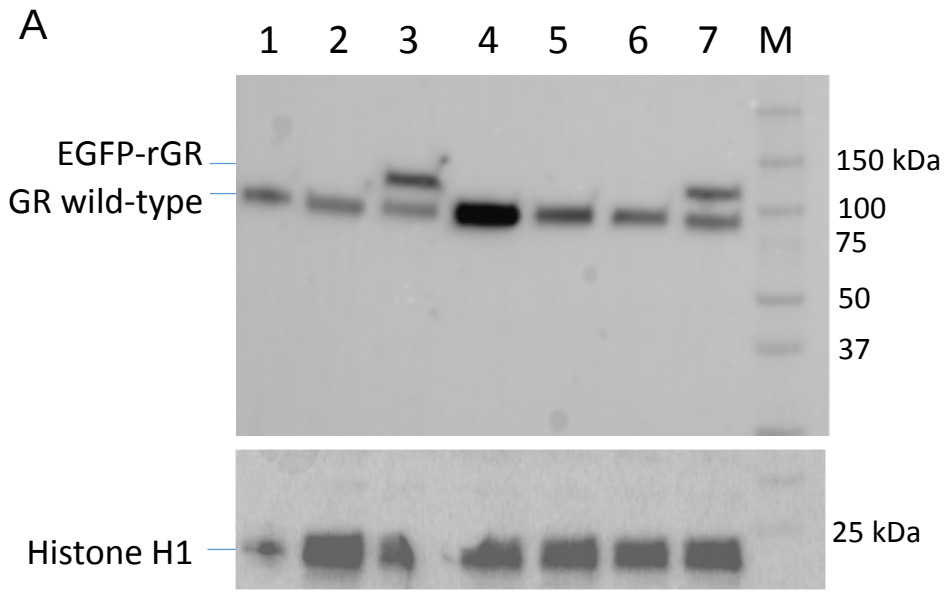
Figure S1. Western blots

Western blots of Neuro 2A (N2A) cell lysates showing expression levels of endogenous and transiently transfected GR and MR. N2A cells in 10 cm plates were transfected with DNA constructs and JetPrime (1:2 w/v). Lysates were prepared (Conway-Campbell BL, McKenna MA, Wiles CC, Atkinson HC, de Kloet ER, Lightman SL., Endocrinology 2007 Nov;148(11):5470-7.) and 20 μg each lysate was loaded and detected with anti-GR (Cell Signalling D6H2L) at 1:1000, anti-MR (1D5) at 1:4000 or anti-Histone H1 (Santa Cruz AE-4) at 1:1000.

(A) Western blot with anti-GR (Cell Signalling D6H2L) and histone H1 (Santa Cruz AE-4)
(B) Western blot with anti-MR (1D5) and histone H1 (Santa Cruz AE-4)



Loading:

1. AtT20 mouse pituitary cells
2. N2A mouse neuroblastoma cells
3. N2A + EGFP-rGR (5 μg plasmid DNA)
4. N2A + rGR wild-type (5 μg)
5. N2A + mCherry-rMR (5 μg)
6. N2A + rMR (5 μg)
7. N2A + EGFP-rGR (2.5 μg) + mCherry-rMR (2.5 μg)

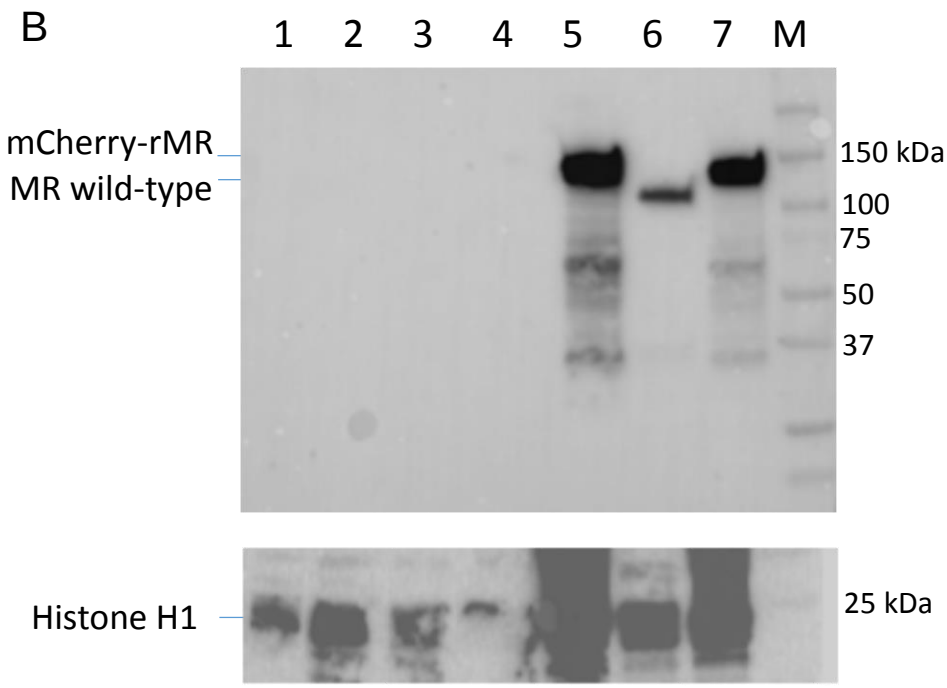
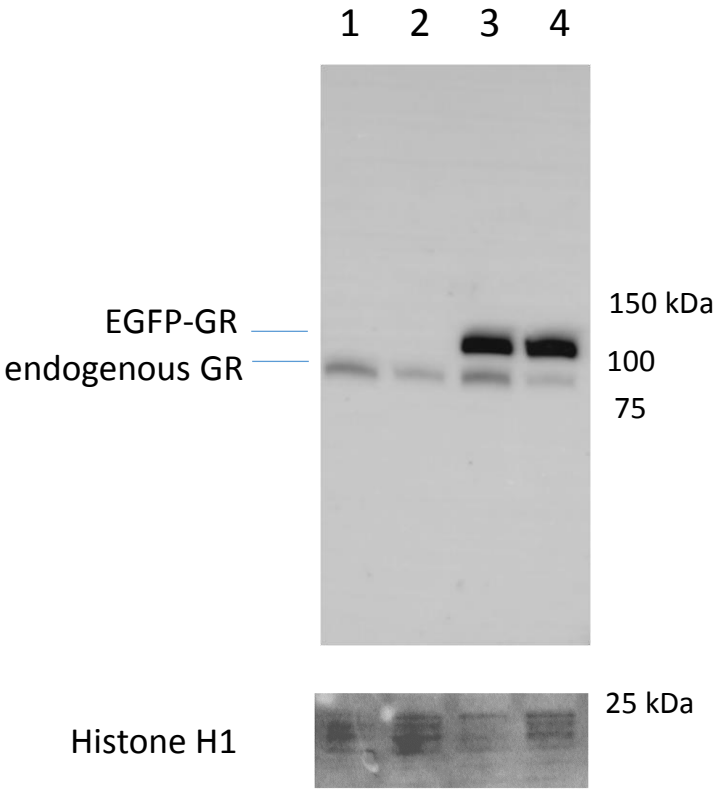


Figure S2. Knockdown of endogenous GR

Western blots of N2A cell lysates showing expression levels of endogenous and transiently transfected GR with non-targeting shControl or shNR3C1-3'UTR. N2A cells in 10 cm plates were transfected with DNA constructs and JetPrime (1:2 w/v). Lysates were prepared (Conway-Campbell BL, McKenna MA, Wiles CC, Atkinson HC, de Kloet ER, Lightman SL., Endocrinology 2007 Nov;148(11):5470-7.) and 20 μg each lysate was loaded and detected with anti-GR (Cell Signalling D6H2L) at 1:1000 or anti-Histone H1 (Santa Cruz AE-4) at 1:1000.



Loading:

1. N2A + shControl (5 μg plasmid DNA)
2. N2A + shNR3C1-UTR (5 μg)
3. N2A + EGFP-rGR + shControl (2.5 + 2.5 μg)
4. N2A + EGFP-rGR + shNR3C1-3'UTR (2.5 + 2.5 μg)

Figure S3. Luciferase reporter gene assays

Induction of MMTV-luciferase reporter in N2A cells transiently transfected with plasmids encoding untagged/tagged GR or MR without/with CORT activation. Plasmids pC1-rGR, pC1-EGFP-rGR (used for ChIP-nexus), pC1-rMR and pC1-mCherry-rMR (used for ChIP-nexus) were tested. Transient transfection of N2A cells was performed with JetPrime (ratio 2:1 (v/w)) and plasmid DNA (0.5 μg control/GR/MR expression vector, 0.5 μg shControl / shRNA-NR3C1-3'UTR vector (targeting the 3'UTR of endogenous GR), 0.99 μg pFC31-luc MMTV-luciferase reporter and 0.01 μg pRL-CMV Renilla control plasmids. Cells were harvested 24 hrs after 100 nM corticosterone (CORT) treatment and assayed with Dual-Luciferase® Reporter Assay System (Promega). Firefly luciferase expression was normalised to Renilla luciferase expression. Data are represented as means -/+ SEM, n=3.

All receptor transfected cells treated with corticosterone had luciferase outputs significantly greater than corticosterone treated cells with empty vector transfection (pcDNA3, $p < 0.001$ in all cases). Two-way anova (shControl + pcDNA3 excluded due to absent matched vehicle) produced a significant main effect of hormone treatment ($F(1,22) = 3831.2$, $p < 0.001$) and a significant main effect of transfection ($F(4,20) = 718.9$, $p < 0.001$). The interaction of hormone and transfection was also significant ($F(4,20) = 707.4$, $p < 0.001$). Post hoc testing was performed using Tukey test, or Games-Howell test as appropriate where the assumption of equality of variance was violated. Comparison of vehicle to CORT stimulated values for each receptor type are independent samples T-tests Bonferroni-corrected for multiple comparisons (P values considered significant at $p < 0.01$; ***$p < 0.001$). For pcDNA3 transfected vehicle versus corticosterone samples, there was no effect of corticosterone ($p = 0.201$).

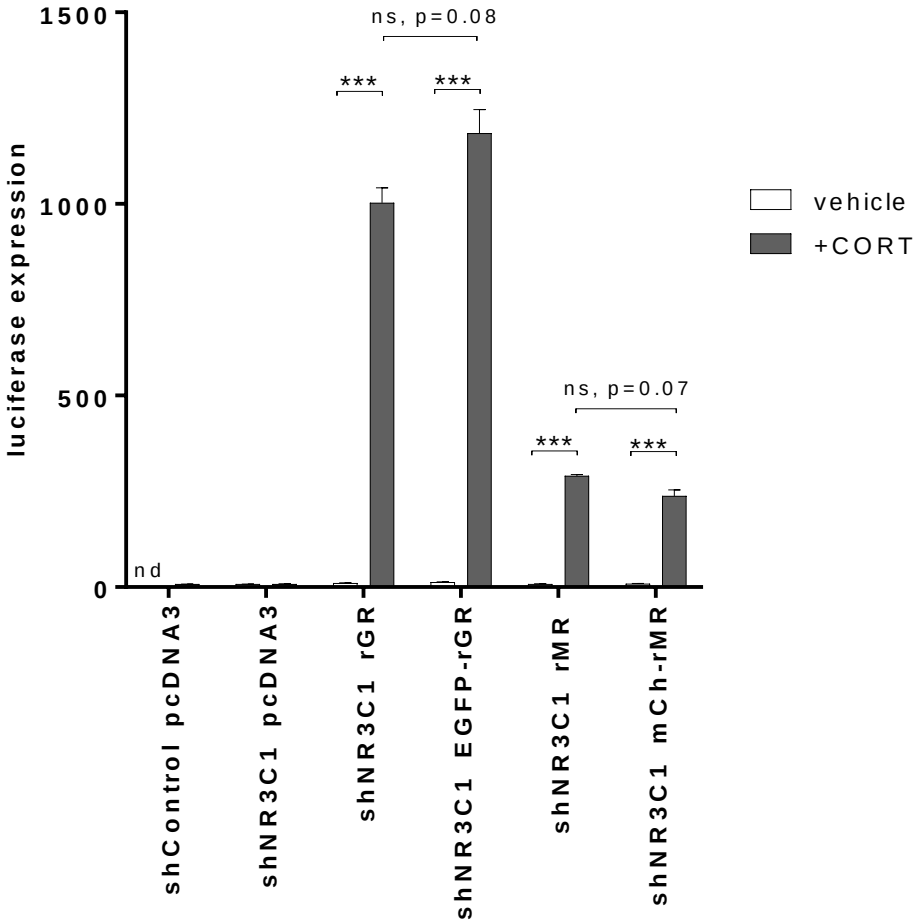
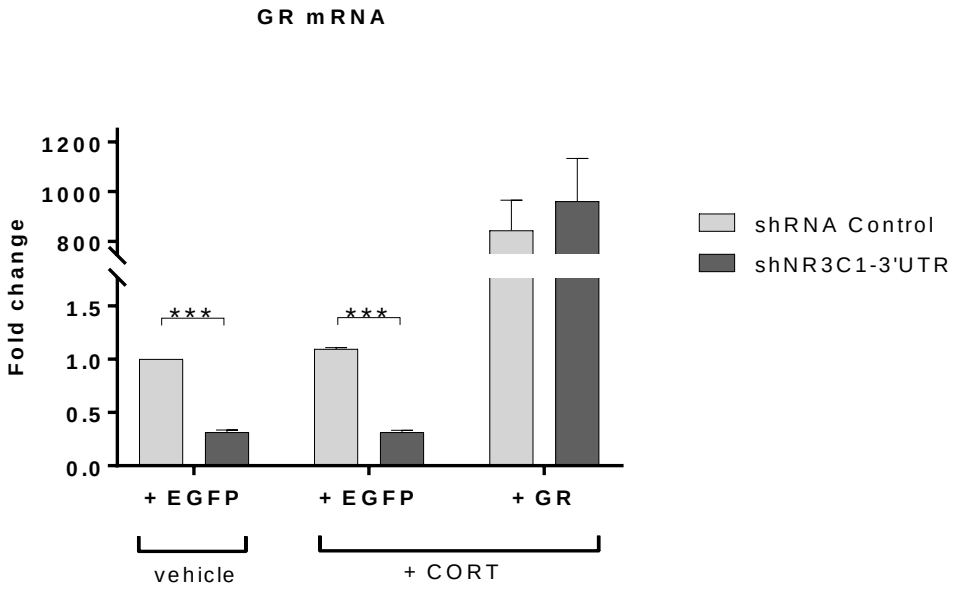


Figure S4. Effects of shRNA NR3C1-3'UTR

(A) Quantitative PCR measurements of mouse GR mRNA levels in N2A cells transfected with EGFP / GR expression plasmids and shRNA Control / NR3C1-3'UTR. Endogenous GR mRNA levels are reduced to 29% by shRNA NR3C1-3'UTR which targets the 3'UTR sequence present only on endogenous transcripts in cells transfected with control EGFP plasmid, both without and with CORT treatment (100 nM 1h). In cells transfected with plasmid mGR (transcripts are not targeted by shNR3C1-3'UTR) GR mRNA levels are not reduced by shNR3C1-3'UTR. Data are represented as means -/+ SEM of 3 independent experiments by ΔΔCt analysis. The Y-axis is split to show both low and high values more clearly.

(B) Quantitative PCR measurements of endogenous Sgk1 mRNA levels with shRNA NR3C1-3'UTR / control in the same samples. Sgk1 mRNA levels are not significantly altered by shNR3C1-3'UTR with either transfected control EGFP plasmid or plasmid mGR (p=0.11), though the trend is downwards. Data are represented as means -/+ SEM of 3 independent experiments by ΔΔCt analysis.

A



B

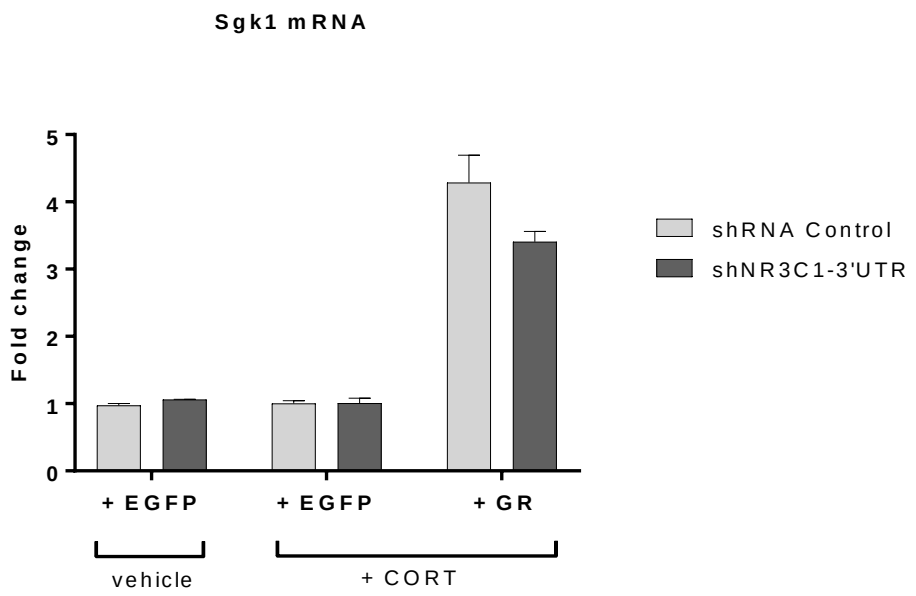


Figure S5.

Motif probability matrices of known motifs used to calculate motif frequency for GRE, GATA3 and AP-1 motif mapping.

>VAGRACAKWCTGTYC GRE(NR),IR3/RAW264.7-GRE-ChIP-Seq(Unpublished)/Homer 7.809859 -4041.196255 0
T:1437.0(71.35%),B:1197.7(2.63%),P:1e-1755 Tpos:101.8,Tstd:27.3,Bpos:98.0,Bstd:69.4,StrandBias:-0.0,Multiplicity:1.13
0.232 0.373 0.231 0.163
0.619 0.006 0.262 0.113
0.058 0.005 0.924 0.013
0.433 0.120 0.287 0.160
0.957 0.008 0.015 0.020
0.002 0.963 0.008 0.027
0.767 0.019 0.135 0.079
0.123 0.231 0.249 0.397
0.259 0.179 0.165 0.397
0.210 0.403 0.228 0.160
0.101 0.044 0.009 0.846
0.006 0.015 0.972 0.007
0.021 0.026 0.018 0.935
0.179 0.288 0.131 0.401
0.041 0.865 0.014 0.080

>AGATAASR GATA3(Zf)/iTreg-Gata3-ChIP-Seq(GSE20898)/Homer 5.938282 -2390.596730 0
T:3368.0(49.21%),B:5753.5(14.09%),P:1e-1038 Tpos:100.1,Tstd:42.5,Bpos:100.6,Bstd:63.6,StrandBias:-0.0,Multiplicity:1.21
0.662 0.066 0.006 0.266
0.001 0.007 0.991 0.001
0.989 0.004 0.001 0.006
0.002 0.023 0.001 0.974
0.825 0.061 0.011 0.103
0.778 0.048 0.129 0.045
0.184 0.401 0.348 0.067
0.433 0.167 0.359 0.041

>GATGACTCATCN Jun-AP1(bZIP)/K562-cJun-ChIP-Seq(GSE31477)/Homer 8.486263 -3.459607e+04 0
74838.0,37419.0,20963.2,20135.0,0.00e+00
0.244 0.149 0.396 0.211
0.473 0.218 0.268 0.041
0.001 0.001 0.001 0.997
0.001 0.001 0.991 0.007
0.997 0.001 0.001 0.001
0.051 0.462 0.440 0.047
0.021 0.001 0.001 0.977
0.024 0.974 0.001 0.001
0.997 0.001 0.001 0.001
0.050 0.258 0.227 0.465
0.210 0.394 0.153 0.243
0.241 0.319 0.211 0.229

Figure S6.

Distribution of sizes of identified MACE regions from ChIP-nexus samples, treatments ii and iii, for MR, GR and background datasets.