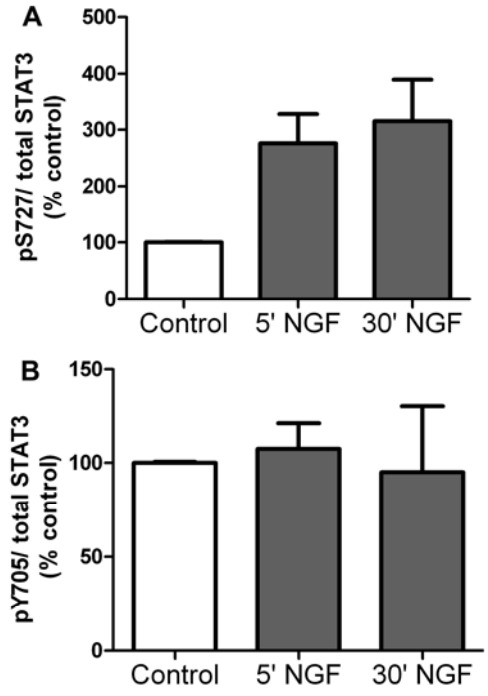
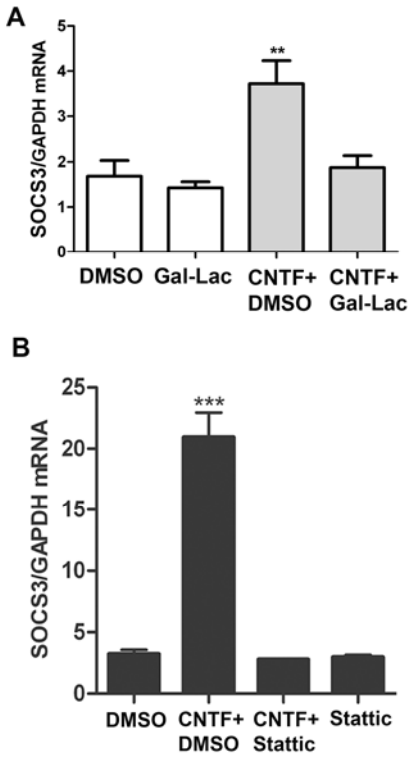
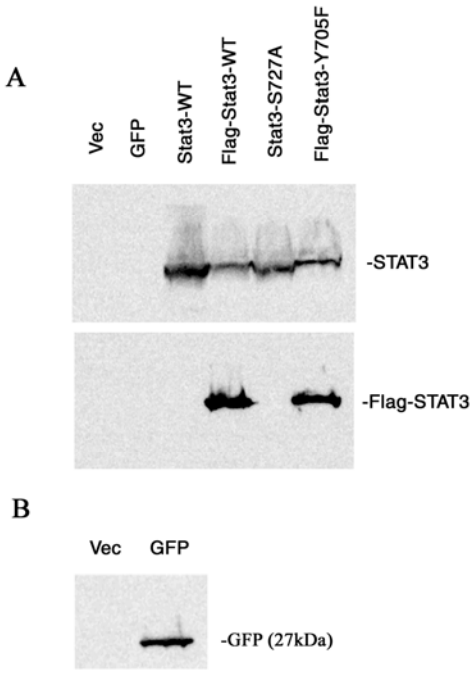
Supplementary Data



Supplementary Figure 1. NGF stimulates significant phosphorylation of S727 (A) on STAT3, but not Y705 (B). Sympathetic neurons maintained in 10ng/ml NGF were stimulated with NGF (100ng/ml) for the indicated time points and western blotting was performed for phospho-STAT3. The ratio of PhosphoSTAT3/total STAT3 was calculated for each residue for 3 independent experiments and averaged as % of control (Mean ± SEM).



Supplementary Figure 2. Blocking STAT3 DNA binding (A) or phosphorylation (B) prevents STAT3 induction of SOCS3 mRNA in sympathetic neurons. (A) Sympathetic neurons were stimulated with CNTF, with or without the drug Gal-Lac to prevent STAT3 DNA binding, for 6 hrs. SOCS3 mRNA was quantified by real-time PCR and normalized to GAPDH mRNA. Data are expressed as the mean ± SEM, n=2, ** $p<0.01$ compared with control. (B) Sympathetic neurons were stimulated with CNTF, with or without the STAT3 inhibitor Stattic, for 30 min. SOCS3 mRNA was quantified by real-time PCR and normalized to GAPDH mRNA. Data are expressed as the mean ± SEM, n=3, *** $p<0.001$ compared with control. Data shown are representative of three independent experiments.



Supplementary Figure 3. Expression of STAT3 constructs. (A) HEK 293 cells were transfected with various STAT3 constructs using Lipofectamine 2000 (750ng per well). The following day, lysates were harvested and blots were probed with antibodies to STAT3 (top panel) and Flag (bottom panel). Similar expression levels were seen for all constructs. (B) pcDNA3 (Vec) and GFP were transfected into HEK 293 cells as in A. The blot was probed with a GFP antibody to demonstrate expression of a protein other than STAT3.