

Supplemental Figure Legends

Supplemental Figure 1. Overall CD4 T cell numbers were decreased in flight mice and numbers of transferred CD45.2⁺CD4⁺ T cells remained higher compared to ground mice after normalization for baseline CD4 T cell numbers. (A) Splenocytes from flight and ground mice were analyzed by flow cytometry for lymphocyte and T cell subsets. T cells were identified as CD3⁺B220⁻, B cells identified as CD3⁻B220⁺, CD4 T cells as CD3⁺CD4⁺, and CD8 T cells as CD3⁺CD4⁻. Ground mice n=8 and flight mice n=7. (B) CD45.2⁺CD4⁺ T cell numbers from flight and ground mice were normalized to total CD4 T cell numbers in each corresponding mouse. All groups n=4, except for Flight Control n=3. Error bars represent standard error. * p<0.05, ** p<0.01 by two-tailed Student's t-test.

Supplemental Figure 2. Fas/Fas ligand expression and activation-induced cell death (AICD) remained intact in mice exposed to spaceflight. (A) Quantitative real-time reverse transcription polymerase chain reaction analysis of Fas and Fas ligand (FasL) expression in freshly collected splenocytes. (B) AICD as measured by DNA fragmentation assay of splenocytes restimulated *in vitro* with OVA peptide for 3 days. All groups n=4, except for Flight Control n=3. Error bars represent standard error. * p<0.05 by two-tailed Student's t-test.

Supplemental Figure 3. Cultures from ground and flight mice produced similar cytokine levels in response to mitogen stimulation. Splenocytes were stimulated with ConA and anti-CD28 for 2 days and multiplex assay was used to measure production of pro-inflammatory (IL-1 β , IL-6, TNF α , and GM-CSF) and T cell subset cytokines (Th1 represented by IFN γ , Th2 represented by IL-4, Th17 represented by IL-17, and Tr1 cells represented by IL-10). All groups n=4, except for Flight Control n=3. Error bars represent standard error.