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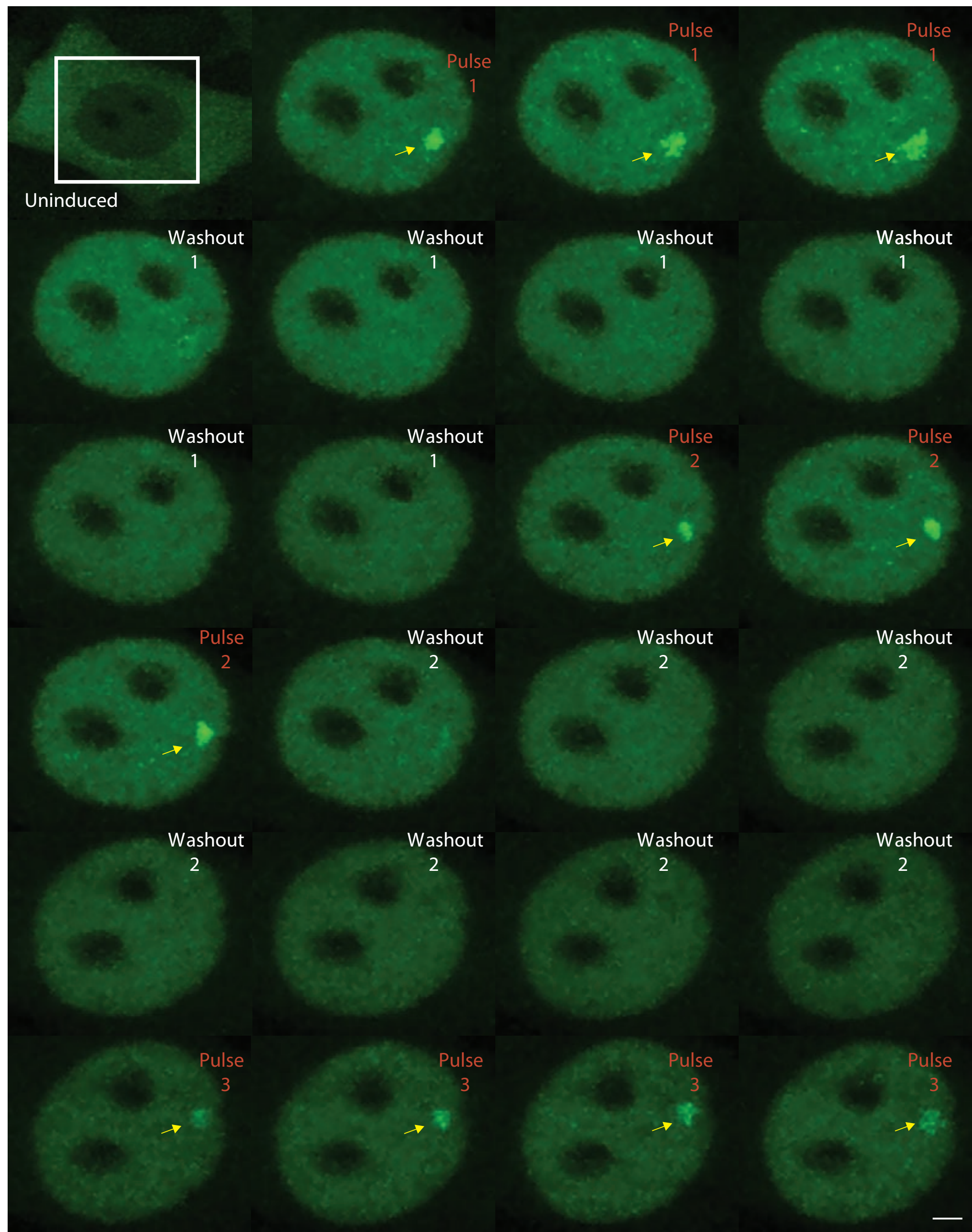

Figure S1 Cyclic GR loading at the MMTV promoter array in vivo in response to ultradian corticosterone treatment. A complete data set is shown for the material summarized in Fig. 1b. Images were obtained from a single cell over a 3 hour experiment, with three induction periods (Pulse 1, 2 and 3) and two hormone withdrawal periods (Washout 1 and 2). Yellow arrows indicate GFP-GR associated with the promoter array locus. Scale bar 2 µm.

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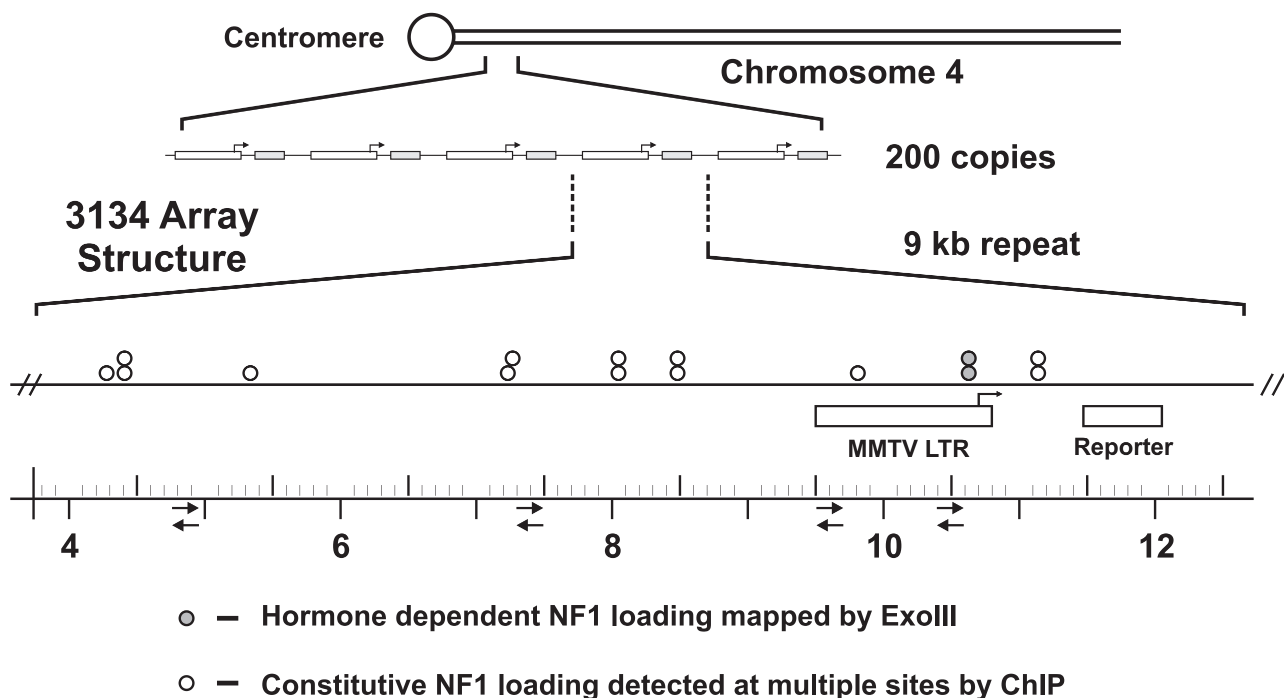

Figure S2 Schematic representation of the MMTV promoter array structure, with multiple NF1 binding sites present throughout the 9 kb repeat. NF1 binds to the site immediately proximal to the promoter in a hormone-dependent manner[1]. In contrast, the extent of NF1 association with the other binding is constitutive (S. John, D. Stavreva, unpublished). Thus, observation of Cherry Red Fluorescent Protein (ChRFP)-tagged NF1 binding to the remaining NF1 sites permits array visualization in both non-induced and induced cells.

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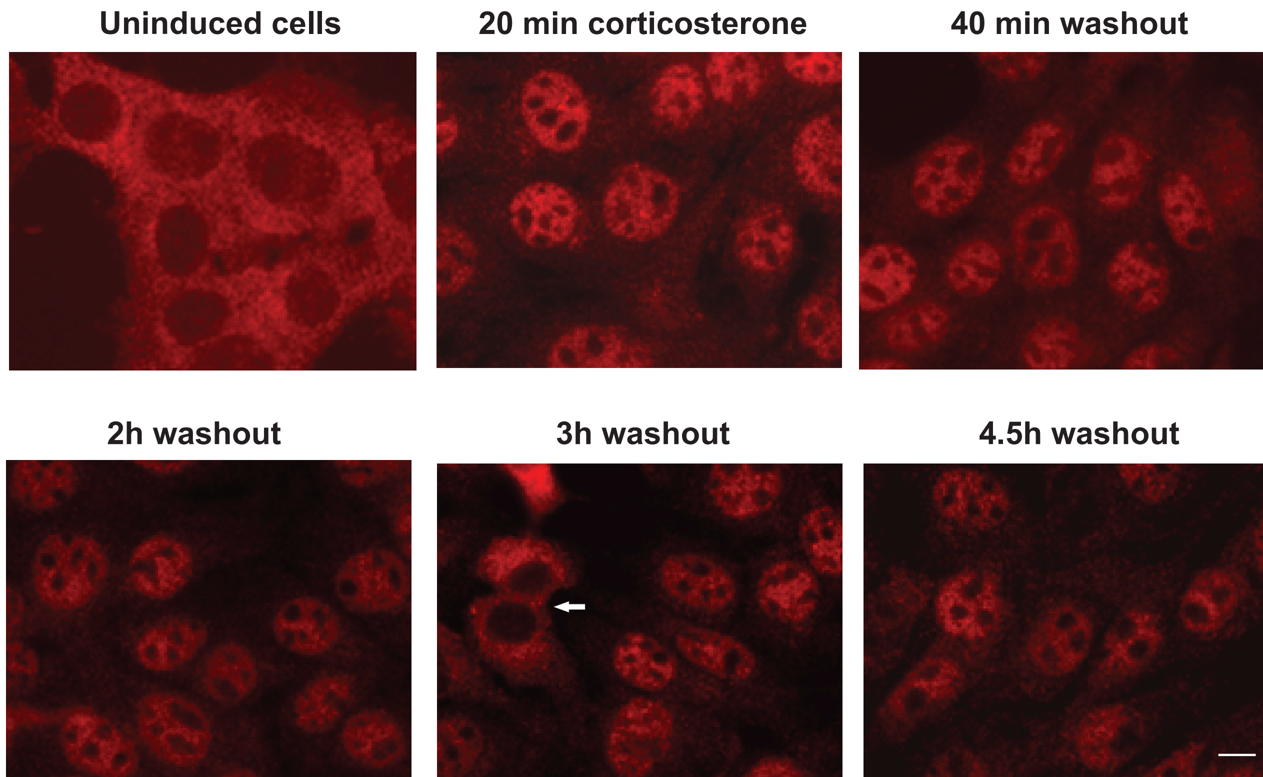

Figure S3 Endogenous GR remains in the nucleus several hours after corticosterone withdrawal. 3134 cells were treated with 100 nM corticosterone for 20 min and fixed at different times after hormone withdrawal. Cells were immunostained with anti-GR antibody (PA1-511A, ABR). As expected, endogenous GR is in the cytoplasm before induction and translocates to the nucleus upon hormone stimulation. With exception of the dividing cells, where resulting daughter cells have cytoplasmic GR (white arrow), cells exhibit predominantly nuclear GR staining more than 4h upon hormone withdrawal. Scale bar 5 μm.

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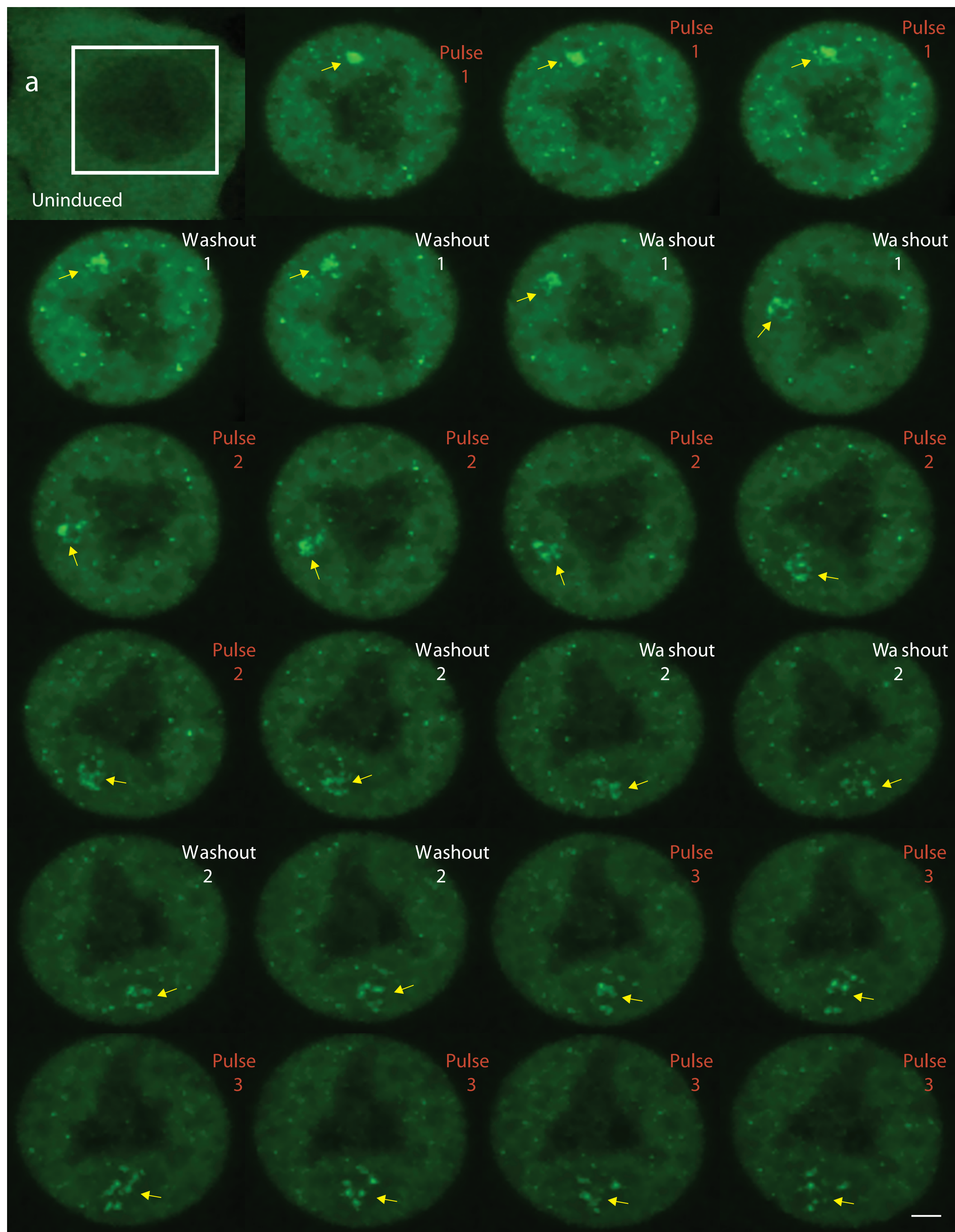

Figure S4a GFP-GR promoter interactions in vivo; ultradian dex treatment. Promoter interactions are insensitive to fluctuations in extracellular dex conc. During withdrawal of dex, GFP-GR array association remains unchanged, and the arrays persist in the decondensed state[2] (yellow arrows). Loss of dex from the receptor is too slow for the receptor to respond to the decreased extracellular hormone level. Scale bar 2 µm.

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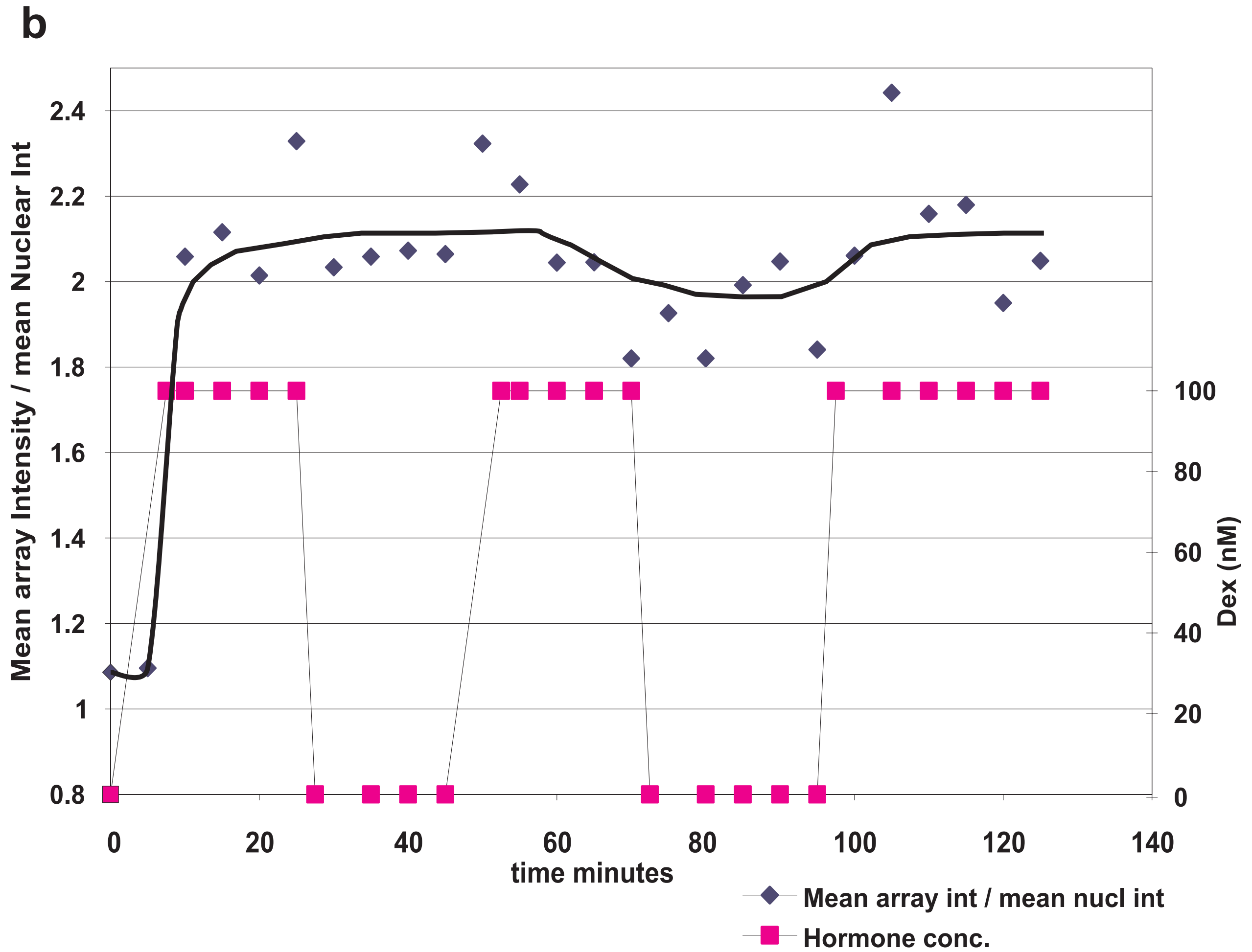

Figure S4b GFP-GR/MMTV promoter interactions in vivo in response to ultradian dexamethasone treatment. Quantitative analysis. GFP-GR association with the array locus over three periods of dexamethasone induction and two hormone withdrawal periods demonstrates a loss of cyclic GR/array association when GR is activated with the synthetic hormone dexamethasone.

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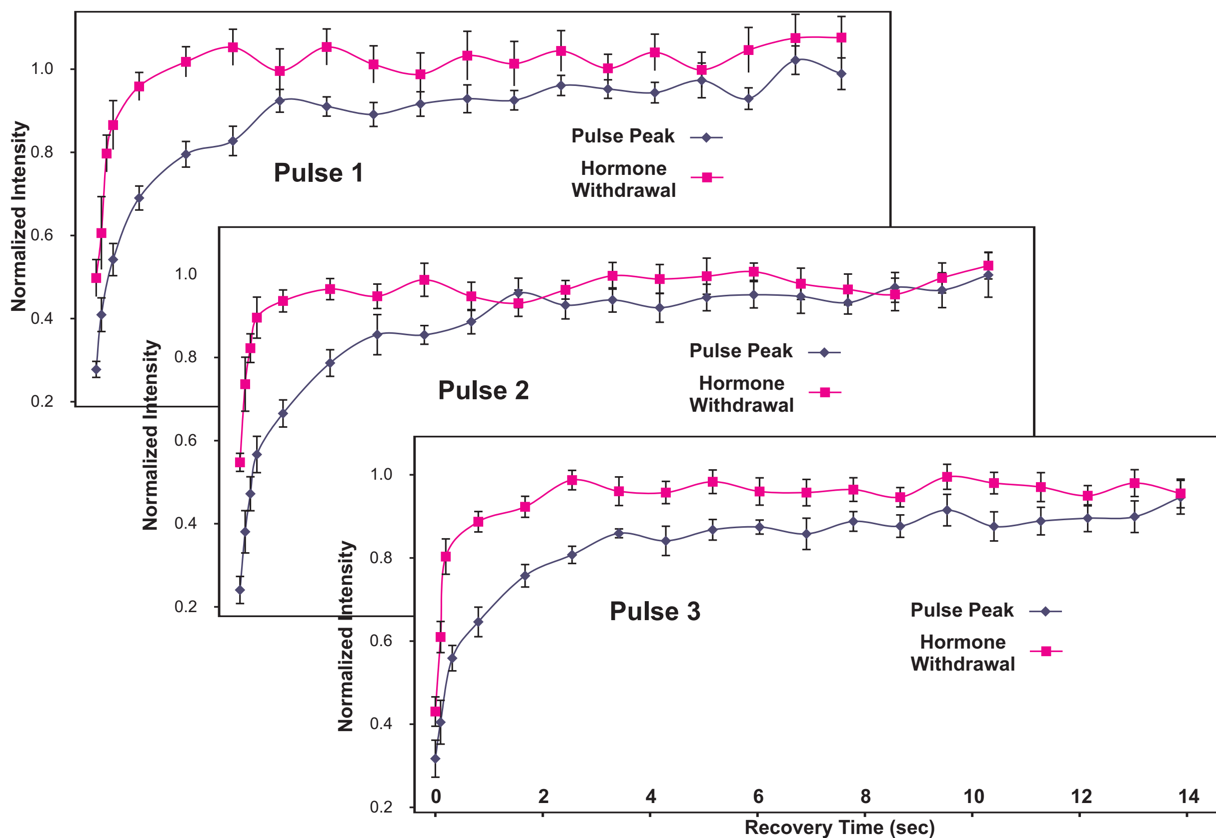

Figure S5 GFP-GR exchange dynamics at the promoter array over three cycles of ultradian corticosterone stimulation, determined by fluorescent recovery after photobleaching (FRAP). Arrays were localized with ChRFP-NF1 during the hormone withdrawal periods. The data points in Fig. 2d were derived from these recovery curves. Data values represent the mean value ± s.e.m., n = 15 cells for each curve. Average $t_{1/2}$ for the recovery of the pulsed and washed samples are 0.28 (±0.0012) and 0.08 (± 0.009) sec., respectively.

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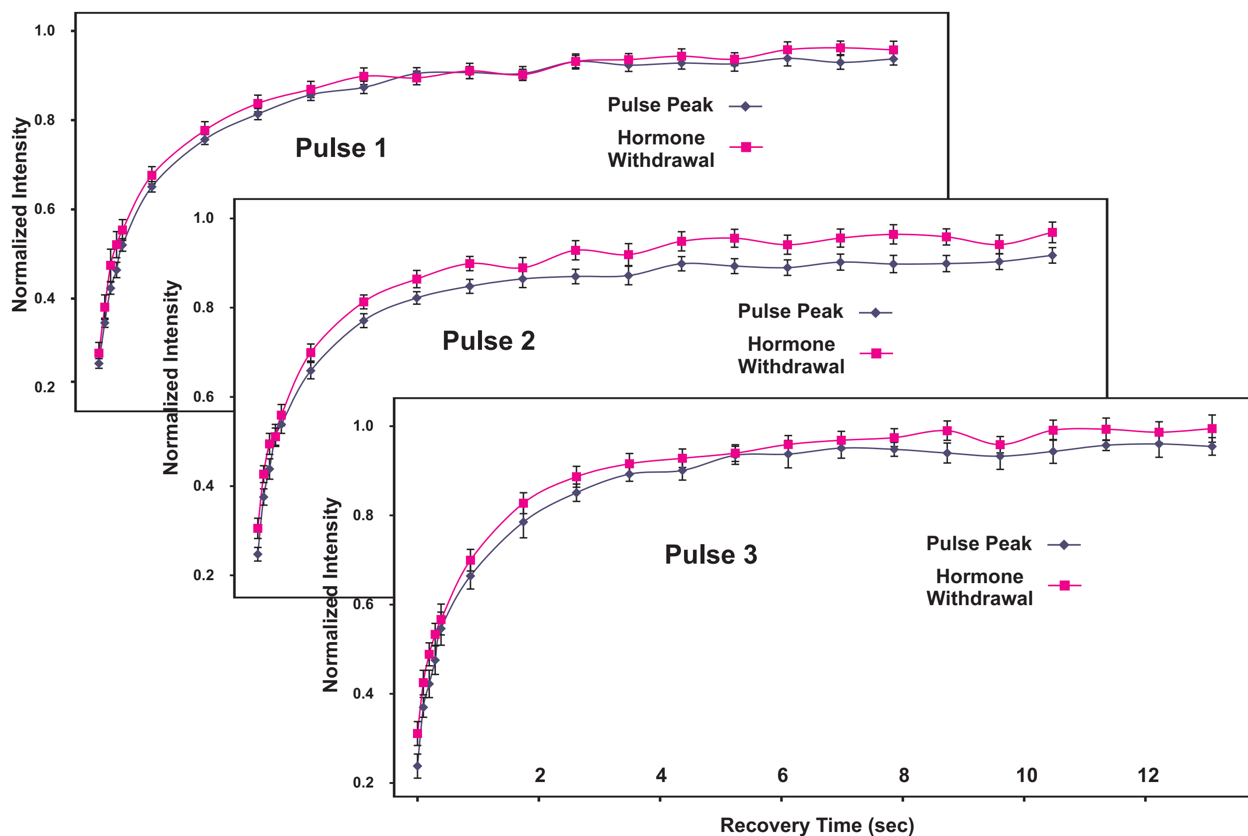

Figure S6 GFP-GR exchange dynamics at the promoter array over three cycles of ultradian dexamethasone stimulation, determined by FRAP analysis. Arrays were localized with ChRFP-NF1 during the hormone withdrawal periods. The data points in Fig. 2h were derived from these recovery curves. Data values represent the mean value ± s.e.m., n = 15 cells for each curve. Average $t_{1/2}$ for the recovery of the pulsed and washed samples are 0.32 (±0.0015) and 0.2 (± 0.003) sec., respectively.

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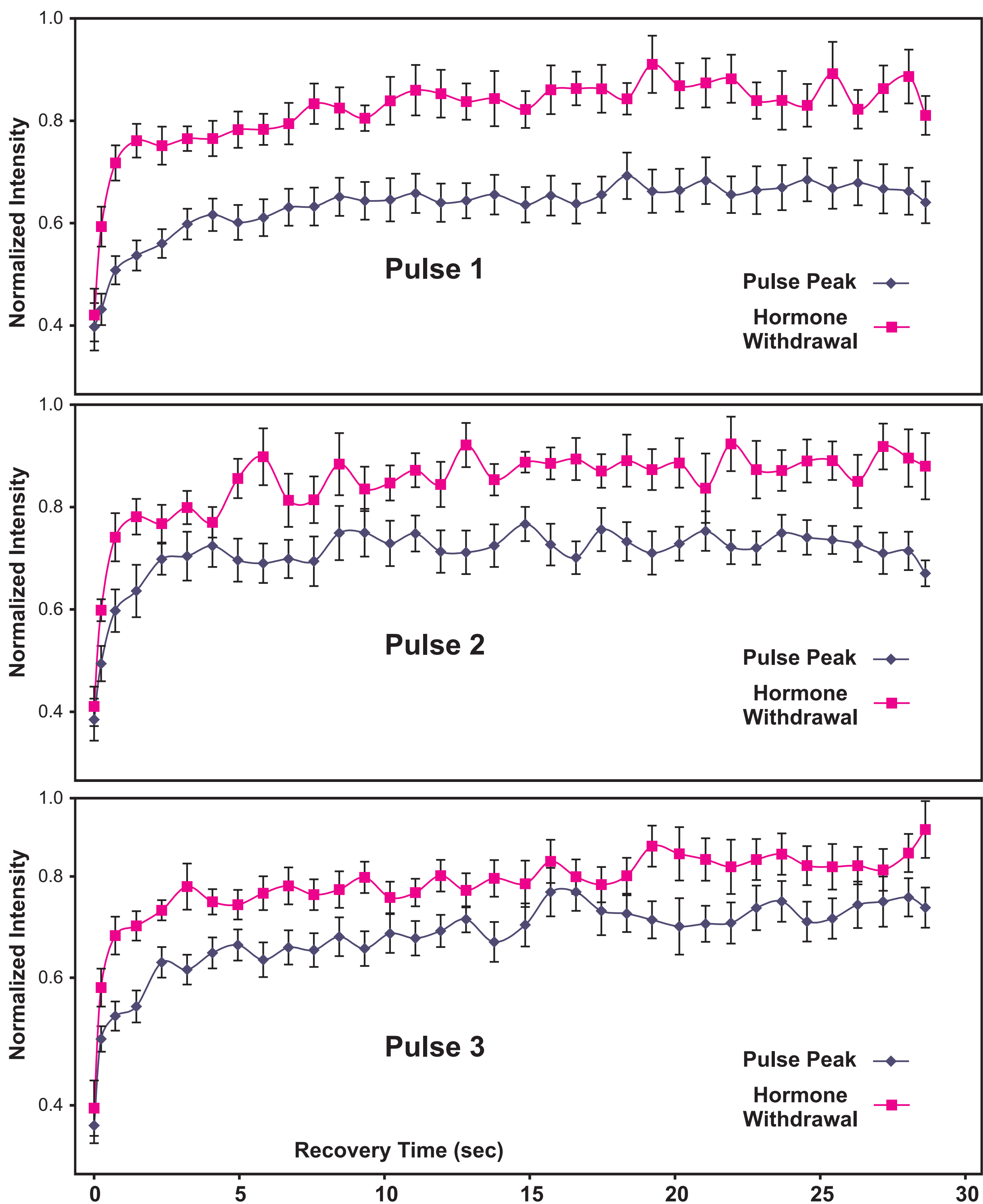

Figure S7 GFP-Pol II exchange dynamics at the promoter array over three cycles of ultradian corticosterone stimulation, determined by FRAP analysis. Arrays were localized with ChRFP-NF1 during the hormone withdrawal periods. The data points in Fig. 3d were derived from these recovery curves. Data values represent the mean value ± s.e.m, n= 12-19 cells for each curve. Average $t_{1/2}$ for the recovery of the pulsed and washed samples are 0.72 (±0.0145) and 0.24 (± 0.0058) sec., respectively.

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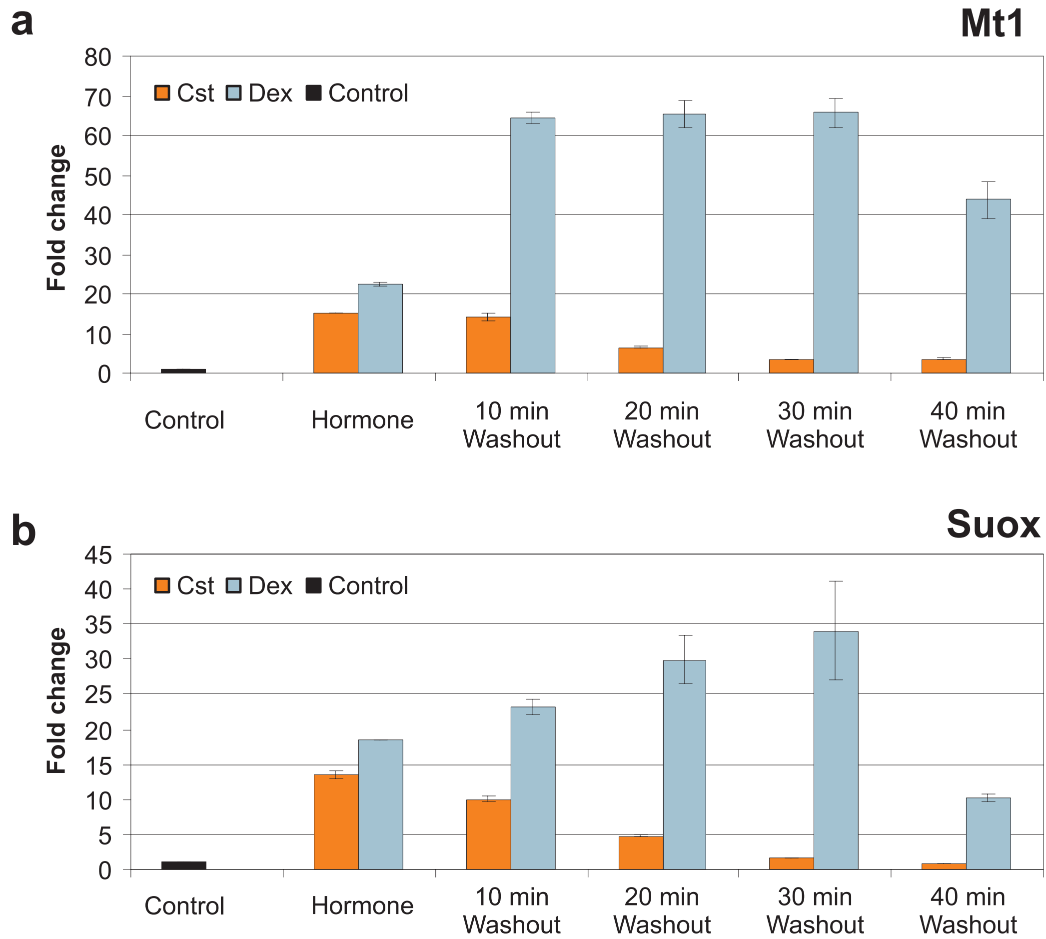

Figure S8 Transcription downregulation upon corticosterone (100 nM) and dexamethasone (100 nM) withdrawal. (a) Mt1 and (b) Suox genes are both induced after 20 minutes of treatment with corticosterone (100 nM) or dexamethasone (100 nM). Upon hormone withdrawal transcription is rapidly downregulated when the activating hormone is corticosterone. However, genes induced with dexamethasone are downregulated at a much slower rate. Data values represent the mean value ± s.e.m., n=3.

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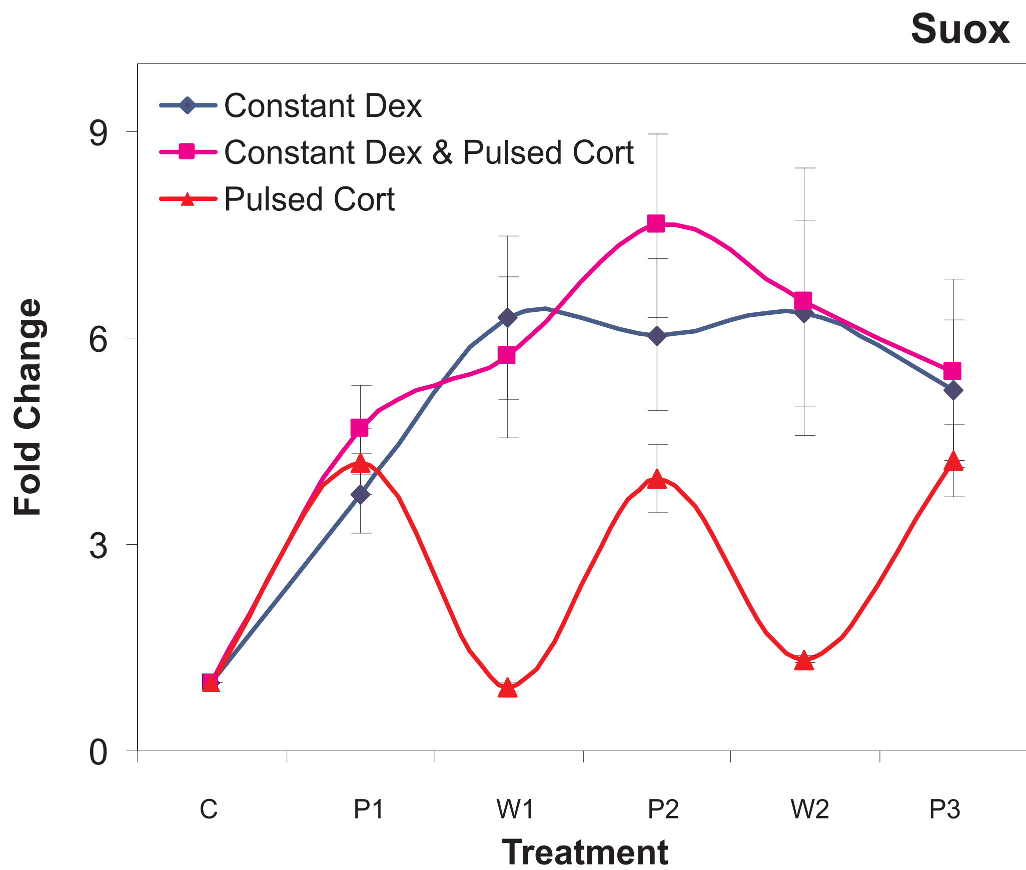

Figure S9 Effect of constant dexamethasone stimulation on ultradian corticosterone treatment. Constant treatment with low doses (25 nM) of dexamethasone leads to constant transcription induction, while ultradian corticosterone treatment (100 nM) induces pusatile release of nascent RNA from a GR-regulated gene (Suox). Combined application of both treatments leads to a constant transcription induction. Data values represent the mean value (±) s.e.m., n=6.

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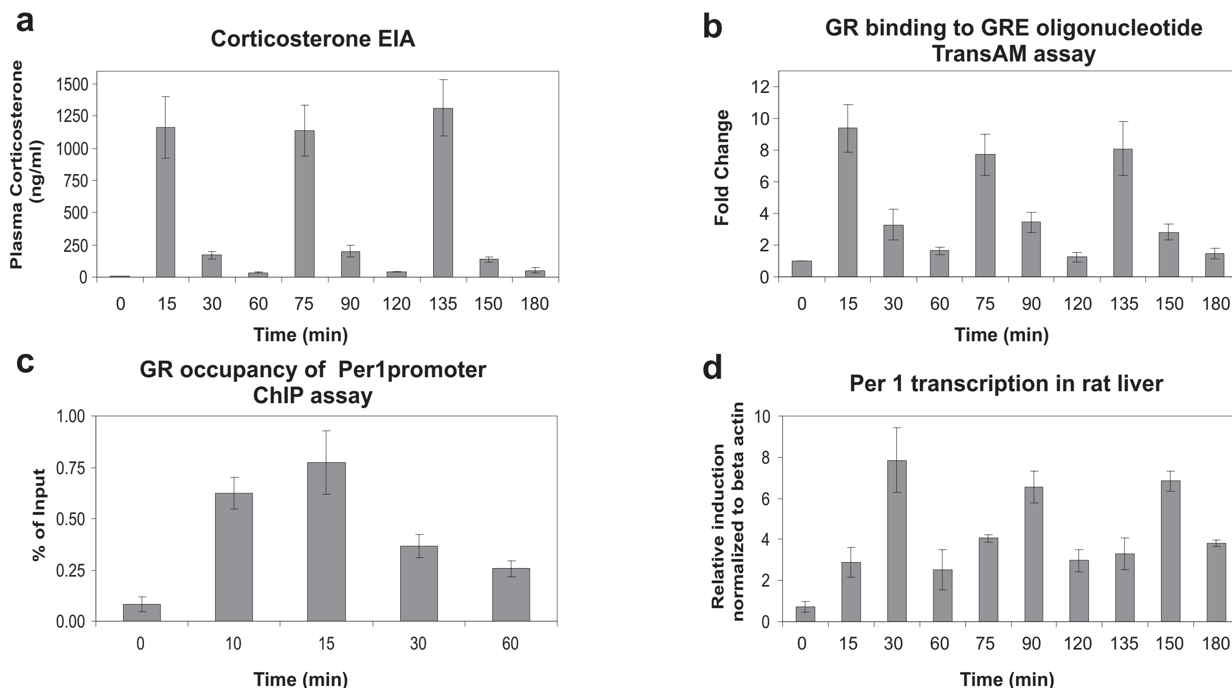

Figure S10 Live animal experiments (**a**) Circulating corticosterone levels were detected maximally in plasma at 1 min after each IV injection (1161 ± 238 ng/ml, 1141 ± 198 ng/ml, 1315 ± 221 ng/ml after pulses 1, 2 and 3 respectively) and then subsequently cleared according to the characterized half-life of corticosterone in blood (t1/2<10min). Data are the mean value ± s.e.m., n= 4 to 12 animals. (**b**) GR binding to GRE containing synthetic oligonucleotides was maximal at 15min post IV corticosterone injection (fold induction relative to time 0 was 9.4 ±1.5; 7.7 ± 1. 3; 8.1 ±1.7 for pulses 1, 2 and 3 respectively) and then declined rapidly and in parallel with ligand clearance from the circulation. At 60 min post injection, GRE binding had decreased to baseline levels. Each subsequent pulse elicited similar maximal levels of GR activation, and a similar timecourse of GRE association then dissociation. Data are the mean value ± s.e.m., n= 4. (**c**) The TransAM GRE binding results were validated, for the timecourse of the first pulse up to 60min, with chromatin immunoprecipitation (ChIP) assays for GR association with a GRE containing promoter region of the rat Period 1 gene. The timecourse of GR:DNA association and dissociation detected with the ChIP assay was consistent with the data obtained from the TransAM assay. Data are the mean value ± s.e.m., n= 4. (**d**) Nascent transcript production from the Period 1 gene occurred rapidly, reaching a maximum at 30min after each injection (fold induction relative to time 0 was 11.0 ± 2.2, 9.2 ± 1.1 and 9.6± 0.7 for pulses 1, 2 and 3 respectively). Consistent with the pattern of GR dissociation from the DNA as ligand was cleared from the circulation, nascent transcript production also decreased in parallel to declining corticosterone levels (albeit delayed) with diminished levels at 60 min. after each pulse. Data are the mean value ± s.e.m., n= 3 to 6. The data points in Fig. 4b were derived from panels a, b and d.

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References

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2. Muller,W.G., Walker,D., Hager,G.L., & McNally,J.G. Large-scale chromatin decondensation and recondensation regulated by transcription from a natural promoter. *J. Cell Biol.* **154**, 33-48 (2001).

Supplementary Movie Legends

Movie S1
Time dependent association kinetics of corticosterone-liganded GFP-GR with the MMTV promoter array over a 2 hour treatment period. The array localized GFP-GR fluorescence signal is plotted in green; corresponding extracellular corticosterone concentration is plotted in red.

Movie S2
Time dependent association kinetics of dexamethasone-liganded GFP-GR with the MMTV promoter array. In the presence of dex, GFP-GR continues to associate with response elements, even during the ligand withdrawal periods. The array also remains decondensed throughout the course of the three hormone pulses.

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