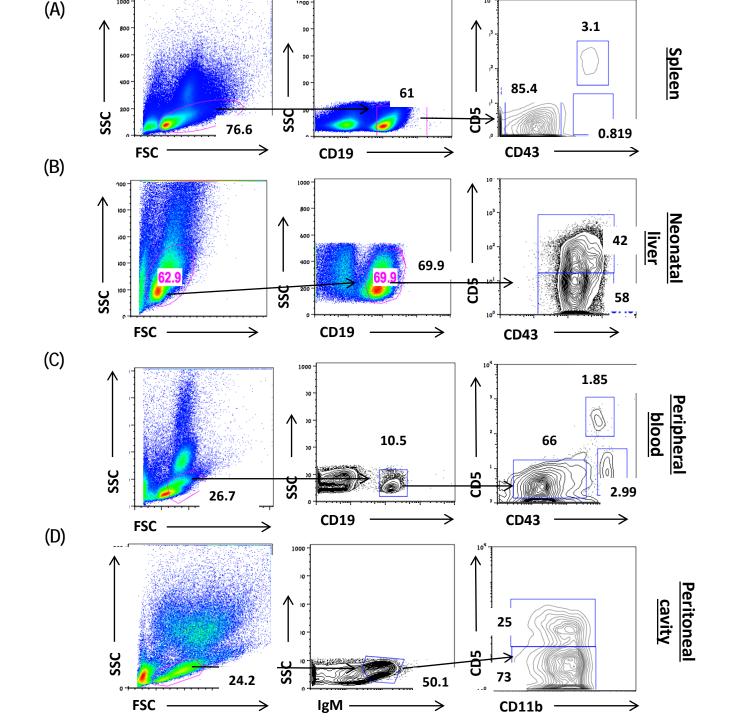
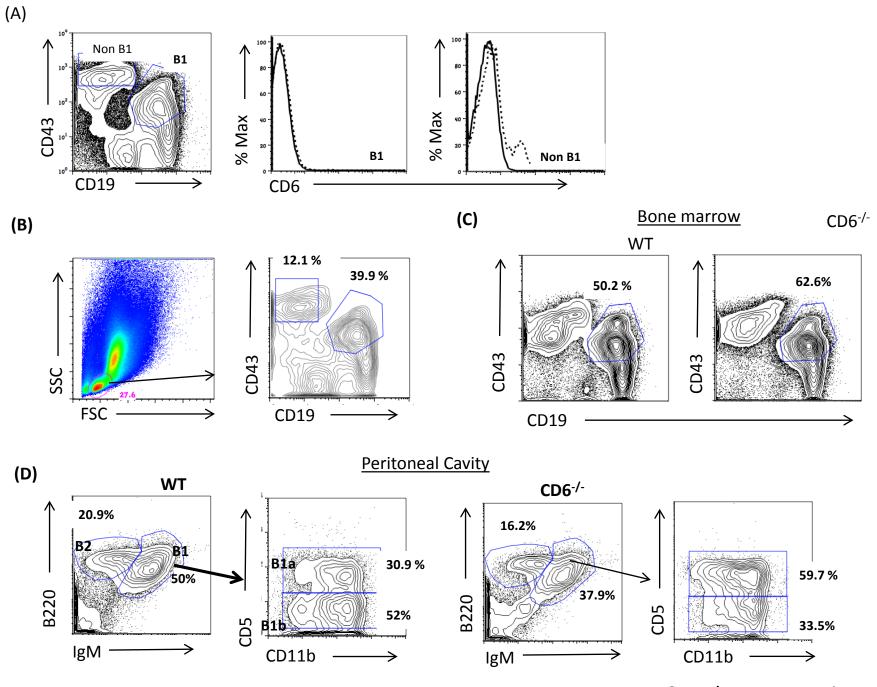
Supplementary figures

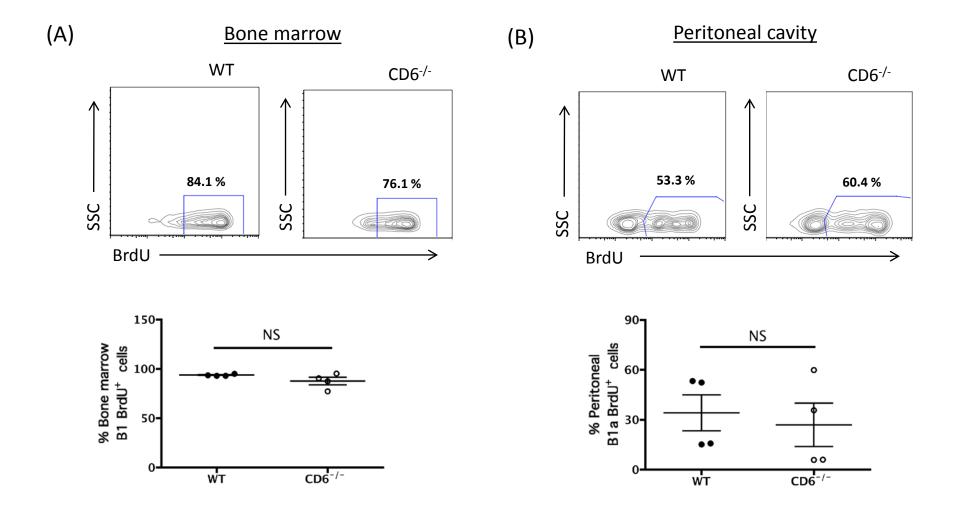


Supplementary Figure 1



Supplementary Figure 2

Table 2			
		Frequency (%)	
	Mice (n)	WT	CD6-/-
Bone marrow B1 cells	5	44.40 ± 8.68	47.20 ± 7.606
Peritoneal B1 cells	10	57.29 ± 4.358	46.71 ± 3.630 *
Peritoneal B1a cells	10	21.92 ± 1.789	38.87 ± 3.22 ***
Peritoneal B1b cells	10	61.97± 4.004	49.92 ± 4.177 *
		Absolute cell numbers	
		WT	CD6-/-
Bone marrow B1 cells	5	2859000 ± 944400	3145000 ± 769200
Peritoneal B1 cells	10	192800 ± 37200	117300 ± 19980*
Peritoneal B1a cells	10	83610 ± 15110	105500 ± 20860
Peritoneal B1b cells	10	209500± 39910	119500 ± 15870 *



Supplementary Figure 1. The flow cytometry gating strategy for B1 cells in different tissue compartments. (A) (B) (C) B1 cell characterization strategy. Cells were first gated on size and singularity to identify the lymphocyte -like population. Live lymphocyte cells were gated on CD19 expression to identify B cells. Finally, B cells were gated for the expression of B1 cell immune phenotype markers CD43 and CD5. (D) In the peritoneal cavity, cells were first gated on size and singularity to identify the lymphocyte-like population. Live lymphocyte cells were gated on IgM high expression to identify B cells. Finally, B cells were gated on peritoneal B1 cell immune phenotype markers CD11b and CD5.

Supplementary Figure 2. CD6 expression on Bone marrow B1 cells and its effect on B1 cell population in the Bone marrow and Peritoneal Cavity. (A) CD6 expression was analyzed on Bone marrow B1 cells. Bone marrow B1 cells are CD19⁺CD43⁺. Data are representative of n=5. (B) Bone marrow B1 cell characterization. Cells were first gated on size and singularity to identify the lymphocyte -like population. Live lymphocyte cells were gated on CD19 and CD43 double positive cells to identify B1 cells. (C) Flow cytometric analysis of Bone marrow B1 cells (CD19⁺CD43⁺). Data are representative of n=11 mice per group. (D) Flow cytometric analysis of peritoneal B1 (B220⁺ IgM⁺), which was further analyzed for peritoneal B1a (B220⁺ IgM⁺CD11b⁺ CD5⁻) and peritoneal B1b (B220⁺ IgM⁺CD11b⁺ CD5⁻). Data are representative of n=10 mice per group.

Table 2. Frequency and absolute numbers of Bone marrow and peritoneal B1/a/b cells for WT and CD6-/-- mice. The numbers are mean values ± standard deviation. * p<0.01, **p<0.001, ***p<0.0001.

Supplementary Figure 3. B1/a cell proliferation are not reduced in the Bone marrow and peritoneal cavity of CD6-/- mice. (A) WT and CD6-/- Bone marrow B1 cells incorporation of BrdU (CD19+CD43+BrdU+) were analyzed by flow cytometry. Data are representative of n= 4 mice per group.

(B) WT and CD6 ^{-/-} Peritoneal B1a cells incorporation of BrdU (B220 ⁺ CD11b ⁺ CD5 ⁺ BrdU ⁺) were analyzed			
by flow cytometry. Data are representative of n= 4 mice per group.			