

## Supplementary Figure Legends

**FIGURE S1.** Knockdown of OGT results in a decrease in global *O*-GlcNAc levels. A549 cells were transfected with various amounts of OGT siRNA (siOGT). The total amount of siRNA oligonucleotide duplex was compensated with scrambled siRNA to final 5 pmol. Two days after transfection, cells were lysed and subjected to Western blotting analysis for OGT proteins and global *O*-GlcNAcylation.  $\beta$ -Actin was used as a loading control.

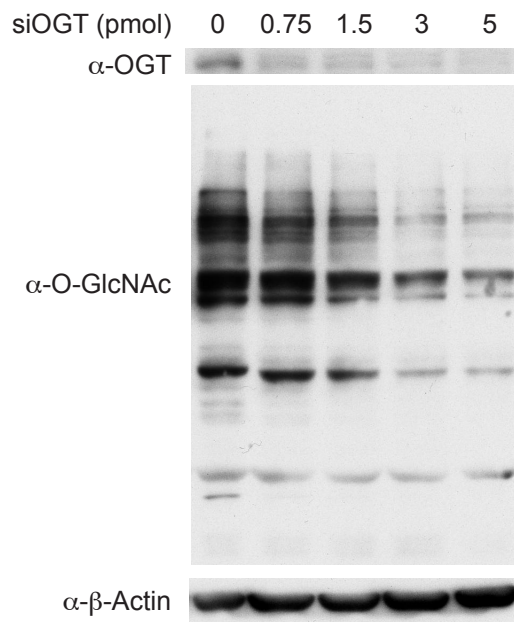
**FIGURE S2.** The interaction between endogenous OGT and GR. Western blot analysis of immunoprecipitates and cell lysates (left panel). HeLa cells were cultured in DMEM containing 10% charcoal-stripped FBS. Upon confluency, cells were treated with 10 ng/ml of TNF $\alpha$  and/or 1  $\mu$ M Dex for 1 hr and lysed. Immunoprecipitation with an  $\alpha$ -GR antibody was carried out at 4°C overnight. OGT bands were detected using  $\alpha$ -OGT (Abcam, ab50270) antibody. Co-immunoprecipitated OGT was quantified by densitometric analysis of  $\alpha$ -OGT blot (right panel).

**FIGURE S3.** Quantitative PCR analysis of RNA pol II protein modifications. (A) Q-PCR analysis of RNA pol II protein modifications in A549 cells. Cells treated with TNF- $\alpha$ , Dex or ethanol vehicle were subject to sequential chromatin immunoprecipitation using  $\alpha$ -RNA pol II CTD antibody (Total pol II), followed by  $\alpha$ -phos-Ser2 pol II CTD (CTD-Phos) or  $\alpha$ -*O*-GlcNAc (*O*-GlcNAc). (B) Q-PCR analysis of RNA pol II protein modifications in A549 cells transfected with OGT siRNA or scrambled siRNA and then treated with TNF- $\alpha$  and Dex. \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , Boferroni's post-test. Q-PCR data were normalized to the input control. Primer sequences are available from the authors on request.

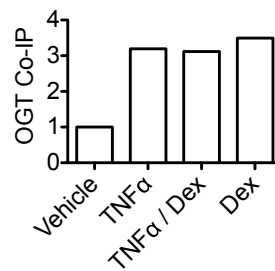
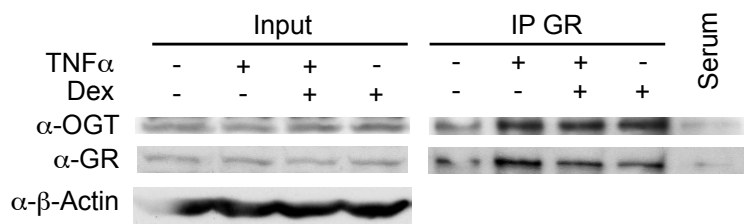
**FIGURE S4.** siRNA knockdown of OGT in CCRF-CEM cells. CCRF-CEM cells were transfected with siRNA oligonucleotide duplex (siCON, scrambled siRNA; siOGT, OGT siRNA). Two days after transfection, cells were lysed and subjected to Western blot analysis for OGT proteins and global *O*-GlcNAcylation.  $\beta$ -Actin was used as a loading control.

**FIGURE S5.** OGT mediates GR-induced transrepression. TNF $\alpha$  activates NF- $\kappa$ B and AP-1 signaling, promoting the expression of inflammatory and/or anti-apoptotic genes. GR recruits OGT to the promoters of NF- $\kappa$ B and AP-1 target genes, where OGT inhibits gene transcription through *O*-GlcNAc modification of the CTD of RNA pol II.

Supplementary Figure 1

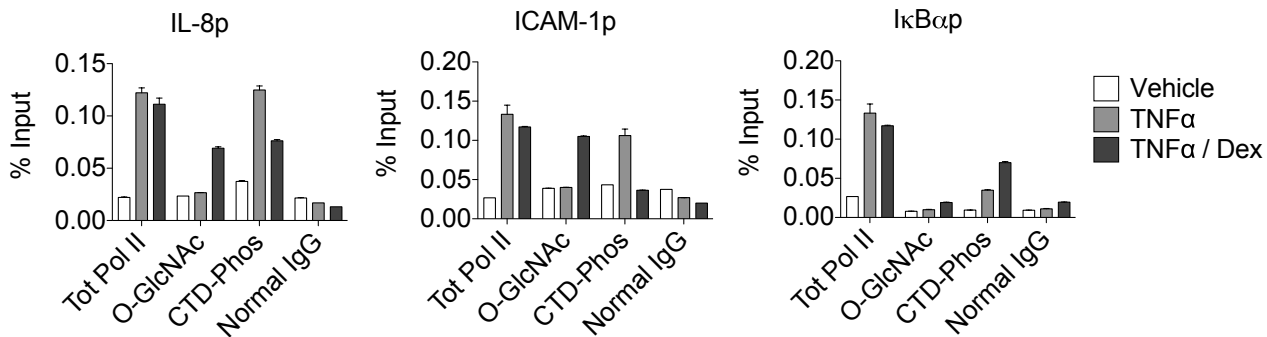


Supplementary Figure 2

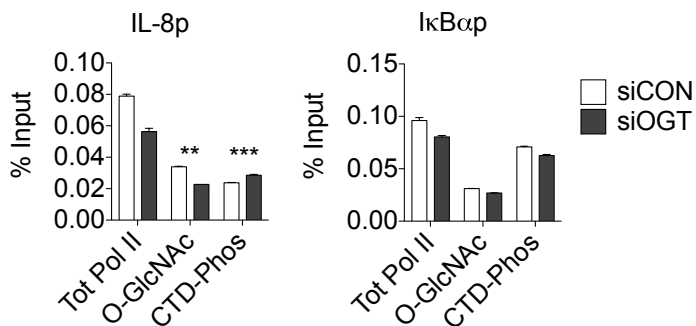


Supplementary Figure 3

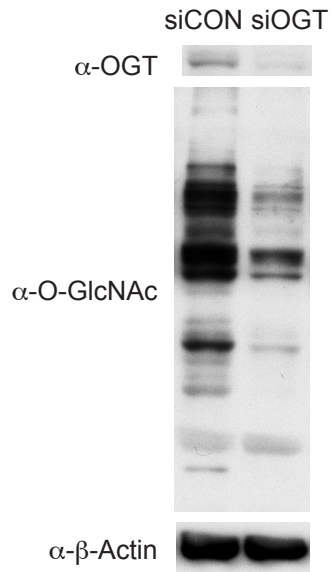
A



B



Supplementary Figure 4



Supplementary Figure 5

