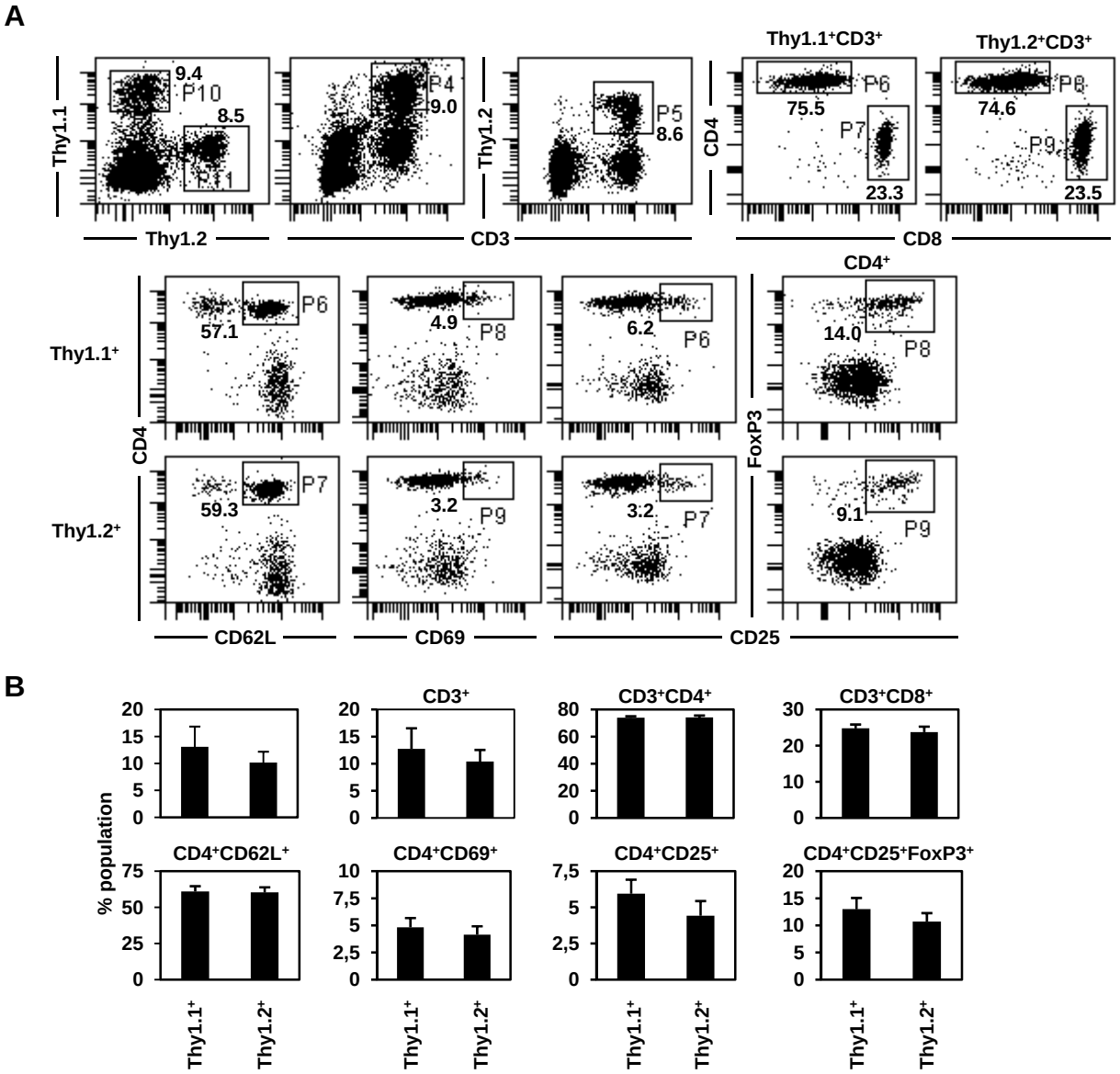
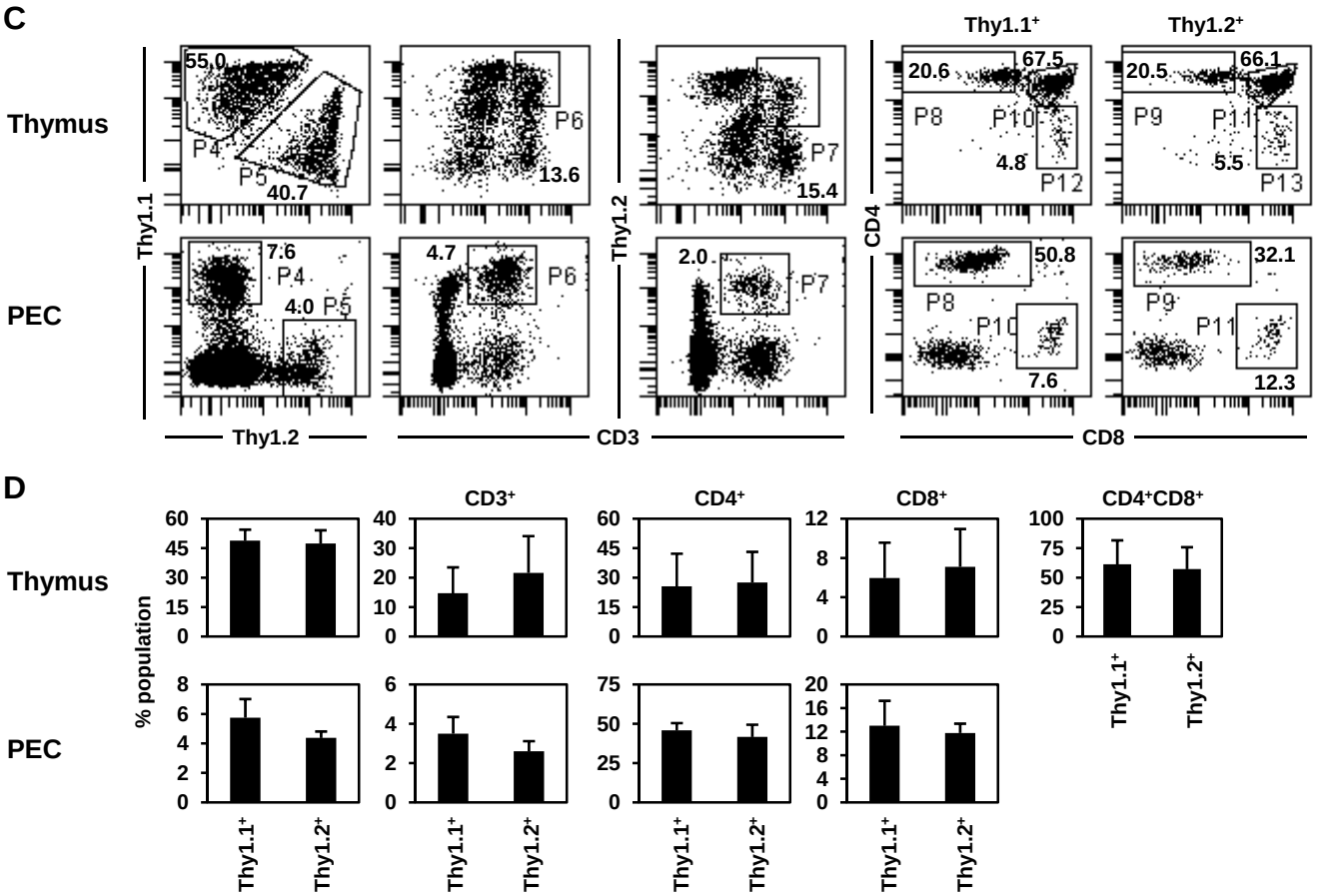
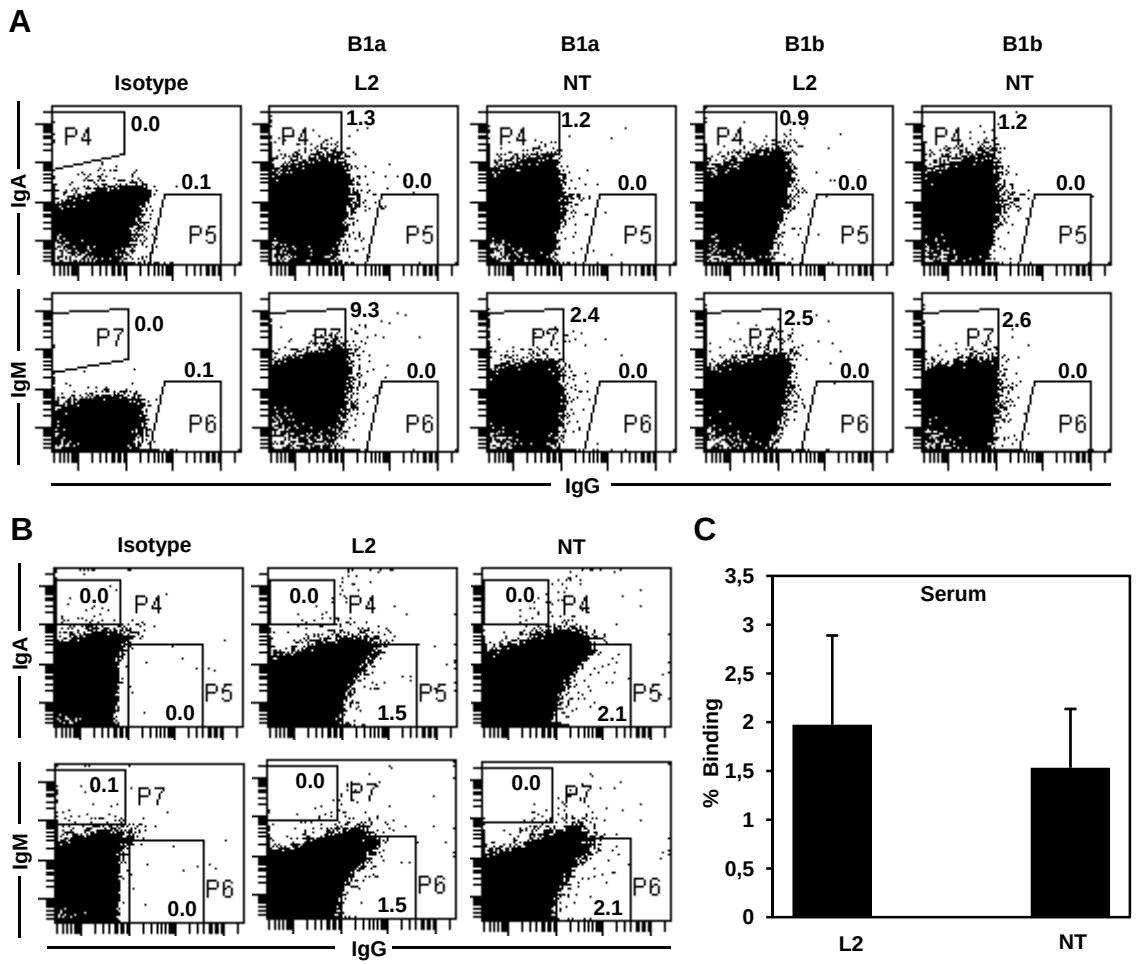


Roy, et. al. Supplemental Figure 1A and 1B



Roy, et. al. Supplemental Figure 1C and 1D



Roy, et. al. Supplemental Figure 2A, 2B and 2C

Supplementary figure legends

Supplemental Figure 1: T cells of L2 origin do not display any developmental defect. Bone marrow chimera generated by transferring a mixture of bone marrow from L2 mice and age matched normal wild type (WT) mice at 1:1 ratio into irradiated (7.5Gy) $Rag1^{-/-}$ mice was analyzed after 6 weeks of adoptive transfer for the presence of T cells of L2 origin in the spleen, peritoneal cavity and thymus of the recipients. T cells of L2 origin expressing Thy1.2 was distinguished by using the congenic Thy1.1 WT mice. (A-B) Flow cytometric analysis of splenocytes (C-D) peritoneal cavity and thymic cells from recipient $Rag1^{-/-}$ mice. The dot plots show the percentages of total ($CD3^+$), naïve ($CD4^+CD62L^+$), activated ($CD4^+CD69^+$ or $CD4^+CD25^+$), and regulatory ($CD4^+CD25^+FoxP3^+$) T cells of L2 and WT origin in the indicated organs of recipient mice. The numbers represent the percentages of gated T cell populations. Data (B, D) are shown as mean ± SD of n = 4 and are representative of 2 independent experiments.

Antibodies: Anti mouse Thy1.1 (eBioscience), Thy1.2 (self made), CD3 (BD), CD4 (BD/eBioscience), CD8 (eBioscience), CD62L (eBioscience), CD69 (BD), CD25 (BD), and FoxP3 (eBioscience) antibodies were conjugated with PECy7/FITC/PE/APC/PerCpCy5.5 or were biotinylated. Biotinylated antibodies were revealed by using PerCpCy5.5 conjugated streptavidin (eBioscience).

Supplemental Figure 2: Binding specificity of IgM antibodies derived from B1 cells of L2 mice to gut bacteria is comparable to that of non transgenic (NT) mice. Binding of secretory IgM/IgA/IgG antibodies present in the culture supernatant (A) of FACS purified peritoneal B1a ($CD19^{hi}CD43^+CD5^+Mac\text{-}1^+$) and B1b ($CD19^{hi}CD43^+CD5^-Mac\text{-}1^+$) cells and in the serum (B-C) of unimmunised L2 and NT mice was tested by flow cytometry. Sorted cells were stimulated with LPS (at 25µg/ml, Sigma) and IL5 (home made from culture supernatant) in culture for 5 days before collecting the supernatant. After incubation of a mixture of gut bacteria isolated from

L2 and NT mice with supernatant/serum for 30 minutes, secretory IgM/IgA/IgG bound to bacteria was revealed by using FITC conjugated anti mouse IgA (BD), PE conjugated anti mouse IgM (BD) and Biotinylated anti mouse IgG (MABTECH) antibodies. Biotinylated antibody was revealed by using APC conjugated streptavidin (BD). For isolating the gut bacteria, colonic content of the mouse was collected after flushing with PBS, effectively mixed by vortexing and centrifuged at 30g for 30 minutes to remove the fecal material. Supernatant containing gut bacteria was collected and used for the experiment. Numbers in each plot represent the percentage of bacteria bound by Ig molecules. Data (C) are shown as mean ± SD of n = 3 and are representative of 2 independent experiments.