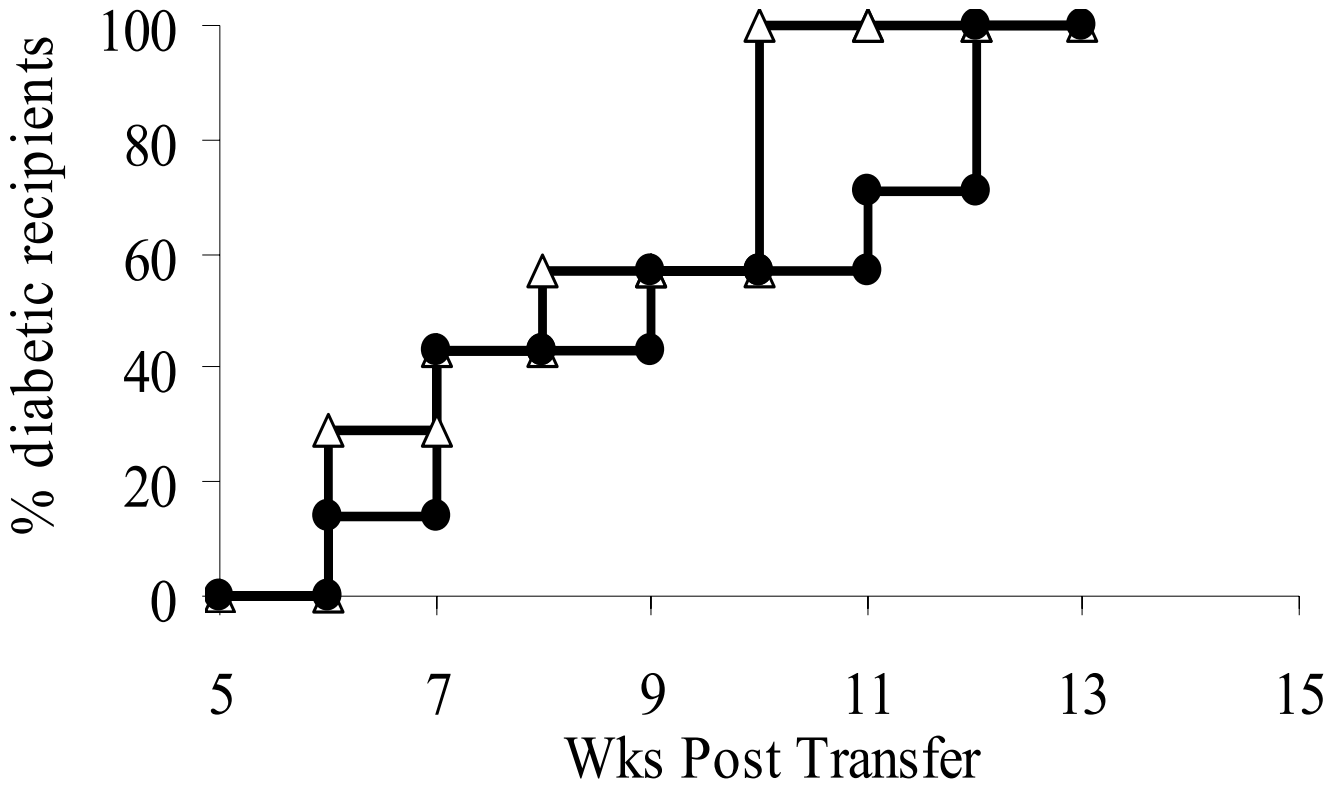
Supplementary figure 1: B7-2 deficiency does not affect the effector phase of diabetes

To examine whether B7-2 played a role in the effector phase of the disease, we purified T cells from the spleen and LN of diabetic NOD females and transferred 7 to 15 x 10^6 T cells into NOD-RAG$^{-/-}$ (open triangles) (*n*=7) or NOD-RAG$^{-/-}$ B7-2$^{-/-}$ (closed circles) (*n*=7) recipients. We measured blood glucose levels weekly to assess the development of diabetes. Diabetic NOD T cells induced diabetes in NOD-RAG$^{-/-}$ and NOD-RAG$^{-/-}$B7-2$^{-/-}$ recipients with similar kinetics, indicating that B7-2 was dispensable for the effector phase of diabetes induced by a polyclonal diabetogenic memory T cell population

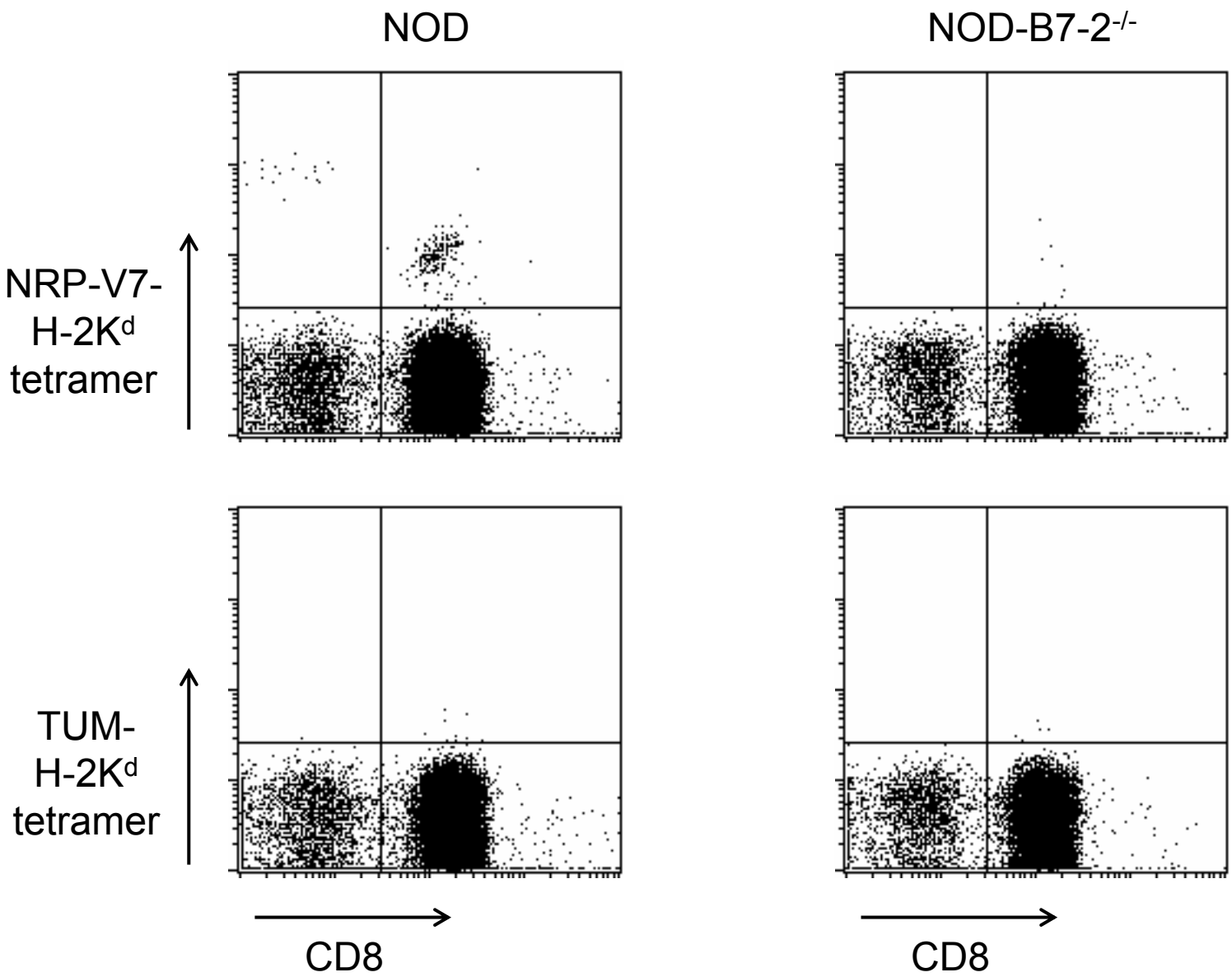
Supplementary figure 1



Supplementary figure 2: B7-2 deficiency affects the activation and/or expansion of a predominant population of pathogenic $CD8^+$ T cells

Single-cell suspensions were prepared from pancreatic LN of 16-18 wk old NOD and NOD-B7-$2^{-/-}$ mice. Cells were stained with CD8 and NRP-V7-H-$2K^d$ tetramers that identify a prevalent population of pathogenic $CD8^+$ T cells (22) or control TUM-H-$2K^d$ tetramers that contain an irrelevant peptide. Results display NRP-V7-H-$2K^d$ tetramer (upper panels) and TUM-H-$2K^d$ control tetramer (lower panels) staining versus CD8 staining in NOD (left panels) and NOD-B7-$2^{-/-}$ mice (right panels) (gated on CD4-negative B220-negative cells).

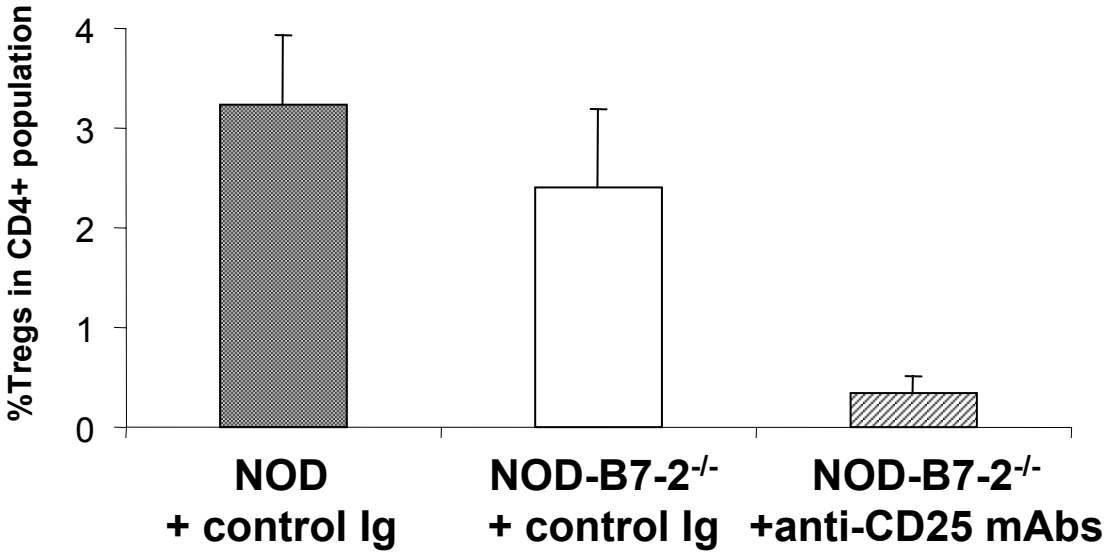
Supplementary figure 2



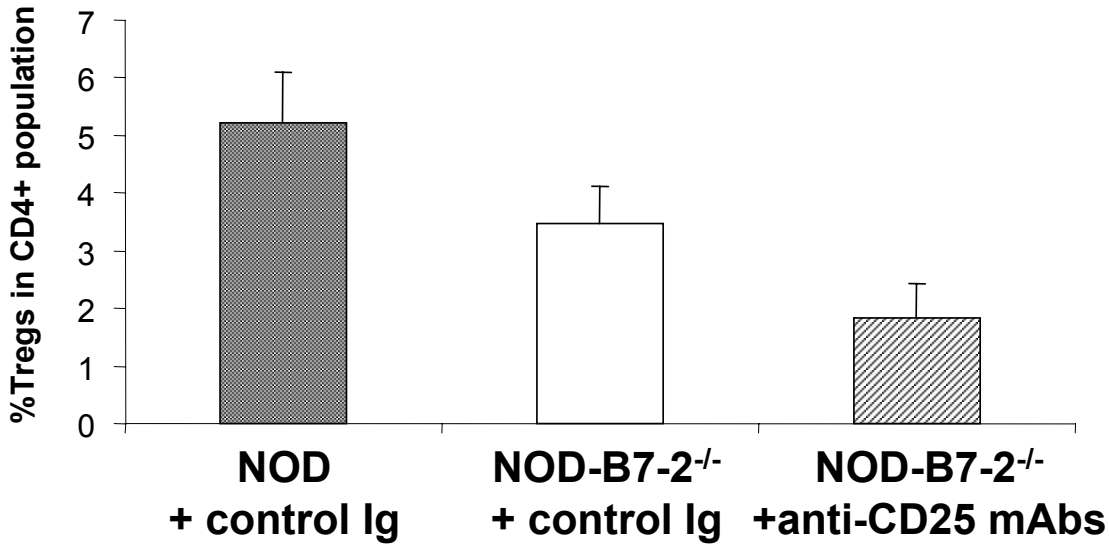
Supplementary figure 3: Tregs depletion after anti-CD25 mAbs treatment

a. All groups of mice were thymectomized prior to mAbs treatment. Two weeks before transfer, NOD mice were treated with control Ig and NOD-B7-2$^{-/-}$ mice were treated with control Ig or anti-CD25 mAbs (PC61). One day after transfer of BDC2.5 cells, the percentage of CD25^{+} CD62L^{hi} Tregs in the CD4^{+} population was analyzed in the blood. Histograms represent the mean value and standard deviation for each group. Results are pooled from two separate experiments. *b*. The percentage of Tregs was analyzed in the lymph nodes of the same groups of mice two weeks after transfer of BDC2.5 cells (i.e. four weeks after mAbs treatment). *c*. Depletion of Tregs does not affect BDC2.5 proliferation in peripheral LN of NOD-B7-2$^{-/-}$ mice, suggesting that deletion of Tregs resulted in islet antigen-specific activation of diabetogenic T cells in the pancreatic lymph nodes rather that non-specific polyclonal activation in all peripheral lymphoid tissues. A representative experiment is shown.

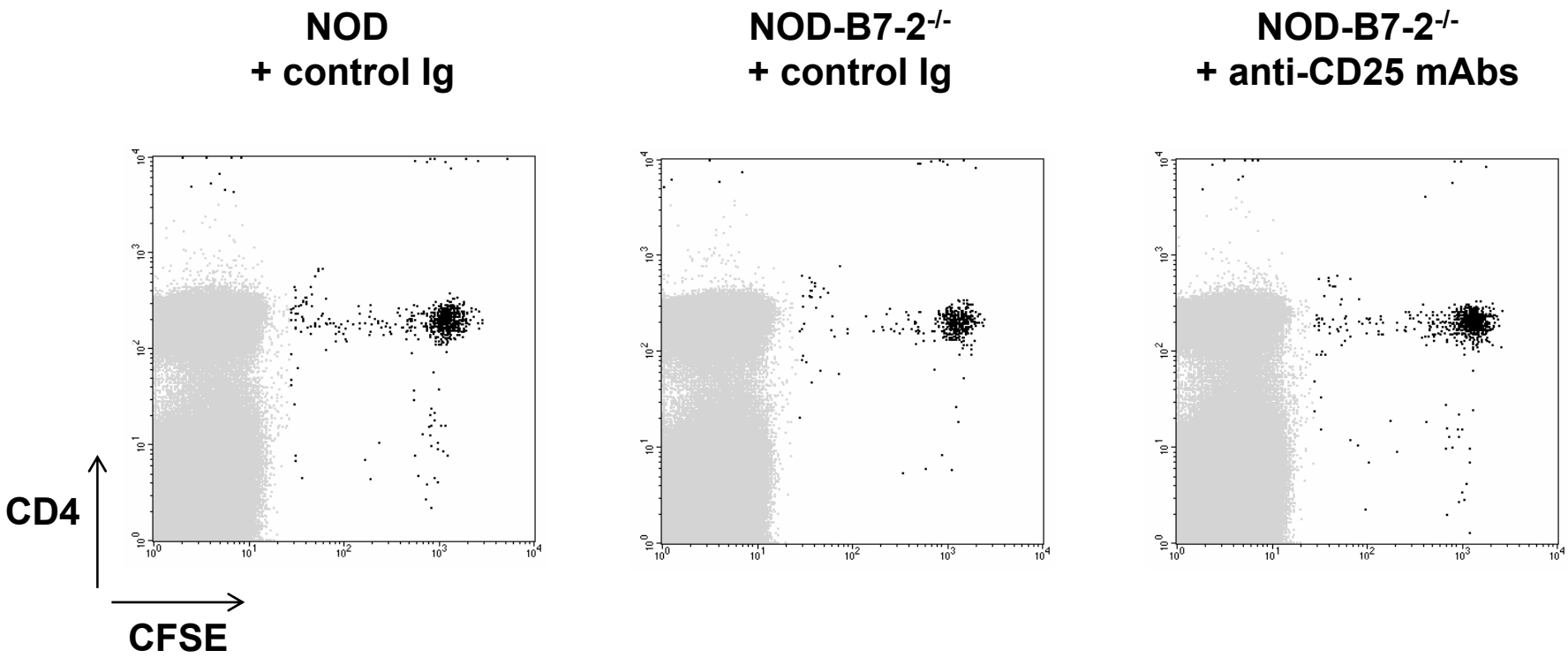
Supplementary figure 3a



Supplementary figure 3b



Supplementary figure 3c

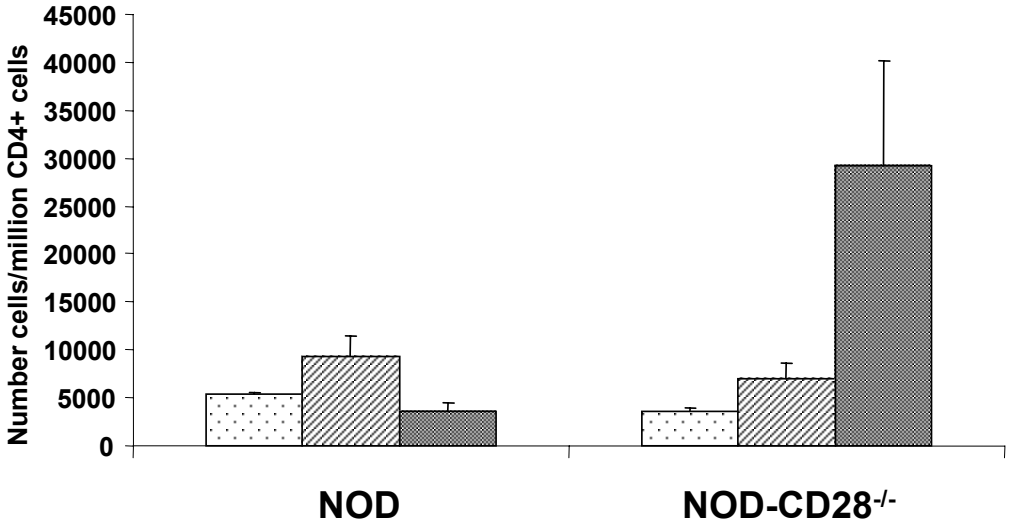


Supplementary figure 4: Increased expansion and numbers of autoreactive cells in the absence of Tregs

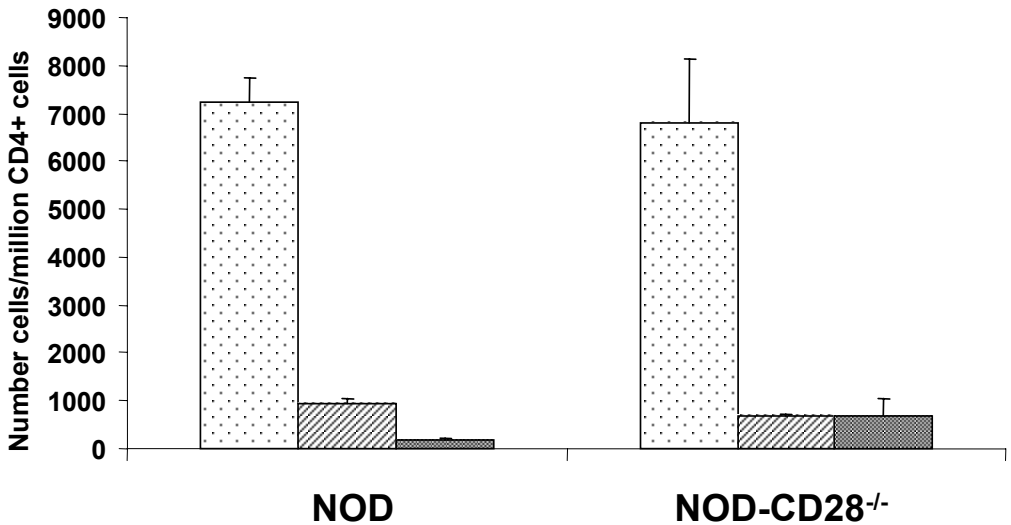
a. CD25-depleted BDC2.5-CD90.1 T cells were labeled with CFSE and transferred into NOD or NOD-CD28$^{-/-}$ mice. Four days later, the proliferation of BDC2.5 cells was assessed in the pancreatic lymph nodes. The histograms represent the number of undivided cells, cells that underwent 1 to 3 divisions, and cells that underwent 4 or more divisions (respectively) per million CD4^{+} cells, in the pancreatic LN (top) or peripheral LN (bottom). *b*. The total number of autoreactive BDC2.5 CD4^{+} CD90.1^{+} in the pancreatic lymph nodes is indicated for NOD and NOD-CD28$^{-/-}$ mice. Each circle represents an individual mouse. Horizontal bars represent the mean value for each group.

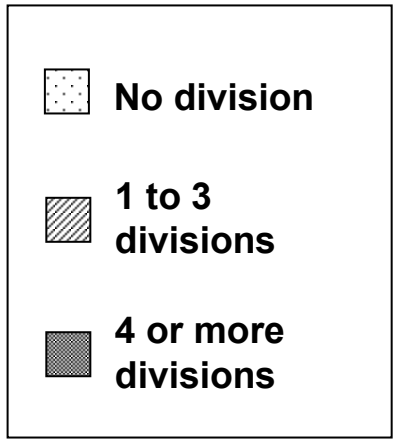
Supplementary figure 4a

Pancr. LN



LN





Supplementary figure 4b