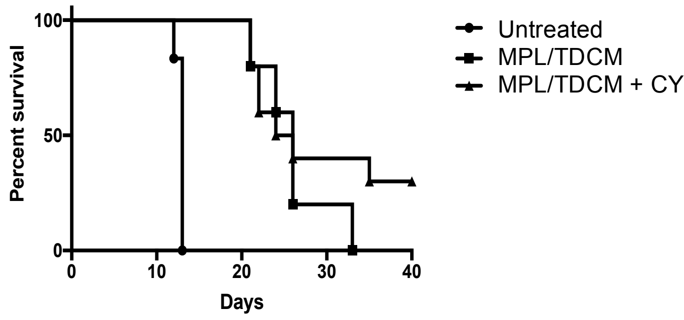
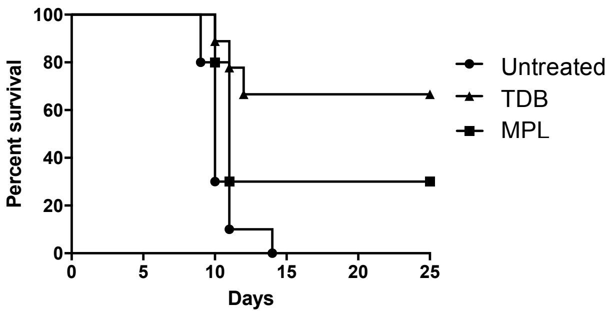
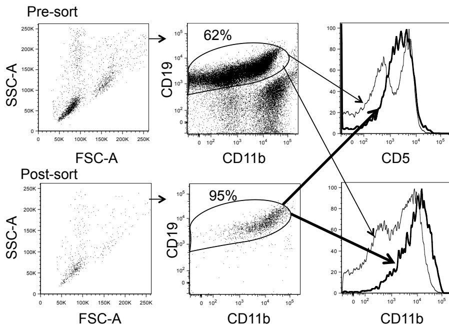
Supplementary Figures. Haro et al.



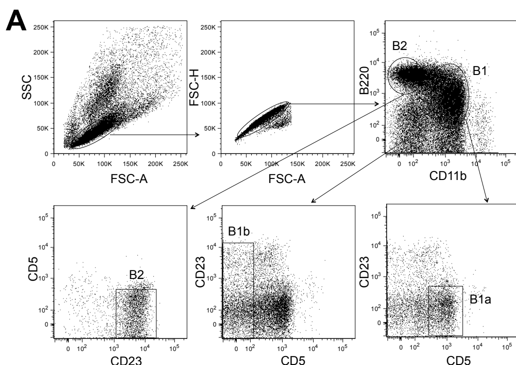
Supplementary Figure S1. TA3-Ha challenge in CAF_1 mice. CAF_1 female mice were challenged i.p. with 10^4 TA3-Ha-GFP-luciferase cells on d0 and either untreated (n=5) or treated with MPL/TCDM (n=5) or MPL/TCDM + 1 mg cyclophosphamide (CY) i.p. on d1 (n=10). Morbidity due to tumor growth requiring euthanasia was considered a death time point. The remaining 3 survivors in the MPL/TDCM + CY group were rechallenged with 10^4 TA3-Ha-GFP-luciferase cells on d40 but did not survive.

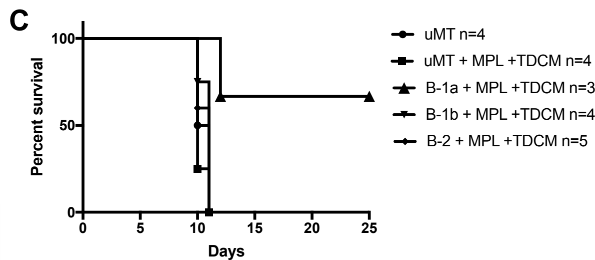


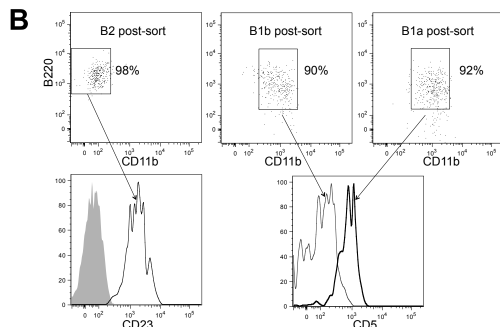
Supplementary Figure S2. MPL or TDB in squalene provide partial protection against TA3-Ha tumor challenge. Wild type female mice were challenged i.p. with 2 x 10^4 TA3-Ha cells and either untreated (n=10) or treated with 10 μg Vaccigrade MPL mixed with 0.2% squalene (n=10) or 10 μg TDB mixed with 0.2% squalene i.p. (n=9). Morbidity due to tumor growth requiring euthanasia was considered a death time point. MPL, TDB, and squalene (Addavax) were from Invivogen.



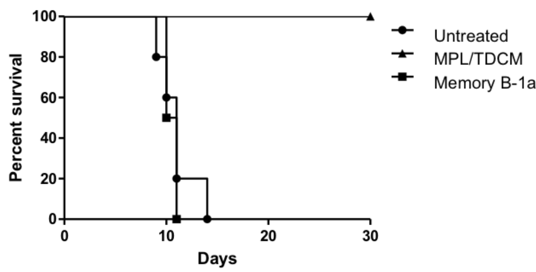
Supplementary Figure S3. Bead purification of peritoneal B-1a cells. Peritoneal non-B cells were depleted using Thy 1.2-, F4/80-, GR1-, DX5-, CD11c-magnetic bead depletion (Dynal), followed by positive anti-Ly5.1 (CD5) bead selection (Miltenyi Biotec). Representative purity achieved with this selection is shown, with a yield of ~95% $CD19^+$ B cells expressing CD11b and CD5.



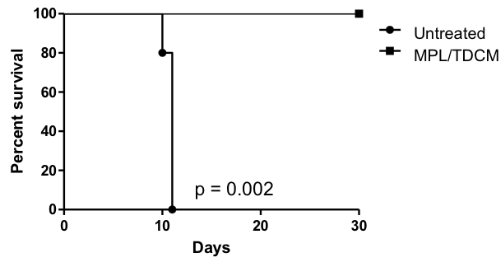




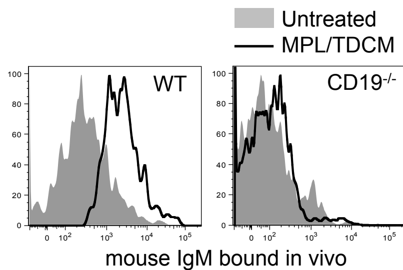
Supplementary Figure S4. FACS-purified B-1a, but not B-1b or B-2, cells reconstitute MPL/TDCM-elicited protection in μMT mice. A-B) Peritoneal cells were harvested from 40 WT female mice. Cells ($2x10^7$/ml) were blocked with Fc Block for 15 minutes and then stained with fluorochrome labeled-mAbs to B220, CD23, CD5, and CD11b. B cells were sorted into $B220^+CD23^{neg}CD11b^+CD5^+$ (B-1a), $B220^+CD11b^+CD5^-$ (B-1b), and $B220^{hi}CD23^{hi}CD11b^-CD5^-$ (B-2) populations using a FACSAria sorter. Gates for sorting are shown in (A) and post-sort purities are shown in (B). **C)** μMT female mice were given $1x10^5$ sort-purified B-1a, B-1b, or B-2 cells ip. One day later, recipient mice were challenged with 10^4 TA3-Ha cells i.p. and treated with MPL/TDCM. Log-rank analysis of B-1a reconstituted vs. non-reconstituted MPL/TDCM-treated μMT mice, p=0.02.



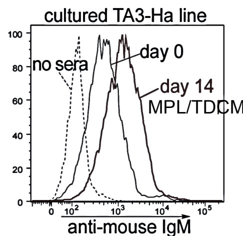
Supplementary Figure S5. Peritoneal "memory" B-1a cells are not sufficient for protection in the absence of MPL/TDCM treatment. Forty days post MPL/TDCM-treatment and TA3-Ha challenge, peritoneal B-1a cells were isolated from surviving WT mice and transferred into naïve WT mice i.p. (3 x10^5 B-1a cells/mouse). The next day, recipient mice (n=4) were challenged i.p. with 2x10^4 TA3-Ha cells. Naïve WT mice, either untreated (n=5) or treated with MPL/TDCM (n=5) were also challenged. Mice were monitored for ascites development and morbidity requiring euthanasia.



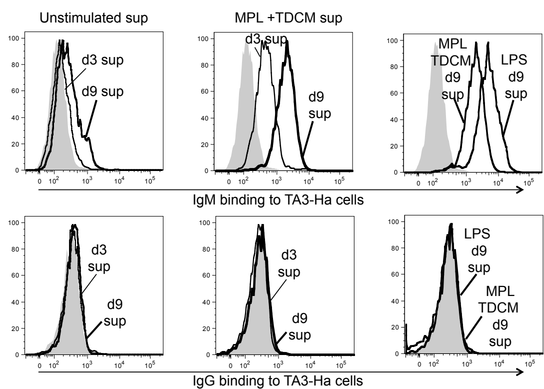
Supplementary Figure S6. MPL/TDCM protects IL-$10^{-/-}$ mice following TA3-Ha challenge. IL-$10^{-/-}$ mice were challenged with 2 x10^4 TA3-Ha cells i.p. (n=10), with half (n=5) treated with MPL/TDCM. Mice were monitored for ascites development and morbidity requiring euthanasia. Log rank analysis was used to assess significant differences between groups, p=0.002.



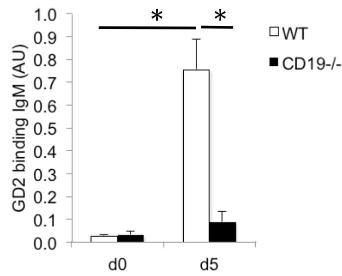
Supplementary Figure S7. IgM bound to TA3-Ha cells harvested from peritoneal cavities of WT and CD$19^{-/-}$ mice 5 days post challenge. WT and CD$19^{-/-}$ mice were challenged with 2 x10^4 TA3-Ha cells i.p., with half treated with MPL/TDCM. On day 5, peritoneal cells were harvested and IgM deposition on recovered TA3-Ha cells ($HPA^+CD138^+CD11b^-FSC^{hi}SSC^{hi}$) was measured by flow cytometry using an F(ab)'$_2$ goat anti-mouse IgM-FITC Ab.



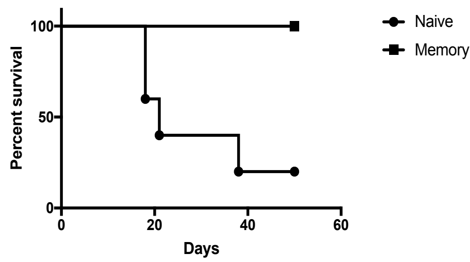
Supplementary Figure S8. WT mice treated with MPL/TDCM produce tumor-reactive IgM in the absence of tumor challenge. Serum was harvested from WT mice 14 days post i.p. MPL/TDCM treatment. Serum IgM binding (1:25 dilution) to cultured TA3-Ha cells was measured by flow cytometry using an F(ab)'$_2$ goat anti-mouse IgM-FITC Ab.



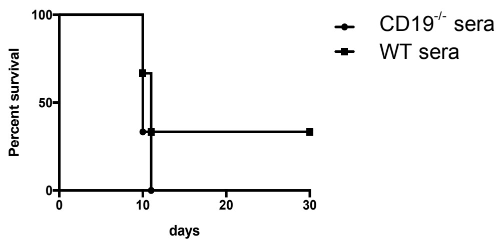
Supplementary Figure S9. Culture of peritoneal cells with MPL/TDCM or LPS induces production of TA3-Ha-reactive IgM. Peritoneal cells were harvested from naive WT mice and cultured at 2x10^6/ml in cRPMI+10%FCS in medium alone (unstimulated) or with 10 µg/ml MPL/TDCM-0.04%squalene. 2 x10^4 cultured TA3-Ha cells in wash buffer (PBS containing 2% FCS) were incubated with peritoneal cell culture supernatant (1:1 dilution; 50 ul final) for 30 min. at RT. Ta3-Ha cells were washed with wash buffer and incubated with F(ab')$_2$ goat anti-mouse IgM-FITC and rat anti-mouse IgG-PE for 25 minutes at RT. Cells were washed and fixed, with IgM and IgG binding measured by flow cytometry.



Supplementary Figure S10. Increased serum IgM binding to GD2 in WT, but not CD19$^{-/-}$ mice 5 days post i.p. MPL/TDCM injection (n=6-7 mice/group). Nunc 96-well Maxisorp plate were coated with 50μl/well of 2 μg/ml GD2 (EMD Millipore) in 95% ethanol and air dried. Plates were blocked with 1% BSA, washed, and serum was added at a 1:50 dilution, with IgM-alkaline phosphatase detection using pNPP. Asterisks (*) indicate significant differences ($p<0.05$). AU=Arbitrary Units, defined as OD_{405} of serum samples minus background OD_{405} values.



Supplementary Figure S11. MPL+TDCM supports the development of protective memory responses against EL4 cells. WT mice that had been treated with MPL+TDCM and survived initial EL4 challenge (10^6 i.p.) were rechallenged 9 weeks later with the same dose of EL4 cells (graphed as "memory") without additional treatment (n=3). Naïve WT mice (n=5) were also challenged with the same dose without treatment. Log rank analysis, p=0.05.



Supplementary Figure S12. Passive transfer of sera from WT and CD19$^{-/-}$ mice treated with MPL+TDCM (d5) into CD19$^{-/-}$ mice following TA3-Ha challenge. Sera (200 ul) was given i.p. on days 1, 3, 5 and 7 post TA3-Ha challenge (2 x 10^4 i.p.), n=6 mice/group. Differences were not significant.