Supplementary figure 1. Binding assay and D-peptides-H-bonds (**A**) The α chain construct contains the DQ α chain fused to the coiled-coil region of the basic leucine zipper domain of Fos. The β chain construct contains the coiled-coil region of the basic leucine zipper domain of JunB. The Jun and Fos dimerization motifs allow the protein to dimerize to form the HLA-DQ8 protein. The α chain has a His6 tag, whereas the β chain has a FLAG epitope tag for purification purposes. (**B**) Recombinant HLA-DQ8 protein was incubated with biotinylated InsB:9-23 peptide, either with or without D-peptides. The ELISA plate was coated with nickel, which captured the 6 histidines of the HLA-DQ8 α chain. If the tested D-peptide blocked the HLA pocket, it would prevent the binding of the InsB:9-23 peptide. Europium-streptavidin was added and gave the fluorescence signal. The level of fluorescence signal was reduced when InsB:9-23 binding to HLA-DQ8 was inhibited by the tested D-peptides. (**C**) Recombinant HLA-DQ8 protein was incubated with biotinylated InsB:9-23, biotinylated RI-CT and biotinylated RI-EXT or their scrambled versions. RI-CT and RI-EXT binding to HLA-DQ8 was significantly stronger compared to scrambled D-peptides and compared to InsB:9-23. (**D**) Comparison of H-bonds in RI, RI-CT and RI-EXT peptides. The bars represent the total number of H-bonds between the specific peptide and the HLA-DQ8. (**E**) and (**F**) Recombinant HLA-DQ8 protein was incubated with biotinylated GAD65, or biotinylated Gliadin or their scrambled versions. (**E**) Both peptides significantly bound to the recombinant HLA-DQ8 (compared to scrambled negative controls) but (**F**) RI-CT and RI-EXT did not inhibit their binding to HLA-DQ8. The results are representative of 3 independent experiments and are expressed as the mean ± SEM. ***$p < 0.001$, by Student's t-test.

Supplementary figure 2. InsB:9-23 activates 5KC cells specifically and dose-dependently. (**A**) BSM cells were loaded with InsB:9-23 or scrambled-InsB:9-23 or Gliadin peptide and 5KC cells were added to the system. IL-2 was detected only when BSM cells and 5KC cells were incubated together with InsB:9-23, and not with scrambled-InsB:9-23 or Gliadin. (**B**) Flow assay showing binding of Gliadin to HLA-DQ8 molecule expressed on BSM cells. Cells were gated on live BSM cells. (**C**) Dose-dependent InsB:9-23-5KC activation. Results are expressed as mean ± SEM from 3 to 4 independent experiments. ***$p < 0.001$, by Student's t-test compared to control cells.

Supplementary figure 3. Proliferation and cytokine production in murine DQ8-lymphocytes. (**A**) Flow cytometry results of inhibition of T-cell proliferation by RI-CT and RI-EXT from a representative SJL-DQ8 mouse injected with InsB:9-23. T-cell proliferation was analyzed by the CFSE assay after stimulation with InsB:9-23 with or without addition of D-peptides. Cells were gated on live splenocytes in singlets. Both RI-CT and RI-EXT significantly suppressed InsB:9-23 induced cell proliferation. (**B, C**) InsB:9-23 is specifically presented to T-cells by human HLA-DQ8 in SJL-DQ8 mice. 10 wild type SJL mice and 10 SJL-DQ8 mice were immunized subcutaneously with InsB:9-23 in CFA on day 1 and with InsB:9-23 in IFA on day 8. 9 days after the second immunization (day 17) mice were sacrificed. Lymphocytes isolated from SJL or SJL-DQ8 mice were stimulated with InsB:9-23 and the supernatants were analyzed by Luminex for (**B**) IL-2 and (**C**) IFN-γ. Only in SJL-DQ8 mice InsB:9-23 significantly induced T-cell activation confirming the essential role of HLA-DQ8 in presenting InsB:9-23 to T-cells. ***$p < 0.001$, by Student's t-test compared to control cells.

Supplementary figure 4. RI-CT and RI-EXT significantly suppress InsB:9-23 induced T-cell proliferation in DQ8-PBMCs isolated from T1D patients. (**A**, **B**) Flow cytometry results of inhibition of T-cell proliferation by RI-CT or RI-EXT from DQ8-PBMCs isolated from a representative new onset T1D patient. T-cell proliferation was analyzed by the CFSE assay after stimulation with InsB:9-23 with or without the addition of D-peptides. Analyzed cells were gated on live T-cells. Both RI-CT and RI-EXT significantly suppressed InsB:9-23 induced T-cell proliferation in DQ8-hPBMCs.