Interleukin 17 treatment prolongs CXCL1 mRNA half-life via TRAF5 and the splicing regulatory factor SF2/ASF

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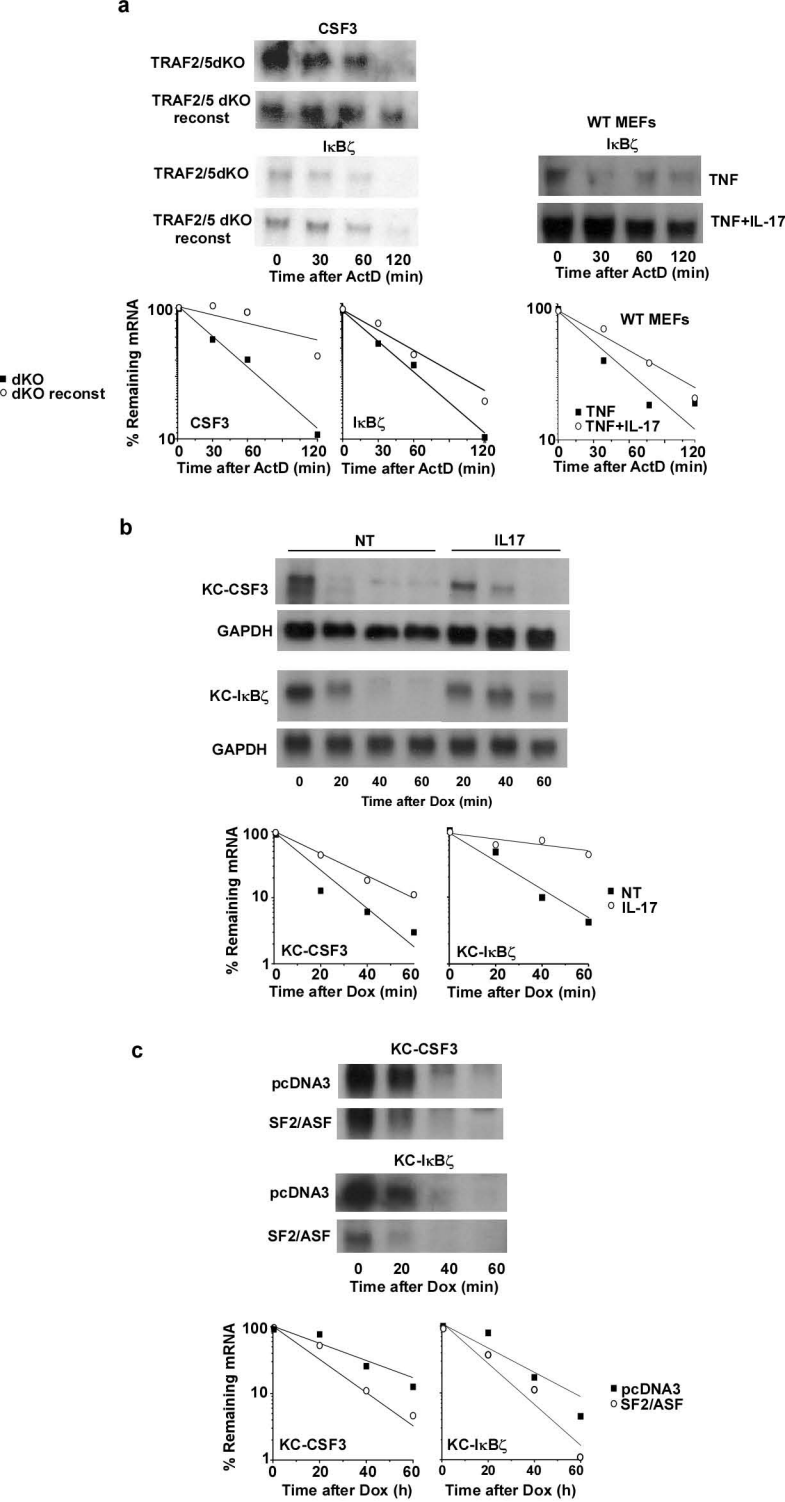
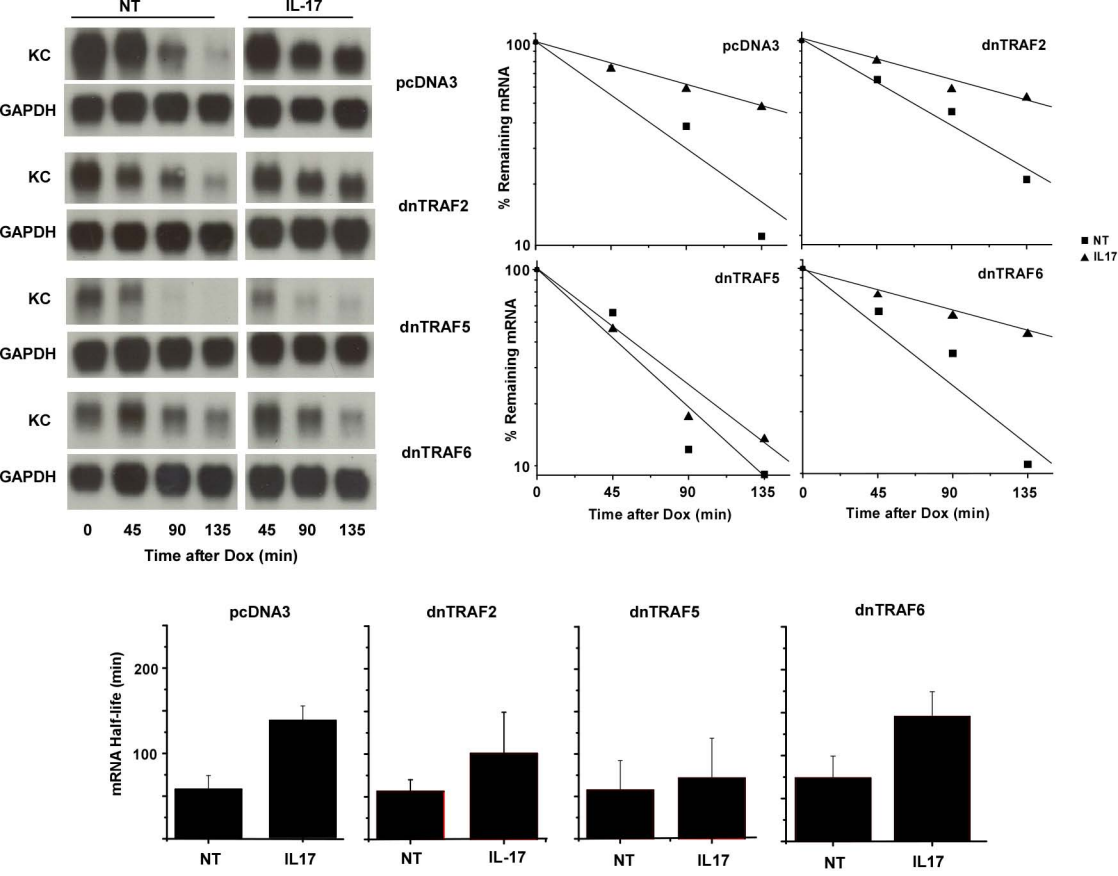
Supplementary Figures

Supplementary Figure 1. Post-transcriptional control of CSF3 and IκBζ mRNA abundance **a.** Wildtype MEFs were treated with TNF (10 ng/ml) alone or TNF+IL17 (25 ng/ml) for 2 hrs while TRAF2/TRAF5 deficient MEFs and TRAF-reconstituted MEFs were treated with TNF + IL-17 for 2 h. ActD (5 μg/ml) was added and after the indicated times abundance of CSF3 and IκBζ mRNA was determined by RNA hybridization. Individual mRNA measurements were normalized to the quantity of 18S ribosomal RNA on the blots. While IκBζ mRNA was readily detected in both wild-type and TRAF-deficient cells, CSF3 mRNA could be detected only in the immortalized TRAF-deficient MEFs. Similar results were obtained in two independent experiments. **b.** HeLa tet-off cells were transfected with KC-CSF3 or KC-IκBζ reporter constructs and treated with Dox (1 μg/ml) alone or in combination with IL-17 (25 ng/ml) for the indicated times prior to analysis of CXCL1 and GAPDH mRNA by RNA hybridization. Reporter mRNA quantity was normalized to GAPDH in the same sample. Similar results were obtained in two independent experiments. **c.** HeLa tet-off cells were co-transfected with KC-CSF3 or KC-IκBζ reporters and either pcDNA3 or expression plasmid encoding full length SF2/ASF. Dox (1 μg/ml) was added and remaining CXCL1 and GAPDH (not shown) mRNA was quantified after the indicated times by RNA hybridization as described in b. Similar results were obtained in two independent experiments.



Supplementary Figure 1

Supplementary Figure 2. dnTRAF5 interferes with IL-17-mediated CXCL1 mRNA stabilization HeLa tet-off cells were co-transfected with KCΔ4 and either pcDNA3 or dn versions of TRAF2, TRAF5 or TRAF6. Dox was added alone or along with IL-17 (25 ng/ml) and the remaining CXCL1 and GAPDH mRNA was measured by RNA hybridization. The autoradiographs were quantified and the half-lives calculated as described in the legend to Figure 1 using results from 2 independent experiments.



Supplementary Figure 2