



Green, C3

(A) Expression of RICTOR, p-AKT(S473), total AKT, p-S6K, pS6RP, p-4EBP1 and actin in flow sorted B220⁺TCRb⁺, B220⁺TCRb⁻, CD4⁺CD25⁺, and CD4⁺CD25⁻ cells was examined by immunoblot. (B) Immunofluorescence staining of IgG (Red) and C3 (Green) on mice kidney sections. Right, the scores of IgG and C3 deposition. * P < 0.05 (one-way ANOVA). Results represent 3 (A), or were pooled from 2 (B), independent experiments. Error bars represent SEM.



(A) Expression of CD38 and IgG1 expression on B220⁺TCRb⁻ B cells. Right, the frequencies of CD38⁺IgG1⁺ B cells (left) and CD38⁺ percentages (right). (NS, not significant; * P < 0.05, ** P < 0.01, **** P < 0.001, one-way ANOVA, Results were pooled from 3 independent experiments. Error bars represent SEM.



(A) Gating strategy for analysis of Tfh cells.(B) Flow cytometry analysis of CXCR5 and PD-1 on B220⁻CD4⁺ T cells. Right, the percentages of PD-1⁺CXCR5⁺ cells. (C) Expression of CXCR5 in Tregs. Right, summary of CXCR5⁺ Tfr cell percentages. (D) Expression of CD25 on FOXP3⁺ Tregs. Numbers indicate the percentages of CD25⁻ Tregs. Right, summary of the percentages of CD25⁻ Tregs. (F) Expression of Tbet and Rorgt on on B220⁻CD4⁺ T cells. Right, summary of Tbet⁺ and Rorgt⁺ cell percentages. Cells were from peripheral lymph nodes (pLNs) of 6 months old B6, Lpr and Lpr.*Rictor* T-KO mice. NS, not significant; ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.001 (one-way ANOVA). Results were pooled from at least 3 independent experiments. Error bars represent SEM.



(A) Expression of *Rictor* in sorted CD25⁺ CD4⁺ T cells from B6, Lpr, and Lpr.*Rictor* Treg-KO mice. (B) Absolute numbers of Tregs in spleens (left) and pLN (right) from B6, Lpr, and Lpr.*Rictor* Treg-KO mice at 6 months old. Data represent 2 (A) or pooled from at least 3 (B) independent experiments.



(A) Flow cytometry analysis of CD44 vs. CD62L in B220⁻CD4⁺ T cells among 2 and 3 months old B6, Lpr and Lpr.*Rictor* T-KO mice, respectively. (B) Summary of cell numbers for B220⁻CD4⁺ T cells with anti-CD3/anti-CD28 activation for 72 hours. Numbers were normalized to B6 control. (C) Flow cytometry analysis of CD73 vs. FR4 expression in B220⁻CD4⁺ T cells among 6 months old B6, Lpr and Lpr.*Rictor* T-KO mice. Right, summaries of frequencies of CD73^{hi}FR4^{hi} populations. (D) Summary of cell numbers for B220⁻CD4⁺ T cells activated with anti-CD3/anti-CD28, and then with secondary anti-CD3/ICOS stimulation. Numbers were normalized to B6 control. NS, not significant; * P < 0.05, ** P < 0.01, *** *P* < 0.001, **** P < 0.0001 (one-way ANOVA). Results were pooled from at least 3 independent experiments. Error bars represent SEM.



(A) Immunoblot analysis of p-STAT1 and p-S6 expression in CD4⁺ T cells from B6 and $Cd4^{cre}Rictor^{fl/fl}$ mice treated with or without IFN α in presence of anti-CD3/anti-CD28 for 6 hours. The relative expressions were normalized to them in B6 CD4⁺ T cells without any stimulation. (B) Immunoblot analysis of RICTOR, p-p70S6K, p-S6RP, p-4EBP1 and Actin in CD4 T cells from B6 and $Cd4^{cre}Rictor^{fl/fl}$ mice activated with anti-CD3/anti-CD28 in the presence or absence of IFN α . (C) Immunoblot analysis of p-AKT473, p-STAT1 and Actin in CD4 T cells from B6 and $Cd4^{cre}Rictor^{fl/fl}$ mice activated with anti-CD3/anti-CD28 in the presence or absence of IFN α . (C) Immunoblot analysis of p-AKT473, p-STAT1 and Actin in CD4 T cells from B6 and $Cd4^{cre}Rictor^{fl/fl}$ mice activated with anti-CD3/anti-CD28 in the presence or absence of IFN β . (D) Blood CD19⁺ B cell counts were determined before and after poly(I:C) treatment in B6 and $Cd4^{cre}Rictor^{fl/fl}$ mice. (E) Expression of CD69 was measured by flow cytometry in blood CD4⁺ T cells from 4-6 months old B6, Lpr and Lpr.*Rictor* T-KO mice. Right, summary of CD69⁺ percentages among blood CD4⁺ T cells. (F) Percentages of CD19⁻CD4⁺ T cells in blood from B6, Lpr and Lpr.*Rictor* T-KO mice among total lymphocytes. NS, not significant; * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.0001 (A, unpaired Student's *t* test, C-E, one-way ANOVA). Results represent, or were pooled from, at least 3 independent experiments. Error bars represent SEM.