Supplementary Material

Nascent transcript analysis of glucocorticoid crosstalk with TNF defines primary and cooperative inflammatory repression

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Running title: Glucocorticoid-mediated inflammatory repression

Supplementary Material

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Cell culture

BEAS-2B cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Corning) containing L-glutamine and 4.5 g/L glucose and supplemented with 10% fetal bovine serum (VWR) and 1% penicillin/streptomycin (Corning). For lentiviral production, following transfection and 72 hr incubation, viral supernatant was collected from HEK cells, centrifuged at 1000 rpm for 10 min at room temperature and filtered with a sterile 0.45 um syringe filter. For transduction, BEAS-2B cells were plated on 10 cm tissue culture dishes and grown to confluence. Viral transduction was performed using a 1:1 ratio of viral supernatant to standard growth media along with 8 ug/ml polybrene. Cells were incubated for 24 hr and then transduced a second time, followed by antibiotic selection with puromycin (1 ug/ml) for 24-48 hr.

Reagents and Western blotting

Dexamethasone (dex; Sigma-Aldrich) was dissolved in sterile 100% ethanol (vehicle) and used at a final concentration of 100 nM. Recombinant Human TNF-alpha Protein purchased from R&D Systems was diluted in 1 × Dulbecco's phosphate buffered saline (DPBS) containing 0.1% bovine serum albumin (BSA) and used at a final concentration of 20 ng/ml. Primary antibodies for Western blotting included mouse anti-TNFAIP3 [59A426] (ab13597), rabbit anti-NFKBIA [E130] (ab32518) and rabbit anti-Beta-Tubulin (ab52901) from Abcam, anti-GAPDH [FL-335] (sc-25778) from Santa Cruz Biotechnology, rabbit anti-CEBPB (PA5-27244) from Thermo Fisher Scientific, and anti-Lamin B1 [D4Q4Z] (12586S) from Cell Signaling Technology. Secondary antibodies were ECL sheep anti-mouse IgG-HRP (95017-332) and donkey anti-rabbit IgG-HRP (95017-330) from GE/Amersham. Western blotting and protein detection were performed using standard procedures (Sasse et al. 2016). Antibodies for GR ChIP-seq were GR-IA1 (Sasse et al. 2017) and GR-356, which was raised in rabbit against the identical epitope as IA1 (QPDLSKAVSLSMGLYMGETETKVMGNDLG) and prepared by Covance.

Plasmids, transfection and reporter assays

Genomic enhancer regions of interest identified by GRO-seq were PCR-amplified, inserted into pCR2.1-TOPO (Life Technologies), then introduced into the pNL3.2 [NlucP/minP] minimal promoter-driven NanoLuc luciferase expression vector from Promega. Sequences of PCR primers used for cloning are as follows:

	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
pNL3.2-*PNRC1*	CTACCACTGCTGGGTGTTT	GCCTGGGATTACTGGAGTTT
pNL3.2-*PGM3*	ATTCACTACCACGAGAAGTATGG	TGCAGCTGCTGTCATATAAGT
pNL3.2-*IER3*	GTCTCCCATGTGTGCTCAA	GGAAAGAAATGACCAAGTGTCTG
pNL3.2-*NGF*	AGTGCCTTTCCAGGGTAATAAA	GGAGAGAATTCCAGCTCCTATC
pNL3.2-*ANGPTL4*	CACAAGTGTCTGATGGTGTAAAG	CACACAGGCCTAGAAGAGTATAG
pNL3.2-*ZBTB16*	CCTCTTCCCTGAATCTGTGTATC	CCCAGGATCCCAATGACAATAA

The pNL3.2-*FKBP5* construct was generated by excising the insert from our previously described *FKBP5* reporter in the pGL3-Promoter backbone (Altonsy et al. 2014) using KpnI/BglII and ligating into the pNL3.2 vector cut with KpnI/BglII. All constructs were verified by Sanger DNA Sequencing provided by Eton Biosciences and will be made available through Addgene.

For transfection experiments, BEAS-2B cells were plated in fresh complete medium on 6 cm tissue culture dishes at 3E6 cells/dish. The next day, each dish was transfected with an 11:1 ratio (8 ug total DNA) of NanoLuc luciferase construct to pGL4.13[luc2/SV40] firefly luciferase expression vector (Promega) as internal control, respectively, complexed with 20 ul Lipofectamine 2000 (Life Technologies). Medium was replaced with fresh complete medium 4-6 hours later and cells were incubated overnight. The next day, cells were detached from each dish by trypsinization and plated at 75K cells/well in 48-well plates in fresh complete medium. Approximately 20 hours later, cells were treated with vehicle, TNF, dex, or TNF+dex in fresh complete medium for 1, 2, or 4 hours. Cells were briefly rinsed in 1 × DPBS and lysed in 1 × Passive Lysis Buffer (Promega) for at least 15 min prior to measuring firefly and NanoLuc luciferase activities with a Tecan Infinite M1000 plate reader using the Nano-Glo Dual-Luciferase Reporter Assay System (Promega) with the following modifications to the manufacturer's protocol: 10 ul of lysate was combined with 40 ul of ONE-Glo EX Reagent for the first measurement, then 40 ul of NanoDLR Stop & Glo Reagent were added for the second measurement.

Global Run-on Sequencing (GRO-seq)

Treatment and nuclear isolation. BEAS-2B cells were plated on 15 cm tissue culture dishes at 3.75E6 cells/dish (2 plates per treatment per time-point) and grown to confluence (~3 days). Cells were treated with vehicle, TNF, dex, or TNF+dex for exactly 10 or 30 minutes. Plates were washed 3 times in ice-cold 1X PBS and cells were then scraped in ice-cold Lysis Buffer (10 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.5% Igepal, 10% glycerol, 1 mM DTT, 1 × EDTA-Free Protease Inhibitor Cocktail (Thermo Fisher Scientific), 4 U/ml SUPERase-In RNase Inhibitor (Invitrogen)). Cells from each treatment (2 plates per treatment per time-point) were pooled, transferred to 50 ml conical tubes, and centrifuged in a fixed-angle rotor at 1000 × g for 15 min at 4°C. Cell pellets were resuspended in fresh Lysis Buffer and centrifuged under the same conditions as above. Pellets were next resuspended in 1 ml of Lysis Buffer and transferred to 1.7 ml low binding microcentrifuge tubes (Corning). Suspensions were pelleted at 1000 × g for 5 min at 4°C, after which pellets were resuspended in 500 ul of Freezing Buffer (50 mM Tris-HCl pH 8.3, 5 mM $MgCl_2$, 40% glycerol, 0.1 mM EDTA pH 8.0, 4 U/ml SUPERase-In). Nuclei were pelleted at 2000 × g for 2 min, then resuspended in ~100 ul of fresh Freezing Buffer. Nuclei from each treatment were counted from 2 ul of suspension diluted in 1 × PBS. Freezing Buffer was then added to generate 4 × 100 ul aliquots containing 10E6 nuclei for each treatment, and samples were flash frozen in a dry ice/ethanol bath and stored at -80°C.

Nuclear run-on and total RNA extraction. After briefly thawing on ice, duplicate 100 ul aliquots of nuclei for each treatment and time-point were added to 100 ul of Reaction Buffer (10 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 1 mM DTT, 300 mM KCl, 0.5 mM each rATP, rGTP, rCTP, and 5-bromouridine 5'-triphosphate (Br-UTP; Sigma), 1% Sarkosyl, 20 Units SUPERase-In) pre-heated to 30°C and incubated for exactly 5 min at 30°C. Reactions were stopped by addition of 1 ml of TRIzol Reagent

(Invitrogen) and samples were completely homogenized by vortexing. To isolate RNA, 100 ul of chloroform were added and samples were centrifuged at max speed for 15 min at room temperature. The aqueous phase was transferred to a fresh tube and RNA was extracted with room temperature acid phenol/chloroform (Invitrogen) once, then chloroform-extracted once, and finally precipitated overnight at -20°C in 3 volumes 100% ethanol containing 1 ul GlycoBlue (Invitrogen).

RNA fragmentation and DNase treatment. RNA was pelleted by centrifugation at max speed for 20 min at room temperature, washed in 75% ethanol, then briefly air-dried prior to resuspension in nuclease-free water. RNA was fragmented to ~200 bp fragments by addition of NEBNext RNA Fragmentation Buffer (part of the Magnesium RNA Fragmentation Module from New England Biolabs) and incubation at 94°C for 5 min. Samples were purified over Bio-Rad P30 Buffer Exchange Columns as instructed by the manufacturer, then added to DNase reactions (1 × RQ1 DNase Buffer (Promega), 2 Units RQ1 RNase-Free DNase (Promega)) and incubated at 37°C for 10 min. Reactions were stopped by addition of DNase Stop Solution (Promega) and incubation at 65°C for 5 min, then tubes were chilled on ice.

Br-UTP-RNA enrichment and nascent RNA extraction. All anti-Br-UTP agarose bead (Santa Cruz) washes were performed in 500 ul of buffer for 5 min at room temperature with end-over-end rotation at ~15 rpm. Following each wash, samples were centrifuged at 1000 × g for 2 min at room temperature and supernatant carefully removed. Beads (60 ul/sample) were initially washed twice in Binding Buffer (0.25 × SSPE, 37.5 mM NaCl, 0.05% Tween-20, 1 mM EDTA, 0.004 U/ul SUPERase-In), after which 500 ul of Blocking Buffer (Binding Buffer supplemented with 0.1% PVP, 1 ug/ml BSA, and 0.04 U/ul SUPERase-In) were added and tubes were incubated for 1 hour with end-over-end rotation. Beads were pelleted as described above, washed twice in Binding Buffer, resuspended in Binding Buffer and then combined with RNA samples from above and incubated for 1 hr at room

temperature with end-over-end rotation. Beads now bound to nascent RNA were next washed in Binding Buffer, Low Salt Wash Buffer (0.2 × SSPE, 1 mM EDTA, 0.05% Tween-20, 0.004 U/ul SUPERase-In), High Salt Wash Buffer (0.25 × SSPE, 1 mM EDTA, 0.05% Tween-20, 137.5 mM NaCl, 0.004 U/ul SUPERase-In; this wash only lasted ~5 sec), and twice in TET Buffer (1 mM EDTA, 0.05% Tween-20, 10 mM Tris-HCl pH 8, 0.004 U/ul SUPERase-In), after which nascent RNA was eluted from the beads 4 consecutive times by incubation in Elution Buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 20 mM DTT, 0.1% SDS, 1 mM EDTA, 0.004 U/ul SUPERase-In) at 42°C. RNA was then extracted with room temperature acid phenol/chloroform once, chloroform-extracted once, and finally precipitated overnight at -20°C in 3 volumes 100% ethanol containing 1 ul GlycoBlue and 300 mM NaCl. The next day, RNA was pelleted, washed in 75% ethanol, then briefly air-dried prior to resuspension in nuclease-free water. Fresh aliquots of anti-Br-UTP beads were washed and blocked for each sample and then combined with resuspended RNA for a second round of nascent RNA enrichment and extraction, performed exactly as detailed above for the first round. RNA was again precipitated overnight at -20°C in 3 volumes 100% ethanol containing 1 ul GlycoBlue and 300 mM NaCl. The next day, RNA was pelleted, washed in 75% ethanol, then briefly air-dried prior to resuspension in nuclease-free water.

Strand-specific RNA library preparation and sequencing. The NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs) was used according to the Protocol for use with NEBNext rRNA Depletion Kit provided by the manufacturer to generate libraries for sequencing. Specifically, random priming was performed by incubating nascent RNA with supplied random hexanucleotide primers at 65°C for 5 min. Primed RNA was then added to First Strand cDNA Synthesis reagents (supplied) containing 0.5 ug of freshly thawed Actinomycin D (Sigma-Aldrich) and incubated for 10 min at 25°C, 15 min at 42°C, and 15 min at 70°C. Reaction products were combined with Second Strand cDNA Synthesis reagents (supplied) and incubated for 1 hr at 16°C. Double-stranded cDNA

was next purified using Mag-Bind RxnPure Plus Beads (Omega Bio-tek) at a relative volume of 1.8X, as instructed by the NEBNext Kit protocol, and eluted in 0.1 × TE Buffer. Purified double-stranded cDNA was then combined with supplied End Repair reagents and incubated for 30 min at 20°C followed by 30 min at 65°C in order to generate 5'-phosphorylated dA-tailed ends. The NEBNext Adaptor from the NEBNext Multiplex Oligos for Illumina Kit (New England Biolabs) was diluted 30-fold in 10 mM Tris-HCl containing 10 mM NaCl and added to the end-repaired products together with the supplied Blunt/TA Ligase Master Mix and incubated 15 min at 20°C. Adaptor-ligated products were then purified using Mag-Bind RxnPure Plus Beads at a relative volume of 1X, as instructed by the NEBNext Kit protocol, and eluted in 0.1 × TE Buffer. Purified adaptor-ligated cDNA was next combined with supplied PCR Enrichment reagents plus Universal PCR Primer and a single NEBNext Index Primer from the NEBNext Multiplex Oligos for Illumina Kit to uniquely barcode the libraries and cycled as follows: 1 cycle at 37°C for 15 min, 1 cycle at 98°C for 30 sec, 15 cycles of 98°C for 10 sec and 65°C for 75 sec, 1 cycle at 65°C for 5 min, then hold at 4°C. Amplified libraries were purified using Mag-Bind RxnPure Plus Beads at a relative volume of 0.9×, as instructed by the NEBNext Kit protocol, and eluted in 0.1 × TE Buffer. Libraries were then pooled and sequenced on an Illumina HiSeq instrument using 50 bp single-end reads by the Microarray and Genomics Core at the University of Colorado-Denver.

GRO-seq computational analysis

Identifying regions with bidirectional transcriptional activity. FStitch (v. 1.0) was first used to filter regions of inactive transcription in all samples to lessen downstream compute processing requirements. FStitch train was used with arguments '-r (sample.positiveStand.bedGraph) -t (trainingFile.bed) -o (outputTrainingParameters.hmminfo)' using the IGV (v. 2.3.75) Region Navigator tool to produce a BED format training file generated from one of the 10 min dex-treated samples. This sample was chosen for training as it was determined to be most representative of the data in terms of

read coverage, complexity, and total reads mapped. All relevant analysis files are included in Supplemental File S7. FStitch segment was then run on all single-strand non-normalized bedGraph files with arguments '-r (sample.pos.bedgraph) -w (outputTrainingParameters.hmminfo) -o (sample.bed)'. Positive and negative strand files were then concatenated and sorted using sortBed in the BEDTools suite (v. 2.25.0). Following FStitch processing, Tfit (v. 1.0) was used to generate the predictions of bidirectionals/eRNAs. BED file annotations of preliminary bidirectional calls were generated using the Tfit bidir old module with arguments '-ij (sample.concatenated.bedGraph) -N (bidir old) -k (sample.concatenatedFStitch.bed) -mle 1 -o (output directory) -logout (output directory) -tss (hg38 RefSeq gene transcription start site file)'. Using the bidirectional predictions file, the Tfit model module was run to get higher confidence calls with arguments '-ij (sample.concatenated.bedGraph) -k (preliminaryBidirectionals.bed) -N (tfit mod) –logout (output directory) -o (output directory) -config (config file)' to produce a final BED annotation file of predicted bidirectionals.

ChIP-seq computational analysis

Data processing, visualization and peak calling

For both antibodies (GR-IA1 and GR-356) at the 1 hour timepoint, two biological paired-end sequencing replicates for each treatment (veh, TNF, dex, and TNF+dex) for both shCtrl- and shGR-transduced cells were processed through a standardized ChIP-seq data pipeline. Samples were trimmed for adapters, minimum length, and minimum quality using the BBMap Suite (v. 38.05) bbduk.sh tool with arguments 'ref = adapters.fa ktrim = r qtrim = 10 k = 23 mink = 23 hdist = 1 maq = 10 minlen= 20 tpe tbo'. Quality of each sample was assessed both pre- and post-trim by FastQC (v. 0.0.13). The FastQC reports suggested the ChIP samples may be contaminated with non-human DNA due to the appearance of two distinct G/C distribution peaks centered at 42% and 68% mean G/C content (Input control samples exhibited a normal distribution). To resolve these two peaks in the ChIP samples, bbsplit.sh (part of the BBMap Suite v. 38.05) was used with argument 'ref = hg38.fa' to

separate reads that mapped to hg38 from those that did not. NCBI BLAST was then used to determine the organism responsible for the unmapped reads, which indicated that the majority of these reads mapped to the Pseudomonas aeruginosa genome. To confirm P. aeruginosa was the source of contamination, the P. aeruginosa genome strain F5677 was downloaded from NCBI (June-29-2018, NCBI Reference Sequence: NZ CP026680.1) and included as an additional reference genome in the bbsplit.sh module with the argument 'ref = hg38.fa, p aeruginosa f5677'. Across all ChIP samples, a mean number of 380706 reads mapped to the P. aeruginosa genome. While this only accounts for a small fraction of total reads sequenced, there are 427 genome strain variants reported by NCBI. Thus, the clear separation of G/C distribution peaks between reads mapping to hg38 (centered at 42% G/C) and those either mapping to P. aeruginosa or remaining unmapped (centered at 68% G/C) suggests the majority of the remaining unmapped reads likely correspond to a different strain of P. aeruginosa. As samples were sequenced deeply in this study, an average of 22.7M reads for each paired-end run still remained even after contaminants were removed. Further, two replicates per treatment condition were sequenced in this experiment, resulting in an average of 39.1M mapped reads per treatment condition, which was more than sufficient for downstream sequencing analysis. The remaining hg38-specific reads were mapped to hg38 using hisat2 (v. 2.1.0) with argument '--no-spliced-alignment' using indexes built with hisat2-build (same version), which resulted in over 91.6% mapping for all ChIP and Input control samples. The full data for percent G/C, reads split to each genome, the remaining FastQC statistics, reads mapping to hg38, and complexity and coverage information is included in a complete report generated by MultiQC (v. 1.6) in Supplemental File S8. Resulting SAM files were converted to sorted and indexed BAM files using samtools (v. 1.8) view, sort, and index, respectively. Complexity for each sample was determined using Preseq (v. 2.0.3) c curve and lc extrap modules with argument '-B'. Read distributions across the hg38 genome were calculated using RSeQC read distribution.py using the same RefSeq reference annotation file that was used for differential transcription analysis. Sorted BAM files were converted to bedGraph format using genomeCoverageBed from bedtools (v. 2.25.0) with arguments

'-bg -ibam -g hg38', read coverage was normalized to counts per million mapped, and bedGraphs were converted to TDF format using igvtools (v. 2.3.75) for visualization in IGV.

The 10 minute ChIP-seq data were processed using a Nextflow pipeline (https://github.com/Dowell-Lab/ChIP-Flow). A full pipeline report of the run as well as a quality control report generated by MultiQC (v. 1.7), including trimming, mapping, coverage, and complexity metrics, is included in Supplemental File S9.

For each ChIP-seq dataset, MACS2 (v. 2.1.1) callpeak with '--SPMR' argument was applied to each biological replicate pair of ChIP and respective Input control samples to call significant peaks (Supplemental File S4 and S10 for 10 minute and 1 hour datasets, respectively).

qRT-PCR

shCtrl- and shGR-transduced BEAS-2B cells were plated at 500K cells/well in 6-well tissue culture dishes and treated with vehicle, TNF, dex, or TNF+dex on the following day for 1, 2, or 4 hours in biologic quadruplicate. RNA preparation and qRT-PCR were performed as previously described with normalization to *RPL19* (Sasse et al. 2013). Primer sequences are listed in Supplemental Table S4.

ChIP-qPCR

shCtrl-transduced BEAS-2B cells were cultured, harvested, and immunoprecipitated in biologic quadruplicate using 12 ug of GR-356 antibody as described for ChIP-seq. Purified ChIP DNA was subjected to qPCR using primer sequences provided in Supplemental Table S5 and relative GR occupancy was calculated as described previously (Sasse et al. 2013).

REFERENCES

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Sasse SK, Mailloux CM, Barczak AJ, Wang Q, Altonsy MO, Jain MK, Haldar SM, Gerber AN. 2013. The glucocorticoid receptor and KLF15 regulate gene expression dynamics and integrate signals through feed-forward circuitry. *Mol Cell Biol* **33**: 2104-2115.

Supplemental Table S2: t-test comparisons of the log2 normalized enrichment within GR-IA1 vehicle and dex ChIP-seq data subsets that co-localize with GRO-seq defined enhancer activity as indicated

ChIP-seq subset comparisons	t-statistic	p-value
IA1_shCtrl_V_down_vs_IA1_shCtrl_D_down*	13.965	1.80E-37
IA1_shGR_V_down_vs_IA1_shGR_D_down	-1.553	1.21E-01
IA1_shCtrl_V_up_vs_IA1_shCtrl_D_up**	4.164	3.31E-05
IA1_shGR_V_up_vs_IA1_shGR_D_up	-10.379	3.59E-24
IA1_shCtrl_V_up_vs_IA1_shCtrl_V_down	-7.497	1.48E-13
IA1_shCtrl_D_up_vs_IA1_shCtrl_D_down	6.601	6.58E-11

* “down” signifies co-localized enhancers that had decreased activity with dex
** “up” signifies co-localized enhancers that had increased activity with dex.

Supplemental Table S3. Sequences of qPCR primers used to span enhancers of interest in MNase chromatin accessibility assay.

PTPRK	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Pair 1	CTCTGACTAGTGGGTAGAGAAA	GGAAACTCTCTGAGGATGGA
Pair 2	CTATTCTTGGCCCTGTGAAA	GGGTATTTCTGTGAGGAAACT
Pair 3	TCCATCCTCAGAGAGTTTCC	TCTTCACAGGATCTCCCTTC
Pair 4	AAGGGAGATCCTGTGAAGAT	GTCTCTTTGGAGAAAGCTACAT
Pair 5	CTTTCTCCAAAGAGACCTGAG	TTTCATTTCCCTGGCTAACA
Pair 6	GCTCCTGAGTGGTGTTAG	TGAGAGATATGTCTGACAATGA
Pair 7	GCATCATTTCCTCATTGTCAG	CTGGAGAGAGGATGTCTAGT
Pair 8	GGCAAGGAAGCTCATGTC	AGGAAACGGAGACCAAGA
Pair 9	CAGGACAATGGTGGAGATTC	AGAAAGAGGGTTTATCAAGCAG
Pair 10	TCTTGGTCTCCGTTTCCT	CTGAGAGAGCTACAAACTTACC
Pair 11	CCTGCTTGATAAACCCTCTT	CAAGTGAGATGACTAATTAGCAAAG
Pair 12	TCTCAGTCCCAGTGACATTA	CTCATAGGACTATCAGTATTTGTGA
Pair 13	CTGATAGTCCTATGAGTCATTC	GATGGTCCTAGCAAAGATT
Pair 14	AATCTTTGCTAGGACCATCTATC	GGCAAGGTCAAACTAGAAGA
Pair 15	CTAGTTTGACCTTGCCCTTAT	CGACCAATCCACCACAAA
Pair 16	TGGATTGGTCGGTGAGG	AAAGCTATGATTGAGCCACTG
Pair 17	GTGGCAGACAGGGTCTT	TTAGCCAGTCATAGTGCATGT
Pair 18	TGCACTATGACTGGCTAAT	TTTAATTCTGTTCCAGCCT

TMEM217	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Pair 1	GATATGAGAAAGCATTGAAGCATAG	GTAAATCTAAGGTGCCGTCAA
Pair 2	AGTTGACGGCACCTTAGA	GCAGCAACATATTCTACTGAGAT
Pair 3	TCAATTGCTCTCATCAAGTT	CCCAGTGTTGCAAGAGAT
Pair 4	TTGCAACACTGGGTTAGG	CCGATATAGATGAATTAGGCTCT
Pair 5	AGAGCCTAATTCATCTATATCGG	CCAGGCTGGTCTGAAAC
Pair 6	AATAGGAGGATCACTTAAGCC	GTTCCTTGCAGCCAAATG
Pair 7	GCAAGGAACAGAGACTCAAA	CACTGCCAGATTTCCTGTTA
Pair 8	TCTTCTTCTAACAGGAAATCTGG	TTCTGTCTCGCCTCTTCTTA
Pair 9	CTAAGAAGAGGCGAGACAGA	GTGACTAATACAAGAGTTCAGAGG
Pair 10	AAGCCTTTAAGTATGTGTGACT	GTGTCTAAGGTTTGAGGTAATTTG
Pair 11	CTCTGTAACAAATTACCTCAAACC	CCCACCTACGCTACTCTT
Pair 12	GAATCCAAGAGTAGCGTAGG	ACAAGTAGAGCCTTTAGATGG
Pair 13	GGTTCTGGTTCAAGGTTTCT	CTTCCCTCCCACAAGTAGA
Pair 14	GGCCATCTAAAGGCTCTACT	GAAAGAACTGTGGCTTCCTG
Pair 15	TCTGACTGTTGGCAGGAA	CATGAGGAAATTCAAGTGGACTA
Pair 16	TCTATAGTCCACTTGAATTTCCTC	GACAGTGCAGCTTCCATT
Pair 17	AGCTGCACTGTCTTAGTAATC	CACCCTTATGTTGTCCTCTC
Pair 18	TAATTGGTCACACTCACTGAC	CCTCAAGTTTGCCTCAATAATC
Pair 19	AGGACAACATAAGGGTGTAAAT	ATACATACATAGAGATGTGATAGCC
Pair 20	CAGGAGGCCAGGATTATT	CATGTTTCTCAAATACACACAC
Pair 21	CCGGCTATCACATCTCTATGTATG	TGGAAGAGTGAATTAGTAACAACCA
Pair 22	CAAATGGTTGTTACTAATTCACTCTTCC	ACCAAATGATAGCAAATGTGTG

SH3RF3	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Pair 1	GCTTACTTCAAGTCCCAGTGT	CGAGGACCAATCATCTTCAGAG
Pair 2	TTTCTGGAGGATCAGAGC	AGAGCCATCCACTTCTTG
Pair 3	AGAGCTCTGAAGATGATTGG	CTTCCAGTTGCCAAGGT
Pair 4	CCACATTTCAAGAAGTGGATG	TACCCTTTCTCGCTCCTT
Pair 5	TTGGCAACTGGAAGGCA	TTCCCTTTCCTCATTCTTTCCC
Pair 6	GAGAAAGGGTAAGGGAGAGA	CTGTGTATCCGTGTCTTGTC
Pair 7	AGAAGGGTAAGGAGTAAGGG	TTCCAGAATGCTTGAAGGG
Pair 8	ACACGGATACACAGGAGA	ACACAAAGTTCCAGCAAGA
Pair 9	CCGAATAATCACCACTCCTTC	CAGAGCAGGATCTGGATTTATG
Pair 10	TGCATTTCCTGTTGAAGTCATA	TGAAATGTTCTGCCTCTGC
Pair 11	GGCAGAGGCAGAACATTT	GTTCAGGCTCCTGGATTC
Pair 12	CAGGCAGACTAGGCAGTT	GCTGATTTAAGCTCAACTCTCA
Pair 13	GTCTTTGCCTGAGAGTTGAG	ACAGGCCCTATTTGTGAAATTA
Pair 14	GTCCATCTTAAGGAAGCTGAAA	CACACTATTACACAGCACAGG
Pair 15	GCCTGTGCTGTGTAATAGTG	CATTTGACTGGCGACTACTG
Pair 16	GCAGTAGTCGCCAGTCAAA	CCTGTGTGCTCAGCTAATGA
Pair 17	GGTCTCCAGCAGAAATAACC	TGAGCTGATCATCTACCTCTTA
Pair 18	CAGGGCACCCACATAAG	TCTGAGTTGGAGAAGGGT
Pair 19	AGGATTCAAATAAGAACCCTTC	TTGATAGCAATAAAGCTGTCTG
Pair 20	CTTCTGGTGCCTGCAAAT	CATTACAAGGTTTGTGCATGG

ANGPLT4 **enhancer**	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Pair 1	CTTCTGAGCTGTGTGACTTT	TCTCAACCTCAAGTCGGT
Pair 2	CAGAAGCTCAATTTATCTCCTTTC	TTTAGTGCCACCTTTACACC
Pair 3	GATTACACAAGTGTCTGATGGT	AGGGCAGACAGCTATGAA
Pair 4	ATGTACGCTGCTACAGTTATT	CTTCCAGTCTGGGAACTTG
Pair 5	GAGGGTGGAAAGGCAAC	CAAGAGCAGGACCTCAAAC
Pair 6	CCCAGACTGGAAGGAATTATG	TCAGGGCAGAAAGGATGA
Pair 7	CCAGATTCTGAGTCATCCTTTC	GCCACACTGTACTCACATC
Pair 8	TGACCTGGATGTGAGTACAG	GGACTATGTAATGGCACATCG
Pair 9	GACACGATGTGCCATTACA	TCTCTCTGGCTTGAGACAT
Pair 10	GGGCTTGTCTGAGAAGAATC	CCTAGACAAAGTGTACAGGAAATA
Pair 11	TGCATGTCTCAAGCCAGA	GATGCTTGACAGAGTCCTAGA
Pair 12	CTTTGTCTAGGACTCTGTCAAG	AGATGTCAATCCCATTAGGTAAG
Pair 13	CTGTCAAGCATCTCTCAGTC	ACAGGCCTAGAAGAGTATAGAA
Pair 14	GCTCCAGGAATCCTTACCTA	CTAGATGGTCAATGCTGCAA
Pair 15	TCTAGGCCTGTGTGCTTAC	GCTCATTGGCACCATGAAT
Pair 16	CCATTGCAGCATTGACC	GTATGATATCAGGAGGCACAT
Pair 17	ATGGTGCCAATGAGCAAA	CCCTTAAGGTGCCTGTTATG
Pair 18	ATCATACACTGAGAAGGACAC	CATTTGTCTGATGCAGATGTT
Pair 19	GGGAAGAAACATCTGCATCA	CCTGGCCAAACACTGATATT

SMURF2	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Pair 1	AATTTGTGGTACTTTGTCTCAGG	CTGTATCGTATGTGATAAAGTATGGAAG
Pair 2	GGAGCCCTGGGAAATGAATA	AACCTGTTGCAGAATGTGTTT
Pair 3	AAACACATTCTGCAACAGGTT	GGGTGCATAAAATGGTCTAG
Pair 4	CCAATTTGGTGATTTAAGCATGGA	TGCTTCATGCTATCGTCTTACAG
Pair 5	TGCTGTAAGACGATAGCATGAA	AGTAGTTGCAGTCGAGTGATG
Pair 6	CATCACTCGACTGCAACTAC	TTGAGGATGATTGGATATGACT
Pair 7	GCAACATGAACAGGCTCAAA	CCATCTCGCCCTTAGAGAATTG
Pair 8	AGTCATATCCAATCATCCTCAA	CCATAAAGACTGCATCGTACA
Pair 9	CAATTCTCTAAGGGCGAGATGG	TGGCCCATATTCTCTGCATTAC
Pair 10	GGGCGGAGAAGTGGAAATA	ATTGACCCACAGGCTTATGA
Pair 11	TGTACGATGCAGTCTTTATGGG	AAGCAATGGTTTGACATTGACC
Pair 12	CAGAGAATATGGGCCAGAATGA	ATGAGCAGCCATTGAGGAAT
Pair 13	GATCAACATGACTCATAAGCCT	TCTCTGGACTGGGTCCT
Pair 14	AATTCCTCAATGGCTGCTCA	GAGGAAATAAGGCTGGAGGTG
Pair 15	CAGGACCCAGTCCAGAGA	GGGCAGAGAAGGAGGAAATAAG
Pair 16	GAGAGCTTTGTGTGGCATTTC	AGAGTCTTGAGTTCTTTGGTACAG
Pair 17	CTCCAGCCTTATTTCCTCCTTC	GAGAAACTGAGCTCCATGAGAC
Pair 18	CCTGTACCAAAGAACTCAAGACT	GTGCCTACTTACTGACTGAAGG
Pair 19	CTCAAGACTCTCATCTTTCAGGTT	GGCCAGACAGATGGGAAAG

Supplemental Table S4. Sequences of primers used for qPCR experiment.

	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
RPL19 (internal control)	ATCGATCGCCACATGTATCA	GCGTGCTTCCTTGGTCTTAG
FKBP5	AGGCTGCAAGACTGCAGATC	CTTGCCCATTGCTTTATTGG
TNFAIP3	GCCCAGGAATGCTACAGATAC	AGTGGAACAGCTCGGATTTC
TNIP1	AACAAGCAGTGGGACCAGCATTTC	TGCTTCTGCAAATCAGCCAGCTTC
IL1A	CATCCTCCACAATAGCAGACAG	GAGTTTCCTGGCTATGGGATAAG
CXCL8	CTTGGCAGCCTTCCTGATTT	GGGTGGAAAGGTTTGGAGTATG
ICAM1	TGGGCAGTCAACAGCTAAA	GGTAAGGTTCTTGCCCACT
LIF	AAGGAGGCAGCAGAGTTGG	AGAGAGCGACAGGGAAAGG
CCL2	GCTCATAGCAGCCACCTTCA	CTTGGCCACAATGGTCTTGA

Supplemental Table S5. Sequences of primers used for ChIP-qPCR experiment.

	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
Negative Control 1	CGGCTACTAGCGGTTTTACG	AAGAAGATGCGGCTGACTGT
Negative Control 2	TGCAGGAGATGAAATACTAAGCAAGTA	AGATTGGAAACTGAGGACTTTAGTTAGAG
Negative Control 3	GGCAAGGACAGAGACAATCATA	CTCTGTGTTCTCGCTTTGGA
FKBP5	TAACCACATCAAGCGAGCTG	GCATGGTTTAGGGGTTCTTG
LIF	TGCCTCTTGTCCAATCATCAG	GCCACTTCCCTCACGTAATC
ICAM1	GGCTAGCGCTATAAAGGATCAC	CATAGCGAGGCTGAGGTTG
SMURF2	TGGGCCAGAATGAGATCAAC	ATGAGCAGCCATTGAGGAAT

Figure S1. Western blots for TNFAIP3, NFKBIA and CEBPB protein expression in BEAS-2B cells treated for indicated times with vehicle (ethanol (E)), TNF (T), dex (D), or TNF+dex (TD). GAPDH served as a loading control.

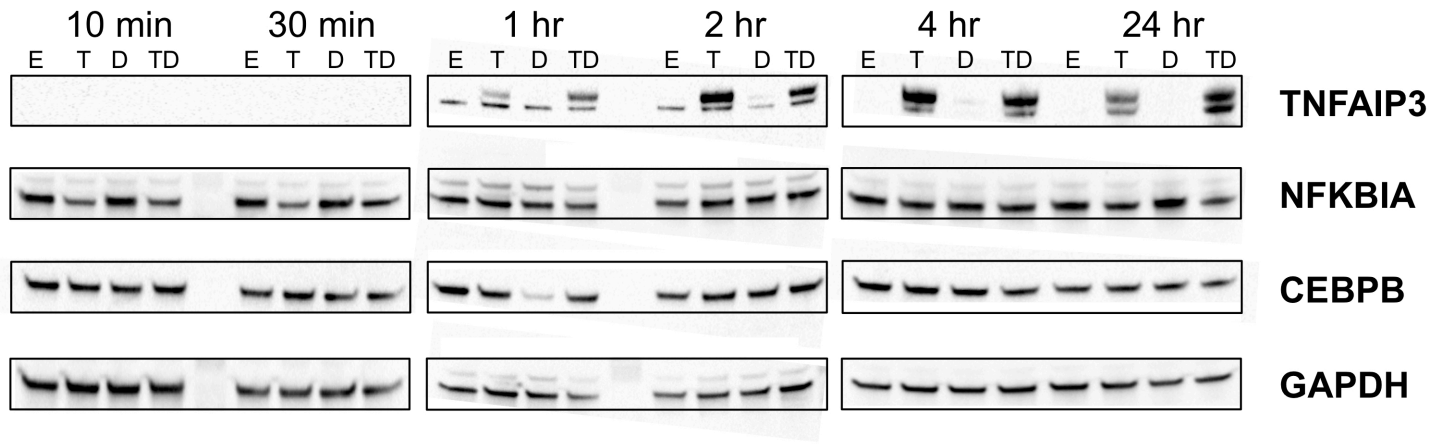
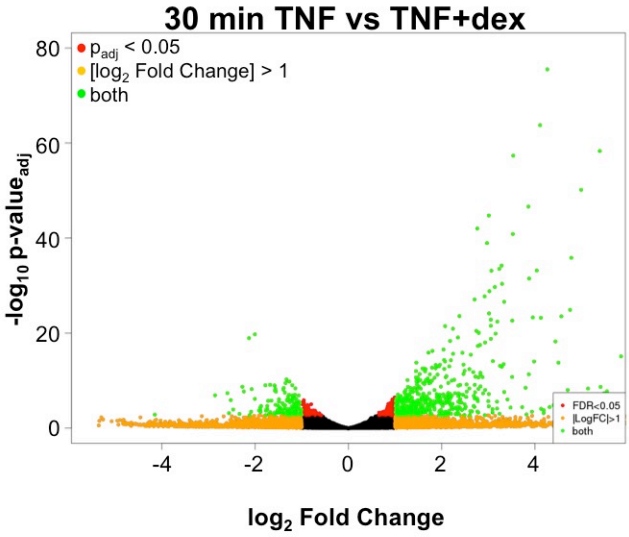
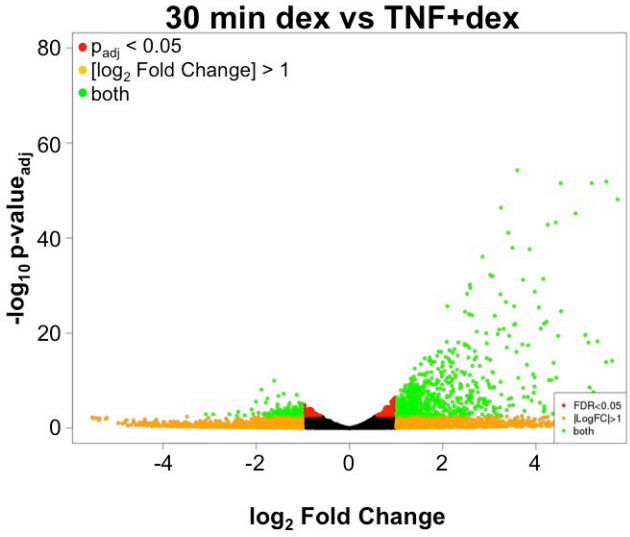


Figure S2. Volcano plots indicating differentially regulated nascent transcripts identified by GRO-seq.



TNF < TNF+dex; n = 609
TNF > TNF+dex; n = 228



dex < TNF+dex; n = 856
dex > TNF+dex; n = 200

Figure S3. Genomic distances from transcripts in each cluster to the nearest eRNA in the analogous cluster.
Clusters are based on $p_{adj} < .05$. the stringency of this cut-off likely impacts the frequency of close genomic associations.

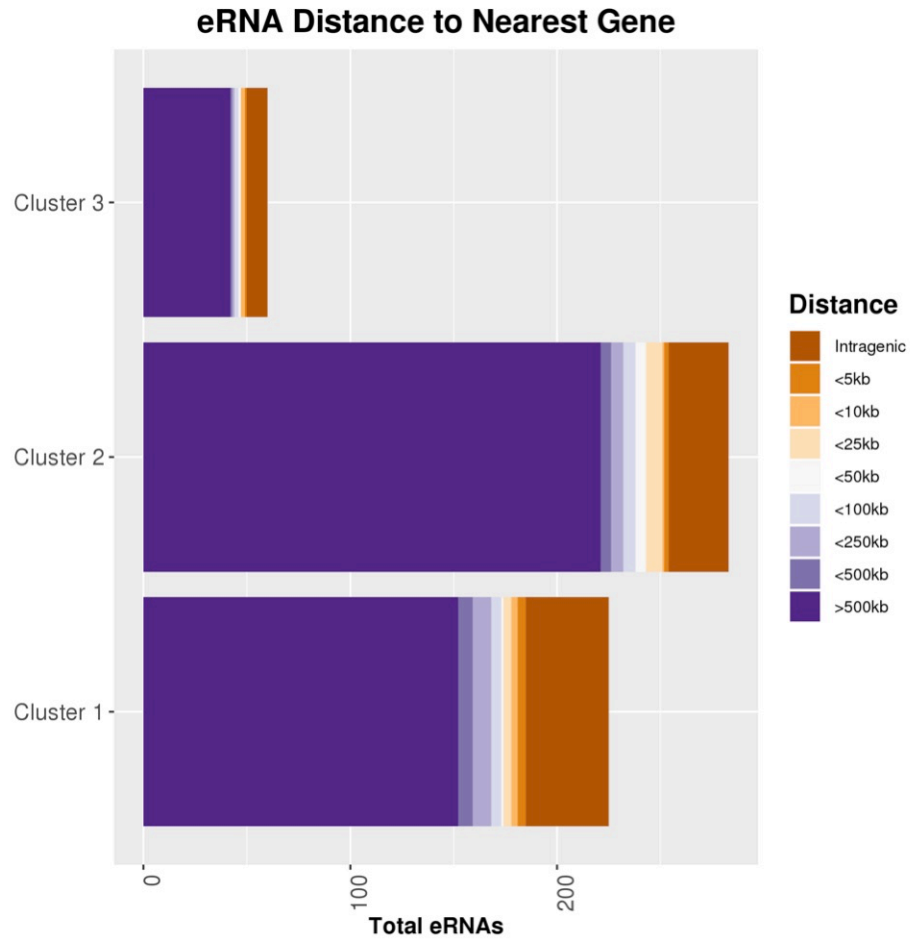


Figure S4. MA plots illustrating statistically different ($p < 0.0000001$) MD scores amongst all 641 HOCOMOCO transcription factor binding motifs tested comparing Tfit-identified enhancers in cells treated for 30 minutes with vehicle or TNF.

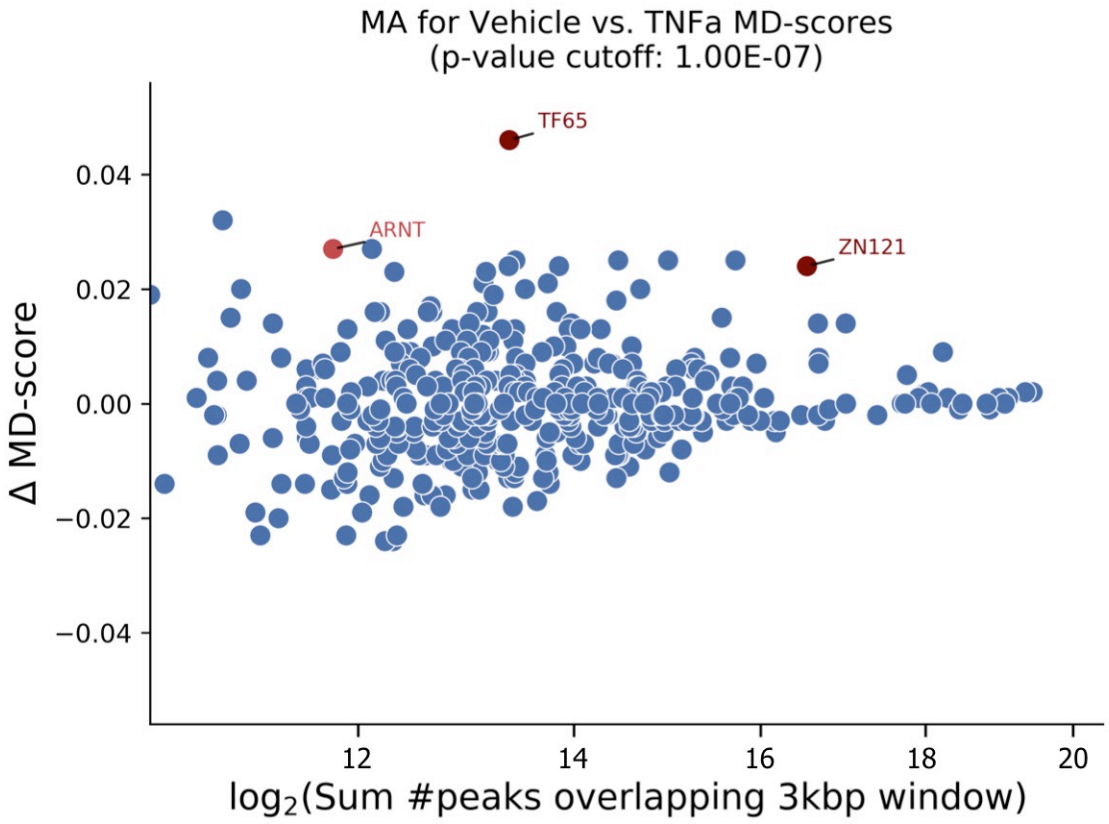


Figure S5. UCSC Genome Browser visualization of presumptive GR ChIP-seq peaks observed under basal culture conditions aligned with marks associated with open chromatin from ENCODE data.

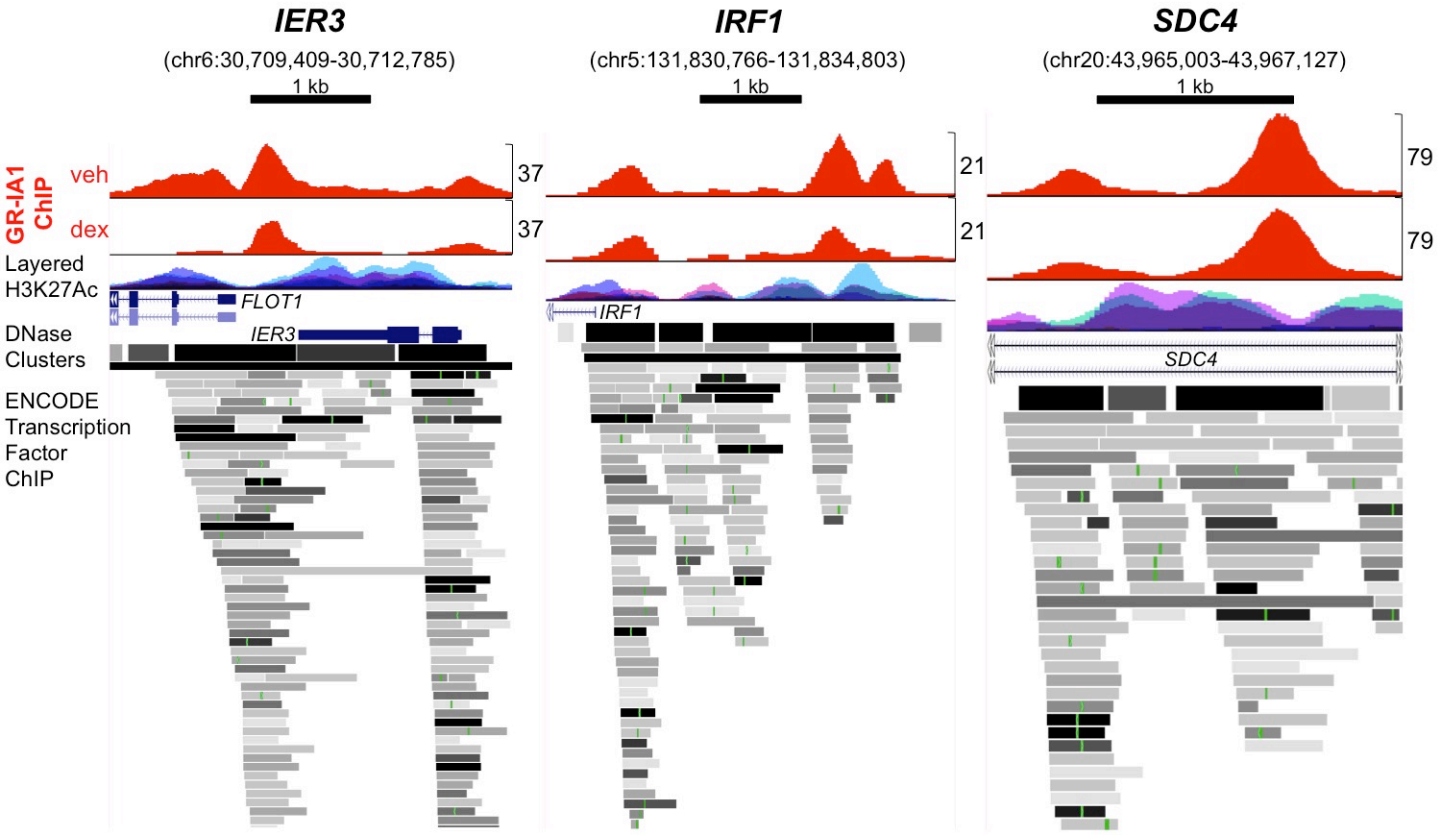


Figure S6. Western blot of GR protein expression in nuclear vs cytosolic fractions isolated from Beas-2B cells treated as indicated for 1 hr. Fraction-specific markers also served as loading controls and included beta-Tubulin (cytosolic) and Lamin (nuclear).

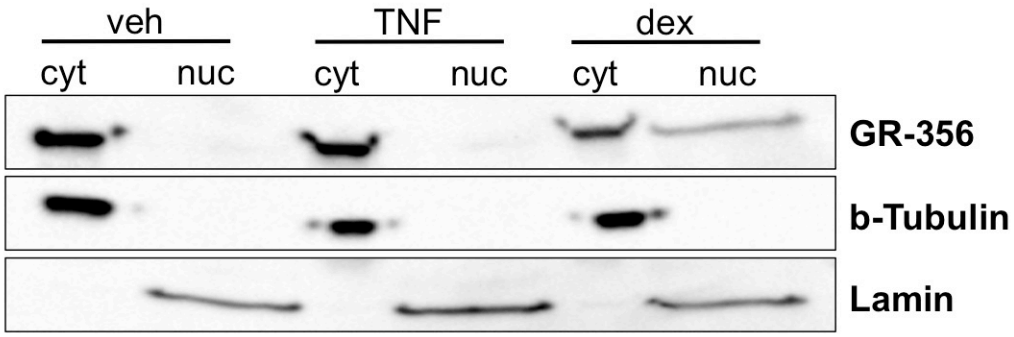


Figure S7. qRT-PCR analysis of dex-induced control (A), TNF+dex cooperatively-induced (B), and TNF-induced, dex-repressed (C) loci in shCtrl and shGR Beas-2B cells treated with TNF (20 ng/ml) and/or dex (100 nM) for indicated times. *$p < 0.05$ vs. same treatment in shCtrl cells.

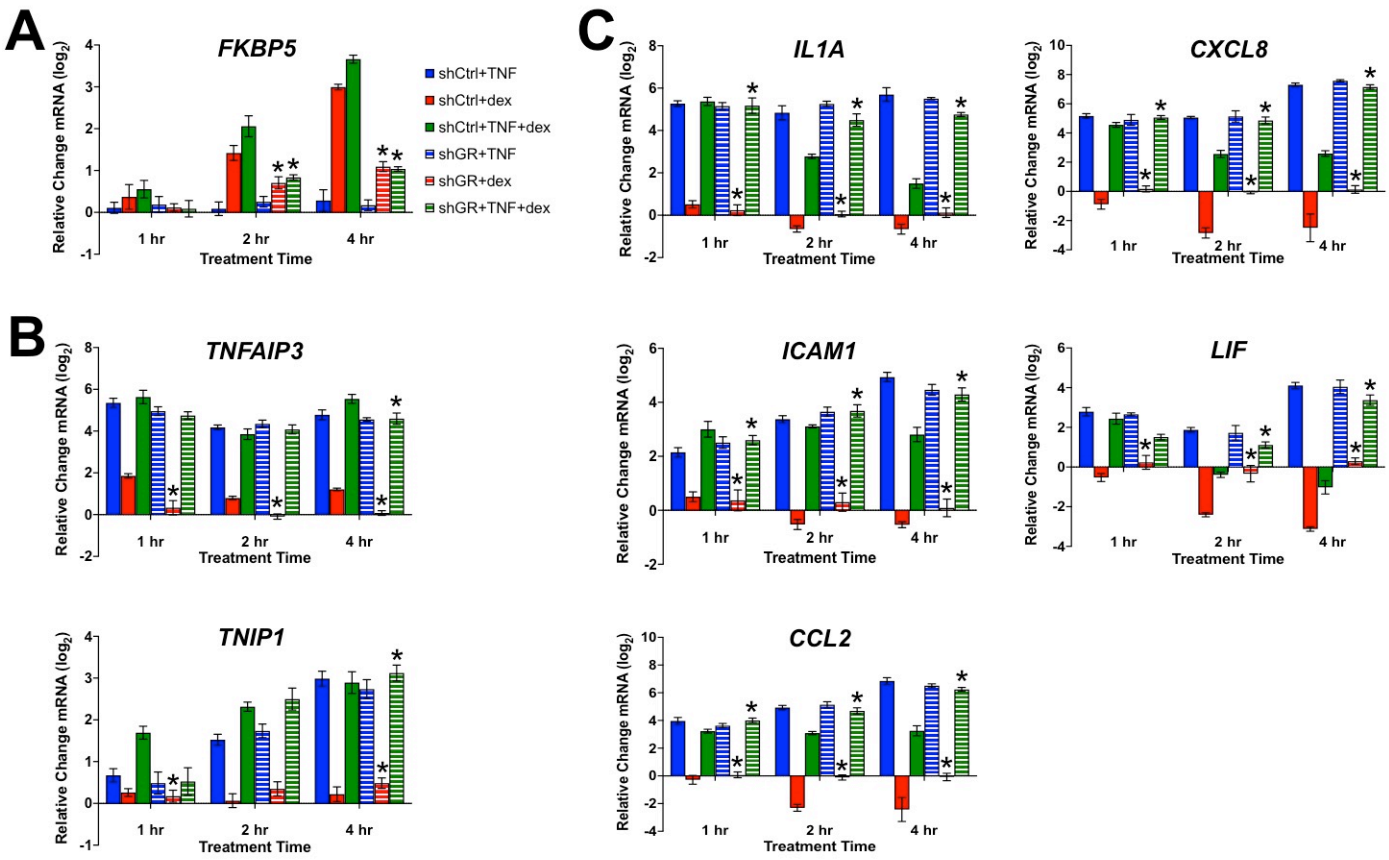


Figure S8. Western blot detection of GR protein with both GR ChIP antibodies in shCtrl- and shGR-transduced cells.

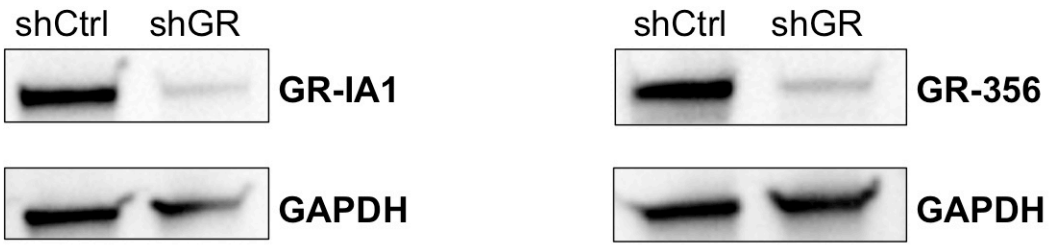


Figure S9. MA plots illustrating statistically different ($p < 0.00001$) MD scores amongst all 641 HOCOMOCO transcription factor binding motifs tested in dex-treated shCtrl vs shGR cells for each GR antibody. Motifs for glucocorticoid receptor (GCR) and progesterone receptor (PRGR), which recognize highly similar sequences, are highlighted in purple and exhibited the largest changes in MD-scores in both ChIP-seq datasets.

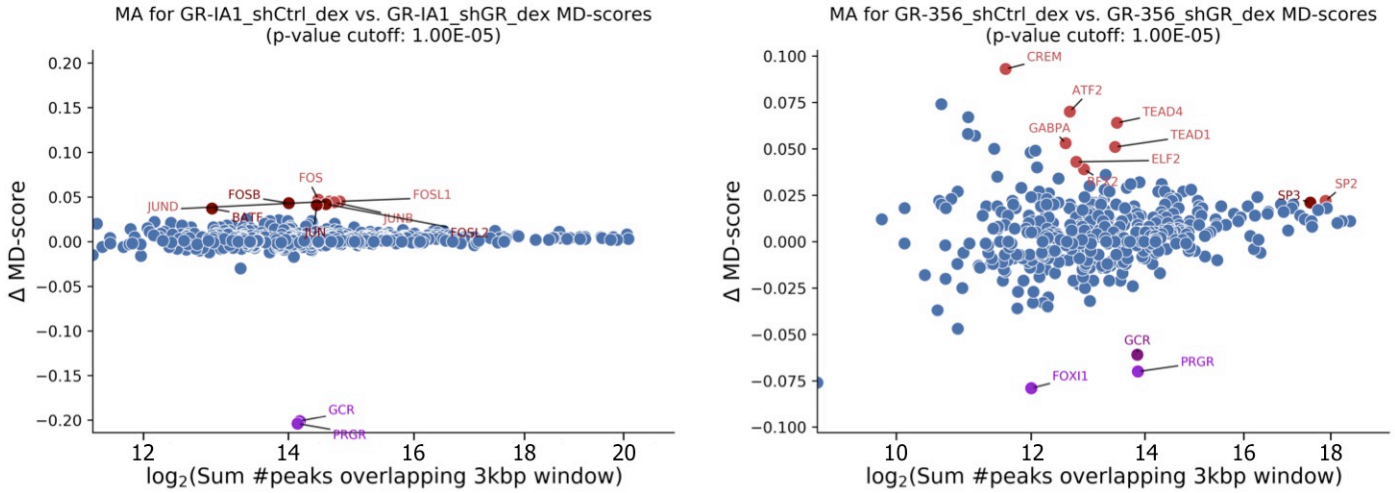


Figure S10. MD analysis of enrichment for indicated binding motifs in specified ChIP-seq datasets.

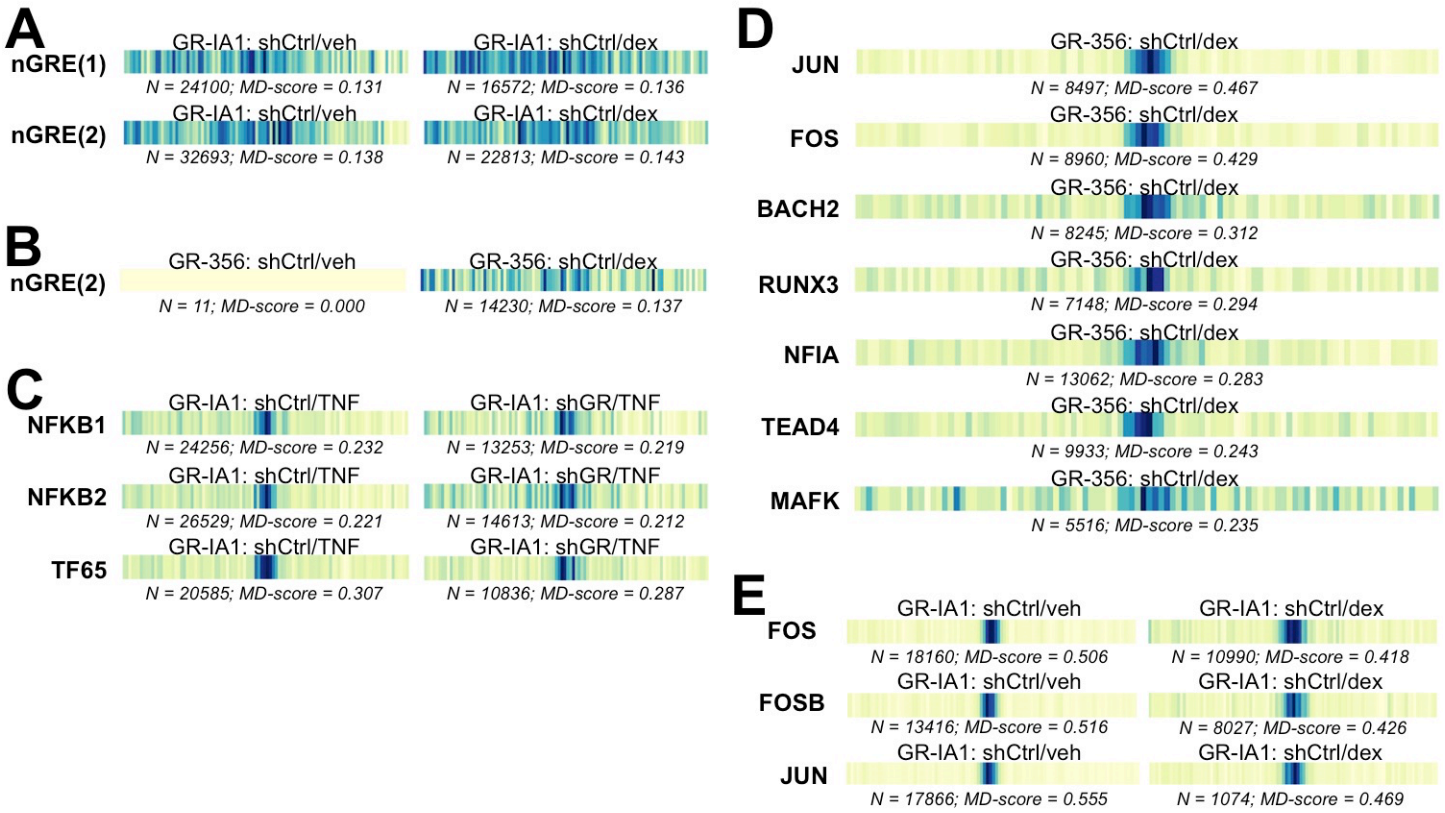


Figure S11. GR-356 ChIP-qPCR analysis in BEAS-2B cells treated for 1 hr with TNF and/or dex.

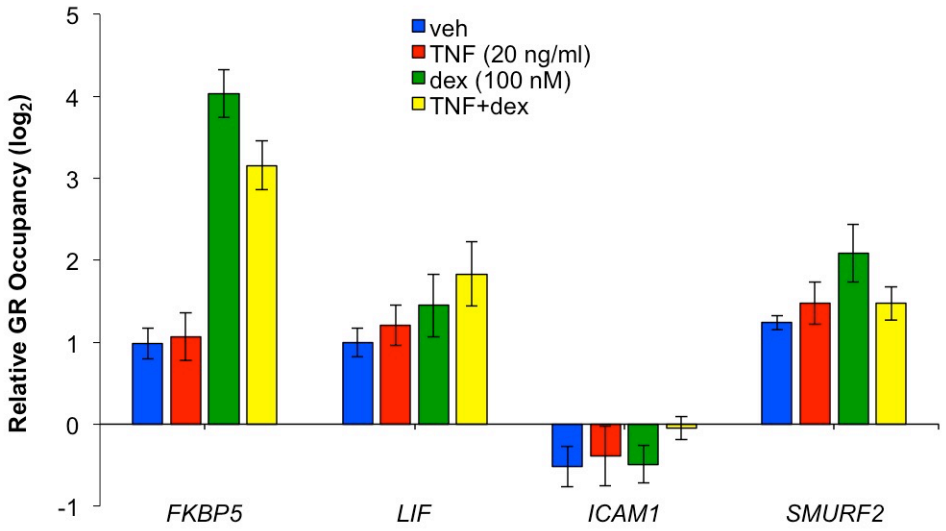


Figure S12. Integrated GRO- and ChIP-seq analysis reveals qualitatively different occupancy patterns between GR-repressed (A) and GR-induced (B) enhancers.

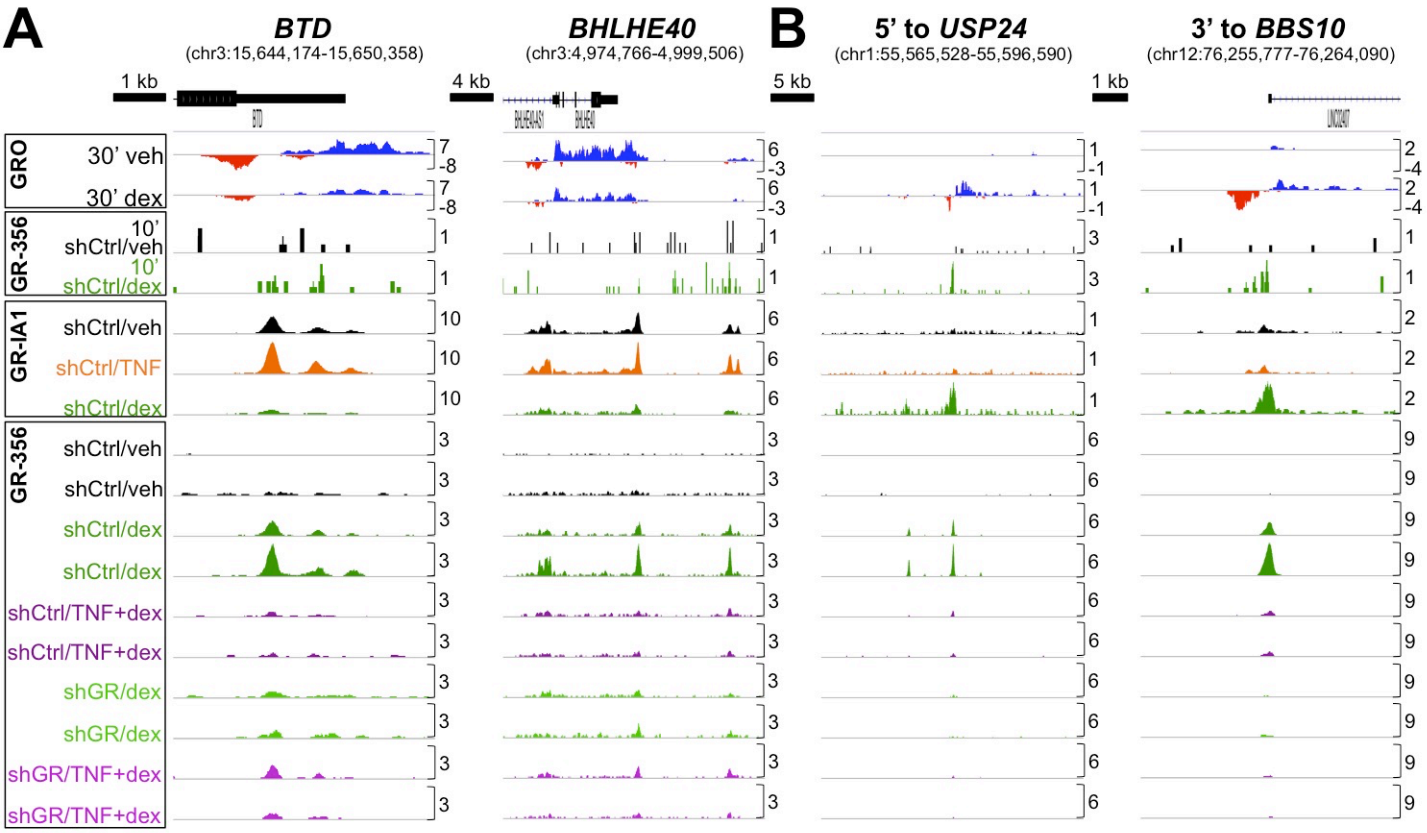


Figure S13. Relative magnitude of ChIP-seq peaks for IA1 vehicle and dex at different classes of enhancers.

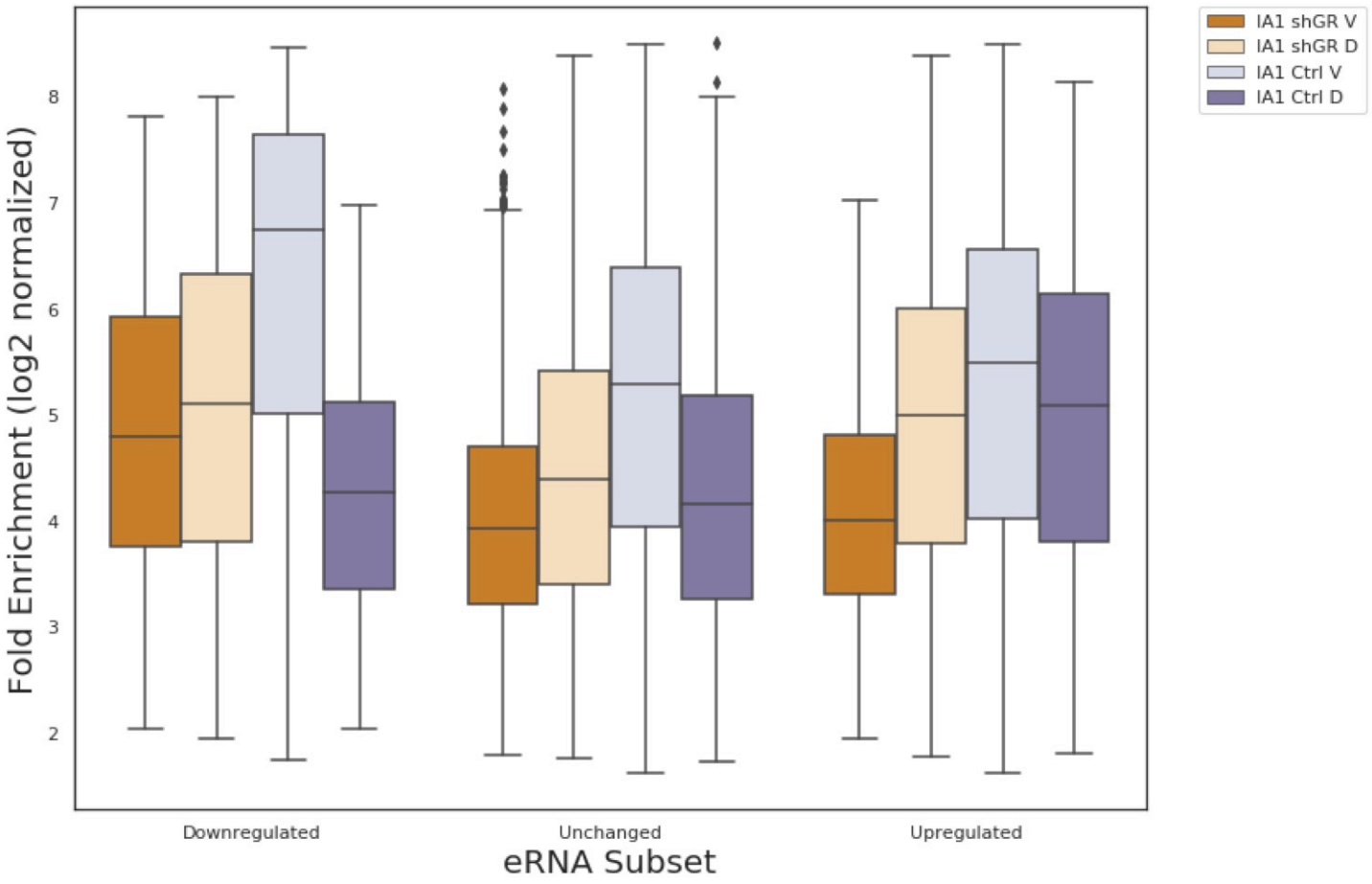


Figure S14. UCSC Genome Browser visualization of dex-repressed enhancers associated with MACS2-called GR ChIP-seq peaks from 10 min GR-356 ChIP-seq experiment aligned with marks associated with open chromatin from ENCODE data. All ENCODE Transcription Factor ChIP data are visible in top screenshots but truncated in bottom screenshots due to space constraints.

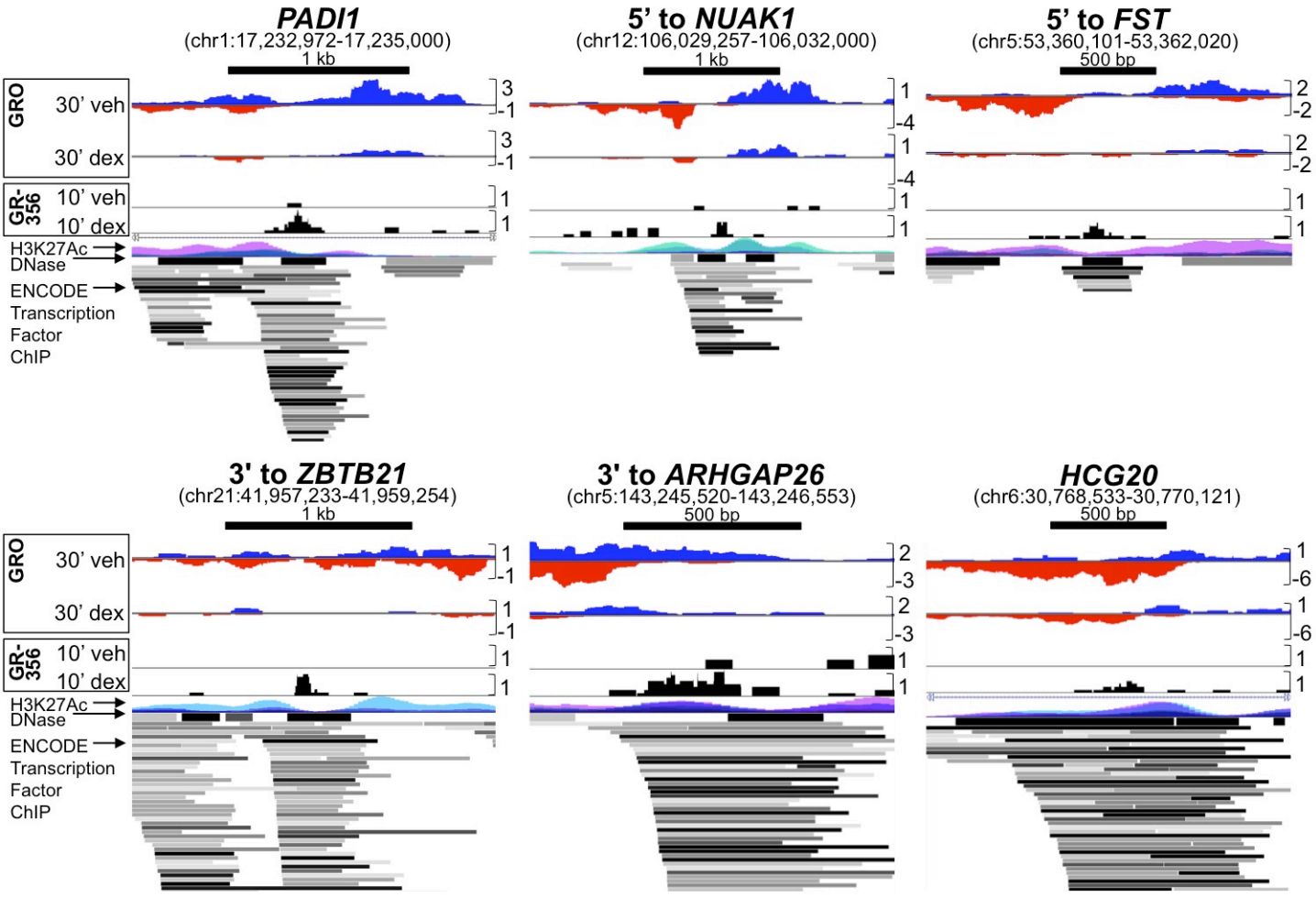


Figure S15. H3K27Ac ChIP-seq in A549 cells at different classes of BEAS-2B enhancers defined by our GRO-seq data. A549 H3K27 acetylation data (ENCSR375BQN) were generated by the Reddy lab within the ENCODE consortium.

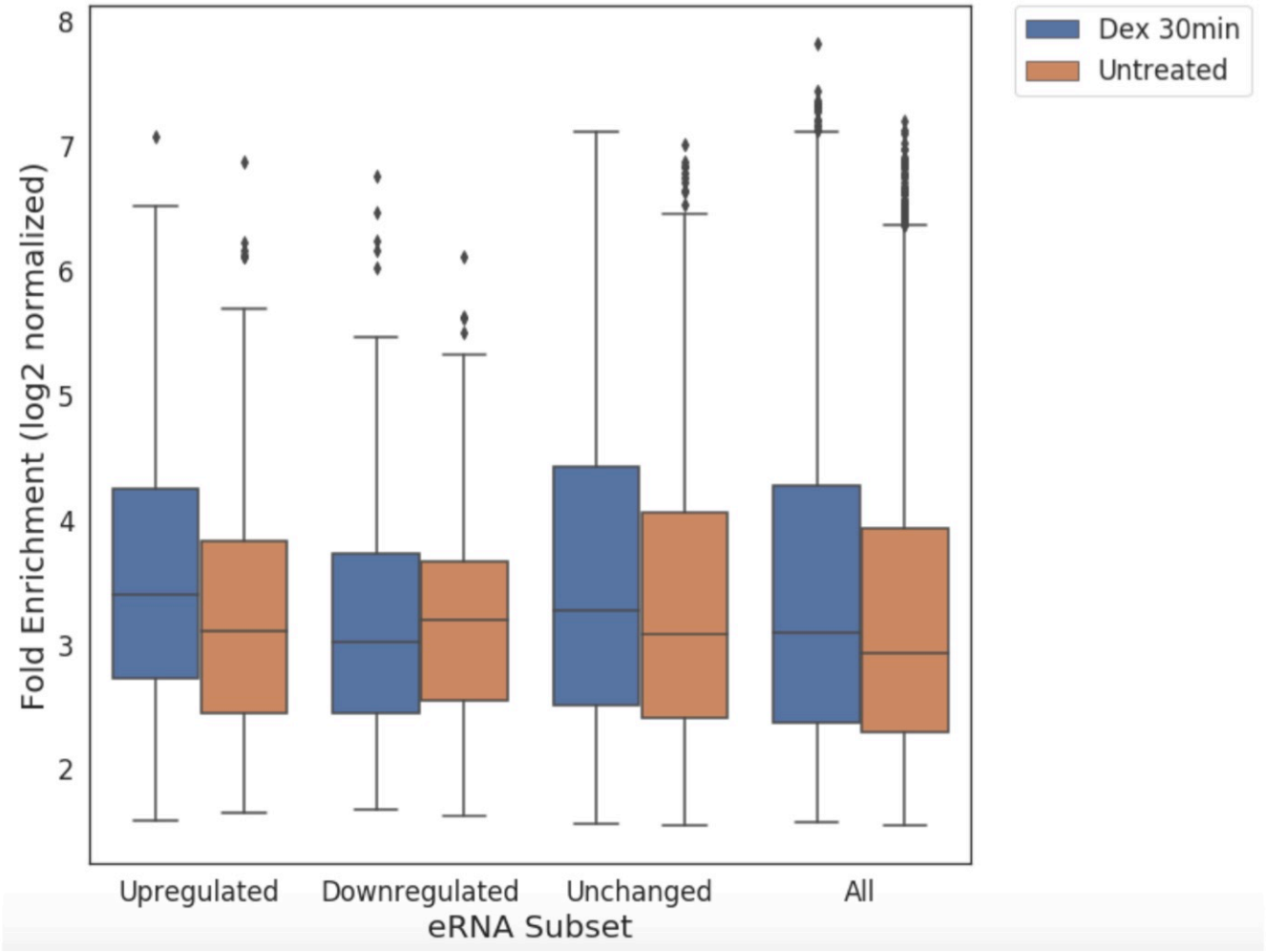


Figure S16. MNase chromatin accessibility assay for dex-repressed *SMURF2* enhancer, as described in the legend for Fig. 6.

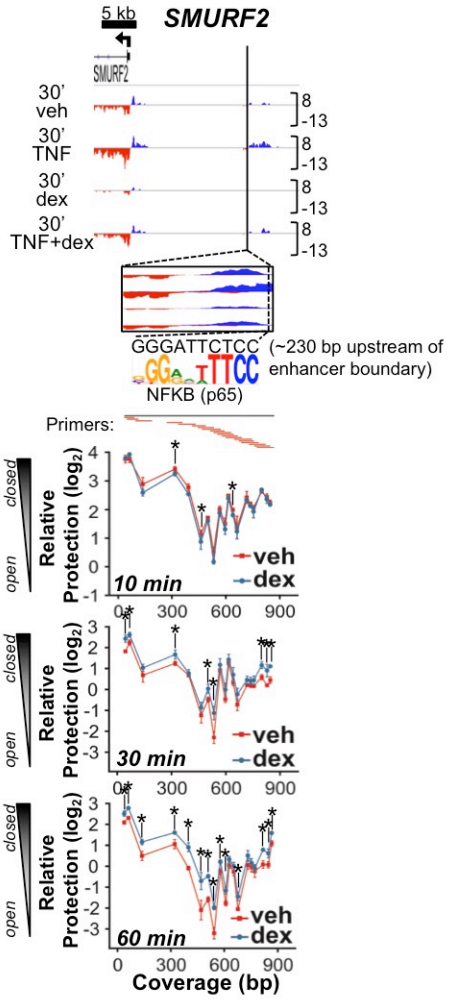


Figure S17. Aligned GRO- and ChIP-seq data for enhancers interrogated with MNase chromatin accessibility assay.

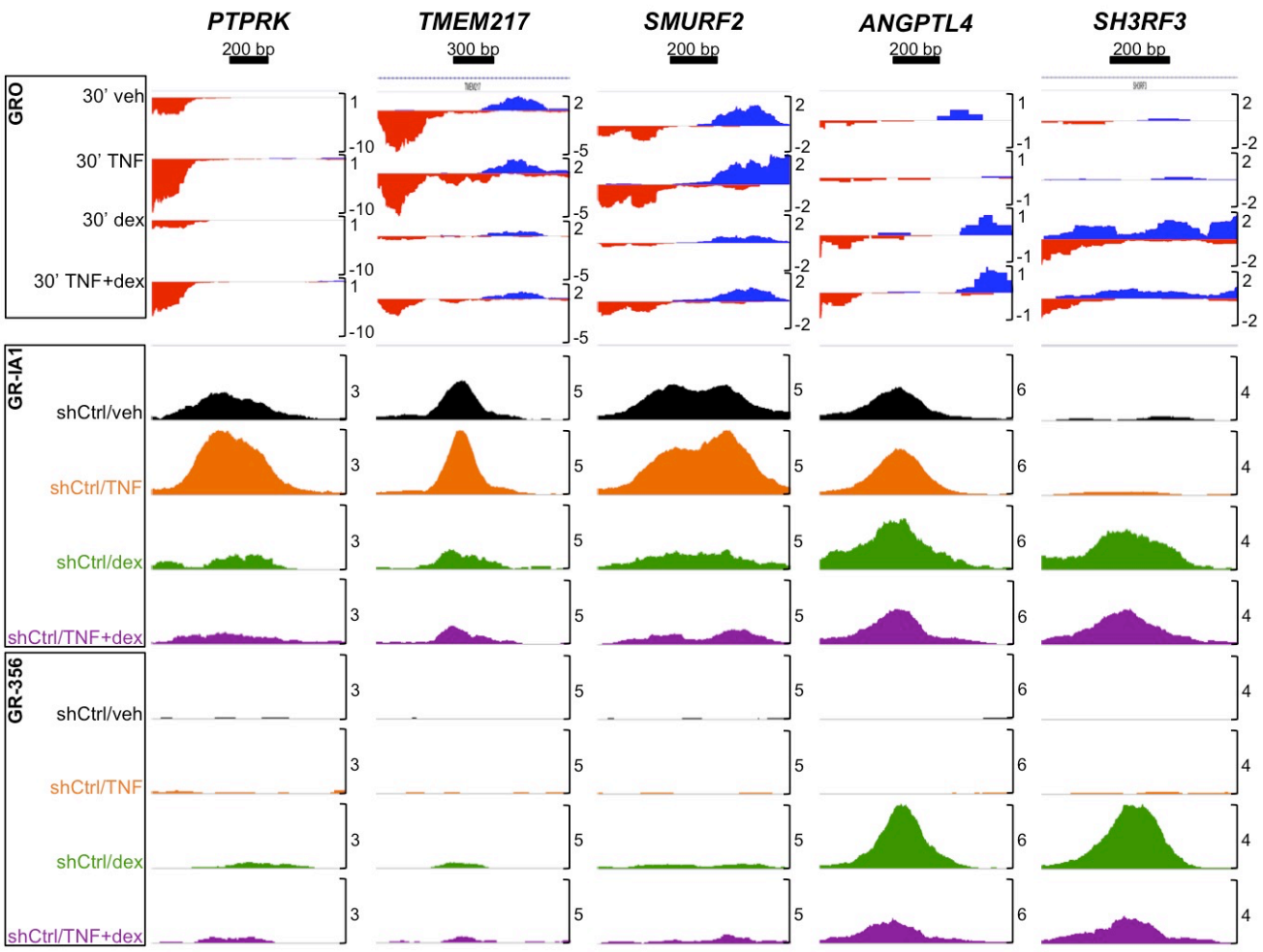


Figure S18. GRO-seq tracks and bar graphs showing relative genomic locations and normalized luciferase activity, respectively, of (A) Cluster 1 (TNF+dex cooperatively induced), (B) Cluster 2 (TNF-induced, dex-repressed) and (C) Cluster 3 (dex-induced, TNF-repressed) enhancers cloned into a destabilized, short half-life luciferase reporter vector and transiently transfected into BEAS-2B cells prior to treatment with vehicle, TNF, dex, or TNF+dex for 1, 2, or 4 hours. Luciferase activity of empty vector control (pNL3.2) is included in the inset of the pNL3.2-PNRC1 graph in Panel A. Bars indicate mean activity (±SD) normalized to that of a firefly luciferase internal control. *p < 0.05 vs. veh; [a]p < 0.05 vs. TNF; [b]p < 0.05 vs. dex.