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Supplemental information

Chronic stress-driven glucocorticoid receptor activation programs key cell phenotypes and functional epigenomic patterns in human fibroblasts

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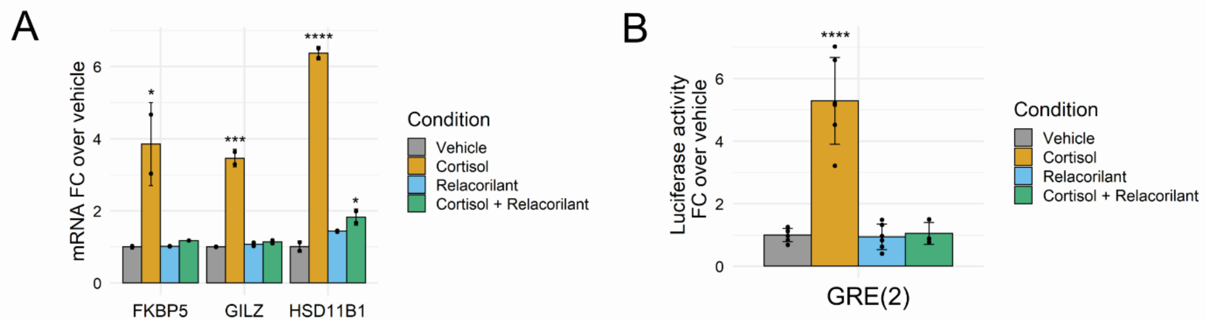

Figure S1, related to Figure 1. Acute transcriptional responses to physiological stress levels of cortisol with concomitant GR antagonism. **A)** qPCR analysis of glucocorticoid-responsive genes *FKBP5*, *GILZ*, and *HSD11B1* in IMR-90 fibroblasts treated with vehicle (DMSO), cortisol, relacorilant, or cortisol + relacorilant (*FKBP5*: $F_{3,6}$ = 11.66, P < 0.05, fold change (FC) cortisol vs. vehicle = 3.85; *GILZ*: $F_{3,6}$ = 171.1, P < 0.001, FC cortisol vs. vehicle = 3.46; *HSD11B1*: $F_{3,6}$ = 447.2, P < 0.0001, FC cortisol vs. vehicle = 6.33; all post-hoc Tukey-adjusted P values (Padj) < 0.05 for cortisol vs vehicle comparisons). Bars represent the mean of 2 biological replicates. **B)** Glucocorticoid receptor element (GRE) luciferase reporter assay in IMR-90 fibroblasts treated with vehicle, cortisol, relacorilant, or cortisol + relacorilant ($F_{3,20}$ = 48.89, P < 0.0001, FC cortisol vs. vehicle = 5.28, Padj < 0.0001 for cortisol vs. vehicle comparison). Bars represent the average of 4 biological replicates. All treatments were for four hours. All error bars represent one standard deviation above and below the mean. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

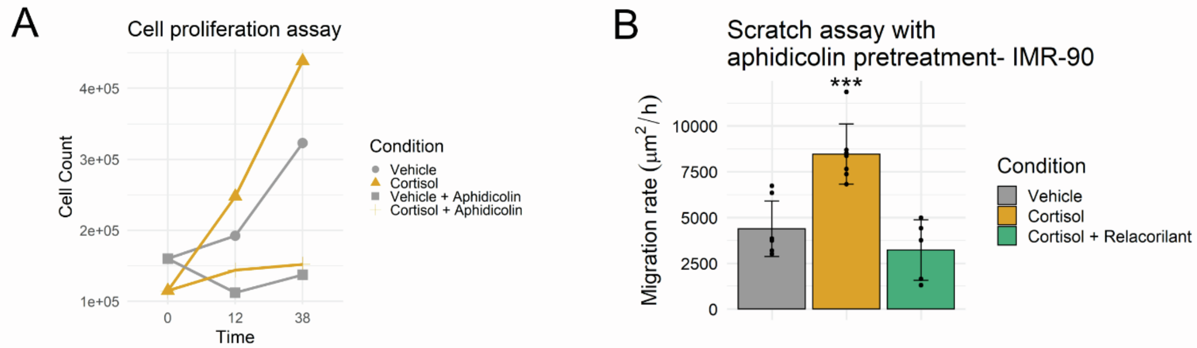

Figure S2, related to Figure 1. Effects of GR activation on cell migration after concomitant inhibition of cell proliferation. **A)** Cell proliferation assay of IMR-90 cells treated continuous with vehicle or cortisol for 35 days and subsequently treated with aphidicolin for 38 hours. Total cell counts were measured at 0, 12, and 38 hours post-aphidicolin treatment. Each point represents one biological replicate. **B)** Scratch assay of IMR-90 cells that have been continuously treated with vehicle, cortisol, or cortisol + relacorilant for 27 days and then treated with aphidicolin for 12 hours prior to scratch generation. Cells were imaged for up to 24 hours ($F_{2,16} = 18.8$, $P < 0.001$, FC cortisol–vehicle = 1.9, Padj < 0.001 for cortisol vs. vehicle comparison). Bars represent the mean of at least 5 biological replicates. Error bars represent one standard deviation above and below the mean. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

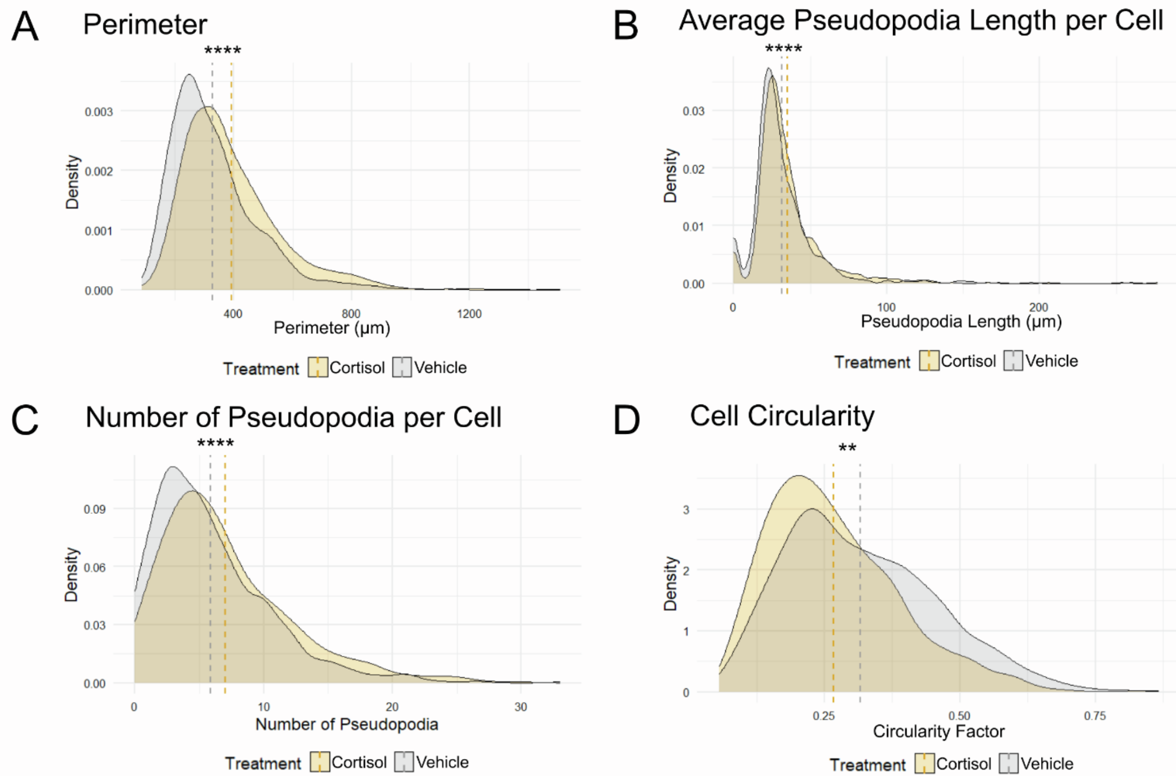

Figure S3, related to Figure 1. Overlapping density plots of cell perimeter of IMR-90 fibroblasts treated with aphidicolin and either vehicle or cortisol for cell perimeter **(A)**, average pseudopodia length per cell **(B)**, number of pseudopodia per cell **(C)**, and cell circularity **(D).** Dashed lines represent the mean for each respective measure. Image analyses included a total of 980 vehicle-treated cells and 1026 cortisol-treated cells. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

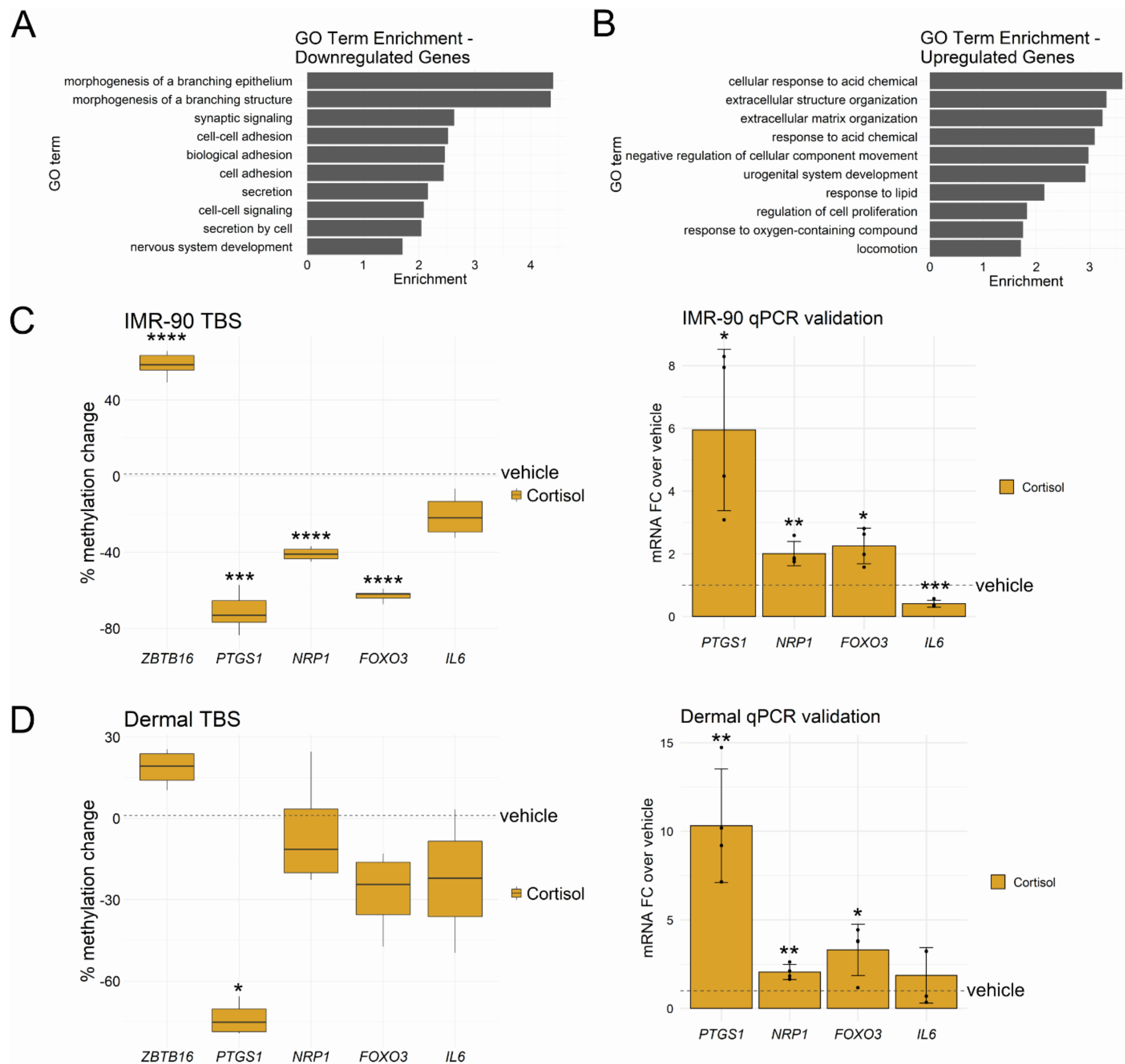

Figure S4, related to Figure 3. Direction-specific gene ontology and targeted gene validation in both IMR-90 cells and dermal fibroblasts. **A)** Gene ontology (GO) biological process analysis of downregulated GR-modulated genes. Top 10 most enriched pathways are shown. **B)** GO biological process analysis of upregulated GR-modulated genes. Top 10 most enriched pathways are shown. **C)** Targeted bisulfite sequencing (TBS) validation of five representative genes (left panel) and qPCR validation of four of these genes (right panel) in IMR-90 cells. **D)** TBS validation of five representative genes (left panel) in dermal fibroblasts and qPCR validation of four of these genes (right panel) in dermal fibroblasts. *B2M* was used as the housekeeping gene for all qPCR analyses. For both panels, *ZBTB16* mRNA fold change (FC) is not shown because they were too low to be detected with qPCR in treatment conditions other than the cortisol group. Bars represent the mean of at least 3 biological replicates. All error bars represent one standard deviation above and below the mean. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

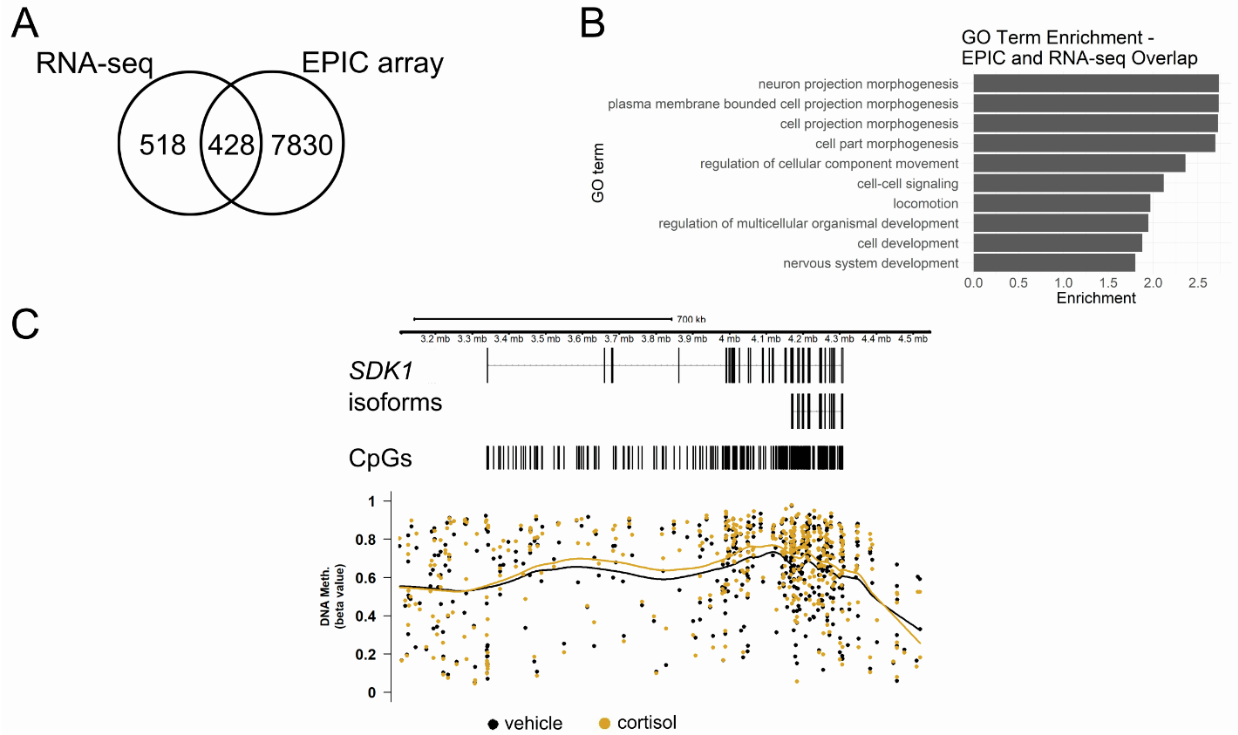

Figure S5, related to Figure 4. Gene ontology analysis of overlapping RNA-seq and EPIC array genes and additional example of GR-driven gene body hypermethylation. **A)** Overlap between GR-modulated genes identified in RNA-seq and EPIC array DNA methylation analysis. **B)** Gene ontology (GO) biological process analysis of the 428 GR-modulated genes that overlap between the RNA-seq and EPIC array analysis. The top 10 most enriched pathways are shown. **C)** Metagene plot showing locally estimated scatterplot smoothing of CpG DNA methylation values across the *SDK1* gene. Yellow points represent DNA methylation in cortisol-treated IMR-90 cells. Black points represent DNA methylation in vehicle-treated IMR-90 cells. Locations of CpGs are indicated.