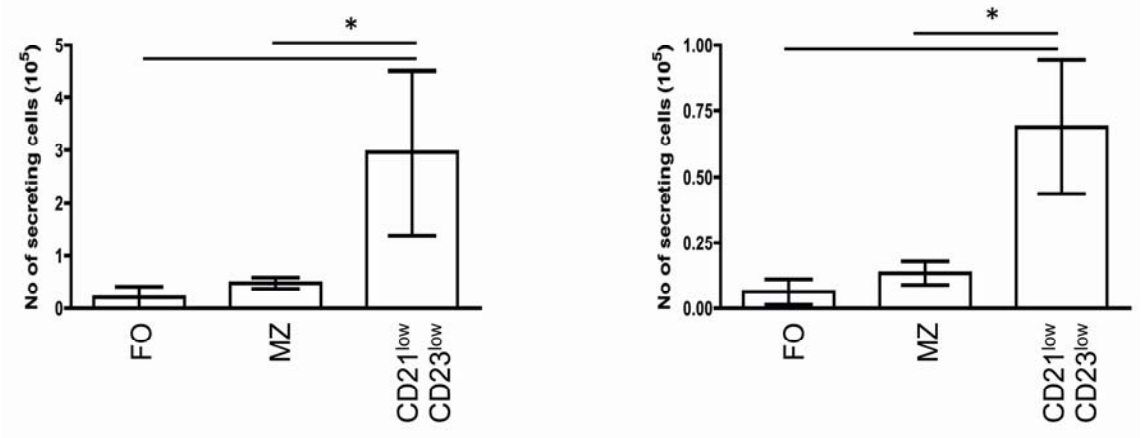
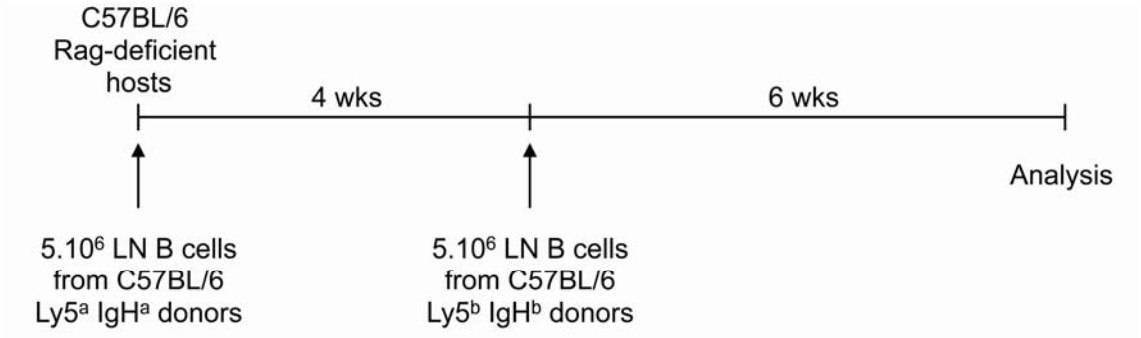
SFigure 1A



Supplementary Figure 1A – Shows the number of Igκ- (left panel) and IgG-secreting cells (right panel) among the CD21$^{int/high}$CD23^{high}, CD21^{high}CD23^{low} and CD21^{low}CD23^{low} B cell subsets recovered from the spleen of host mice 16 weeks after B cell transfer. The Ig-secreting cells detected by intra-cytoplasmic Ig staining were most numerous among the CD21^{low}CD23^{low} B cells. Each bar represents the mean of values for 3 individual mice.

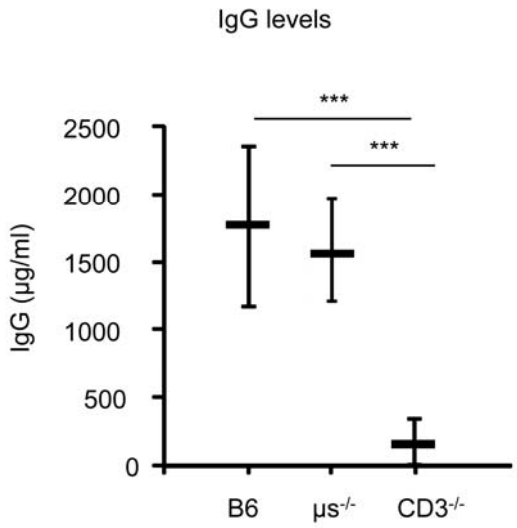
Supplementary Note 1 – Please note that we did not consistently recover significant numbers of B1 cells in the peritoneal cavity of the hosts upon transfer of lymph node (LN) cells into immune-deficient hosts in the experimental conditions used in this study. Our findings also indicated that most of the IgM and IgG-secreting cells were CD21^{low}CD23^{low} B cells recovered from the host spleen, as no Ig-secreting cells were detected in the host bone marrow, but could not exclude their presence in other less accessible sites (flat bones BM, mucosa, etc.). The serum IgM level was therefore considered the most reliable indicator of the state of B cell activation and of the number of IgM-secreting B cells in the whole mouse.

SFigure 1B



Supplementary Figure 1B – Sequential B cell transfers. Rag-deficient hosts were sequentially injected fi rst with LN cells containing 5×10^6 $Ly5^aIgH^a$ B cells and 4 weeks later with LN cells containing 5×10^6 $Ly5^bIgH^b$ B cells or vice-versa. Host mice were sacrificed 6 weeks after the second cell transfer.

SFigure 2

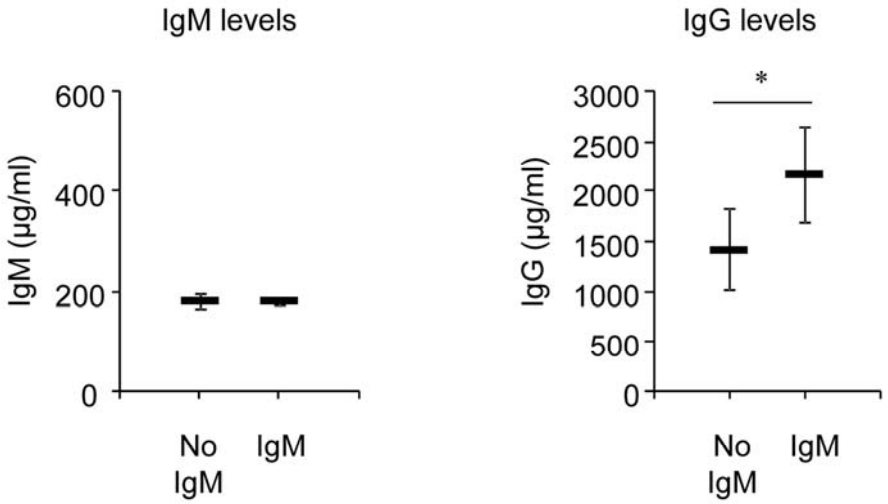


Supplementary Figure 2 – The IgG concentrations in mice injected with LN cells from WT B6 (left), $\mu s^{-/-}$, or T cell–deficient $CD3\varepsilon^{-/-}$ donor mice (*** $p < 0.001$). Each bar represents the mean ± SD of values for 8-9 mice. Note that in the absence of T cells, IgG levels in the host before the transfer of the second B cell population were negligible.

Supplementary Note 2 – In the absence of T cells, B cells are unable to produce significant levels of IgG. T cell-deficient mice have very low or absent IgGs in the serum. Thus, to completely prevent IgG production by the first population of B cells, we transferred B cells from $CD3\varepsilon^{-/-}$ T cell-deficient mice. It should be pointed out that by transferring double-sorted B cells from WT donors and by testing individual host mice for the presence of T cells and IgG before the transfer of the 2^{nd} B cell population, we obtained results similar to those observed using B cells from $CD3\varepsilon^{-/-}$ donors (not shown). In contrast, in the presence of a few contaminating T cells, these T cells readily expanded and promoted IgG production by the

transferred B cells. When that occurred, IgG levels similar to those observed in normal donors were attained in the host mice. Since IgG levels were dependent on the variable presence of T cells, we chose IgM levels as a more reliable indicator of the state of activation of the second B cell population.

SFigure 3

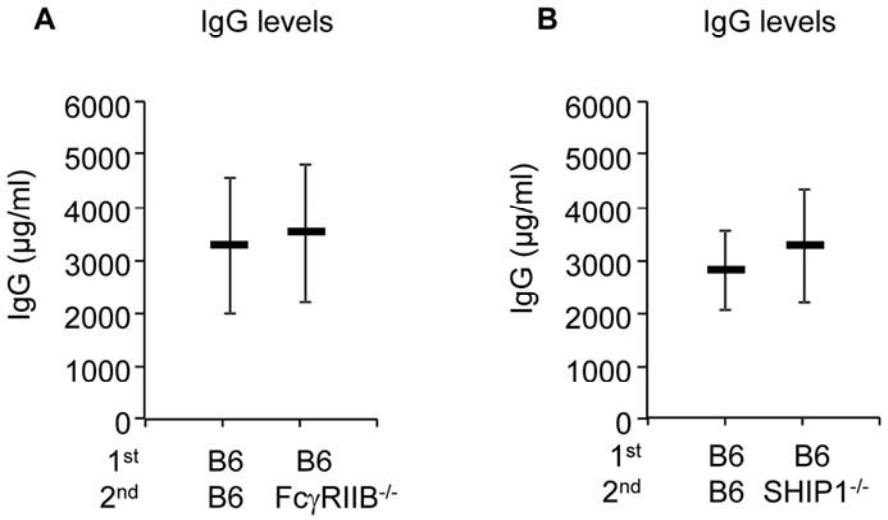


Supplementary Figure 3 –The IgM (left) and IgG (right) produced by B cells when a LN population was transferred alone into untreated naïve mice or into mice injected i.v. with 200µg purified mouse IgM (Innovative Research, Inc.) the day before LN cell transfer and thereafter intraperitoneally every 3 days for 4 weeks. Each bar represents the mean ± SD of values for 10 mice. Statistically significant differences are shown (* $p < 0.05$).

Supplementary Note 3 – Pretreatment of immuno-deficient hosts prior to cell transfer with mouse IgMs did not alter IgM production but induced an increased IgG synthesis by transferred cells (Supplementary Fig. 3). It should also be pointed out that since IgG pre-treatment did not alter expansion of the transferred LN T cells, these results exclude the possible role of a differential T cell expansion in feedback regulation of IgM production. Indeed, upon the sequential transfer of two LN populations, we observed that in the presence of a first T cell population, T cells from the second LN cell population proliferated less, but were still able to expand to replace about 50% of the first T cell population (not shown). Moreover, mutant B cells either from SHIP1-deficient or FcγRIIB$^{-/-}$ mice were not inhibited

in the same experimental settings thus excluding the involvement of differential T cell expansion in the observed feedback regulation.

SFigure 4



Supplementary Figure 4 – a, IgG concentrations recovered from host mice injected with WT and/or FcRγIIB$^{-/-}$ LN cells. Note that IgG levels were identical in all groups of mice; therefore, the lack of inhibition of IgM production by the FcRγIIB$^{-/-}$ B cells could not be attributed to lower IgG levels. **b,** IgG concentrations recovered from host mice injected with WT and/or SHIP1$^{-/-}$ LN cells. Note that IgG levels were identical in all groups of mice; therefore, the lack of inhibition of IgM production by the SHIP1$^{-/-}$ B cells could not be attributed to lower IgG levels. Statistically significant differences are shown: (** $p < 0.01$). Each bar represents the mean ± SD of values for 6 mice.

Supplementary Note 4 – In SHIP1-deficient mice IgM levels were significantly higher than in FcγRIIB$^{-/-}$ mice (Fig. 4e), probably as a result of the additional status of inflammation due to the simultaneous lack of negative regulation of myeloid cells. Consequently, B cells from SHIP1 donors also show a higher propensity to be activated and produced more IgM upon transfer into the immune-deficient hosts. Both SHIP1 and FcγRIIB-deficient donors were

used at 4-5 weeks of age before any signs of autoimmune disease to minimize transfer of activated self-reactive B or T cells.

Supplementary Note 5 – The absence of overt autoimmune manifestations in T cell-deficient or CD40/CD40L-deficient mice suggests that in mice T cells may be required for excessive B cell activation and hyper IgM production, most likely as part of the "inflammatory" or innate response to environmental antigens. Notably, serum IgM titers were lower in the absence of T cells than in the presence of T cells.