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Supplemental data:

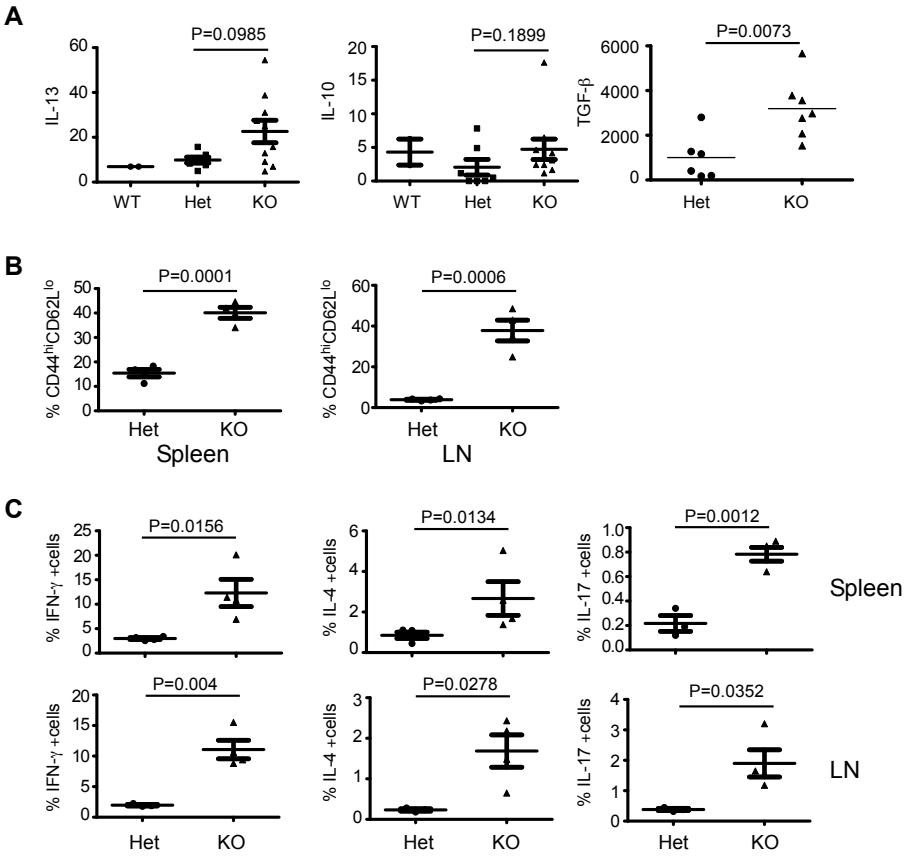
gp96/grp94 Chaperones GARP and Controls Functions of Regulatory T Cells

Yongliang Zhang[1], Bill X Wu[1], Alessandra Metelli[1], Jessica Thaxon[1], Feng Hong[1], Saleh Rachidi[1], Ephraim Ansa-Addo[1], Shaoli Sun[2], Chenthamarakshan Vasu[1], Yi Yang[1], Bei Liu[1] and Zihai Li[1#]

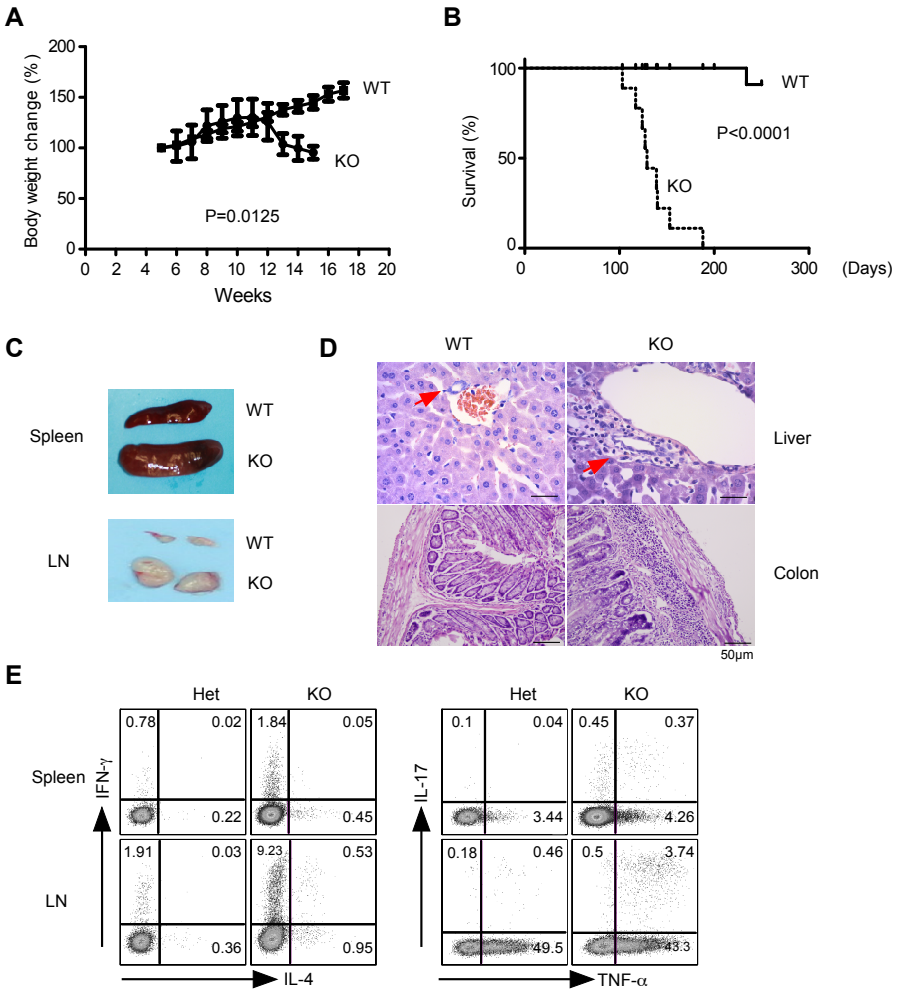
[1]Department of Microbiology & Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA
[2]Department of Pathology & Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425

[#]Corresponding author: Zihai Li, Medical University of South Carolina, 86 Jonathan Lucas Street, Charleston, SC 29425. Phone: 843-792-1034; Fax: 843-792-9588; E-mail: zihai@musc.edu

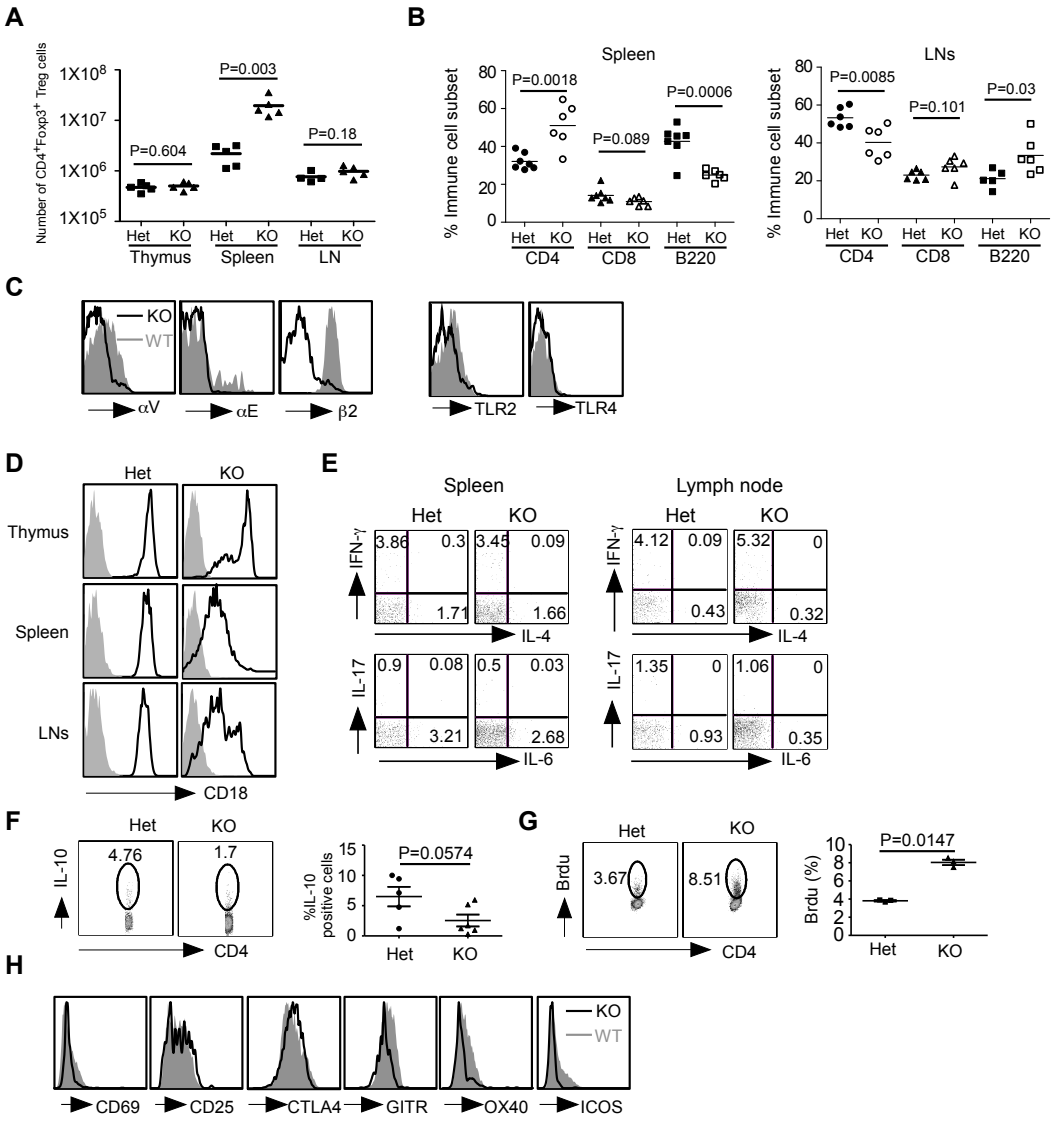
6 Figures



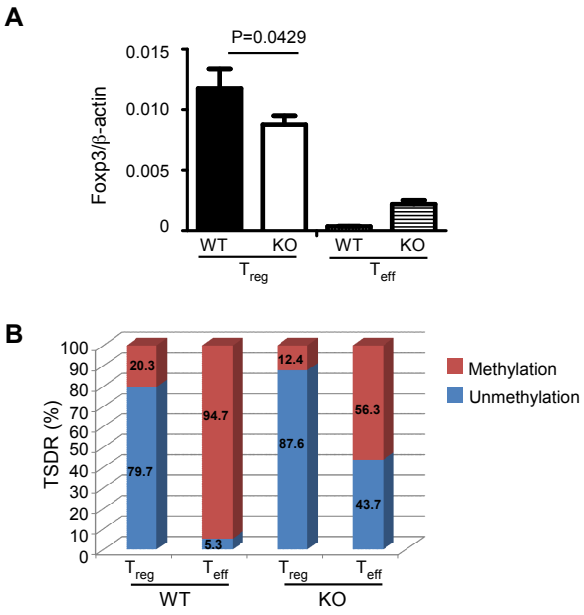
Supplement Figure 1. Uncontrolled systemic inflammatory diseases in *Hsp90b1*$^{flox/flox}$*Foxp3-EGFP*^{cre} mice. (**A**) Serum inflammatory cytokine level (pg/ml) of IL-13, IL-10, TGF-β in WT, Het and KO mice. Each dot represents value from one individual mouse. Bars: means± SEM. (**B**) Statistical analysis of $CD4^+$ cells with high CD44 and low CD62L expression in 6-week-old KO mice and Het littermates. Bars: means±SEM. (**C)** Flow cytometry analysis of intracellular IFN-γ, IL-4, IL-17, IL-6 production, in response to PMA/ionomycin, by splenic $CD4^+$ T cells from KO mice and Het NOD mice. Bars: means±sem. Two-tailed Student's *t* test was used for comparison of experimental groups.



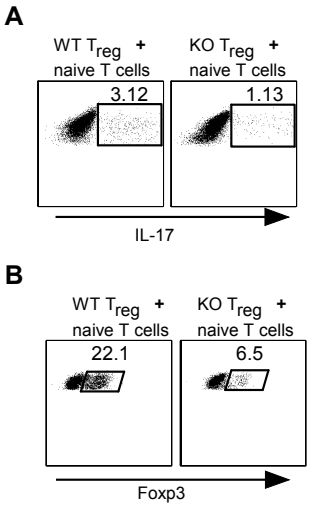
Supplement Figure 2. CD4-cre mediated deletion of gp96 in C57B/L6 mice results in systemic inflammatory diseases. (**A, B**) Rapid body weight loss and death of *Hsp90b*$^{flox/flox}$*CD4*^{cre} mice (n=13) compared with littermate control, *Hsp90b1*$^{flox/wt}$*CD4* ^{cre} (n=11). Bars (A): mean±sem. (**C**) Representative images from multiple mice demonstrate the evidence of splenomegaly and lymphoadenopathy in KO mice. (**D**) Representative images of Haematoxylin and Eosin staining of liver and colon sections from 20-week-old *Hsp90b1*$^{flox/flox}$*CD4*^{cre} and Het littermate. Scale bar: 100 μm (colon), 50 μm (liver). (**E**) Flow cytometry analysis of cytokine production intracellularly by splenic or LN CD4^{+} T cells from KO and Het littermates. Shown are representative data from two independent experiments. Statistic difference of body weight change in (A) was confirmed with two-way ANOVA, and mouse survival differences in (B) were analyzed with log-rank (Mantel-Cox) test.



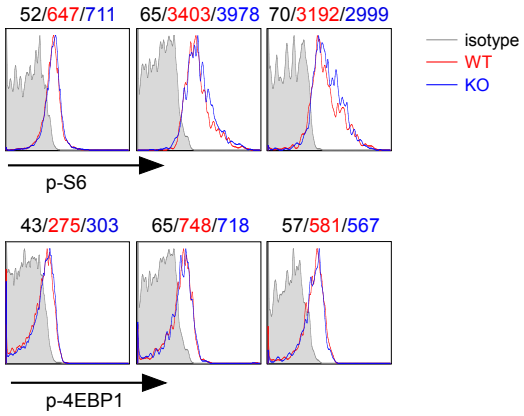
Supplement Figure 3. Characterization of T_{reg} cells and other subsets of immune cells in mice with T_{reg}-specific deletion of gp96. (**A**) Absolute T_{reg} cell number in different immune organs. Bar: mean ± sem. (**B**) Immune cell subset distribution in the spleen and lymph node. Bar: mean ± sem. (**C**) Flow cytometry analysis of indicated cell surface integrins and TLRs of T_{reg} cells from *Hsp90*$^{flox/flox}$ *Foxp3-EGFP*^{cre} mice and WT littermate. Data represent two independent experiments. (**D**) Flow cytometry analysis of CD18 expression by CD4$^+$Foxp3$^+$ cells from thymus, spleen and Lymph nodes. (**E**) Flow cytometry analysis of intracellular IFN-γ, IL-4, IL-17 and IL-6 expression by CD4$^+$Foxp3$^+$ T cells from the spleen and LNs**.** Numbers represent percentage of cells in each quadrant. Data represent three independent experiments. **(F).** Flow cytometry analysis of intracellular IL-10 expression (%) by CD4$^+$Foxp3$^+$ T cells from spleen and LNs. (**G**) Flow cytometric analysis of BrdU incorporation (%) by Het and KO CD4$^+$Foxp3$^+$ T cells from the spleens. (**H**) Flow cytometric analysis of splenic T_{reg} cells for the indicated cell surface markers from bone marrow chimeric mice transplanted with 1:1 mixture of CD45.2$^+$ WT and CD45.1$^+$ KO bone marrows. Data represent three independent experiments. The two-tailed Student's *t* test was used for comparisons between Het and KO mice.



Supplement Figure 4. Quantification of Foxp3 mRNA expression level and *Foxp3* promoter methylation status. $CD4^+CD25^+$ T_{reg} cells and $CD4^+CD25^-$ T effector cells were isolated by MACS beads followed by isolation of mRNA and genomic DNA. (**A**) Quantification of Foxp3 mRNA by q-RT-PCR. Data represent two independent experiments. Two-tailed Student's *t* test were used for comparisons between WT and KO mice. (**B**) DNA demethylation in the TDSR region was quantified with demethylation-specific probe by the Taqman Real time-PCR as described in Materials and Methods. Four independent experiments were performed with similar findings. Data represent mean ± sem (A) and mean (B).



Supplement Figure 5. Compromised activity of gp96 KO T_{reg} cells in driving T_{h17} cells and iT_{reg} cell differentiation *in vitro*. (**A, B**) $CD4^+CD25^+$ T_{reg} cells were activated with plate bound anti-CD3 antibody (2 μg/ml) for 2-3 days, followed by irradiation (2000 cGy) and co-culturing with CFSE-labeled $CD4^+CD25^-$ naïve T cells for 3 days, in the presence of anti-CD3 antibody and IL-2 for T_{reg} cells, and additional IL-6 for T_{h17} cells as described in Materials and Methods. Intracellular staining of IL-17 and Foxp3 were performed, followed by flow cytometry. Data represent more than three independent experiments.



Supplement Figure 6. gp96-deficient T cells exhibit normal mTORC1 activity. Phosphorylation of p-S6 (upper panel) and 4E-BP1 (lower panel) in freshly isolated naïve T cells ($CD4^+CD44^{low}Foxp3^-$, Left), T_{reg} cells ($CD4^+Foxp3^+$, Middle), and memory-phenotype (MP) T cells ($CD4^+CD44^{high}Foxp3^-$, Right). Numbers above the plots indicate mean fluorescent intensity (MFI). Two independent experiments were performed with similar findings.