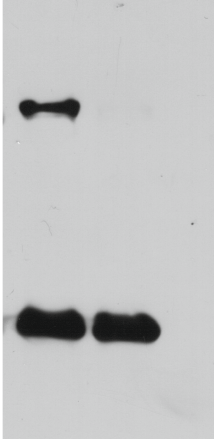


Supplemental Data

1) Knockdown of KSRP protein in CD4⁺ T cells

We performed western blot analyses to demonstrate loss of KSRP protein expression in CD4⁺ T cells derived from KSRP^{-/-} (KO) mice. Therefore, CD4⁺ T cells from KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice were isolated by magnetic bead separation as described in the method section of the main manuscript. To obtain protein extracts for western blot experiments, CD4⁺ T cells were lysed in RIPA buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2mM EDTA, 10% glycerol, 1% NP40, 1x complete EDTA-free protease and phosphatase inhibitor cocktail) using an ultrasonic homogenizer. Protein concentration was determined using Protein Assay Dye Reagent (Bio-Rad, München, Germany) as described by the manufacturer and 100 µg of CD4⁺ T cell extract were separated by SDS-gel electrophoresis. KSRP protein expression was analyzed with a specific anti-KSRP antibody (abcam, ab229660). For normalization, the expression of GAPDH was analyzed in parallel using a specific anti-GAPDH antibody (Santa Cruz Biotechnology, 32233, Dallas, USA). The immunoreactive proteins on the blot were visualized by the enhanced chemiluminescence detection system (Thermo Fisher Scientific, Darmstadt, Germany).

WT	KO	
		
		KSRP (75 kD)
		GAPDH (36 kD)

Sup. Figure 1: Knockdown of KSRP protein in CD4⁺ T cells

Expression of KSRP in CD4⁺ T cells from KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice, isolated by magnetic bead separation, was analyzed by western blot experiments using a specific anti-KSRP antibody. For normalization, the expression of GAPDH was analyzed in parallel using a specific anti-GAPDH antibody. The blot is representative of two blot showing similar results.

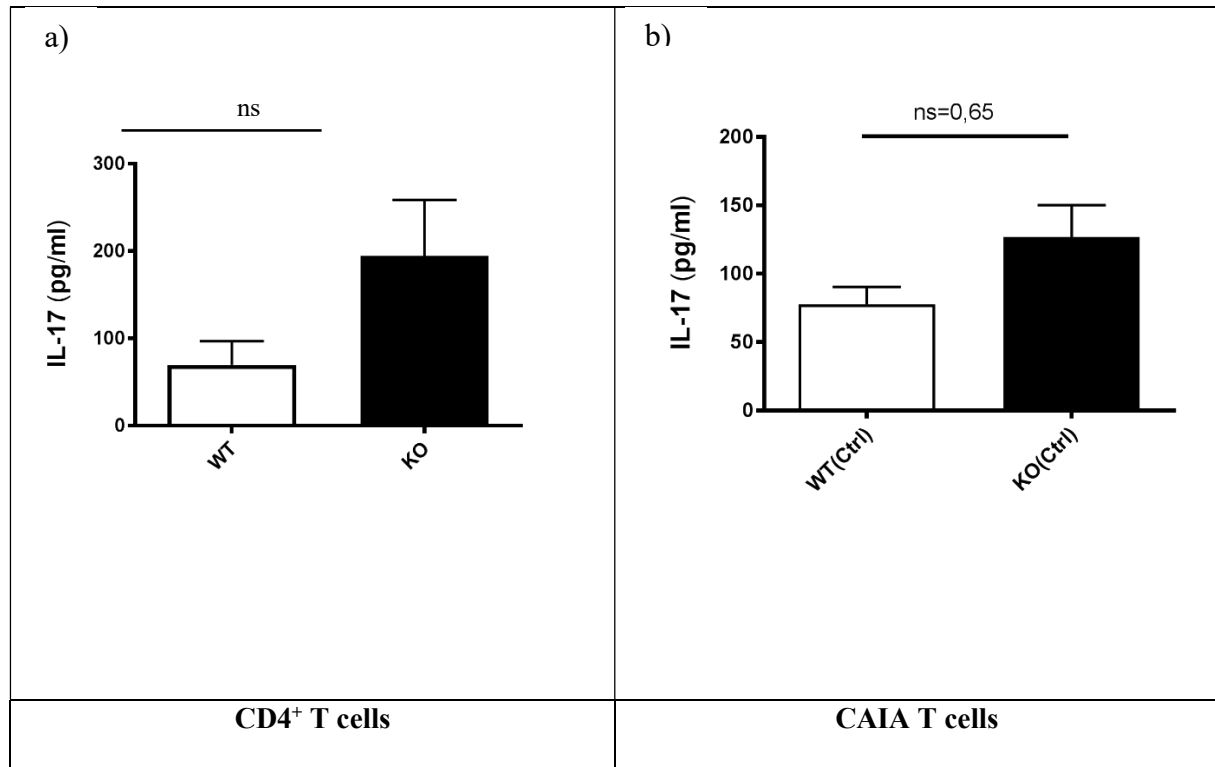
2) T cell numbers in KSRP^{-/-} mice

To figure out whether KSRP deficiency effect the T cell compartment we isolated total spleen cells from KSRP^{+/+} (**WT**) and KSRP^{-/-} (**KO**) mice. Spleens were removed and erythrocytes within the spleen cell suspension were lysed by incubation of cells for 1 min in hypotonic buffer (155 mM NH₄Cl, 10 mM KHCO₃, 100 μM EDTA-disodium, pH 7.4). All cells were incubated with PE-anti-CD3, APC-anti-CD4, eFluor450-anti-CD8 mAbs and FITC-anti-CD25 mAb. Cells were analyzed using a FACS Canto II. Data are shown are the mean MFI ± SEM (n=2-3 animals per genotype) and are pooled from 2-3 independent experiments. (ns = not significant, unpaired T-test).

	KSRP WT	KSRP^{-/-}	
CD 4⁺	13348.67 ± 4403.45	12799.33 ± 4655.84	ns
CD 8⁺	1957.00 ± 117.95	1837.00 ± 49.15	ns

Sup. Table 1: Mean Fluorescence Intensity (MFI) of CD4⁺ and CD8⁺ T cells in KSRP^{-/-} mice

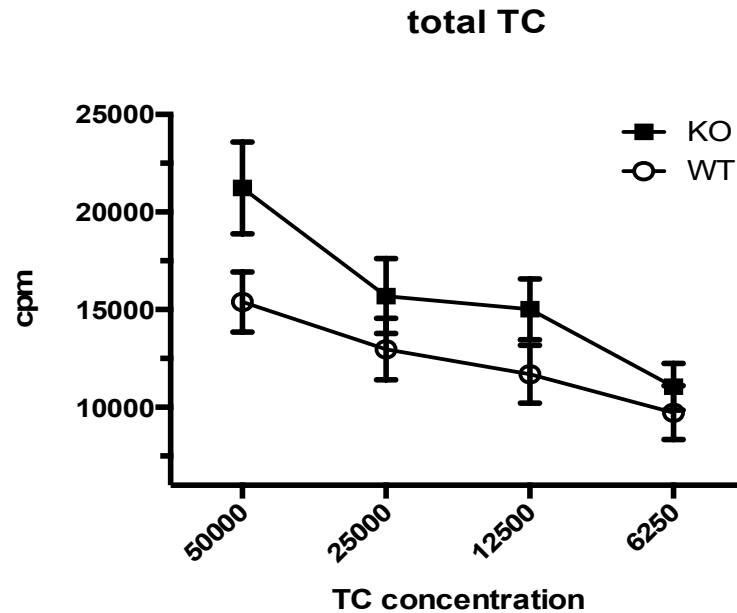
3) IL-17 production in KSRP^{-/-} T cell



Sup. Figure 2: Effect of inactivation of the KSRP gene on T cell mediated IL-17 production

(a) In supernatants of polyclonally stimulated CD4⁺ T cells isolated by magnetic bead separation from KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice the IL-17 content was measured by CBA. Shown data are the mean + SEM (n=3 animals per genotype). No statistically significant differences were detected. (b) Nylon wool-enriched T cells isolated from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice treated without CII-specific mAbs (Ctrl) were polyclonally stimulated with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) specific antibodies for 96 h. IL-17 expression was measured in the supernatant of the cells using CBA. Data are shown are the mean IL-17 production in pg/ml + SEM (n=10-15 animals per genotype and are pooled from four independent experiments. (ns. = not significant different from wildtype mice; unpaired T-test).

4) Proliferation of total splenic T cells

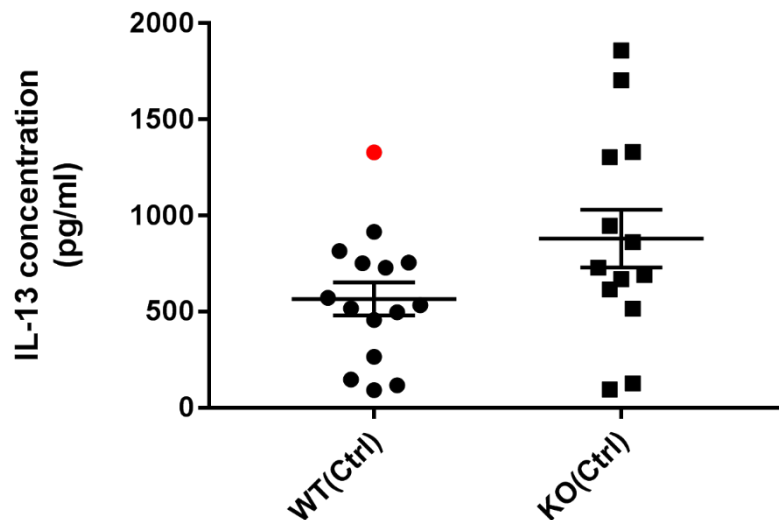


Sup. Figure 3: Proliferation of total T cells isolated from spleen

Inactivation of the KSRP gene enhances proliferation of polyclonally stimulated T cells

T cells were isolated from spleen cells of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice via nylon wool enrichment and were polyclonally stimulated with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) specific antibodies for 96h. In order to assess the proliferation of T cells, ³H thymidine (0.5 µCi/well) was applied for the last 16-18h of culture incubation. Total ³H thymidine uptake is presented. Data are shown are the mean ± SEM (n=20-25 animals per genotype) and are pooled from five independent experiments.

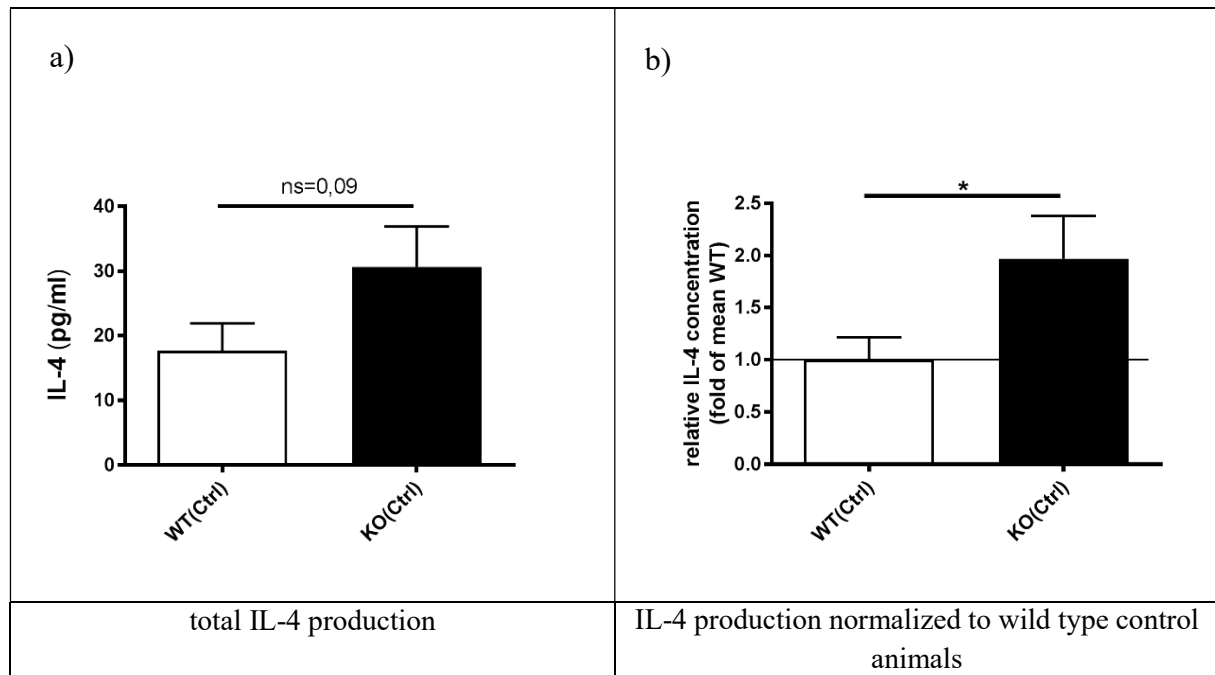
5) IL-13 production in KSRP^{-/-} T cells



Sup. Figure 4: Effect of KSRP knockdown on T cell mediated IL-13 cytokine production in CAIA-treated mice

KSRP^{+/+} (WT) and KSRP^{-/-} (KO) were treated on day 0 with a cocktail of 5 different collagen-II-specific mAbs (AB) or with PBS (Ctrl) as control. On day 3 the animals were treated with LPS (50 µg/animal) or PBS as control. At day 5 and day 9 mice (n = 10-15) of each treatment group were killed for subsequent analyses. Nylon wool-enriched T cells isolated from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice treated with (AB) or without (Ctrl) CII-specific mAbs were polyclonally stimulated with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) specific antibodies for 96 h. IL-13 expression was measured in the supernatant of the cells using CBA. Data shown are the mean ± SEM (n=10-15 animals per genotype) and are pooled from four independent experiments. Without red marked value statistical difference (* = p < 0.05 from wildtype mice, unpaired T-test).

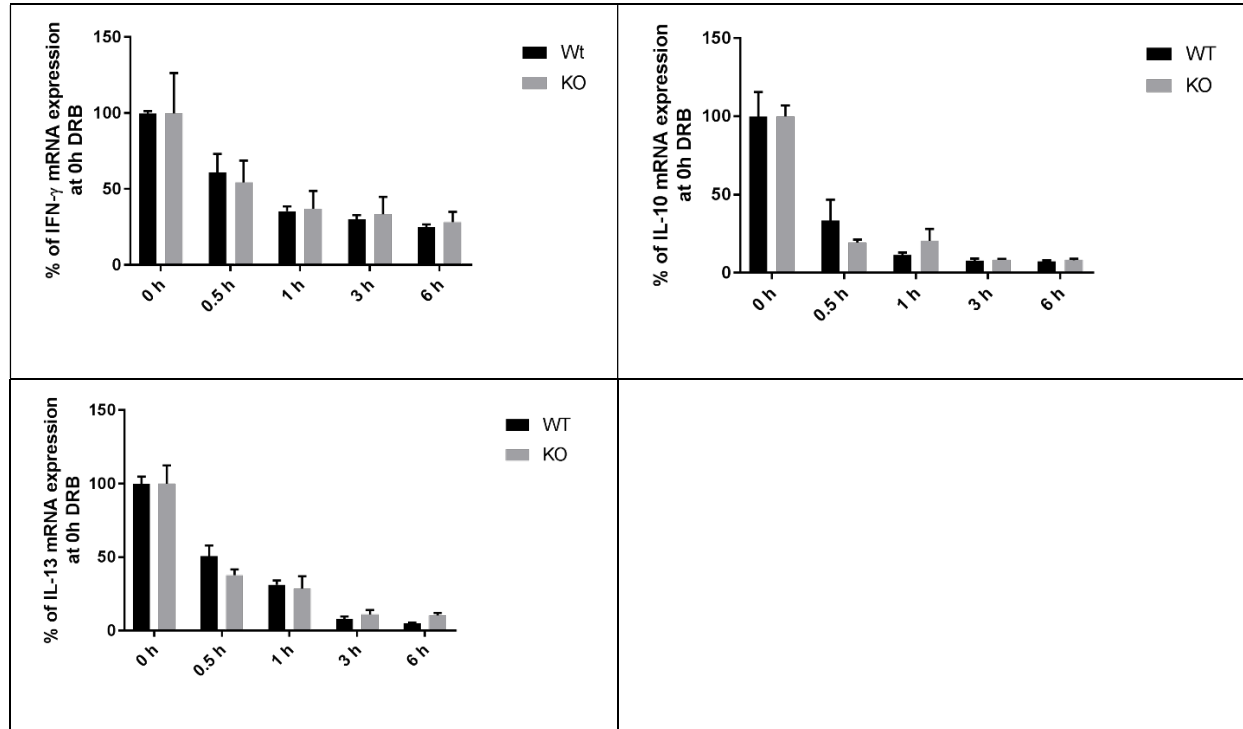
6) IL-4 production in KSRP^{-/-} T cells



Sup. Figure 5: Effect of KSRP knockdown on T cell mediated IL-4 production in control mice of CAIA experiments

Nylon wool-enriched T cells isolated from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice treated without (Ctrl) CII-specific mAbs were polyclonally stimulated with anti-CD3 (1 µg/ml) and anti-CD28 (2 µg/ml) specific antibodies for 96 h. IL-4 expression was measured in the supernatant of the cells using CBA (a). Data are shown are the mean IL-4 production in pg/ml + SEM (n=10-15 animals per genotype and are pooled from four independent experiments. (ns. = not significantly different from wildtype mice; unpaired T-test). (b). To normalize for the inter-individual cytokine production, IL-4 expression of KSRP^{-/-} (KO) T cells was normalized to that of KSRP^{+/+} (WT) T cells. The IL-4 expression of KSRP^{+/+} T cells was set to 1. Data are shown are the mean + SEM (n=10-15 animals per genotype and are pooled from four independent experiments. (* = p < 0.05 from wildtype mice, unpaired T-test).

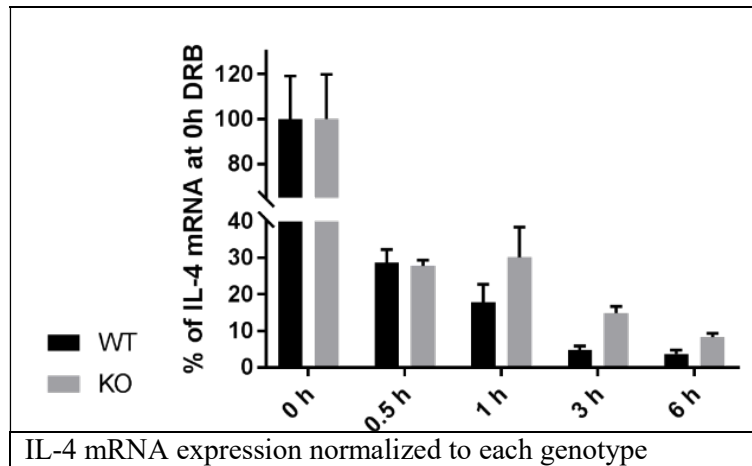
7) mRNA decay analyses in KSRP^{-/-} T cells



Sup. Figure 6: mRNA decay analyses

CD4⁺ T cells from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice were isolated by magnetic bead separation, and were polyclonally stimulated with CD3/CD28 antibodies for 24h. Then 25 μ g/ml 6-dichloro-1-ribofuranosylbenzimidazole (DRB) (Sigma, Deisenhofen, Germany) was added to examine the influence of KSRP on the stability of IFN- γ , IL-10 or IL-13 mRNA. RNAs were prepared 0, 0.5, 1, 3 and 6 h thereafter. mRNA amount was determined by qRT-PCR by normalizing to 18 S rRNA and GAPDH expression. The relative mRNA amount at 0 h DRB was set at 100% for each genotype. Data shown are the means + SEM of two analyses.

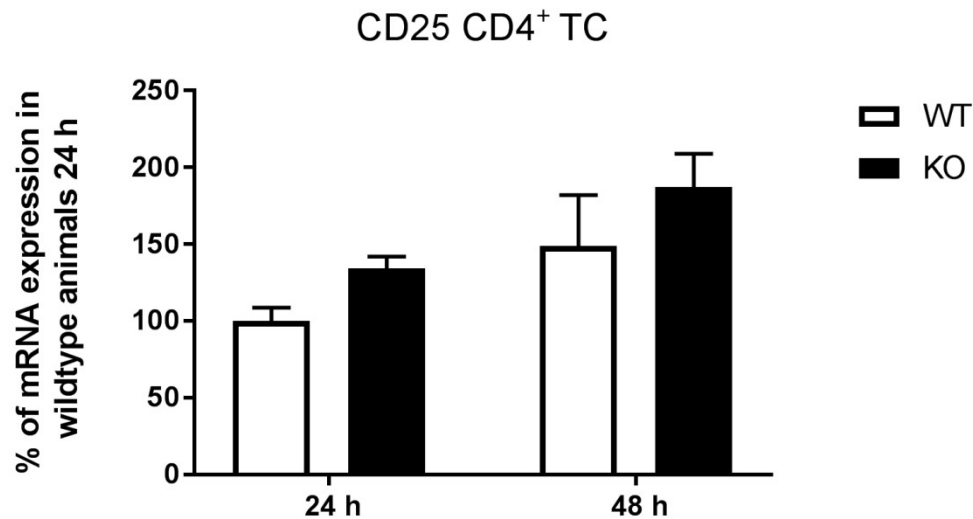
8) IL-4 mRNA decay in KSRP^{-/-} T cells



Sup. Figure 7: *IL-4 mRNA stability analyses*

CD4⁺ T cells from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice were isolated by magnetic bead separation, and were polyclonally stimulated with CD3/CD28 antibodies for 24h. Then 25 µg/ml 6-dichloro-1-ribofuranosylbenzimidazole (DRB) (Sigma, Deisenhofen, Germany) was added to examine the influence of KSRP on the stability of IL-4 mRNA. RNAs were prepared 0, 0.5, 1, 3 and 6 h thereafter. mRNA amount was determined by qRT-PCR by normalizing to 18 S rRNA and GAPDH expression. The relative mRNA amount at 0 h DRB was set at 100% for each genotype.

9) CD25 mRNA expression KSRP^{-/-} T cells



Sup. Figure 8: *CD25* mRNA expression in CD4⁺ T cells of KSRP^{-/-} mice

In CD4⁺ T cells from KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice mRNA expression of CD 25 measured by qRT-PCR 24h and 48h after polyclonal stimulation of the cells. The mRNA expression was normalized to Pol2a mRNA expression. The mRNA expression of KSRP^{+/+} mice polyclonal stimulated for 24h was set to 100%. Shown data are the mean + SEM (n=3-4 animals per genotype) and are pooled from two technical replications.