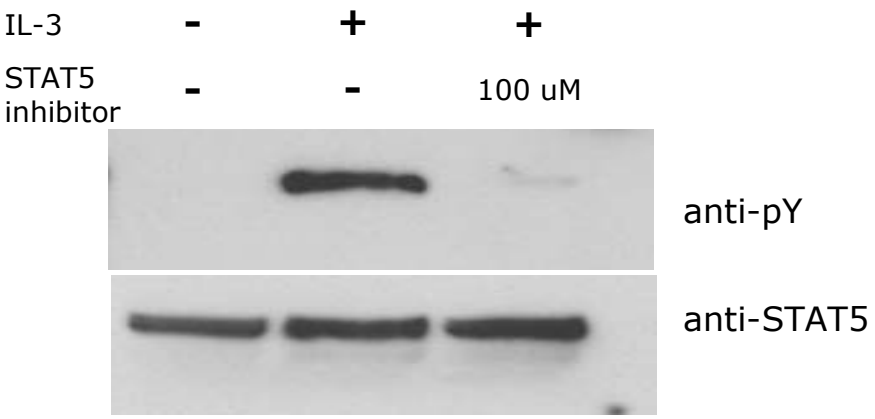
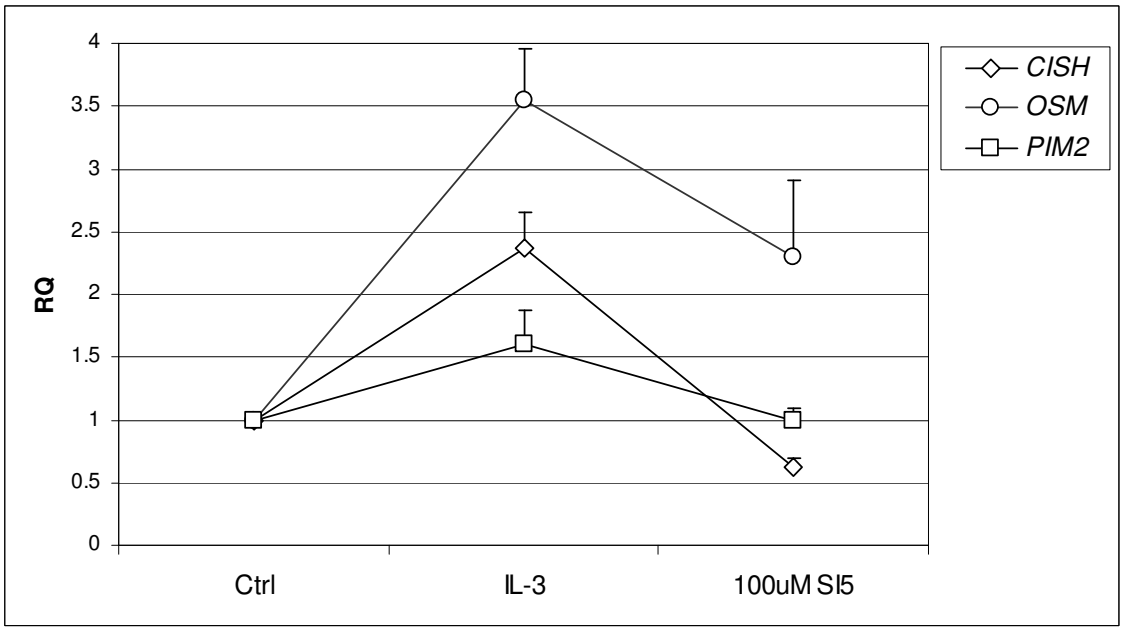
Supplementary results and methods

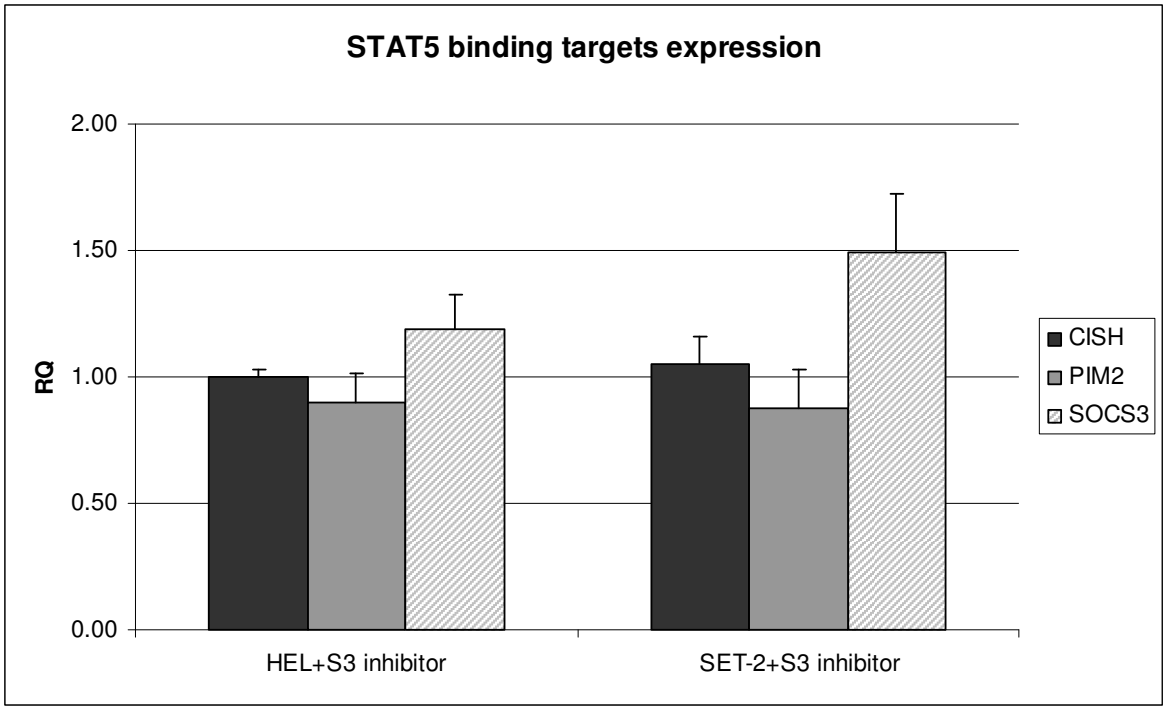
<u>Optimization of treatment with STAT5 inhibitor</u>

Several concentrations of STAT5 inhibitor (SI5) were tested. As shown below, 100uM was chosen because it was the lowest concentration that showed good inhibition of IL3-mediated STAT5 activation in M07e cells, assayed by immunoprecipitation with a polyclonal STAT5 antibody followed by western blot either with anti-phosphorylated tyrosine (pY) or with monoclonal anti-STAT5. The bottom panel shows inhibition assays for three known STAT5-regulated genes. Higher concentrations of the STAT5 inhibitor did not improve inhibition.





Specificity of inhibition was also tested using a STAT3 inhibitor from the same vendor and measuring expression of STAT5-regulated genes in HEL and SET-2 cells, in which JAK2/STAT5 is constitutively active due to activating mutations in JAK2. As shown below, none of the three genes tested was affected by treatment with STAT3 inhibitor (all RQ values are referred to untreated cells):



STAT5 phosphorylation in HEL and SET-2 cells

An immunoprecipation with a polyclonal anti-STAT5, followed by western blot using an anti-phosphotyrosine or a monoclonal anti-STAT5, is shown below. Two independent inhibition experiments are shown for HEL and SET-2 cells, either untreated (C) or treated with 100uM STAT5 inhibitor (SI5). These protein extracts belong to the same experiment in which *LIF*, *OSM* and *CEBPD* expression levels were measured, shown in Figure 1D in the main manuscript.