

Supplementary Figure 1. Dixit et al



Supplementary Figure 2. Dixit et al.



Supplementary Figure 3 Dixit et al



Supplementary Figure 4 Dixit et al.





Ghrelin +1+ Ghrelin -1-

0

GHS-R+IK GHS-R-I

Supplementary Figure 1. (**A**) Thymocyte subsets populations were sorted and real time PCR analysis revealed both ghrelin and GHSR mRNA expression in all of the cells examined. The Ct values from 4 repeats were collapsed and normalized to GAPDH and expressed as average fold change. (B) Ghrelin infusion results in an increase in the GH immunopositivity in the pituitary glands of 14-month old mice. (C) Similar to aged BALB/c mice, 2 week long ghrelin infusion into aged C57BL/6 mice doubled the number of thymocyte in the thymi. (**D**) The increased in thymic cellularity of ghrelin treated mice is not associated with significant changes in the apoptosis in the thymus. TUNEL labeling of frozen thymic sections from 14-month old sham-treated and ghrelin- or des-acyl ghrelin-infused mice revealed no significant differences in cellular apoptosis. Images were acquired on a Zeiss Axiovert fluorescent microscope using SPOT advanced software.

Supplementary Figure 2. Real-time PCR analysis of aire mRNA expression revealed no significant difference between sham and ghrelin infused old mice (n = 4).

Supplementary Figure 3. CDR3 length analysis in peripheral CD4⁺ and CD8⁺ cells derived from 14-month old mice post ghrelin infusions. Ghrelin significantly improved the TCR diversity in V_8 family along with modest effects on V_7, V_19 and the V_20 regions. Note that ghrelin infusion also altered relatively polyclonal TCR repertoire of V_11 to a more oligoclonal profile and skewed the V_3.1 profile in CD8⁺ T cells, while similarly improving it in CD4 cells.

Supplementary Figure 4. TCR spectratyping of total splenic T cells revealed a similar profile of TCR diversity as observed in purified CD4⁺ and CD8⁺ cell. Note that ghrelin also significantly reduced the V_11 diversity compared to control mice.

Supplementary Figure 5. (A) The 12m old GHS-R ^{-/-} mice display a reduction in CD3+ T cells in peripheral blood. (B) The ghrelin-deficient mice do not show perturbation in T cell development and DN stages. (C) Both ghrelin and GHS-R knockout mice display reduction in LSK cell in the bone marrow. 24m old mice were utilized in these studies and the data is represented at mean \pm SEM of Ghrelin^{-/-} (n = 5),GHS-R ^{-/-} (n = 5) and four wild type littermates of each genotype.