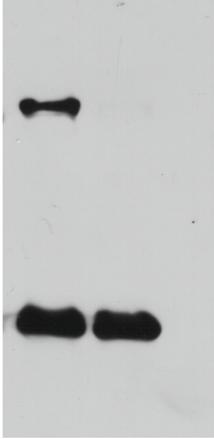


Supplemental Data

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	
		<p>Sup. Figure 1: Knockdown of KSRP protein in CD4⁺ T cells</p> <p>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.</p>
		KSRP (75 kD)
		GAPDH (36 kD)

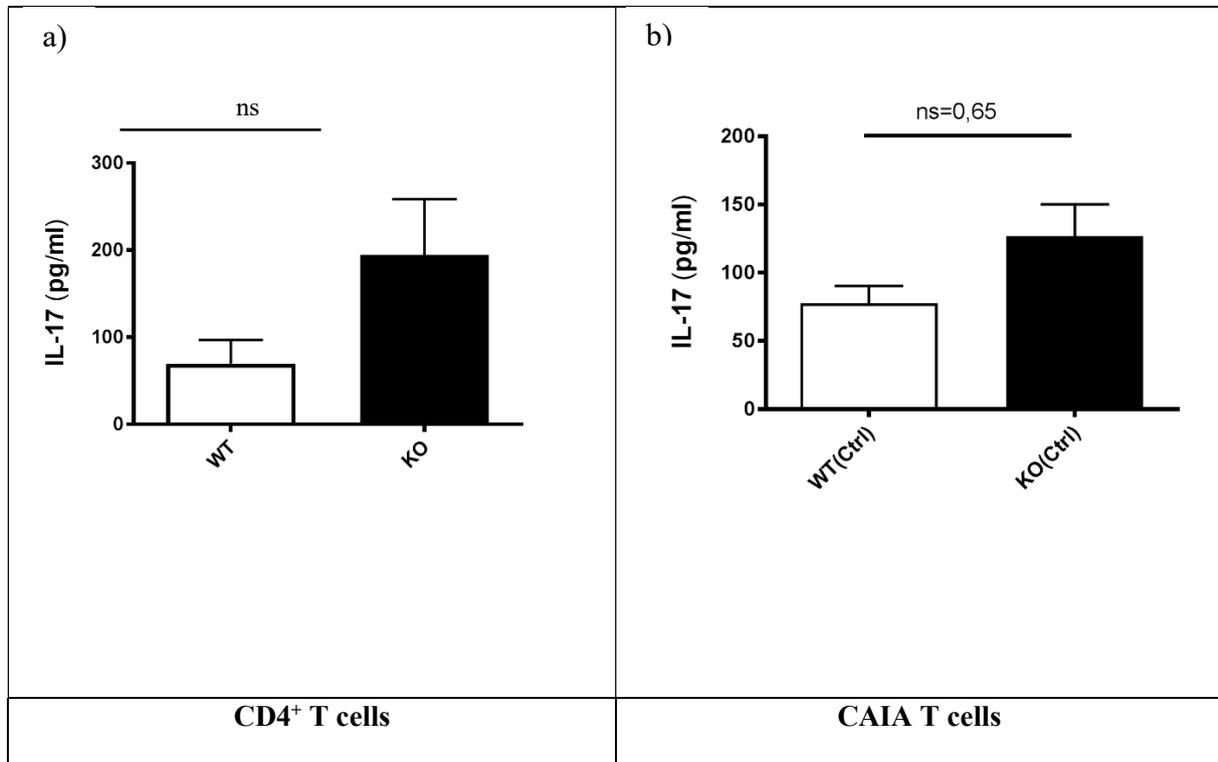
2) T cell numbers in KSRP^{-/-} mice

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 nM 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

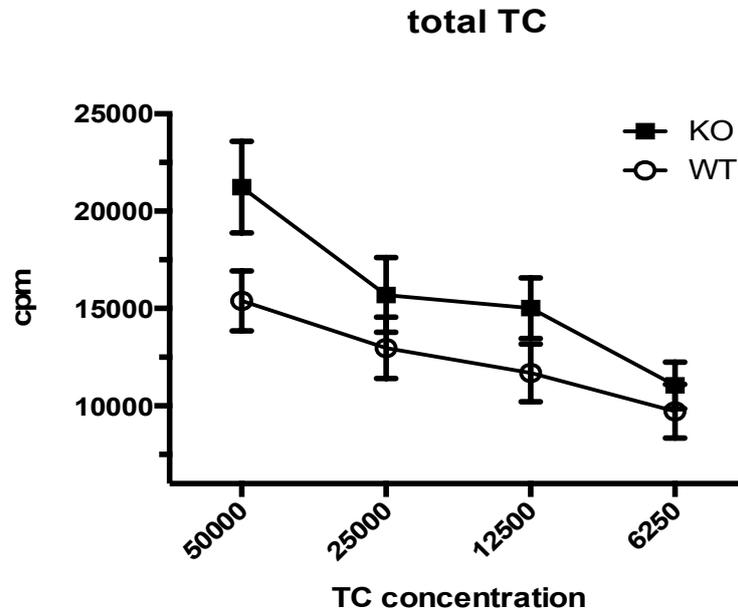
3) IL-17 production in KSRP^{-/-} T cell



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

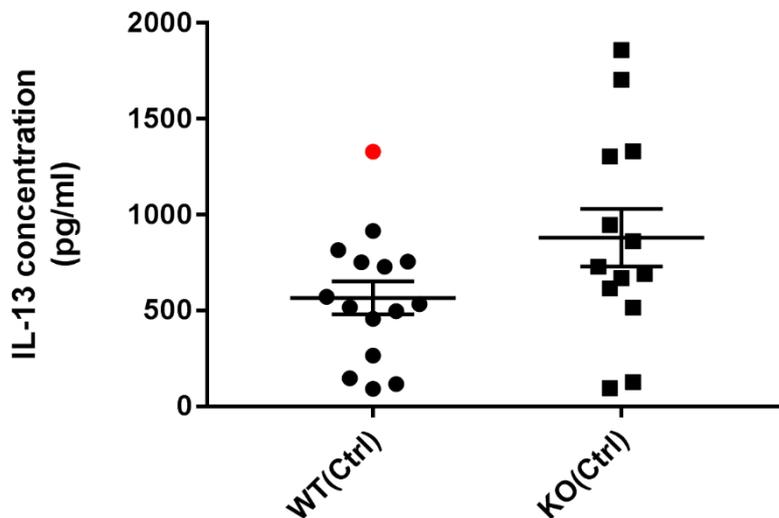


Sup. Figure 3: Proliferation of total T cells isolated from spleen

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.

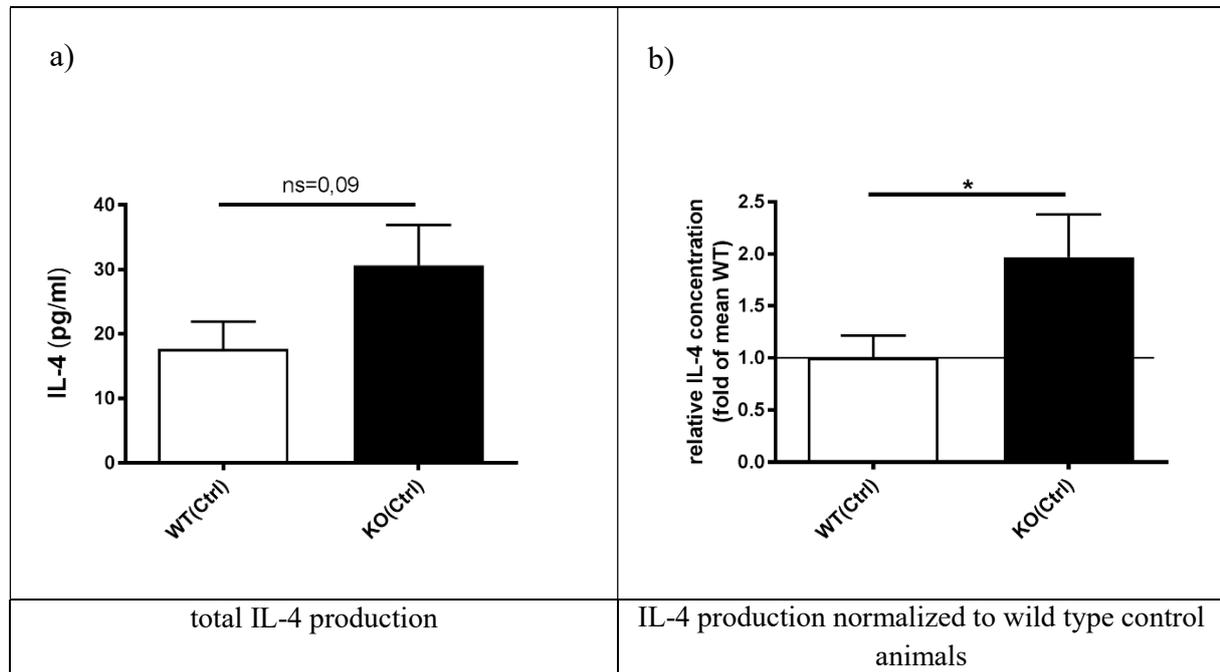
5) IL-13 production in KSRP^{-/-} T cells



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).

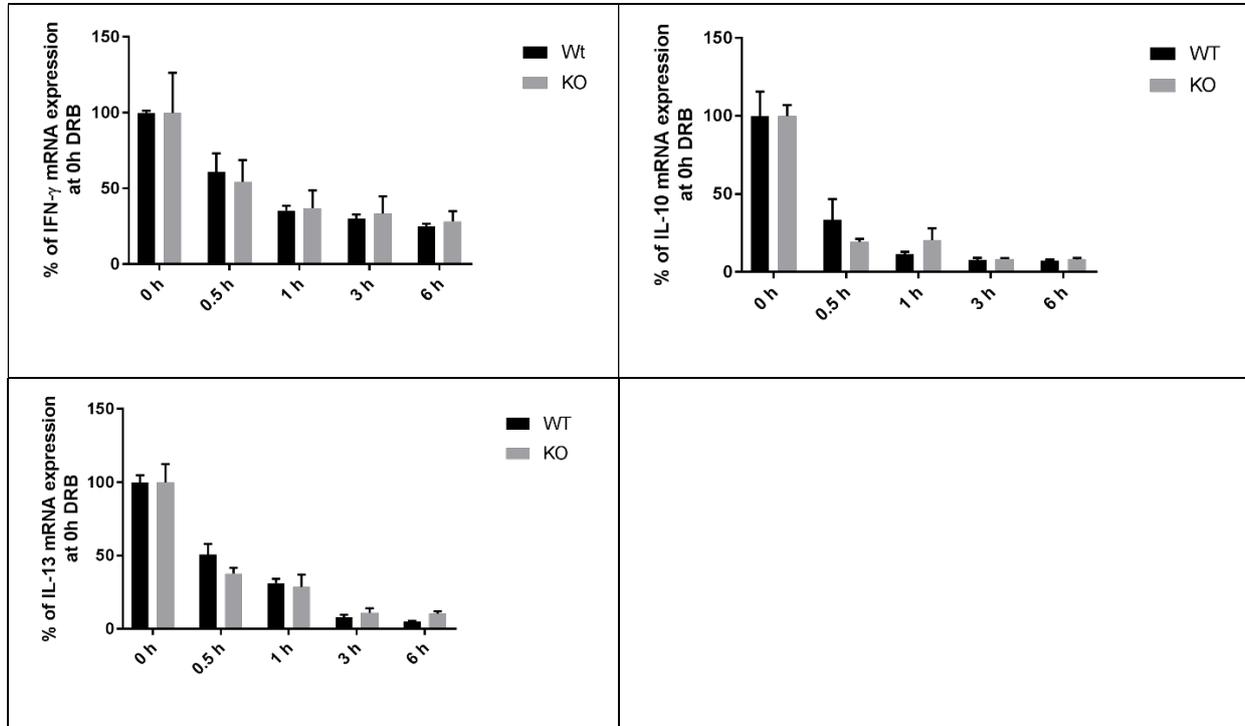
6) IL-4 production in KSRP^{-/-} T cells



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).

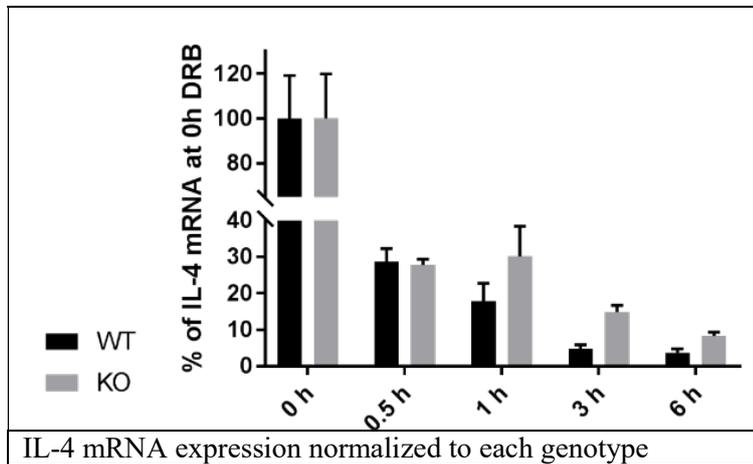
7) mRNA decay analyses in KSRP^{-/-} T cells



Sup. Figure 6: mRNA decay analyses

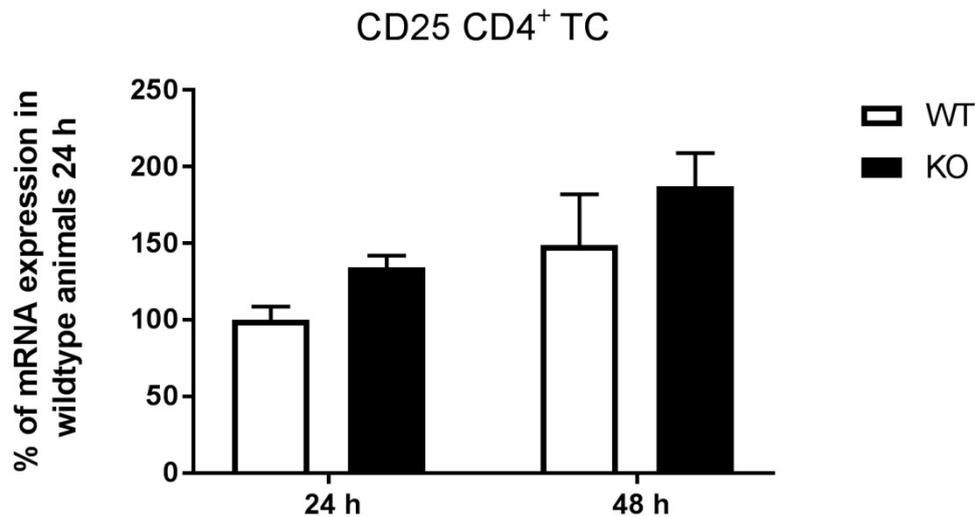
CD4⁺ T cells from spleens of KSRP^{+/+} (WT) and KSRP^{-/-} (KO) mice were isolated by magnetic bead separation, and were polyclonally stimulated with CD3/CD28 antibodies for 24h. Then 25 µg/ml 6-dichloro-1-ribofuranosylbenzimidazole (DRB) (Sigma, Deisenhofen, Germany) was added to examine the influence 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.