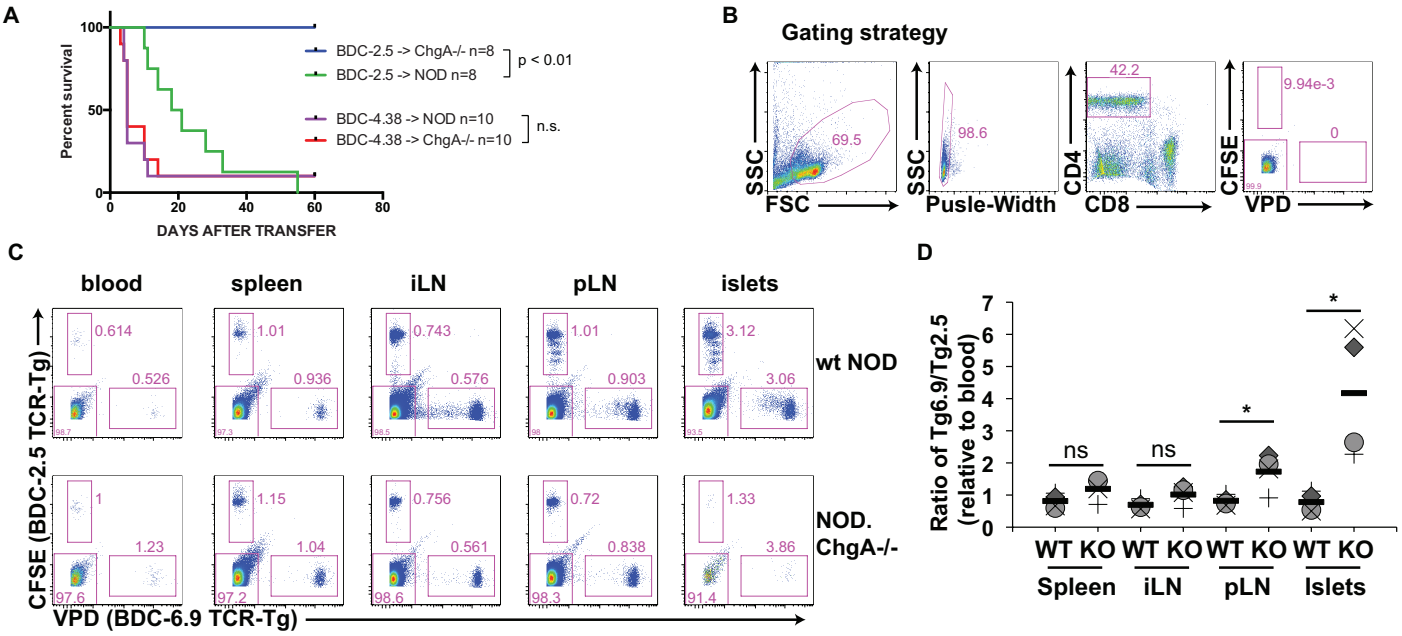
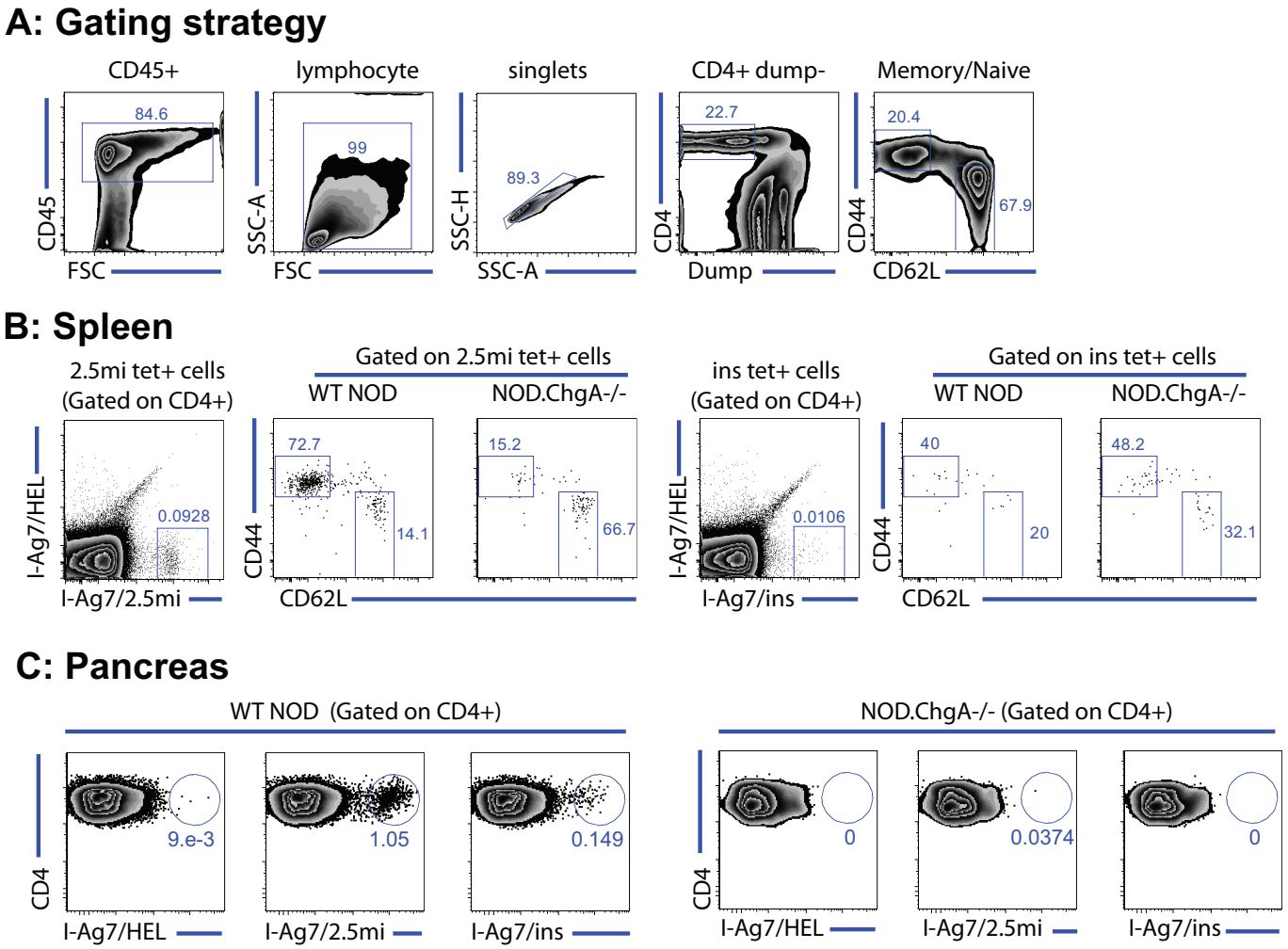
Supplemental Fig. 1: ChgA-deficient mice lack the antigen for BDC-2.5 CD4 T cells.



(**A**) To test for the presence of antigen in vivo in ChgA-deficient mice, very young (< 14 days old) wt NOD (n = 8), or NOD.ChgA-/- (n = 8) mice were injected i.p. with 1 x 10^7 BDC-2.5 T cells and as another control, young wt NOD (n = 10) or NOD.ChgA-/- (n = 10) mice were injected i.p. with 1 x 10^7 insulin-reactive BDC-4.38 T cells. Mice were then monitored for development of hyperglycemia. Data summarize three experiments for BDC-2.5 and three experiments for BDC-4.38. (**B, C & D**) To test for activation of ChgA-reactive T cells in ChgA-deficient mice, CD4 T cells were isolated from BDC-2.5 TCR-Tg and BDC-6.9 TCR-Tg NOD mice and labeled with either CFSE (BDC-2.5 TCR-Tg) or VPD (BDC-6.9 TCR-Tg). Since the CFSE dye is more sensitive at detecting proliferation, dyes were switched in repeat experiments. BDC-2.5 TCR-Tg (5 x 10^6 cells) and BDC-6.9 TCR-Tg (5 x 10^6 cells) cells were then co-injected i.v. into NOD (WT) or NOD.ChgA-/- (KO) mice. Two days after transfer, cell suspensions from spleen, inguinal lymph nodes (iLN), pancreatic lymph nodes (pLN), blood and hand-picked islets were analyzed by flow cytometry. **(B)** Gating strategy is shown for an uninjected mouse: gates were set on the lymphocyte gate, singlets, CD8- and CD4+ cells. (**C**) Representative flow cytometric graphs from the blood, spleen, inguinal lymph node (iLN), pancreatic lymph node (pLN) and islets (wt NOD vs NOD.ChgA-/-) are shown for wt NOD (top) and NOD.ChgA-/- (bottom) recipient mice. **(D)** Data are expressed as a ratio of the percentage of BDC-6.9 TCR-Tg over BDC-2.5 TCR-Tg CD4 T cells relative to the ratio present in the blood. Data are representative of two independent experiments with 2 mice per group in each experiment. Each symbol represents an individual mouse and averages are indicated as a black horizontal bar.

Supplementary Fig. 2: 2.5mi tetramer-positive CD4 T cells do not accumulate in the pancreas of NOD.ChgA-/- mice.



Single cell suspensions from spleen and pancreas were stained with tetramers specific for T cells reactive to HEL, ChgA (2.5mi), or insulin (Insp8G). After 1h incubation, cells were surface stained with anti-CD4, anti-CD44, anti-CD62L, anti-CD45, 7AAD and anti-CD8, anti-CD11b, anti-CD11c, anti-CD19, anti-F4/80 and analyzed by flow cytometry. (**A**) Gating strategy is shown: gates were set on CD45+ cells, the lymphocyte gate, singlets, CD4+ and dump- (CD8, CD11b, CD11c, CD19, F4/80 and 7AAD). Memory CD4 T cells were defined as $CD44^{hi}$ $CD62L^{lo}$ and Naïve CD4 T cells were $CD44^{lo}$ $CD62L^{hi}$ (**B** and **C**) Representative flow plots are shown from WT NOD vs NOD.ChgA-/- mice: (**B**) spleen, (**C**) pancreas. Data is representative of three (**B**) and four (**C**) independent experiments with 2 mice per group per experiment.