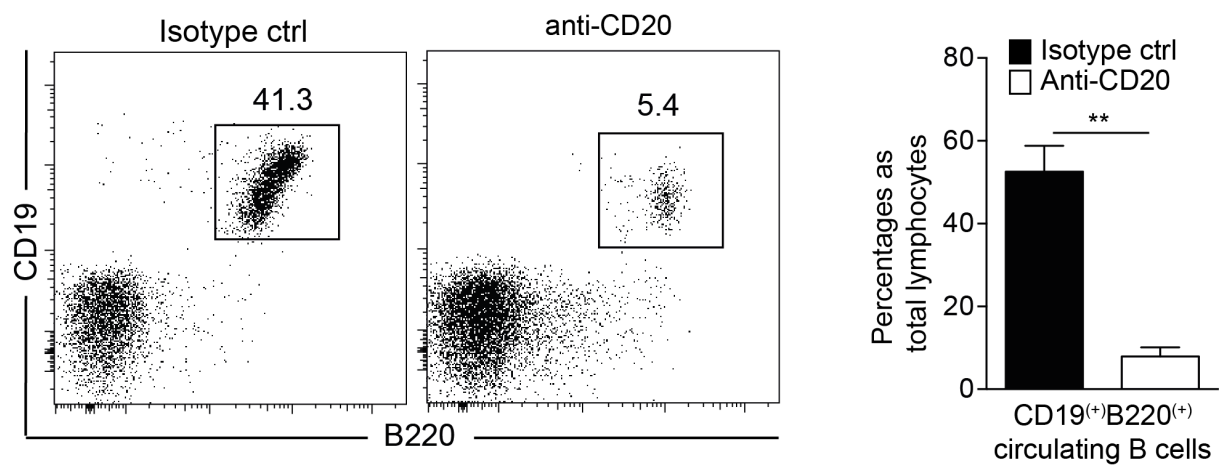


**Intravital imaging reveals improved Kupffer cell-mediated phagocytosis as
a mode of action of glycoengineered anti-CD20 antibodies**

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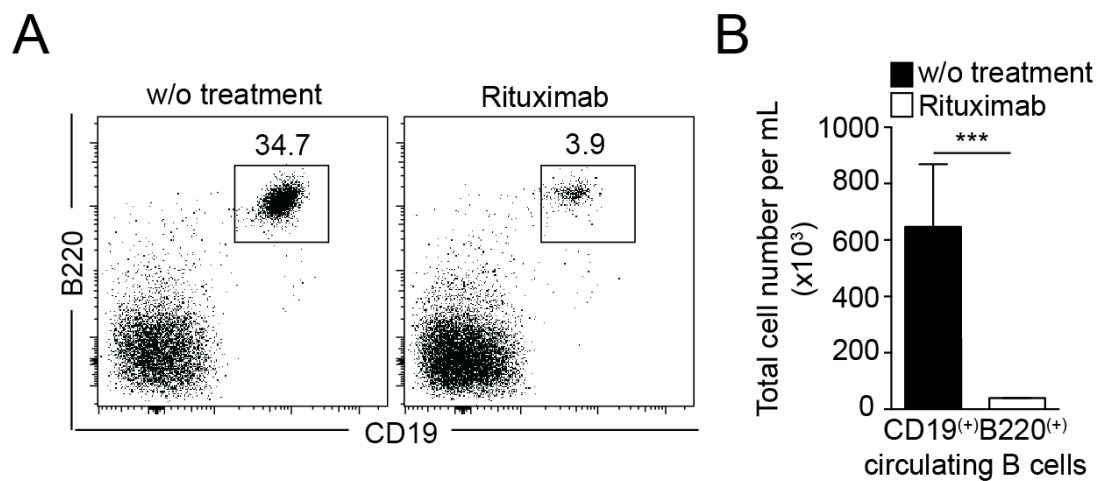
Supplemental materials



Supplementary Figure 1. Efficient B cell depletion by the 5D2 mouse anti-mouse CD20

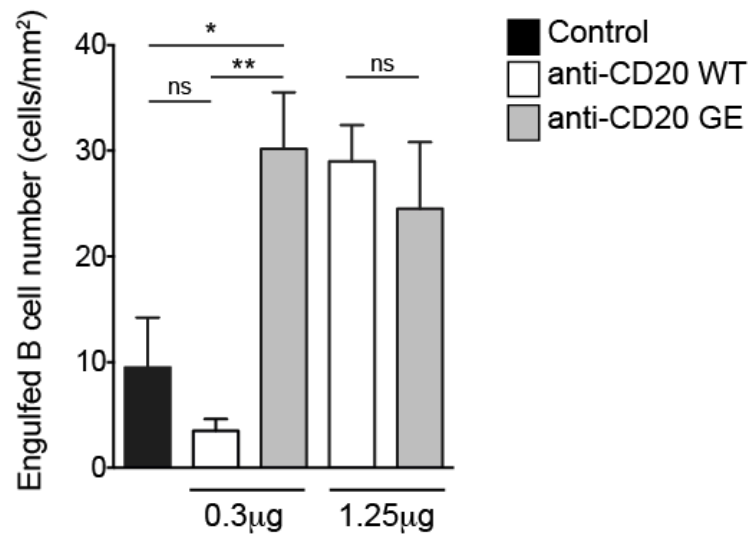
Ab. WT mice were treated with 50 μ g of anti-CD20 (5D2) or isotype control (HY1.2) for 24h.

Summary bar charts showing percentages of B cells in the blood of treated mice.



Supplementary Figure 2. Rituximab efficiently depletes B cells in hCD20Tg mice

hCD20Tg mice were treated with 200 μ g Rituximab for 5hrs or left untreated. **A)** Representative flow cytometry profiles of B cells in blood. **B)** Summary bar charts showing B cells/ml of blood in untreated or Rituximab treated mice.



Supplementary Figure 3. Glycoengineered anti-CD20 Ab (18B12) triggers enhanced Ab-dependent phagocytosis by Kupffer cells *in vivo*.

WT or glycoengineered anti-CD20 mAbs (clone18B12) were used to treat WT mice at the indicated doses. Frozen liver sections were stained using PE-labeled anti-F4/80 Ab and FITC-labeled anti-B220 Ab. Summary bar charts showing the density of engulfed B cells for the indicated conditions.

Movie legends

Movie S1. Intravital two-photon imaging of Kupffer cell-mediated B cell phagocytosis following rituximab injection.

hCD20Tg mice were injected i.v. with 0.2µg F4/80-FITC and B220-PE to visualize Kupffer and B cells respectively. Mice were prepared for intravital liver imaging and subsequently treated with 200µg rituximab.

Movie S2. Efficient Kupffer cell-mediated B cell phagocytosis triggered by low doses of glycoengineered anti-CD20 Ab.

MAFIAxCD19^{cre}xRosaRFPxCFP mice were used to visualize B cell (red) phagocytosis by Kupffer cells (green) following 0.3µg GE anti-CD20 injection. The liver architecture appears in blue.

Movie S3. Low doses of GA101 trigger robust Kupffer cell-mediated B cell phagocytosis

hCD20Tg mice were injected i.v. with 2µg FITC-conjugated anti-F4/80 Ab and B220-PE Fab fragments to visualize Kupffer and B cells respectively. Mice were prepared for intravital liver imaging and subsequently treated with 0.4µg GA101.

Movie S4. Low doses of rituximab fail to induce B cell phagocytosis in the liver

hCD20Tg mice were injected i.v. with 2µg FITC-conjugated anti-F4/80 Ab and B220-PE Fab fragments to visualize Kupffer and B cells respectively. Mice were prepared for intravital liver imaging and subsequently treated with 0.4µg rituximab.