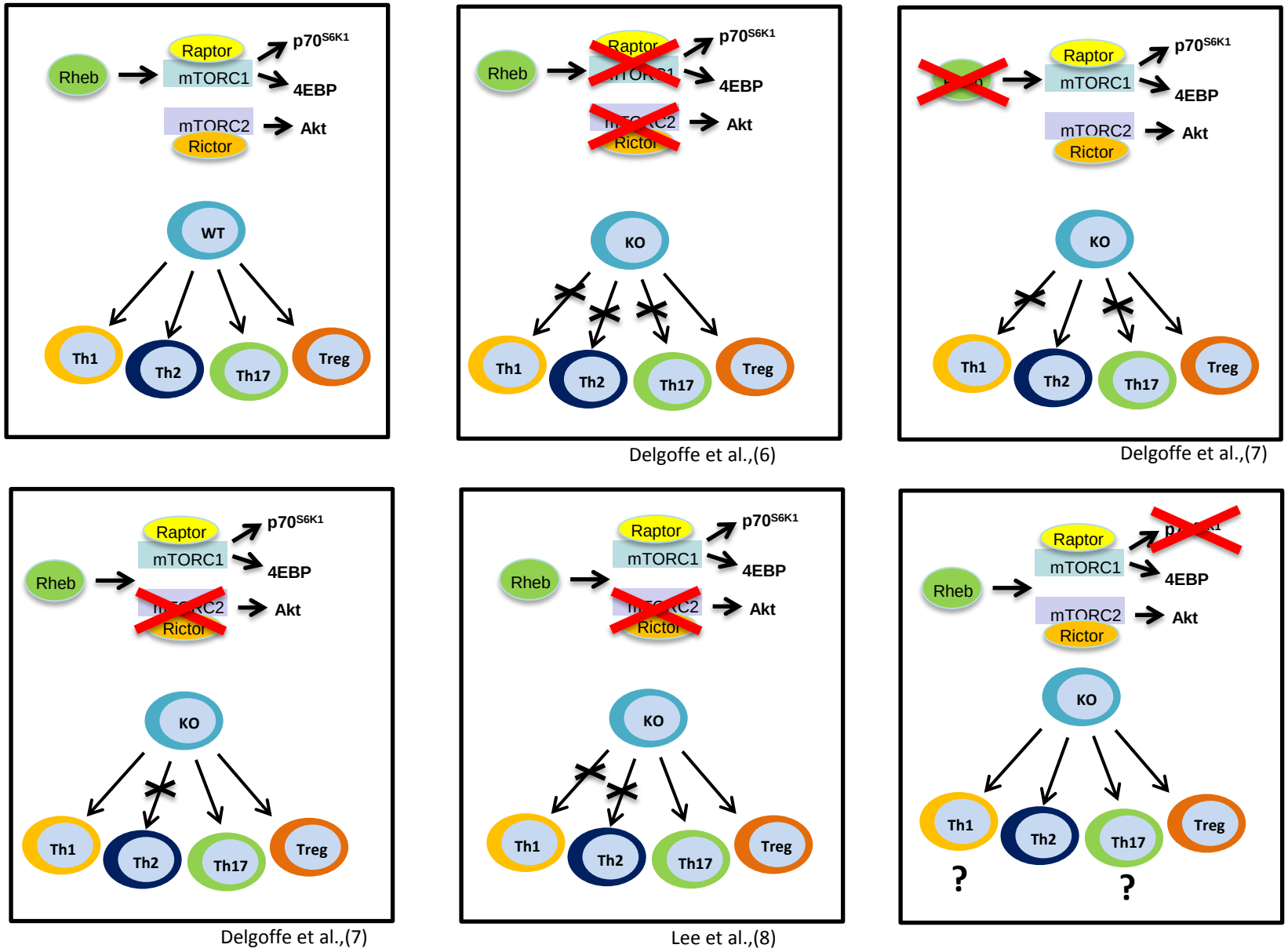
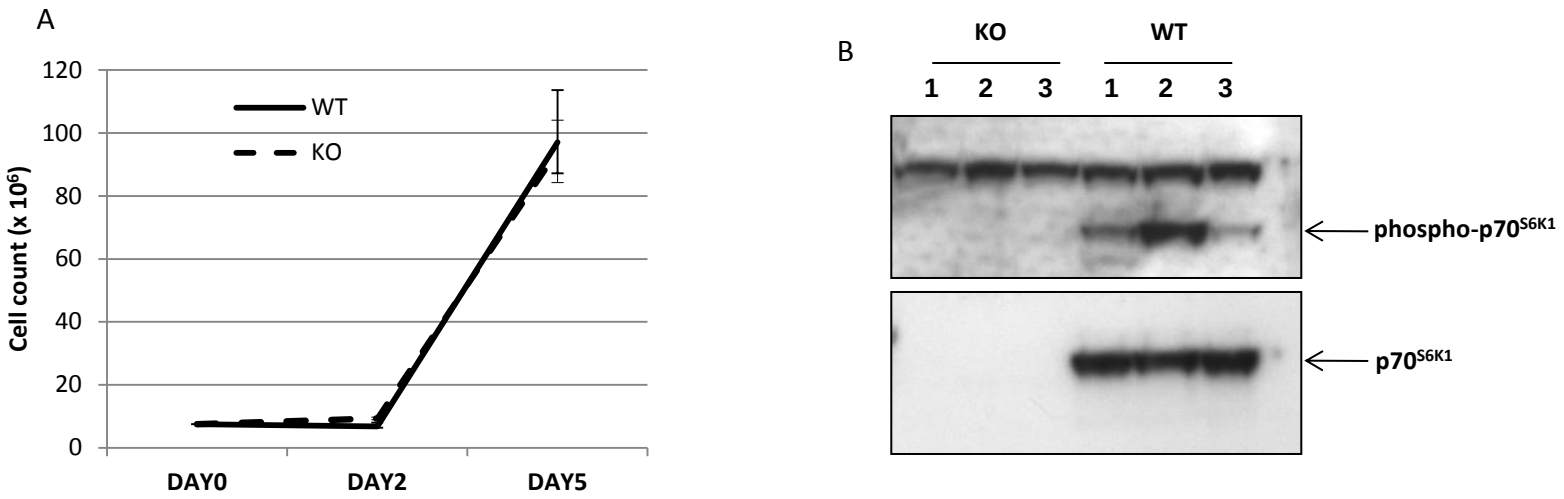
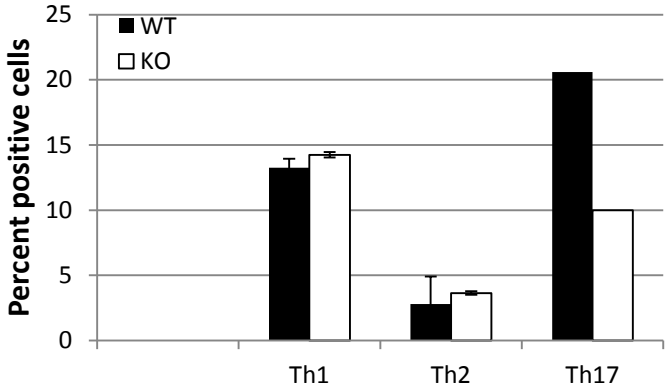


Supplemental Figure 1. Schematic representation of the effects of different knockout mice on the differentiation of naïve $CD4^+$ T cells.



Supplemental Figure 2. Measurement of proliferation and the status of phospho-p70^{S6K1} (Thr 389) in naïve activated CD4^{+} T cells. Naive splenic CD4^{+}T cells from wild type and p70^{S6K1} knockout mice (3 mice/group) were activated with plate-bound anti-CD3 and anti-CD28 either for 5 days to measure the proliferation (A), or activated for 24 hours for whole cell lysate preparation for western blot analysis (B). Equal proteins (20 μg) from whole cell lysates were used to detect the status of phospho- and total-p70^{S6K1}.

A



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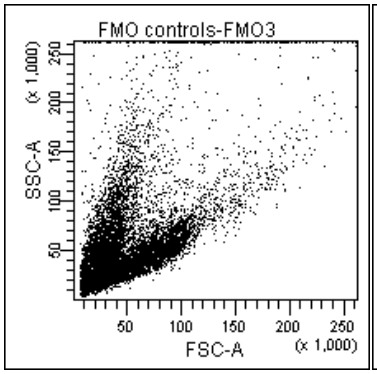
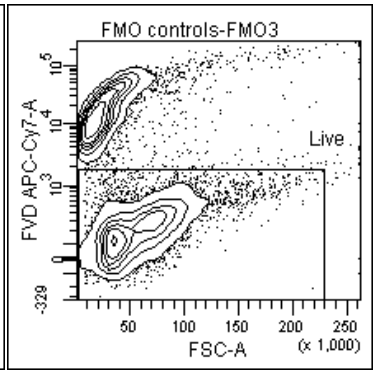
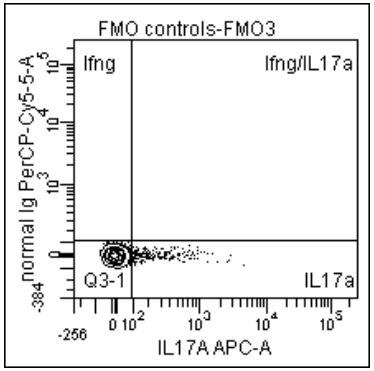
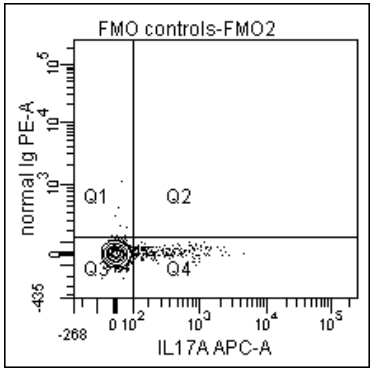
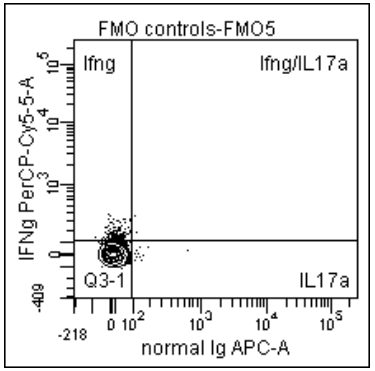
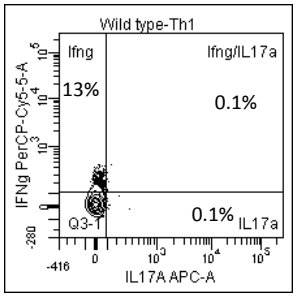


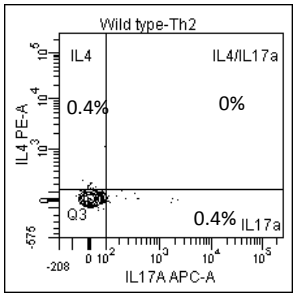


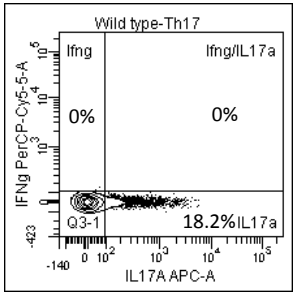


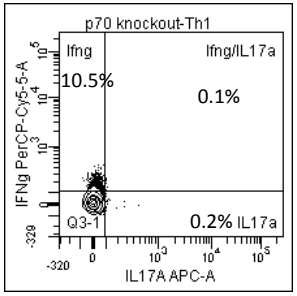


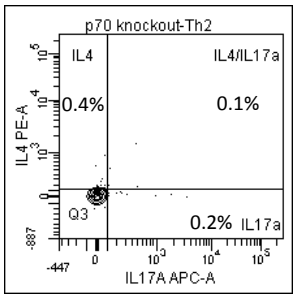


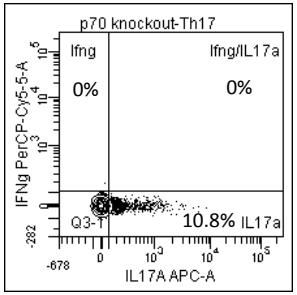






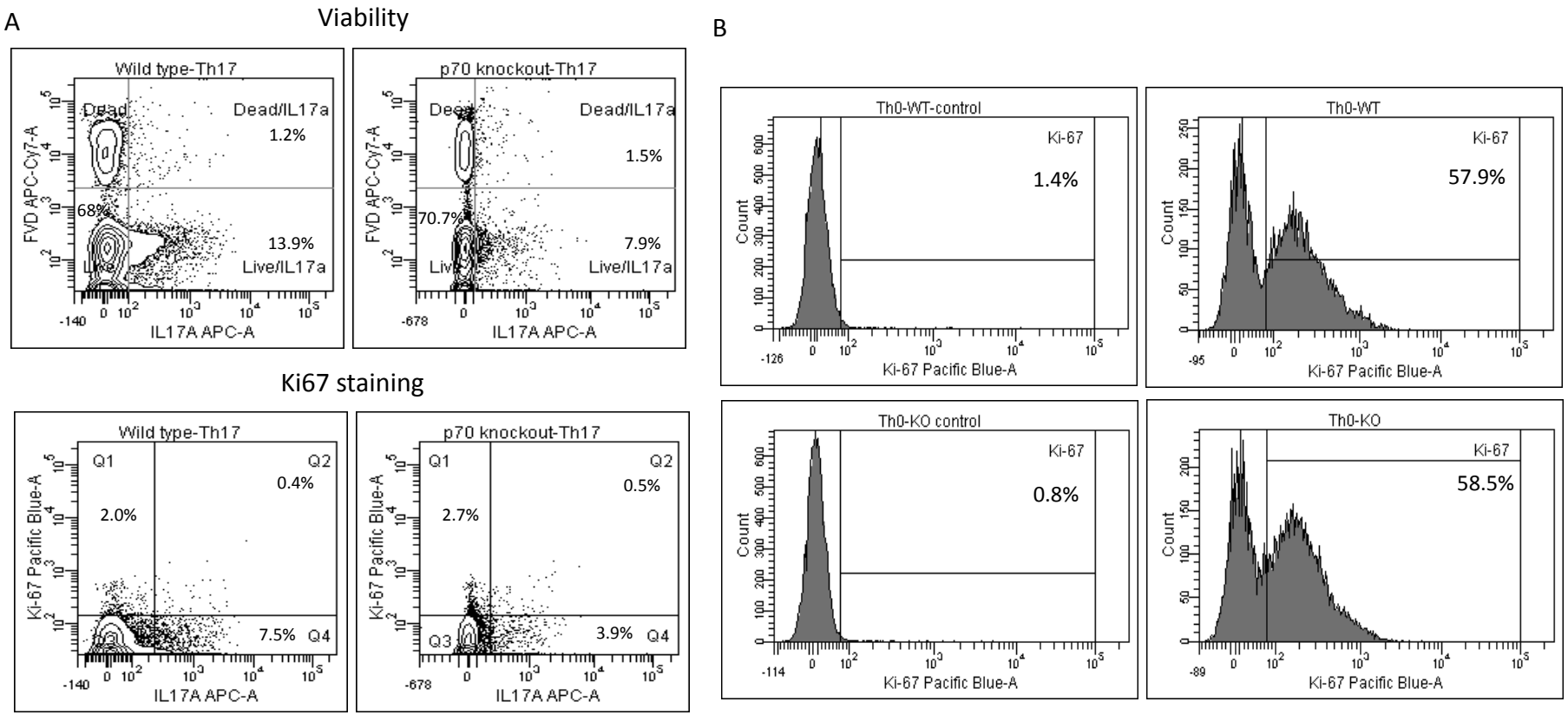




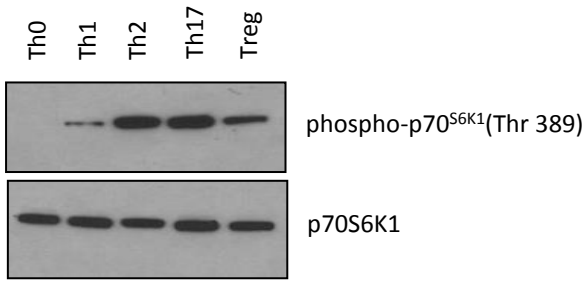


Supplemental Figure 3

Supplemental Figure 3. Differentiation of Naïve $CD4^+$ T cells from wild type (WT) and knockout (KO) spleens for three days. (A) Naïve $CD4^+$T cells ($CD4^+CD62L^+$) were enriched from murine splenocytes from wild type and $p70^{S6K1}$ knockout by negative selection for CD3/CD4 and then positive selection for CD62 ligand (pooled of 3 mice per group). The enriched T cells were then activated by plate-bound anti-CD3 and anti-CD28 (5 µg/ml each) for 3 days under the following skewing conditions: for Th1, IL-12 (10 ng/ml) & anti-IL4 (10 µg/ml); for Th2, IL-4 (10 ng/ml) & anti-IFN-γ (10 µg/ml); for Th17, IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IL4 (10 µg/ml), & anti-IFN-γ (10 µg/ml); for Treg, TGFß (5ng/ml), anti-IL-4 (10 µg/ml), & anti-IFN-γ (10 µg/ml). Following *in vitro* differentiation, T cells were stimulated with PMA and ionomycin for 4 hours with last 2 hours in the presence of monensin. Stimulated T cells were stained with the following antibodies: for Th1, IFNγ; for Th2, IL-4; and for Th17, IL17a. Data were obtained by flow cytometry using FACS Canto II, and the results are presented as percent positive cells. Results from two independent experiments are shown here. (B) Gating strategy for panel A. *In vitro* differentiated cells were stained with the Fixable Viability Dye (FVD) eFluor® 780 (eBioscience) and subsequently stained with the appropriate normal Ig control (FMO, fluorescent minus one). FMO2, FMO3, and FMO5 represent different isotype matched Ig controls. These controls were initially gated for viability based on the exclusion of the Fixable Viability Dye (FVD) (top second panel from the left). The FMO controls were used to establish the gates for IFNγ, IL-17a and IL4 staining.



Supplemental Figure 4. Measurement of viability and proliferative response in *in vitro* differentiated Th17 cells from wild type and knockout mice. (A) Naïve splenic $CD4^{+}$T cells from wild type and $p70^{S6K1}$ knockout mice were activated with plate-bound anti-CD3 and anti-CD28 (5 µg/ml each) under Th17 skewing conditions for three days: for Th17, IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IL4 (10 µg/ml). Following *in vitro* differentiation, T cells were stimulated with PMA and ionomycin for 4 hours, last 2 hours in the presence of monensin. Stimulated T cells were stained with cell viability (Fixable Viability Dye (FVD) eFluor® 780, eBioscience) and with the following antibodies: IL17a (APC) and Ki-67 (eFluor® 450, eBioscience). Data were obtained by FACS Canto II and analyzed by FACS DIVA. Representative of two independent experiments is shown here. (B) Naïve splenic $CD4^{+}$T cells from wild type and $p70^{S6K1}$ knockout mice were activated with either medium alone or plate-bound anti-CD3 and anti-CD28 for three days. Cells were then stained with Ki-67 (eFluor® 450, eBioscience), and data were obtained by FACS Canto II. Representative of two independent experiments is shown here.



Supplemental Figure 5. Measurement of phospho-p70^{S6K1} (Thr 389) in *in vitro* differentiated T effector cells. Naïve CD4^{+}T cells were activated with plate-bound anti-CD3 and anti-CD28 (5 µg/ml each) under the following skewing conditions for 3 days: for Th1, IL-12 (10 ng/ml) & anti-IL4 (10 µg/ml); for Th2, IL-4 (10 ng/ml) & anti-IFNγ (10 µg/ml); for Th17, IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IL4 (10 µg/ml), & anti-IFN-γ (10 µg/ml); for Treg, TGFß (5ng/ml), anti-IL-4 (10 µg/ml), & anti-IFN-γ (10 µg/ml). Whole cell lysates (20 µg) were analyzed by western blot analysis. Representative data from two independent experiments are shown here.

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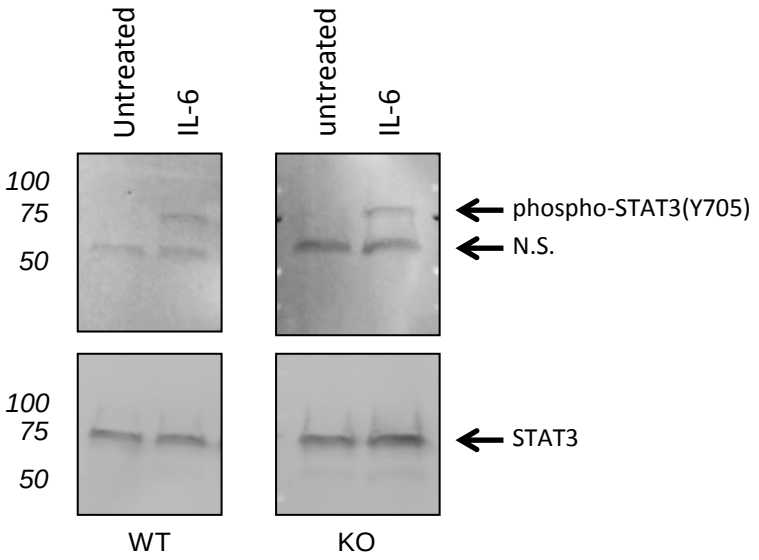

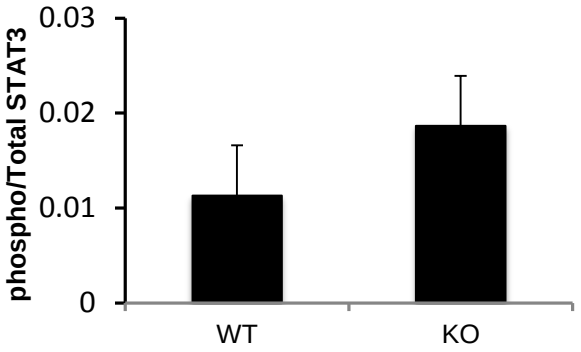

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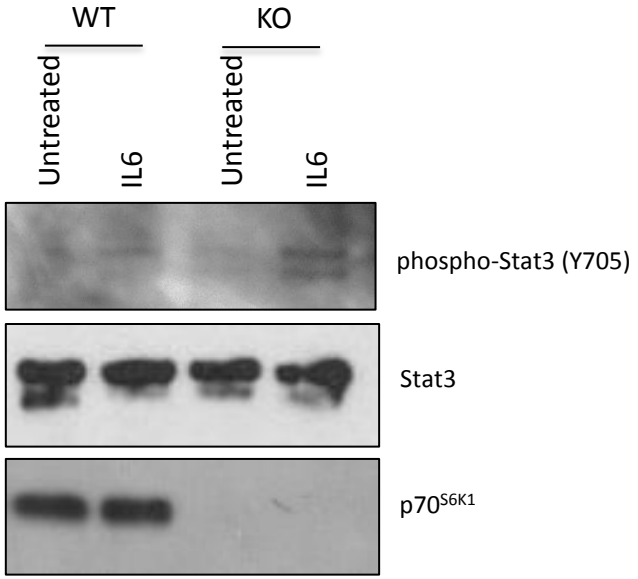

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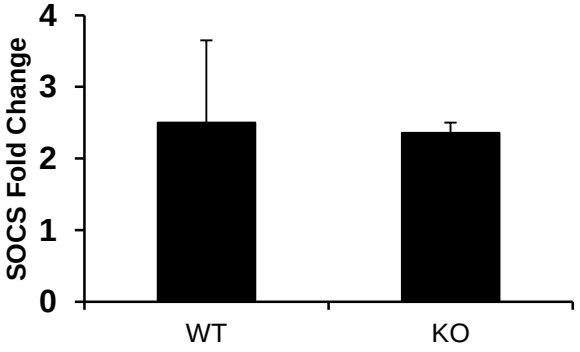
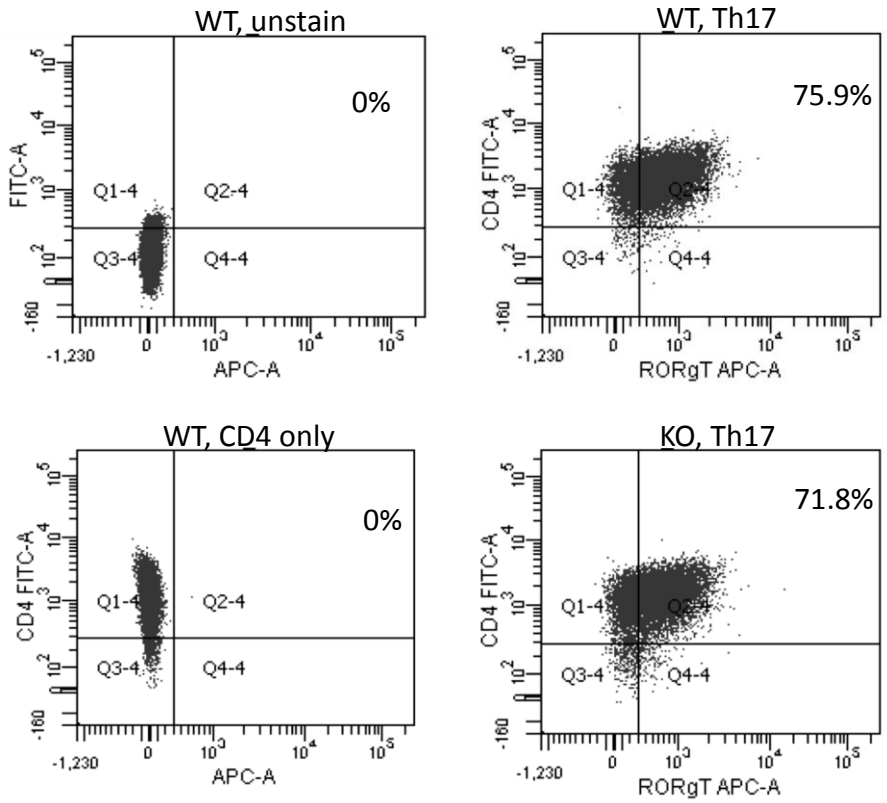
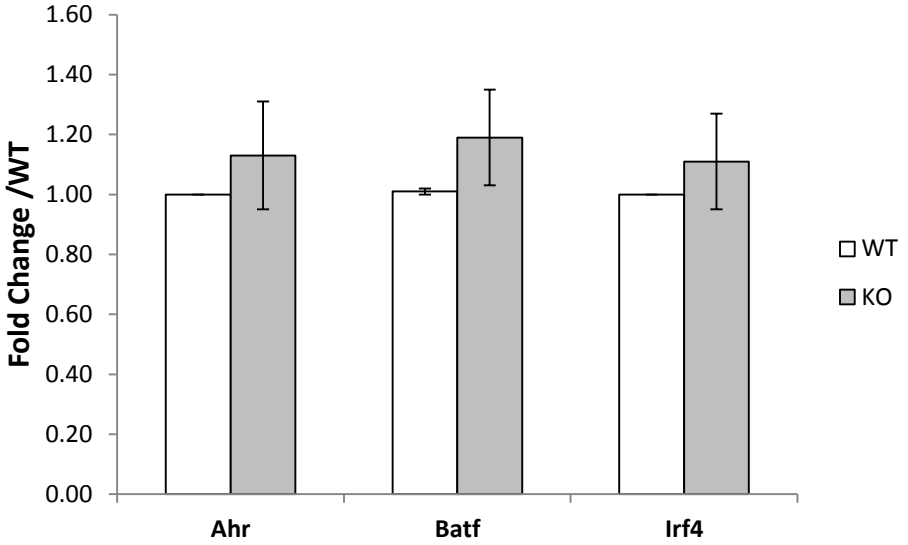


Supplemental Figure 6

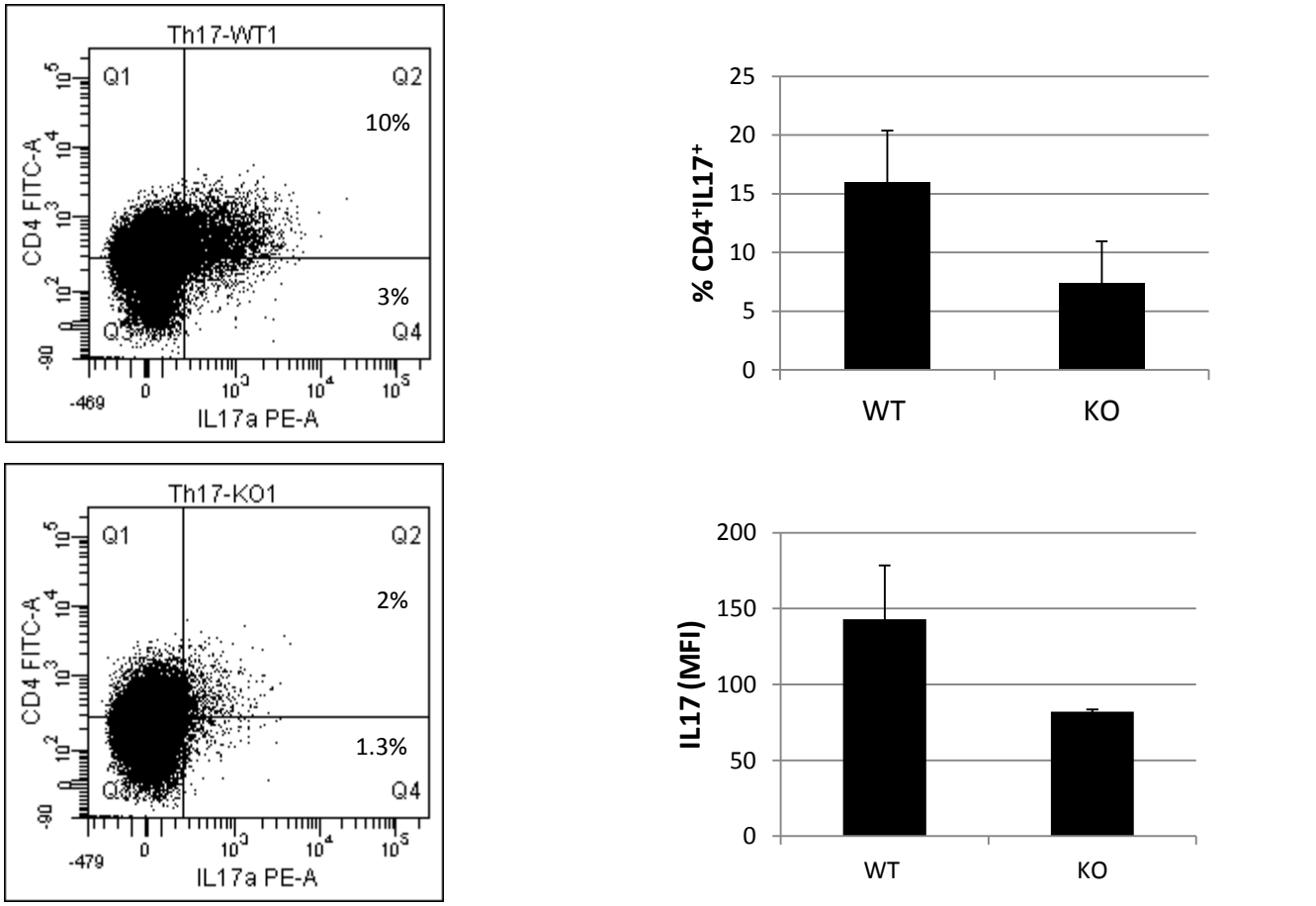
Supplemental Figure 6. Role of $p70^{S6K1}$ in IL-6 signaling and the expression of suppressor of cytokine signaling (SOCS). (A) $CD4^{+}$T cells were harvested from spleens of either wild type or $p70^{S6K1}$ knockout mice by negative selection. After incubating in RPMI medium without serum for 30 minutes, the cells were treated with recombinant mouse IL-6 (10 ng/ml) for an additional 30 minutes. Whole cell lysates from the treated cells were prepared and analyzed by western blot analysis. The membranes were initially blotted with antibodies against phospho-STAT3 (Y705) and then reblotted with total STAT3. NS = non-specific band detected by phospho-STAT3 antibody. Densitometric analysis of IL-6-mediated phosphorylation of STAT3 (Y705) normalized with total STAT3 from two independent experiments is shown in the right panel. (B) Naïve $CD4^{+}$ T cells were cultured for 24 hours in Th17 skewing conditions followed by IL-6 starvation for 1 hour, and subsequent treatment with either medium alone or with IL-6 for 1 hour. Whole cell lysates were analyzed by western blot analysis. Representative of two independent experiments is shown here. (C) Total RNA from *in vitro* differentiated Th17 cells from wild type (WT) and $p70^{S6K1}$ knockout (KO) spleens was collected using the RNeasy Mini Kit and used to generate complementary DNA with the VILO superscript kit. The cDNA was then analyzed by quantitative PCR to measure transcript levels. These experiments were normalized to GAPDH. Then the normalized results were compared to Th0 cells to determine the fold change. Summary of two independent experiments is shown here.



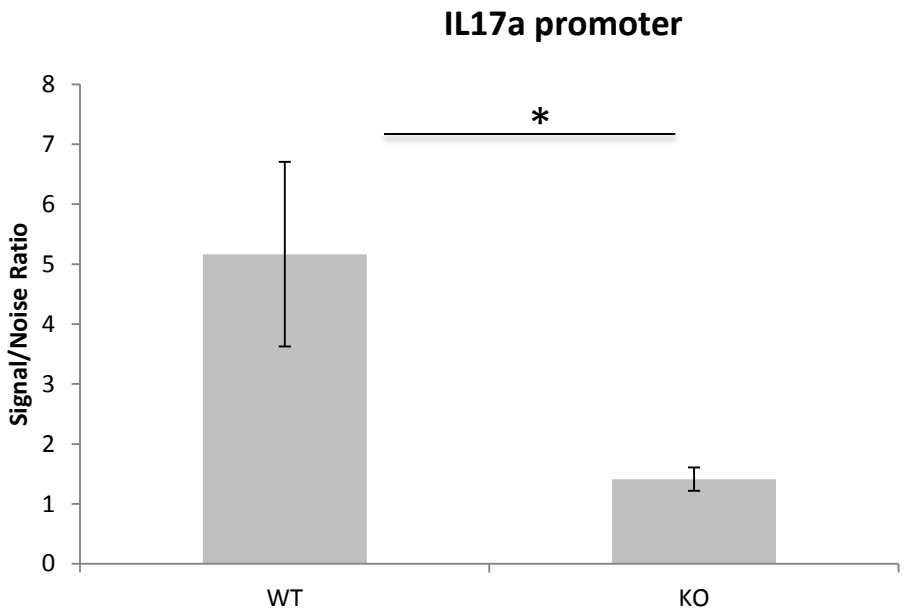
Supplemental Figure 7. Measurement of RORγT levels in *in vitro* differentiated Th17 cells from wild type (WT) and $p70^{S6k1}$ knockout (KO) mice. Naïve splenic $CD4^+$ T cells ($CD4^+CD62L^+$) were activated by plate-bound anti-CD3 and anti-CD28 (5 µg/ml each) under Th17 skewing conditions for three days: IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IFNγ (10 µg/ml), and anti-IL4 (10 µg/ml). Following *in vitro* differentiation, T cells were stimulated with PMA and ionomycin for 4 hours, the last 2 hours in the presence of monensin. Stimulated T cells were stained with antibodies against CD4 (FITC) and RORγT (APC). Data were obtained by FACS Canto II and analyzed by FACS DIVA. Representative from three independent experiments is shown here.



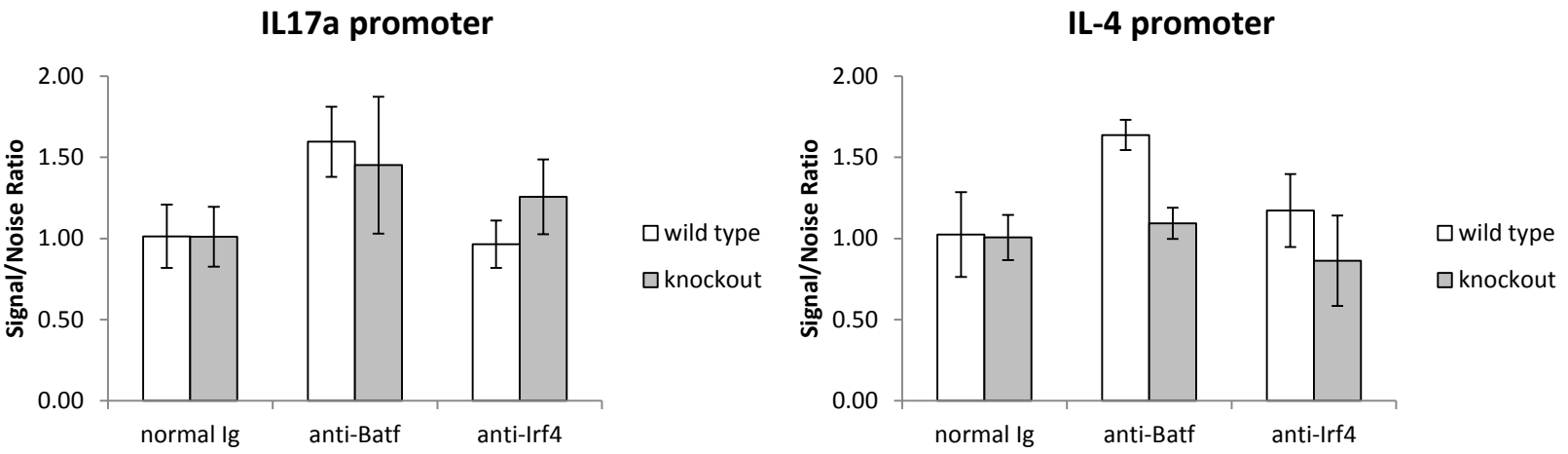
Supplemental Figure 8. Measurement of Ahr, Batf, and Irf4 expression in *in vitro* differentiated Th17 cells. Naïve splenic $CD4^+$T cells ($CD4^+CD62L^+$) from wild type (WT) and knockout (KO) mice were activated by plate-bound anti-CD3 and anti-CD28 (5 µg/ml each) under Th17 skewing conditions for 3 days: IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IFNγ (10 µg/ml), and anti-IL4 (10 µg/ml). Following *in vitro* differentiation, T cells were stimulated with PMA and ionomycin for 4 hours. Total RNA from differentiated cells was collected using the RNeasy Mini Kit and used to generate complementary DNA with the VILO superscript kit. The cDNA was then analyzed by quantitative PCR to measure transcript levels. These experiments were normalized to β-actin. Then the normalized results were compared to wild type Th17 cells to determine the fold change. Summary of two independent experiments is shown here.



Supplemental Figure 9. Measurement of IL17 production by *in vitro* differentiated Th17 cells. Naïve splenic $CD4^+$T cells ($CD4^+CD62L^+$) from wild type (WT) and $p70^{S6K1}$ knockout (KO) mice (pooled of 3 mice per group) were activated by plate-bound anti-CD3 and anti-CD28 under Th17 skewing conditions for 3 days: IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IFNγ (10 µg/ml), and anti-IL4 (10 µg/ml). Following *in vitro* differentiation, T cells were stimulated with PMA and ionomycin for 4 hours with last 2 hours in the presence of monensin. Stimulated T cells were stained with the following antibodies: CD4 (FITC) and IL17a (PE). Percent of $CD4^+IL17^+$ T cells and the mean fluorescence intensities (MFI) for IL17 were obtained by FACS Canto II and analyzed by FACS DIVA. The numbers in the boxes indicate the percentages of antibody-stained cells relative to the total number of cells. Summary of three independent experiments is shown at the right panel.



Supplemental Figure 10. Measurement of RNA polymerase II recruitment to the IL17a promoter in wild type (WT) and $p70^{S6K1}$ knockout (KO) Th17 cells. Naïve splenic $CD4^+$T cells ($CD4^+CD62L^+$) from wild type and $p70^{S6K1}$ knockout mice were activated by plate-bound anti-CD3 and anti-CD28 under Th17 skewing conditions for 3 days: IL-6 (10 ng/ml), TGFß (5 ng/ml), anti-IFNγ (10 µg/ml), and anti-IL4 (10 µg/ml). The recruitment of RNA polymerase II to the IL-17a promoter was measured by chromatin immunoprecipitation analysis. Sheared chromatins from differentiated T cells were incubated with antibodies against RNA polymerase II. Immune complexes were precipitated with Protein A/G agarose beads, and the captured DNA was precipitated with Chelex beads. The immunoprecipitated chromatin was used in quantitative PCR . The relative level of recruitment of RNA polymerase was expressed as a Signal/Noise Ratio, which was calculated as $2^{\wedge}(Ct_{\text{normal Ig}} - Ct_{\text{anti-RNA polII}})$. Bar graphs and error bars represent an average and standard error, respectively, of four experiments. * $p = 0.05$ based on Student's t-test.



Supplemental Figure 11. Chromatin Immunoprecipitation analyses of B-ATF and IRF-4 recruitment to the IL-17a and IL-4 promoter. The recruitment of B-ATF and IRF-4 to the IL-17a and IL-4 promoter were measured by chromatin immunoprecipitation as previously described [31] with the following exceptions. Sheared chromatins from differentiated Th17 cells were incubated with antibodies against either mouse normal Ig, mouse anti-B-ATF (Santa Cruz, sc-100974), or goat anti-IRF-4 (Santa Cruz, sc-6059X). The relative level of recruitment of B-ATF or IRF-4 expressed as a Signal/Noise Ratio was calculated as $2^{\wedge}(Ct_{\text{normal Ig}} - Ct_{\text{specific antibody}})$. Bar graphs and error bars represent an average and standard deviation, respectively, from two different experiments

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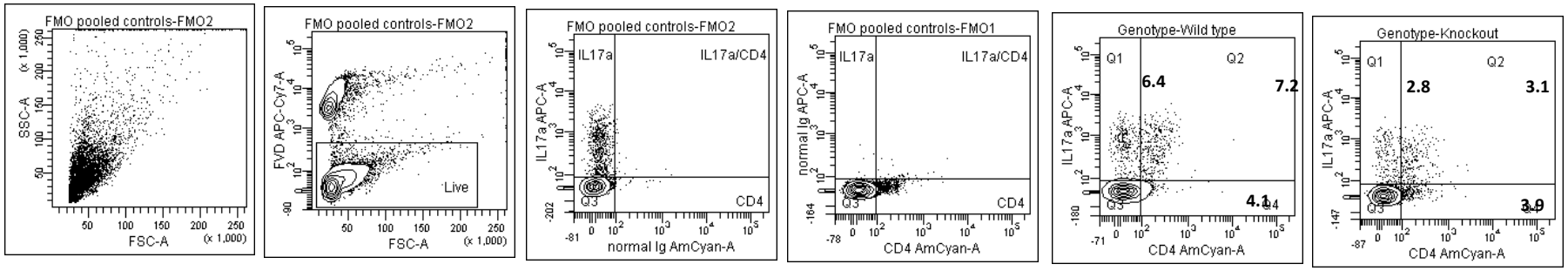

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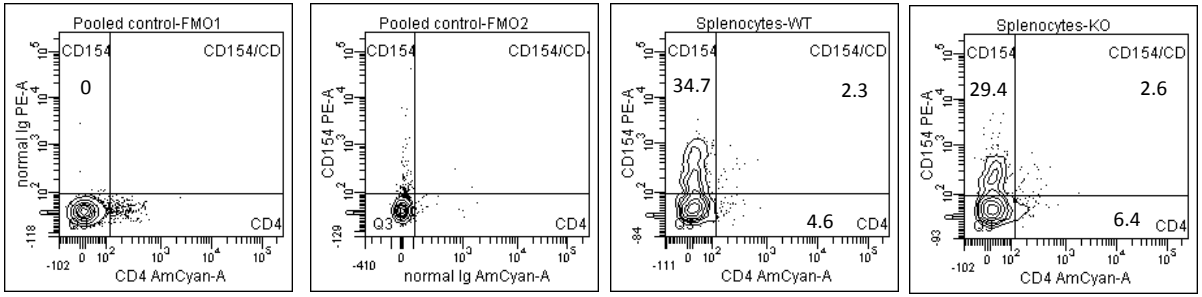
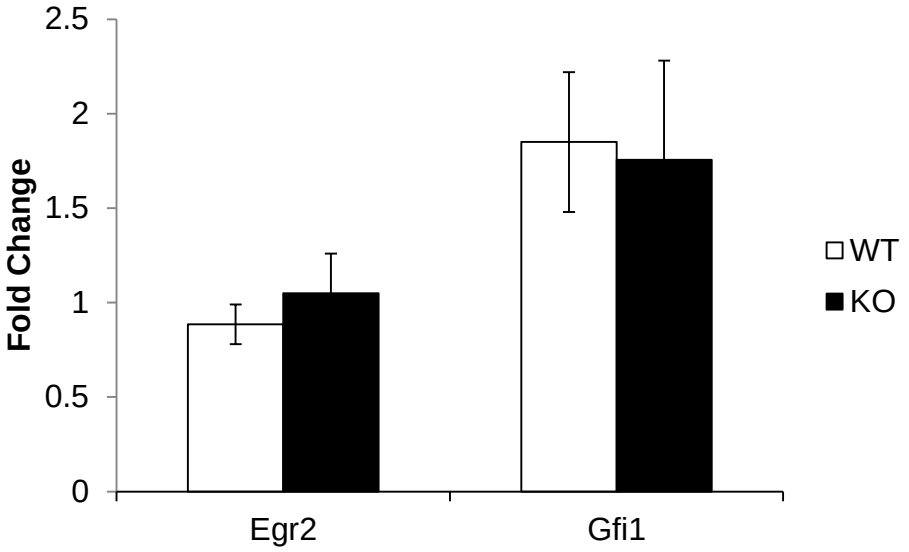


Supplemental Figure 12. Wild type and p70^{S6K1} knockout mice (7 mice/group) were immunized with MOG in complete Freund's adjuvant (CFA) without administering pertussis toxin. Splenocytes from individual mice were harvested 10 days after immunization, and were cultured in the presence of MOG (20 μg/ml), IL-23 (20 ng/ml), and anti-IFN-γ antibody (10 μg/ml) for three days. (A) The *in vitro* cultured splenocytes were stimulated with PMA/ionomycin for 4 hours with last 2 hours with monensin. Splenocytes were then stained with the Fixable Viability Dye (FVD) eFluor® 780 (eBioscience) and subsequently stained with the appropriate normal Ig control (FMO, fluorescent minus one). FMO1 and FMO2 represent different isotype matched Ig controls. These controls were initially gated for viability based on the exclusion of the Fixable Viability Dye (FVD) (top second panel from the left). The FMO controls were used to establish the gates for IL-17a and CD4 staining. (B) The *in vitro* cultured splenocytes were cultured overnight with monensin. Splenocytes were then stained with the appropriate normal Ig control (FMO, fluorescent minus one). FMO1 and FMO2 represent different isotype matched Ig controls. The FMO controls were used to establish the gates for CD154 and CD4 staining.



Supplemental Figure 13. Measurement of Egr2 and Gfi1 expression in *in vitro* differentiated Th17 cells. Naïve splenic $CD4^+$T cells ($CD4^+CD62L^+$) from wild type (WT) and $p70^{S6K1}$ (KO) knockout mice were activated by plate-bound anti-CD3 and anti-CD28 under Th17 skewing conditions for 3 days. Total RNA from differentiated cells was collected using the RNeasy Mini Kit and used to generate complementary DNA with the VILO superscript kit. The cDNA was then analyzed by quantitative PCR to measure transcript levels. These experiments were normalized to GAPDH. Then the normalized results were compared to Th0 cells to determine the fold change. Summary of two independent experiments is shown here.

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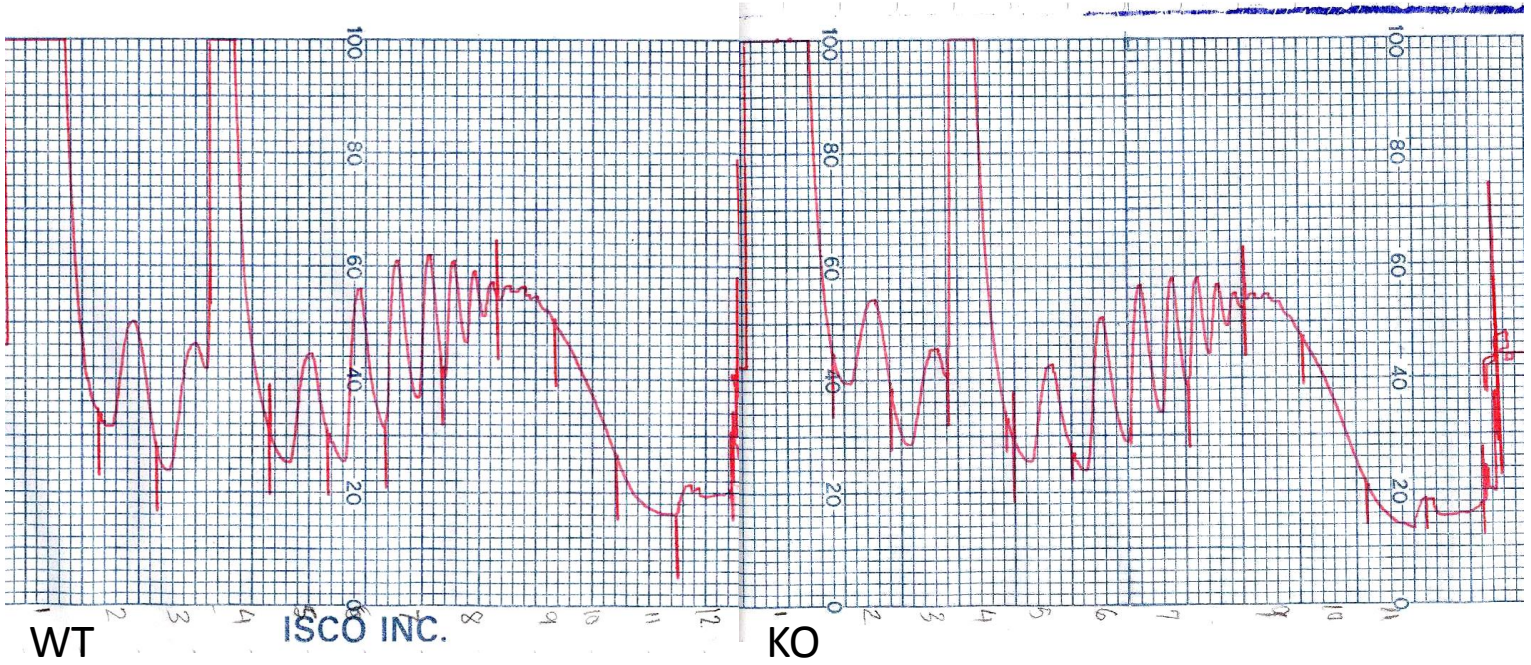

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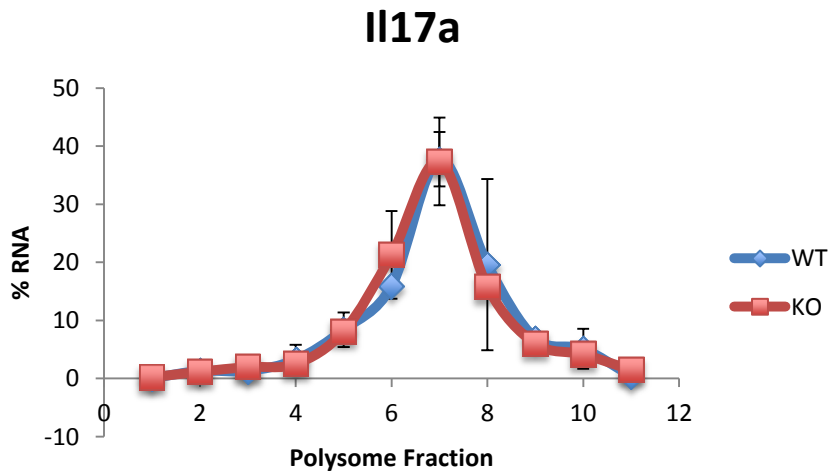

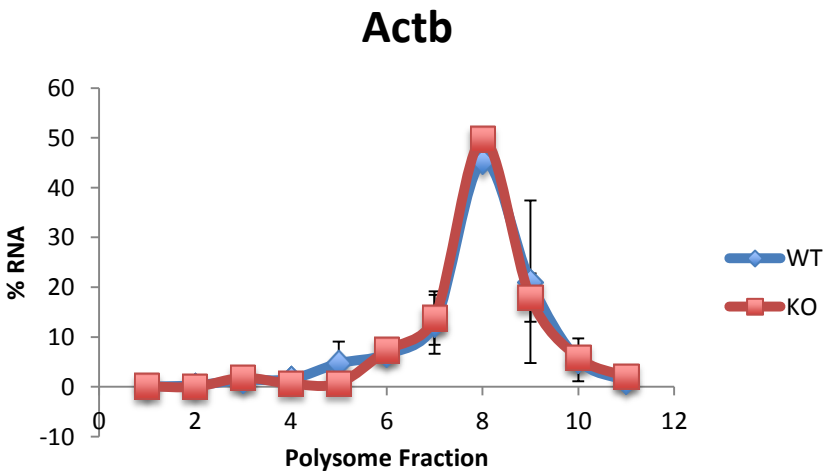

Supplemental Figure 14

Supplemental Figure 14. Measurement of distribution of polysome-associated IL17a mRNA in *in vitro* differentiated Th17 cells. (A) Whole cell lysates from *in vitro* differentiated Th17 cells were fractionated through sucrose gradients according to the protocol described by Yoon et al., (Mol. Cell, 2012, 47, 648-655). In general, the lower fractions (1-5) contain mainly monosomes whereas the higher fractions (6-11) are associated with polysomes that are translationally active. (B) Complementary DNA was generated from isolated RNA from each fraction and analyzed by RT-qPCR for either IL17a or beta-actin (Actb). The data points and error bars represent the average and standard deviation, respectively, from two different experiments.