Supplementary Materials for **"The Hypersensitive Glucocorticoid Response Specifically Regulates Period 1 and Regulates Expression of Circadian Genes"**

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Supplementary Figures:

Figure S1

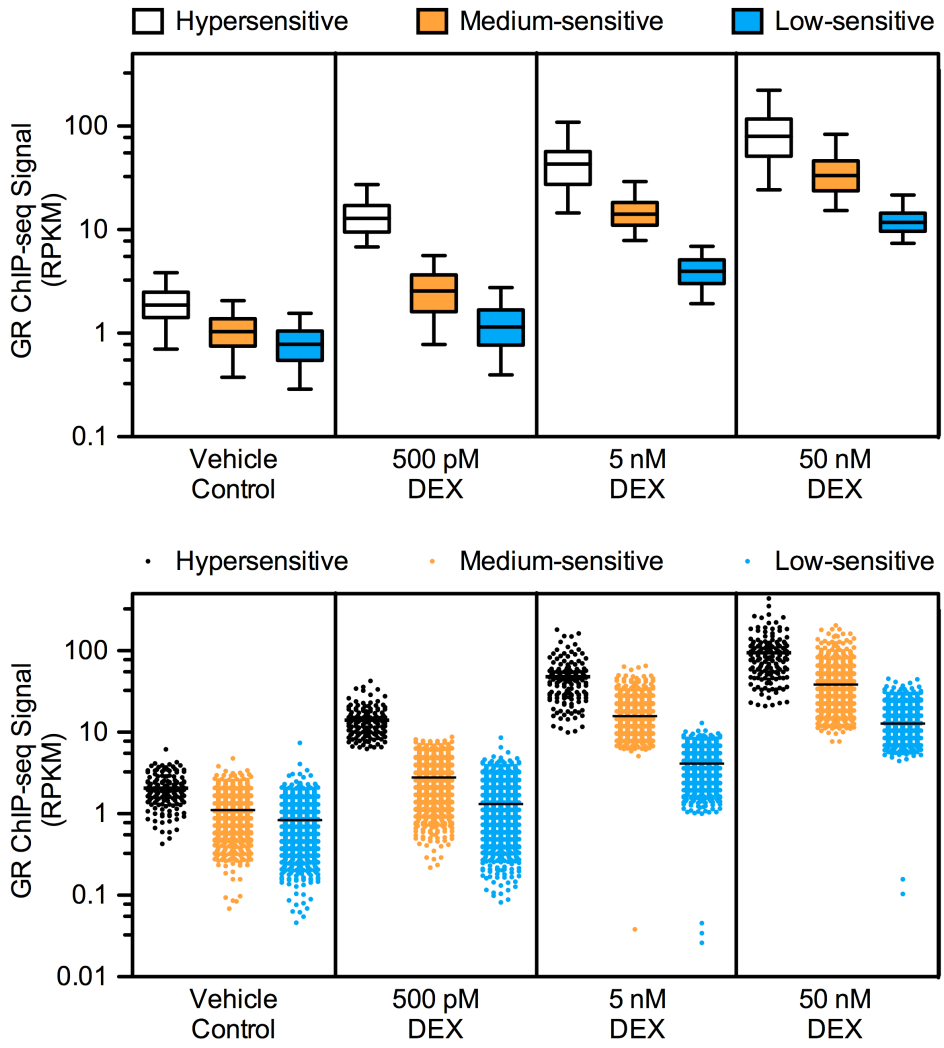


Figure S1: Distribution of ChIP-seq signal for each class of GR binding site. In each panel, the x-axis indicates the concentration of DEX used. Within each panel, the distribution of ChIP-seq signal is shown for the hypersensitive (white/black), medium-sensitive (orange), and low-sensitive GR (blue) GR binding sites. Whiskers in the top plot indicate the 5%-95% range of the data. In the bottom plot, each point is a GR binding site.

Figure S2

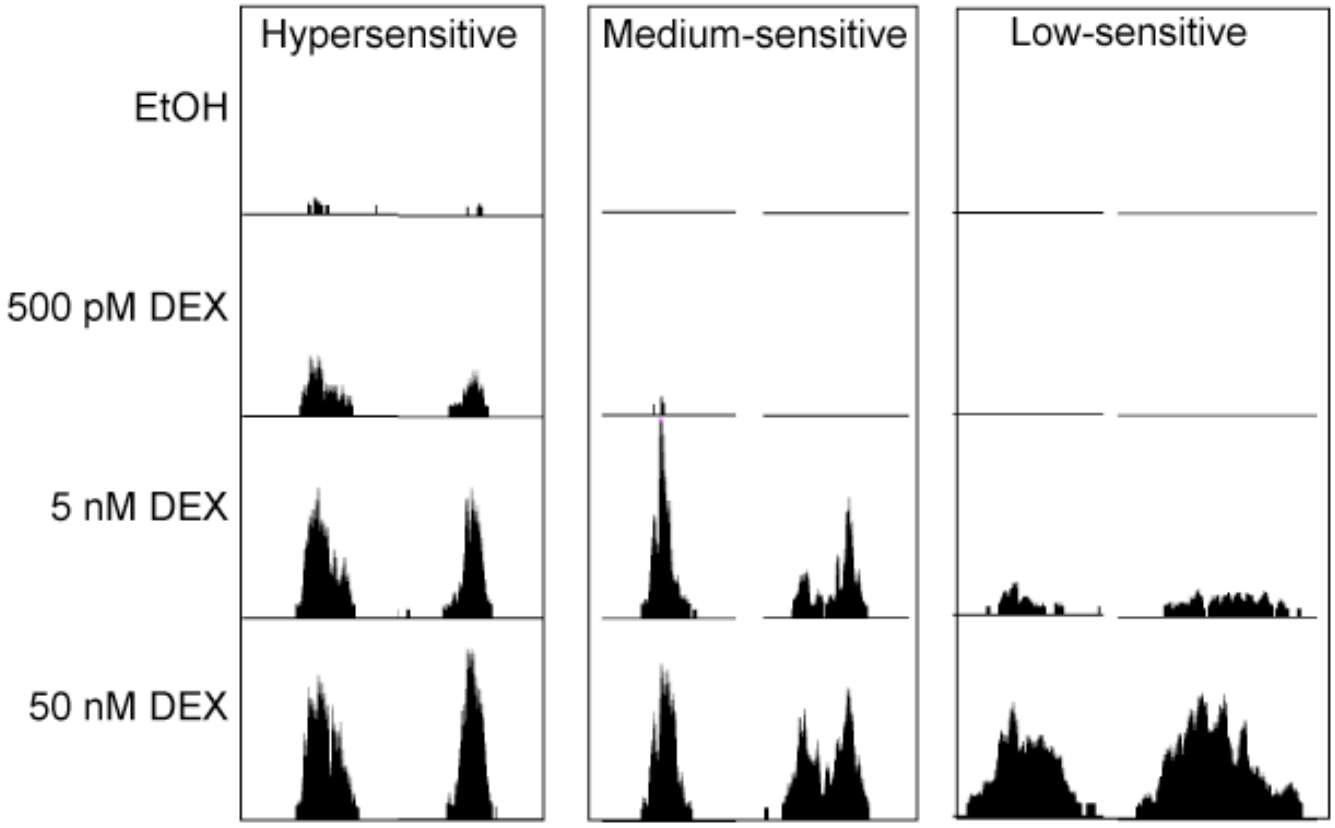
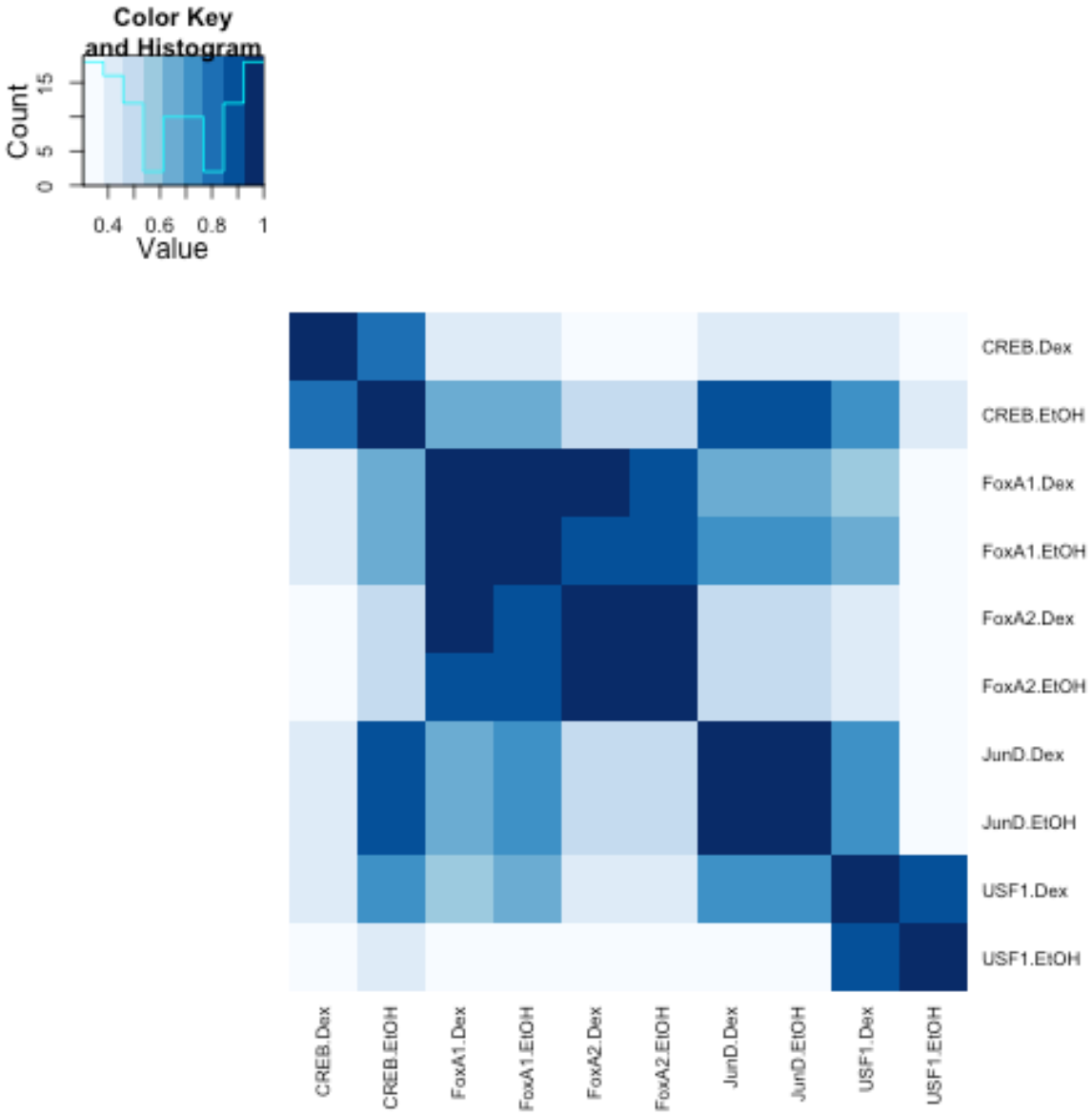


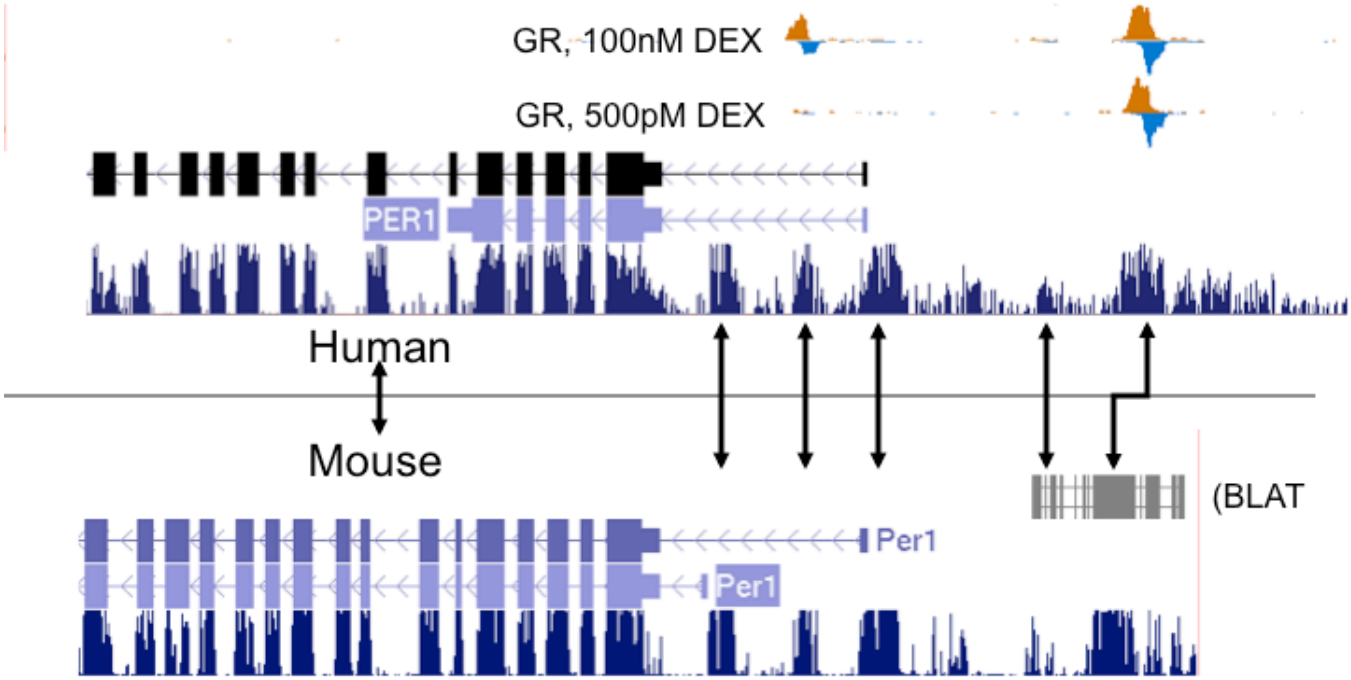
Figure S2: Examples of hypersensitive, medium-sensitive, and low-sensitive GR binding sites from the genome. Sites were selected such that they have similar ChIP-seq signal strength at 50 nM DEX.

Figure S3



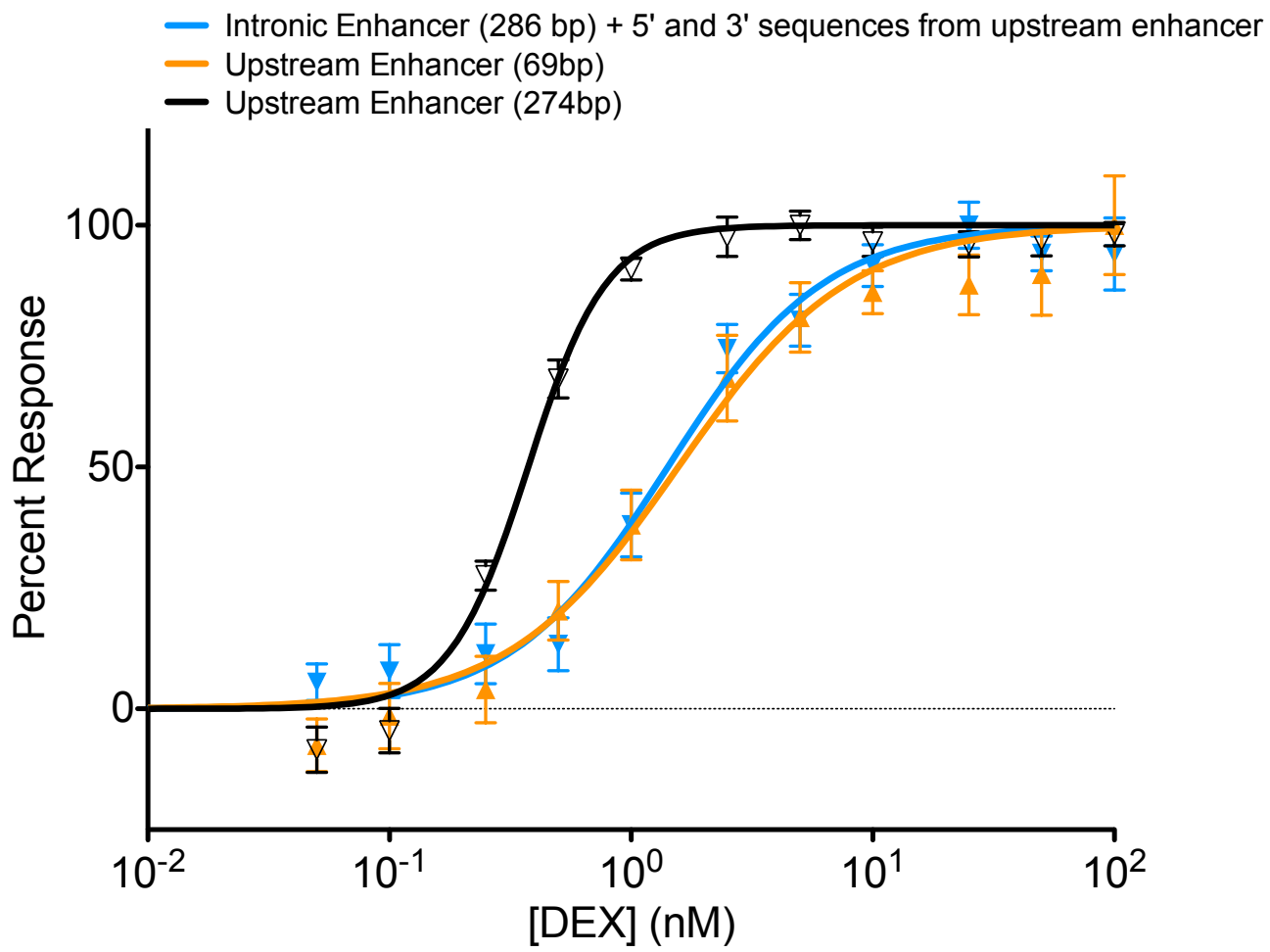
Pearson correlation of ChIP-seq signal, measured in aligned reads per kilobase of genomic sequence and per million aligned reads (RPKM) within 5 kb of genomic transcription start sites. ChIP-seq signal for the same factor but under different conditions were highly correlated, as was ChIP-seq signal between *FOXA1* and *FOXA2*. Correlated occupancy was also observed between *JUND* and *CREB*, and may be indicative of co-regulation between the two factors.

Figure S4:



Conservation of GR binding sites surrounding PER1 between human and mouse. Both the intronic and upstream GR binding sites occur in regions of conservation (indicated by blue graph beneath gene diagrams). In mouse, the upstream GR binding site is closer to the PER1 transcription start site.

Figure S5:



Dose response of enhancer element derived from the PER1-intronic (normally-sensitive) GR binding site, but with flanking sequences mutated to match the sequence of the PER1-upstream (hypersensitive) enhancer region. The nucleotide sequence of the modified intronic enhancer region is listed below, where blue indicates the GR binding sequence, and bold and underlined sequence indicates sequences that match the corresponding location relative to the GR binding sequence in the hypersensitive enhancer element.

CCGGTCTTCTTGCTCGTTACTCGAGGGGCCCCAGGTTTGCCCCGGTACCAGGACCCTAT
TAGGCTTTCAGCGCTCCCCGTGTCTCTGTTCTCCAGTCCCTGGCCCTGCGCCGCCTGTG
ATGT**TGGGCCACCAGCCAA**T**AGAACATCCCGTTCC**CA**GCGCTGCTGGCCGCCGCCCTC**
CACAAC**CTGGCCGCCTCCCAA**GGCGCTCAGAAAATGCTCAGTAGTAGGGGTGTGGTTGG
ACGGGGAGTAGGGGAAGAAAGATCTGTTGGCTGTTGTGTGTCTTCT

Figure S6:

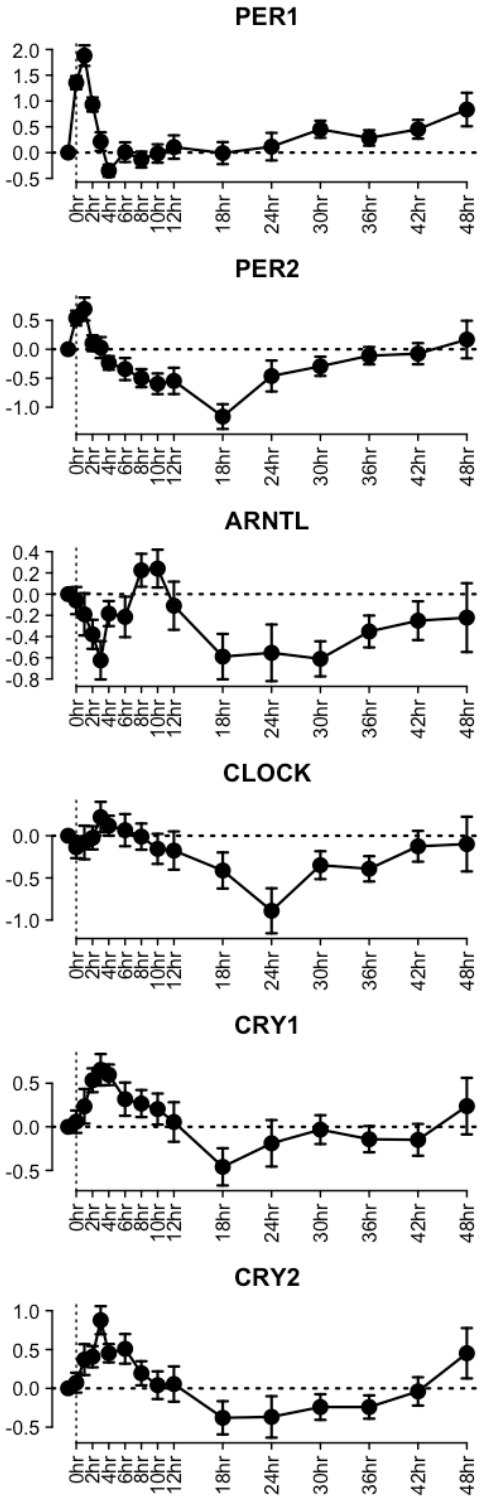


Figure S6: 48 time-course after treatment with 0.5 nM DEX for one hour, followed by removing the DEX for the number of hours indicated on the x-axis. The data here combine experiments shown in Figure 7 as well as an additional 6 biological replicates (4 technical replicates ea.) of the following time points: No Dex, 0 hr, 1 hr, 3 hr, 6 hr, 12 hr, 18 hr, 24 hr, 30 hr, 36 ht, 42 hr, and 48 hr). Expression of each gene was measured using TaqMan RT-QPCR assays. Expression was normalized both to GAPDH and GR expression, both of which we expect to be constant, using linear regression. The y-axis shows the log-fold expression change relative to no DEX treatment. Error bars indicate 95% confidence intervals on the fold changes.

Supplementary Tables:

Table S1: Numbers of dose-specific GR binding sites

	Aligned Sequence Reads	
[DEX] (nM)	Rep1	Rep2
0	18,022,468	18,412,092
0.5	16,799,279	12,740,831
5	18,078,035	18,349,363
50	16,504,811	14,615,434

Table S2: Dose-specific GR Binding Sites

(Table included as external Excel spreadsheet)

Table S3: Numbers of dose-specific GR binding sites

Class	# binding sites	# w/ GRE (fraction)	# w/ rev. GRE (fraction)	Fold enrichment*
0.5 nM	145	86 (0.59)	24 (0.17)	3.58
5 nM	1447	891 (0.62)	250 (0.17)	3.56
50 nM	4295	2466 (0.57)	685 (0.16)	3.60

Class	# binding sites	# GREs	GREs/site
0.5 nM	145	155	1.068965517
5 nM	1447	1639	1.132688321
50 nM	4295	4647	1.081955763

* Fold enrichment is defined as the number of sites with a GRE divided by the number of sites with a reverse GRE.

Table S4: Aligned Sequencing Reads for ChIP-seq of GR Co-factors

		Aligned Sequencing Reads			
Factor	Antibody*	EtOH, Rep1	EtOH, Rep2	100nM DEX, Rep1	100nM DEX, Rep2
FoxA1	sc-101058	22,847,803	18,192,774	23,260,471	16,932,805
FoxA2	sc-6554	27,442,050	54,840,111	42,044,987	14,820,581
CREB	sc-240	16,603,386	17,608,941	24,436,090	16,164,577
JunD	sc-74	18,776,549	25,296,785	21,053,673	16,120,795
USF1	sc-229	15,087,543	12,330,014	20,814,482	11,521,793

* All antibodies were supplied by Santa Cruz Biotechnology

	EtOH	100nM DEX
Input Control, 1 PCR	18,140,684	13,273,349

Table S5: ChIP-seq Determined Binding Sites for GR Cofactors

(Table included as external Excel spreadsheet)

Table S6: Gene-expression Changes in Response to Low-dose DEX

(Table included as external Excel spreadsheet)

Supplementary Methods:

Mutation screening of hypersensitive GR enhancer upstream of PER1

To screen the entire hypersensitive GR enhancer for mutations that impact hypersensitivity, we synthesized wild-type and 29 mutant versions of a 235 bp regions surrounding the hypersensitive GR binding sites upstream of PER1 (Genscript). Each mutant had 10 nucleotides of the wild type sequence replaced with the 10 bp nucleotide sequence ACACACACAC. The mutations were introduced every 7 bp along the enhancer so that 6 bp of every mutant sequence overlapped another mutant enhancer. The enhancer sequences were subcloned from a cloning vector into a pGL4.24 luciferase reporter vector with a minimal promoter (pGL4.14 from Promega) and verified with Sanger sequencing. Each construct was then miniprepped, and transfected into A549 cells, assayed in response to increasing concentrations of DEX, and analyzed as described in the main text. The exact sequence of each enhancer used is as follows:

>Wild-type
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_2
GGCCTAACTGGCCGGGGGGAA**CACACACACAC**GGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_3
GGCCTAACTGGCCGGGGGGAGGTGTGG**CACACACACAC**CCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_4
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGG**CACACACACAC**TACTAATCCTCTCTCC
AGGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_5

GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCT**ACACACACAC**CCTCTCTCC
AGGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_6
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACT**ACACACACAC**CC
AGGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_7
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTC**ACACA**
CACACTCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_8
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
G**ACACACACAC**CTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_9
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCAC**ACACACACAC**AGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_10
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GGAATCCCTGGCTTT**ACACACACAC**GGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_11
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GGAATCCCTGGCTTTGGGACAG**ACACACACAC**GTTTTGTTGGGGGAGGCGCCCAGGCTGG
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_12
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GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_13
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
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GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_14

GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGG**ACACACACAC**AGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_15
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCG**ACACACACAC**
GTGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_16
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGC**ACAC**
ACACACTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_17
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGC**ACACACACAC**CCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCC
CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_18
GGCCTAACTGGCCGGGGGGAGGTGTGGGGAGGGGGCCCCCTTCCTACTAATCCTCTCTCCA
GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCT**ACACACACAC**CCAAGAGAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_19
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TGCATGTCCTGGGCC**ACACACACAC**ACAACATGATGTTCCCAAGTGCGCTGGCCGCCGCCC
TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
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TCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_21
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CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_22
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CTCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC
>mut_23

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GGAATCCCTGGCTTTGGGACAGGACGGCTGTCGTTTTGTTGGGGGAGGCGCCCAGGCTGGG
TGCATGTCCTGGGCCACCAGCCAAGAGAACATGATGTTCCCAAGTGCGCTGGAC**ACACAC**
ACCTCGGGCTGGCCGCCTCCCAAACCGCTGCCTCTCCAGTGGCCTCGGCGGCC

>mut_25
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>mut_27
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>mut_28
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TCTCGGGCTGGCCGCCTCC**ACACACACAC**CCTCTCCAGTGGCCTCGGCGGCC

>mut_29
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>mut_30
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