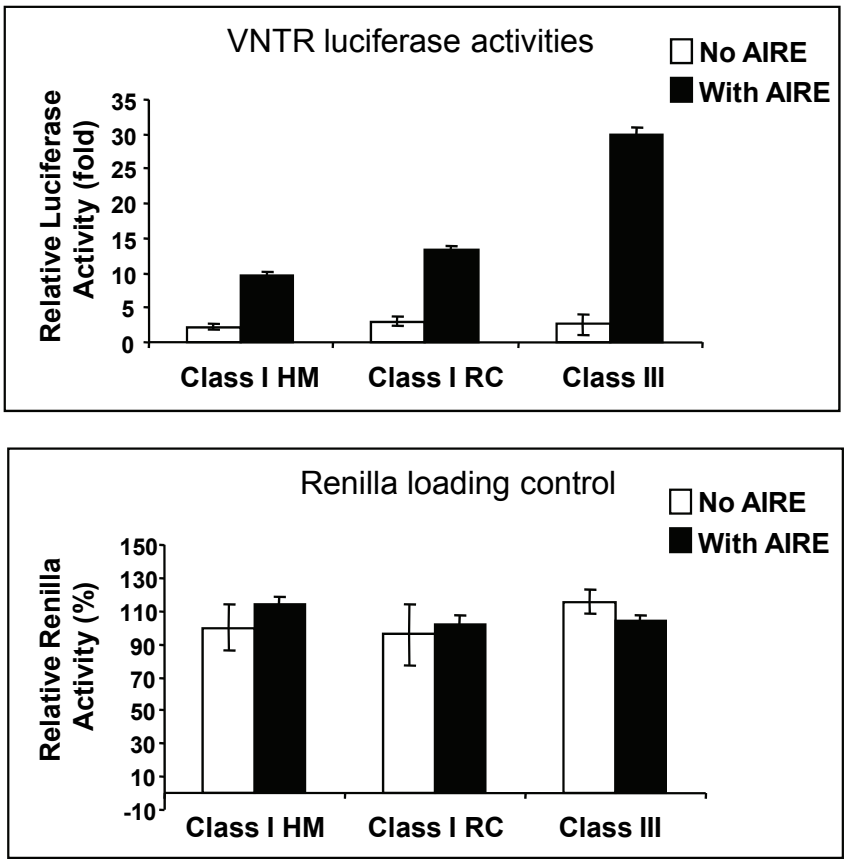
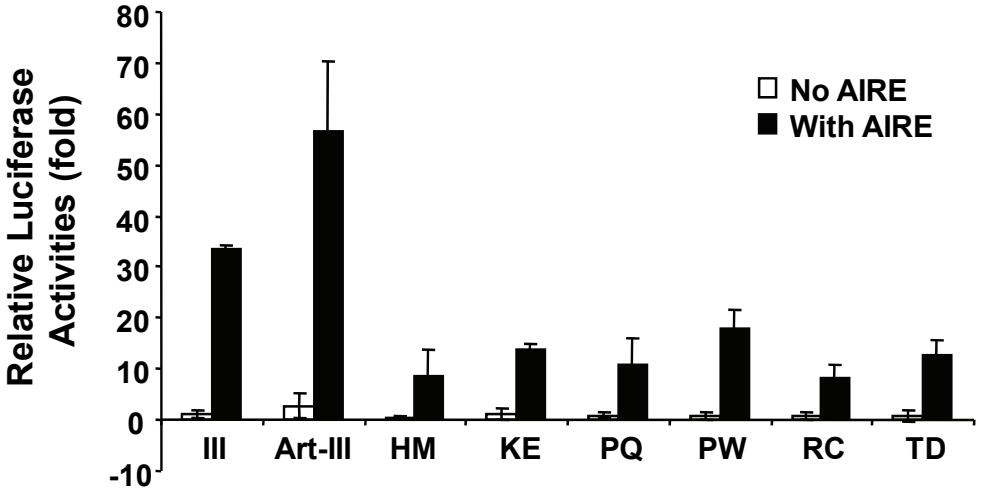
Supplemental Figure 1. Dual reporter system for transient transfection assay. The null-Renilla reporter gene was inserted downstream of the class I (HM and RC) or class III pGL3 luciferase-reporter construct. Equal molar of DNA was transfected into hTEC cells in the presence of AIRE or β-gal expression vector. Post-transfected cells were subjected to luciferase and renilla activity measurements. The relative luciferase or renilla activity was presented from average of three experiments. The relative Renilla activity uses class I HM (no AIRE) as 100% and the other activities are presented as relative to class I HM (no AIRE).



Supplemental Figure 2. Comparison of artificial class III (Art-III) against the natural class I and III VNTRs. An Art-III was constructed by adding four different class I VNTR sequences 5'-upstream of the human insulin basal promoter in the pGL3 basic vector sequentially. An equal molar of reporter vector (~50 ng) was transfected into hTEC with or without AIRE (50 ng) with a null-Renilla internal control (2 ng). The relative luciferase activities are shown by the normalization with renilla activities



Supplemental Figure 3 Additional VNTRs responded to AIRE in a human thymic epithelial cell line. Two class I VNTRs (HM and RC), a natural class III, and an Art-III VNTR reporter construct (50 ng) were co-transfected with AIRE expression vector or β-gal control vector into a hTEC line. The amount of AIRE expression vector (ng) added to each sample of a 24-well plate format is indicated.