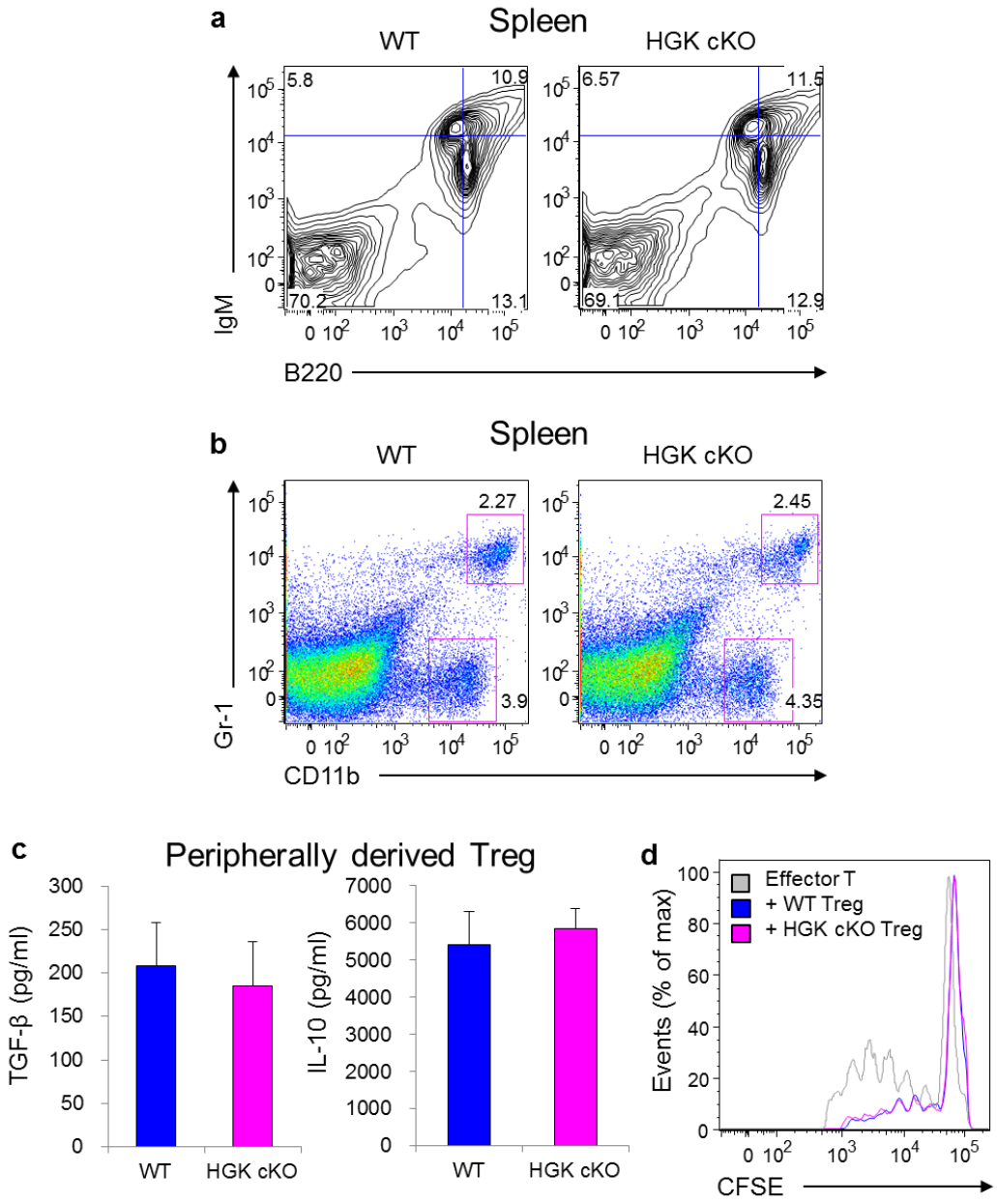
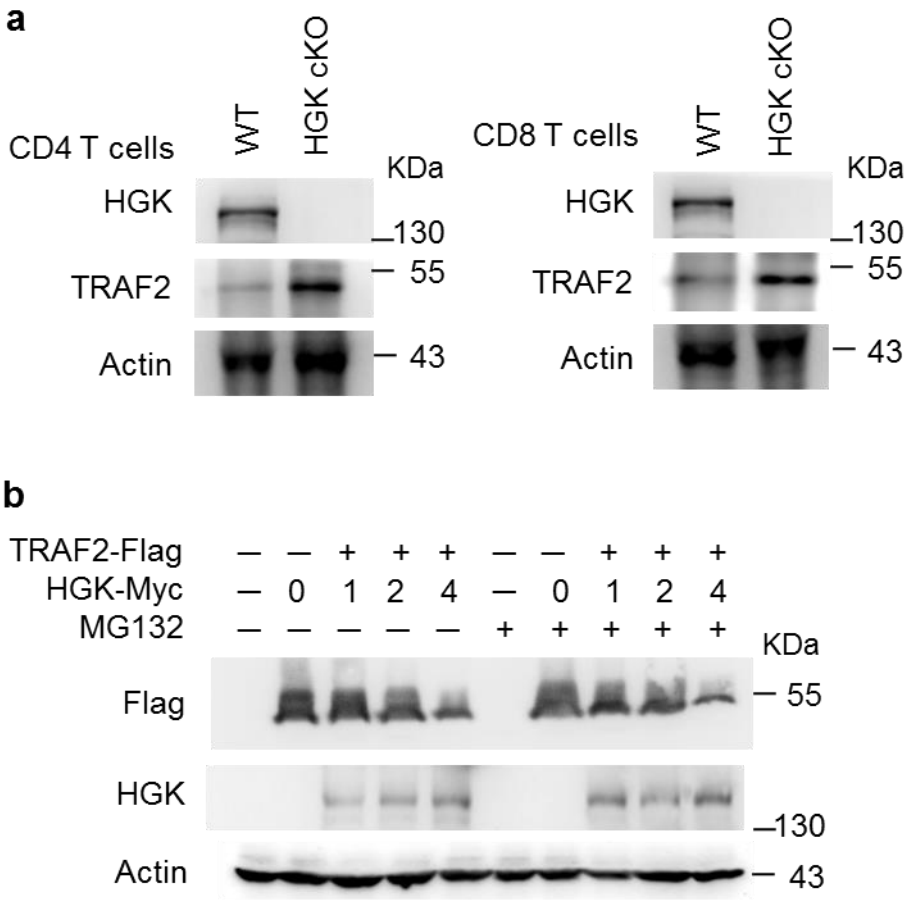
Supplementary Figures

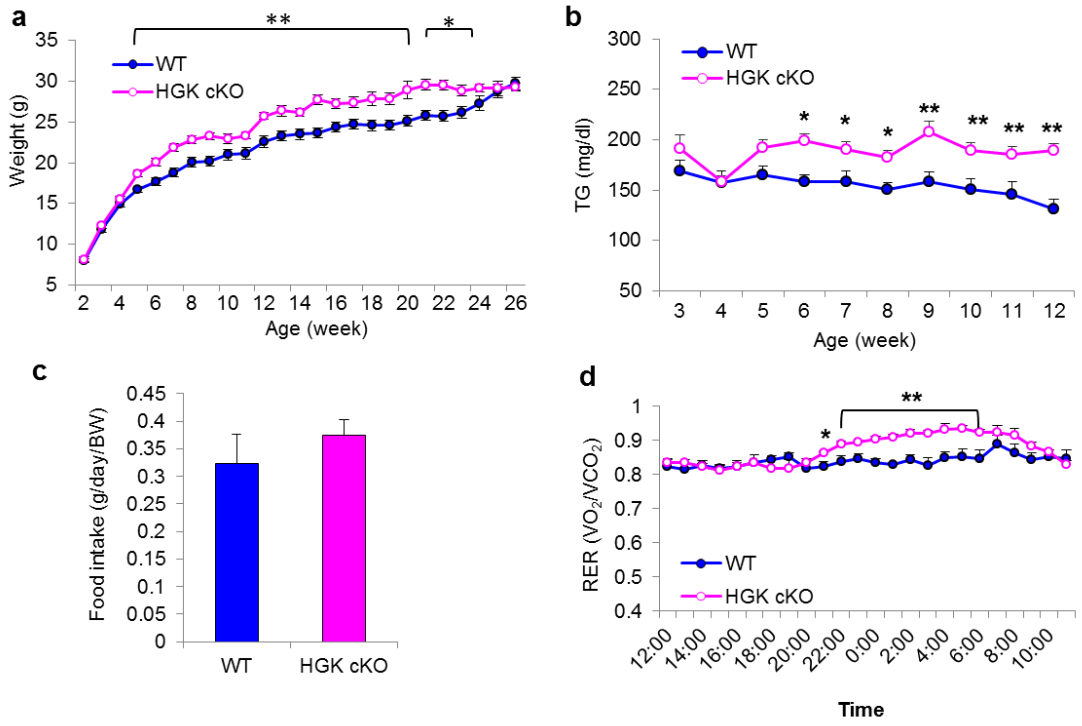


Supplementary Figure 1. Normal B-cell or monocyte development and normal Treg function in T-HGK cKO mice. (**a** and **b**) Flow cytometry analyses of $B220^{+}$ B cells (a), $CD11b^{+}Gr\text{-}1^{high}$ neutrophils, and $CD11b^{+}Gr\text{-}1^{low}$ macrophages (b) from the spleen of wild-type (WT) and T-HGK cKO (HGK cKO) mice. Data shown are

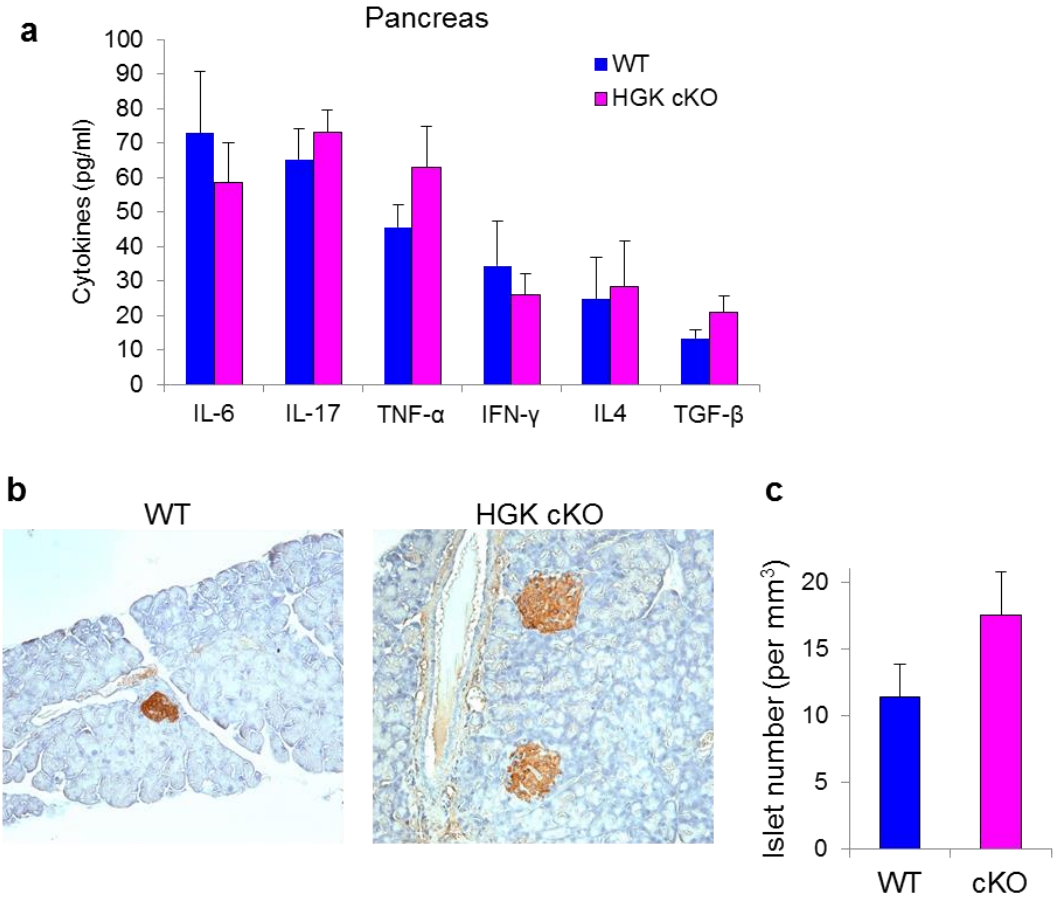
representatives of three independent experiments. (**c**) The TGF-β and IL-10 levels in the supernatants of anti-CD3 stimulated peripherally derived Treg cells from the spleen of WT and T-HGK cKO (HGK cKO) mice were determined by ELISA assays. n = 6 per group. Means ± SEM are shown. (**d**) Suppression of CFSE-labeled effector T cells by WT or HGK cKO Treg cells, presented as CFSE dilution in responding T cells cultured at a ratio of 3:1 with Treg cells plus anti-CD3-coated beads. Numbers in or adjacent to outlined areas indicate percent cells in each throughout.



Supplementary Figure 2. HGK-induced TRAF2 degradation is not mediated through a proteasome-independent pathway. (**a**) The protein levels of HGK, TRAF2, and actin in splenic $CD4^+$ or $CD8^+$ T cells of wild-type (WT) and T-HGK cKO (HGK cKO) mice were determined by immunoblotting analyses. (**b)** Immunoblotting analyses of indicated molecules in lysates of HEK293T cells transfected with TRAF2 (2 μg) and/or HGK (various amounts) and treated with the proteasome inhibitor MG132 (25 μM).

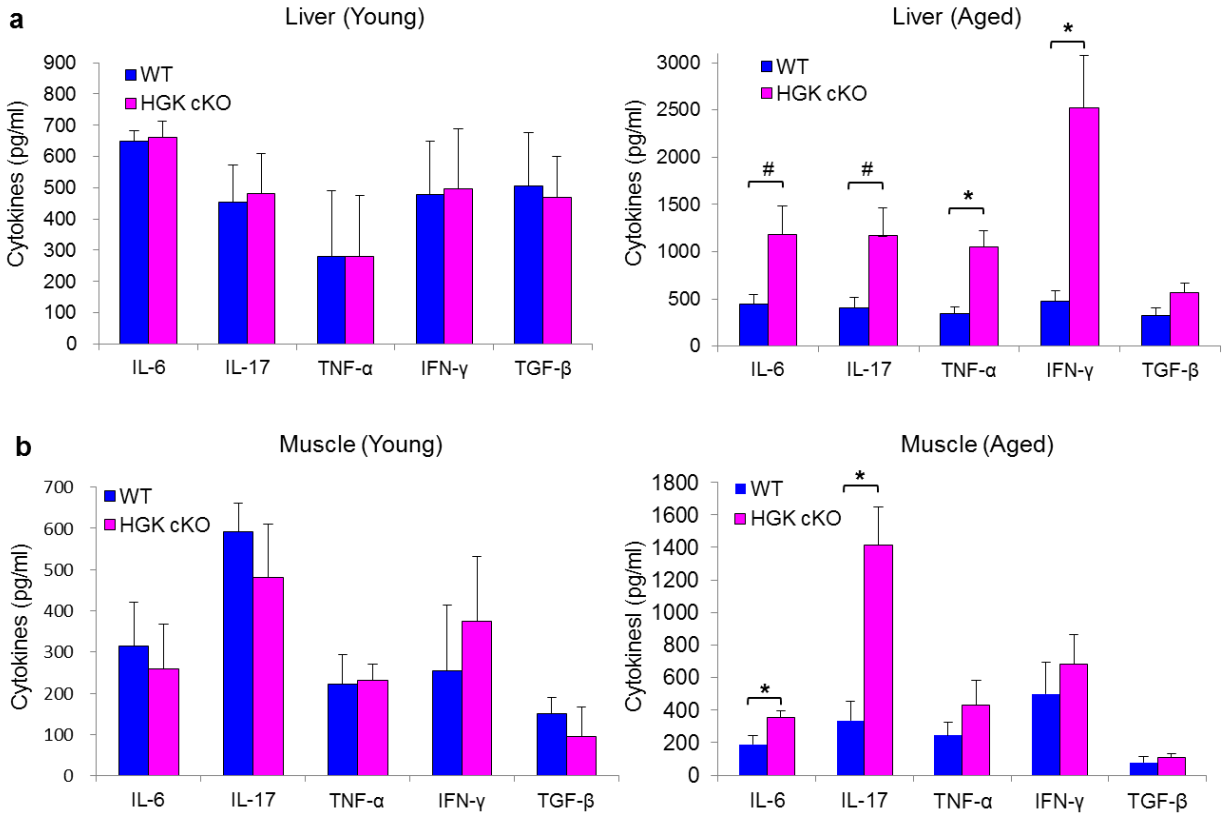


Supplementary Figure 3. (**a**) Body weights of WT and T-HGK cKO mice. n = 17–32. Means ± SEM are shown. n = 18-23 per group. WT, littermate controls ($HGK^{f/f}$ mice or CD4-Cre mice); HGK cKO, T-HGK cKO mice. (**b**) Fasting triglyceride (TG) levels in the sera of WT and T-HGK cKO mice. n = 18-23 per group. (**c**) Food intake over 24 h normalized to body weight (BW) of in 20-week-old mice. n = 4 per group. (**d**) Respiratory exchange ratios (RERs) were measured during the course of the day. VO_2, oxygen consumption; VCO_2, carbon dioxide production. n = 4 per group.*, *P* value < 0.05; **, *P* value < 0.01.

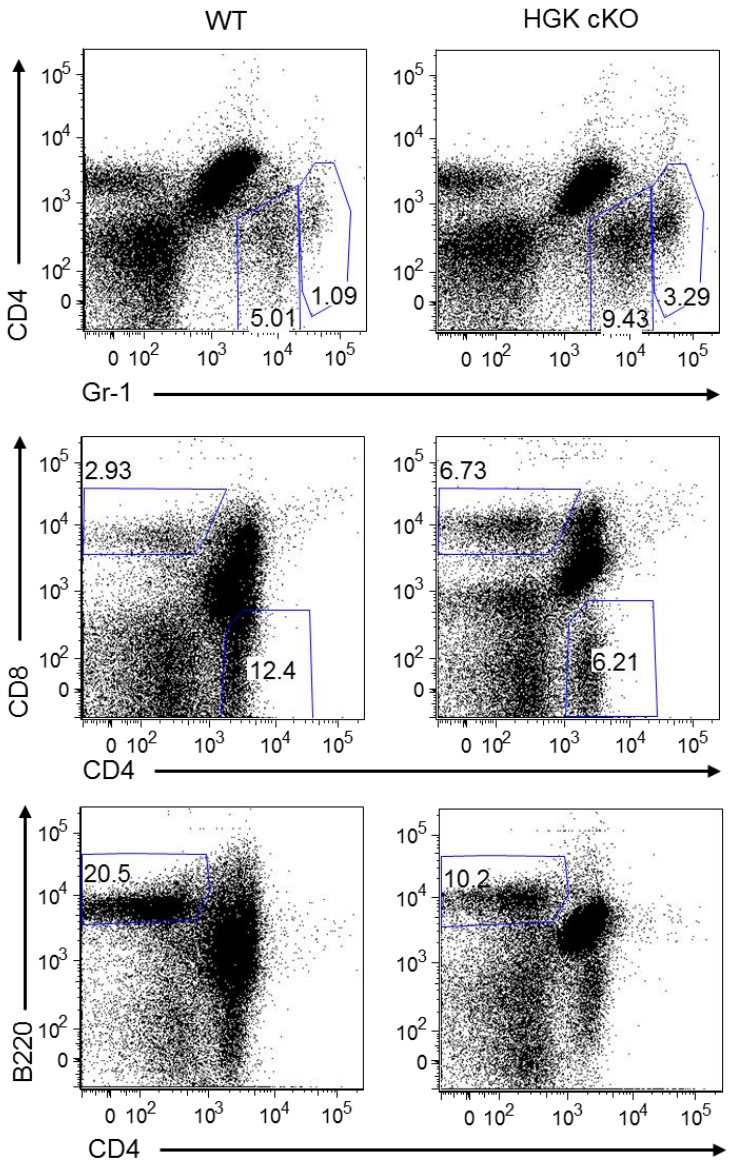


Supplementary Figure 4. Normal function of the pancreas in T-HGK cKO mice.

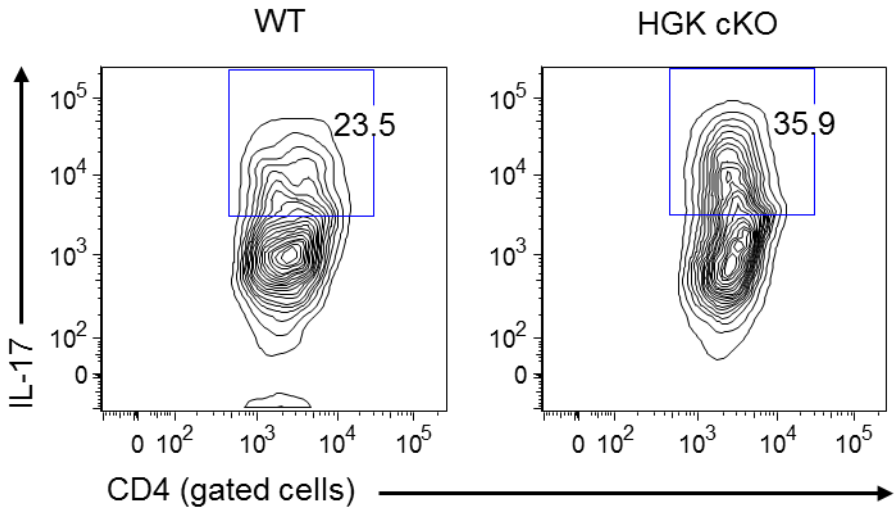
(**a**) The levels of indicated cytokines from the pancreas of wild-type (WT) or T-HGK cKO (HGK cKO) mice were determined by ELISA assays. WT, n = 4; T-HGK cKO, n = 3. (**b**) Representative anti-insulin-stained sections of the pancreas from wild-type (WT) or T-HGK cKO (HGK cKO) mice. (**c**) Statistical analyses of the islet number from (b). WT, n = 6; T-HGK cKO, n = 5. Means ± SEM are shown. WT, littermate controls ($HGK^{f/f}$ mice); HGK cKO, T-HGK cKO mice.



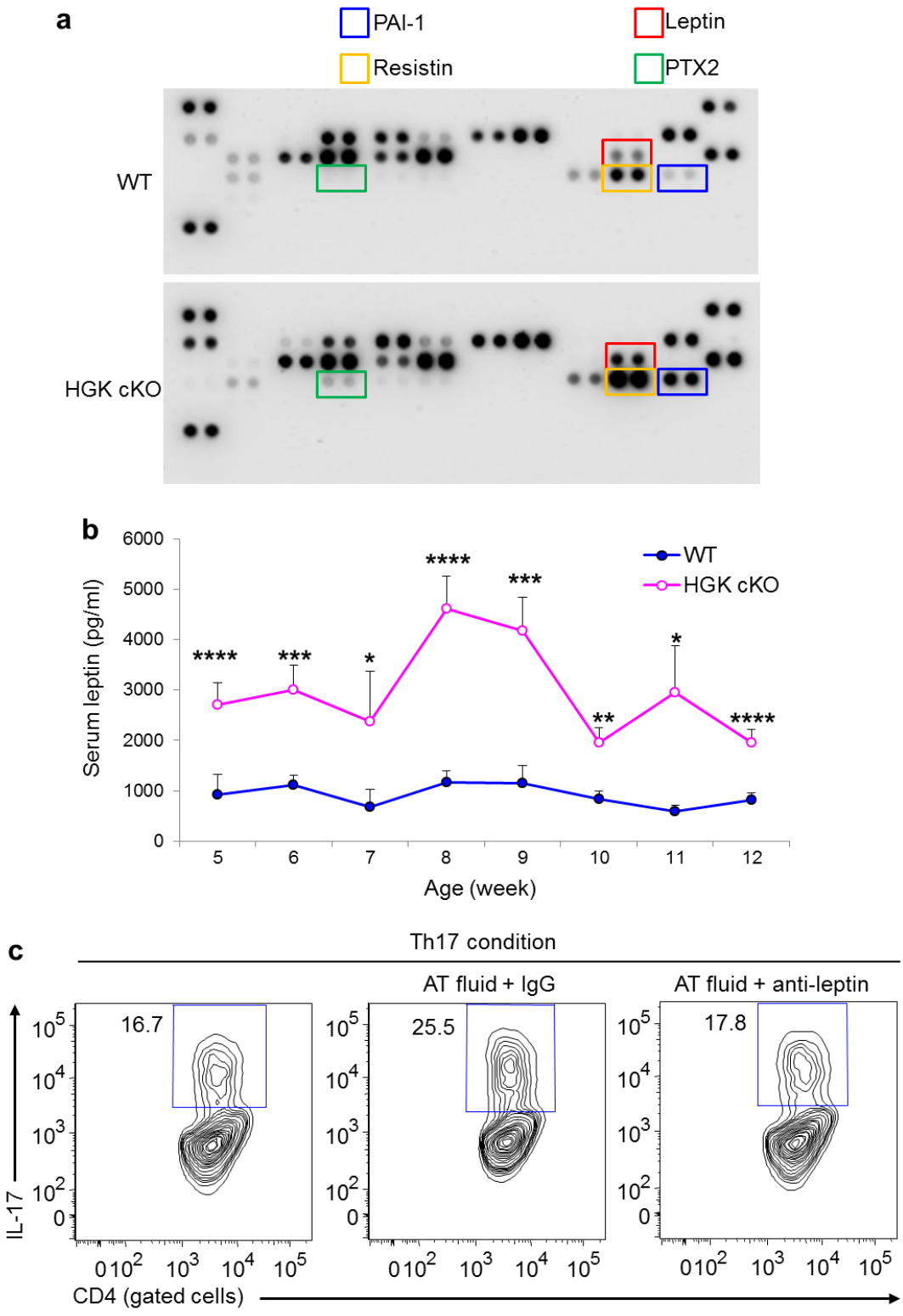
Supplementary Figure 5. Increased inflammatory cytokine levels in the liver of aged but not young T-HGK cKO mice. (**a** and **b**) The levels of indicated cytokines from the liver (a) or muscle (b) of young (5-7-week old; left panel) or aged (17-week old, right panel) wild-type (WT) and T-HGK cKO (HGK cKO) mice were determined by ELISA assays. n = 4 per group. *, *P* value < 0.05 (two-tailed Student's t-test); #, *P* value < 0.05 (one-tailed Student's t-test).



Supplementary Figure 6. Infiltrating immune cells in the liver of T-HGK cKO mice. Cells were isolated from the liver of 20-week-old wild-type (WT) or T-HGK (HGK cKO) cKO mice. Cells (without stimulation) were stained for expression of CD45, CD4, CD8, B220, or Gr-1, and cells were then analyzed by flow cytometry. Representative dot plots show percentages of Gr-1^{high} neutrophils, Gr-1^{low} macrophages, CD4^{+} T cells, CD8^{+} T cells, or B220^{+} B cells from the liver of WT and T-HGK cKO mice.

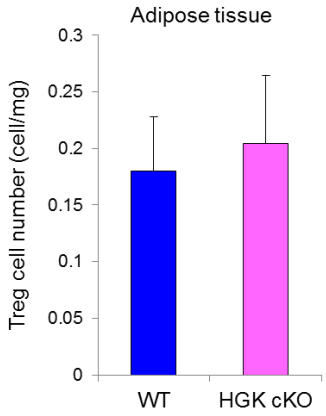


Supplementary Figure 7. Enhanced *in vitro* Th17 differentiation of T cells from T-HGK cKO mice. $CD4^+CD25^-$ T cells were purified from spleens of wild-type (WT) or T-HGK cKO (HGK cKO) mice and cultured for 4 days under Th17 differentiation conditions.



Supplementary Figure 8. Leptin levels are enhanced in T-HGK cKO mice. (**a**) Adipose tissue fluids from WT and T-HGK cKO mice were subjected to the adipokine array analyses. The adipokines whose levels were increased in T-HGK cKO mice are

labeled by colored rectangles. (b) Fasting leptin levels in the sera of WT and T-HGK cKO mice. n = 18-23 per group. Means ± SEM are shown. WT, littermate controls (HGKf/f mice or CD4-Cre mice); HGK cKO, T-HGK cKO mice. *, P value < 0.05; **, P value < 0.01; ***, P value < 0.005; ****, P value < 0.001 (two-tailed Mann-Whitney U-test). (**c**) $CD4^{+}CD25^{-}$ T cells were purified from spleens of wild-type mice and cultured for 4 days under Th17 differentiation conditions in the presence or absence adipose tissue fluids, anti-leptin antibodies (1 μg), and isotype control (IgG).



Supplementary Figure 9. Normal numbers of Treg cells in T-HGK cKO mice.

Determination of the Treg cell number in the fat tissues (per mg) of WT or T-HGK cKO mice by flow cytometry analyses. n = 6 per group.

Figure 1b

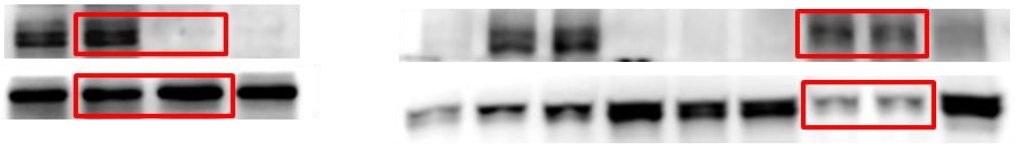


Figure 3a

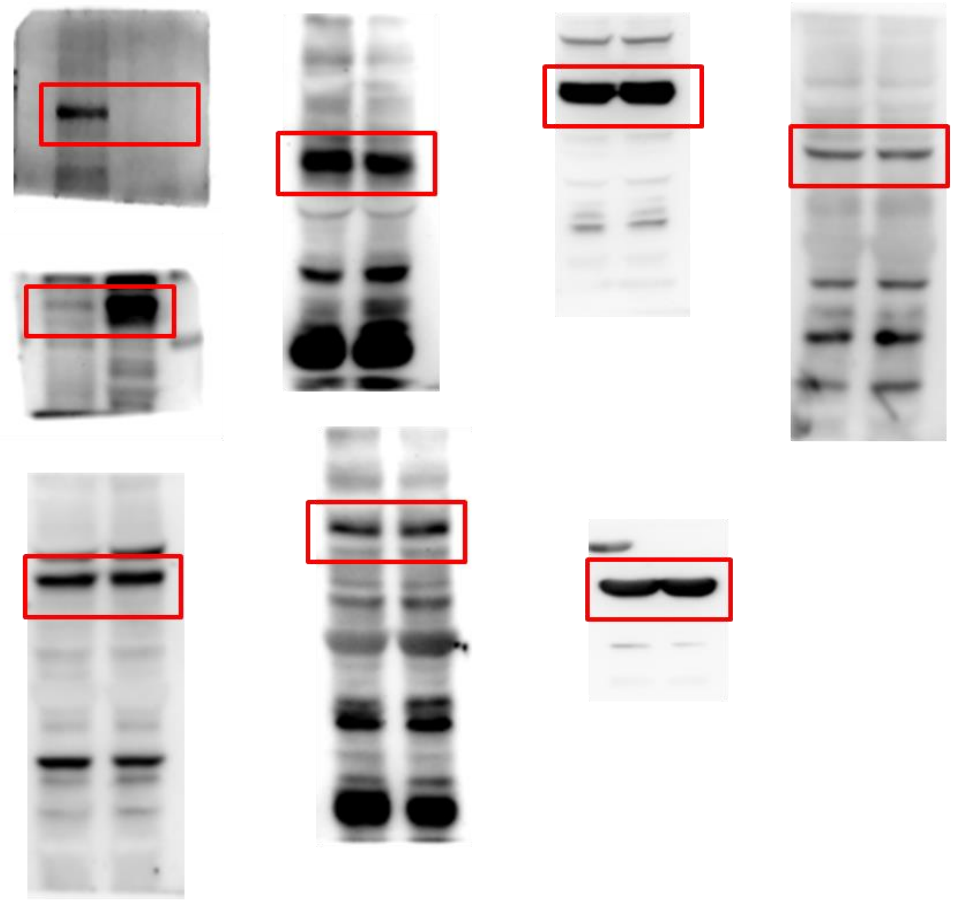
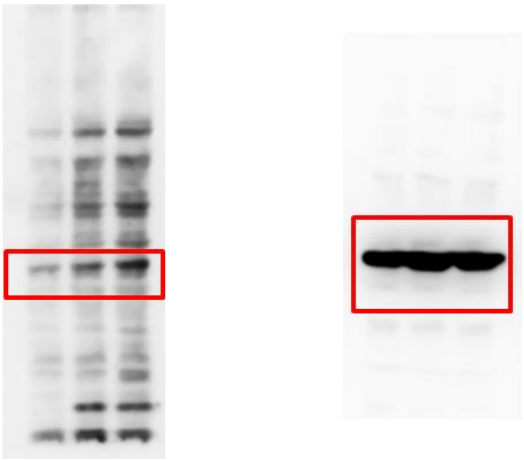


Figure 3c



Supplementary Figure 10. Full immunoblots with indicated areas of selection.

Figure 3d

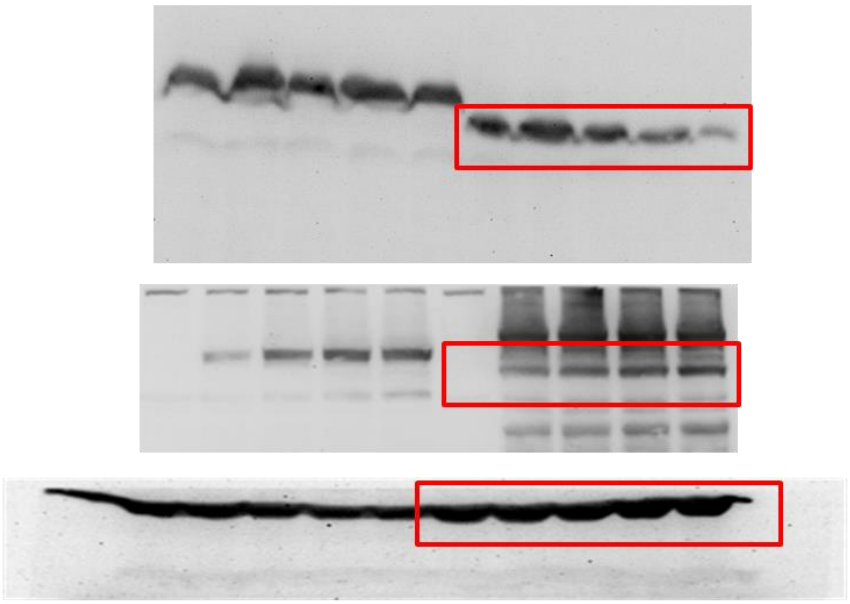
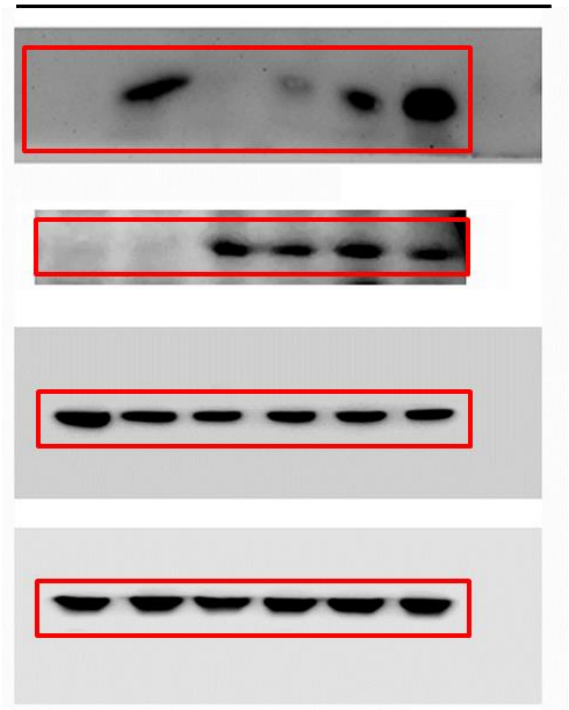
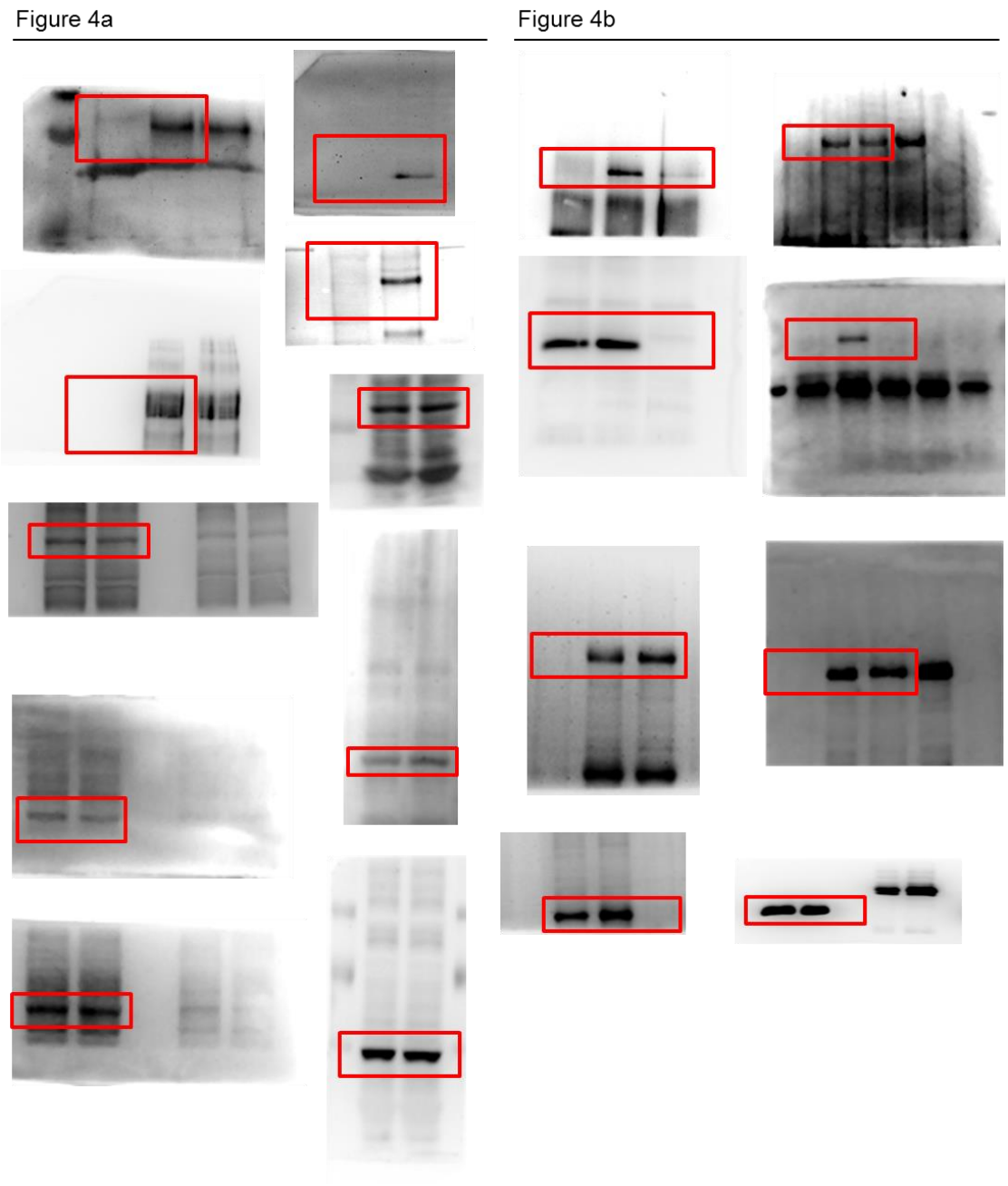


Figure 3d



Supplementary Figure 10. continued.



Supplementary Figure 10. continued.

Figure 4d

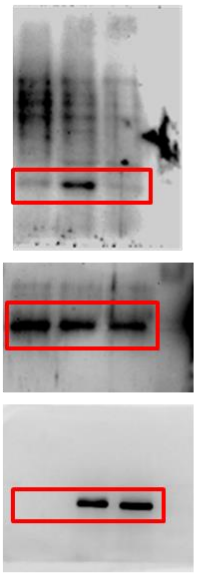


Figure 4f

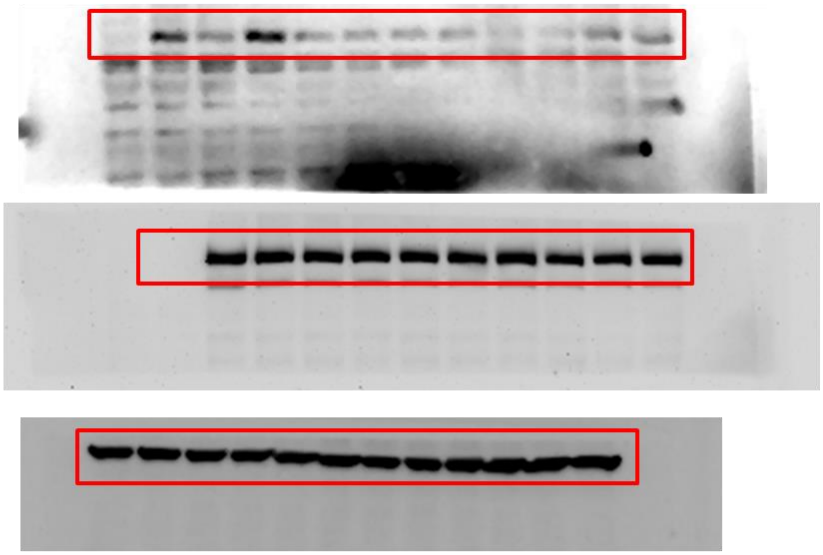
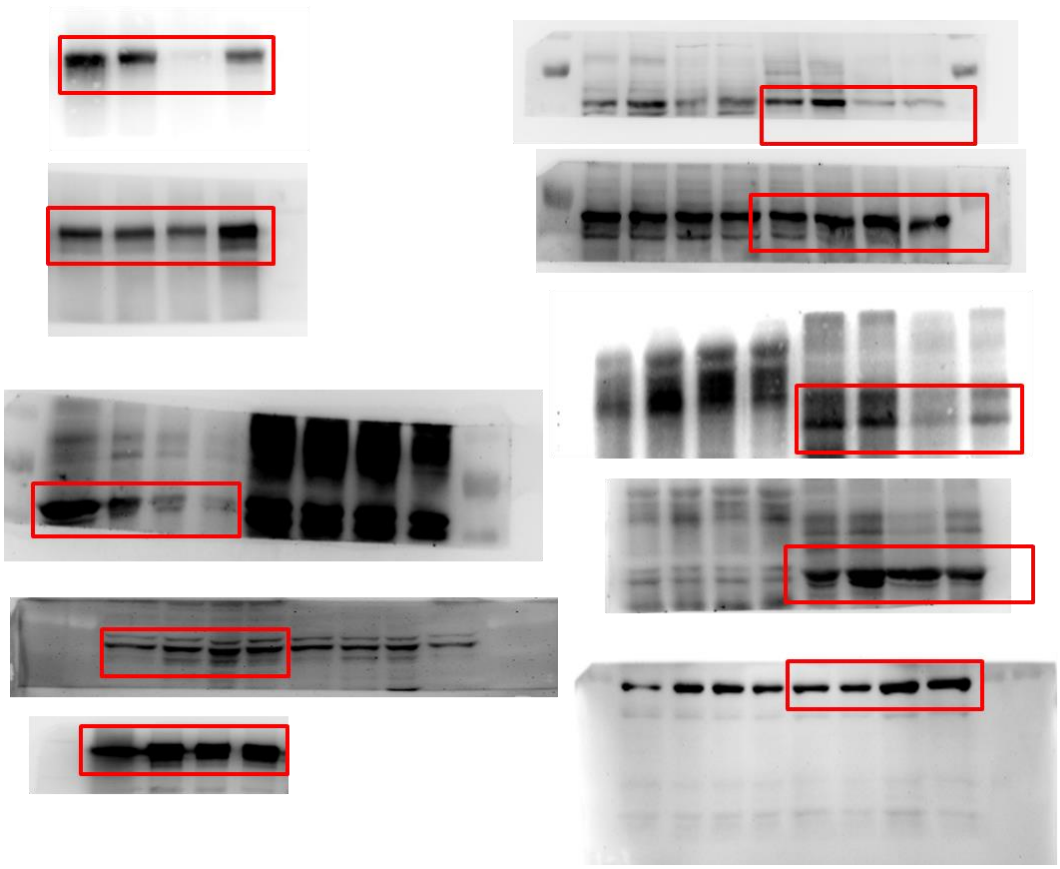


Figure 5e



Supplementary Figure 10. continued.

Figure 5e

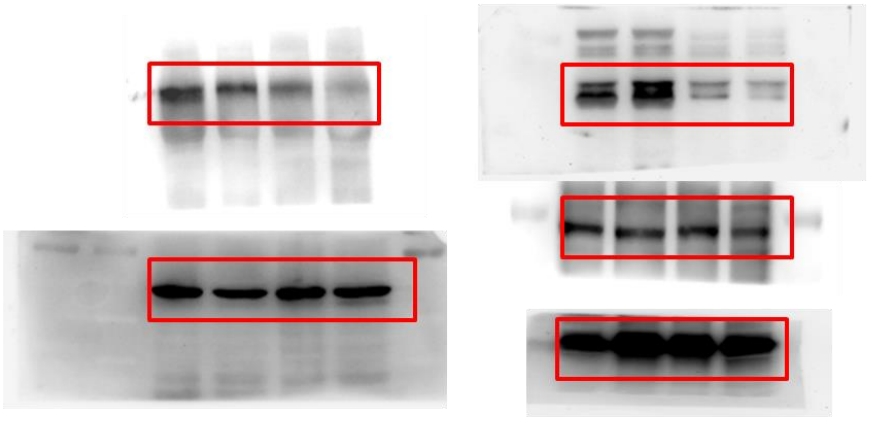
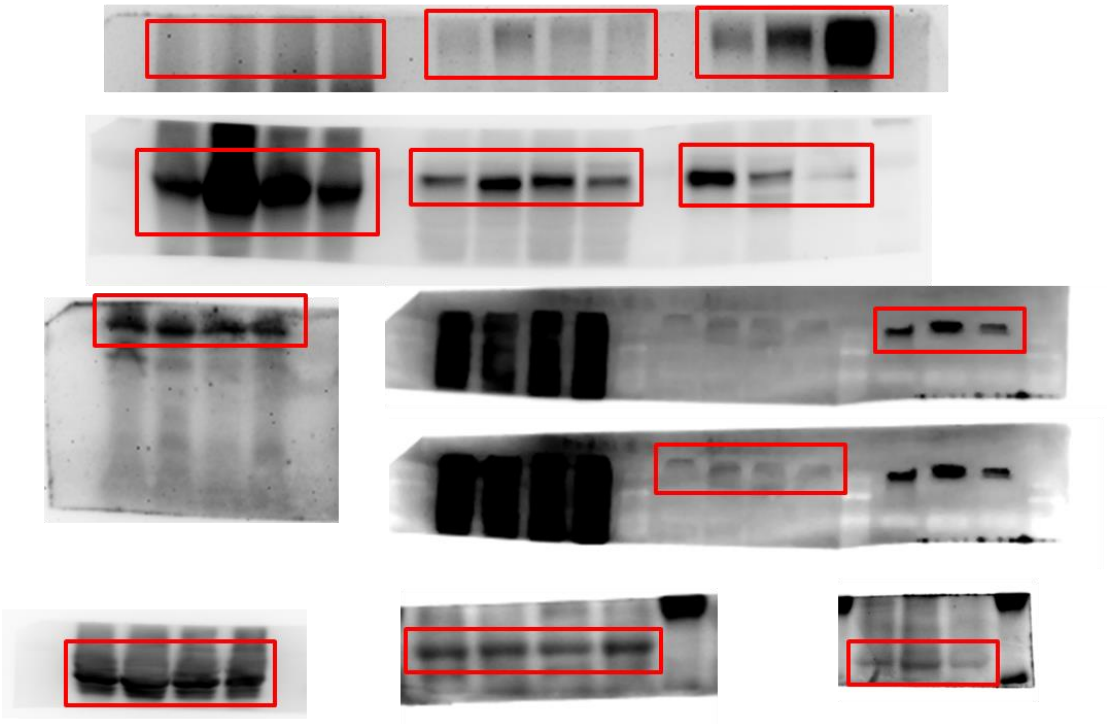
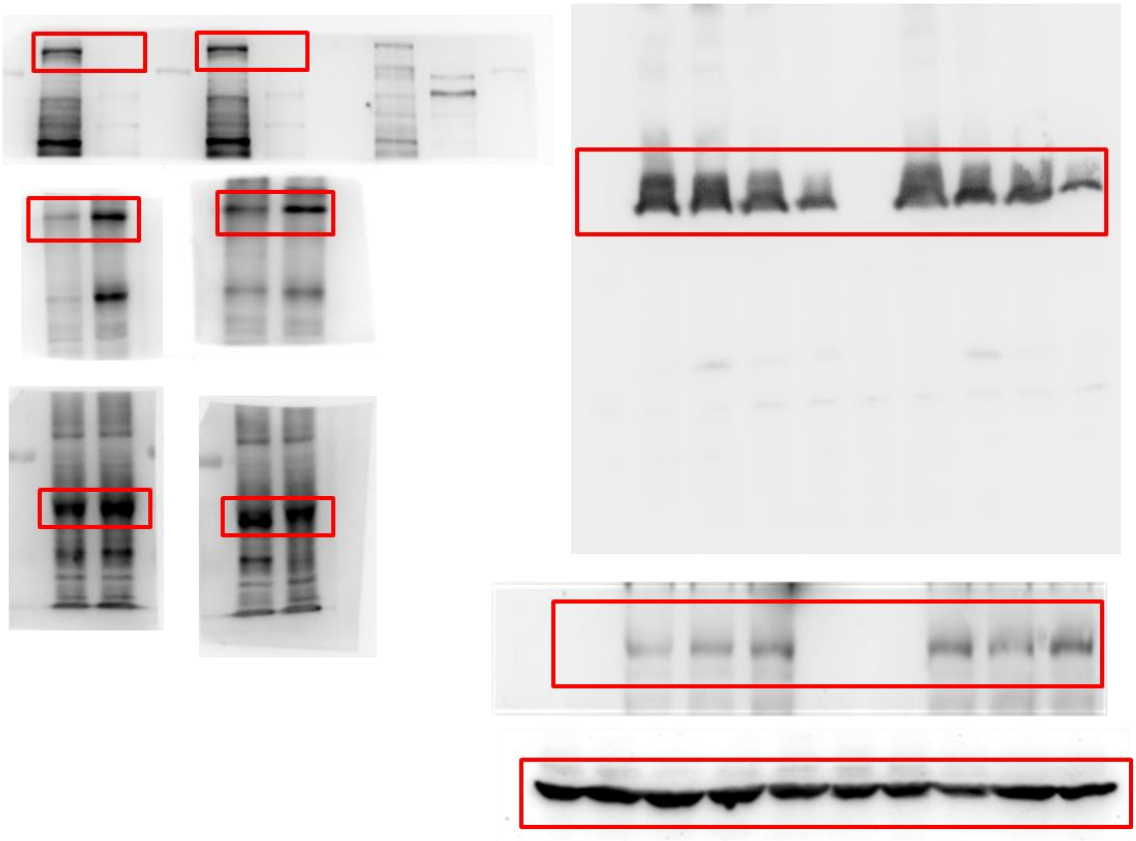


Figure 5f



Supplementary Figure 10. continued.



Supplementary Figure 10. continued.