Supplementary Information

Figure 1: a) Ribbon representation of the overlay between the unphosphorylated STAT5a (blue) and phosphorylated STAT1 (orange) core fragments. The significantly different areas are marked with dashed circles. b) Overlay between the antiparallel dimers of the STAT5a (blue) and STAT1 (orange) core fragments.

Figure 2: Transactivation potential of CFP- and YFP-STAT5a fusion proteins.
a) HEK293-cells were transfected with ß-Casein-promoter-luciferase reporter plasmid (1,25 µg) and expression vectors for the prolactin receptor (25 ng), STAT5a or STAT5a fusion proteins (100 ng) as indicated and SV40-lac Z expression plasmid (12.5 ng). Cells were treated with prolactin for 16 h or left untreated as indicated. Luciferase activities were determined in cell lysates and normalized against ß-galactosidase activities. The values represent the average from three independent experiments. CFP-STAT5a as well as YFP-STAT5a are able to transactivate the ß-Casein reporter plasmid. Both STAT5a fusion proteins show an enhanced basal transactivation potential without prolactin stimulation.
b) STAT5, CFP-STAT5a and YFP-STAT5a-YFP are phosphorylated after prolactin treatment. HEK293-cells (10 cm dish) were transfected with expression vectors for the prolactin receptor (0.5 µg) and STAT5a or CFP- and YFP-STAT5a fusion proteins (3 µg). Cells were treated with prolactin for 30 min or left untreated as indicated. Whole cell lysates were prepared and analyzed by SDS-PAGE and Western blotting. STAT5a and Phospho-STAT5a were detected with specific antibodies (STAT5a: BD Transduction Lab S21520, P-STAT5a: NEB #9351).These data indicate that STAT5 as well as STAT5 fusion proteins are only phosphorylated after prolactin induction of the cells. Please note, that STAT5 fusion proteins are much better expressed. The higher expression levels observed with the CFP/YFP-STAT5a expression plasmids correspond to the higher transactivation level observed in a).

Figure 3: STAT5a, CFP-STAT5a and YFP-STAT5a bind DNA after prolactin treatment. HEK293-cells were transfected with expression vectors for the prolactin receptor (0.5 µg), STAT5a or STAT5a fusion proteins (3 µg) as indicated. Cells were treated with prolactin or left untreated as indicated. Whole cell extracts were prepared and DNA binding was analyzed by electrophoretic mobility shift assay. Based on the expression levels of CFP-STAT5a, YFP-STAT5a and wildtype STAT5a (see Fig. 4b), equal amounts of proteins were used for the bandshift assay. Hereby the total protein amount of cell extract was kept constant by adding whole cell extract from untransfected HEK293 cells. For upshift experiments antibodies recognizing STAT5a (R&D, PA-ST5A) were added. These data indicate that STAT5a as well as the CFP- and YFP-STAT5a fusion proteins are able to bind to the ß-Casein response element. Interestingly, a weak DNA binding activity of CFP- and YFP-STAT5a was already detected in untreated cells. This is in line with the observed basal transactivation activity of both fusion proteins (see Fig. 4a).

Figure 4: Quantification of purified, bacterially expressed STAT5a core fragment (lane 1) and cell extracts from HeLa cells overexpressing STAT5a before (lane 2) and after (lane 3) activation with prolactin. The amounts loaded on the gel for the Western Blot were identical to the amounts used for the bandshift shown in Fig. 5 of the main text. To create comparable assay conditions, also here the purified bacterial sample was combined with whole cell extract from unstimulated cells. According to densitometric measurements the HeLa cell extracts contained about five times less full-length protein.

Figure 1a:

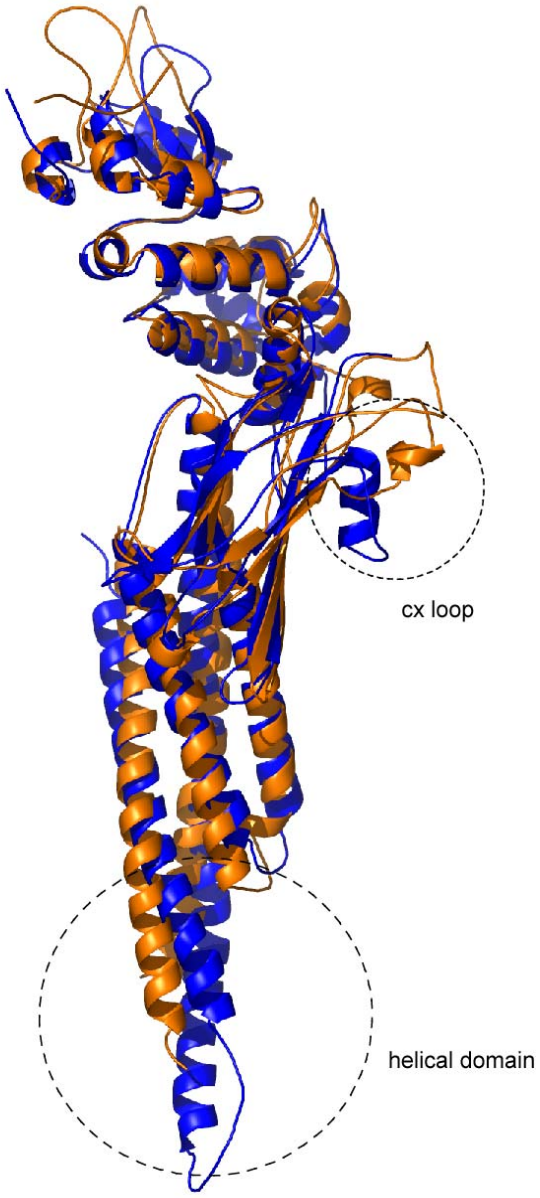


Figure 1b:

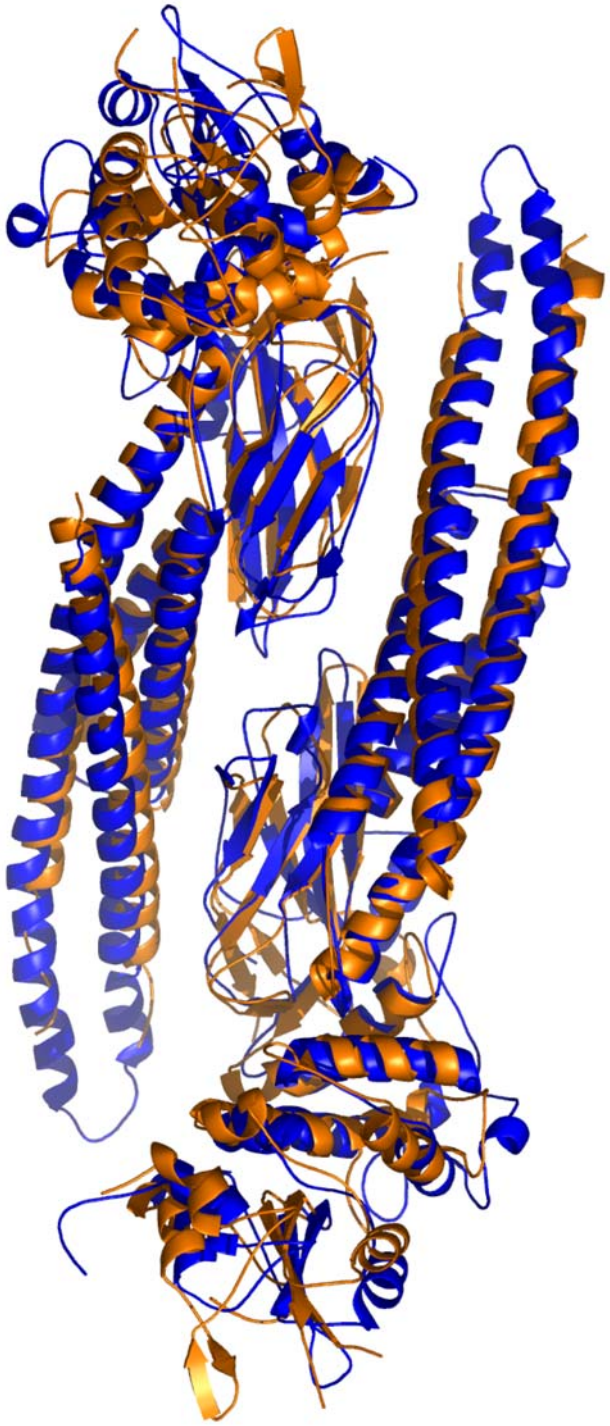
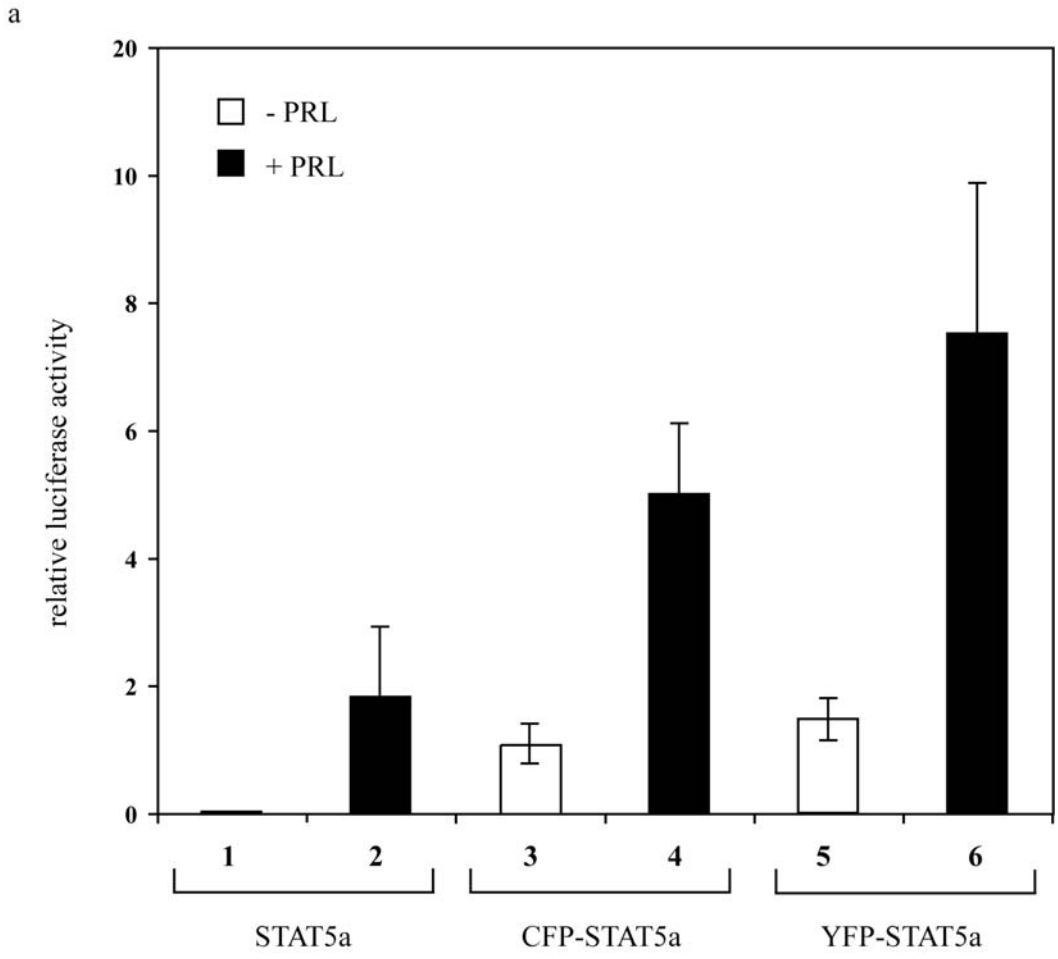


Figure 2:


a
20
10
8
6
4
2
0
- PRL
+ PRL
relative luciferase activity
1
2
3
4
5
6
STAT5a
CFP-STAT5a
YFP-STAT5a

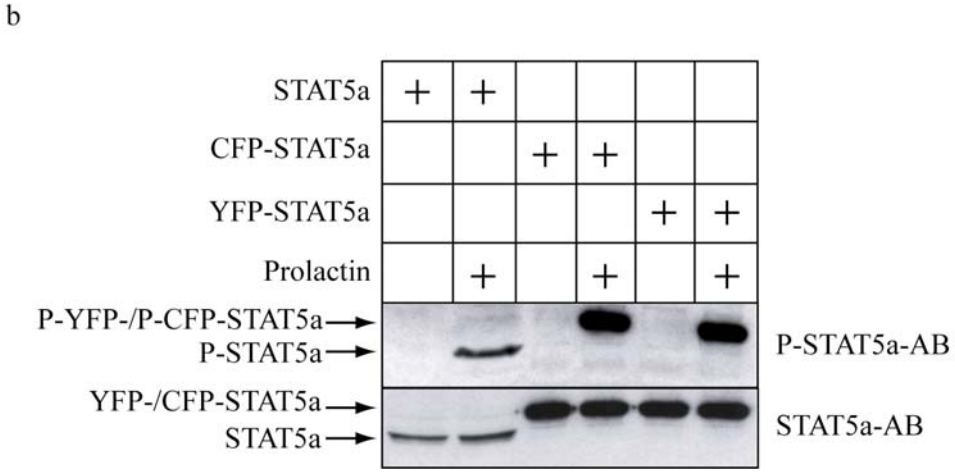

b
STAT5a
CFP-STAT5a
YFP-STAT5a
Prolactin
P-YFP-/P-CFP-STAT5a
P-STAT5a
YFP-/CFP-STAT5a
STAT5a
P-STAT5a-AB
STAT5a-AB

Figure 3:

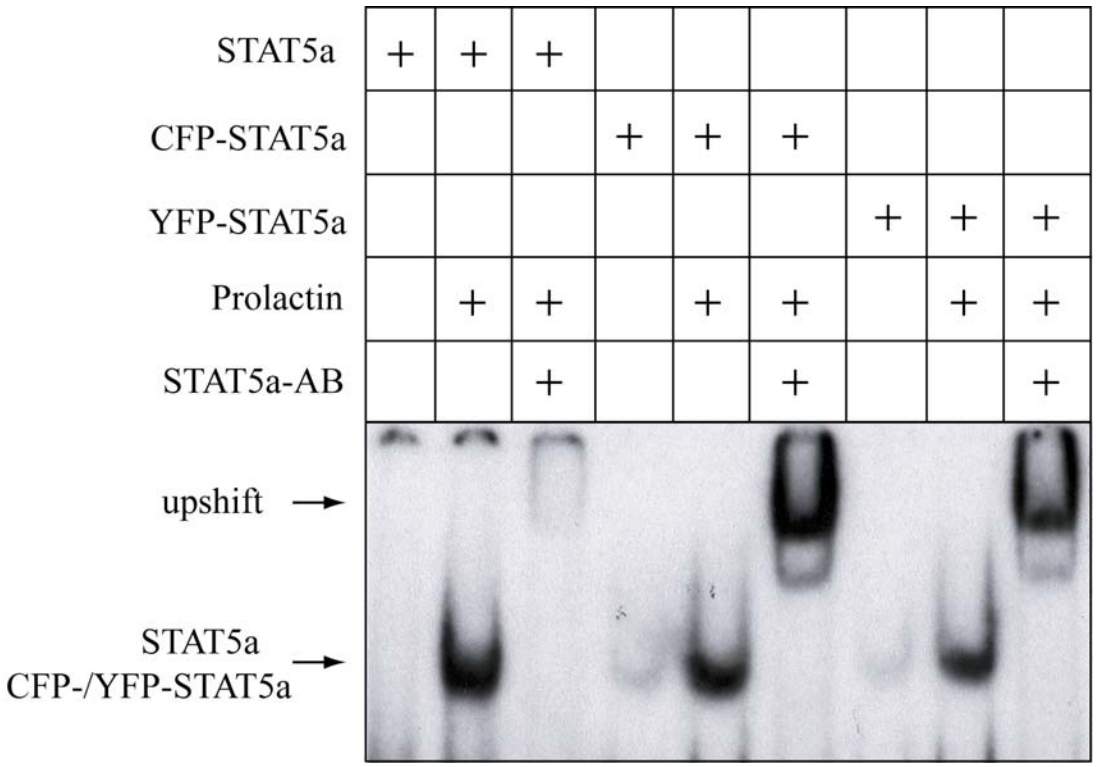


Figure 4: