

Supplementary Materials for **Intracellular Delivery of a Cell-Penetrating SOCS1 that Targets IFN- γ Signaling**

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Fig. S4. Transfection of HEK 293T cells with plasmids encoding non-CP-SOCS1 or CP-SOCS1 inhibits IFN- γ -induced production of chemokines.

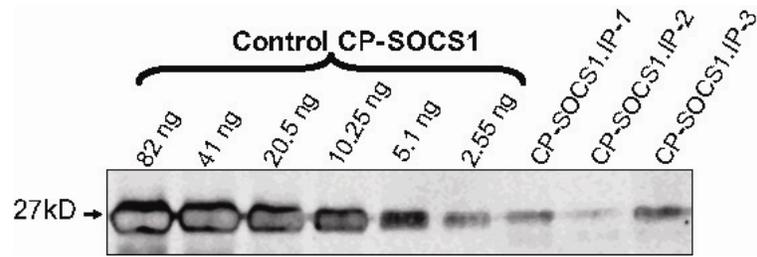


Fig. S1. Quantification of intracellular CP-SOCS1. CP-SOCS1 immunoprecipitated from treated cells following proteinase K treatment and washing was quantified by analysis of Western blots with a polyclonal antibody specific for SOCS1 in comparison with known concentrations of purified CP-SOCS1.

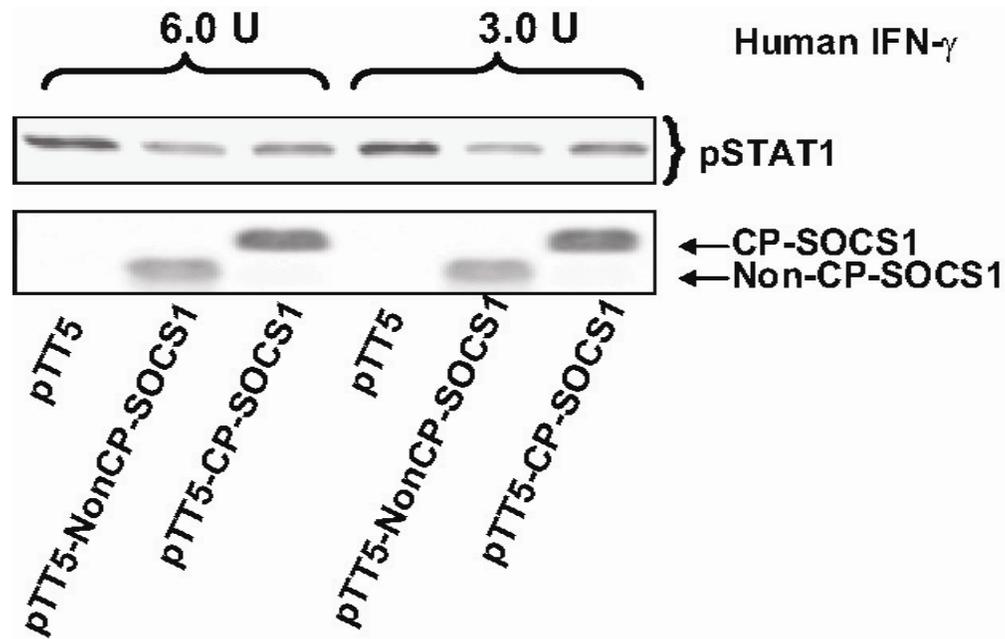


Fig. S2. Transfection of HEK 293F cells with plasmids encoding CP-SOCS1 or non-CP-SOCS1 results in a similar inhibition of STAT1 phosphorylation. HEK 293F cells were transiently transfected with the plasmids pTT5, pTT5-non-CP-SOCS1, or pTT5-CP-SOCS1 followed by stimulation with the indicated amounts of human IFN- γ . Cells containing non-CP-SOCS1 or CP-SOCS1 displayed similar inhibition of IFN- γ -induced phosphorylation of STAT1, whereas cells transfected with the control plasmid did not show inhibition of STAT1 phosphorylation in response to IFN- γ .

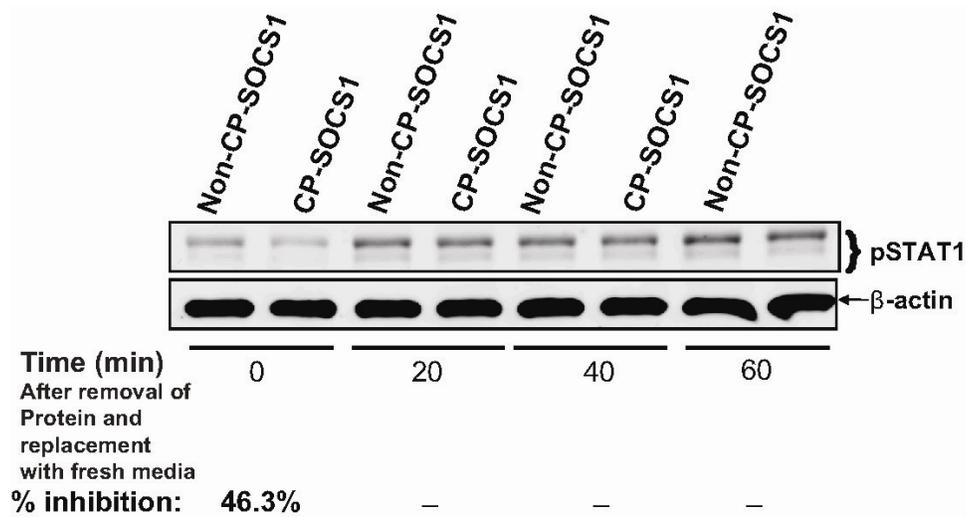


Fig. S3. The action of intracellular CP-SOCS1 is short-lived and reversible. AMJ2.C8 cells were treated with non-CP-SOCS1 (~2.5 μ M) or CP-SOCS1 (~2.5 μ M) for one hour at 37°C. Following incubation, the proteins were removed and replaced with tissue culture medium and the cells were stimulated with 2 U/ml of IFN- γ at the indicated time points. The inhibition of IFN- γ -induced phosphorylation of STAT1 by CP-SOCS1 was time-limited and reversible following removal of the recombinant protein.

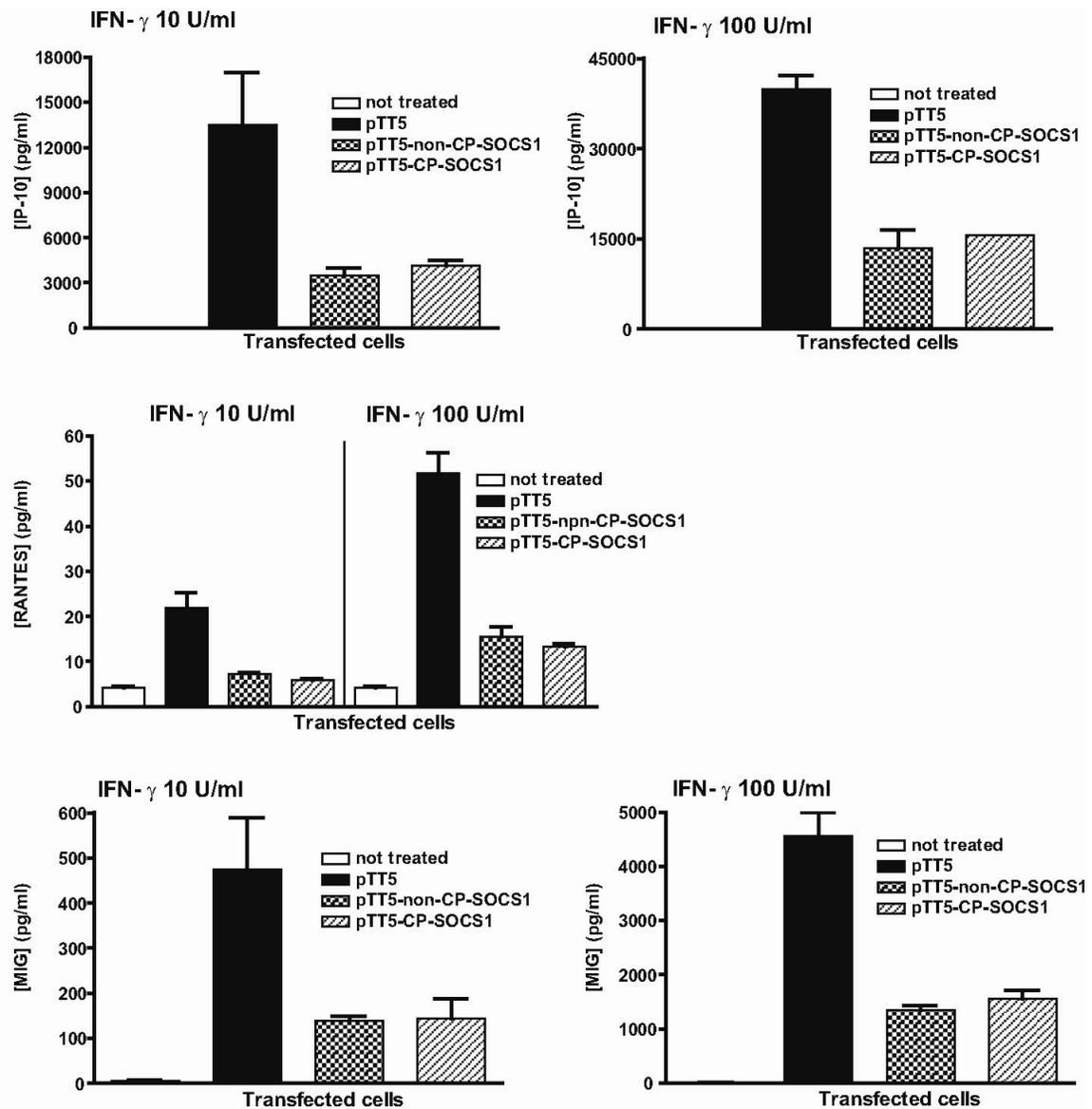


Fig. S4. Transfection of HEK 293T cells with plasmids encoding non-CP-SOCS1 or CP-SOCS1 inhibits IFN- γ -induced production of chemokines. HEK 293T cells were transiently transfected with pTT5, pTT5-non-CP-SOCS1, or pTT5-CP-SOCS1 and then stimulated with the indicated amounts of human IFN- γ and 0.1 ng/ml of IL-1 β . Supernatants were sampled after 24 hours and analyzed with a human chemokine cytometric bead array kit. Chemokine production by cells transfected with non-CP-SOCS1 or CP-SOCS1 was inhibited when compared to that of cells transfected with the control plasmid.