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Supplementary Materials for

T cell receptor stimulation impairs IL-7 receptor signaling by inducing expression of the microRNA *miR-17* to target Janus kinase 1

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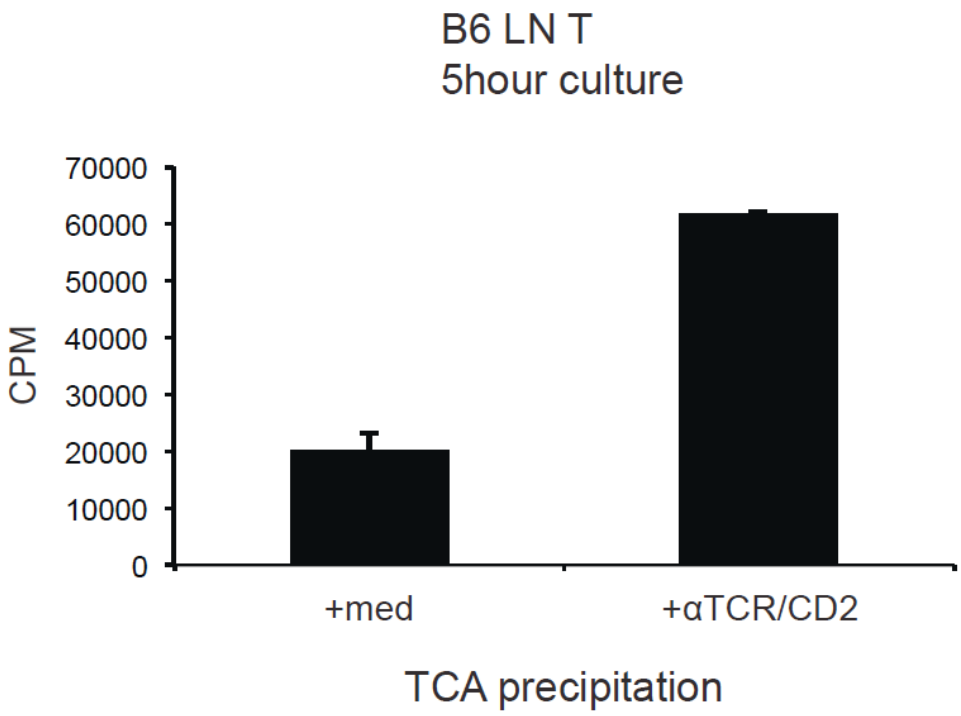

Fig. S1. Analysis of total protein synthesis in unstimulated and TCR-stimulated cells. LN T cells from B6 mice were cultured either with medium (med) or with αTCR/CD2 for 5 hours and then were metabolically labeled for 30 min. TCA-precipitable counts were obtained from cell lysates and are displayed as mean cpm ± SD from three independent experiments.

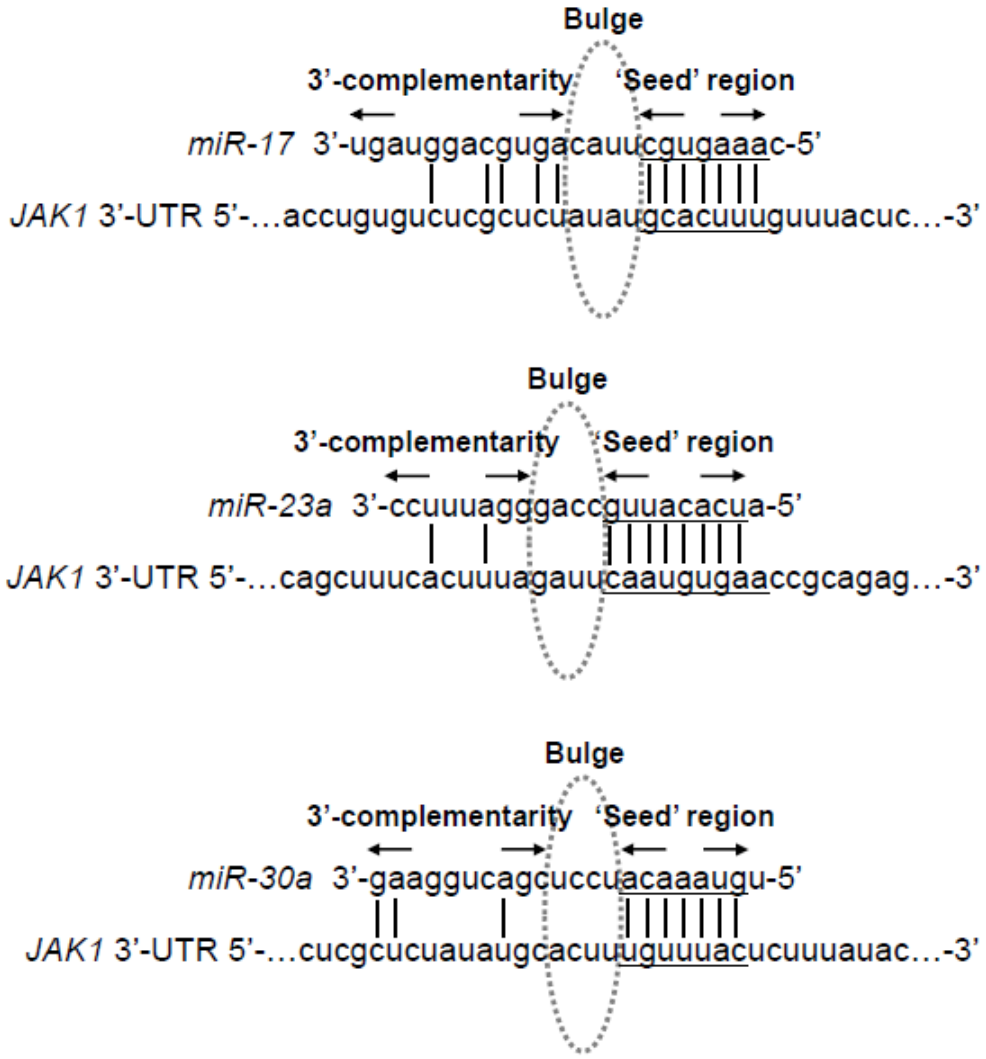

Fig. S2. Imperfect match for microRNAs that potentially bind to the 3′UTR of *Jak1* mRNA. Shown are "seed" regions (underlined), predicted bulges (dotted ovals), and 3′-complementarity sequences for *miR-17*, *miR-23a*, and *miR-30a*. Vertical lines indicate nucleotide bonding [adapted from (16)].

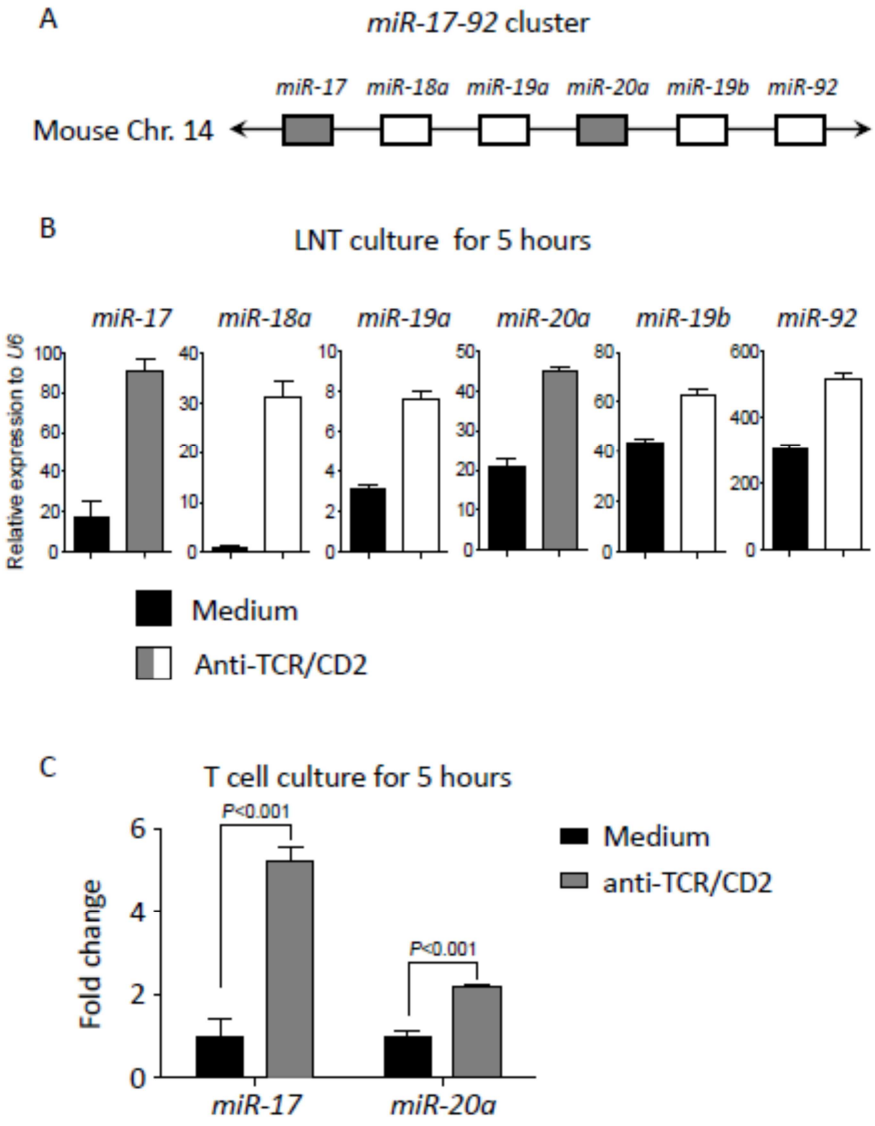

Fig. S3. Effect of TCR signaling on the expression of microRNAs in the *miR-17~92* cluster. (**A**) Schematic of the mouse *miR-17~92* cluster on chromosome 14. Gray shading indicates that *miR-17* and *miR-20a* are both members of the *miR-17* family and contain the same seed region. (**B**) LN T cells were cultured for 5 hours in medium alone or with αTCR/CD2. The relative abundances of the indicated microRNAs in the *miR-17~92* cluster, relative to that of snoRNA U6, were determined by quantitative PCR analysis. (**C**) The fold-increases in the abundances of *miR-17* and *miR-20a* in TCR-stimulated cells relative to that in cells cultured in medium alone were determined. Data in (B) and (C) are means ± SEM of quadruplicate samples from a single experiment, which is representative of two independent experiments.

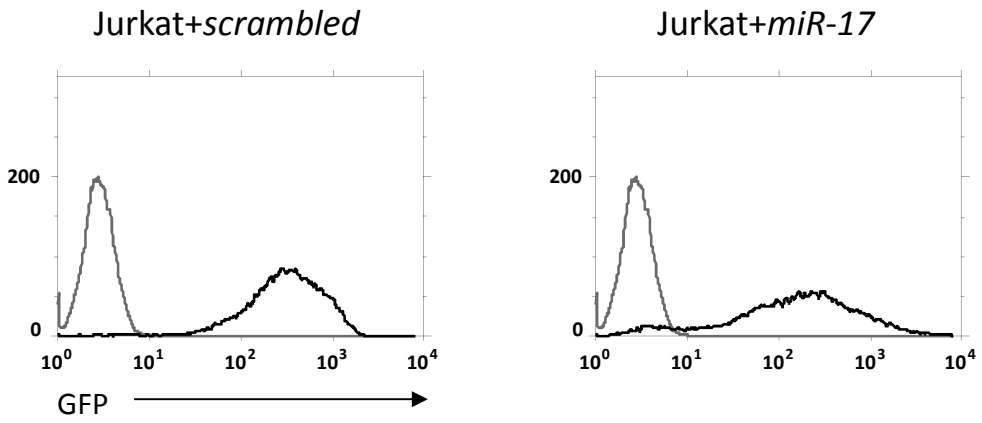

Fig. S4. Analysis of GFP abundance in Jurkat cells stably transfected with plasmids encoding GFP and either scrambled control microRNA or *miR-17*. Cells were analyzed by flow cytometry. Data are from the same cells shown in Fig. 4C. The gray line indicates untransfected cells. Data are representative of four independent experiments.

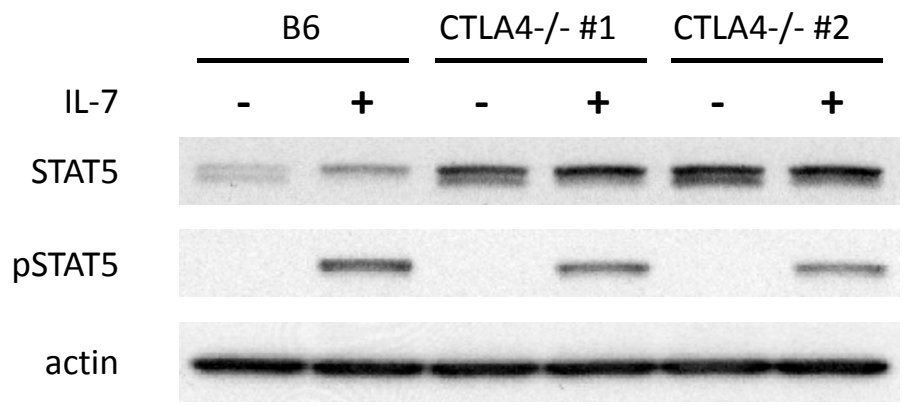

Fig. S5. IL-7 signaling is impaired in *Ctla4*$^{-/-}$ mice. LN T cells isolated from B6 mice and two individual *Ctla4*$^{-/-}$ mice were left untreated (-) or were incubated with IL-7 for 20 min. Cell lysates were analyzed by Western blotting with antibodies against the indicated proteins. Quantification of the relative abundance of pSTAT5 normalized to that of total STAT5 is displayed in Fig. 5D. Western blots are representative of two independent experiments.

Table S1. Primers used for real-time PCR analysis. F, forward; R, reverse.

Primer	Sequence
mJAK1-F	5'-AGAACCTGAGTGTGGCTGCT-3'
mJAK1-R	5'-TGTTGTTGGCTGCTTTTCTG-3'
mRPL13-F	5'-CGAGGCATGCTGCCCCACAA-3'
mRPL13-R	5'-AGCAGGGACCACCATCCGCT-3'
hJAK1-F	5'-TACCTCTATGGCGTCTGTGTC-3'
hJAK1-R	5'-TGGTAAGGACATCGCTTTTCC-3'
hRPL13-F	5'-CCCTTCCCGAGGCCCCTACC-3'
hRPL13-R	5'-GGCGGTGGGATGCCGTCAAA-3'