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Supplemental Information

T-Bet^{+} IgM Memory Cells Generate Multi-lineage Effector B Cells

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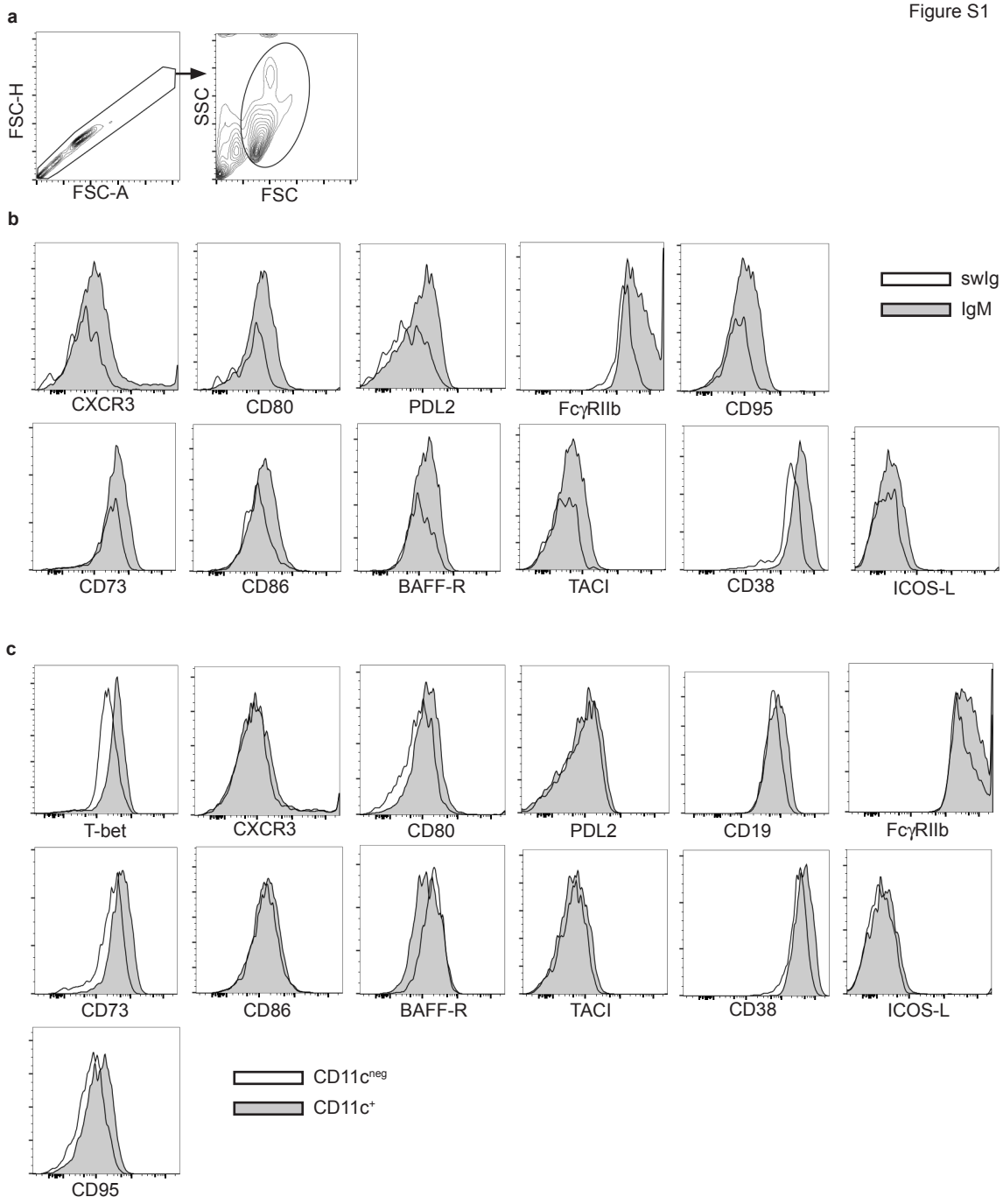

FIGURE S1. Related to Figure 1. Phenotype of *Aidca*-expressing memory B cell subsets.

E.muris-infected (AID-creER^{T2} X ROSA26-eYFP) F_1 mice were administered tamoxifen on day 7 and 10 post-infection and splenocytes were analyzed day 70 post-infection.

(**a**) Representative plots of the gating strategy used to select singlet events and lymphocytes prior to analysis of the lymphocytes.

(**b**) eYFP$^+$ GL7^{neg} CD138^{neg} IgM$^+$ memory cells (shaded histograms) and eYFP$^+$ GL7^{neg} CD138^{neg} IgM^{neg} memory cells (open histograms) were analyzed for expression of indicated surface markers.

(**c**) eYFP$^+$ GL7^{neg} CD138^{neg} IgM$^+$ CD11c$^+$ memory cells (shaded histograms) and eYFP$^+$ GL7^{neg} CD138^{neg} IgM$^+$ CD11c^{neg} memory cells (open histograms) were analyzed for expression of the indicated markers. The data are representative of two experiments containing 4 mice each.

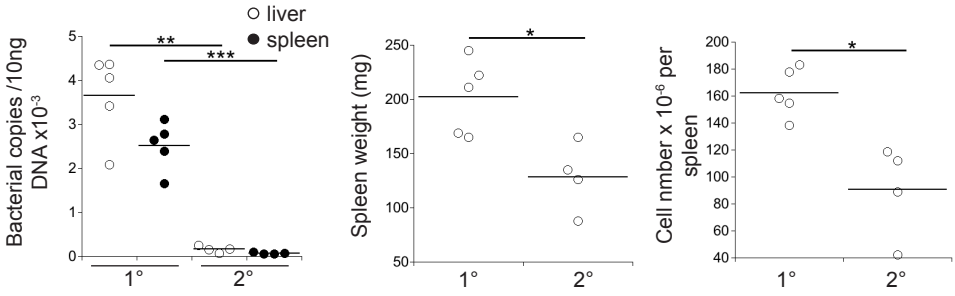

FIGURE S2. Related to Figure 2. Infected mice were resistant to a secondary challenge infection.

C57BL/6 mice that had been infected for 98 days, or naïve mice, were infected with *E. muris,* and bacterial copy number in the liver and spleen was quantified 10 days later. The statistical data are as follows. Left panel, liver: P=0.0012, t=8.125, df=4.063; spleen, P=0.0006, t=9.971, df=4.0017; middle panel, spleen weights: P=0.013, t=3.335, df=6.804; right panel, cell number: P= 0.0175, t=3.749, df=4.308. The data are from one experiment that used groups of 4 or 5 mice. Statistical significance was determined using an unpaired two-tailed *t* test with Welch's correction.

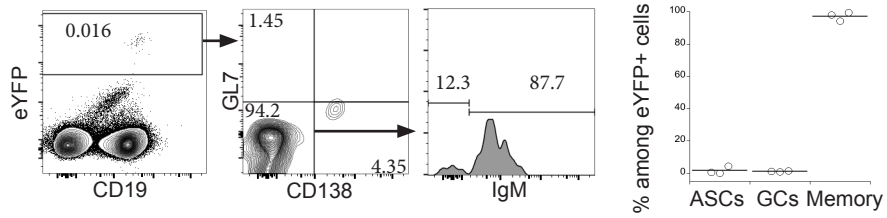

FIGURE S3. Related to Figure 2. IgM memory cells did not differentiate following transfer into naive mice.

Purified EYFP$^+$ IgM memory cells were transferred into naive mice and splenocytes were analyzed 30 days post-transfer. GL7- and CD138-negative eYFP$^+$ donor cells were analyzed for IgM expression. The percentages of eYFP$^+$ cells that expressed markers characteristic of ASCs, GC B cells, and IgM memory cells (as described in Figure 2), are shown in the plot on the right. The data are from one experiment that utilized 3 mice. Statistical significance was determined using a Friedman test (P=0.1944) with Dunn's multiple comparisons.

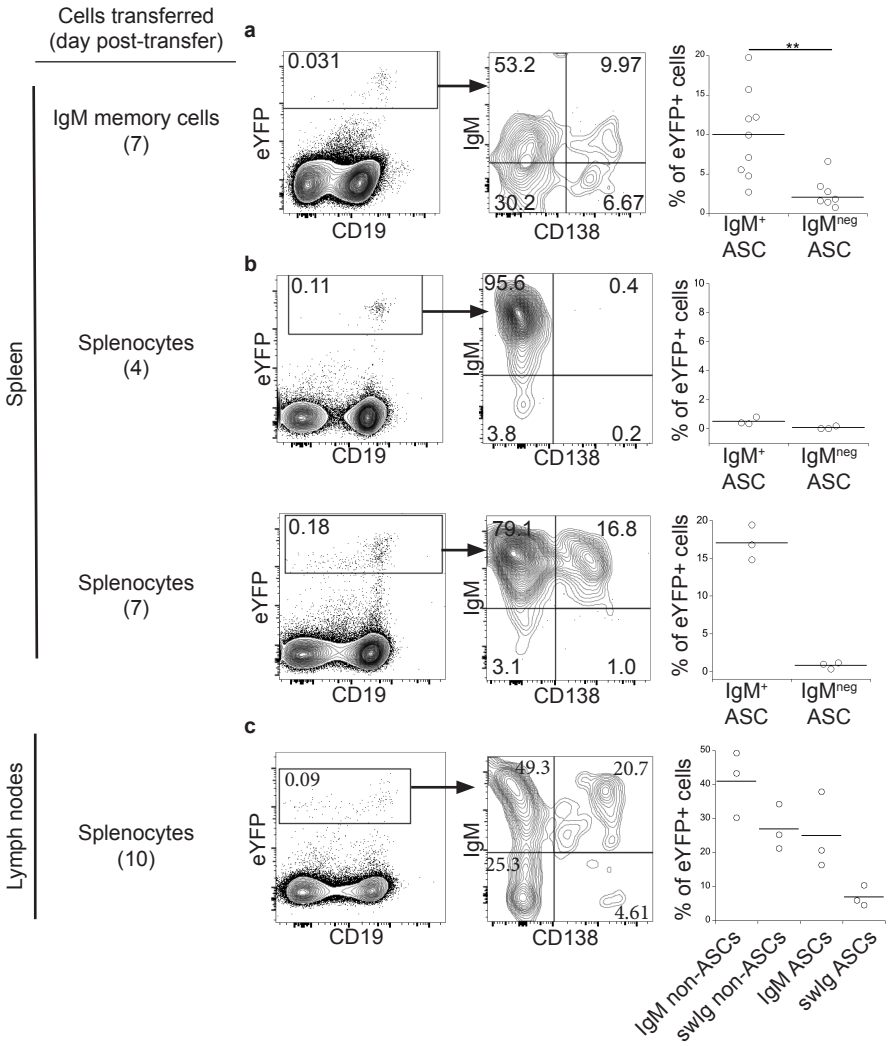

FIGURE S4. Related to Figure 2. IgM memory cells were also detected in lymph nodes.

Spleen cells from infected (AID-creER^{T2} ROSA26-eYFP) F_1 mice, or flow cytometrically purified IgM memory cells, were transferred into naïve mice. The recipient mice were then infected and IgM and CD138 expressing eYFP$^+$ donor cells were monitored in the indicated tissues.

(**a**) Representative flow cytometry dot plots of eYFP$^+$ donor cells identified in the spleen on day 7 post-transfer of purified IgM memory cells. eYFP$^+$ donor cells were analyzed for IgM and CD138 expression (middle plot). The percentage of CD138$^+$ IgM$^+$ and IgM^{neg} cells, within the eYFP$^+$ gate, is quantified in the plot on the far right. Statistical significance was determined using a two-tailed paired *t* test (P=0.0018, t=4.585, df=8).

(**b**) Representative flow cytometry dot plots of eYFP$^+$ donor cells identified in the spleen on day 4 (top) and 7 (bottom) post-transfer of unseparated splenocytes. eYFP$^+$ donor cells were analyzed for IgM and CD138 expression (middle plot).The percentage of eYFP$^+$ cells that expressed CD138 is quantified to the right. Statistical significance was determined using Wilcoxon matched-pairs signed rank test (top, P=0.25; bottom, P=0.25).

(**c**) Representative flow cytometry dot plots of eYFP$^+$ donor cells in the inguinal and mesenteric lymph nodes day 10 post-transfer of all splenocytes. The percentage of eYFP$^+$ cells that expressed CD138 and/or IgM is quantified to the right; swIg cells were IgM^{neg}, and ASCs were identified by expression of CD138. Statistical significance was determined using a Friedman test (P=0.0747) with Dunn's multiple comparisons. The data are from one experiment containing 3 mice per time point for the transfer of unseparated splenocytes and two experiments containing 4 or 5 mice per experiment for analysis of day 7 post-transfer of purified IgM memory cells.

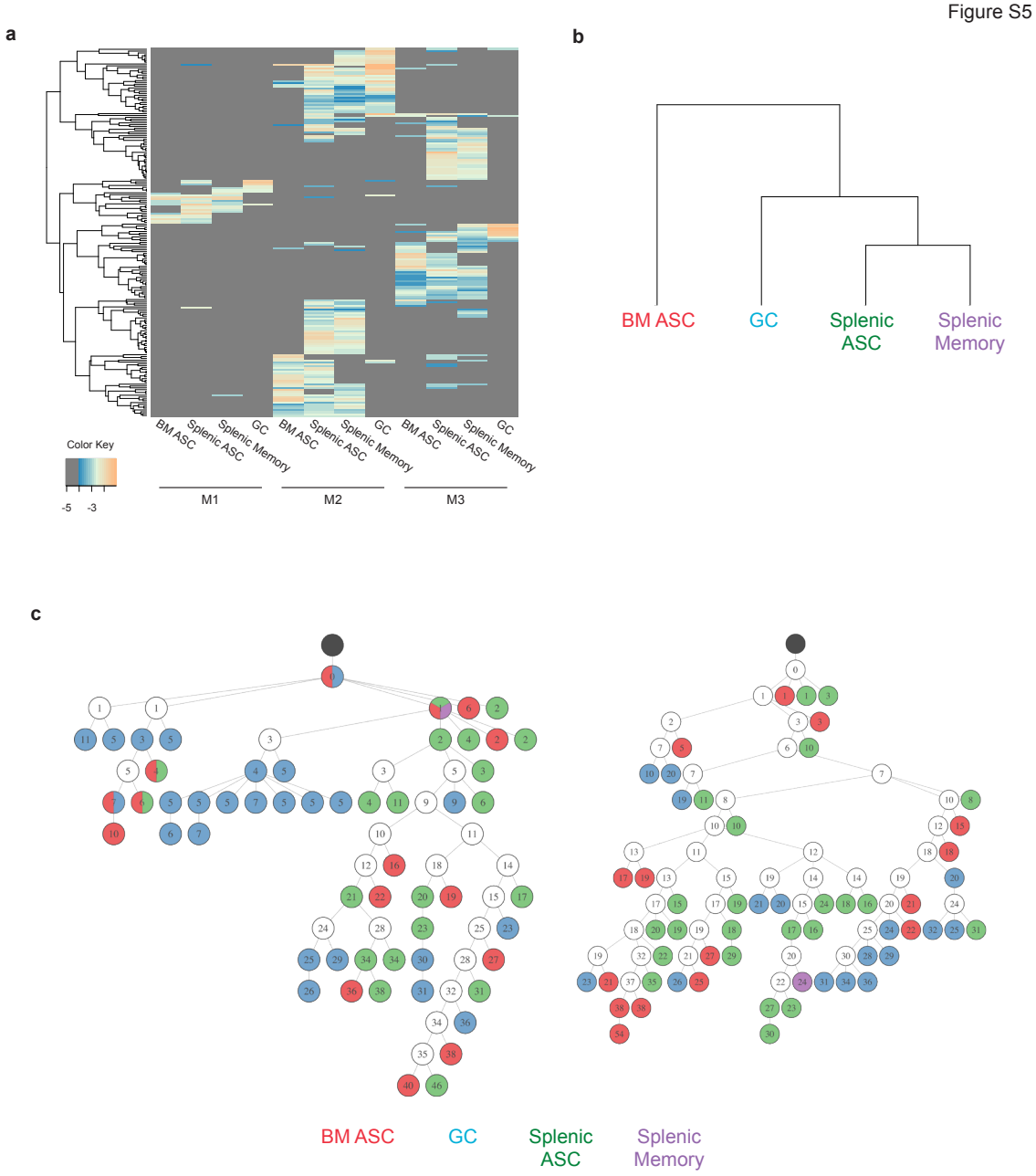

FIGURE S5. Related to Figure 6. Clonal relationship between recipient mice and lineages.
Clones shared between B cell populations from three recipient mice.
(**a**) The VDJTools (Shugay et al., 2015) TrackClonotypes function was used to analyze clones that appeared in at least two different populations; only top 200 are visualized. Color indicates the frequency of each clone.

(**b**) Dendrogram of the clonal relationship between the different effector populations generated with VDJTools ClusterSamples function.

(**c**) Lineage trees of a common clone identified in all of the isolated B cell populations. Black circles represent a germline clone, and white circles represent inferred clones. Daughter clones are colored based on the effector population in which they were identified. The number of mutations from germline is indicated in each clone.

Table S1. Related to Figure 1: Surface Marker Expression on $CD11c^{+}$ and $CD11c^{neg}$ IgM Memory Cells (MFI)[a]

Surface marker	Cell population		Fold-difference
	$CD11c^{pos}$	$CD11c^{neg}$	
T-bet	3662	2002	**1.8**[b]
CXCR3	1048	894	1.2[c]
CD11b	9094	1738	**5.2**
CD73	5411	2985	**1.8**
CD86	1599	1519	1.1[c]
CD80	1425	893	**1.6**
PD-L2	1000	992	1.0
FcγRIIb	50417	32594	**1.5**
CD95	1409	883	**1.6**
BAFF-R	1378	1933	**-1.4**
TACI	564	500	**1.1**
CD19	7876	6154	**1.3**
CD38	48441	33631	1.4[c]
ICOS-L	206	158	**1.3**

[a]MFI values represent the mean determined from the analysis of 4 mice on day 70 post-infection.
[b]Bold type indicates statistical significance, as determined using a paired *t* test.
[c]A non-parametric Wilcoxon test was used to determine statistical significance.