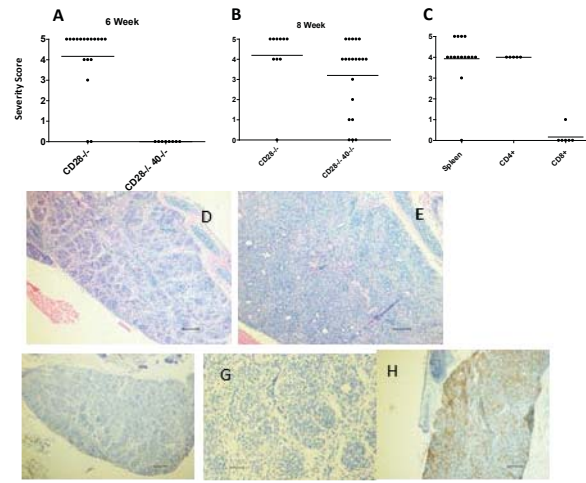


## Supplementary Figure Legends

**Supplementary Figure 1.** Inflammatory cell infiltration in thyroids of IFN- $\gamma$ <sup>-/-</sup> CD28<sup>-/-</sup> mice with severe TEC H/P. A-D, both normal (arrows in C, D) and proliferating TEC in mice with severe TEC H/P are TTF-1 positive, indicating that proliferating cells derive from thyroid epithelial cells. F-H, many infiltrating cells in thyroids with severe TEC H/P are CD3<sup>+</sup> T cells, including both CD8<sup>+</sup> (I, J) and CD4<sup>+</sup> (K, L) T cells. CD8<sup>+</sup> T cells are more numerous than CD4<sup>+</sup> T cells. E, negative control (no primary Ab) for CD3 staining. Thyroids of mice with mild or no TEC H/P express almost no CD40 (M), whereas thyrocytes of mice with severe TEC H/P highly express CD40 (N, O, P). M, N, O: paraffin sections stained with sc975. P, frozen section stained with 1C10 as in earlier studies (19). Images are representative examples of at least 6 thyroids examined for each group. A-H and M-O are paraffin sections; I-L and P are frozen sections. Magnification: 100X (A, B, E-G, I, K and N-P or 400X (C, D, H, J, L, M).



**Supplementary Figure 2.** CD40 is not required for development of severe TEC H/P in CD28<sup>-/-</sup> mice. A, B, IFN- $\gamma$ <sup>-/-</sup>CD40<sup>-/-</sup>CD28<sup>-/-</sup> or IFN- $\gamma$ <sup>-/-</sup> CD28<sup>-/-</sup> (CD40-positive) NOD.H-2h4 mice, 8 wk of age, both sexes, were given NaI water for 6 (A) or 8 (B) wk before thyroids were removed. CD40<sup>-/-</sup> mice did not develop TEC H/P after 6 wk on NaI water (A), but after 8 wk on NaI water, many of them had severe (4-5+) TEC H/P (B). TEC H/P severity at 6 wk was significantly reduced compared to sex and age matched CD28<sup>-/-</sup> CD40-positive mice CD40 (p < 0.0001), while severity scores at 8 wk were not significantly different from those of CD40-positive mice (p = 0.08). A, N=18 for CD40-positive mice and N=8 for CD40<sup>-/-</sup> mice. B, N=10 for CD40-

positive and N= 20 for CD40<sup>-/-</sup> mice. C, Splenocytes from CD28<sup>-/-</sup>CD40<sup>-/-</sup> mice with severe TEC H/P were cultured, separated into CD4<sup>+</sup> or CD8<sup>+</sup> subsets using magnetic beads as described in Methods and transferred to SCID recipients (recipients were not CD40-deficient). Thyroids were removed 28 days later. CD4<sup>+</sup> T cells were sufficient for development of severe TEC H/P; CD8<sup>+</sup> T cells did not transfer TEC H/P. N= 12 (spleen), 5 (CD4<sup>+</sup>) and 6 (CD8<sup>+</sup>). D, E, H&E stained thyroid sections from CD28<sup>-/-</sup>CD40<sup>-/-</sup> mice with severe TEC H/P. F, G, CD40 stained thyroid from a CD28<sup>-/-</sup>CD40<sup>-/-</sup> mouse demonstrating absence of CD40 staining. H, CD3 stained thyroid from a CD28<sup>-/-</sup> CD40<sup>-/-</sup> mouse with severe TEC H/P. Thyroids in F, G and H were stained at the same time as thyroids in Fig. 3 E and F and M, N, O. Paraffin sections. Magnification: D-F and H, X100; G, X400.