SUPPLEMENTARY INFORMATION

Glucocorticoid Receptor Binding to Chromatin is Selectively Controlled by Coregulator Hic-5 and Chromatin Remodeling Enzymes

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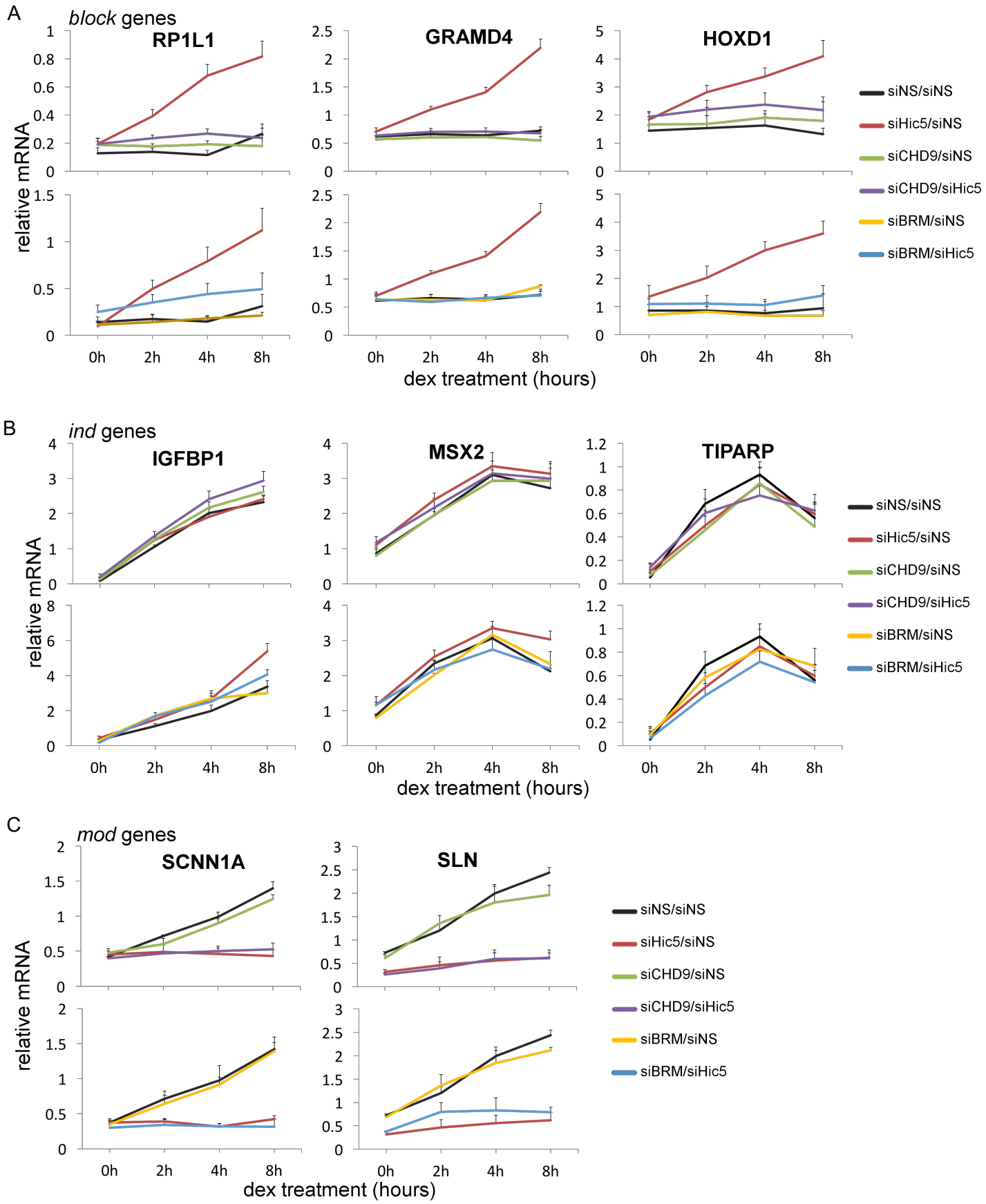
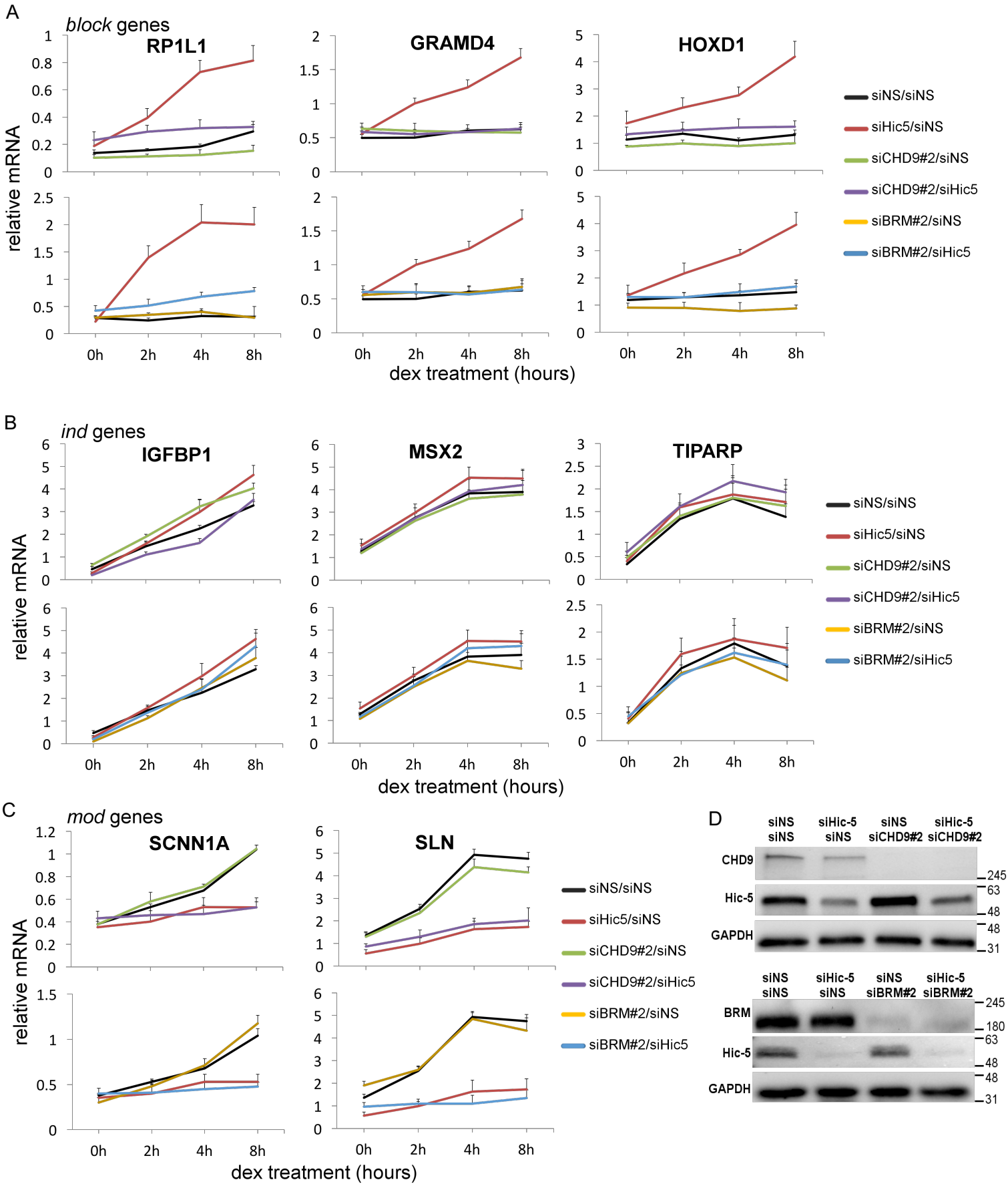
Supplementary Figure S1-6

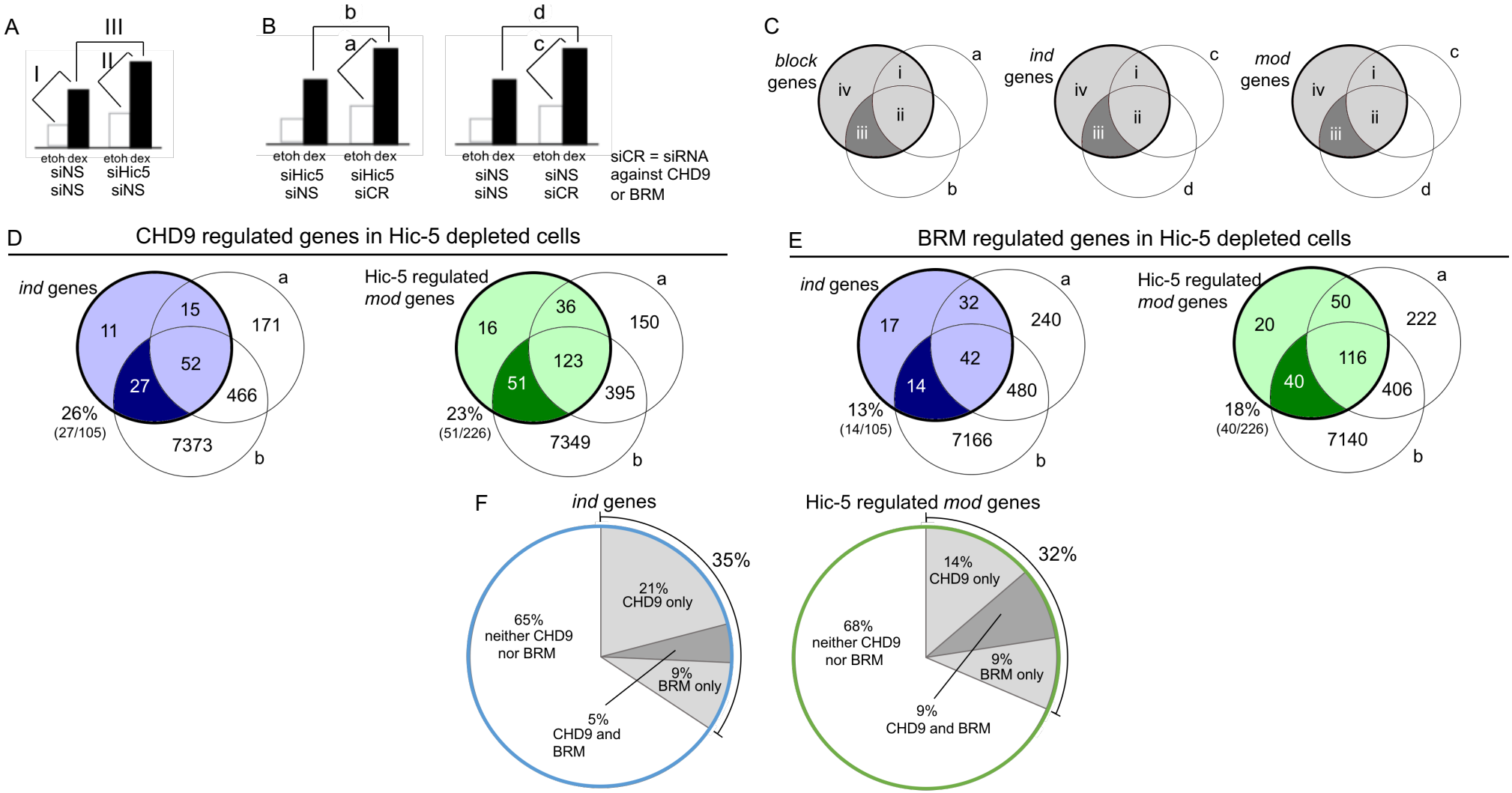
Supplementary Dataset S1-4



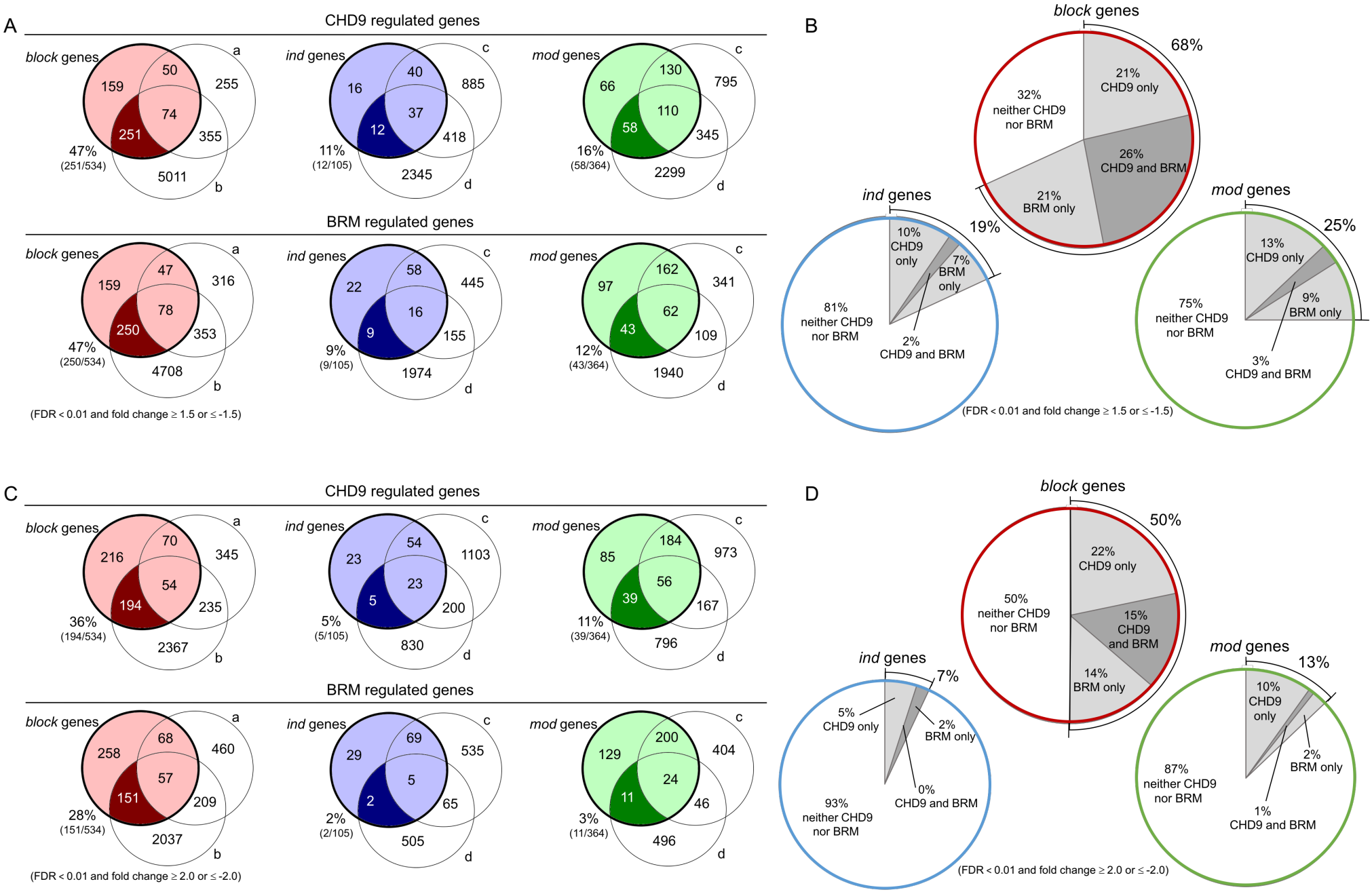
Supplementary Figure S1. Temporal profile of dex-induced gene expression in cells depleted of Hic-5, chromatin remodelers, or both. Cells were transfected with the indicated combinations of siRNAs against Hic-5, CHD9, and BRM, and non-specific siRNA (siNS) was used as control. Quantitative RT-PCR data for the indicated mRNAs from representative genes in the *block* (A), *ind* (B) and *mod* (C) gene classes is shown as mean ± SD for 3 biological replicates conducted on different days.



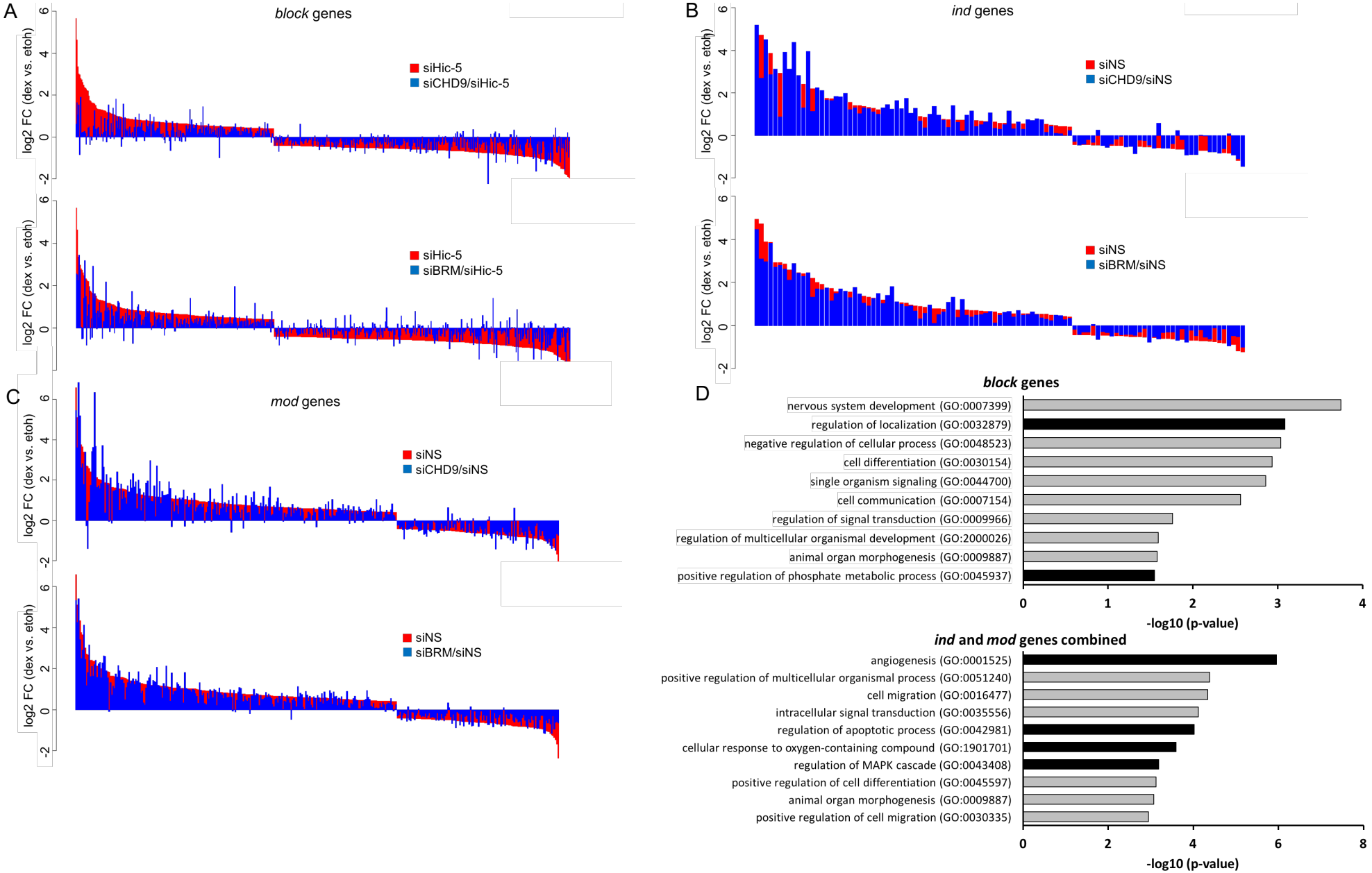
Supplementary Figure S2. Temporal profile of dex-induced gene expression in cells depleted of Hic-5, chromatin remodelers, or both, using a second siRNA for CHD9 and BRM. (A-C) Experiments were conducted as in Figure S1, but with a second set of siRNAs directed against CHD9 and BRM. (D) siRNA depletion of chromatin remodelers and Hic-5. Immunoblots for the indicated chromatin remodelers and Hic-5 are shown with GAPDH as an internal control.



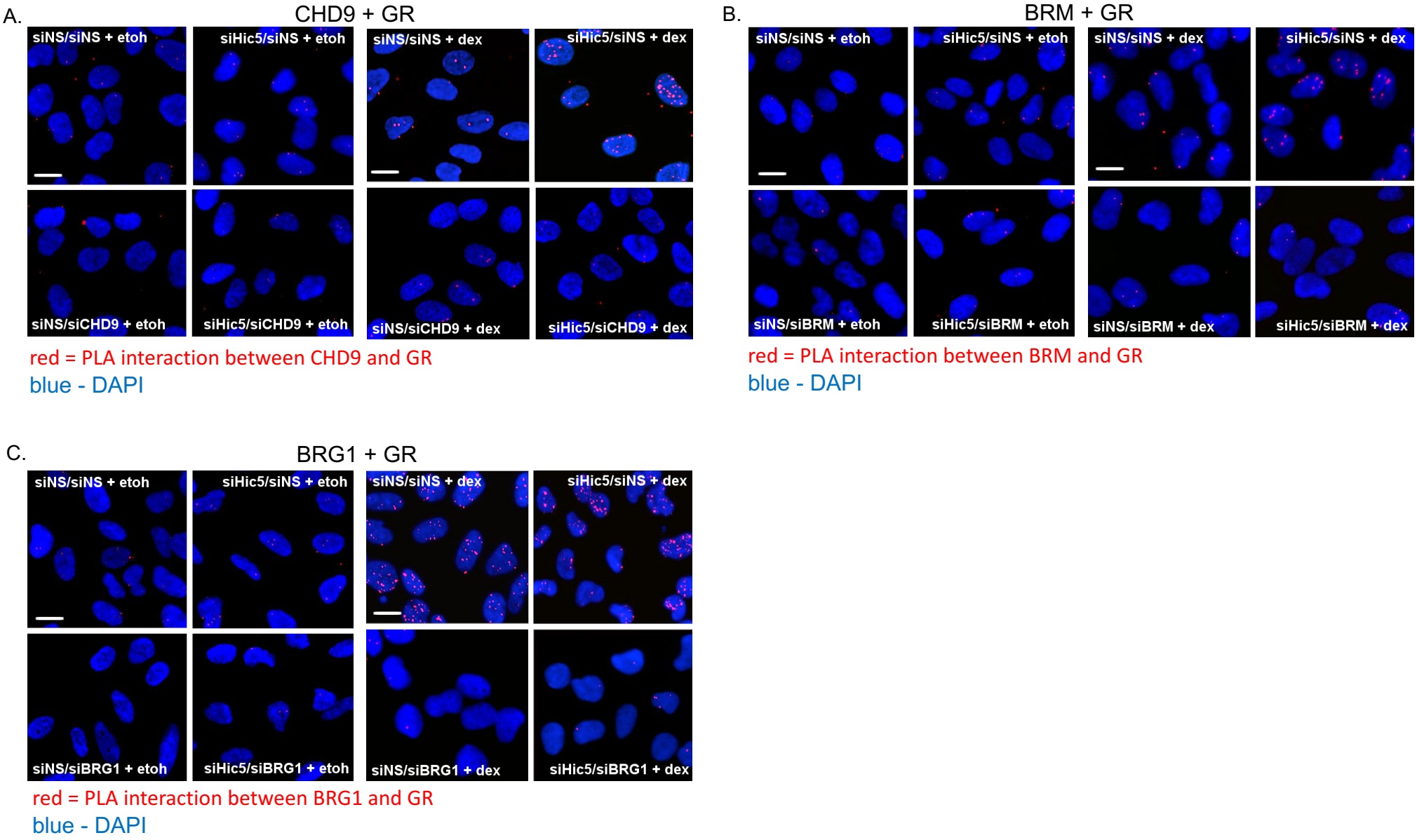
Supplementary Figure S3. Comparisons from RNA-seq data used to determine the three gene classes and the genes within each class that depend on CHD9 or BRM for dex-regulated expression. (A) Diagram shows which samples were compared to define the three gene sets that were overlapped to identify the *block*, *ind*, and *mod* genes, as described by the algorithms in Experimental Procedures. This strategy for overlapping of gene sets was used to generate the data in Figure 3A. Differentially expressed genes in each of the three comparisons were identified using edgeR with FDR adjusted p-value threshold of 0.05 and a 1.3 fold change cutoff. (B) Diagrams show which samples were compared to define gene sets that were overlapped with the *block*, *ind*, or *mod* gene sets to identify genes that require CHD9 or BRM for their dex-regulated expression, as described in the algorithms in Experimental Procedures: a and b compare mRNA levels in cells depleted of Hic-5 which contain or are depleted of CHD9 or BRM; c and d compare mRNA levels in cells containing Hic-5 and either containing or depleted of CHD9 or BRM. (C) Diagrams show which gene sets were overlapped to determine the *block*, *ind*, and *mod* genes that require CHD9 or BRM for their dex regulated expression, as described in the algorithms in Experimental Procedures. The strategies for overlapping of gene sets shown here were used to generate the data in Figure 3B-E. (D-E) As an alternative to the overlapping set strategy specified in C above, *ind* genes or the subset of *mod* genes that were still dex-regulated after depletion of Hic-5 (226 genes in the central sector of Figure 3A) were overlapped with sets a and b described in B above. This strategy indicates the effect of depleting CHD9 or BRM in cells that are also depleted of Hic-5. Dark regions identify the number and percent of genes from the *ind* genes or from the *mod* genes subset that are dependent on CHD9 (D) or BRM (E) for dex-regulated expression. (F) Pie chart summarizing the percent of *ind* genes and of the *mod* gene subset that are dependent on CHD9, BRM, or both, derived from the data in Figure S3D-E



Supplementary Figure S4. Genes dependent on CHD9 and BRM for dex-regulated expression, defined with more stringent parameters. The analyses in Figure 3B-D were repeated, but more stringent fold change and FDR cutoffs were used for comparisons b and d (from Figure S3B), i.e. the effects of CHD9 or BRM depletion. The genes from the *block*, *ind* and *mod* gene classes that required CHD9 and/or BRM for their dex-regulated expression were determined using FDR < 0.01 and fold change ≥ 1.5 or ≤ -1.5 (A-B), or FDR < 0.01 and fold change ≥ 2.0 or ≤ -2.0 (C-D) for comparisons b and d.



Supplementary Figure S5. Genome-wide effect of CHD9 and BRM depletion on dex-regulated gene expression. The $\log_2$ fold change in mRNA levels from dex treatment is shown for cells containing (red bars) or depleted of (blue bars) CHD9 or BRM for all genes from the *block* (A), *ind* (B), and *mod* (C) gene classes (from Figure 3A). Red bars are arranged (left to right) from most positive to most negative $\log_2$ fold change caused by dex treatment, and blue bars for the same gene are superimposed on the red bars. Thus, genes that are positively regulated by dex when neither CHD9 or BRM is depleted are shown on the left, and genes that are negatively regulated by dex are shown on the right. Fold change values were determined in cells transfected with the indicated siRNAs. (D) Gene ontology analysis for *block* genes and for the combined *ind* and *mod* genes. The analysis was conducted using the web-based Gene Ontology Consortium software (1,2). The 10 categories with the lowest p values are shown for each gene set. Enriched gene categories that are distinct in the *block* genes versus the combined *ind* and *mod* genes (and vice-versa) are indicated by black bars.



Supplementary Figure S6. PLA images of GR interactions with CHD9 (A), BRM (B), and BRG1 (C). Cells transfected with the indicated siRNAs were treated with ethanol (etoh) or dex for 1 h. Red fluorescent dots indicate a single interaction between two molecules. DAPI (blue) staining specifies nuclei. Images from dex-treated cells are the same ones shown in Figure 6, but here the ethanol-treated cells are also shown for comparison. Scale bar = 20 µm

Supplementary Dataset Legends

Supplementary Dataset S1-3
The datasets contain the RNA sequencing data with the full gene list (15,186 genes) analyzed for each comparison as described in separate sheets. Within each sheet column A indicates the Entrez Gene ID, column B represents the gene symbol as determined by the HGNC (HUGO Gene Nomenclature Committee), column C shows the full gene name, column D is the log2 fold change for the comparison as indicated on the sheet name, column E shows the p-values for the comparison and column F indicates the FDR adjusted p-values.

Supplementary Dataset S1
This dataset contains three comparisons: sheet 1 compares siNSsiNSdex vs. siNSsiNSetoh to determine the set of dex-regulated genes in the control cells (Supplementary Figure S3A, set I), sheet 2 compares siHic5siNSdex vs. siHic5siNSetoh to determine the set of dex-regulated genes in cells depleted of Hic-5 (Supplementary Figure S3A, set II), and sheet 3 compares siHic5siNSdex vs. siNSsiNSdex to determine the set of genes in the dex treated cells that are differentially expressed upon Hic-5 depletion (Supplementary Figure S3A, set III). 1.3 fold change cut off and FDR value < 0.05 were applied to each comparison to determine the differentially expressed genes that are significant in each comparison. The sets of genes obtained from each comparison (set I-III) were overlapped to determine the three gene classes (*block*, *ind*, and *mod*, Figure 3A) as described in the Experimental Procedures.

Supplementary Dataset S2
This dataset contains four comparisons used to determine sets a-d for CHD9 depleted cells. Sheet 1 compares siCHD9siNSdex vs. siCHD9siNSetoh to determine the set of dex-regulated genes in cells depleted of CHD9 only (Supplementary Figure S3B, set c), sheet 2 compares siCHD9siNSdex vs. siNSsiNSdex to determine the set of genes in the dex treated cells that are differentially expressed upon CHD9 depletion (Supplementary Figure S3B, set d), sheet 3 compares siCHD9siHic5dex vs. siCHD9siHic5etoh to determine the set of dex-regulated genes in cells doubly depleted of CHD9 and Hic5 (Supplementary Figure S3B, set a), and sheet 4 compares siCHD9siHic5dex vs. siHic5siNSdex to determine the set of genes in the dex treated cells that are differentially expressed upon CHD9 depletion in the absence of Hic-5 (Supplementary Figure S3B, set b). 1.3 fold change cut off and FDR value < 0.05 were applied to each comparison to determine the significantly differentially expressed genes in each comparison. The sets of genes obtained from each comparison (set a-d) were overlapped with each gene class to determine the genes within each class that are dependent on CHD9 for dex-regulated expression (Figures 3B and Supplementary Figure S3D) as described in the Experimental Procedures.

Supplementary Dataset S3
This dataset contains four comparisons used to determine sets a-d for BRM depleted cells. Sheet 1 compares siBRMsiNSdex vs. siBRMsiNSetoh to determine the set of dex-regulated genes in cells depleted of BRM only (Supplementary Figure S3B, set c), sheet 2 compares siBRMsiNSdex vs. siNSsiNSdex to determine the set of genes in the dex treated cells that are differentially expressed upon BRM depletion (Supplementary Figure S3B, set d), sheet 3 compares siBRMsiHic5dex vs. siBRMsiHic5etoh to determine the set of dex-regulated genes in cells doubly depleted of BRM and Hic5 (Figure S3B, set a), and sheet 4 compares siBRMsiHic5dex vs. siHic5siNSdex to determine the set of genes in the dex treated cells that are differentially expressed upon BRM depletion in the absence of Hic-5 (Supplementary Figure S3B, set b). 1.3 fold change cut off and FDR value < 0.05 were applied to each comparison to determine the significantly differentially expressed genes in each comparison. The sets of genes obtained from each comparison (set a-d) were overlapped with each gene class to determine the genes within each class that are dependent on BRM for dex-regulated expression (Figures 3C and Supplementary Figure S3E) as described in the Experimental Procedures.

Supplementary Dataset S4
This file lists all the genes in each of the 3 gene classes (*block*, *ind*, and *mod*) as indicated on the sheet names. For each sheet, column A represents the Entrez Gene ID, column B shows the HUGO gene symbol, column C provides the full gene name, column D indicates whether or not ("TRUE" or "FALSE") the gene is dependent on CHD9 for dex-regulated expression with 1.3 fold change and 0.05 FDR p-value cutoff, and column E indicates whether or not the gene is dependent on BRM for dex-regulated expression with 1.3 fold change and 0.05 FDR p-value cutoff.

References

1. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* **25**, 25-29
2. The Gene Ontology Consortium. (2017) Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Res* **45**, D331-D338