**Supplementary Figure 1. (A)** Aligned protein sequences of human and mouse GAD65 and GAD67. Light to dark shades, low to high similarity. Star, residue identical in all 4 sequences. Dot, residue identical in 2 out of 4 sequences. Black rectangle, $GAD65_{115\text{-}127}$ epitope. **(B)** Numbers of tetWT+ and tet120E+ CD4+ T cells detected from pooled secondary lymphoid organs (SLOs, pooled from spleen, ancillary lymph nodes, brachial lymph nodes, cervical lymph nodes, inguinal lymph nodes, popliteal lymph nodes) of 10-12wk (n=6) and 18-20wk old (n=5) untreated DR4Tg females. **(C-G)** Cells from spleens and inguinal lymph nodes of p115.WT/CFA and p115.120E/CFA immunized DR4Tg mice were harvested 12 days after immunization and cultured *ex vivo* for 2 weeks with APCs, 20U/ml hIL-2 and 10μM of p115.WT or p115.120E (the same peptide as immunization). (**C)** After 2 weeks, *ex vivo* expanded polyclonal T cells were stimulated for 5hrs with indicated peptides and Flt3l induced DCs in the presence of Brefeldin A and monensin. Intracellular IFNγ was measured as the readout of activation. Representative flow plots of T cells stimulated with 1μM of indicated peptides are shown. Gated on Foxp3- CD4+ T cells. **(D-G)** *Ex vivo* expanded polyclonal CD4+ T cells were stained with tetWT, tet120E or CLIP tetramer conjugated with APC or PE. For cells co-stained with tetWT and tet120E, two fluorophore combination conditions (tetWT-PE&tet120E-APC, tetWT-APC&tet120E-PE) were included to eliminate fluorophore caused bias. **(D, E)** Representative flow plots and **(F, G)** summary of frequencies of tetramer+ cells in CD4+ T cells. Data are plotted as means ± SEM and are pooled from (B, F, G) 2 or (C) 3 independent experiments. **, $p \leq 0.01$; *, $p \leq 0.05$; ns > 0.05 by (B) two-way ANOVA or (F, G) one-way ANOVA followed by Benjamini, Krieger and Yekutieli's test.

**Supplementary Figure 2. (A, B)** HEK293Ts were co-transfected with (A) WT-TCR or (B) 120E-TCR TCR and CD3ε expression plasmids and stained with PE conjugated tetWT, tet120E or control CLIP tetramer. Cells were also stained for surface CD3 expression. **(C)** Surface CD3 expression of WT-TCR or 120E-TCR TCR transduced 4G4 thymoma cells after CD3+ MACS enrichment. WT-TCR expressing cell line without CD3 staining (WT-TCR NS) was used as negative control. Representative plots of 3 independent experiments.

**Supplementary Figure 3. (A, B)** $1.2x10^7$ Flt3l induced DCs generated in DR4Tg.RagKO mice were labeled with CFSE and transferred intravenously to DR4Tg mice. Thymi of recipient mice were harvested 3 days after the transfer. **(A)** Representative flow plots of CFSE+ donor cells in transferred and non-transferred (NA) thymi.

Gated on Zombie red-CD3-CD11c+ cells. **(B)** Numbers of CFSE+CD11c+HLA-DR+ donor DCs. **(C-H)** Indicated peptide pulsed DCs were transferred (IV) into WT-TCR and 120E-TCR Hu-Rg mice. Hu-Rg mice received two DC transfers (7 days apart) and were sacrificed one week after the second transfer (6-8 weeks after BM transfer). Data were pooled from 3 independent experiments (n=7-10). **(C)** Frequencies and **(D)** numbers of Ametrine+ cells within Lin-Sca-1+ hematopoietic precursors of single TCR Hu-Rg mice that received HA pulsed DC transfer. **(E)** Representative flow plots of Ametrine+ thymocytes. (**F)** Frequencies and numbers of immature Ametrine+MHCI$^{lo/-}$ thymocytes of WT-TCR and 120E-TCR Hu-Rg mice. **(G)** Representative flow plots of activated caspase 3 expression in CD4 SP thymocytes of WT-TCR and 120E-TCR Hu-Rg mice. **(H)** Representative flow plots of thymic Tregs in WT-TCR and 120E-TCR Hu-Rg mice. Gated on Ametrine+CD73-CD4 SP. Data are plotted as mean ± SEM. **, $p \leq 0.01$; *, $p \leq 0.05$; ns > 0.05 by (B-D) Mann-Whitney test and (F) two-way ANOVA and Benjamini, Krieger and Yekutieli's test.

**Supplementary Figure 4. (A-D)** WT-TCR and 120E-TCR expressing Hu-Rg mice were sacrificed for analysis 8 weeks after bone marrow transfer. **(A, B)** Frequencies of Foxp3+ Tregs in CD4+ T cells in spleens of Hu-Rg mice expressing indicated TCRs. **(C-E)** CD4+ T cells in spleens, ndLNs, pLNs and pancreatic islets (Gated on CD4+CD5+CD3+Ametrine+). **(F-I)** Hu-Rg mice received two peptide pulsed DC transfers (7 days apart) and were sacrificed one week after the second transfer (6-8 weeks after bone marrow transfer). Numbers of splenic CD4+ T cells (Ametrine+CD4+CD3+CD5+) in **(F)** WT-TCR and **(G)** 120E-TCR Hu-Rg mice that received DCs pulsed with indicated peptide. **(H, I)** Frequencies of Foxp3+ Tregs among splenic CD4+ T cells. Data are plotted as means ± SEM and are pooled from 2 or 3 independent experiments (n=6-10). **, $p \leq 0.01$; *, $p \leq 0.05$; ns, $p > 0.05$ by Mann-Whitney test (B) and Kruskal-Wallis ANOVA (F, G) or Welch ANOVA (H, I). Outliers were excluded from analysis using ROUT test.

**Supplementary Figure 5.** CD4+ T cells were isolated from spleens of Hu-Rg mice, stimulated with PMA and ionomycin and expanded in vitro for 2 weeks with 1000U/mL hIL-2. Expanded cells were then stimulated with antiCD3 or APC and 10μM indicated peptide for 5 hours in the presence of BFA and monensin. **(A)** Representative flow plots and **(B, C)** summary of Intracellular interferon gamma production. Data are plotted as

means ± SEM pooled from 3 independent experiments (n=5-11). **, $p \leqslant 0.01$; *, $p \leqslant 0.05$; ns > 0.05 by Welch ANOVA followed by Dunnett's test.