Supplementary data

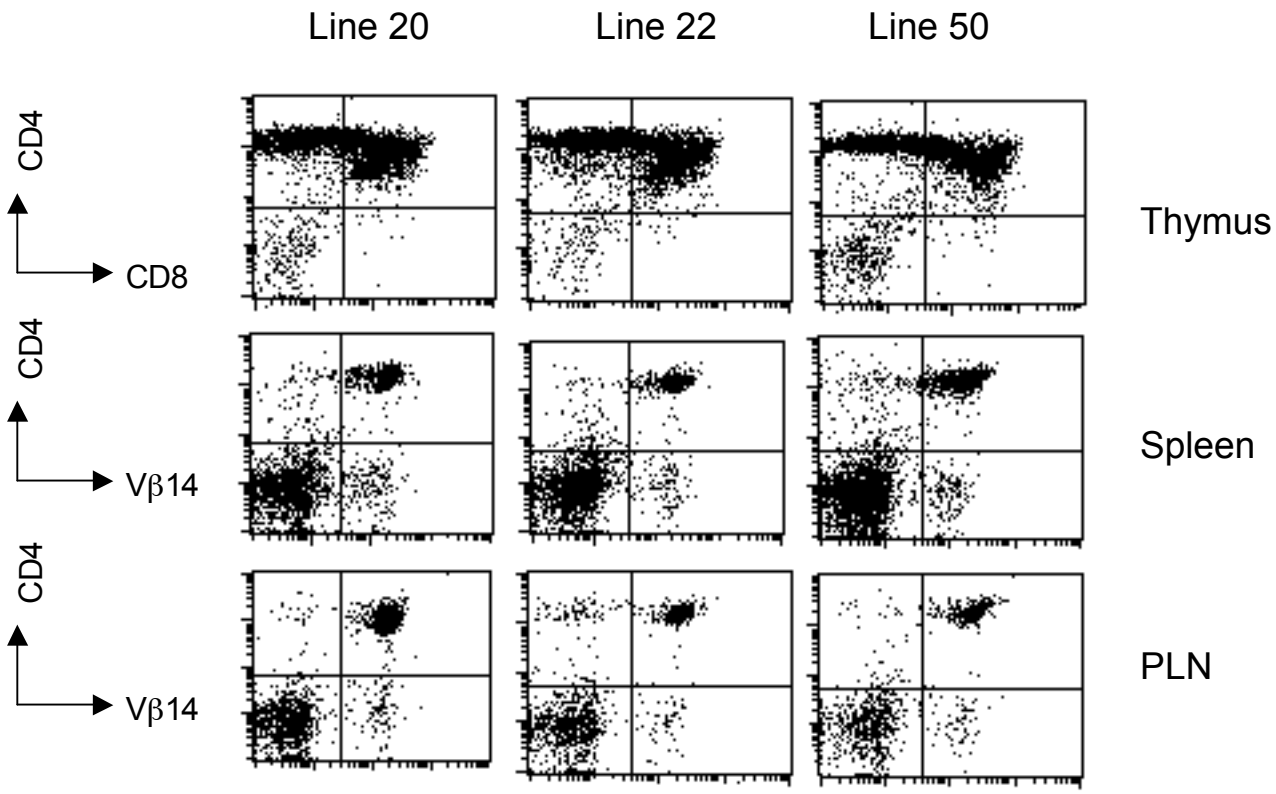

Fig. S1A

Supplementary data

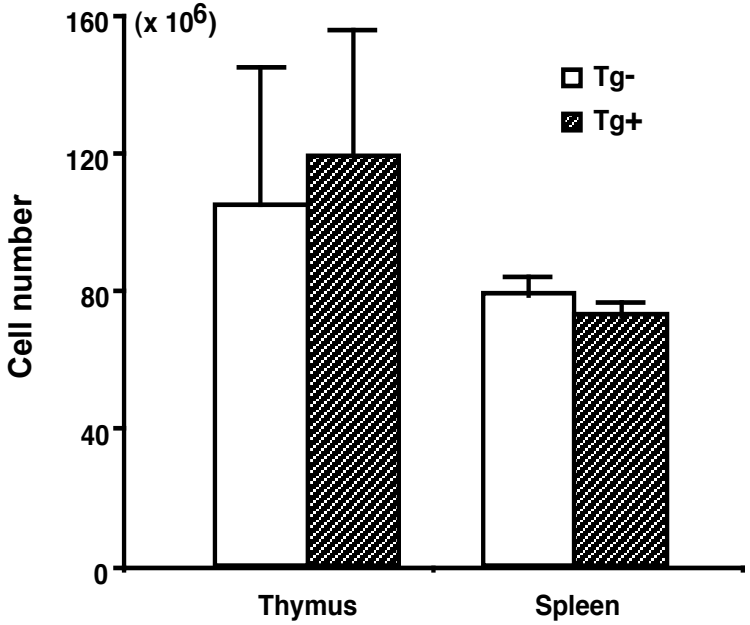

Fig. S1B

Supplementary data

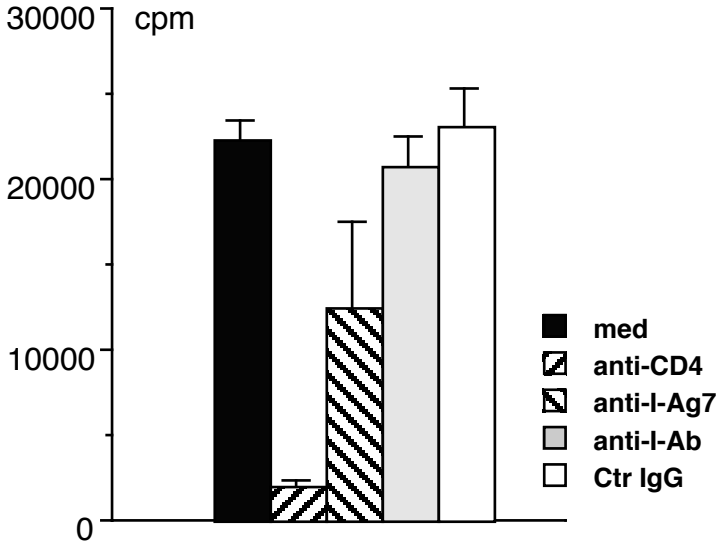

Fig. S2

Supplementary data

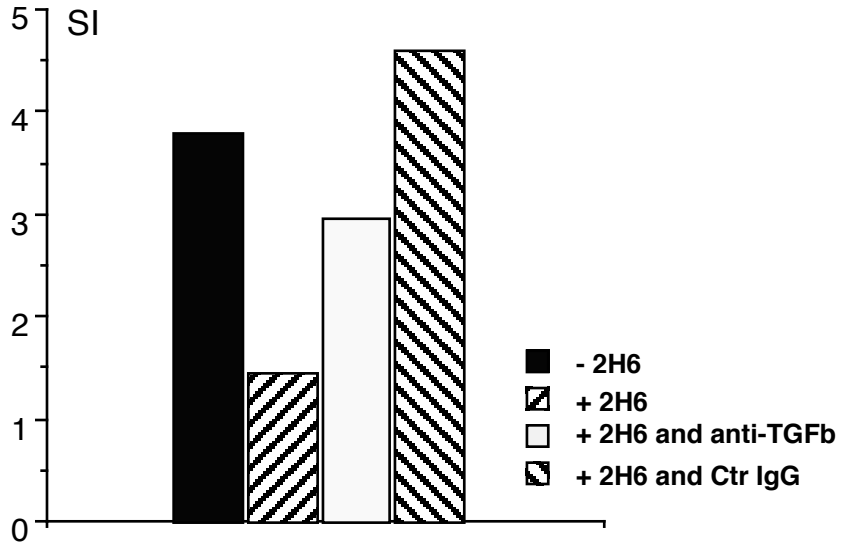

Fig. S3

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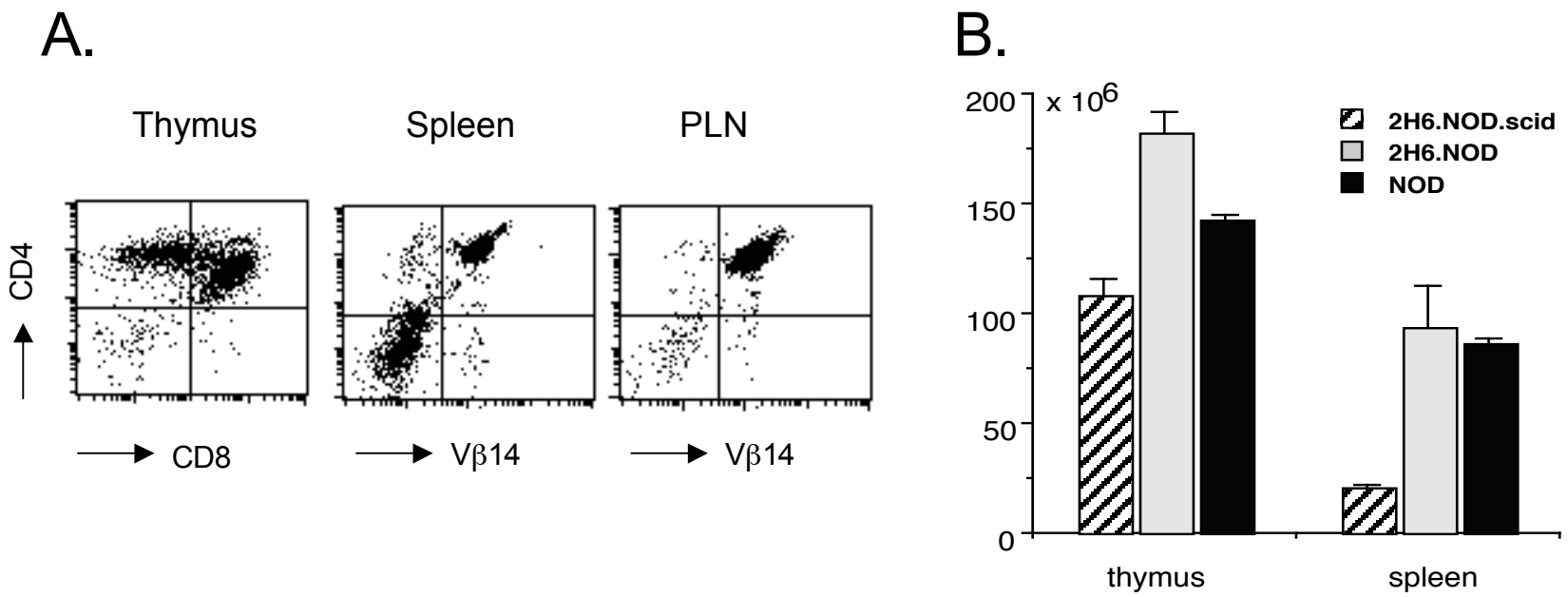

Fig. S4

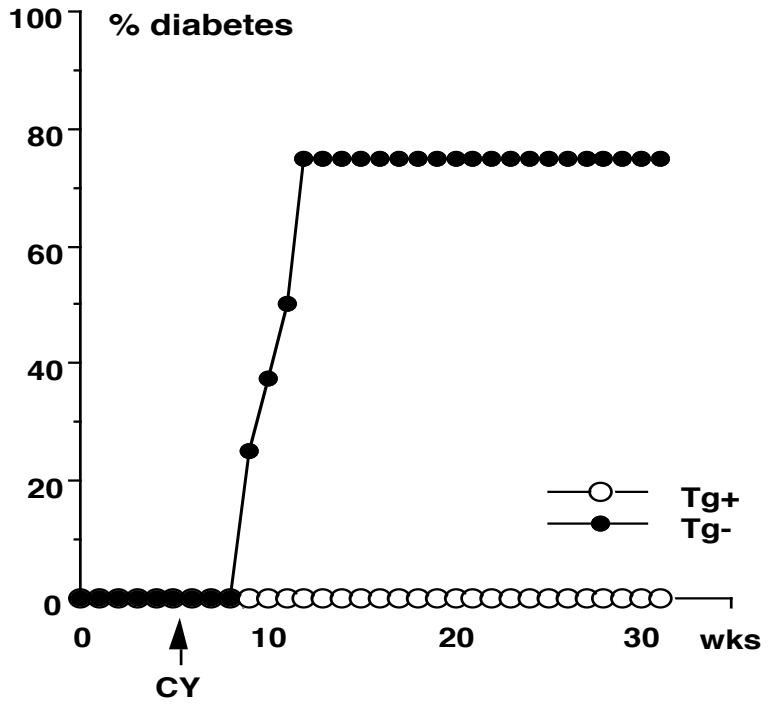

Fig. S5

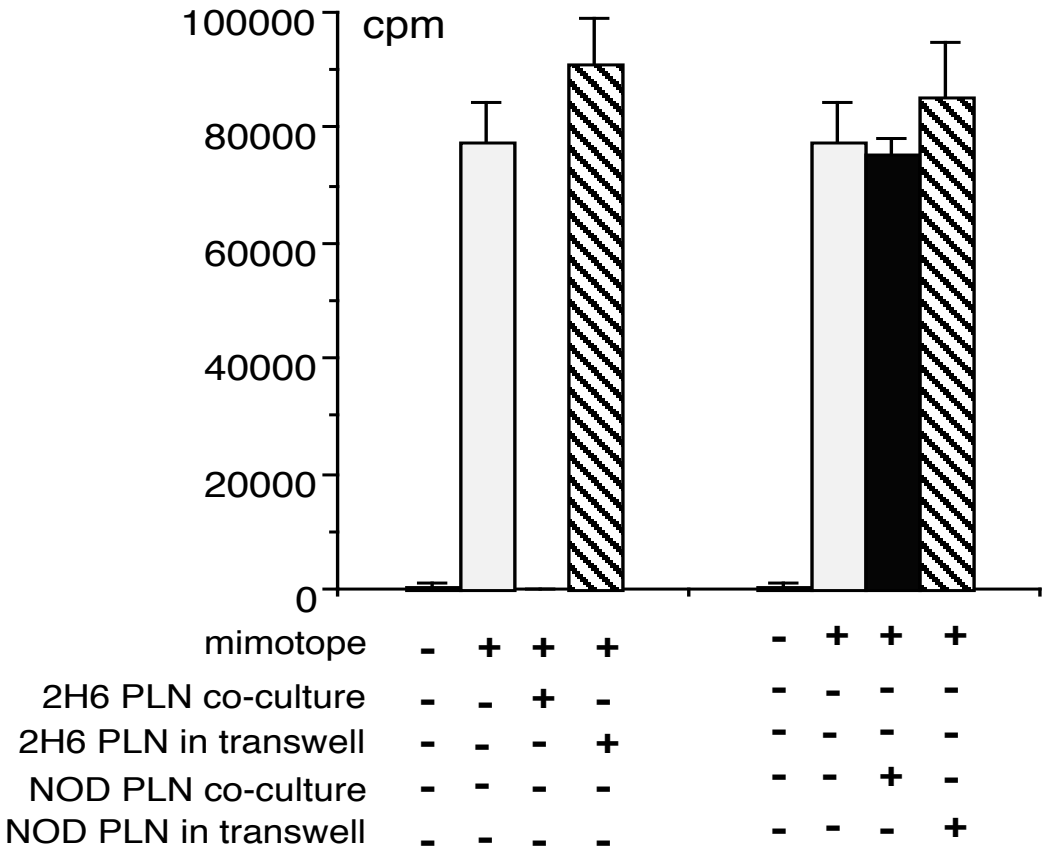
100000
cpm
80000
60000
40000
20000
0
mimotope
2H6 PLN co-culture
2H6 PLN in transwell
NOD PLN co-culture
NOD PLN in transwell

Fig. S6

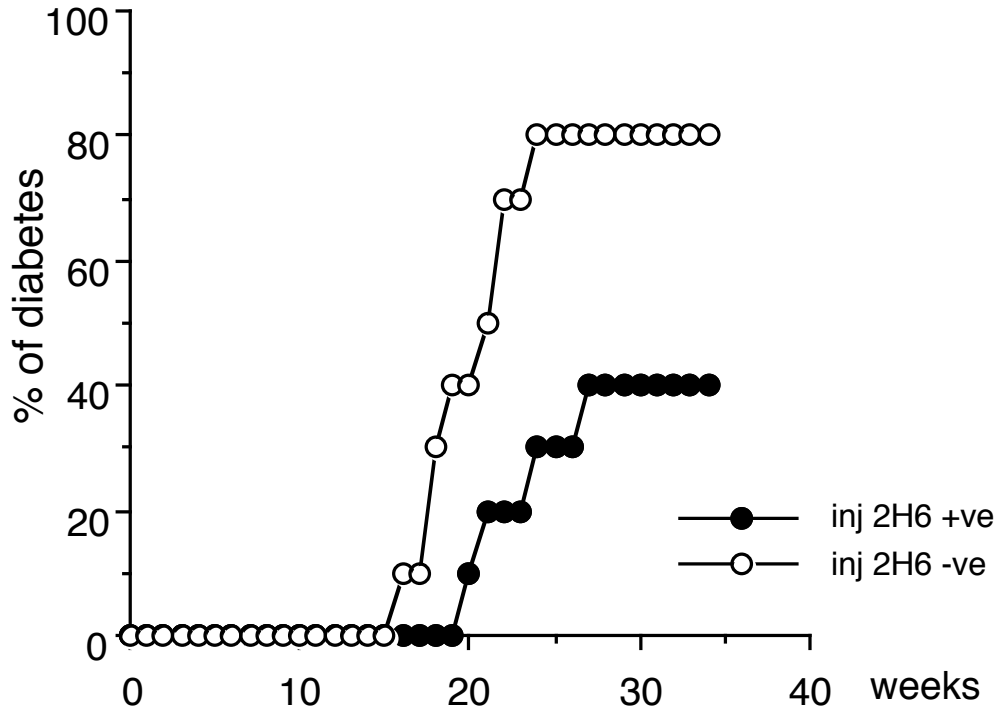
100
80
60
40
20
0
% of diabetes
0
10
20
30
40
weeks
inj 2H6 +ve
inj 2H6 -ve

Fig. S7

Supplementary data

Figure S1. Phenotypic and cellularity analyses of three 2H6 TCR transgenic lines.

A. Expression of the 2H6 TCR transgene. Thymocytes, splenocytes and pancreatic lymph node cells were stained with anti-Vβ14, and-CD4 and anti-CD8 mAbs and analyzed by flow cytometry.

B. Thymic and splenic cellularity of 2H6 transgene-positive and negative NOD mice. Thymocytes and splenocytes were harvested from 6 weeks old 2H6 transgene-positive (n = 5) and negative (n = 6) NOD mice and counted with a hemocytometer.

Figure S2. Inhibition of autoreactivity of 2H6 TCR transgenic cells. 2H6 splenoctyes (10^5 cells/well) were cultured in either Click's medium with 5% FCS or serum free medium X-Vivo in 96-well plate in the presence and absence of monoclonal antibodies to CD4 (GKi.5), I-A^{g7} (10.2.16), I-A^b (25-9-17) or purified control IgG for 72 hours (in triplicates). Anti-CD4, I-A^{g7} and I-A^b were used at 1:30 of the hybridoma supernatants and purified control IgG was used at 10 μg/ml). ^{3}H-thymidine was added for the last 16-18 hours of culture and the cells were harvested and counted in a β-plate counter. The experiments were repeated 4 times using Click's medium with 5% FCS and twice with serum free medium X-Vivo. This figure represents one of the 4 experiments.

Figure S3. Inhibiton of the regulatory effect of 2H6 TCR transgenic cells by anti-TGF-β. BDC2.5 splenoctyes (10^5 cells/well) were cultured with or without 3 μg/ml mimotope in the presence or absence of 2H6 splenocytes (10^5 cells/well) and purified monoclonal antibody to TGF-β (10 μg/ml) or purified control IgG (10 μg/ml). ^{3}H-thymidine was added for the last 16-18 hours of 72 hours culture and the cells were harvested and counted in a β-plate counter. The results were converted to simulation index (SI), in which the cpm from experimental proliferation was divided by the cpm from background proliferation. The experiments were repeated 3 times. This figure represents one of the 3 experiments.

Figure S4. Phenotypic and cellularity analyses of 2H6.scid mice.

A) Thymocytes, splenocytes and pancreatic lymph node cells were stained with anti-Vβ14, and-CD4 and anti-CD8 mAbs and analyzed by flow cytometry.

B) Thymic and splenic cellularity of 2H6 NOD.scid, 2H6 NOD and NOD mice. Thymocytes and splenocytes were harvested from 5 week old mice (n = 2 to 5 for each group) and counted with a hemocytometer.

Figure S5. Diabetes development post Cyclophosphamide (CY) treatment. Both 2H6 TCR transgene negative (n=8) and positive (n=6) NOD

mice were treated with CY (see Materials and Methods). The treated mice were then observed for diabetes development by screening for glycosuria weekly and diabetes was confirmed by blood glucose measurement (>13.9 mmol/l). The experiment was terminated when the mice were 32 weeks old.

Figure S6. 2H6 cells from pancreatic lymph nodes potently inhibit proliferation of BDC2.5 T cells. BDC2.5 splenoctyes (10^6 cells/ml) were cultured with or without 3 μg/ml mimotope in the presence or absence of 2H6 or NOD PLN cells (0.5 x 10^6 cells/ml) in direct contact (co-culture) or separated by a 0.4 μm membrane (transwell). ^{3}H-thymidine was added for the last 16-18 hours of 72 hours culture and the cells were harvested and counted in a β-plate counter. The experiments were repeated twice and the figure represents one of the 2 experiments.

Figure S7. 2H6 cells inhibit the development of spontaneous diabetes in NOD mice. Twenty 4 to 5 week old female NOD mice were intravenously injected either 2H6 transgene positive or negative splenocytes (n = 10 for each) 5 times at weekly intervals. The mice were then screened for diabetes development. The experiment was terminated when the mice reached 35 weeks of age. Eighty percent of the mice that received transgene negative splenocytes developed diabetes (open circles) whereas only 40% of mice became diabetic after receiving 2H6 splenocytes (solid circles).