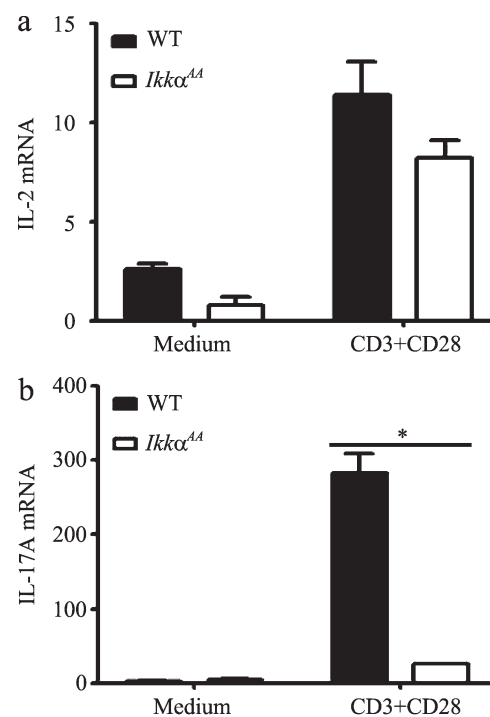
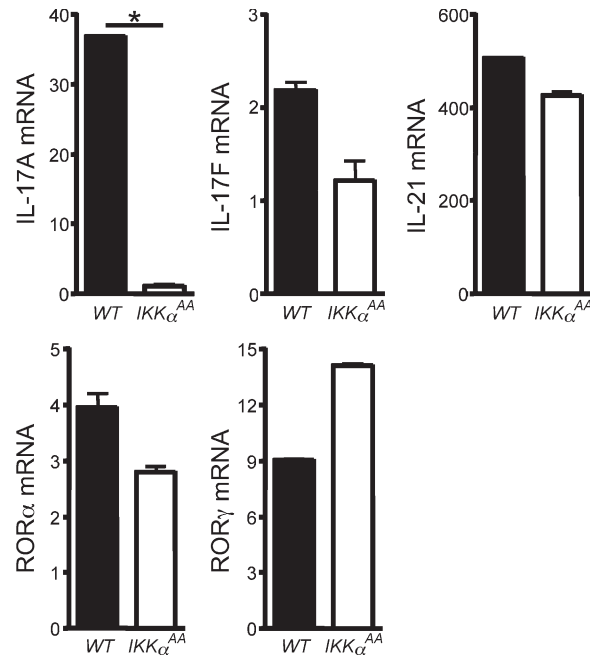


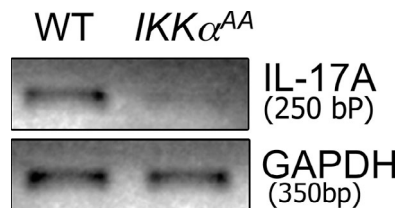
## SUPPLEMENTAL MATERIAL

Li et al., <http://www.jem.org/cgi/content/full/jem.20091346/DC1>

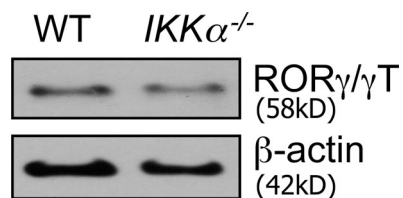
**Figure S1. Reduced IL-17A mRNA expression in *Ikkα<sup>AA</sup>* CD4<sup>+</sup> T cells.** CD4<sup>+</sup> T cells isolated from naive WT ( $n = 5$ ) and *Ikkα<sup>AA</sup>* ( $n = 5$ ) mice were cultured with or without 1  $\mu\text{g/ml}$  plate-bound anti-CD3 and soluble 2  $\mu\text{g/ml}$  anti-CD28. 24 h later, total RNA was isolated and cytokine mRNA levels were assessed by real-time RT-PCR. Data are presented as means  $\pm$  SD of triplicate cultures and are representative of two independent experiments. \*,  $P < 0.001$ .



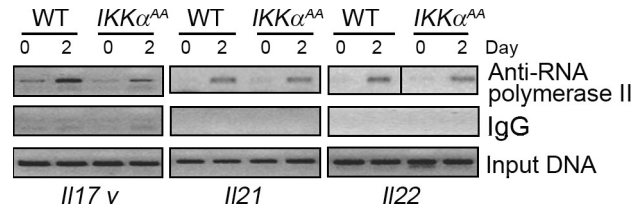
**Figure S2. Defective IL-17A expression in *Ikkα<sup>AA</sup>* CD4<sup>+</sup> T cells during Th17 differentiation.** Splenic CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>low</sup>CD62L<sup>+</sup> T cells from WT ( $n = 3$ ) and *Ikkα<sup>AA</sup>* ( $n = 3$ ) mice were isolated by FACS and cultured under the Th17-inducing condition as described in Materials and methods. 24 h later, total RNA was isolated and mRNA levels of the Th17 lineage genes were assessed by real-time RT-PCR. The lowest expression level of each gene was set to 1. Data are representative of two independent experiments. \*,  $P < 0.001$ .



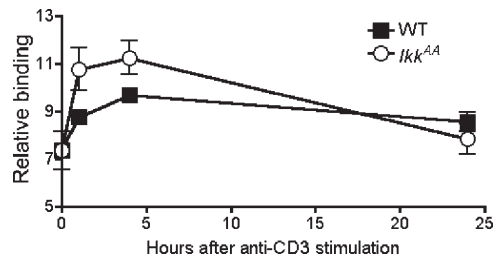
**Figure S3. Reduced IL-17A hnRNA expression in *Ikkα<sup>AA</sup>* CD4<sup>+</sup> T cells.** Flow-sorted CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>low</sup>CD62L<sup>+</sup> T cells from WT ( $n = 3$ ) and *Ikkα<sup>AA</sup>* ( $n = 3$ ) mice were cultured under the Th17-inducing condition. 24 h later, total RNA was isolated and treated with RNase-free DNase I. Reverse transcription was performed using hexamers, and IL-17A hnRNA levels were assessed by PCR using *Il17a* intron-specific primers that gave rise to a 250-bp product. The experiments were repeated three times with similar results.



**Figure S4. Normal RORγ/RORγt protein expression in *Ikkα<sup>AA</sup>* CD4<sup>+</sup> T cells.** Flow-sorted CD4<sup>+</sup>CD25<sup>-</sup>CD44<sup>low</sup>CD62L<sup>+</sup> T cells from WT ( $n = 3$ ) and *Ikkα<sup>AA</sup>* ( $n = 3$ ) mice were cultured under the Th17-inducing condition. 36 h later, cell lysates were prepared and Western blot was performed using antibodies to β-actin, or RORγ and RORγt (mol wt 58 kD). Data are representative of two independent experiments.



**Figure S5. Binding of RNA polymerase II to the *IL17*, *IL21*, and *IL22* promoters.** Purified naive CD4<sup>+</sup> T cells from 8-wk-old WT ( $n = 4$ ) and *IKKα<sup>AA</sup>* mice ( $n = 4$ ) were cultured under Th17 differentiation conditions, as described in Materials and methods. At the indicated times, cells were fixed and ChIP was performed using antibodies to RNA polymerase II or control IgG. Primers used to perform ChIP-PCR were designed to cover the core promoters of murine *IL17*, *IL21*, and *IL22* genes that gave rise to products of 167p, 150, and 180 bp, respectively. Data are representative of two independent experiments.



**Figure S6. Nuclear RelA/p65 activities as determined by ELISA.** Purified naive CD4<sup>+</sup> T cells from WT ( $n = 3$ ) and *IkKα<sup>AA</sup>* ( $n = 3$ ) mice were stimulated with 10 μg/ml plate-bound anti-CD3 and 10 μg/ml plate-bound anti-CD28 for the indicated times. Nuclear protein extracts were added to streptavidin-coated plates bound with biotinylated NF-κB consensus nucleotides. The captured active NF-κB was then detected with anti-RelA/p65 and HRP-conjugated secondary antibody. Results are representative of three independent experiments. The differences between the two groups are not statistically significant. Error bars represent the SEM.