Supplemental Table 1. Mouse strains utilized in these studies.

Strain (common name)	Source	Notes (all strains bred at Trudeau)
C57BL/6J (B6)	Jackson Laboratory	
BALB/c	Jackson Laboratory	
B6.129S2-IgH-6^{tm1Cgn}/J (μMT)	Jackson Laboratory	B cell deficient on B6 genetic background
BALB/c-Il4ra^{tm1Sz}/J (IL-4Rα$^{-/-}$)	Jackson Laboratory	
B6.129S2-H2$^{d1Ab1-Ea}$/J (MHCII$^{-/-}$)	Jackson Laboratory	
B6;129S-Tnf^{tm1Gki}/J (TNFα$^{-/-}$)	Jackson Laboratory	
B6;129S2-Lta^{tm1Dch}/J (LTα$^{-/-}$)	Jackson Laboratory	
B6.129P2-Il2^{tm1Hor}/J (IL-2$^{-/-}$)	Jackson Laboratory	
B6.129P2-Il4^{tm1Cgn}/J (IL-4$^{-/-}$)	Jackson Laboratory	
B6.129P2-Il10^{Tm1Cgn}/J (IL-10$^{-/-}$)	Jackson Laboratory	
C.IgH-J^{tm1Dhu} (JHD)	Taconic	B cell deficient mice on BALB/c genetic background
C57BL/6-Tg(IghelMD4)4Ccg/J IgH-6^{tm1Cgn}/J (MD4μMT)	Dr. J. Kearney (Univ. of Alabama)	MD4 BCR transgenic mice (1988 Nature 334:676) were crossed to μMT mice to generate mice with a monoclonal B cell population specific for Hen Egg Lysozyme (HEL)
C.129-Il4^{tm1Lky}/J (BALB/c 4get)	Dr. M. Mohrs (Trudeau Institute)	A knockin replaces the endogenous IL-4 gene with an Il4/IRES/EGFP gene (IL4 activity remains intact). This leads to generation of a bicistronic transcript under the control of endogenous regulatory elements. Cells activated to express IL4 mRNA will express EGFP. IL-4 reporter backcrossed to BALB/c (2001 Immunity 15:303).
B6.129-Il4^{tm1Lky}/J (B6 4get)	Dr. M. Mohrs (Trudeau Institute)	A knockin replaces the endogenous IL-4 gene with an Il4/IRES/EGFP gene (IL4 activity remains intact). This leads to generation of a bicistronic transcript under the control of endogenous regulatory elements. Cells activated to express IL4 mRNA will express EGFP. IL-4 reporter backcrossed to B6 (2001 Immunity 15:303).
B6.129-Il4^{tm1Lky}/J IgH-6^{tm1Cgn}/J (4getμMT)	Dr. M. Mohrs (Trudeau Institute)	The IL-4 reporter mouse on the B6 background (see above) was crossed to μMT mice to generate IL-4 reporter mice that lack B cells.
C.129-Il4^{tm1Lky}/J Il4ra^{tm1Sz}/J (4getIL4Rα$^{-/-}$)	Dr. M. Mohrs (Trudeau Institute)	The IL-4 reporter mouse on the BALB/c background (see above) was crossed to IL-4Ra knockout mice to generate IL-4 reporter mice that are also IL-4Ra deficient.
μs$^{-/-}$	Dr. R. Corley (Boston Univ.)	Mice that lack the secretory exon for IgM (1998 J. Immunol. 160:4776). Are incapable of secreting IgM antibody but can secrete class-switched antibodies
AID$^{-/-}$	Dr. R. Gerstein (Univ. Mass.)	Mice deficient in activation induced cytidine deaminase (AID) (2000 Cell 102:541). Mice cannot class-switch and cannot secrete class-switched antibodies
μs$^{-/-}$AID$^{-/-}$	Dr. Troy Randall (Trudeau Institute)	Mice that lack AID and the secretory exon for IgM. These mice cannot secrete antibodies of any isotype.

Supplemental Figure Legends- Wojciechowski et al

Supplemental Figure 1. Protective immunity to *Hp* challenge infection is not controlled by B cell dependent lymphoid tissue organogenesis.

(A) Lethally irradiated μMT recipients (950 rads) were reconstituted with either B6 or μMT BM (1 x 10^7 cells/recipient). Since the organogenesis of Peyer's patches and the development of the splenic T cell compartment is controlled by B cells during fetal ontogeny, all of the chimeras (all made in μMT hosts) have missing or abnormal Peyer's patches and spleen. (B) μMT mice reconstituted with B6 BM contain B cells while μMT mice reconstituted with μMT BM are B cell deficient. (C) The reconstituted mice were exposed to two rounds of *Hp* infection and drug treatment and were then challenged with 200 L3 *Hp*. Three weeks later, parasite burdens were determined for individual animals.

Supplemental Figure 2. Generation of bone marrow chimeras to assess the role of B cells in regulating Th2 primary and memory responses to *Hp*. (A) IL-4 reporter mice (4get mice) were generated by replacing the endogenous IL-4 gene with an IL-4/IRES/EGFP cassette that permits normal IL-4 expression and activity. Cells activated to express IL-4 mRNA will also express the bicistronic EGFP transcript under the control of endogenous IL-4 regulatory elements. To address the role of B cells in Th2 responses, the 4get mice were intercrossed with μMT mice to generate B cell deficient IL-4 reporter animals (4getμMT). BM chimeric animals were then generated by lethally irradiating B cell deficient hosts (μMT) and reconstituting the mice with either 100% 4get BM (4get chimeras) or with 100% 4getμMT BM (4getμMT chimeras). The 4get chimeras express all hematopoietic cell lineages while the 4getμMT chimeras lack B cells. The IL-4 reporter cassette (EGFP) is knocked into the IL-4 locus of

100% of the hematopoietic cells that are present in the chimeric mice. Therefore, 100% of T cells present in both the 4get and 4getμMT chimeras are competent to express the IL-4 reporter cassette, allowing us to use EGFP expression to follow Th2 development in vivo in the presence and absence of B cells. (B) MLN cells from 4get and 4getμMT chimeras that were infected 8 days earlier with L3 *Hp* were examined by flow cytometry for the expression of EGFP and CD62L on $CD4^+$ gated cells. Representative FACS plots are shown and the percentage of cells in each quadrant is indicated. (C) MLN cells were isolated from day 8 post-*Hp* infection 4get and 4getμMT mice. The cells were stimulated in vitro for 4 hrs with platebound anti-CD3 in the presence of Brefeldin A. Cells were then subjected to intracellular cytokine staining and flow cytometric analysis. EGFP and IL-4 expression in $CD4^+EGFP^+$ gated cells was analyzed. Representative FACS plots are shown and the percentage of cells in each quadrant is indicated.

Supplemental Figure 3. Generation of mixed bone marrow chimeras to address the role of antibody in Th2 memory responses to *H. polygyrus*. To assess whether secreted antibody regulates Th2 responses to *H. polygyrus*, we generated mixed BM chimeras. B cell deficient μMT mice were lethally irradiated and reconstituted with different mixtures of BM (1×10^7 total BM cells/recipient). Mice reconstituted with 75% 4getμMT BM + 25% μMT BM are unable to generate B cells and are referred to as μMT chimeras. Mice reconstituted with 75% 4getμMT BM + 25% B6 BM are able to generate all hematopoietic lineages, including B cells. The B cells in this chimera must develop from the B6 BM and are therefore normal wild type B cells. These mice are referred to as B-WT chimeras. Mice reconstituted with a mixture of 75% 4getμMT BM plus 25% $\mu s^{-/-}AID^{-/-}$ BM are able to generate all hematopoietic lineages, including B lymphocytes. The B cells in this mouse must all develop from the $\mu s^{-/-}AID^{-/-}$ BM and thus are

unable to secrete antibody of any isotype. These mice are referred to as B-$\mu s^{-/-}$AID$^{-/-}$ chimeras. 75% of all other hematopoietic cells (i.e. all cells but the B cells) in each of these chimeras must develop from the 4getμMT BM. Thus, 75% of all T cells in each chimera contain the IRES-EGFP reporter knocked into the 3' untranslated region of the IL-4 locus. These T cells are still competent to produce IL-4 and when the IL-4 locus is transcribed in these T cells, EGFP protein is produced.

Supplemental Figure 4. Generation of mixed bone marrow chimeras to assess the role of B cell-derived MHCII in protection to *Hp*. (A) An analysis of mixed BM chimeras was performed to address whether antigen presentation by B cells is necessary for immune protection to *H. polygyrus*. B cell deficient μMT mice were lethally irradiated and reconstituted with different mixtures of BM (1 x 10^7 total BM cells/recipient). Mice reconstituted with 100% μMT BM are unable to generate B cells and are referred to as μMT chimeras. Mice reconstituted with 75% μMT BM + 25% B6 BM are able to generate all hematopoietic lineages, including B cells. The B cells in this chimera must develop from the B6 BM and are therefore normal wild type B cells. These mice are referred to as B-WT chimeras. Mice reconstituted with a mixture of 75% μMT BM plus 25% MHCII$^{-/-}$ BM are able to generate all hematopoietic lineages, including B lymphocytes. However, the B cells in these mice are unable to express MHCII. These mice are referred to as B-MHCII$^{-/-}$ chimeras. (B-C) The B cell reconstitution profile was analyzed in the (B) peripheral blood and (C) the spleen after 8 weeks of reconstitution. Panel B shows a representative flow cytometric analysis of μMT, B-WT and B-MHCII$^{-/-}$ chimeras. The percentage of CD19$^+$ B cells is indicated. No differences were observed in the proportion of B cells present in blood of B-WT and B-MHCII$^{-/-}$ chimeras. (C) The number of CD19$^+$IgM$^+$ B cells

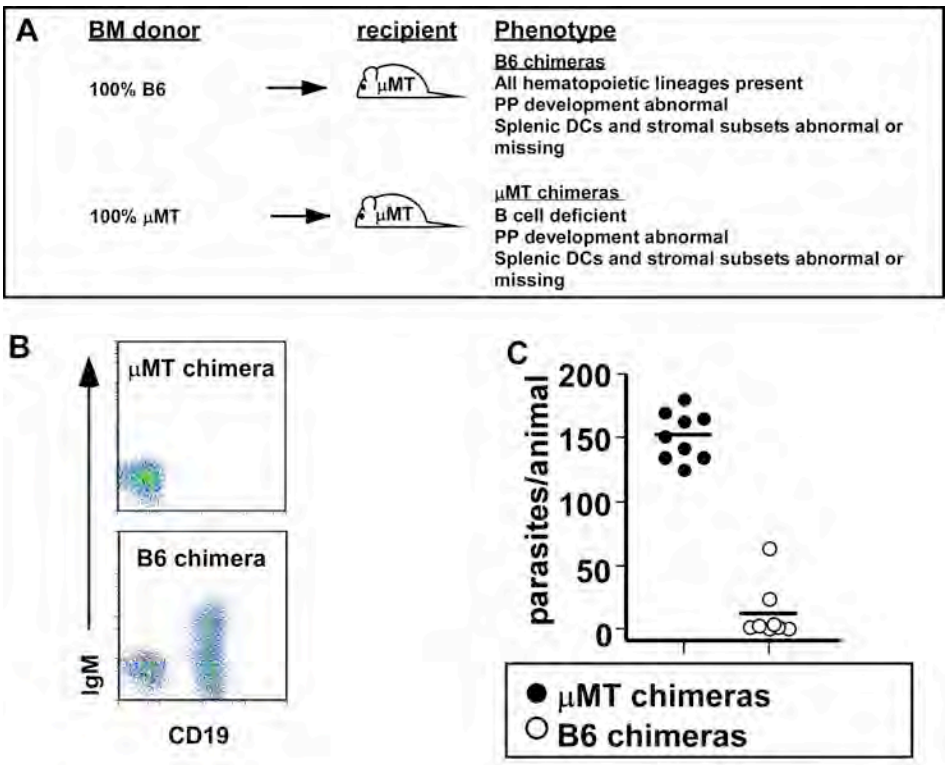
present in the spleen of the chimeric mice was determined by flow cytometry and counting. No B cells were found in the μMT chimeras but equivalent numbers of B cells were found in the B-WT and B-MHCII$^{-/-}$ chimeras. Data are shown for individual mice (n=14 mice/group). (D) To assess class II expression on the various cell populations, spleen cells from reconstituted μMT, B-WT and B-MHCII$^{-/-}$ chimeras were stained with antibodies to CD19 and I-A^{b}. Representative FACS plots are shown and the percentage of cells in each quadrant are indicated. As expected, B cells in the B-MHCII$^{-/-}$ chimeras do not express class II while B cells from the B-WT chimeras are classII^{+}. The percentage of I-A^{b+}CD19^{neg} cells (non-B APCs) is very similar in all three groups of mice.

Supplemental Fig 5. Generation of mixed BM chimeras to determine whether *Hp* protective B cells must be activated by IL-4/IL-13. (A) To determine whether B cells must receive an IL-4Rα-dependent signal to mediate protection to *Hp,* B cell deficient mice (JHD) were lethally irradiated and reconstituted with different mixtures of BM (1 x 10^7 total BM cells/recipient). Mice reconstituted with a mixture of JHD BM (80% of mixture) + 4get BM (20% of mixture) are able to generate all hematopoietic lineages and all of the lineages are competent to express IL-4Rα. The B cells in this chimera (referred to as B-4get chimeras) must develop from the 4get (IL-4 reporter mouse) BM. Therefore, the 3' untranslated region of the IL-4 gene present in all B cells has been replaced by a bicistronic IRES-EGFP reporter cassette. All of the B cells in the B-4get chimeras are still competent to produce IL-4, however when the IL-4 locus is transcribed in these B cells, the EGFP cassette is also transcribed and EGFP protein is produced. Likewise, irradiated B cell deficient mice reconstituted with a mixture of 80% JHD BM + 20% 4getIL-4Rα$^{-/-}$ BM are also able to generate all hematopoietic lineages. The B cells in

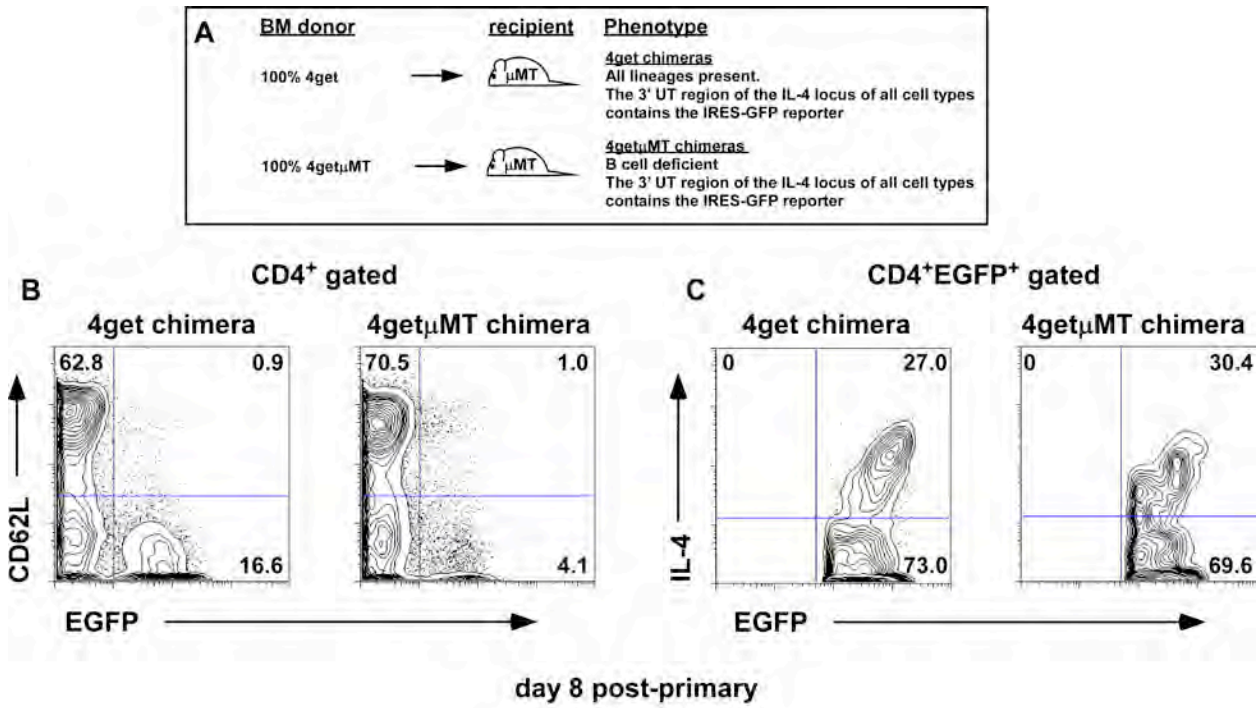
this chimera (referred to as B-4getIL-4Rα$^{-/-}$ chimeras) must develop from the 4getIL-4Rα$^{-/-}$ BM. Again, the 3' UT region of the IL-4 gene present in all B cells contains a bicistronic IRES-EGFP reporter cassette. All of the B cells in the B-4getIL-4Rα$^{-/-}$ chimeras are still competent to produce IL-4, and when the IL-4 locus is transcribed in these B cells, the EGFP cassette is also transcribed and EGFP protein is produced. However, none of the B cells in the B-4getIL-4Rα$^{-/-}$ chimeras can express IL-4Rα, leaving them unable to respond to IL-4 or IL-13. (B) Eight weeks post reconstitution, MLN cells from B-WT and B-IL4Rα$^{-/-}$ chimeras were isolated and analyzed by flow cytometry. The CD19^{+} cells were gated from the live (PI^{neg}), lymphoid gated CD3^{neg} population and IL-4 expression (EGFP) was analyzed. A representative histogram for each group is shown. The percentage of B cells expressing EGFP is indicated. As controls for FACS gating, we included histograms from MLN cells isolated from uninfected 4get and 4getIL-4Rα$^{-/-}$ mice. The percentage of B cells competent to produced IL-4 was < 0.01% and was below the limit of detection of our assay (~0.01%).

Supplemental Figure 6. Generation of mixed bone marrow chimeras to assess the in vivo role of cytokine-producing B cells. To analyze the importance of cytokines expressed by B lineage cells in protection to *H. polygyrus*, we generated mixed BM chimeras. B cell deficient mice (μMT) were lethally irradiated and reconstituted with different mixtures of BM (1 x 10^7 total BM cells/recipient). Mice reconstituted with 100% μMT BM are unable to generate B cells and are referred to as μMT chimeras. Mice reconstituted with a mixture of μMT BM (75% of mixture) + B6 BM (25% of mixture) are able to generate all hematopoietic lineages, including B cells. The B cells in this chimera must develop from the B6 BM and are therefore normal wild type B cells. These mice are referred to as B-WT chimeras. Mice reconstituted with a mixture

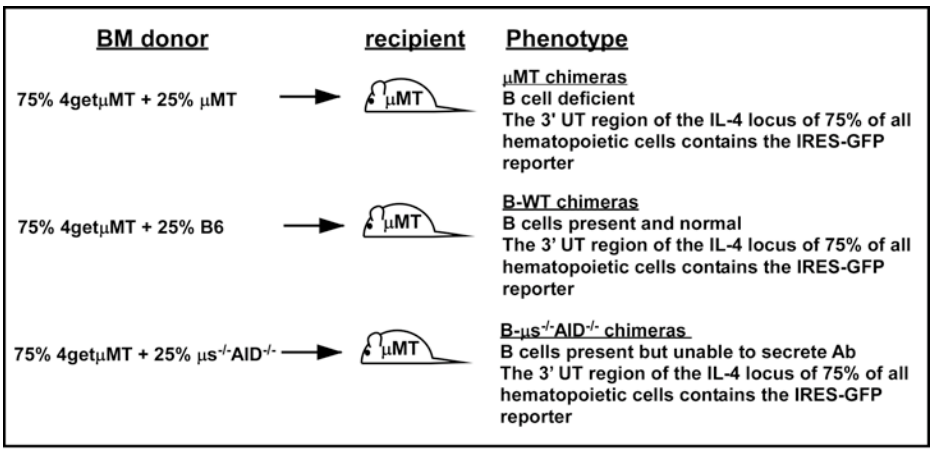
of 75% μMT BM plus 25% cytokine deficient BM are able to generate all hematopoietic lineages, including B lymphocytes. The B cells in this mouse must all develop from the cytokine deficient BM and thus are unable to express the particular cytokine. These mice are referred to as B-cytokine$^{-/-}$ chimeras. Mixed BM chimeras of this type were made to address the role of various B cell-derived cytokines including IL-4, IL-2 and TNFα. As an additional control, mice were reconstituted with 100% IL-4$^{-/-}$, IL-2$^{-/-}$ or TNFα$^{-/-}$ BM. These mice are able to generate all hematopoietic lineages including B cells. However, all hematopoietic cells in these mice are unable to express the relevant cytokine. These mice are referred to as IL-4$^{-/-}$ chimeras, IL-2$^{-/-}$ chimeras or TNFα$^{-/-}$ chimeras.



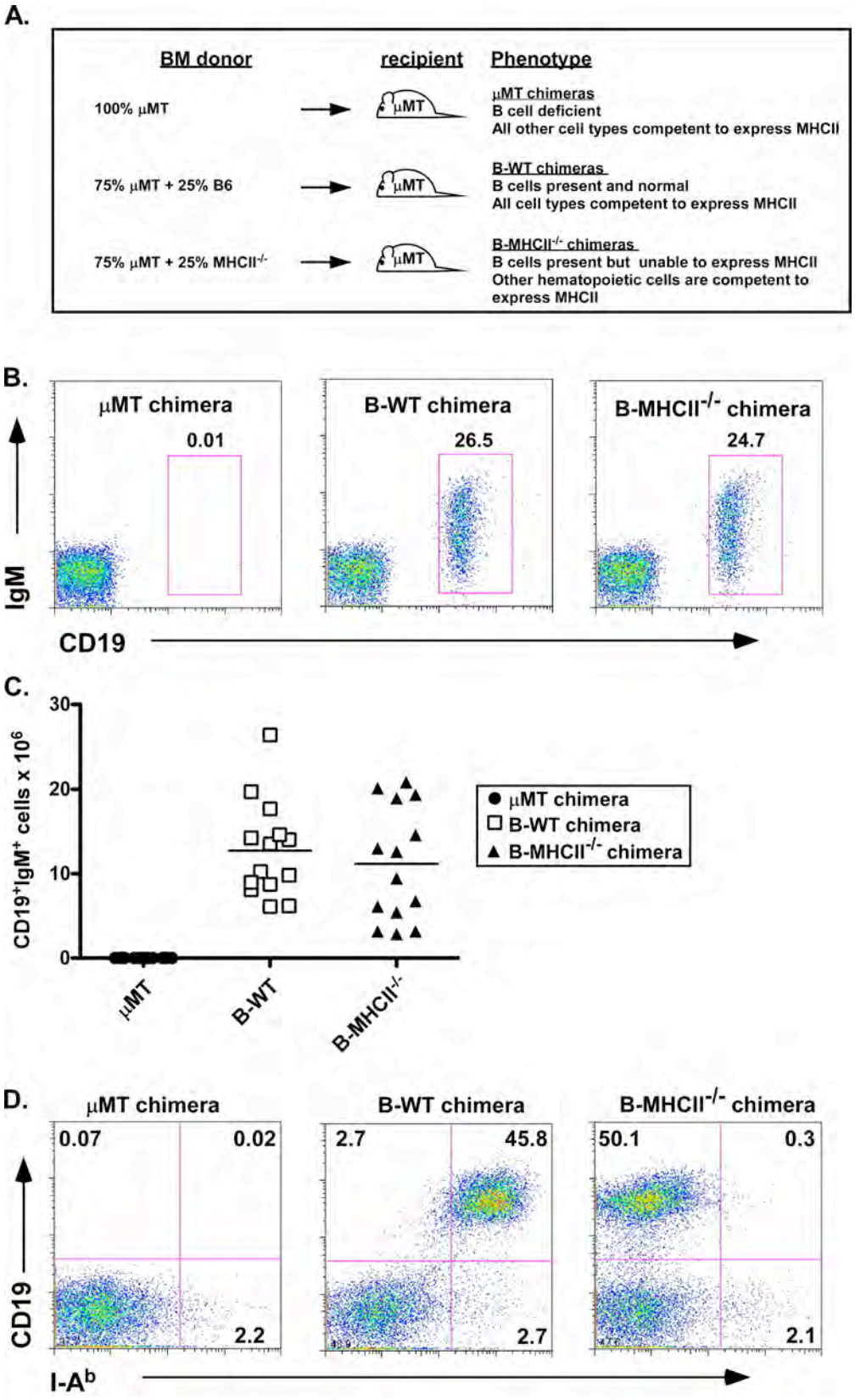
Supplemental Fig. 1 Wojciechowski et al



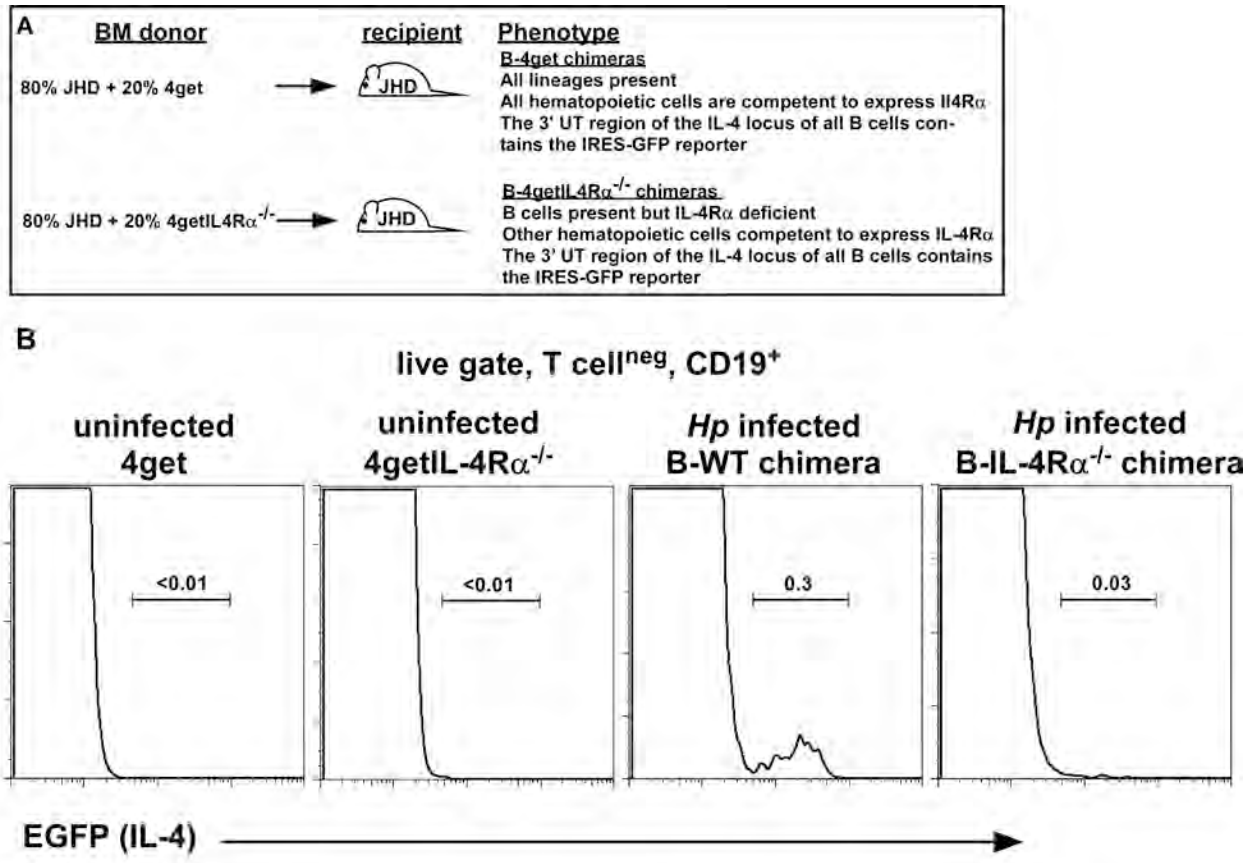
Supplemental Figure 2 Wojciechowski et al



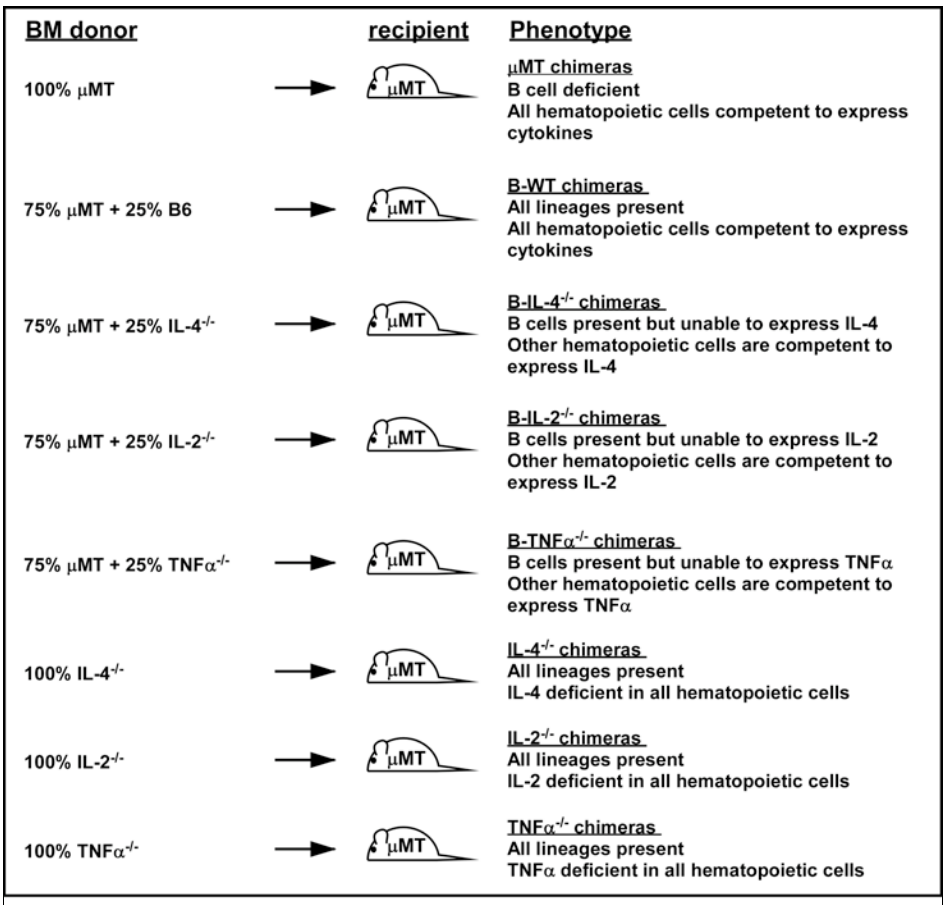
Supplemental Figure 3 Wojciechowski et al



Supplemental Figure 4 Wojciechowski et al



Supplemental Figure 5 Wojciechowski et al



Supplemental Figure 6 Wojciechowski et al