Supplemental Figure 1. (A) A representative plot of the splenic B cells remaining after anti-CD20 Ab depletion of tumor-bearing mice (shown in Fig.2B). It depicts that the increased proportion of CD81^{hi}CD25^{+} cells within CD19^{+} cells in tumor-bearing mice is further enhanced after anti-CD20 Ab depletion. (B) A representative FACS staining histogram CD20^{+} cells within CD19^{+} gated cells. It shows the decrease in CD20 of ex vivo-generated tBregs (broken line, B) as compared with B cells incubated in BAFF (continuous line, B). Control Ig staining is shaded area. All data shown here were reproduced at least three times. (C) B cells infiltrate human primary breast cancer and its metastatic site, the lungs. Shown a representative immunohistochemistry staining of samples, indicated, of a patient with stage III/IV breast cancer. (D,E) Normal human donor peripheral blood B cells are effectively converted into tBregs if treated with conditioned media (CM) of human breast cancer MDA-MB-231 cells (B-MDA) and colon cancer SW480 cells (B-SW480), but not control CM from breast cancer MCF-7 (B-MCF7) and melanoma 938-mel cells (B-938mel). Shown, % of suppression of T cells stimulated with anti-CD3 Ab (D) and MFI of CD20 expression on the surface of CD19^{+} B cells (E) treated with CM. % of suppression is calculated relative to proliferation of T cells mixed with mock-treated B cells (no suppression, B-Mock).

Supplemental Figure 2. (A) The suppressive activity (for CD4^{+} and CD8^{+} T cells) of human tBregs was retained within FACS-sorted CD20^{Low} B cells (enrichment >95%) shown is a representative dot plot of proliferating (numbers show % Ki67^{+}) CD4^{+} and CD8^{+} T cells of triplicate experiments (B). All data shown here were reproduced at least three times. (B) Ex vivo generated human tBregs (B-MDA) retained their suppressive
activity (on both CD4⁺ and CD8⁺ T cells) after in vitro anti-CD20 Ab/magnetic bead–mediated depletion of CD20hi cells (p<0.05, B-MDA CD20lo vs. B-Mock, or B-MDA CD20lo vs. B-MDA CD20hi). (C) CD20 expression on ex vivo generated -tBregs (B-MDA) after in vitro depletion of CD20hi cells (continuous line) with anti-CD20 Ab–coupled beads. Cells were stained with PE conjugated anti-CD20 Ab.

**Supplemental Figure 3.** (A) tBregs were generated in the presence of anti-IgM (10 μg/ml), or TLR ligands, such as 5 μg/ml ssRNA40, 10 μg/ml Pam3CSK4 or FSL-1, 10ug/ml, or 5 μg/ml LPS, 5ug/ml, or 1 μg/ml ODN1826 PS (CpG) or control ODN1826K PS (CpG K). After 48h incubation, cells were tested for expression of tBreg markers (CD81hiCD25⁺ within CD19⁺ B cells). (B ,C) CpG oligonucleotide (1 μg/ml ODN2006 PS) also blocked the phenotype (CD20Low within CD19⁺ cells, B-MDA, B) and suppressive activity of ex vivo –generated human tBregs (B-MDA, C). (D) Schematic depiction of BLC-arp, murine CXCL13 fused with the RNA binding domain of HBV (RBD) via a short spacer sequence (SP). RBD allows binding of oligonucleotides (Oligo) in physiologic buffers. Representative FACS plot (E) of triplicate experiment ± SEM (F) that shows that suboptimal doses of CpG (50 ng/ml) activate murine splenic CD19⁺ B cells (up regulate CD25) in vitro, if coupled with BLC-arp. CpG K is for control non-stimulatory oligonucleotide. Numbers in B show % of cells within the gate, depicted.
Supplemental Figure 4. BALB/c mice were s.c. challenged with 4T1.2 cells (1x10^5) in the fourth mammary gland and then treated intravenously with BLC-Arp (30 μg) coupled with 5 μg CpG (ODN1826 PS) or control CpG K (ODN1826K PS) on days 3, 7, 12, 16 and 21 post tumor challenge. While B cells from 4T1.2 tumor-bearing mice decrease proliferation of CD8^+ T cells (tumor bearing, A), B cells from BLC-arp/CpG -treated tumor-bearing mice stimulated proliferation of CD8^+ T cells (tumor bearing + BLC-arp/CpG, A), as shown in a representative FACS plot. LN B cells and T cells were mixed at 0.5:1 effector : target ratio and proliferation is assessed after 5 days of anti-CD3 Ab stimulation. Numbers represent % of CD8^+ T cells with diluted eFluor670 (proliferated). While tBregs suppress CD8^+ T cell activity, CpG-treated (but not control CpG K) tBregs efficiently activate expression of granzyme B (GrzB) in target CD8^+ T cells in vitro (B, C). Shown, % of GrzB (intracellular) in CD8^+ T cells ± SEM (B) and a representative FACS plot of triplicate experiments repeated twice.

Supplemental Figure 5. μMT (A, B) or pmel (C-E) mice s.c. challenged with B16-F10 melanoma cells (1x10^5) were adoptively transferred with mock (JHT-tBreg) or CpG -treated (5 μg/ml ODN1826 PS, JHT-tBreg+CpG) congeneic tBregs (5x10^6) from C57BL/6 mice at days -1 and 5. Control mice were WTC57BL/6 challenged with B16-F10 melanoma (C57-PBS, A,B). To assess antigen-specific expansion of effector CD8^+ cells in mice, draining lymph node cells (B,E) and splenocytes (C,D) were isolated and stimulated ex vivo for 5-7 days with 5 μg melanoma gp100_25-32 peptide. The transfer of mock-treated tBregs (JHT-tBreg, A) significantly enhances the retarded B16 melanoma
growth in μMT mice (A) and reduces numbers of gp100 \textsubscript{25-32} –specific IFNγ -producing CD8\textsuperscript{+} cells in the secondary lymphoid organs of μMT (B) and pmel mice (E), such as draining lymph nodes (B, E) and spleens (C,D). In contrast, these effects were blocked and reversed when mice were adoptively transferred with tBregs treated with CpG. B and C show IFNγ intracellular staining within CD8\textsuperscript{+} (B) and Ki67\textsuperscript{+} (C-E) CD8\textsuperscript{+} cells.

Supplemental Figure 6. Compared with LPS-stimulated B cells (B-LPS), ex vivo-generated murine tBregs reduce surface expression of 4-1BBL (A). Similarly, as compared with naïve mouse cells, less 4-1BBL is expressed on B cells from 4T1.2 cancer-bearing BALB/C mice, which was further reduced (B), if mice were treated with anti-CD20 Ab (B). Shown is a representative FACS plot (A) of 4-1BBL\textsuperscript{+} within CD25\textsuperscript{+} CD19\textsuperscript{+} B cells of a triplicate experiment.