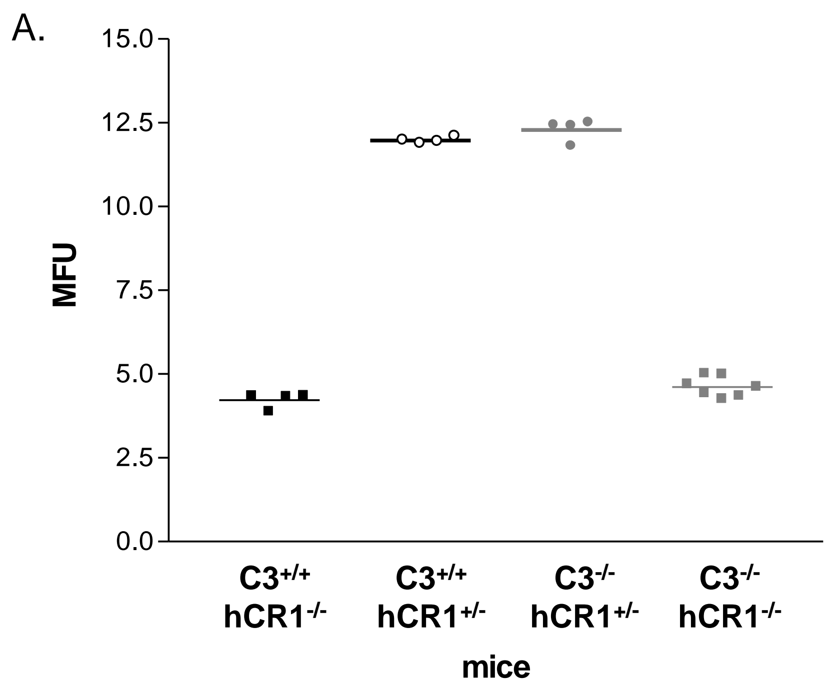
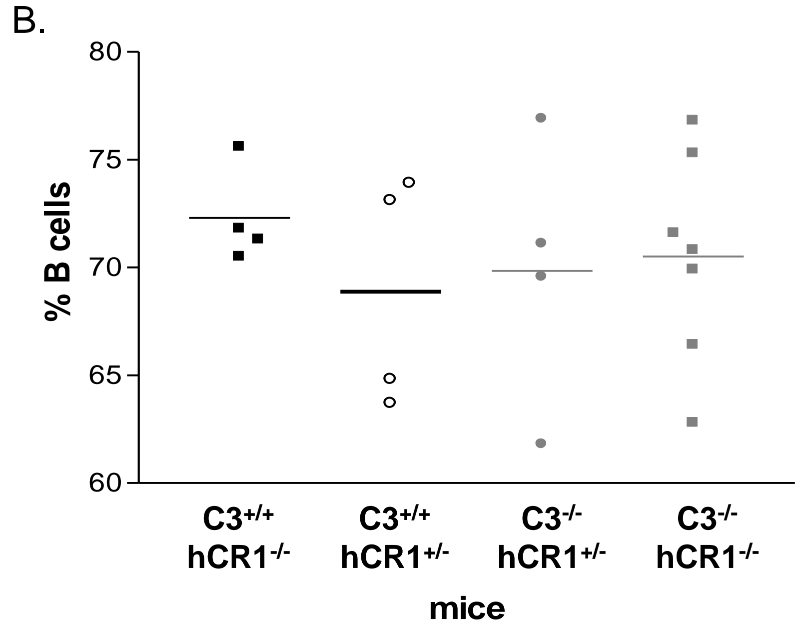
Supplement Figure 1.





Supplemental Figure 2.

A

hCR1 -/-
hCR1+/-

%

Splenic population

B cells | Follicular | Immature/Transitional | Marginal Zone

B

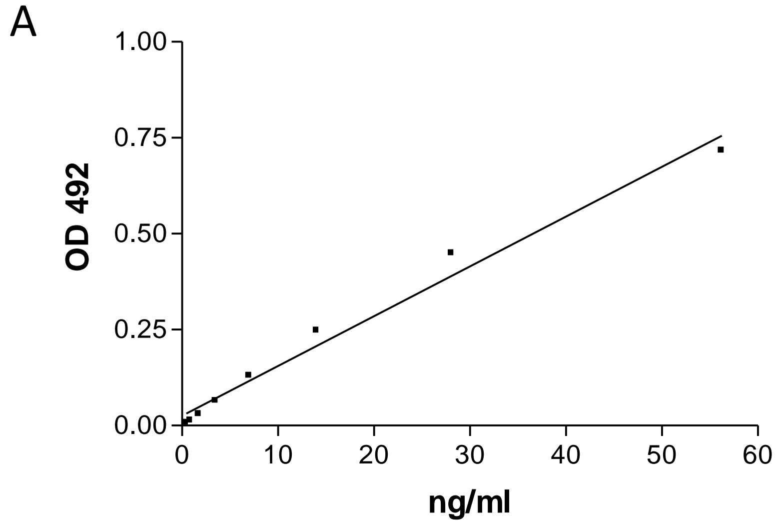
hCR1 -/-
hCR1+/-

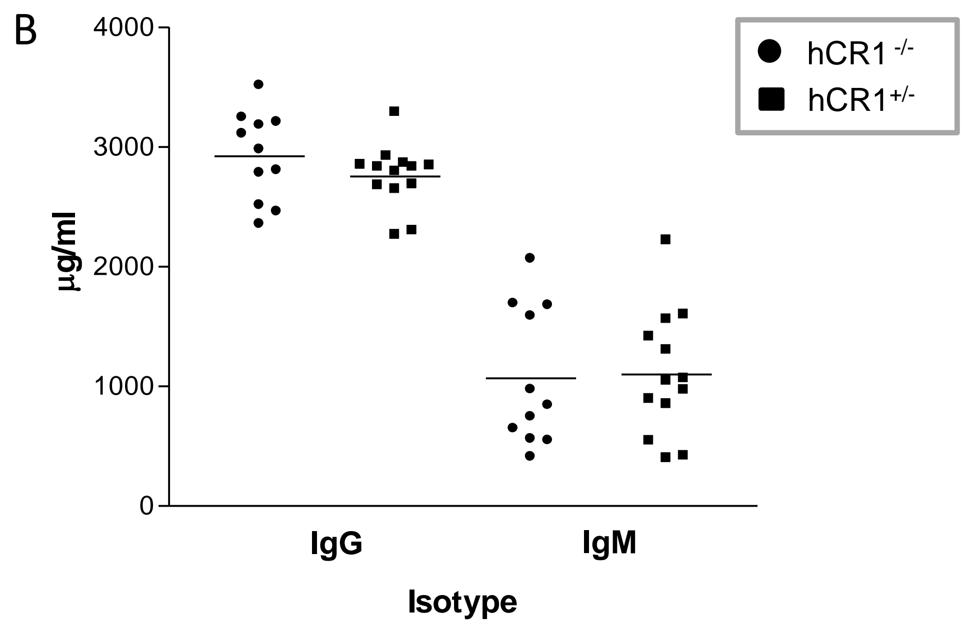
%

BM B cell population

Pro/Pre | Immature | Mature | Total

Supplemental Figure 3.



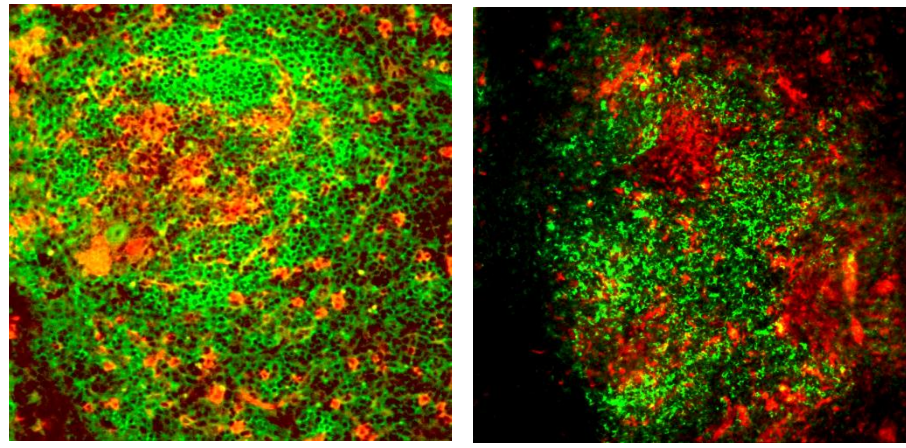


Supplemental Figure 4

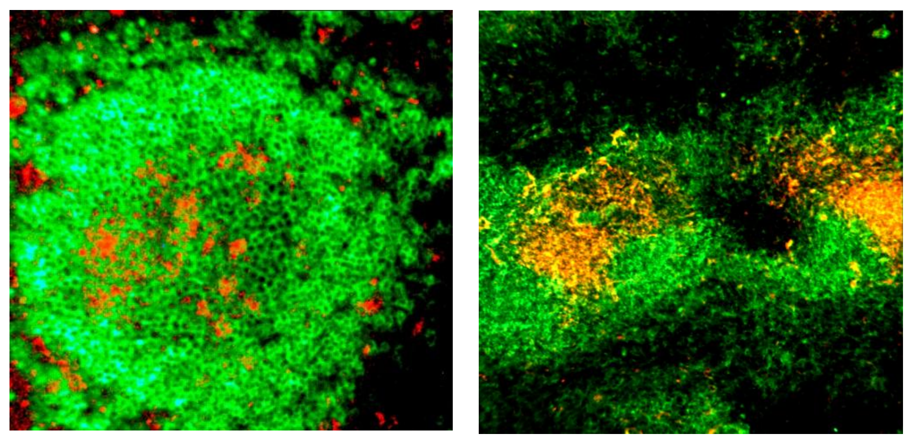
IgD+ PNA+

a).

hCR1$^{+/+}$

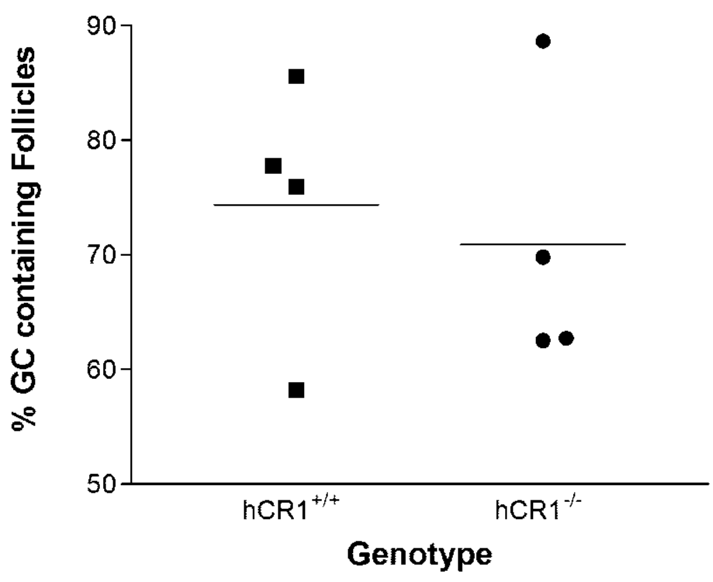


hCR1$^{-/-}$



10x

b).

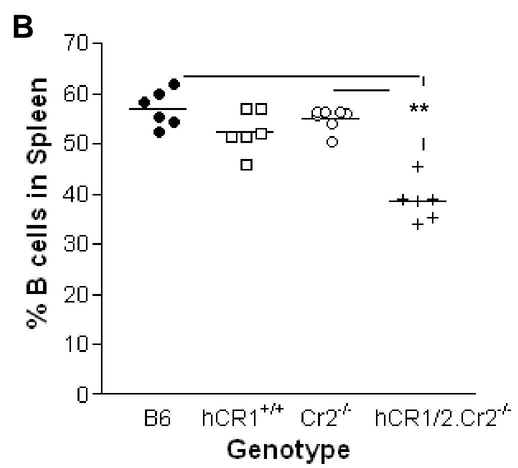


Supplemental Figure 5.

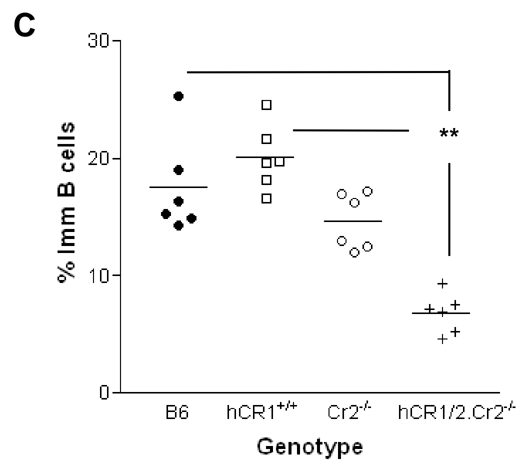
A

SSC-H
FSC-H
R1
Events
B220 - PerCP
R2
M1
Cr2-/-
hCR1/2.*Cr2*-/-
CD1d - PE
CD24 - Fitc
MZ
FO
Imm

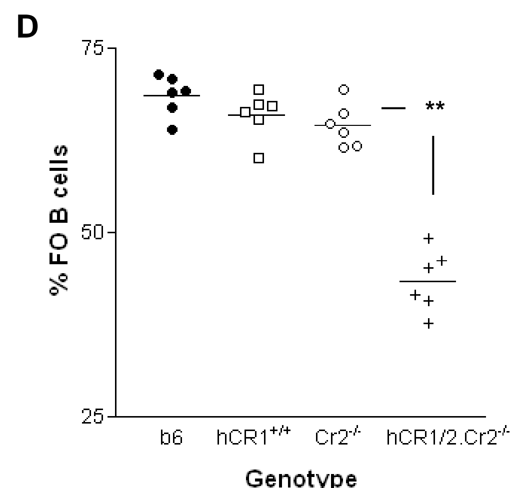
B



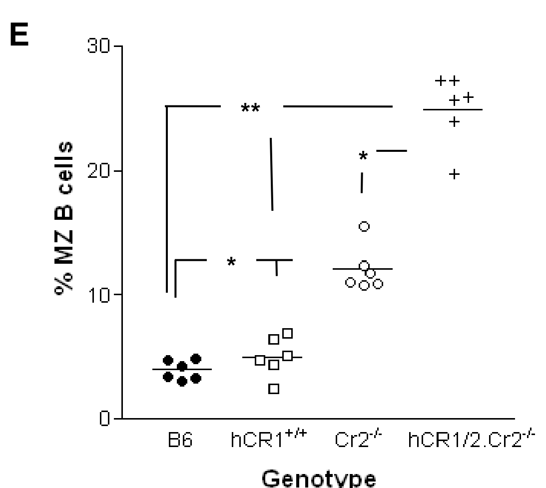
C



D



E



Supplemental Figure 6.

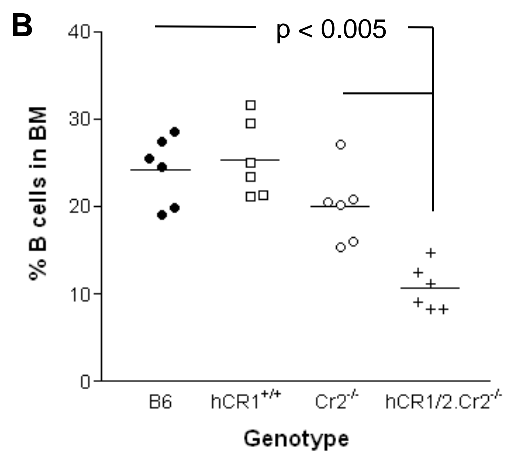
A

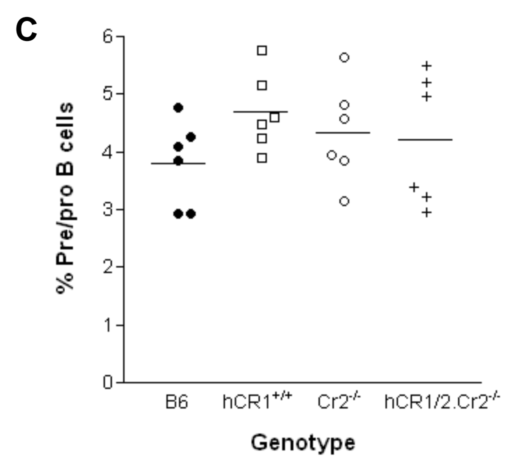
SSC-H
FSC-H
R1

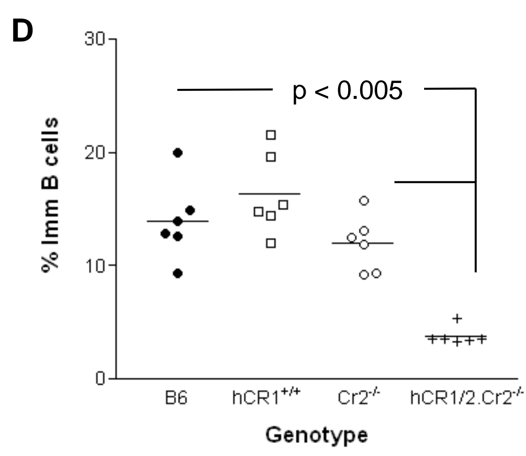
Cr2-/-

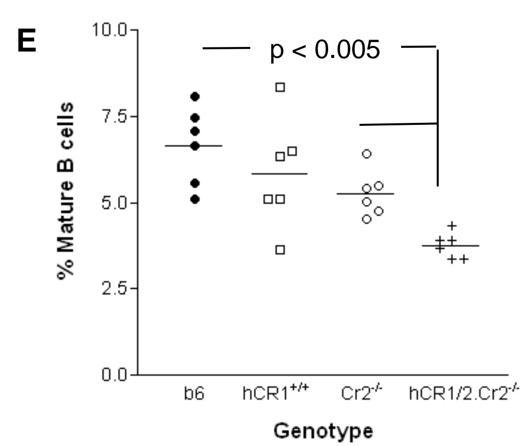
hCR1/2.*Cr2*-/-

CD43 - PE
FL2-H
Pro/pre
Imm
Mature
B220 -PerCP









Supplemental data, Figure legends.

Figure 1. Expression of hCR1 in $C3^{-/-}$ mice is unchanged.

PBL were isolated from F4 hCR1 mice back crossed onto the $C3^{-/-}$ background (two generations) by tail bleed and red cells were lysed. PBL were then stained with (a) B220-FITC and anti-hCR1 or (b) percentages of B cells (B220+) were calculated based on the total number of cells in the lymphocyte gate as determined by forward and side scatter. At least 5000 events were collected and results are representative of triplicate analysis.

Figure 2. hCR1 expression level has no effect on B cell development.

Cells were isolated from the bone marrow and spleen of hCR1 F4 heterozygote transgenic mice and red cells were lysed. Cells were identified during flow cytometry as B cells from their forward and side scatter profile and $B220^{+}$phenotype. (A) The percentage of B220+ cells was established within total lymphocytes in the spleen and cells were further delineated as follows; follicular ($CD24^{int}$, $CD1d^{lo}$), marginal zone ($CD24^{int}$, $CD1d^{high}$) and immature/transitional B cells ($CD24^{high}$, $CD1d^{int}$) . All results are presented as a mean percentage of $B220^{+}$ events. At least eight mice were used in each group. (B) Bone marrow Cells were stained with B220 PerCP, CD43 PE and biotinylated CD25/SA allophycocyanin and delineated as follows: total B cells are B220+ cells, pro/pre-B cells are $B220^{int}CD43^{+}CD25^{-}$, immature B cells are $B220^{int}CD43^{-}$, and mature B cells are $B220^{high}CD43^{-}$. All results are presented as a mean percentage of all live cells in the bone marrow isolate. Student's *t* test analysis was used to generate *p* values. At least eight mice were used in each group. 10,000 events were collected and results are representative of two experiments and triplicate analysis.

Figure 3. hCR1 mice have normal baseline serum Ig levels .

Baseline Ig in F5 hCR1 transgenic mice was established using a standard sandwich ELISA; plates were coated with 5ug/ml goat anti- mouse IgG/M (Caltag, UK). Mouse serum (1/100) dilution was incubated on the plates and bound mouse IgG or IgM was detected using specific secondaries (goat anti-mouse IgG –AP and goat anti-mouse IgM – AP; Caltag, UK) as appropriate. (A) A standard curve was established using mouse reference serum ( ) and linear regression. (B) Shows the levels of IgG and IgM as indicated in hCR1+ (open circle) and hCR1 –ve (black square) littermates. At least 11 mice from each genotype were analysed.

Figure 4. Analysis of hCR1 tg mouse spleens reveal a normal germinal center ratio.

Mice were injected with $5x10^8$ SRBC and tissues were collected from mice into isopentane on dry ice at day 8. (A) Splenic section (8µm) from $hCR1^{+/+}$ (or $hCR1^{-/-}$ littermates were stained with anti-IgD-Fitc, to label B cells (follicles), and biotin PNA (followed by SA-Rhodamine), to label the germinal centers, 2 sections are shown for each background as indicated (10x magnification). The sections displayed are representative of the 6 discontinuous sections per spleen and 4 animals per group that were analysed. (B) The number of germinal center containing follicles versus total number of follicles present in the sections were analysed for each animal shown. At least 100 follicles were viewed per animal.

Figure 5. Analysis of hCR1.hCR2.*Cr2* $^{-/-}$ splenic B cell populations.

Splenocytes were collected and RBC were lysed. Cells were identified during flow cytometry as B cells from their forward and side scatter profile and $B220^{+}$phenotype. (a) Illustrates delineation of cells into (B) follicular (FO; $CD24^{int}$, $CD1d^{lo}$), (C) marginal zone (MZ, $CD24^{int}$, $CD1d^{high}$) and (D) immature/transitional B cells (Imm; $CD24^{high}$, $CD1d^{int}$). (E) The percentage of B220+ cells collected in the total lymphocytes gate (based on forward and side scatter). All results are presented as a mean percentage of $B220^{+}$ events. At least six mice were used in each group. *$p<0.05$; **$p<0.001$

Figure 6. Analysis of hCR1.hCR2.*Cr2* $^{-/-}$ BM B cell populations.

Bone marrow cells were collected and the RBC were lysed. (A) Cells were stained with B220 PerCP, CD43 PE and biotinylated CD25/SA allophycocyanin and delineated as shown. Cells were distinguished as follows: (B) total B cells are B220+ cells, (C) pro/pre-B cells are $B220^{int}CD43^{+}CD25^{-}$, (D) immature B cells are $B220^{int}CD43^{-}$, and (E) mature B cells are $B220^{high}CD43^{-}$. All results are presented as a mean percentage of all live cells in the bone marrow isolate. Student's *t* test analysis was used to generate *p* values. At least six mice were used in each group.