Supplemental Figures

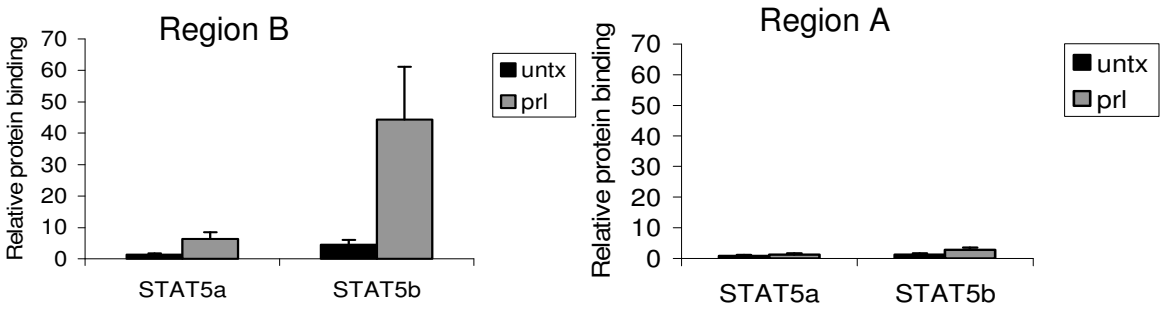


FIG S1. STAT5a and STAT5b bind to region B of BCL6. ChIP was performed in SK-BR-3 cells stimulated with prolactin using antibodies to the individual isoforms STAT5a and STAT5b. Binding was analyzed by ChIP for the indicated regions.

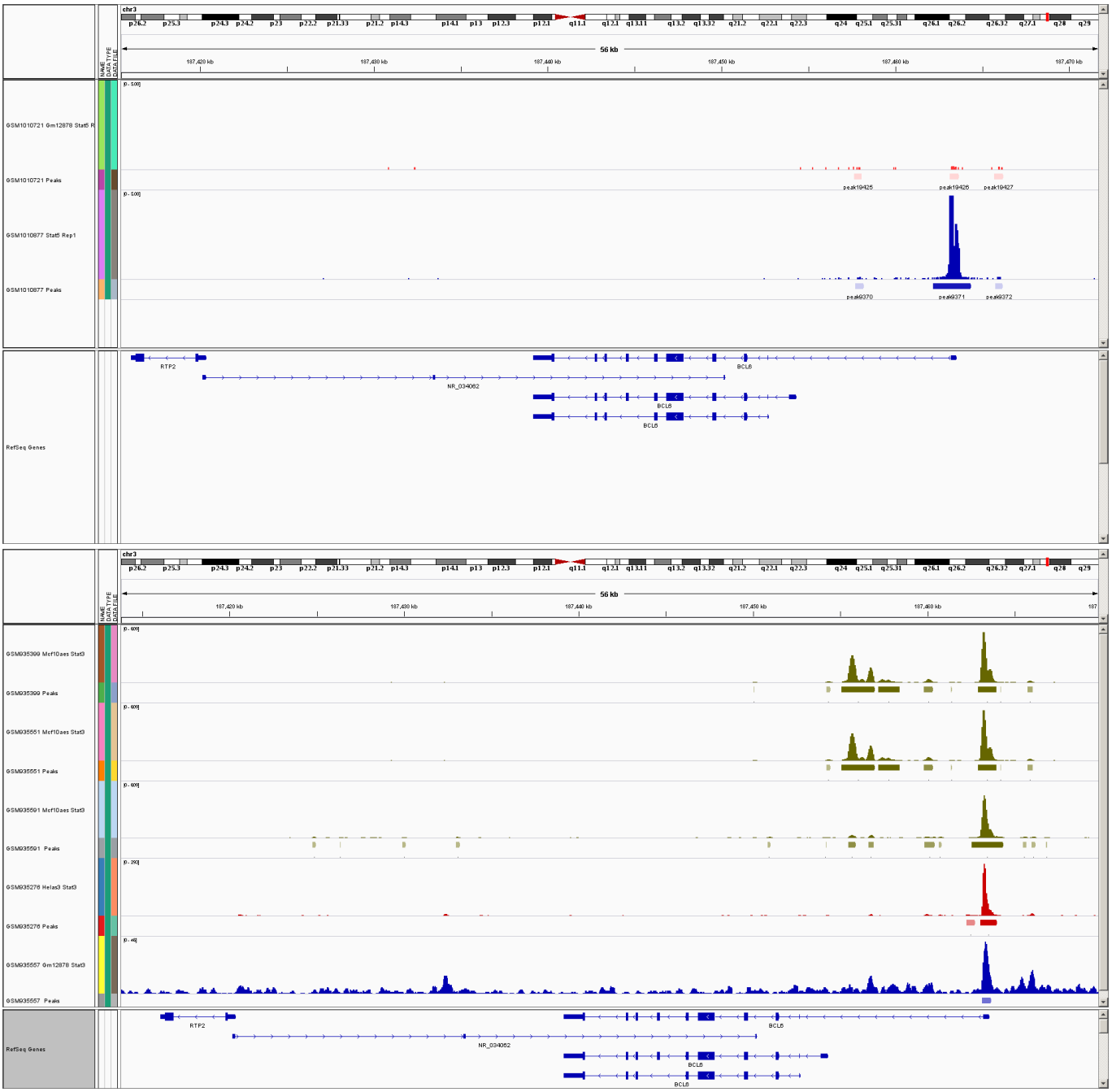
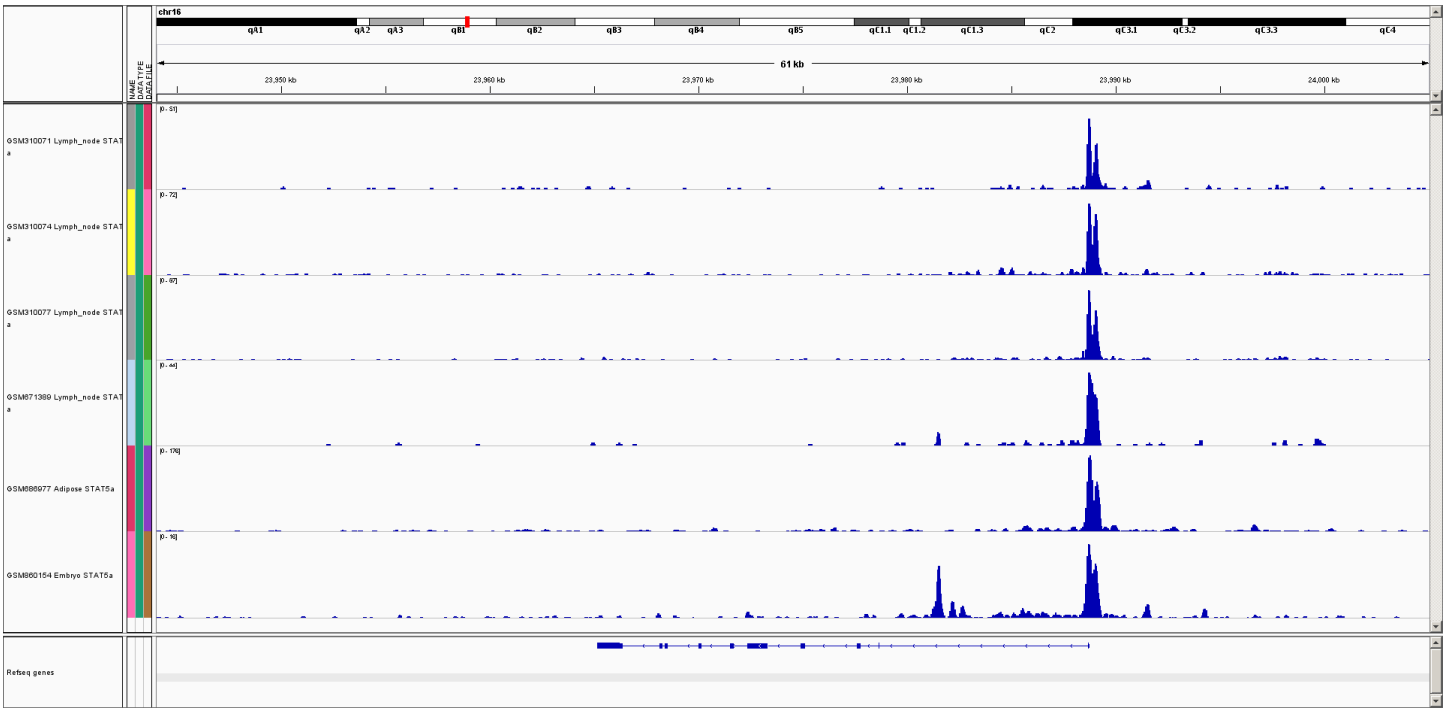


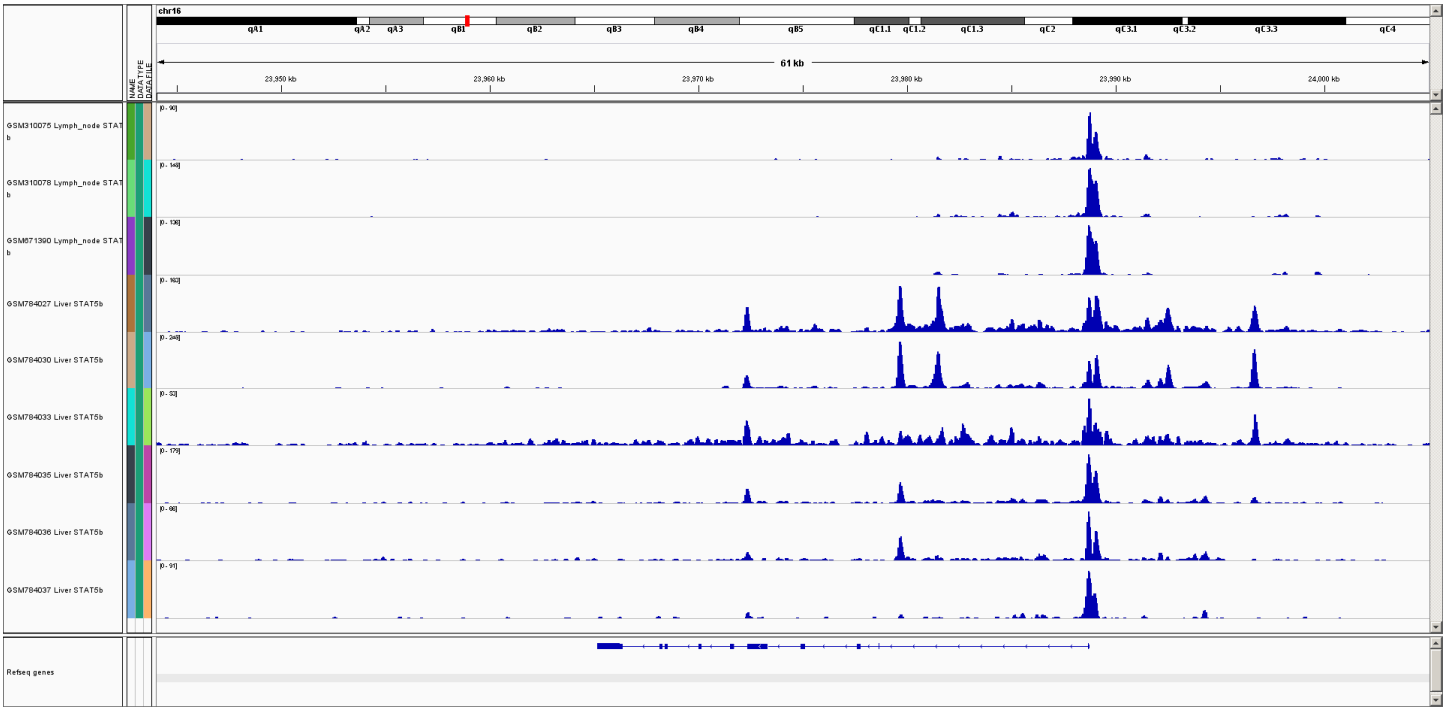
Fig S2. Whole genome ChIP-seq analysis demonstrates that in human cells STAT5 binds to region B, whereas STAT3 binds to both regions A and B. The data were downloaded from the ENCODE project and the peak regions were defined using the method reported http://genome.ucsc.edu/cgi-bin/hgTrackUi?db=hg19&g=wgEncodeSydhTfbs.

FIG S3.

STAT5a



STAT5b



STAT3

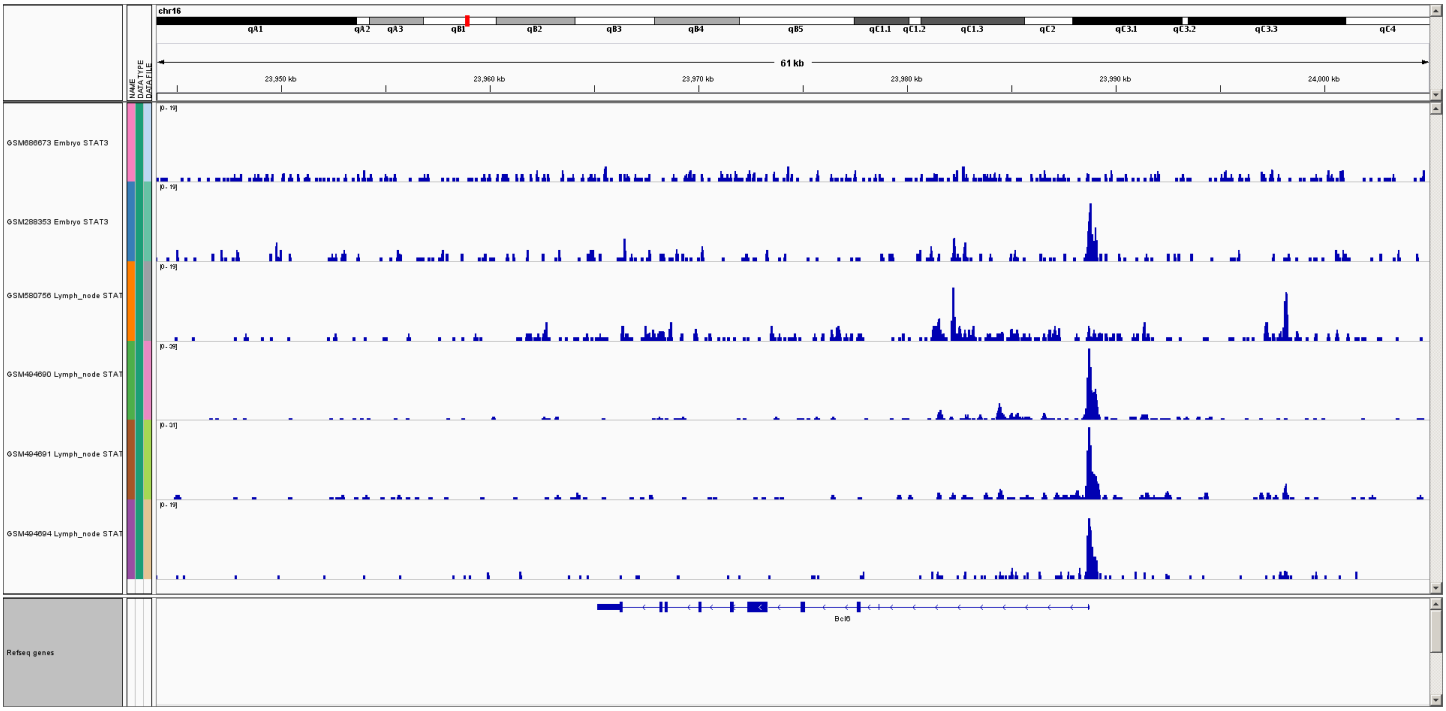


FIG S3. Mouse STAT5 and STAT3 bind to region B in the BCL6 gene. The mouse ChIP-seq data were download from the CistromeFinder Platform (http://cistrome.org/finder/). Datasets that passed the QC based on conservation and #peaks(>1000) were used.

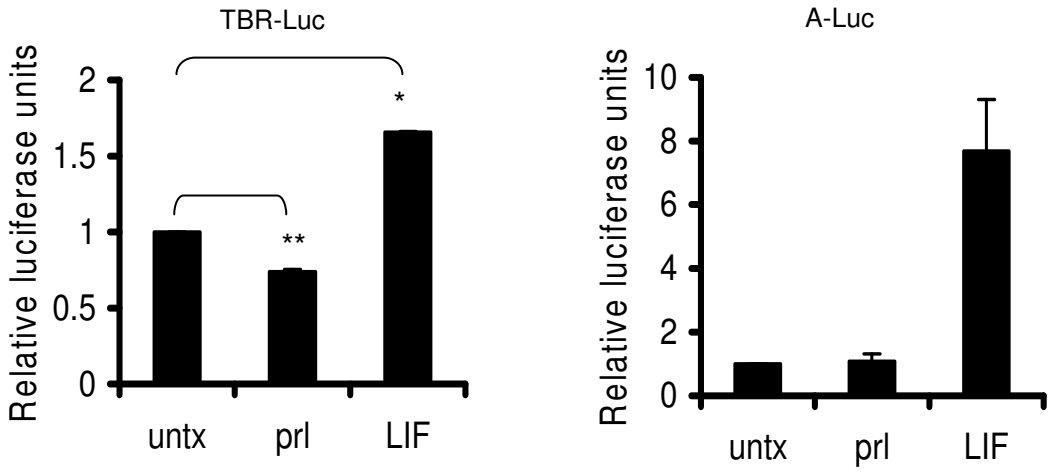


FIG S4. Region B of BCL6 is STAT5 and STAT3 responsive. SK-BR-3 cells were transfected with TBR-Luc or A-Luc and then stimulated with prolactin or LIF for 6 hours. Luciferase activity was measured and analyzed relative to renilla values. N=2. *$p<0.05$, ** $p<0.01$

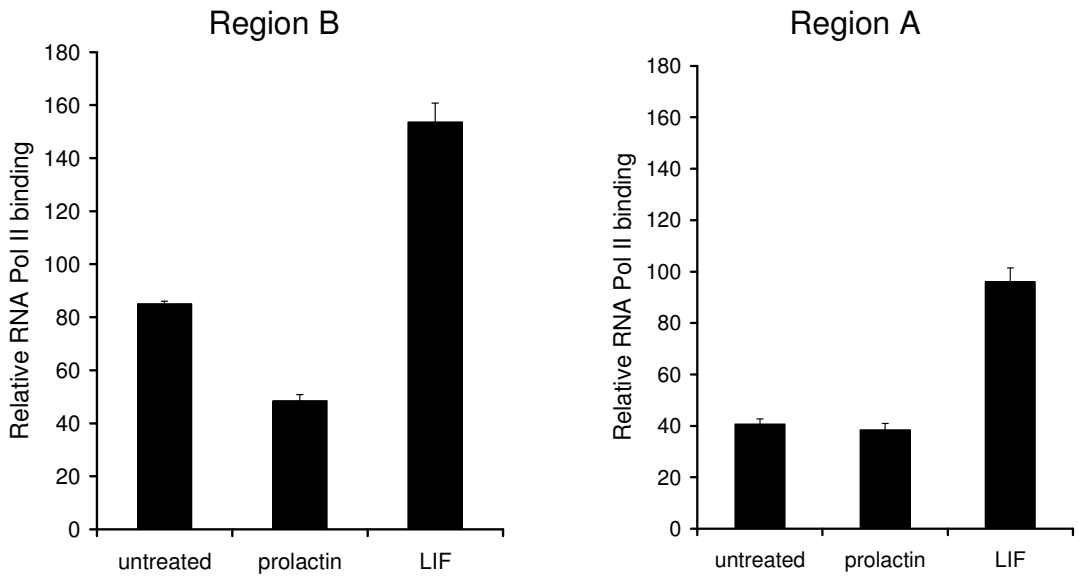


FIG S5. STAT5 and STAT3 oppositely modulate RNA polymerase II binding. SK-BR-3 cells were stimulated with prolactin or LIF and RNA polymerase II (pol II) binding was analyzed by ChIP.

BCL6

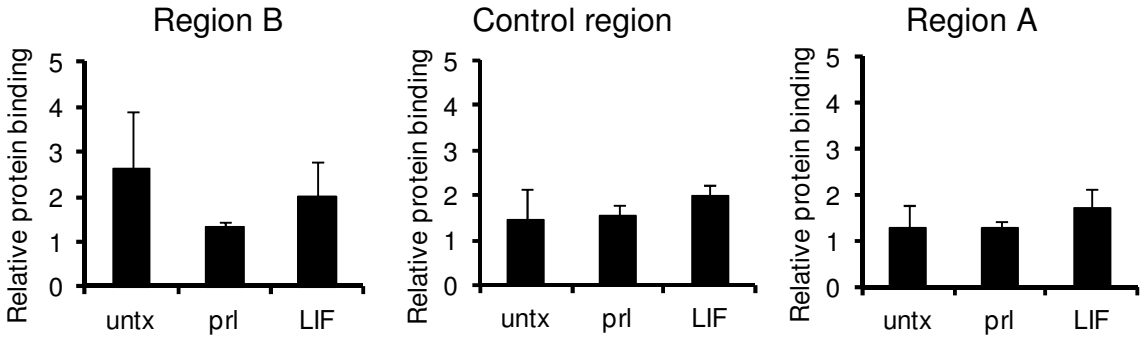


FIG S6. BCL6 binding is lost upon STAT binding to region B. SK-BR-3 cells were stimulated with prolactin or LIF and analyzed for BCL6 binding by ChIP using an antibody to the C-terminus. (N=2)

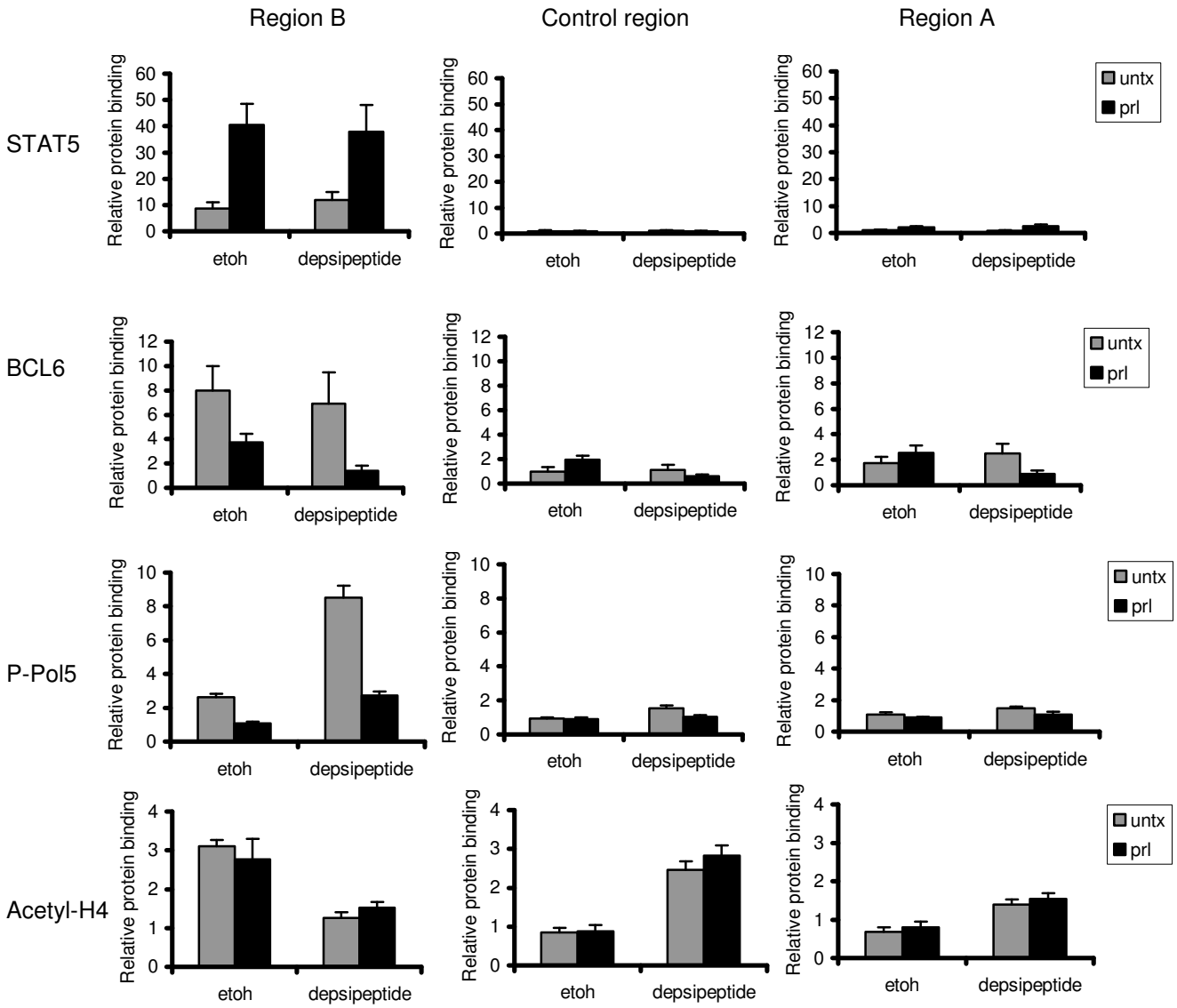


FIG S7. HDAC inhibition leads to BCL6 transcription initiation, but does not affect STAT5 mediated repression of BCL6. SK-BR-3 cells were pretreated with 10 nM depsipeptide for 2 hours and then stimulated with prolactin. ChIP was performed with the indicated antibodies.

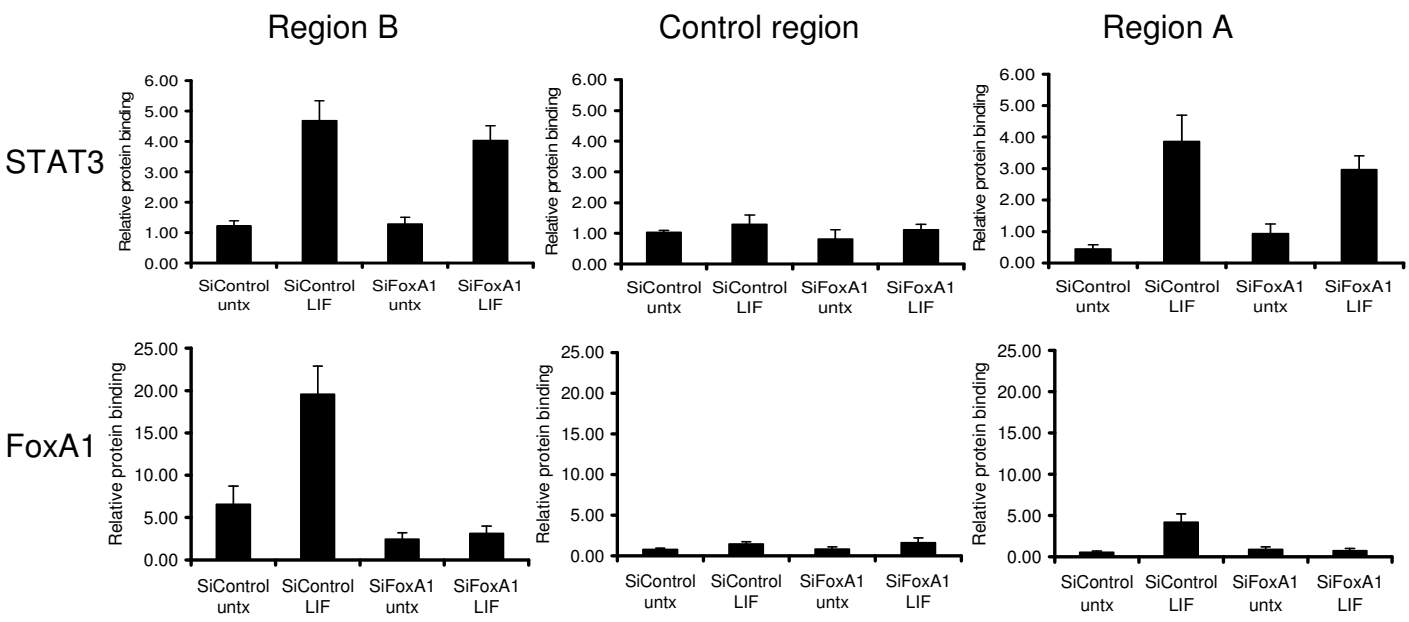


FIG S8. Reducing FoxA1 levels does not affect STAT3 binding to region B. SK-BR-3 cells were transfected with siRNA to FoxA1 and then stimulated with LIF to activate STAT3. ChIP was then performed to STAT3 and FoxA1 at region B, A, and the intervening control region.

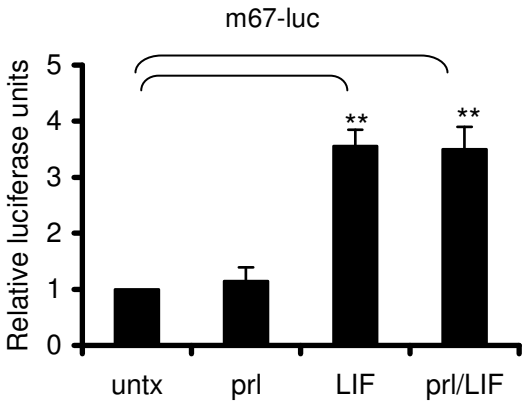


FIG S9. STAT5 does not globally affect STAT3 activity. SK-BR-3 cells transfected with the general STAT3 dependent luciferase construct m67-Luc were stimulated with the indicated cytokines for 6 hours and luciferase activity was measured. N=3. **p<0.01.

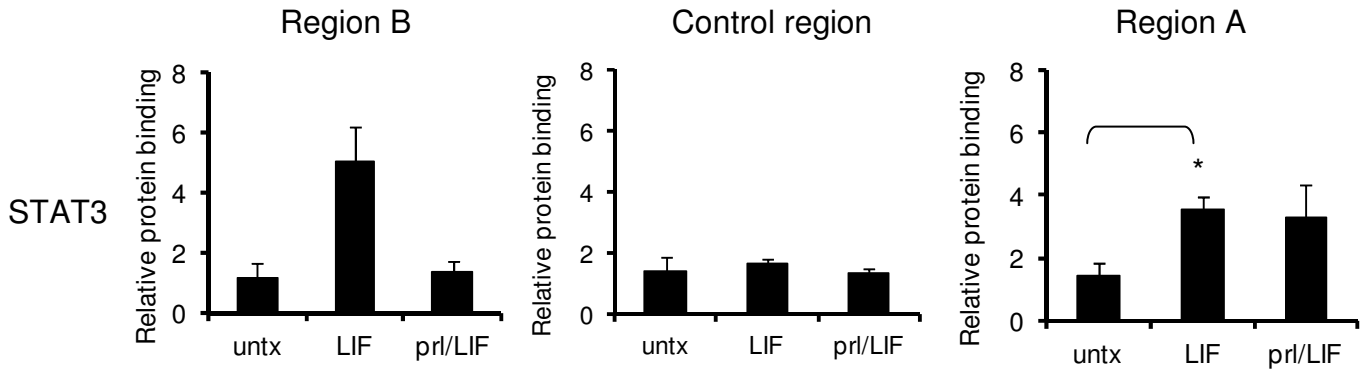


FIG S10. STAT3 binding is lost upon STAT5 binding to region B. SK-BR-3 cells were stimulated with LIF or the combination of LIF and prolactin, and STAT3 binding was analyzed by ChIP using an antibody to the N-terminus of STAT3. (n=2) * $p<0.05$.

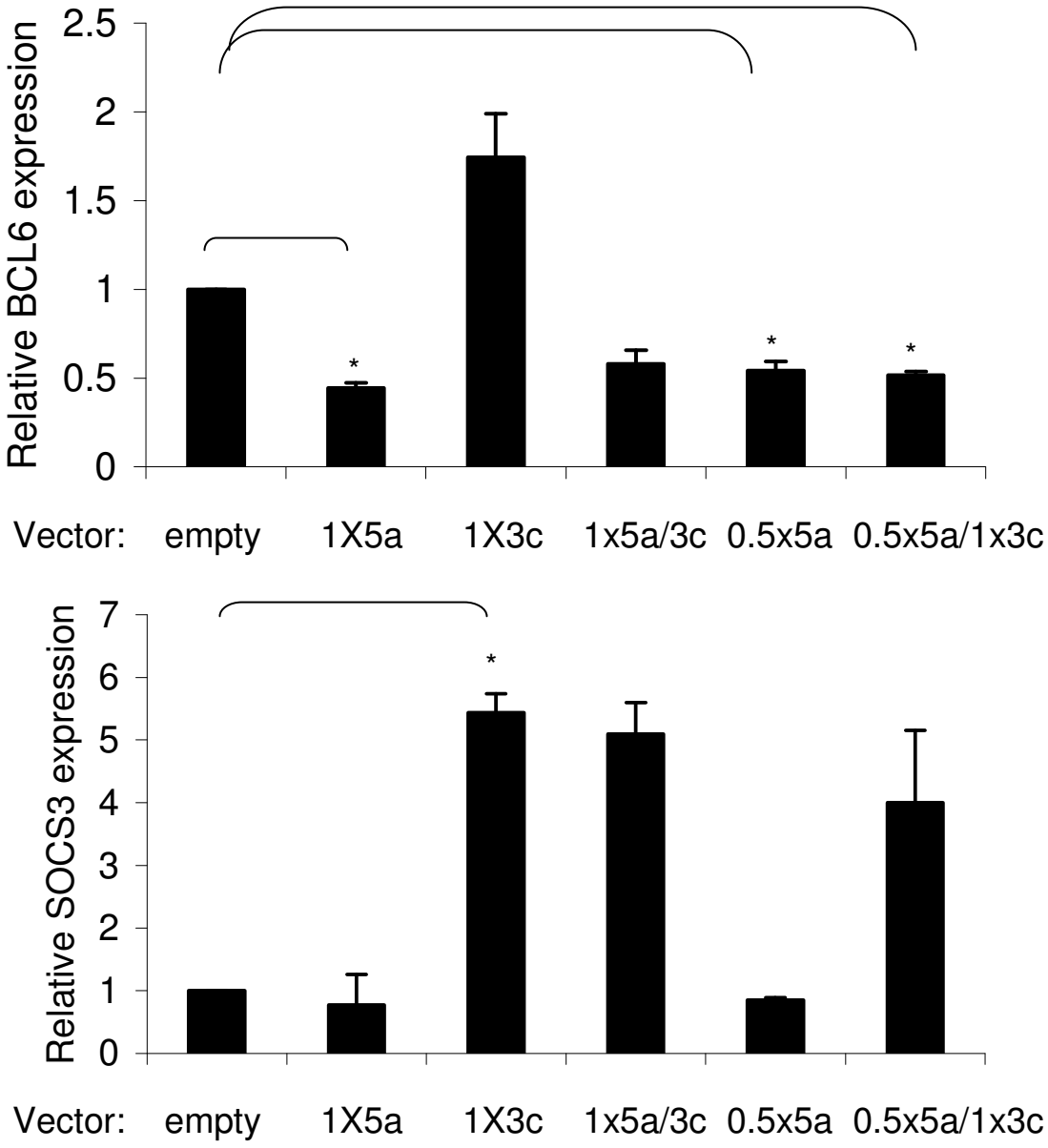


FIG S11. STAT5 is dominant over STAT3 on regulating BCL6 expression even when transfected with 50% less of the construct. Constitutively active STAT5 (STAT5a1*6 (5a)) and STAT3 (STAT3C (3C)) were transfected at the indicated amount into SK-BR-3 cells. BCL6 mRNA expression (top) and SOCS3 mRNA expression (bottom) were analyzed relative to GAPDH. (N=2). * $p<0.05$.