Supplementary data

Fig. S1. Basic characterization of human CD20 (hCD20) NOD Transgenic mice

(A) Expression of hCD20 is restricted to B cells. Splenocytes from hCD20 transgenic mouse were stained with anti-B220, anti-CD19 and anti-human CD20 and analyzed by flow cytometry. The expression of B220 and hCD20 was shown in the FACS plot after gating on CD19+ cells.

(B) Expression of hCD20 does not affect natural immunoglobulin (Ig) production. Serum samples from transgenic (n= 9) and non-transgenic NOD mice (n = 7) were tested for different Ig isotypes by ELISA. The level of serum IgM was also comparable (data not shown). Note: IgG2a in the NOD strain may represent IgG2c (1).

(C) Expression of hCD20 does not affect anti-insulin autoantibody production. Serum samples from transgenic (n = 7) and non-transgenic NOD mice (n = 8) were tested for anti-insulin autoantibody by Luminex assay. The number of anti-insulin antibody positive mice was comparable between hCD20 transgenic and non-transgenic NOD mice.

(D) Expression of hCD20 does not affect B cell function. Splenocytes from hCD20 transgenic and non-transgenic mice were stimulated with different concentrations of anti-Ig (top panels) or anti-CD40 mAb (FGK45 supernatant) treatment (lower panels) and the percentage of CD40 (HM 40-3)-expressing (left panels) or CD86-expressing (right panels) B220+ B cells was measured by flow cytometry. No differences were seen between transgenic and non-transgenic mice.

Fig. S2. B cell depletion one hour after the first injection. hCD20/NOD mice (n = 3 each group) were treated with 0.5 mg of 2H7 or IgG. Peripheral blood was taken before and one hour after injection and stained with mAbs to B220 and mouse CD22. This figure illustrates B cell depletion from one of the 3 2H7-treated mice.

Fig. S3. B cell depletion in different anatomical sites. NOD or hCD20/NOD mice (n = 2 each group) were treated with one course of 2H7 (4 injections). Mice were sacrificed 4 days after the last injection. Cells harvested from different anatomical sites were stained with mAb to B220 and CD22 and analyzed by flow cytometry. The percentage of B220+ B cells in total cells analyzed is shown. Blood and spleen cells were stained after removal of erythrocytes.

Fig. S4. Newly generated B cells have similar immune responses to OVA immunization. hCD20 transgenic mice were immunized with OVA two months after anti-hCD20 or IgG treatment. Anti-OVA antibodies were detected by ELISA. Anti-hCD20 treatment did not affect the B cell response to OVA immunization. Note: IgG2a in NOD strain may represent IgG2c (1).

Fig. S5. Newly generated B cells express less anti-insulin autoantibody. Randomly selected serum samples from anti-hCD20 or control IgG treated transgenic mice were tested for anti-insulin autoantibody by Luminex assay at different time points after treatment. The number of anti-insulin autoantibody positive mice was markedly lower in hCD20-treated mice compared with IgG-treated control mice.

Fig. S6. Pancreatic islet histology following 2H7 or IgG treatment after clinical diabetes onset. H+E stained sections of pancreas illustrating islets were taken over 3 months after treatment with 2H7 (top panel, euglycemic and not under insulin treatment) or IgG antibody (lower panel, hyperglycemic and under insulin treatment). Three mice in each group were examined. Magnification X 200.

Fig. S7. Transitional (T2) B cells are more highly represented in newly generated B cells. T2 B cells were examined according to methods previously described (2-4). Four months after treatment with 2H7 or IgG, splenocytes from treated mice (n=3 each group) were stained with anti-IgM, anti-B220, anti-CD23 and anti-CD21 and analyzed by flow cytometry. The expression of CD21 and CD23 were shown in the FACS plot after gating on IgM+ and B220+ cells (A). Newly generated B cells showed an increased frequency of T2 cells and decreased frequency of MZ B cells (A). There was a small increase in the frequency of follicular B cells (FO). A summary of the percentage of T2 cells in the three groups (n=3 each) is shown in (B). * comparison of diabetic IgG-treated mice with cured mice; ** comparison of non-diabetic IgG-treated mice with cured mice.

References:

1. Martin, R.M., Silva, A., and Lew, A.M. 1997. The Igh-1 sequence of the non-obese diabetic (NOD) mouse assigns it to the IgG2c isotype. *Immunogenetics* 46:167-168.
2. Su, T.T., and Rawlings, D.J. 2002. Transitional B lymphocyte subsets operate as distinct checkpoints in murine splenic B cell development. *J Immunol* 168:2101-2110.
3. Chung, J.B., Silverman, M., and Monroe, J.G. 2003. Transitional B cells: step by step towards immune competence. *Trends Immunol* 24:343-349.

4. Quinn, W.J., 3rd, Noorchashm, N., Crowley, J.E., Reed, A.J., Noorchashm, H., Naji, A., and Cancro, M.P. 2006. Cutting edge: impaired transitional B cell production and selection in the nonobese diabetic mouse. *J Immunol* 176:7159-7164.

Material and methods

Detection of OVA-specific immunoglobulins by ELISA: 96 well plates were coated overnight at 4 ℃ with 10 μg/ml OVA in $NaHCO_3$ buffer (PH9.6, 0.1M). Plates were washed with washing buffer (PBS containing 0.05% Tween 20) three times. Then plates were blocked with 1% BSA in PBS for 1 hour at room temperature. Sera samples (1:50 dilution) were added into the plates. After 2 hours incubation at room temperature, the plates were washed three times. Then alkaline phosphatase (AP) conjugated antibodies (anti-immunoglobulin isotype) were added into wells, respectively, and incubated for 2 hours at room temperature. After washing 3 times with wash buffer, AP substrate pNPP was added into the wells and incubated for 0.5~1 hour at room temperature. 2N NaOH was added to stop the reaction. Plates were read at OD405 by ELISA reader from Biorad.

Fig. S1 A-C

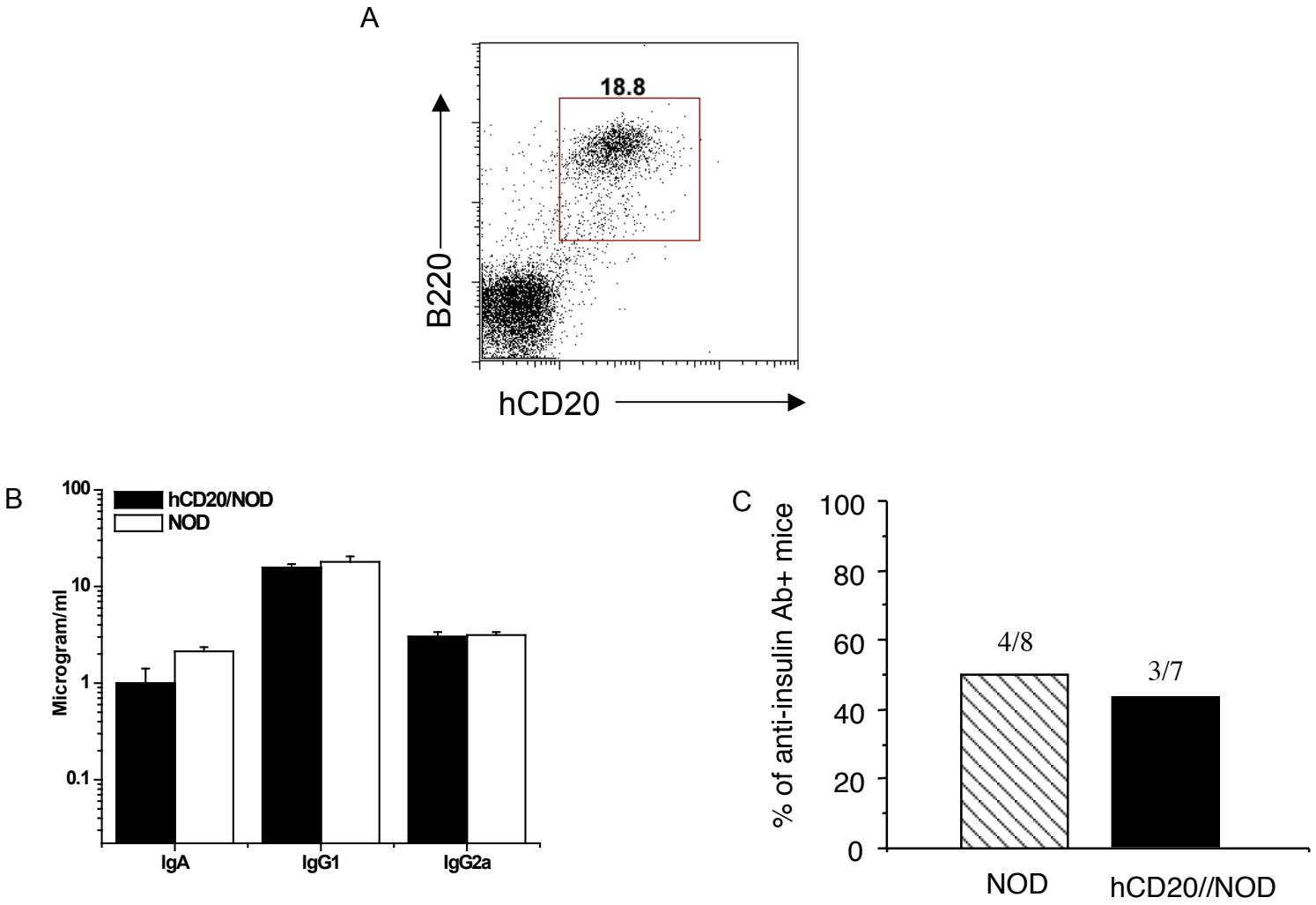
A
18.8
B220
hCD20
B
hCD20/NOD
NOD
100
10
1
0.1
Microgram/ml
IgA
IgG1
IgG2a
C
% of anti-insulin Ab+ mice
100
80
60
40
20
0
4/8
3/7
NOD
hCD20//NOD

Fig. S1 D

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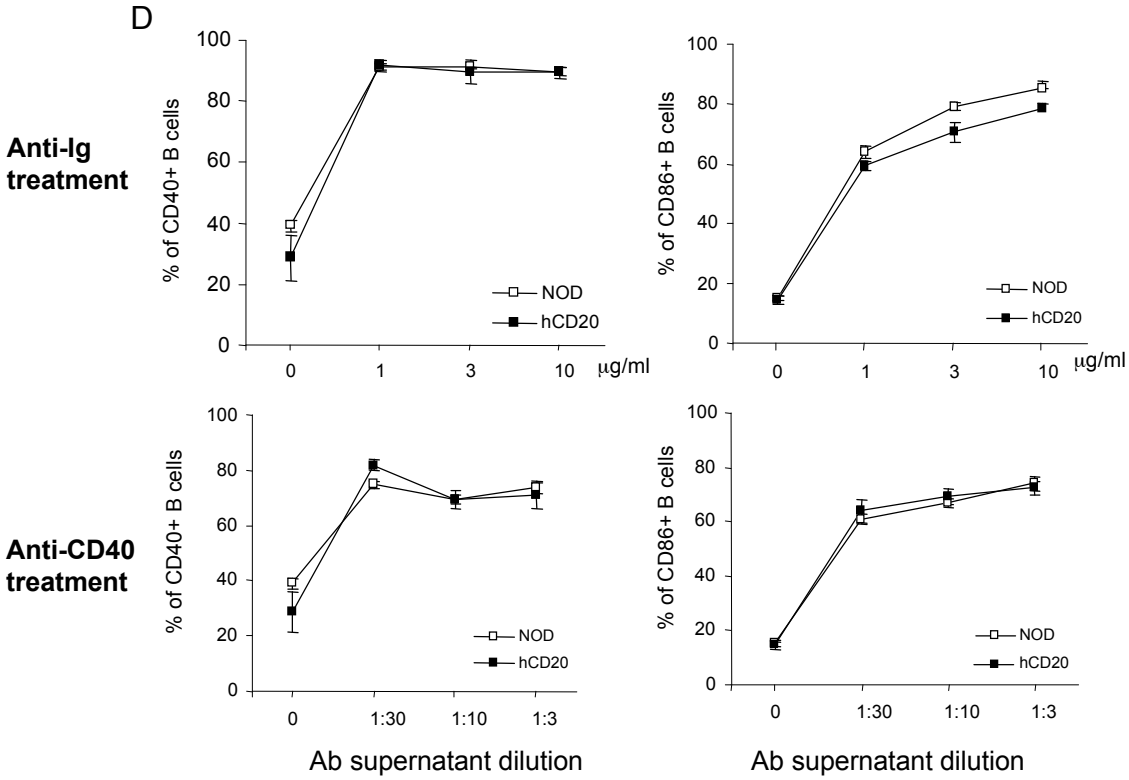


Fig. S2

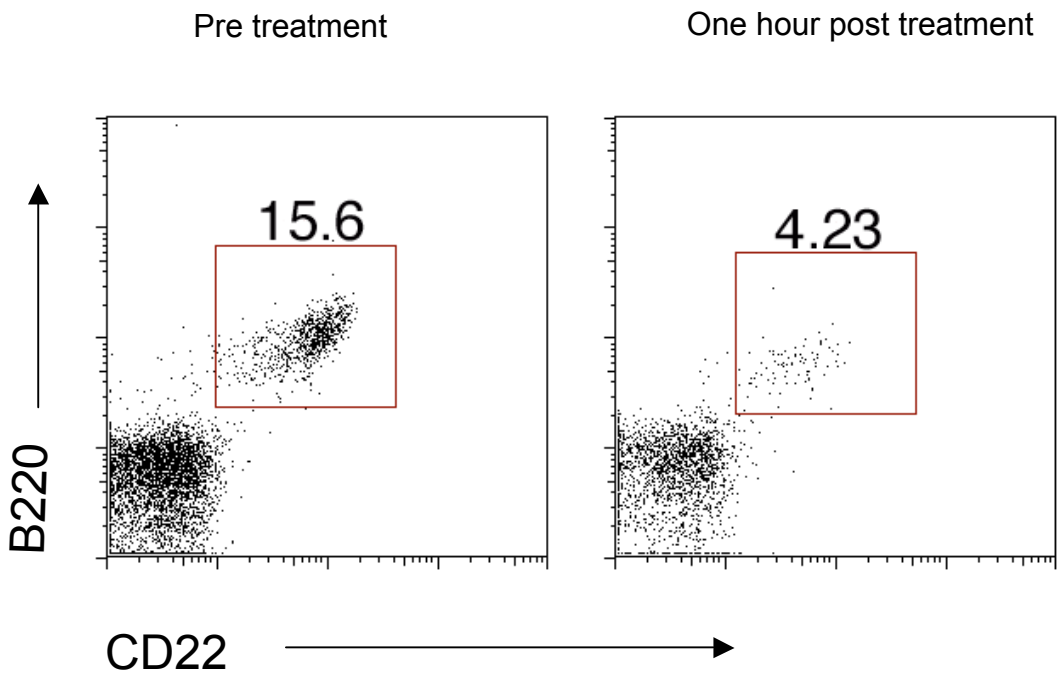


Fig. S3

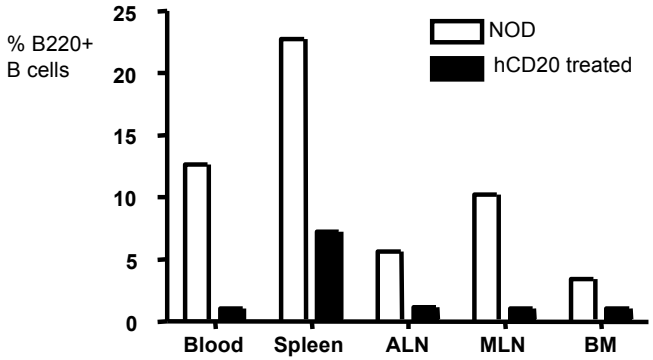

% B220+
B cells
25
20
15
10
5
0
NOD
hCD20 treated
Blood
Spleen
ALN
MLN
BM

Fig. S4

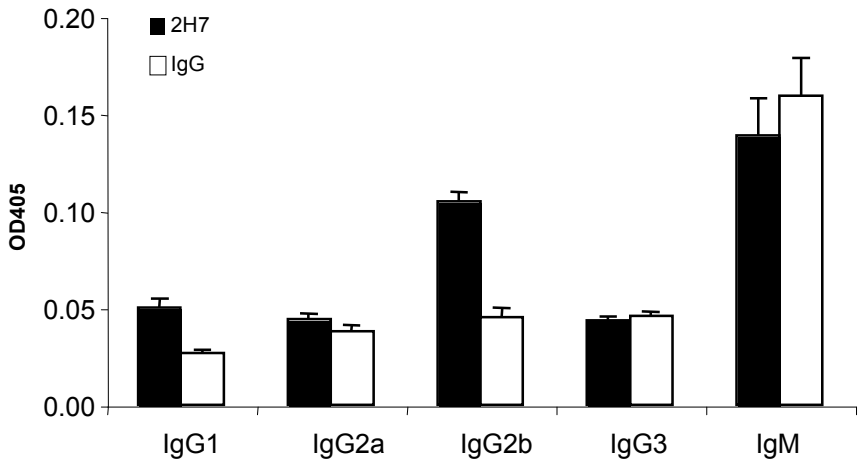


Fig. S5

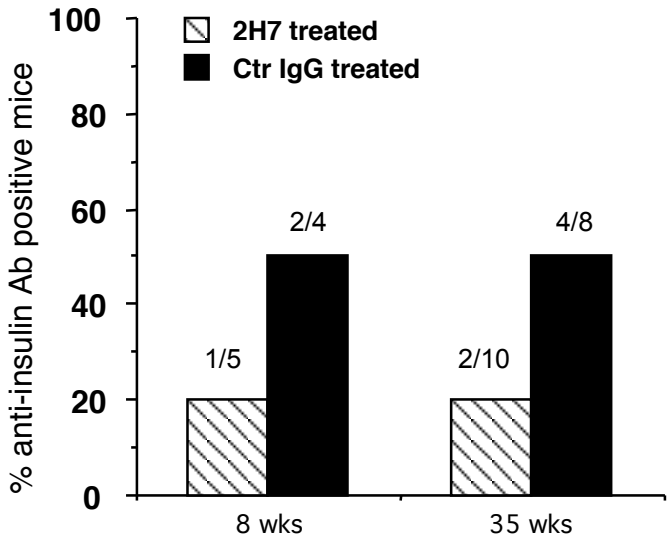
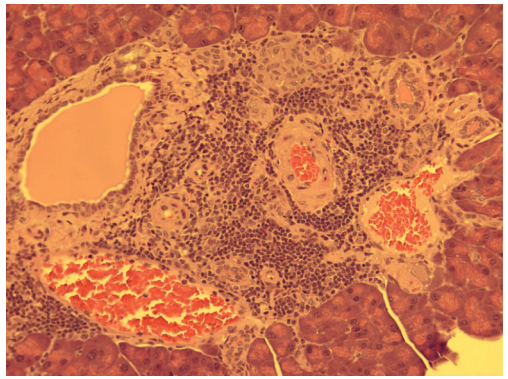

100
80
60
40
20
0
% anti-insulin Ab positive mice
2H7 treated
Ctr IgG treated
1/5
2/4
2/10
4/8
8 wks
35 wks

Fig. S6

cured



control

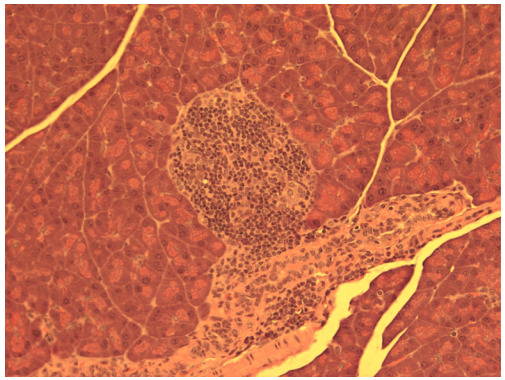
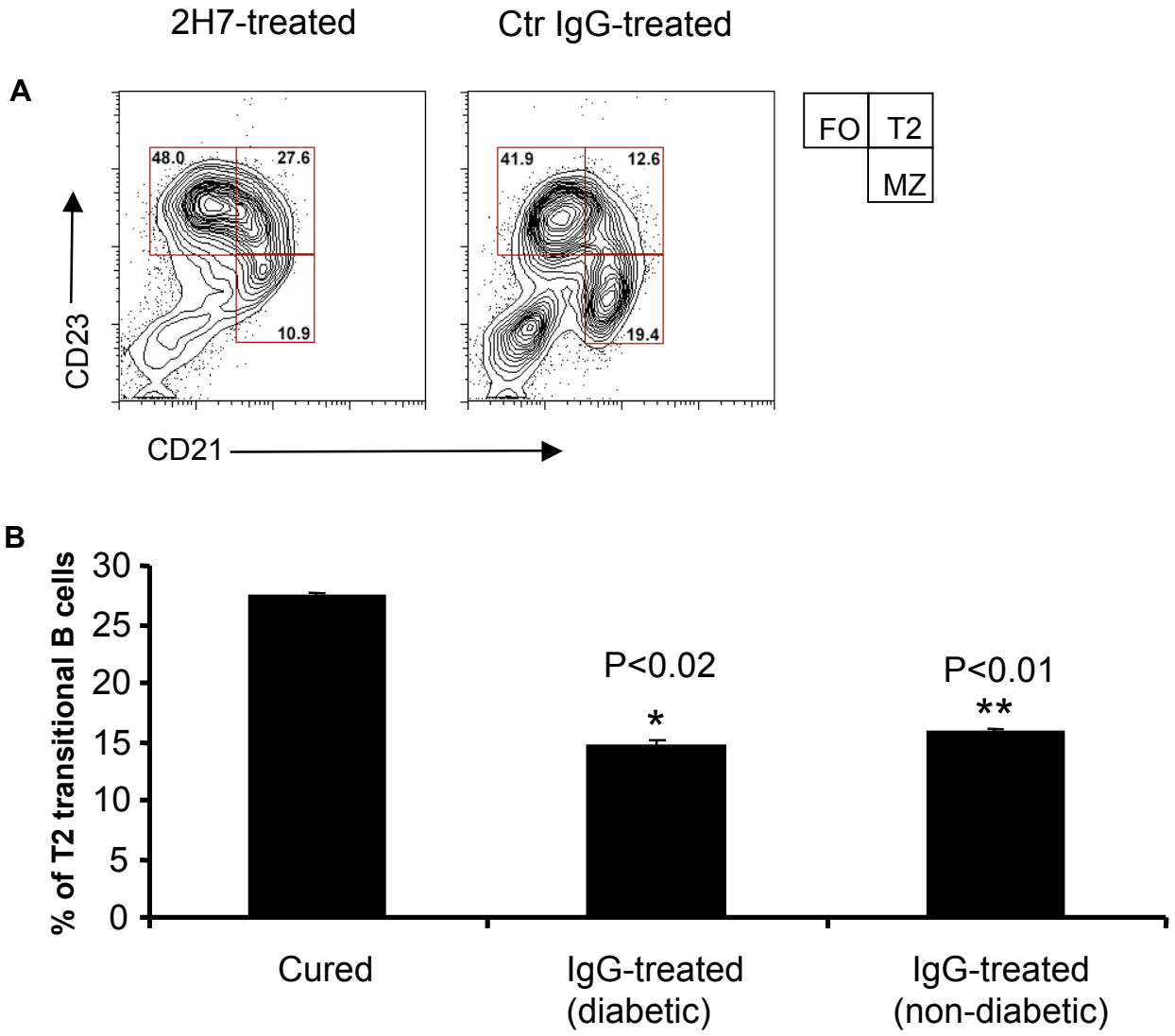


Fig. S7


2H7-treated
Ctr IgG-treated
A
48.0
27.6
10.9
41.9
12.6
19.4
FO
T2
MZ
CD23
CD21
B
% of T2 transitional B cells
30
25
20
15
10
5
0
P<0.02
*
P<0.01
**
Cured
IgG-treated (diabetic)
IgG-treated (non-diabetic)