**Supplemental Figure Description**

Supplemental Figure 1. **ER stress chaperone grp78 is upregulated in response to TCR stimulation** (A) Quantitative RT-PCR analysis of grp786 expression in naive CD4+ or (B) CD8+ T cells isolated from WT spleens stimulated with CD3/28 for 0, 6, or 18 hours. Data points represent separate mice. Data are mean ± SEM. \**P* <0.05, \*\**P* < 0.01, \*\*\**P* <0.001*, \*\*\*\*P* <0.0001, 2-tailed Student’s *t* test.

Supplemental Figure 2. **Efficient deletion of gp96 in CD4*creHsp90b1flox/flox* mice and assessment of TCRv chains** (A) Quantitative RT-PCR analysis of gp96 expression and (B) western blot analysis of gp96 protein expression in naive CD4+ thymocytes or splenocytes from CD4*cre+* heterozygotes *(f/+)* or homozygotes *(f/f) mice*. Data are mean ± SEM and data points represent individual mice. Western blot was repeated in samples from 3 mice. \*\**P* < 0.01, 2-tailed Student’s *t* test. (C) FACS analysis gated on CD4+/Foxp3- splenic or thymic populations from WT or 96KO mice. Graphs represent two mice per group.

Supplemental Figure 3. C**haracterization of WT contaminant cells in CD4*creHsp90b1flox/flox* mice** (A) Absolute number quantification of CD4+ or CD8+ splenocytes from WT or 96KO mice. Data are mean ± SD of 7 mice per group. \*\*\*\**P* < 0.0001, 2-tailed Student’s *t* test. (B) Representative FACS plots gated on CD4+ T cells for CD18+/CD44+ T cell subsets from WT or 96KO mice. Similar data were obtained for 7 mice or more per group.

Supplemental Figure 4. **Transfer of CD4+ T cells from CD4*creHsp90b1flox/flox* mice to immuno-deficient hosts indicates activation defect**. 2x106 CD4+ T regulatory cell (Treg) depleted WT or 96KO T cells were isolated, labeled with CFSE (2 M), and i.v. transferred to irradiated (5 Gy) *Rag1*-/- mice. 14 days post transfer recipients were sacrificed. (A) Representative histogram data of CFSE divisions of CD4+ T cells from WT or 96KO mice. (B) Quantification of mean florescent intensity (MFI) of CFSE gated on CD4+ population. (C) Representative FACS plot gated on CD4+ T cells from WT or 96KO mice. (D) Quantification of mean florescent intensity (MFI) of CFSE gated on CD4+/CD62L+ population. Data are mean ± SD of 3 mice per group. \*\**P* < 0.001, 2-tailed Student’s *t* test.

Supplemental Figure 5. C**ytosolic Ca2+ levels discriminate CD8+ T cell population.** (A) Quantification of WT CD8+ gated splenocytes differentiated by CD62L/CD44 and probed with cytosolic Ca2+ dye Fluo-4. Data are means from 4 mice. \*\**P* < 0.01, 2-tailed Student’s *t* test. (B) OT-1 TCR transgenic CD8+ T cells were peptide activated for 3 days and cells further differentiated with lineage specific cytokines (IL-2 or IL-15) for 4 more days. Cytosolic Ca2+ content was assessed as in A. Experiment was performed twice and data represent biological triplicates. \**P* < 0.05, 2-tailed Student’s *t* test.

Supplemental Figure 6. **Cytosolic Ca2+ levels discriminate among human CD4+ T cell populations.** Peripheral blood mononuclear cells from 4 patients undergoing routine surgery were differentiated by CD4/CD62L/CD45RO and probed with Ca2+ dye Fluo-4. Data are mean ± SEM of individual patient values normalized to naïve CD4+ T cell population. \**P* < 0.05, \*\**P* < 0.01, 2-tailed Student’s *t* test.