Supplementary Material

An Analysis of Glucocorticoid Receptor-mediated Transcription in Human Airway Epithelial Cells Identifies Distinct, Ligand-directed, Gene Expression Fingerprints with Implications for Asthma Therapeutics

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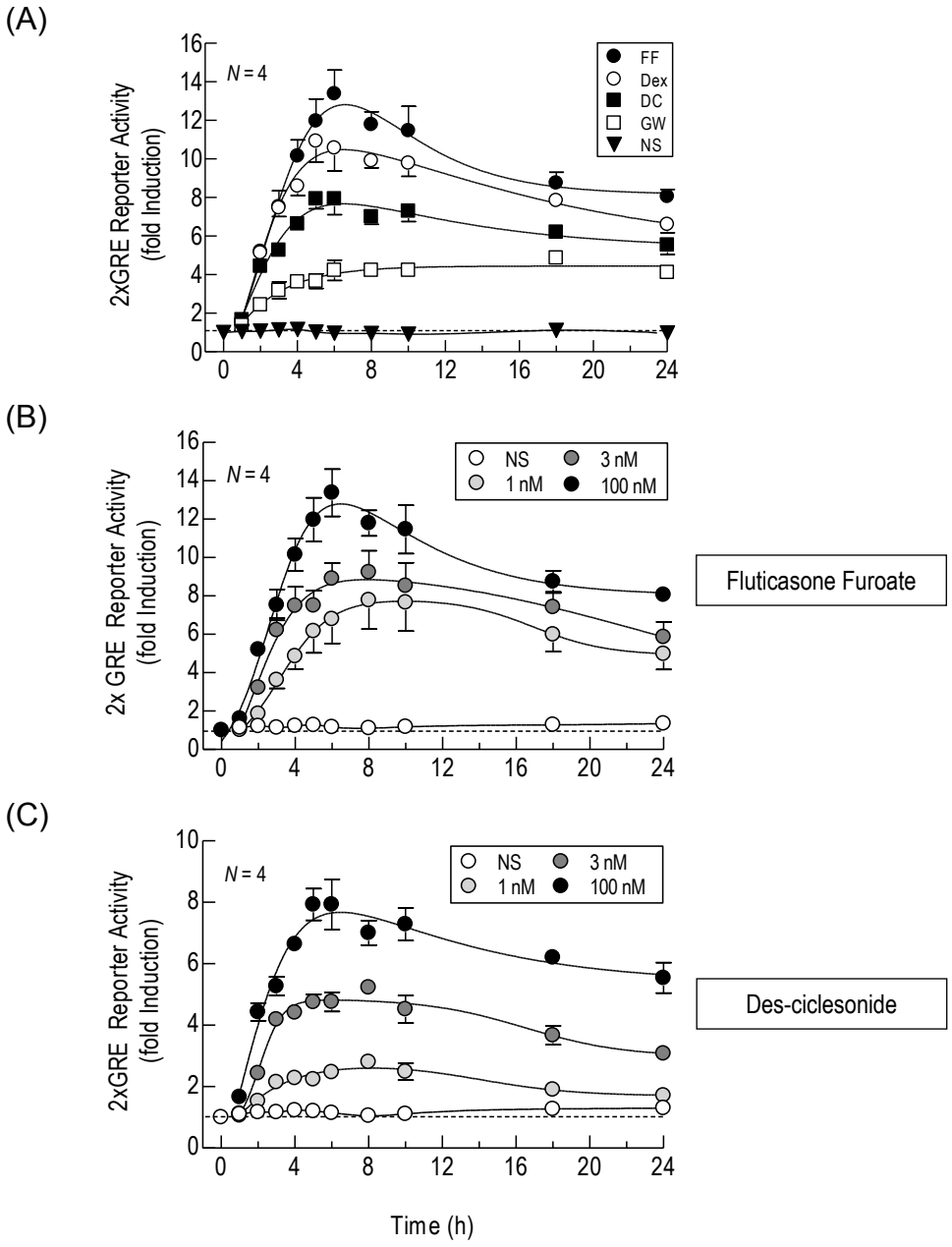
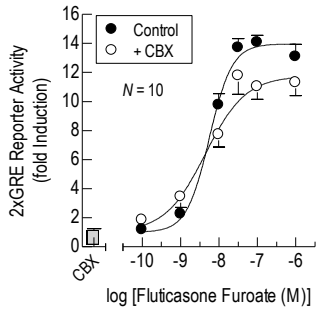
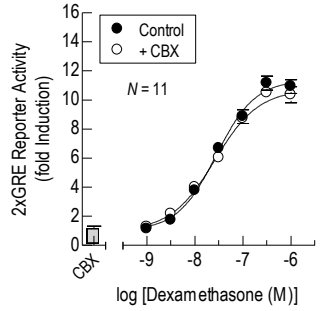


Figure S1. Kinetics of GRE-dependent transcription. Panel *A*: 2×GRE BEAS-2B reporter cells were treated with fluticasone furoate (FF; 100nM), dexamethasone (Dex; 1µM), des-ciclesonide (DC; 100nM), GW 870086X (GW; 1µM) or vehicle (NS). Panels *B* and *C*: cells were treated with FF, DC (at 1nM, 3nM or 100nM) or vehicle (NS). At the times indicated, cells were harvested for the determination of luciferase activity. Data points represent the mean ± s.e. mean of *N* independent determinations. The dashed line in each panel defines baseline luciferase expression.

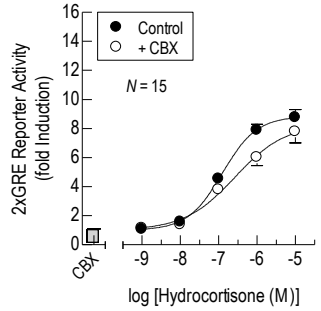
(A)



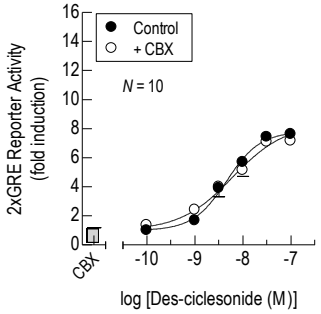
(B)



(C)



(D)



(E)

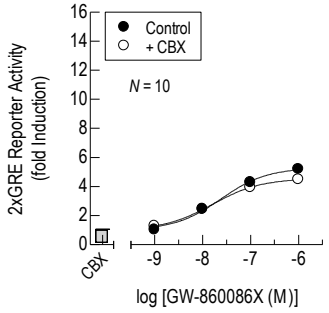


Figure S2. Effect of carbenoxolone on GRE-dependent transcription. 2×GRE BEAS-2B reporter cells were pre-treated with carbenoxolone (CBX; 1µM for 30min) or its vehicle and *E*/[A] curves were constructed to fluticasone furoate (A), dexamethasone (B), hydrocortisone (C), des-ciclesonide (D) and GW-870086X (E). After 6h, cells were harvested for the determination of luciferase activity. Data points represent the mean ± s.e. mean of *N* independent determinations. The dashed line in each panel defines baseline luciferase expression.

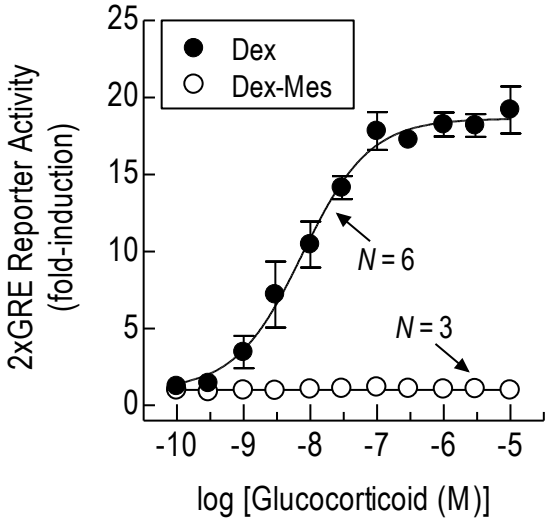


Figure S3. Effect of dexamethasone (Dex) and dexamethasone mesylate (Dex-Mes) on GRE-dependent transcription. 2×GRE BEAS-2B reporter cells were treated with Dex or Dex-Mes at the concentrations indicated. At 6h cells were harvested for the determination of luciferase activity. Data points represent the mean ± s.e. mean of *N* independent determinations.

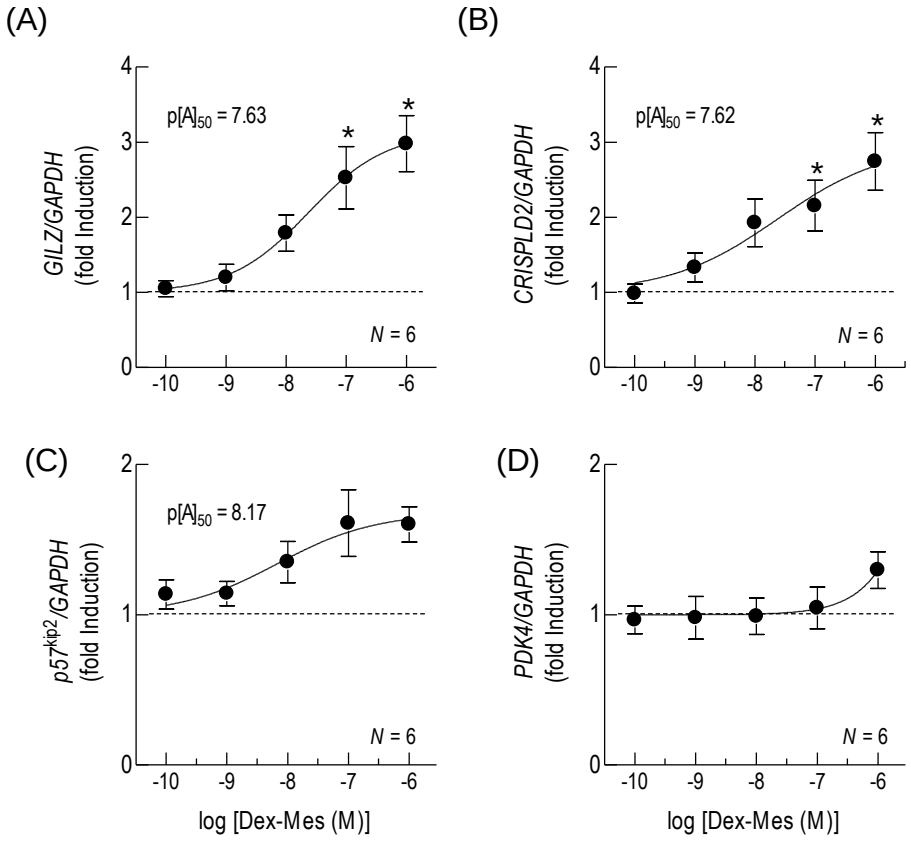


Figure S4. Effect of dexamethasone 21-mesylate (Dex-Mes) on gene expression. 2×GRE BEAS-2B reporter cells were treated with Dex-Mes at the concentrations indicated. At 6h, total RNA was extracted, reverse transcribed and the resulting cDNA subjected to real-time PCR using primer pairs specific for *GILZ* (panel *A*), *CRISPLD2* (panel *B*), *p57*^{kip2} (panel *C*) and *PDK4* (panel *D*). Data are expressed as the mean ± s.e. mean of *N* independent determinations and are expressed as a ratio to *GAPDH*. The dashed line in each panel defines baseline gene expression.

**P* < 0.05, significant induction relative to untreated cells; one-way ANOVA/Tukey's multiple comparison test on untransformed data.