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Supplementary Materials for

MUC1-C Oncoprotein Promotes STAT3 Activation in an Autoinductive Regulatory Loop

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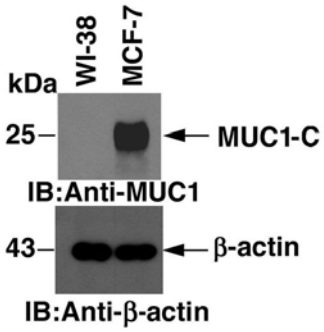
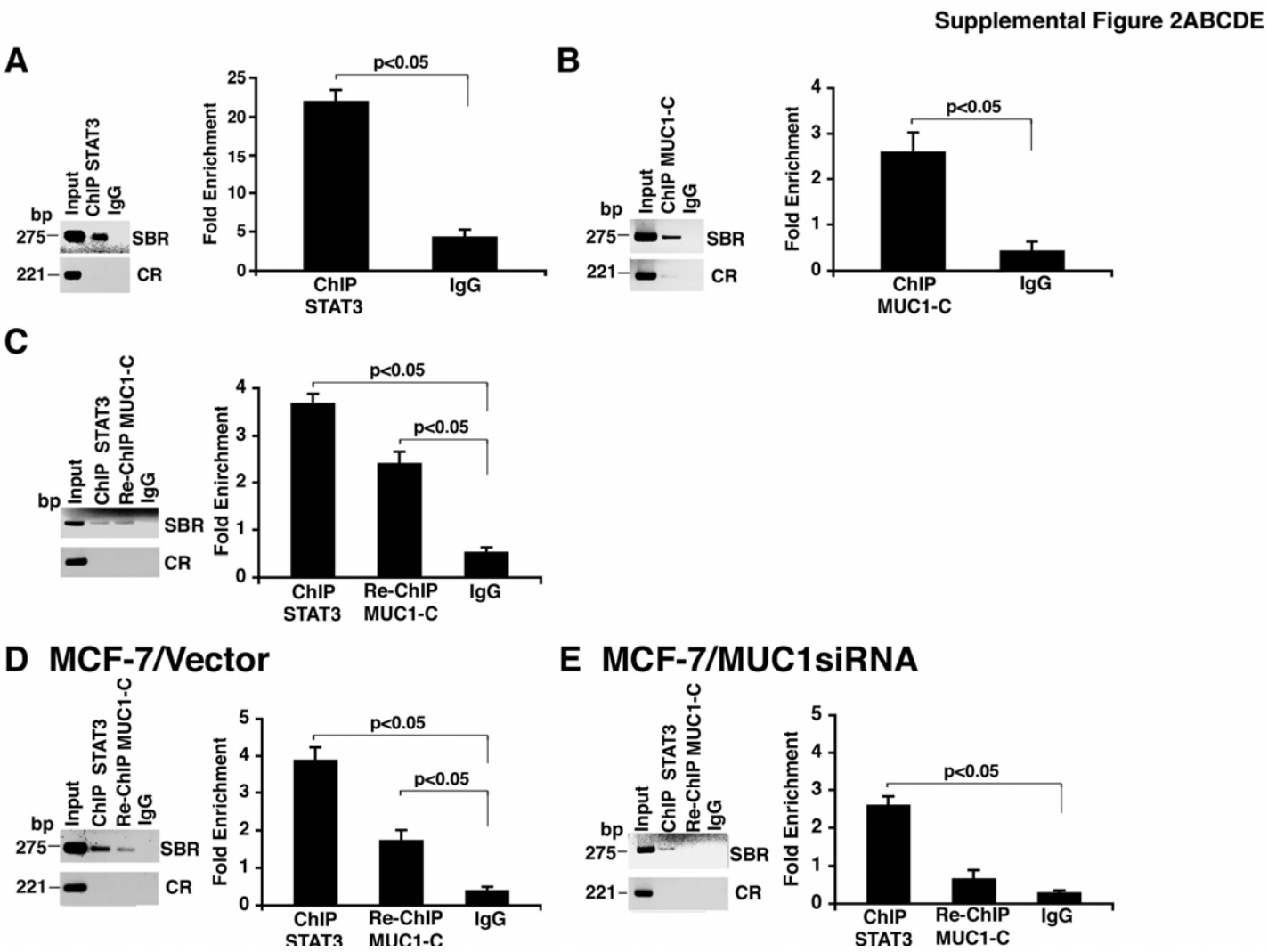
The PDF file includes:

Supplemental Figure Legends

Supplemental Fig. S1

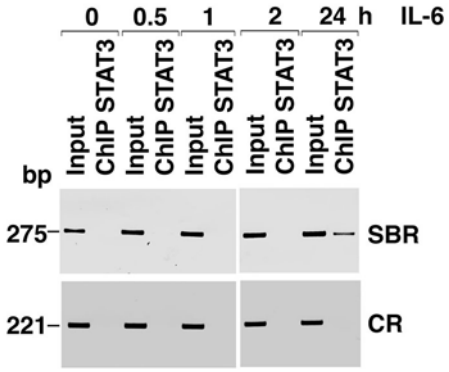


Supplemental Fig. S1. Immunoblot analysis of MUC1-C in lysates from WI-38 fibroblasts and MCF-7 breast cancer cells. Lysates from WI-38 and MCF-7 cells were immunoblotted with the indicated antibodies.



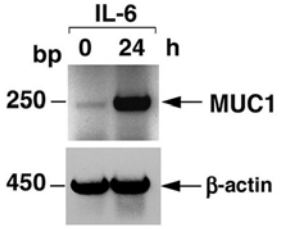
Supplemental Fig. S2. Association of STAT3 and MUC1-C with the STAT binding region of the *MUC1* promoter in MCF-7 cells. A. Soluble chromatin from MCF-7 cells was immunoprecipitated with anti-STAT3 or an IgG control. Final DNA samples were amplified by PCR with pairs of primers flanking the STAT binding region (SBR; -559 to -284) or control region (CR; +4596 to +4817) (left). The precipitated chromatin was also analyzed by qPCR (right). B. Soluble chromatin from MCF-7 cells was immunoprecipitated with anti-MUC1-C or an IgG control and analyzed for *MUC1* promoter SBR and CR sequences (left). The precipitated chromatin was also analyzed by qPCR (right). C. Soluble chromatin from MCF-7 cells was precipitated with anti-STAT3, released, reimmunoprecipitated with anti-MUC1-C and then analyzed for *MUC1* promoter SBR and CR sequences (left). The precipitated chromatin was also analyzed by qPCR (right). D and E. Soluble chromatin from MCF-7/vector (D) and MCF-7/MUC1siRNA (E) cells was immunoprecipitated with anti-STAT3, released, reimmunoprecipiated with anti-MUC1-C and then analyzed for *MUC1* promoter SBR and CR sequences (left).

Supplemental Fig. S3

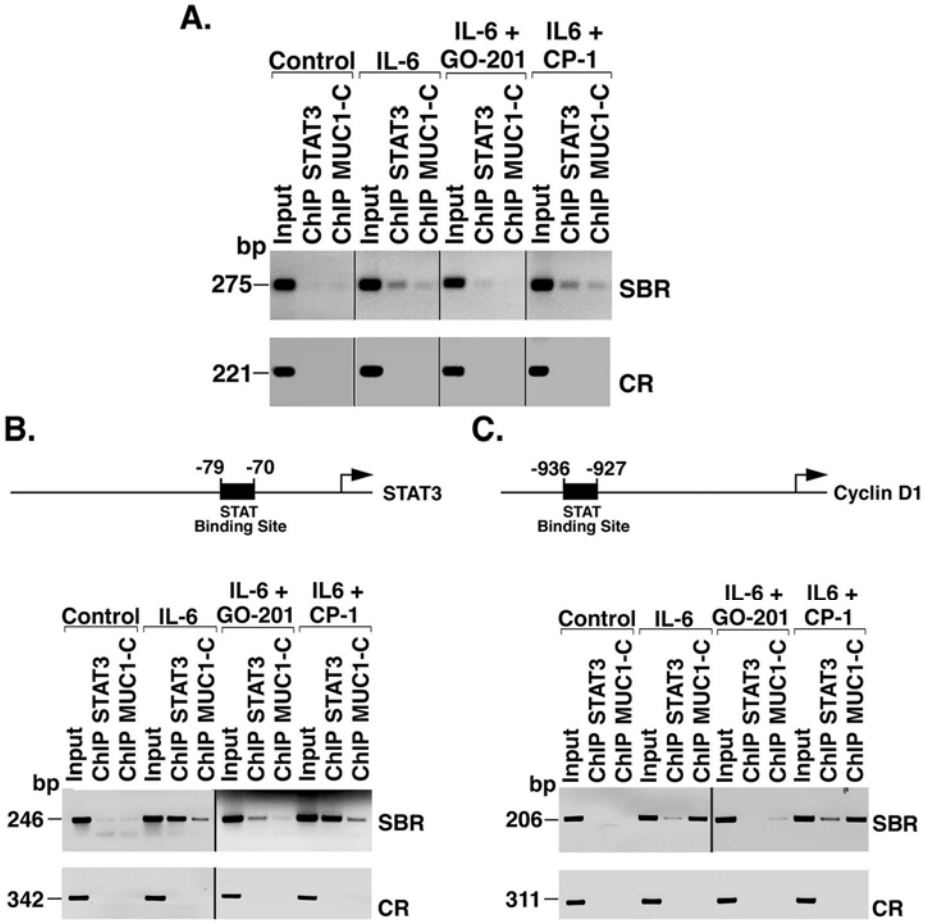


Supplemental Fig. S3. IL-6 induces STAT3 association with the *MUC1* promoter in MCF-10A cells. Soluble chromatin from MCF-10A cells stimulated with IL-6 for the indicated times was precipitated with anti-STAT3 and analyzed for *MUC1* promoter SBR and CR sequences.

Supplemental Fig. S4

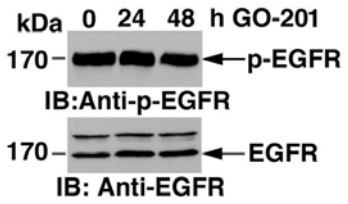


Supplemental Fig. S4. IL-6 increases MUC1 mRNA in MCF-10A cells. MCF-10A cells were stimulated with IL-6 for 24 h. MUC1 and β-actin mRNA levels by RT-PCR.



Supplemental Fig. S5. Effects of GO-201 on IL-6-induced association of STAT3 and MUC1-C with the *MUC1*, *STAT3*, and *cyclin D1* promoters in MCF-10A cells. A-C. MCF-10A cells were left untreated (Control) or stimulated with IL-6 in the presence of 5 μM GO-201 or CP-1 added each 24 h for 72 h. A. Soluble chromatin was precipitated with anti-STAT3 or anti-MUC1-C and analyzed for *MUC1* promoter SBR and CR sequences. B. Schema of the *STAT3* promoter region with the STAT binding site. Soluble chromatin was immunoprecipitated with anti-STAT3 or anti-MUC1-C. The final DNA extractions were amplified by PCR with pairs of primers flanking the STAT binding region (SBR; -316 to -70) or control region (CR; +5005 to +5347). C. Schema of the cyclin *D1* promoter region with the STAT binding site. Soluble chromatin was immunoprecipitated with anti-STAT3 or anti-MUC1-C. The final DNA sampless were amplified by PCR with pairs of primers flanking the STAT binding region (SBR; -1045 to -839) or control region (CR; +2234 to +2545).

Supplemental Figure S6



Supplemental Fig. S6. Effects of GO-201 on EGFR phosphorylation in ZR-75-1 cells. ZR-75-1 cells were left untreated (Control) or treated with 5 μM GO-201 for 24 or 48 h. Lysates were immunoblotted with anti-p-EGFR and anti-EGFR.