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Supplemental Information

GR and LSD1/KDM1A-Targeted Gene Activation Requires Selective H3K4me2 Demethylation at Enhancers

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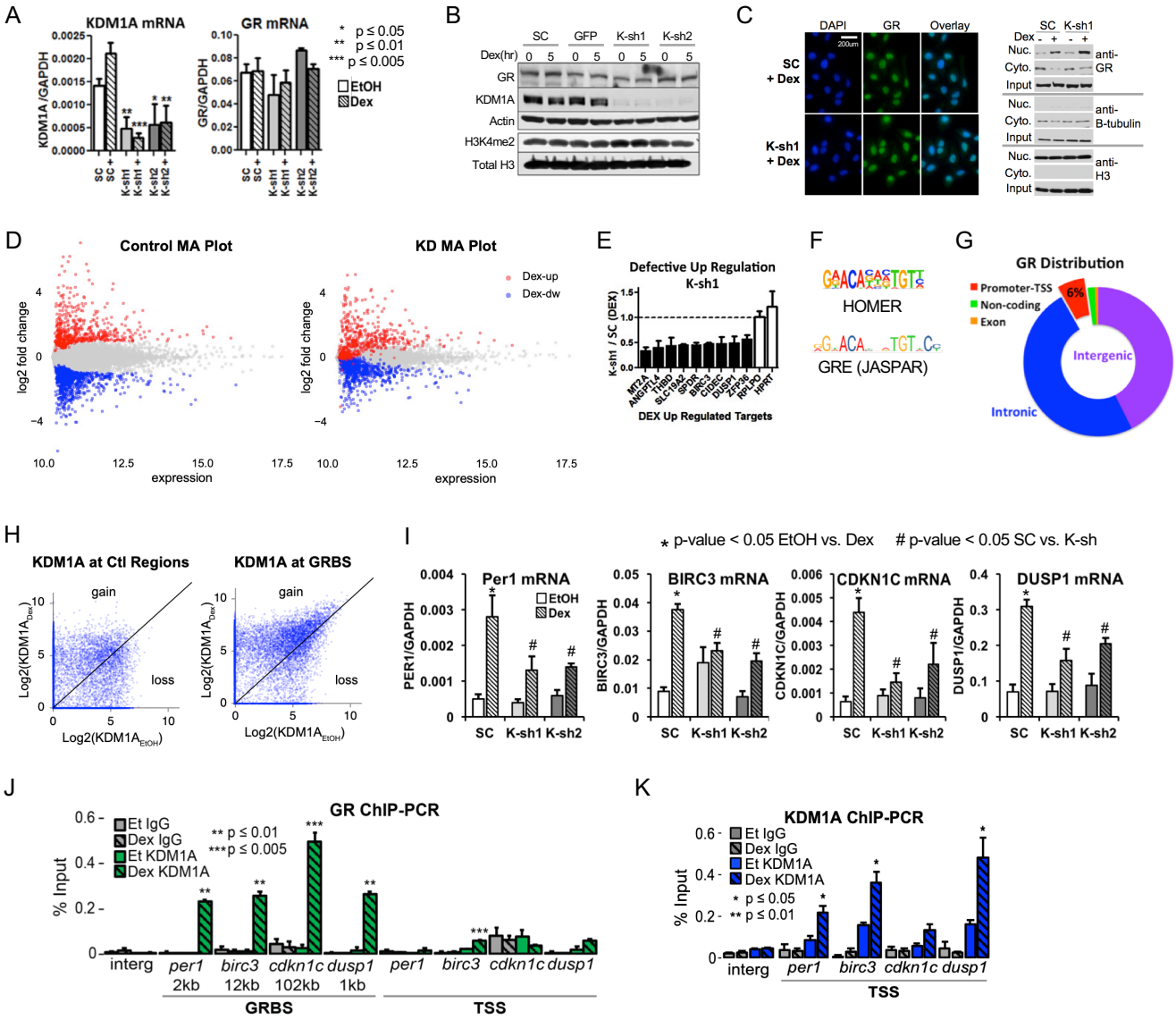


Related to Figure 1. Figure S1. KDM1A is a key GR co-regulator and is recruited to GRBSs.

(A-B) KDM1A KD in A549 by transduced shRNA measuring (A) mRNA by RT-qPCR and (B) protein by Western blot. Antibody used indicated on the left. Two shRNA constructs against KDM1A were tested (K-sh1 and K-sh2). Scramble (SC) and GFP shRNAs used as controls. RNA normalized to GAPDH; N=3; bars plot mean + SE; p-values, Student's t-test comparing SC to K-sh within treatment. No significance was found between SC and K-sh1 for GR mRNA.

(C) Left panel, immunofluorescence staining of GR in Dex treated A549 cells transduced with control (SC) or KDM1A (K-sh1) shRNA. Right panel, Western blot of whole cell lysate (Input), nuclear (Nuc.) or cytoplasmic fractions (Cyto.) from Dex (+) or ethanol (-) treated cells transduced with control (SC) or KDM1A (K-sh1) shRNA.

(D) MA plots of genes with transcript counts >10. Dex-responsive genes are colored by up or down regulation in control.

(E) RT-qPCR confirmation of nine Dex-induced genes co-dependent on KDM1A. Two control genes not regulated by Dex or KDM1A shown in white. Dashed line indicates no change in Dex-response. Normalized to GAPDH. Bars plot mean + SE of biological triplicates.

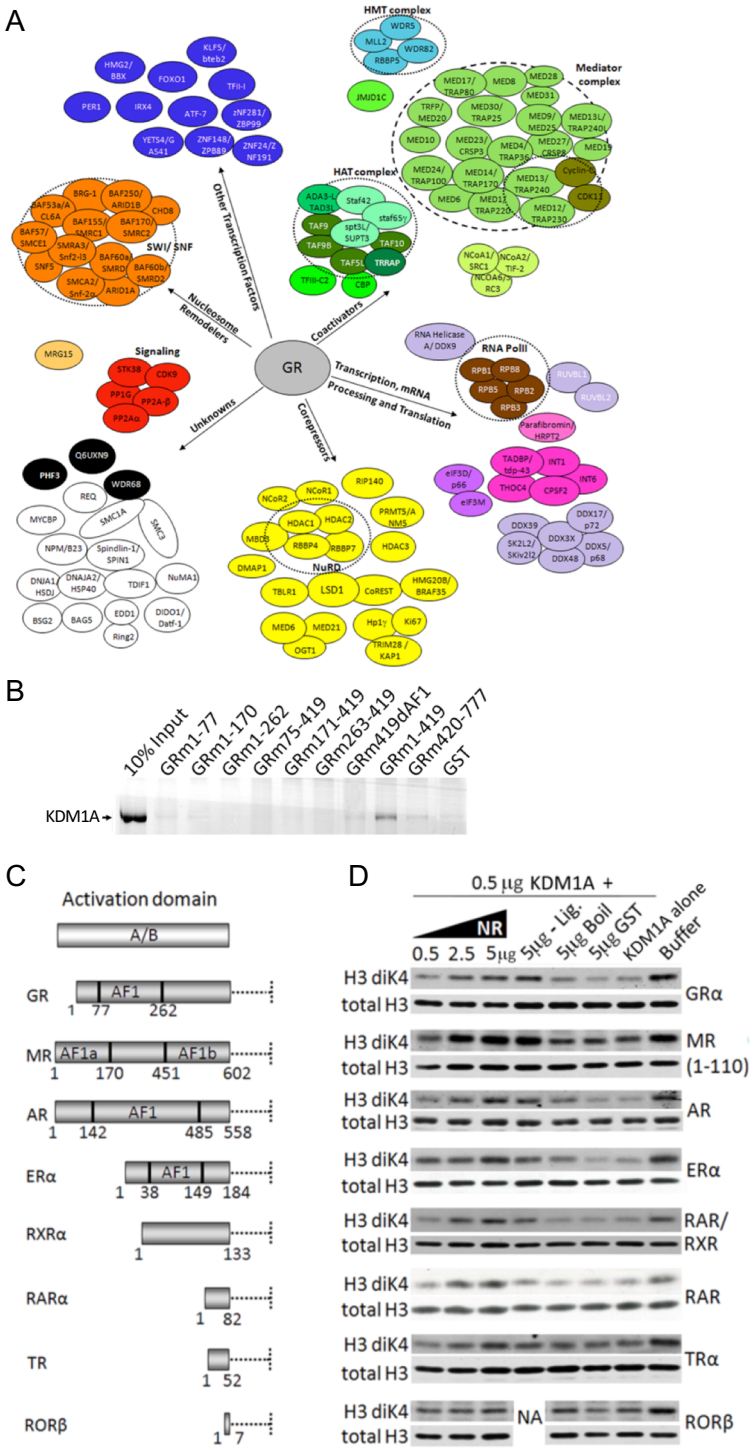
(F) Top *de novo* motif identified from GRBSs by HOMER and the consensus GR motif from Jaspar.

(G) Genome-wide distribution of GRBSs. Promoters defined as 2 kb upstream of an annotated TSS.

(H) KDM1A density in Dex versus EtOH treated cells. Left panel shows control regions and right panel shows all GRBSs. Solid line indicates 0 change, points above gain KDM1A, and points below lose KDM1A.

(I) RT-qPCR of Dex-induced genes. Cells transduced with control (SC) or KDM1A shRNAs (K-sh1 or Ksh2). P-values by Student's t-test.

(J-K) ChIP-qPCR for GR (J) and KDM1A (K) at the TSS and nearby GRBSs as in Figure 1E. Bars plot mean + SE of 3-4 replicates; p-values by Student's t-test between EtOH and Dex.



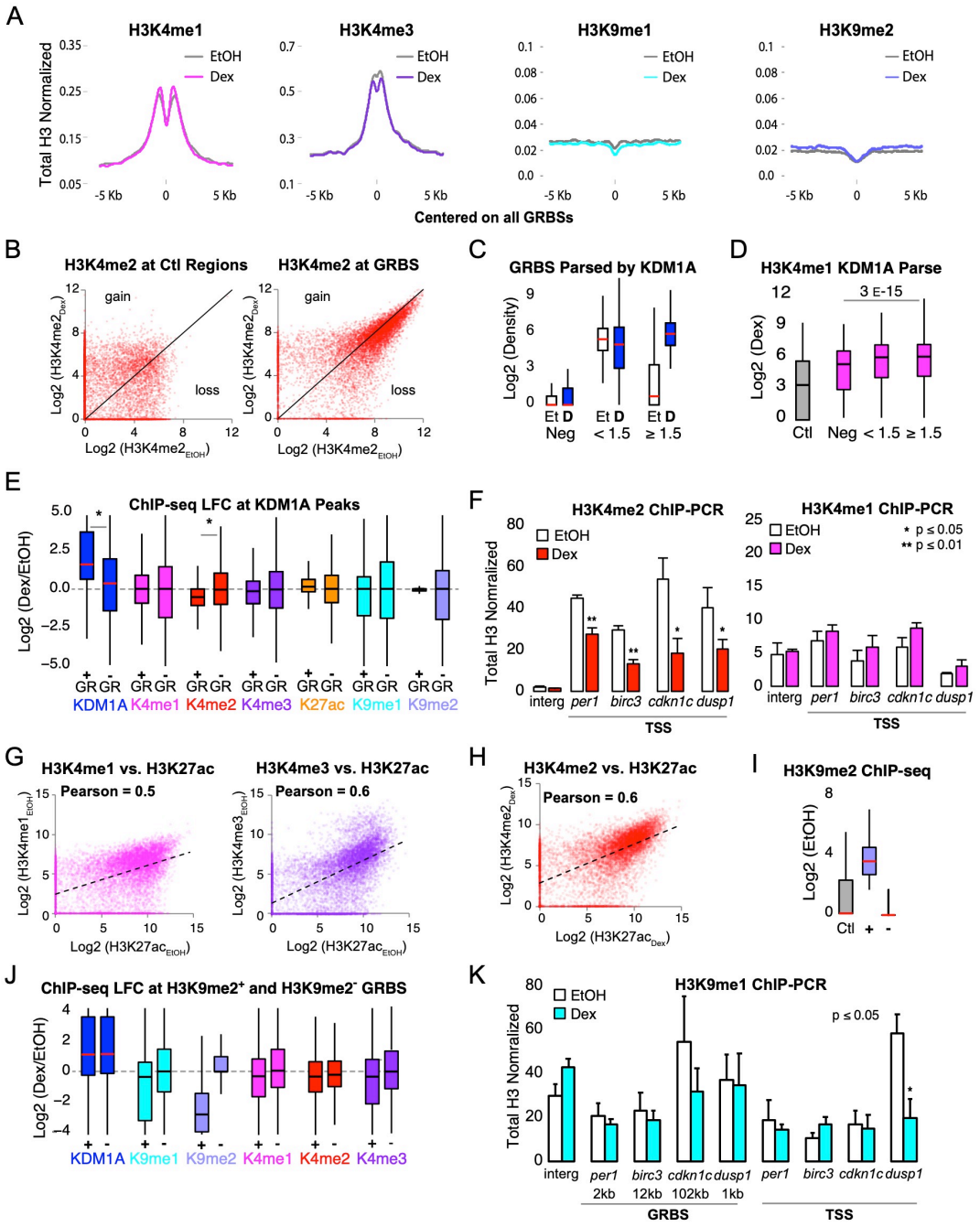
Related to Figure 2. Figure S2. GR complex components and the conserved function of many NRs to inhibit KDM1A.

(A) Diagram of components identified by MS/MS in TAP GR sample.

(B) Silver stain of KDM1A pulldown by GST-tagged GR N-terminal activation domain truncation mutants.

(C) Diagram showing homologous N-terminal domains of each NR.

(D) Most NRs inhibit KDM1A histone demethylase activity. Western blot with modification specific antibodies from cell-free HDM assays. Antibodies indicated on left. The NR added to each reaction indicated on right. Full-length NR used in all reactions except for MR, which contained only the first 110 amino acids. RAR/RXR indicates both proteins added to form heterodimers. All reactions contain the relevant ligand.



Related to Figure 3. Figure S3. H3K9me2 demethylation at a small subset of GRBSs.

(A) Average H3K4me1 (magenta), H3K4me3 (purple), H3K9me1 (cyan), H3K9me2 (lavender) ChIP-seq density.

(B) H3K4me2 density in Dex versus EtOH at all GRBSs and control regions as in Figure S1H.

(C-D) Boxplots of KDM1A (E) and H3K4me1 in Dex (F) at GRBSs parsed by KDM1A status, as in Figure 3C. P-value from Mood's Median test between indicated groups. Et, ethanol control; D, Dex.

(E) Boxplots of ChIP-seq LFC at KDM1A peaks overlapping GRBSs (+ GR) or not overlapping GRBSs (- GR). Dotted line is LFC=0, * indicates p-value < 1 E-74 by Mood's Median test.

(F) ChIP-qPCR of H3K4me2 (red) and H3K4me1 (magenta) at the TSS of four GR-KDM1A co-regulated genes. Bars plot mean + SE of 3-4 replicates; p-values by Student's t-test.

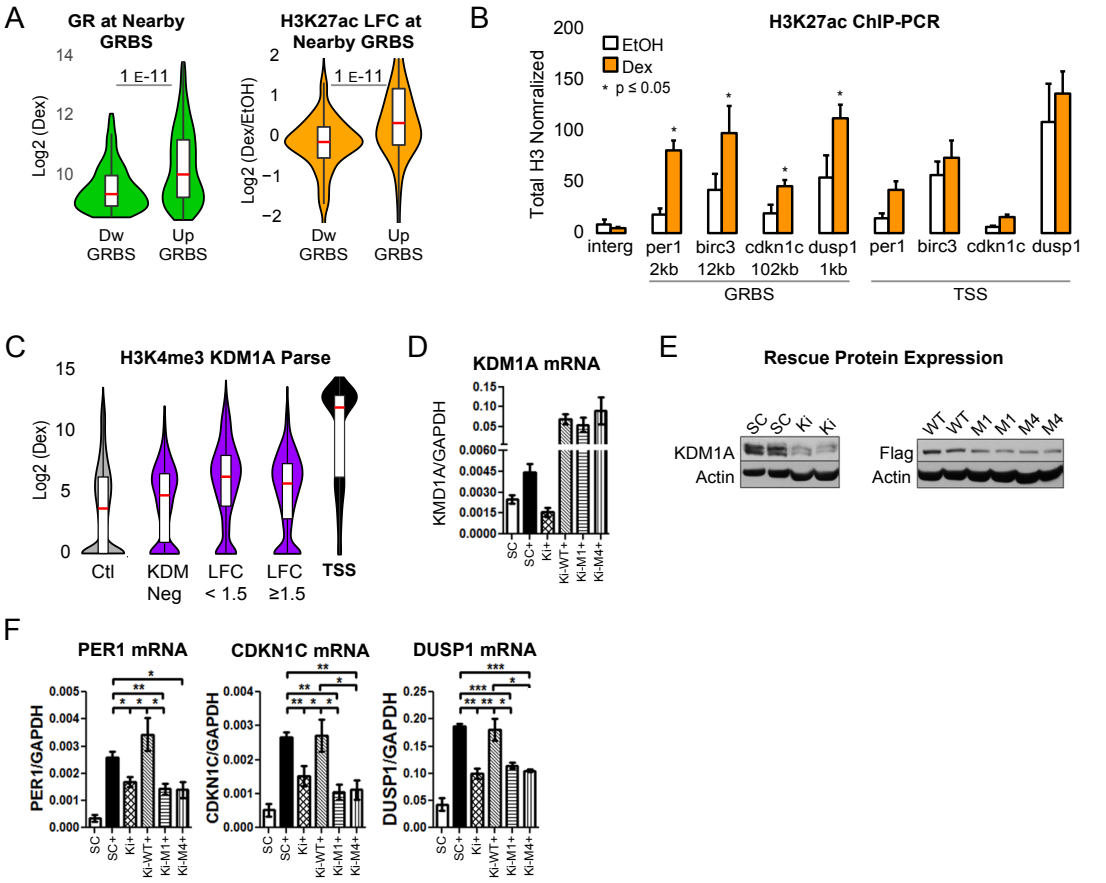
(G) H3K4me1 (magenta) or H3K4me3 (purple) versus H3K27ac at all GRBSs after EtOH.

(H) H3K4me2 versus H3K27ac density at all GRBSs after Dex.

(I) Boxplots of H3K9me2 ChIP-seq density after EtOH at control regions (Ctl) and H3K9me2^{+} (+) or H3K9me2^{-} (-) GRBSs. H3K9me2^{+} sites defined as > mean control (n=2322).

(J) Boxplots of Dex-induced changes at H3K9me2^{+} and H3K9me2^{-} GRBSs. Dotted line indicates LFC=0.

(K) H3K9me2 ChIP-qPCR as in Figure 1E. N=3-4, bars plot mean + SE, p-value Student's t-test EtOH vs. Dex.



Related to Figure 4. Figure S4. KDM1A functions at active enhancers to remove H3K4me2 and rescue experiments show KDM1A activity is required for co-regulation.

(A) Violin boxplots of GR density (green) and H3K27ac LFC (orange) at GRBSs near Dex-induced (Up) and Dex-repressed (Dw) genes. P-values by Mood's Median test.

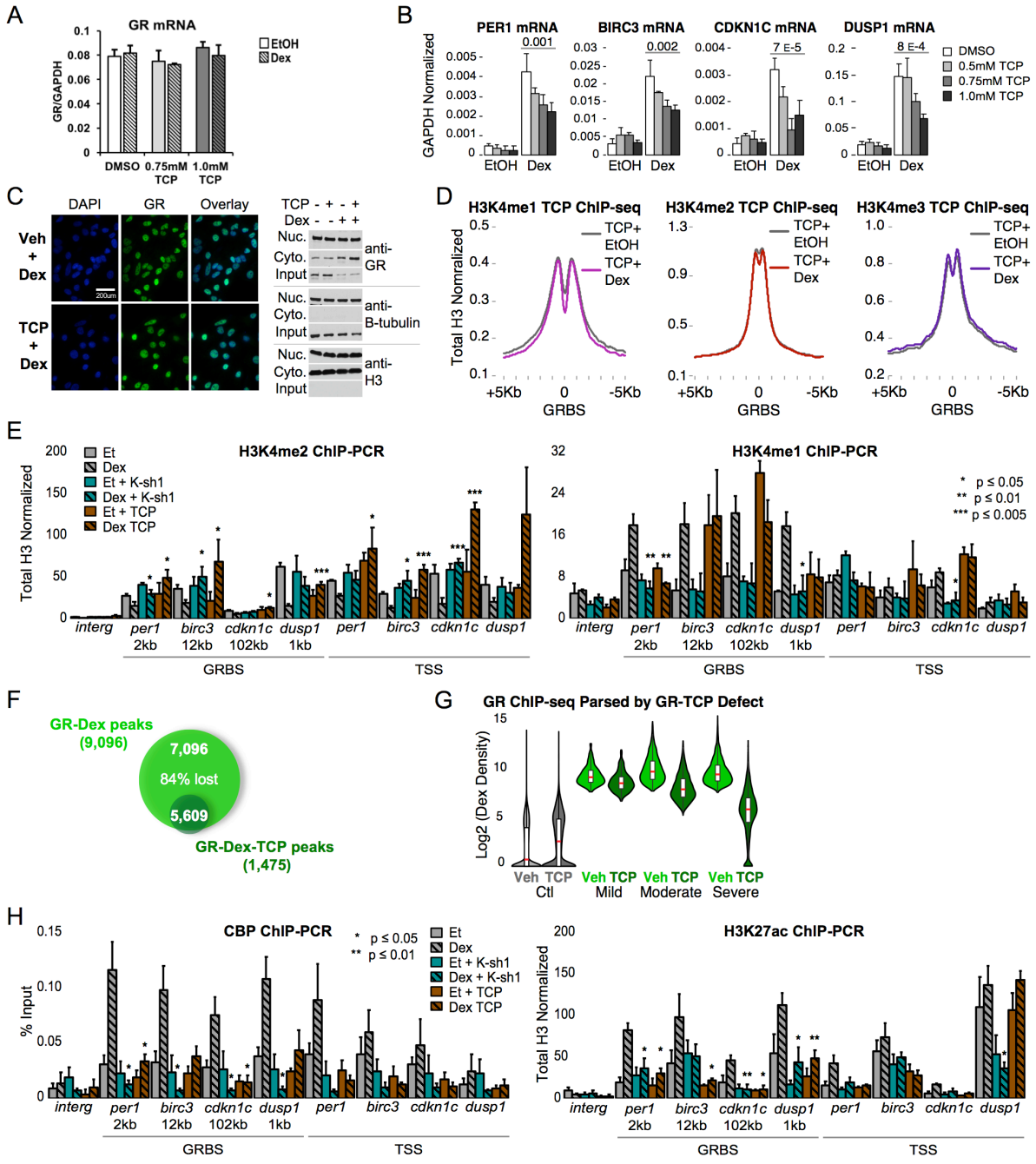
(B) ChIP-qPCR for H3K27ac at TSS and nearby GRBSs of GR-KDM1A co-regulated genes. N=3-4, bars plot the mean + SE, p-value by Student's t-test between EtOH and Dex.

(C) Violin boxplots of H3K4me3 at GRBSs parsed by KDM1A status (as in Figure 3C) and TSS of control genes (black violin) for comparison.

(D-F) Rescue experiments using KDM1A cDNA resistant to a lentiviral RNAi construct against KDM1A (Ki) and two KDM1A catalytic mutants.

(D-E) Expression of WT and mutant KDM1A measured by RT-qPCR (D) and Western blot (E). Ki-WT denotes resistant wild-type KDM1A expressed with KDM1A RNAi. Flag antibody was used to detect tagged KDM1A. M1 has a mutation in the FAD domain (E308A) and M4 in the amine oxidase domain (A814T). M1 and M4 are RNAi resistant and lack HDM activity.

(F) RT-qPCR of three GR-KDM1A co-regulated genes. Ki blocks induction, expression of WT KDM1A rescues induction but neither M1 nor M4 rescue. N=3-4, bars show mean + SE, p-value by Student's t-test.



Related to Figure 5. Figure S5. KDM1A-mediates H3K4me2 demethylation promoting GR and cofactor binding in the genome.

(A-B) RT-qPCR from cells treated with the KDM1A inhibitor, TCP, prior to Dex or EtOH treatment. 0mM TCP treated with DMSO. N=3, bars plot mean + SE, p-value by ANOVA between Dex treated samples.

(C) A549 cells were treated with TCP or DMSO (Veh) for 24 h followed by 1 h Dex treatment. Left panel, immunofluorescence staining of GR; Right panel, Western blot of whole cell lysate (Input), nuclear (Nuc.) and cytoplasmic fractions (Cyto.).

(D) H3K4me1 (dark magenta), H3K4me2 (dark red), H3K4me3 (dark purple) ChIP-seq density at all GRBSs in cells treated with TCP for 24 h followed by two-hour Dex or EtOH treatment.

(E) ChIP-qPCR for histone modifications at the TSS and nearby GRBSs of co-regulate genes, as in Figure 5F.

(F) Venn diagram of GR peaks identified in Dex and Dex-TCP treated cells using the same peak calling parameters.

(G) Violin boxplots of GR density at GRBSs parsed by TCP-induced GR loss, as in Figure 5G.

(H) ChIP-qPCR for CBP and H3K27ac at co-regulate genes and nearby GRBSs, as in (E).