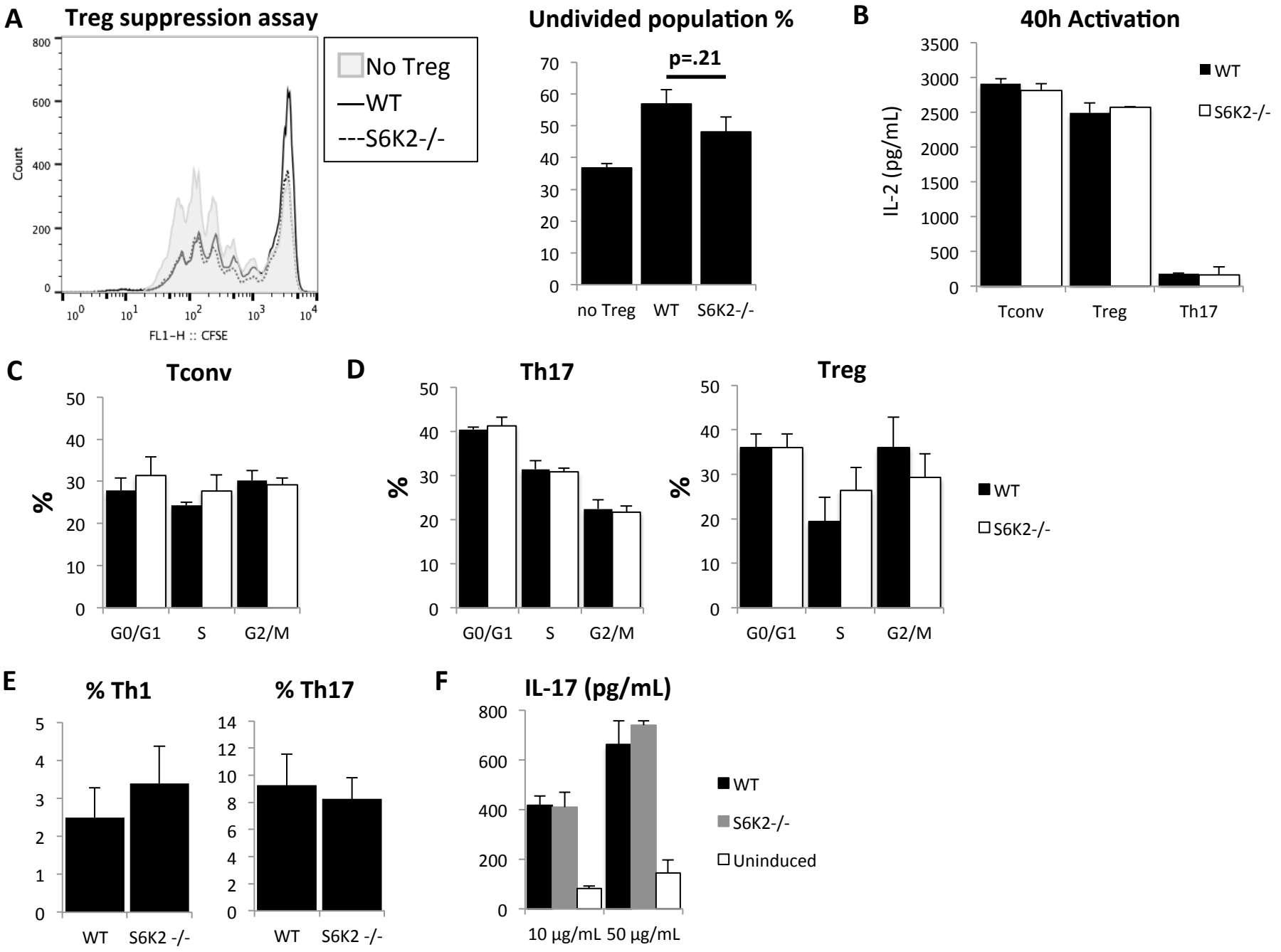
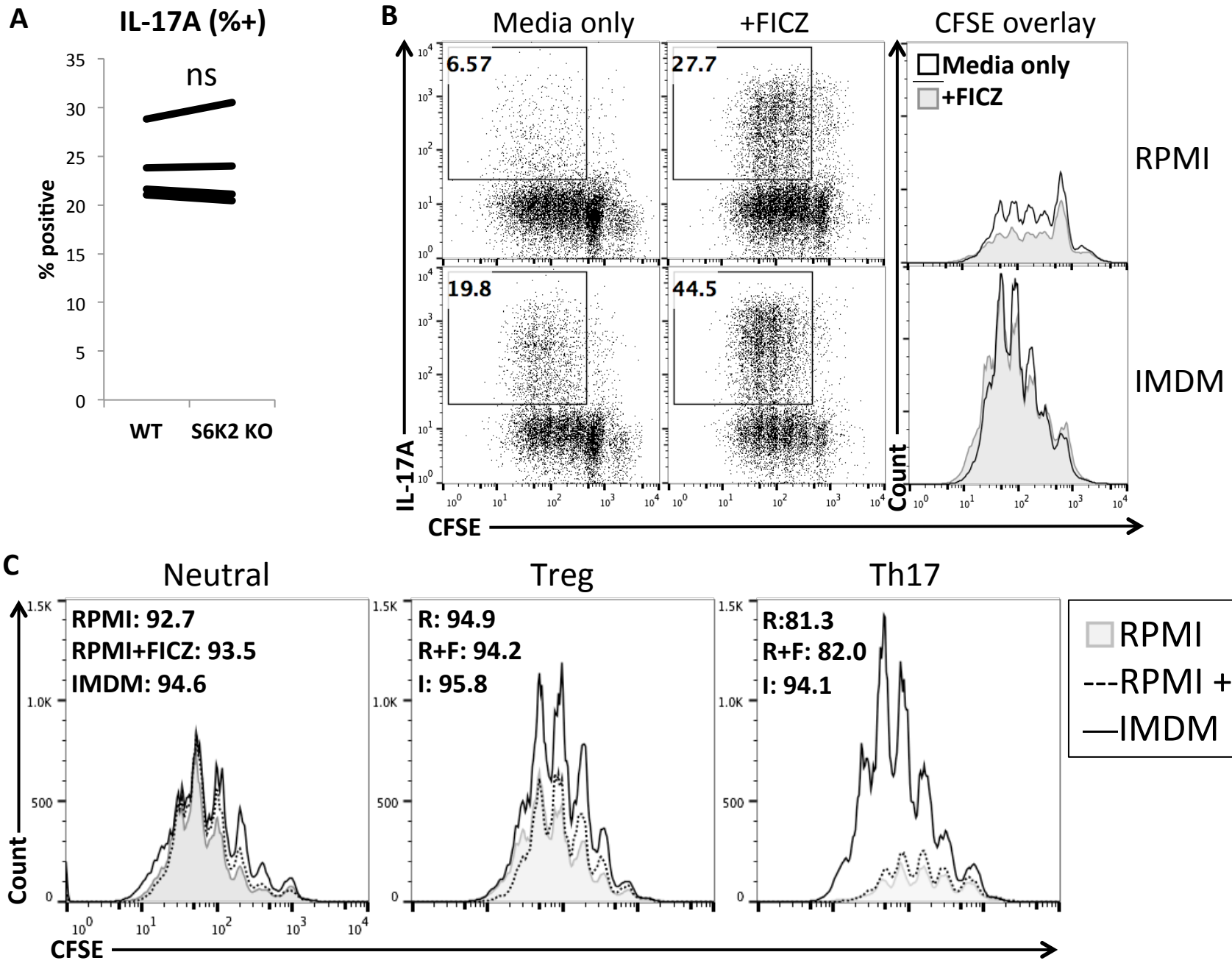
Supplemental Fig. 1



Supplemental Figure 1: Additional nTreg, early activation and EAE data. **(A)** Histogram overlay of a representative Treg suppression assay comparing CFSE labeled WT Tconv cells cultured with isolated WT or *S6K2*$^{-/-}$ nTregs for 3 days. The average undivided population percentage over multiple experiments is graphed by gating on the initial CFSE peak (mean +/- SEM, n = 3 independent experiments). **(B)** IL-2 cytokine measurement by ELISA in the supernatant of 40h activated WT and *S6K2*$^{-/-}$ CD4^{+} T cells in Tconv, Th17, or Treg culturing conditions (mean +/- SEM, n = 3 independent experiments). **(C)** Cell cycle analysis of WT and *S6K2*$^{-/-}$ CD4^{+} T cells by PI staining after 40h stimulation in Tconv culturing conditions (mean +/- SEM, n = 3 independent experiments). **(D)** Cell cycle analysis of WT and *S6K2*$^{-/-}$ CD4^{+} T cells by PI staining after 40h stimulation in Th17 and Treg culturing conditions (mean +/- SEM, n = 3 independent experiments). **(E)** Graphed Th1 and Th17 population percentages from EAE experiment 1 (Fig. 5A) by IFNγ and IL-17A cytokine expression, respectively (n = 3 for each group). Cells were isolated from the brain and gated on CD4^{+} expression. Error bars indicate SEM. **(F)** IL-17 cytokine production in the supernatant of 3 day MOG restimulated splenocytes at 10 and 50μg/mL concentrations from 10 day MOG immunized WT, *S6K2*$^{-/-}$, and MOG uninduced mice (mean +/- SEM, n = 3 for each group).

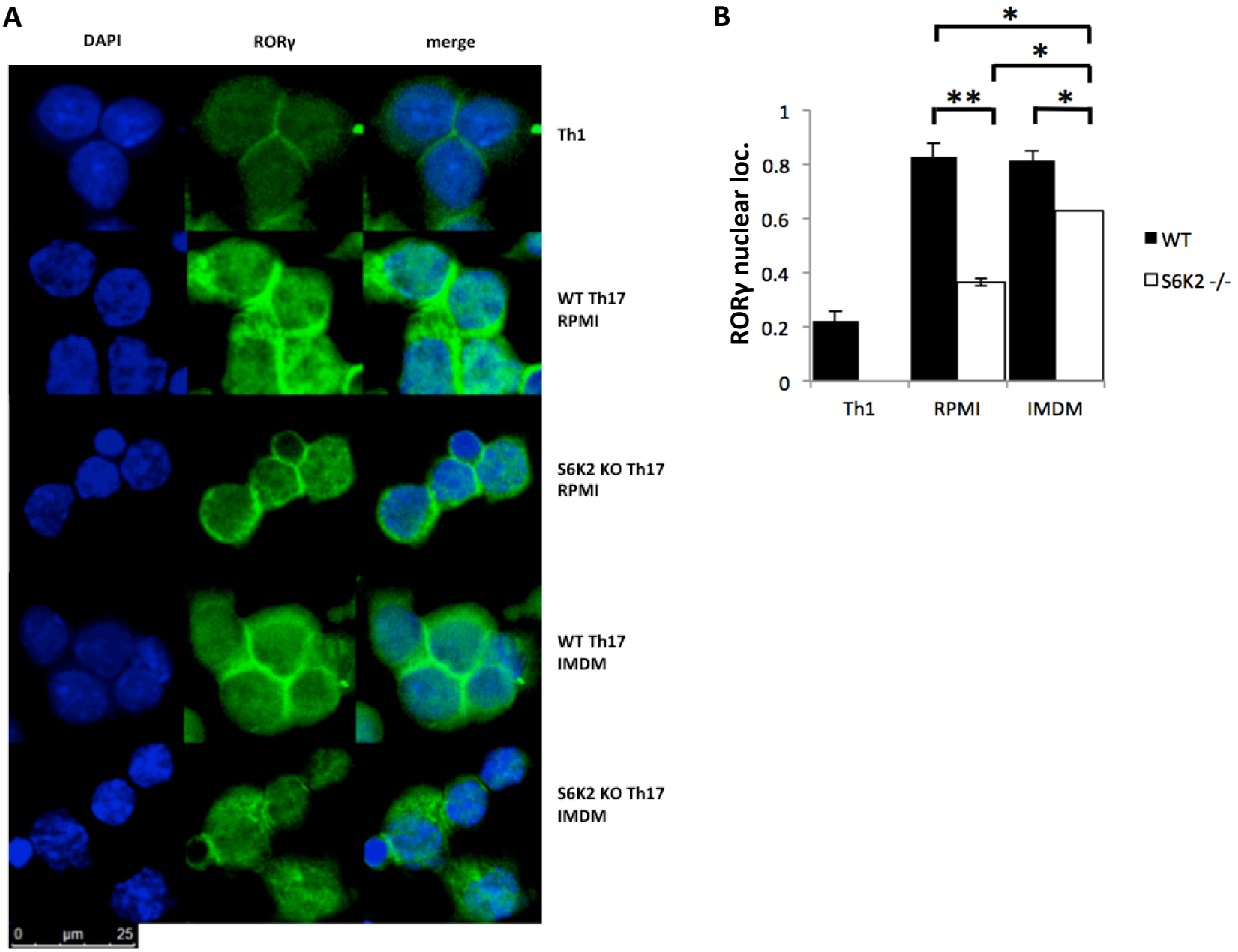
Supplemental Fig. 2



Supplemental Figure 2: FICZ boosts Th17 differentiation in both RPMI and IMDM

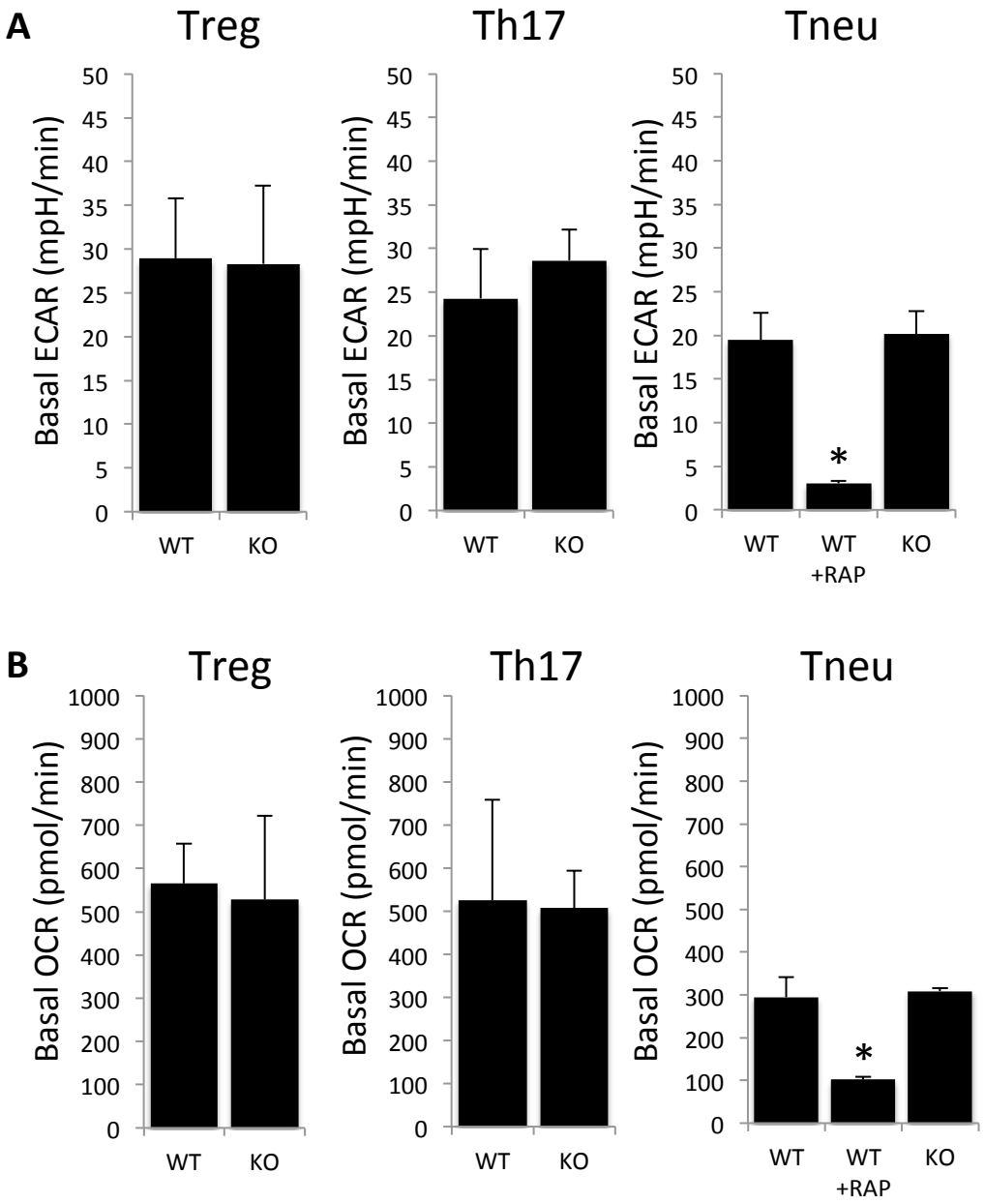
(A) Flow cytometry analysis of IL-17A and CFSE positive cells. Naïve WT $CD4^+$ T cells were stimulated under Th17 conditions for four days in RPMI and IMDM, with or without FICZ (mean +/- SEM, n = 4 independent experiments). **(B)** CFSE histogram overlay of naïve WT or *S6K2*$^{-/-}$ $CD4^+$ T cells that were cultured under indicated conditions for 4 days in RPMI, RPMI +FICZ, or IMDM. Plots and histogram overlays are representative of 3 independent experiments. **(C)** Th17 population percentages from Figure 6A and similar experiments, graphed by IL-17A cytokine expression (n = 4 independent experiments). The Wilcoxon signed-rank test was used to assess p value. ns = not significant ($p > 0.05$).

Supplemental Fig. 3



Supplemental Figure 3: *S6K2*$^{-/-}$ T cells show significantly reduced RORγ nuclear localization compared to WT T cells. **(A)** Immunofluorescence microscopy analysis of RORγ localization in WT or *S6K2*$^{-/-}$ CD4^{+} T cells, representative of 3 independent experiments. Cells were cultured in IMDM or RPMI for 40 hours under Th1 or Th17 differentiation conditions. **(B)** Quantification of RORγ nuclear localization from (A) using the Manders colocalization coefficient. Data represent at least 3 independent experiments, mean +/- SEM. * $p < 0.05$. For statistical analysis, 20 cells were counted in each sample.

Supplemental Fig. 4



Supplemental Figure 4: *S6K2*$^{-/-}$ and WT CD4^{+} T cells have similar metabolic profiles under various T cell skewing conditions. Naïve CD4^{+} T cells were stimulated for 40 hours under the indicated conditions. **(A)** Glycolytic rate (ECAR) **(B)** Oxidative phosphorylation rate (OCR). Data shown are representative of at least 3 independent experiments with n = 3 per group, mean +/- SEM. * $p < 0.05$.