.71E-16	-0.41793	0.359	0.491	1.15E-11
Blood	Cd24a	5.89E-16	-0.26179	0.612	0.73	1.83E-11
Blood	Brd2	1.53E-15	-0.25055	0.69	0.783	4.76E-11
Blood	Tuba1c	1.77E-15	-0.27681	0.476	0.598	5.48E-11
Blood	Dnajb9	1.90E-15	-0.25202	0.209	0.358	5.89E-11
Blood	Hvcn1	3.13E-15	-0.3098	0.552	0.683	9.72E-11
Blood	Cyth1	3.49E-15	-0.27233	0.543	0.669	1.09E-10
Blood	Klf6	5.47E-15	-0.3303	0.299	0.434	1.70E-10
Blood	Satb1	8.06E-14	-0.25392	0.369	0.511	2.50E-09
Blood	Trim25	1.67E-12	-0.2787	0.462	0.575	5.19E-08
Blood	Jun	2.70E-12	-0.31286	0.147	0.267	8.40E-08
Blood	Gm26532	3.39E-12	-0.27249	0.217	0.342	1.05E-07
Blood	Elmsan1	5.62E-12	-0.25504	0.31	0.431	1.75E-07
Blood	Bambi	8.07E-11	-0.25115	0.093	0.194	2.51E-06
Blood	Cd83	2.72E-08	-0.25628	0.667	0.704	8.44E-04

Blood	Rrad	7.50E-08	-0.26229	0.33	0.423	0.002329
Blood	Gm26917	1.09E-06	-0.25718	0.452	0.54	0.0338
Liver	Rps24	3.36E-59	0.250408	0.95	0.952	1.04E-54
Liver	Uba52	2.70E-57	0.259568	0.95	0.956	8.40E-53
Liver	Ccr7	7.59E-45	0.50543	0.931	0.897	2.36E-40
Liver	Cd83	9.13E-45	0.548889	0.808	0.66	2.84E-40
Liver	Srgn	3.34E-36	0.317417	0.936	0.937	1.04E-31
Liver	mt-Atp8	6.27E-34	0.289551	1	1	1.95E-29
Liver	mt-Nd4l	1.67E-30	0.310218	1	0.999	5.17E-26
Liver	Hspa8	1.98E-28	0.264203	0.952	0.948	6.15E-24
Liver	Stk17b	5.62E-28	0.300251	0.948	0.942	1.74E-23
Liver	Plaur	3.21E-27	0.415535	0.809	0.716	9.97E-23
Liver	Cxcr5	2.69E-26	0.409898	0.606	0.469	8.35E-22
Liver	Junb	1.16E-24	0.280433	0.973	0.96	3.61E-20
Liver	Nr4a1	3.92E-24	0.378965	0.891	0.775	1.22E-19
Liver	mt-Co2	1.49E-23	0.256408	1	1	4.62E-19
Liver	Nfkbid	1.11E-20	0.368216	0.62	0.483	3.46E-16
Liver	Rel	2.40E-20	0.379812	0.792	0.73	7.45E-16
Liver	Jund	5.14E-20	0.296905	0.867	0.843	1.60E-15
Liver	mt-Nd2	1.36E-19	0.269796	0.98	0.985	4.22E-15
Liver	Fosb	7.16E-19	0.308426	0.289	0.148	2.22E-14
Liver	Gimap6	9.22E-19	0.293027	0.856	0.83	2.86E-14
Liver	Bcl2a1b	9.01E-17	0.390127	0.339	0.21	2.80E-12
Liver	Gpr183	1.15E-16	0.380672	0.431	0.301	3.57E-12
Liver	Txnip	2.61E-16	0.256408	0.816	0.756	8.10E-12
Liver	Smim14	3.22E-14	0.259431	0.747	0.712	9.99E-10
Liver	Btg1	3.25E-14	0.263282	0.933	0.926	1.01E-09
Liver	Dennd4a	5.21E-14	0.320645	0.681	0.621	1.62E-09
Liver	Fos	2.68E-13	0.383479	0.4	0.27	8.31E-09
Liver	Lbh	1.41E-12	0.276509	0.475	0.372	4.37E-08
Liver	Sell	2.87E-12	0.266596	0.7	0.639	8.91E-08
Liver	Gimap3	3.92E-12	0.264901	0.602	0.517	1.22E-07
Liver	Adgre5	7.04E-12	0.286508	0.667	0.598	2.19E-07
Liver	Myc	1.70E-10	0.547639	0.262	0.164	5.27E-06
Liver	4930523C07Rik	2.77E-10	0.253586	0.641	0.598	8.60E-06
Liver	Rilpl2	8.31E-10	0.326314	0.517	0.437	2.58E-05
Liver	Irf4	1.20E-09	0.272905	0.308	0.215	3.72E-05
Liver	Vps37b	3.47E-09	0.251064	0.784	0.74	1.08E-04
Liver	Rasgef1b	1.28E-08	0.270129	0.353	0.272	3.98E-04
Liver	Klf6	4.39E-07	0.286535	0.445	0.383	0.013632
Lung	Txnip	1.19E-50	-0.52631	0.67	0.809	3.69E-46
Lung	Arhgdib	7.70E-32	-0.26024	0.931	0.928	2.39E-27
Lung	Shisa5	5.96E-31	-0.31016	0.883	0.909	1.85E-26
Lung	mt-Nd4l	5.54E-30	-0.2663	0.998	1	1.72E-25
Lung	Ypel3	8.39E-25	-0.3092	0.732	0.778	2.61E-20
Lung	mt-Nd2	2.99E-22	-0.25025	0.984	0.984	9.29E-18

Lung	Slfn2	4.72E-22	-0.42303	0.496	0.619	1.47E-17
Lung	Gimap6	6.86E-22	-0.29175	0.79	0.854	2.13E-17
Lung	Gimap1	1.37E-18	-0.30793	0.483	0.58	4.27E-14
Lung	Ltb	1.96E-17	-0.34838	0.666	0.734	6.07E-13
Lung	Gimap3	9.19E-17	-0.31288	0.47	0.563	2.85E-12
Lung	Iglc3	1.52E-14	-0.28554	0.705	0.732	4.71E-10
Lung	Ucp2	1.07E-13	-0.25267	0.782	0.799	3.31E-09
Lung	Fcer2a	2.62E-12	-0.29271	0.666	0.725	8.13E-08

Supplemental Table 6: KEGG pathway analysis of genes differentially expressed between blood and organ-associated B cells. Genes were analyzed for Immune System and Signal Transduction Pathways.

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value
KEGG_MAPK_SIGNALING_PATHWAY	267	6	0.0225	1.1E-5
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	75	3	0.0400	3.82E-4
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	88	3	0.0341	6.1E-4
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	102	3	0.0294	9.36E-4

Supplemental Table 7: Gene list of cluster 0 markers.

Gene name	P value	avg_logFC	pct.1	pct.2	Adj. p value
Nr4a1	8.62E-96	0.610281	0.963	0.761	2.68E-91
Junb	3.4E-103	0.492133	0.994	0.955	1.06E-98
Fos	8.52E-28	0.468779	0.449	0.286	2.65E-23
Dusp1	2.36E-39	0.438132	0.618	0.413	7.33E-35
Ppp1r15a	2.04E-51	0.438004	0.763	0.561	6.34E-47
Sertad1	7.26E-57	0.437974	0.826	0.613	2.25E-52
Plaur	6.32E-48	0.386336	0.899	0.703	1.96E-43
Vps37b	4.07E-43	0.383584	0.871	0.736	1.26E-38
Ier2	5.31E-47	0.336228	0.964	0.87	1.65E-42
H3f3b	2.66E-69	0.32224	0.999	0.951	8.25E-65
Plk2	8.86E-12	0.303037	0.294	0.201	2.75E-07
Cebpb	9.58E-24	0.298806	0.516	0.36	2.98E-19
Ubc	1.8E-47	0.291393	0.986	0.917	5.59E-43
Hspa5	1.13E-39	0.289261	0.952	0.844	3.51E-35
Pnrc1	1.9E-40	0.283858	0.98	0.903	5.9E-36
Eif1	4.22E-64	0.282373	0.999	0.946	1.31E-59
Litaf	1.74E-16	0.270325	0.636	0.542	5.41E-12
Hspa8	3.67E-30	0.263399	0.997	0.935	1.14E-25
Tuba1c	3.94E-19	0.263097	0.659	0.572	1.22E-14
Rrad	1.21E-10	0.261136	0.491	0.409	3.77E-06
Hs3st1	7.44E-14	0.251497	0.412	0.313	2.31E-09
Blk	1.02E-18	-0.25126	0.623	0.74	3.16E-14
Samhd1	4.58E-19	-0.25499	0.716	0.803	1.42E-14
mt-Nd5	8.68E-20	-0.25617	0.928	0.961	2.69E-15
Foxp1	2.42E-19	-0.25699	0.923	0.926	7.51E-15
Arpc5l	1.39E-22	-0.25938	0.384	0.564	4.32E-18
Tcf3	1.52E-19	-0.2602	0.509	0.671	4.73E-15
Ikzf3	7.27E-19	-0.26023	0.453	0.618	2.26E-14
Gm30211	1.07E-08	-0.26027	0.397	0.492	0.000332
Ifi27l2a	2.94E-08	-0.26094	0.564	0.641	0.000913
Arhgap45	2.33E-28	-0.26776	0.839	0.913	7.25E-24
Pld4	1.77E-17	-0.27788	0.282	0.438	5.5E-13
Hck	2.19E-22	-0.27822	0.128	0.282	6.79E-18
mt-Atp8	3.3E-28	-0.27952	1	1	1.03E-23
Pou2f2	8.74E-23	-0.28255	0.502	0.651	2.72E-18
mt-Co1	2.24E-42	-0.28654	1	1	6.95E-38
mt-Co2	1.32E-46	-0.29202	1	1	4.11E-42
mt-Co3	3.14E-41	-0.29514	1	1	9.76E-37
Igll3	1.33E-24	-0.30279	0.687	0.777	4.14E-20
Siglecg	1.5E-24	-0.30608	0.54	0.71	4.65E-20
Ahnak	6.5E-16	-0.30851	0.186	0.317	2.02E-11
Ucp2	5.31E-25	-0.31292	0.782	0.829	1.65E-20

mt-Nd4l	3.58E-45	-0.31824	0.997	1	1.11E-40
Ptprcap	4.24E-35	-0.31833	0.683	0.808	1.32E-30
mt-Cytb	4.79E-47	-0.32138	1	1	1.49E-42
Tagln2	2.53E-13	-0.32167	0.553	0.664	7.85E-09
Cnp	5.96E-29	-0.32296	0.552	0.724	1.85E-24
Ptpn6	4.49E-24	-0.3329	0.525	0.694	1.39E-19
Fam129c	4.46E-36	-0.33974	0.07	0.258	1.38E-31
Vim	6.69E-15	-0.34997	0.638	0.728	2.08E-10
Dnajc7	1.08E-21	-0.35351	0.398	0.572	3.37E-17
Spib	3.99E-23	-0.37505	0.353	0.536	1.24E-18
Vpreb3	2.41E-12	-0.38554	0.434	0.546	7.5E-08
Iglc1	2.04E-40	-0.39631	0.115	0.332	6.34E-36
S100a6	3.72E-10	-0.40707	0.046	0.114	1.15E-05
Iglc2	1.85E-27	-0.41949	0.681	0.777	5.73E-23
Crip1	3.84E-16	-0.42509	0.732	0.792	1.19E-11
Tnfrsf13c	1.61E-34	-0.42599	0.363	0.58	4.99E-30
Ltb	1.74E-42	-0.45077	0.635	0.791	5.4E-38
Ighm	1.99E-62	-0.46319	0.851	0.907	6.17E-58
Ms4a1	1.53E-34	-0.4731	0.854	0.89	4.74E-30
Ly6d	2E-33	-0.52303	0.91	0.892	6.22E-29
Slfn2	5.62E-63	-0.56886	0.431	0.696	1.75E-58
Cd79b	4.1E-114	-0.66661	0.943	0.942	1.3E-109

Supplemental Table 8: KEGG pathway analysis of genes differentially expressed in cluster 0. Genes were analyzed for Immune System and Energy Metabolism pathways.

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value
KEGG_OXIDATIVE_PHOSPHORYLATION	131	7	0.0534	1.27E-9
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	75	3	0.0400	2.1E-4

Supplemental Table 9: Antibodies used in flow cytometry experiments.

Specificity	Brand	Clone	Fluorophore	Cat #
CD45	BioLegend	30-F11	PerCP-Cy5	103132
CD19	BioLegend	1D3/CD19	APC	152410
IgD	BioLegend	11-16c.2a	BV605	405727
CD11b	BioLegend	M1/70	PE	101208
IgM	BioLegend	RMM-1	Alexa 488	406522
CD5	BioLegend	53-7.3	PE-Cy7	100622
CD21/CD35	BioLegend	7E9	BV421	123422
CD23	BioLegend	B3B4	APC-Cy7	101630

Supplemental Table 10: Oligo-tagged antibodies used for scRNA seq analysis.

Specificity	Brand	Clone	Catalog#
TotalSeqTM-A0014 anti-mouse/human CD11b	BioLegend	M1/70	101265
TotalSeqTM-A0108 anti-mouse CD23	BioLegend	B3B4	101635
TotalSeqTM-A0107 anti-mouse CD21/CD35 (CR2/CR1)	BioLegend	7E9	123427
TotalSeqTM-C0301 anti-mouse Hashtag 1	BioLegend	M1/42	155861
TotalSeqTM-C0302 anti-mouse Hashtag 2	BioLegend	M1/42	155863
TotalSeqTM-C0303 anti-mouse Hashtag 3	BioLegend	M1/42	155865
TotalSeqTM-C0304 anti-mouse Hashtag 4	BioLegend	M1/42	155867

Supplemental Movies Legends

Supplemental Video 1: Z-stack of a myocardial section of CD19-tdTomato mouse heart at embryonic day 18 (E18). A B cell is visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI

Supplemental Video 2: Z-stack of a myocardial section of CD19-tdTomato mouse heart at post-neonatal day 7 (P7). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 3: Z-stack of a myocardial section of CD19-tdTomato mouse heart at post-neonatal day 14 (P14). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 4: Z-stack of a myocardial section of CD19-tdTomato mouse heart at 5 weeks of age. B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 5: Z-stack of a section of CD19-tdTomato mouse liver at post-neonatal day 7 (P7). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 6: Z-stack of a section of CD19-tdTomato mouse liver at post-neonatal day 14 (P14). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 7: Z-stack of a section of CD19-tdTomato mouse liver at 5 weeks of age. B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 8: Z-stack of a section of CD19-tdTomato mouse lung at post-neonatal day 7 (P7). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 9: Z-stack of a section of CD19-tdTomato mouse lung at post-neonatal day 14 (P14). B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 9: Z-stack of a section of CD19-tdTomato mouse lung at 5 weeks of age. B cells are visible in the intravascular space. Red: B cell, Green: CD31, Blue: DAPI.

Supplemental Video 11: Intravital microscopy of B cells in the lung of adult CD19-tdTomato mice. Lung B cells are mostly intravascular. Some B cells are seen rapidly flowing through blood vessels, while some other B cells move slowly or even paused in the lung endothelial. Blood vessels were marked with intravenous injection of high molecular weight fluorescent dextran. B cells are shown in green; Blood vessels in red; DAPI: blue.

Supplemental Video 12: Intravital microscopy of B cells in the lung of adult CD19-tdTomato mice. Blood vessels were marked with intravenous injection of high molecular weight fluorescent dextran. B cells are shown in green; Blood vessels in red; DAPI: blue.

Supplemental Video 13: Intravital microscopy of B cells in the lung of adult CD19-tdTomato mice. In some regions of the lung, B cells were found stopped or moving slowly through the endothelium. Blood vessels were marked with intravenous injection of high molecular weight fluorescent dextran. B cells are shown in green; Blood vessels in red; DAPI: blue.

Reference

1. Adamo L. Rocha-Resende C. Lin CY. Evans S. Williams J. Dun H. et al. Myocardial B cells are a subset of circulating lymphocytes with delayed transit through the heart. *JCI Insight*. 2020;5(3).