

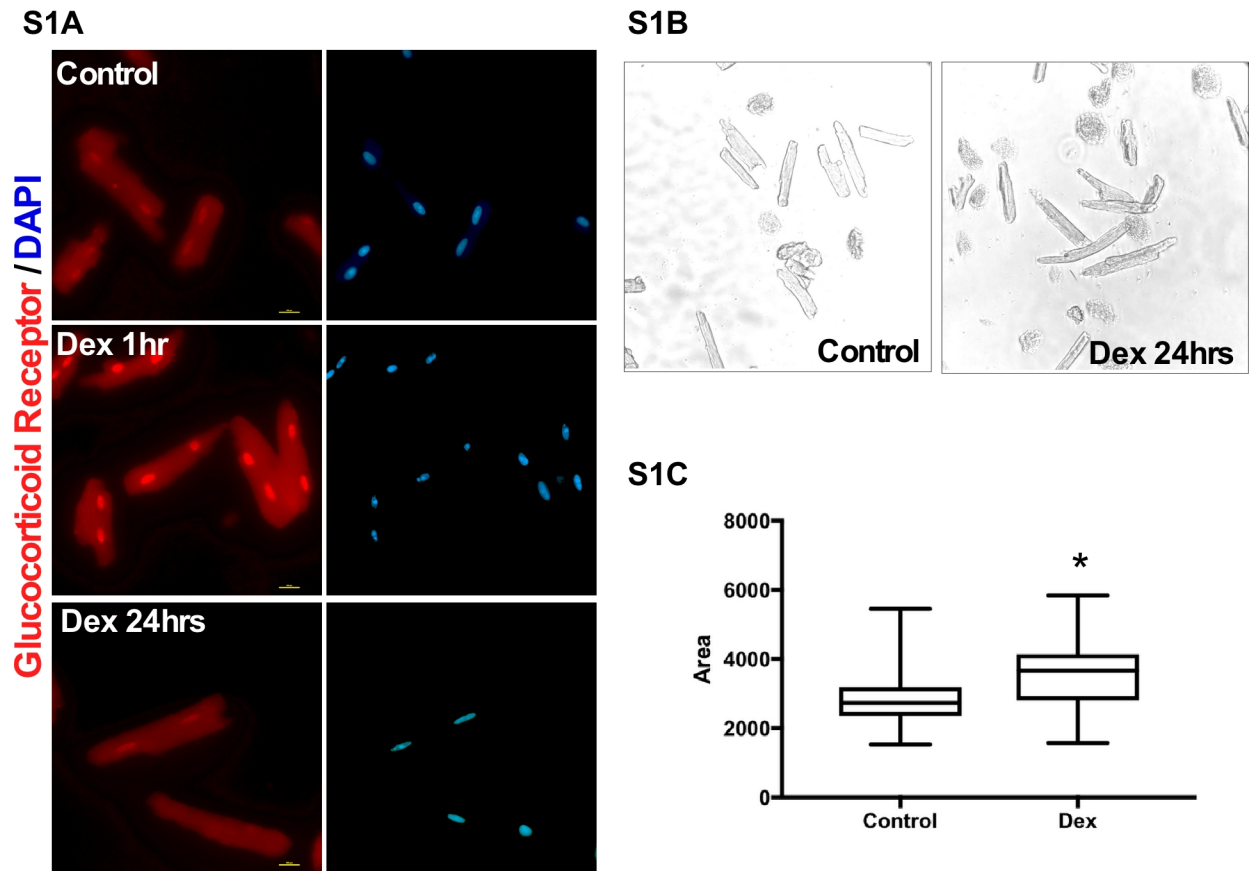
# SUPPLEMENTAL MATERIAL

**Supplemental Tables (see Excel files)**

**Table S1.** List of genes from RNAseq used for creating Heatmap (Figure 3).

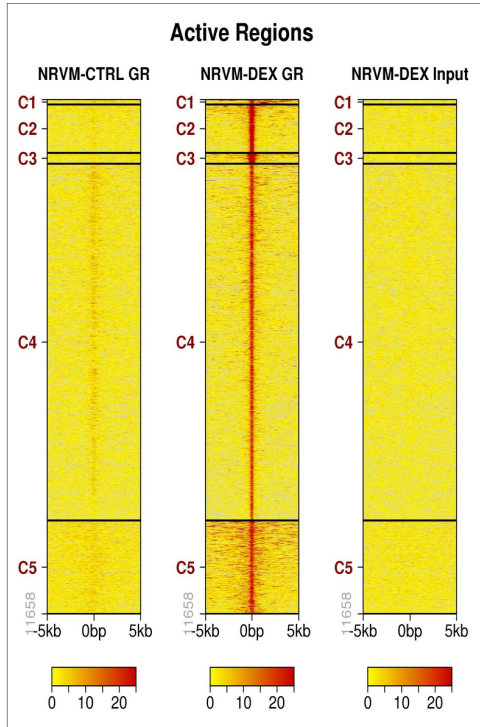
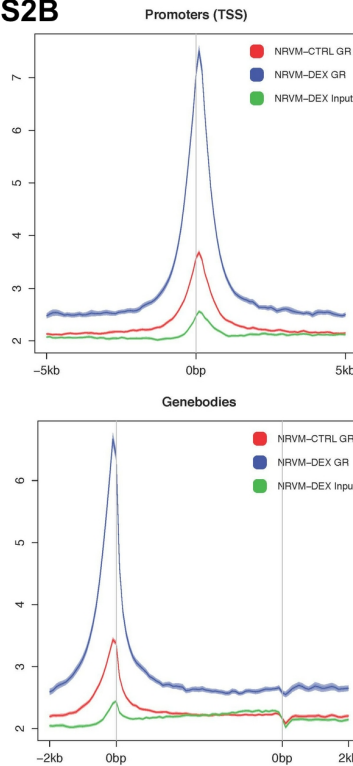
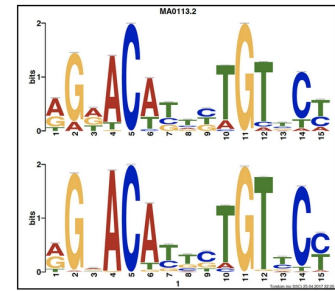
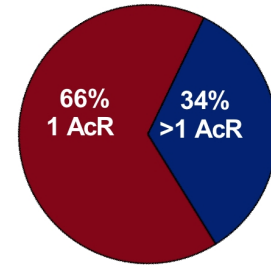
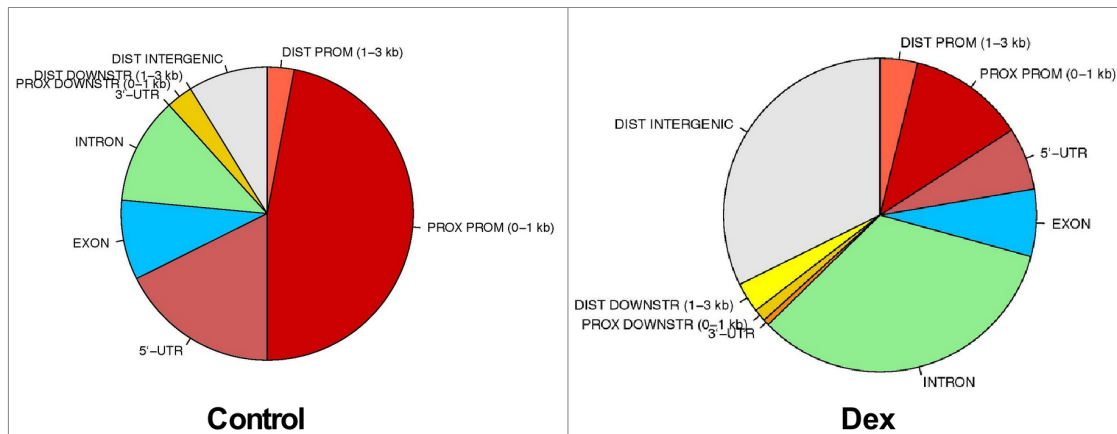
**Table S2.** Genes differentially regulated after 24hrs of Dex treatment with Log<sub>2</sub>FC values.

**Table S3.** Taqman gene assays used for quantitative PCR (qPCR).



**Figure S1. Isolated adult mouse ventricular cardiomyocytes treated with Dex exhibit GR nuclear translocation and cardiomyocyte hypertrophy.**

A. Cardiomyocytes were treated with Ethanol (control) or Dex (100nM) for increasing time periods of 1hr or 24hrs, as indicated. Cardiomyocytes were fixed, and stained for GR (red) and DAPI (blue). Scale bar 10 $\mu$ M **B.** Isolated cultured adult mouse cardiomyocytes were treated with Ethanol or Dex for 24hrs. Phase contrast images are shown for cell size and quality of cardiomyocytes. **C.** Image J was used to measure cardiomyocyte surface area, from two independent cultures, tabulated and graphed as box plot using Prism7. \* represents  $p < 0.05$ .

**S2A****S2B****S2C****S2D****S2E**

**Figure S2. GR binding and distribution across cardiac genome in neonatal cardiomyocytes treated with Dex.**

**A.** Heatmap showing the tag distributions across active regions (values in z-axis/color, active regions in y-axis) in Control, Dex treated and Input samples. The data is presented in 5 clusters (default), C1 to C5 and sorted. **B.** Average plots of tag distributions of active regions at the transcription start site (TSS) and gene bodies are shown for Control (red), Dex treated (blue) or Input sampled (green). **C.** MEME/TOMTOM motif search identifies GRE in top 1000 peaks from GR-ChIP-seq. **D.** Pie chart showing the percent of genes associated with single active region (AcR) which represents genomic GR binding site vs. multiple Active Regions (AcR). **E.** Pie Chart showing locations of GR binding peaks relative to genomic annotations is presented as observed in Control and Dex treated samples.

S3


## Genes regulated after 1hr of Dex treatment

with Gr binding						no Gr binding		
Gene.Name	# ActReg	Ave. peak value	Ave.highest ActRegs peak	RNAseq		Gene.Name	RNAseq	
		Log2Ratio Ave.	Log2Ratio Acr	Log2FC.Dex1h	Log2FC.Dex24h		Log2FC.Dex 1hr	Log2FC. Dex 24hrs
Pdk4	1	1.91	1.994	3.014	1.397	Cldn1	3.420	5.323
Arrdc3	2	2.09	2.382	2.556	0.706	Flrt3	2.112	2.090
Per1	3	4.35	4.421	2.489	2.188	Rgs1	1.926	0.335
Abra	1	4.17	4.130	2.398	1.184	Fam110c	1.435	1.198
Sox8	4	2.22	2.970	2.358	1.957	Zfp697	1.425	1.603
Aspa	1	#DIV/0!	#DIV/0!	2.251	1.506	Foxq1	1.420	1.870
Fam46b	2	4.74	5.034	2.150	3.845	Pcdh20	1.378	1.869
Klf15	3	4.38	4.622	2.129	1.987	LOC689064	1.256	1.303
Arrdc2	2	4.97	5.140	1.996	0.472	Klhl38	1.225	0.765
Sesn1	4	3.79	5.122	1.975	1.281	Zfp697	1.224	1.299
Sgk1	2	3.23	3.637	1.949	2.240	Adrb2	1.193	0.755
Cyb561	1	5.13	5.212	1.937	3.334	LOC1001348	1.184	1.157
Cpa6	2	3.46	3.920	1.757	3.218	C5ar2	1.169	2.119
Nfkbia	4	3.49	4.010	1.743	1.133	Hbb-b1	1.099	1.108
Slc10a6	1	6.17	5.151	1.639	3.247	Hba1	1.017	1.094
Rgcc	2	4.32	5.030	1.588	4.187	Gcnt1	0.975	0.438
Pkfb3	3	2.25	3.102	1.568	1.303	Hba2	0.967	1.117
Zfp189	1	4.86	4.970	1.567	0.661	Lrrc10	0.896	1.175
Errfi1	4	3.11	4.047	1.463	0.970	RGD130936z	0.873	1.227
Ptgs2	1	4.39	4.170	1.419	1.648	Lrrc10	0.871	1.095
Rasd1	2	3.12	3.660	1.331	0.850	Dgke	0.831	0.250
Klf9	5	3.70	4.977	1.323	1.569	Rnd1	-0.746	0.022
Cebpd	1	1.49	2.115	1.254	0.743	Fzd8	-0.756	-0.055
F3	3	4.62	5.401	1.222	1.363	Gja1	-0.822	-0.641
Acer2	2	3.27	4.343	1.199	2.082	Foxc2	-0.901	-0.463
Cish	4	3.53	3.950	1.106	0.268	Efnal	-1.089	-0.161
RGD1309079	2	2.10	3.265	1.104	1.550	Arc	-1.128	0.385
Slc28a2	1	2.54	2.747	1.103	2.608	G0s2	-1.129	-1.123
Relt	1	2.50	2.913	1.097	0.225	Col2a1	-1.433	-2.732
Spsb1	1	4.75	4.285	1.085	1.588	Dusp5	-1.745	-0.819
Ppp1r3b	1	1.17	1.467	0.991	-0.345			
Tob2	2	3.72	3.916	0.971	0.589			
Adamts1	1	2.25	2.713	0.935	0.896			
Dhrs3	2	2.29	3.269	0.929	0.627			
Usp53	4	1.85	3.282	0.917	1.109			
Fkbp5	6	4.13	5.079	0.904	2.686			
Tsc22d3	1	4.78	4.306	0.900	1.386			
Glul	3	2.76	3.610	0.892	1.758			
Mt2A	4	3.16	4.324	0.888	1.418			
Tsku	1	1.87	2.063	0.843	0.857			
Nt5dc3	3	3.87	4.984	0.832	1.083			
Rnf144b	3	3.37	4.363	0.831	0.500			
Cdc42ep2	3	3.42	3.976	0.812	0.414			
Cebpb	1	2.00	2.481	0.802	1.166			
Rhob	3	1.97	2.922	0.795	0.734			
RGD1304884	1	2.81	2.667	0.791	0.388			
Chst3	5	3.30	4.812	0.786	0.767			
Lpin1	3	4.13	4.475	0.771	0.336			
Mtus1	4	2.75	3.170	0.701	1.194			
Ddit4	2	1.63	3.174	0.699	0.565			
Vmp1	6	3.45	4.786	0.695	0.794			
Egr1	1	2.75	2.902	-0.715	0.038			
Ier5	2	1.31	1.793	-0.731	-0.020			
Junb	6	2.06	3.369	-0.763	0.108			
Irs1	5	2.64	4.088	-0.792	-0.186			
Irf1	2	1.62	1.922	-0.797	-0.553			
Arid5a	1	1.84	2.663	-0.871	-0.388			
Nuak2	1	3.19	2.701	-0.921	-0.016			
Klf10	2	1.56	2.118	-0.983	0.057			
Mycn	1	2.81	2.979	-0.998	-0.417			
LOC310926	2	1.73	2.681	-1.059	-1.452			
Ier3	2	2.22	2.609	-1.141	-0.516			
Hes1	5	2.28	3.329	-1.155	-0.241			
Plk2	1	1.07	1.327	-1.327	-0.757			
Lif	1	2.64	3.441	-1.360	-0.547			
Socs3	2	1.85	2.451	-1.421	-0.468			
Phlda1	1	1.78	2.252	-1.464	-0.483			
Cxcr4	1	1.42	2.121	-1.525	0.012			

**Figure S3. Genes differentially regulated after 1hr of Dex treatment in neonatal cardiomyocytes.**

A. Chart showing genes that are regulated after 1hr of Dex treatment with and without GR binding. Log<sub>2</sub> fold change (Log<sub>2</sub>FC) is shown for GR-ChIP-seq and RNAseq data. Color scheme shows the increase (green) or decrease (red), with the color intensity corresponding to the Log<sub>2</sub>FC values. Since some genes have multiple binding sites of GR, the average peak values are shown for gene and the active region with highest peak value.

## S4 Screenshot of DAVID showing functional annotation with genes regulated after 1hr Dex



**DAVID Bioinformatics Resources 6.8**  
Laboratory of Human Retrovirology and Immunoinformatics (LHRI)

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### Functional Annotation Chart

[Help and Manual](#)

**Current Gene List:** 02162018 Sig Diff 1hr  
**Current Background:** Rattus norvegicus  
**92 DAVID IDs**

**Options**

**76 chart records**

[Download File](#)

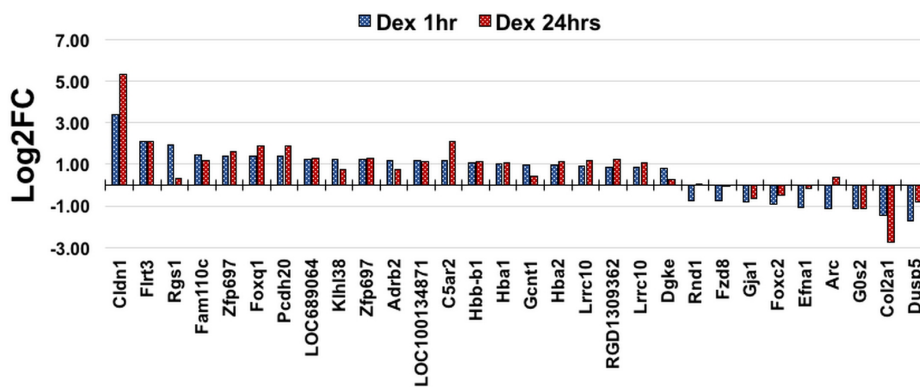
Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of transcription from RNA polymerase II promoter</a>	RT		19	20.7	1.6E-6	1.5E-3
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of cell death</a>	RT		5	5.4	1.4E-4	6.1E-2
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">response to lipopolysaccharide</a>	RT		8	8.7	4.2E-4	1.2E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">regulation of gene expression</a>	RT		7	7.6	9.8E-4	2.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to mechanical stimulus</a>	RT		5	5.4	1.7E-3	2.7E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of transcription, DNA-templated</a>	RT		10	10.9	1.8E-3	2.4E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">apoptotic process</a>	RT		8	8.7	2.0E-3	2.3E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">embryo implantation</a>	RT		4	4.3	2.2E-3	2.2E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">liver regeneration</a>	RT		4	4.3	2.4E-3	2.2E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to insulin stimulus</a>	RT		5	5.4	3.0E-3	2.5E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to peptide</a>	RT		3	3.3	3.8E-3	2.7E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">negative regulation of transcription, DNA-templated</a>	RT		9	9.8	4.2E-3	2.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">regulation of cell proliferation</a>	RT		6	6.5	5.6E-3	3.3E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">decidualization</a>	RT		3	3.3	9.3E-3	4.6E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of gene expression</a>	RT		7	7.6	9.3E-3	4.4E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to drug</a>	RT		4	4.3	1.2E-2	5.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">brown fat cell differentiation</a>	RT		3	3.3	1.2E-2	4.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">negative regulation of insulin receptor signaling pathway</a>	RT		3	3.3	1.3E-2	5.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">regulation of calcium ion transport</a>	RT		3	3.3	1.4E-2	5.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of vascular wound healing</a>	RT		2	2.2	1.4E-2	4.9E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">reactive oxygen species metabolic process</a>	RT		3	3.3	1.5E-2	4.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to peptide hormone stimulus</a>	RT		3	3.3	1.5E-2	4.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">response to insulin</a>	RT		4	4.3	1.5E-2	4.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">negative regulation of JAK-STAT cascade</a>	RT		3	3.3	1.8E-2	5.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">negative regulation of tyrosine phosphorylation of Stat1 protein</a>	RT		2	2.2	1.9E-2	5.3E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">circadian rhythm</a>	RT		4	4.3	2.1E-2	5.5E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">spongiotrophoblast differentiation</a>	RT		2	2.2	2.4E-2	5.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">response to peptide hormone</a>	RT		4	4.3	2.5E-2	5.8E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to tumor necrosis factor</a>	RT		4	4.3	2.7E-2	6.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">endothelial tube morphogenesis</a>	RT		2	2.2	2.9E-2	6.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cellular response to hypoxia</a>	RT		4	4.3	2.9E-2	6.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">cytokine-mediated signaling pathway</a>	RT		4	4.3	3.0E-2	5.9E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">insulin receptor signaling pathway</a>	RT		3	3.3	3.1E-2	6.0E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">positive regulation of protein catabolic process</a>	RT		3	3.3	3.3E-2	6.2E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">glomerular visceral epithelial cell differentiation</a>	RT		2	2.2	3.3E-2	6.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">regulation of auditory receptor cell differentiation</a>	RT		2	2.2	3.3E-2	6.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">response to progesterone</a>	RT		3	3.3	3.4E-2	6.1E-1
<input type="checkbox"/>	GOTERM_BP_DIRECT	<a href="#">negative regulation of cell proliferation</a>	RT		6	6.5	3.5E-2	6.1E-1

**Figure S4. Functional annotation of genes regulated after 1hr of Dex in neonatal cardiomyocytes using DAVID bioinformatics resource.**

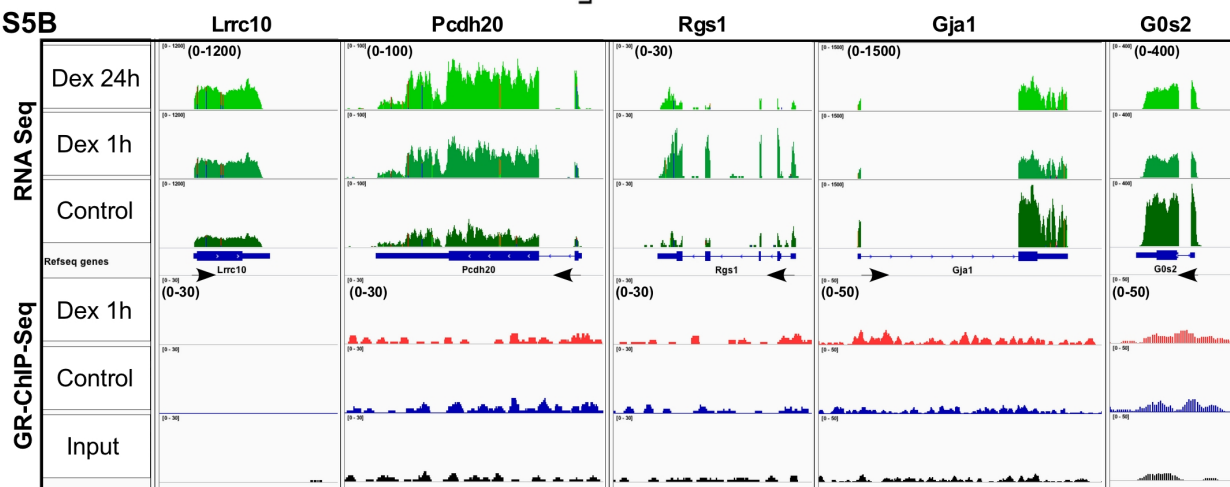
Genes differentially regulated after 1hr of Dex treatment were uploaded onto the DAVID Bioinformatics resources for functional annotation. The screenshot shows the top 38 GOTERM identified by DAVID.



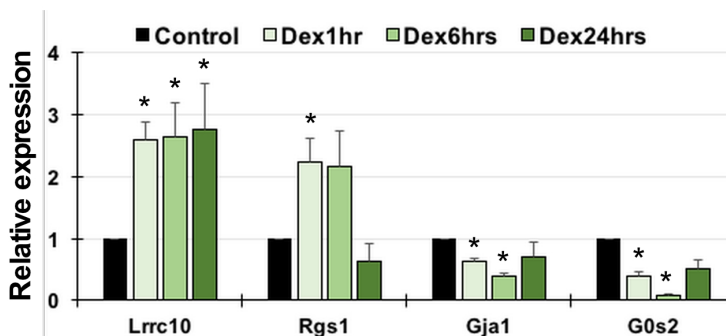
S5A



S5B



S5C



S5D

Functional Annotation of genes differentially regulated after 1 hr of Dex, but with no Gr binding

Functional Annotation Groups		Genes	P value
Embryonic heart development		2	2.70E-02
Cell-cell junction organization		2	2.80E-02
Response to dexamethasone		2	4.10E-02
Regulation of calcium ion transport		2	4.50E-02
Positive regulation of vasodilation		2	4.90E-02
Collagen fibril organization		2	4.90E-02
Heart development		3	5.00E-02
Liver regeneration		2	6.70E-02
Heart morphogenesis		2	6.90E+02
Functional Annotation Clustering (cluster 1)		Genes	P value
GOTERM_CC_Direct	integral component of plasma membrane	5	2.30E-02
GOTERM_CC_Direct	apical plasma membrane	3	5.30E-02
GOTERM_BP_Direct	G-protein coupled receptor signaling pathway	4	4.80E-01

**Figure S5. Genes differentially regulated after 1hr of Dex treatment with no GR association in neonatal cardiomyocytes.**

**A.** Graph represents genes that show significant differential regulation at 1hr and 24hrs of Dex treatment but are not associated with genomic GR binding. **B.** Integrated Genomic Viewer (IGV) screenshots of selected representative genes with aligned RNAseq and GR-ChIP-Seq data, after Dex treatment compared to control (ethanol) cardiomyocytes. Arrows indicate the direction of transcription of that genes, numbers in brackets in the Y axis indicate the values on signal tracks for GR-ChIP-Seq and RNAseq for each gene. The values were kept same within the samples for each gene. **C.** Transcript abundance of selected genes as measured by qPCR in cardiomyocytes treated with Dex for 1hr, 6hrs or 24hrs. Error bars represent SEM, \* is  $p < 0.05$  compared to control,  $n=3$ . **D.** Functional annotation of genes that showed significant differential regulation after 1hr of Dex treatment and were not associated with GR, analyzed using DAVID bioinformatics resource.

## S6 Screenshot of DAVID showing Functional Annotation with genes regulated after 24hrs of Dex



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### Functional Annotation Chart

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Current Gene List: only 24hrs  
Current Background: Rattus norvegicus  
617 DAVID IDs

Options

42 chart records

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Axon guidance</a>	RT	<input type="checkbox"/>	20	3.2	7.6E-8	1.7E-5
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Focal adhesion</a>	RT	<input type="checkbox"/>	24	3.9	9.5E-7	1.0E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">ECM-receptor interaction</a>	RT	<input type="checkbox"/>	15	2.4	2.0E-6	1.5E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Pentose and glucuronate interconversions</a>	RT	<input type="checkbox"/>	9	1.5	2.5E-5	1.4E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Metabolism of xenobiotics by cytochrome P450</a>	RT	<input type="checkbox"/>	12	1.9	2.6E-5	1.1E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Ascorbate and aldarate metabolism</a>	RT	<input type="checkbox"/>	8	1.3	2.8E-5	1.0E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Drug metabolism - cytochrome P450</a>	RT	<input type="checkbox"/>	12	1.9	3.0E-5	9.4E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">PI3K-Akt signaling pathway</a>	RT	<input type="checkbox"/>	28	4.5	4.6E-5	1.3E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Chemical carcinogenesis</a>	RT	<input type="checkbox"/>	13	2.1	6.8E-5	1.7E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Porphyrin and chlorophyll metabolism</a>	RT	<input type="checkbox"/>	9	1.5	6.9E-5	1.5E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Retinol metabolism</a>	RT	<input type="checkbox"/>	12	1.9	1.3E-4	2.6E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Complement and coagulation cascades</a>	RT	<input type="checkbox"/>	11	1.8	1.8E-4	3.3E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Protein digestion and absorption</a>	RT	<input type="checkbox"/>	12	1.9	2.4E-4	4.1E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Leukocyte transendothelial migration</a>	RT	<input type="checkbox"/>	14	2.3	2.6E-4	4.1E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Drug metabolism - other enzymes</a>	RT	<input type="checkbox"/>	9	1.5	6.5E-4	9.5E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Steroid hormone biosynthesis</a>	RT	<input type="checkbox"/>	10	1.6	1.9E-3	2.6E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Renin secretion</a>	RT	<input type="checkbox"/>	9	1.5	2.1E-3	2.8E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Dilated cardiomyopathy</a>	RT	<input type="checkbox"/>	10	1.6	2.5E-3	3.0E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Proximal tubule bicarbonate reclamation</a>	RT	<input type="checkbox"/>	5	0.8	6.4E-3	7.2E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Hypertrophic cardiomyopathy (HCM)</a>	RT	<input type="checkbox"/>	9	1.5	6.5E-3	6.9E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Platelet activation</a>	RT	<input type="checkbox"/>	12	1.9	7.3E-3	7.4E-2
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Adrenergic signaling in cardiomyocytes</a>	RT	<input type="checkbox"/>	12	1.9	1.3E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Mineral absorption</a>	RT	<input type="checkbox"/>	6	1.0	1.3E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Rap1 signaling pathway</a>	RT	<input type="checkbox"/>	15	2.4	1.8E-2	1.6E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Pathways in cancer</a>	RT	<input type="checkbox"/>	23	3.7	2.1E-2	1.7E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Hematopoietic cell lineage</a>	RT	<input type="checkbox"/>	8	1.3	2.2E-2	1.7E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Oxytocin signaling pathway</a>	RT	<input type="checkbox"/>	12	1.9	2.4E-2	1.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Insulin secretion</a>	RT	<input type="checkbox"/>	8	1.3	3.0E-2	2.1E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Arrhythmogenic right ventricular cardiomyopathy (ARVC)</a>	RT	<input type="checkbox"/>	7	1.1	3.7E-2	2.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Bile secretion</a>	RT	<input type="checkbox"/>	7	1.1	3.7E-2	2.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Gastric acid secretion</a>	RT	<input type="checkbox"/>	7	1.1	4.1E-2	2.7E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Pertussis</a>	RT	<input type="checkbox"/>	7	1.1	4.1E-2	2.7E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Calcium signaling pathway</a>	RT	<input type="checkbox"/>	12	1.9	5.8E-2	3.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Serotonergic synapse</a>	RT	<input type="checkbox"/>	9	1.5	6.7E-2	3.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Aldosterone synthesis and secretion</a>	RT	<input type="checkbox"/>	7	1.1	7.2E-2	3.9E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Cell adhesion molecules (CAMs)</a>	RT	<input type="checkbox"/>	11	1.8	7.7E-2	4.1E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Regulation of actin cytoskeleton</a>	RT	<input type="checkbox"/>	13	2.1	7.8E-2	4.0E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Hippo signaling pathway</a>	RT	<input type="checkbox"/>	10	1.6	8.6E-2	4.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Chemokine signaling pathway</a>	RT	<input type="checkbox"/>	11	1.8	9.0E-2	4.3E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">p53 signaling pathway</a>	RT	<input type="checkbox"/>	6	1.0	9.6E-2	4.4E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Renin-angiotensin system</a>	RT	<input type="checkbox"/>	4	0.6	9.9E-2	4.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Prion diseases</a>	RT	<input type="checkbox"/>	4	0.6	9.9E-2	4.5E-1

**Figure S6. Functional annotation of genes regulated after 24hrs of Dex in neonatal cardiomyocytes using DAVID bioinformatics resource.**

Genes differentially regulated only at 24hr time point after Dex treatment compared to control cardiomyocytes were uploaded onto DAVID Bioinformatics Resources for functional annotation. The screenshot shows the 42 pathways associated with the genes as identified by KEGG pathway.

## S7 Screenshot of Functional annotation of genes with GR binding and no sig diff regulation



\*\*\* Welcome to DAVID 6.8 \*\*\*  
\*\*\* If you are looking for DAVID 6.7, please visit our [development site](#). \*\*\*

### Functional Annotation Chart

[Help and Manual](#)

Current Gene List: **Gr binding Incremental**  
Current Background: **Rattus norvegicus**  
4818 DAVID IDs

Options

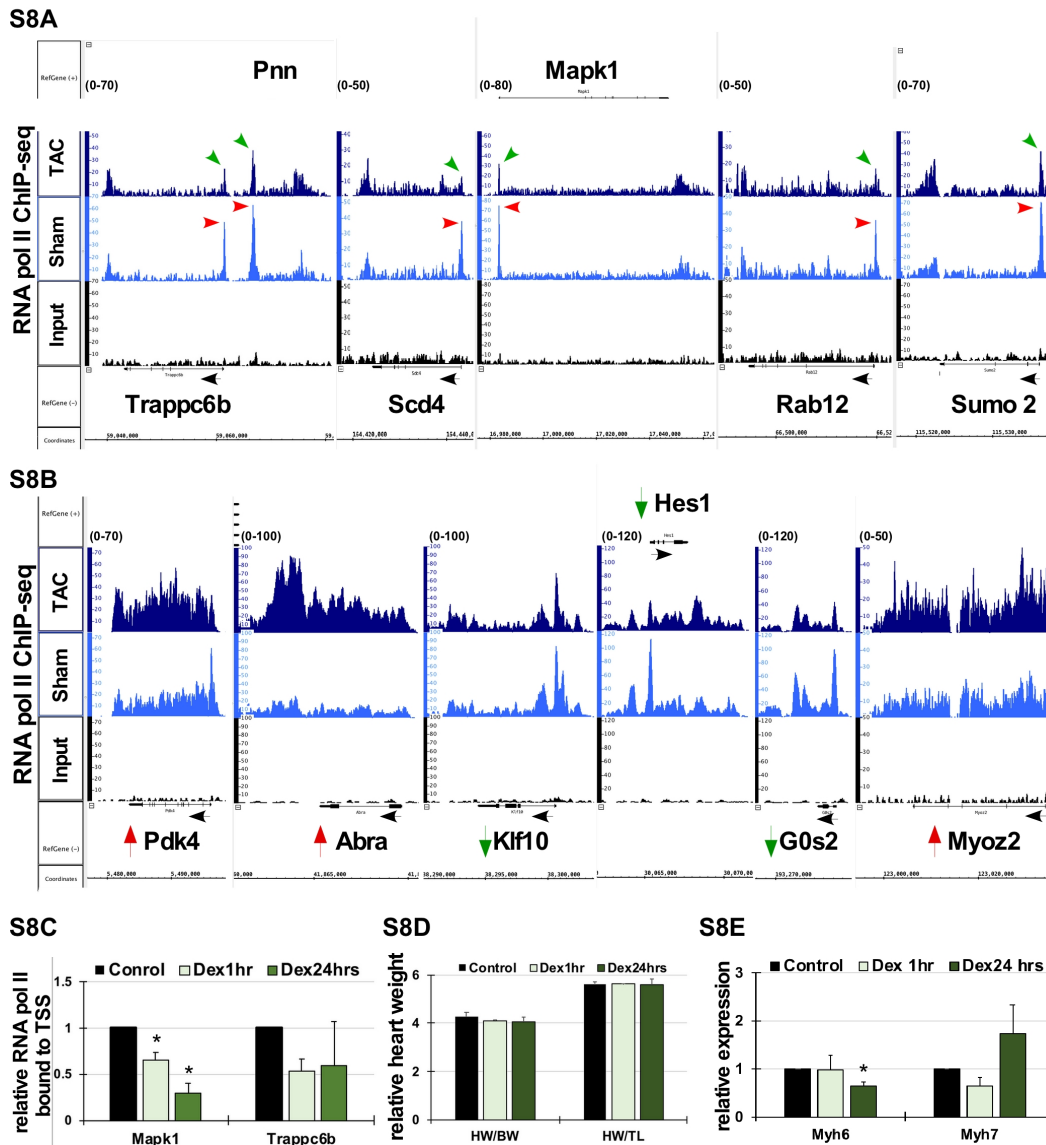
116 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Protein processing in endoplasmic reticulum</a>	RT		86	1.8	9.1E-13	2.6E-10
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Spliceosome</a>	RT		73	1.5	3.1E-12	4.4E-10
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">MAPK signaling pathway</a>	RT		114	2.4	4.5E-11	4.3E-9
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">MicroRNAs in cancer</a>	RT		72	1.5	2.1E-10	1.5E-8
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">RNA transport</a>	RT		79	1.6	3.9E-10	2.2E-8
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Thyroid hormone signaling pathway</a>	RT		60	1.2	1.4E-9	6.9E-8
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Proteoglycans in cancer</a>	RT		84	1.7	3.4E-7	1.4E-5
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Proteasome</a>	RT		29	0.6	6.0E-7	2.1E-5
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">cGMP-PKG signaling pathway</a>	RT		73	1.5	6.4E-7	2.1E-5
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Pathways in cancer</a>	RT		144	3.0	7.8E-7	2.2E-5
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Non-alcoholic fatty liver disease (NAFLD)</a>	RT		69	1.4	1.9E-6	5.0E-5
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">FoxO signaling pathway</a>	RT		60	1.2	2.6E-6	6.2E-5
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Ubiquitin mediated proteolysis</a>	RT		61	1.3	3.5E-6	7.6E-5
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Neurotrophin signaling pathway</a>	RT		56	1.2	4.5E-6	9.2E-5
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">AMPK signaling pathway</a>	RT		56	1.2	6.0E-6	1.1E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Cell cycle</a>	RT		56	1.2	6.0E-6	1.1E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Alzheimer's disease</a>	RT		74	1.5	6.1E-6	1.1E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Phosphatidylinositol signaling system</a>	RT		45	0.9	8.8E-6	1.5E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Glucagon signaling pathway</a>	RT		46	1.0	1.3E-5	2.0E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Parkinson's disease</a>	RT		63	1.3	1.6E-5	2.4E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Adrenergic signaling in cardiomyocytes</a>	RT		61	1.3	1.7E-5	2.4E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Metabolic pathways</a>	RT		387	8.0	2.4E-5	3.3E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Oxytocin signaling pathway</a>	RT		65	1.3	2.5E-5	3.2E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Huntington's disease</a>	RT		78	1.6	3.6E-5	4.5E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Citrate cycle (TCA cycle)</a>	RT		20	0.4	4.3E-5	5.1E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Epstein-Barr virus infection</a>	RT		85	1.8	4.6E-5	5.3E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Wnt signaling pathway</a>	RT		58	1.2	4.8E-5	5.3E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Endocytosis</a>	RT		103	2.1	5.1E-5	5.4E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Dopaminergic synapse</a>	RT		54	1.1	5.2E-5	5.3E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Prostate cancer</a>	RT		40	0.8	7.1E-5	7.1E-4
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Regulation of actin cytoskeleton</a>	RT		81	1.7	9.7E-5	9.3E-4
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Estrogen signaling pathway</a>	RT		42	0.9	1.3E-4	1.2E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Renal cell carcinoma</a>	RT		32	0.7	1.5E-4	1.3E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Insulin signaling pathway</a>	RT		56	1.2	1.6E-4	1.4E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">PI3K-Akt signaling pathway</a>	RT		116	2.4	1.7E-4	1.4E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Acute myeloid leukemia</a>	RT		28	0.6	1.7E-4	1.4E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Biosynthesis of antibiotics</a>	RT		80	1.7	2.0E-4	1.6E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Ribosome</a>	RT		65	1.3	3.0E-4	2.3E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Insulin resistance</a>	RT		45	0.9	4.4E-4	3.3E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Colorectal cancer</a>	RT		29	0.6	9.3E-4	6.8E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">GnRH signaling pathway</a>	RT		38	0.8	1.1E-3	7.8E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Hippo signaling pathway</a>	RT		57	1.2	1.1E-3	7.7E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Focal adhesion</a>	RT		74	1.5	1.2E-3	8.0E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Long-term potentiation</a>	RT		29	0.6	1.2E-3	8.3E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Glioma</a>	RT		29	0.6	1.2E-3	8.3E-3
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Oxidative phosphorylation</a>	RT		54	1.1	1.3E-3	8.4E-3
<input type="checkbox"/>	KEGG_PATHWAY	<a href="#">Chronic myeloid leukemia</a>	RT		32	0.7	1.6E-3	1.0E-2
<input checked="" type="checkbox"/>	KEGG_PATHWAY	<a href="#">Circadian entrainment</a>	RT		39	0.8	1.7E-3	1.0E-2

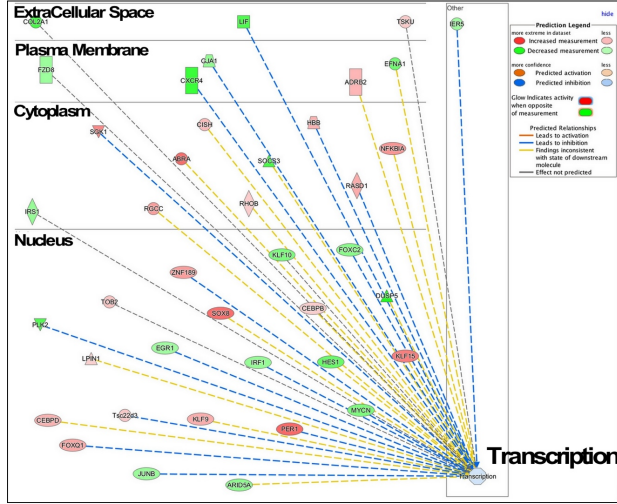
**Figure S7. Functional annotation of genes with associated GR binding, but no significant change in transcript abundance.**

Genes that were associated with GR binding but did not show significant differential change in transcript abundance on RNAseq were loaded onto DAVID Bioinformatics Resources for functional annotation. Screenshot shows top 48 pathways associated with genes as identified KEGG pathway.

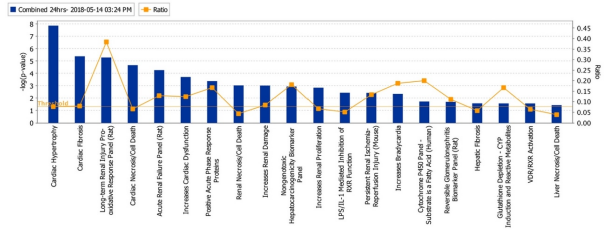


**Figure S8.** A. Screenshot of representative genes from RNA pol II ChIP-Seq data in mice subjected to sham or TAC operations, showing promoter paused RNA pol II peaks (red arrows) in sham hearts versus promoter clearance of these peaks (green arrow) in TAC hearts. B. RNA pol II occupancy and dynamics on genes after sham or TAC operations in mice that show significant change in transcript abundance in neonatal cardiomyocytes after Dex. These data have been described in detail in previous publication (27), and has been uploaded to GEO series GSE50637. C. RNA pol II-ChIP-qPCR was performed encompassing transcription start site (TSS) for Trappc6b and Mapk1. The graph shows relative fold enrichment of bound RNA pol II/IgG at TSS with Dex treatments for 1hr or 24hrs vs. control. Error bars represents SEM and \* is  $p < 0.05$ . D. Graph represents heart weight (HW) to body weight (BW) or tibia length (TL) from mice injected with Dexamethasone for 1hr or 24hrs. E. Graph represents relative myh6 (alpha myosin heavy chain) and myh7 (beta myosin heavy chain) transcript abundance in mice hearts injected with Dexamethasone for 1hr or 24hrs, as indicated. Error bars for C and D indicate SEM, and \* is  $p < 0.05$ .  $n = 3-4$ .

**S9A Genes regulated after 1hr of Dex vs. control**

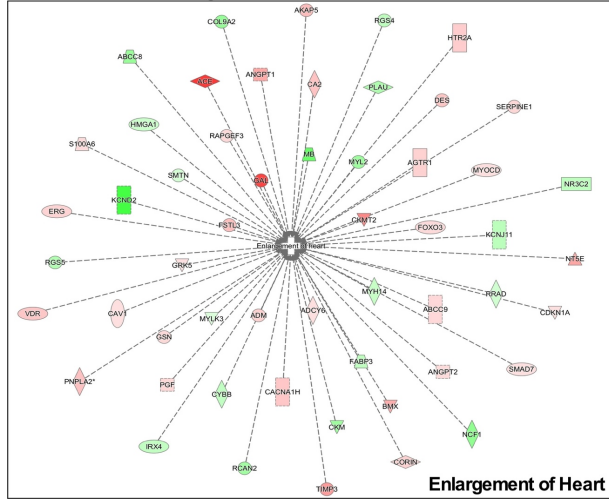


**S9B**



Top Tox Lists from IPA	
Name	P Value
Cardiac Hypertrophy	7.41E-13
Cardiac Failure	4.34E-10
Cardiac Necrosis/Cell Death	6.65E-08
Renal Necrosis/Cell Death	4.00E-06
Acute Renal Failure Panel (rat)	4.52E-06

**S9C Genes regulated after 24hrs of Dex vs. control**



**S9D**

Top Tox Cardiotoxicity Related Functions		
Name	P-value	# Molecules
Cardiac Enlargement	7.67E-02 - 2.05E-20	54
Heart Failure	4.72E-01 - 2.08E-17	44
Cardiac Congestive Cardiac Failure	2.62E-02 - 4.13E-13	25
Cardiac Dysfunction	2.92E-01 - 8.21E-12	28
Cardiac Infarction	1.25E-01 - 5.85E-11	30

Top Diseases and Disorders		
Name	P-value	# Molecules
Metabolic Disease	3.49E-07 - 6.11E-24	134
Cardiovascular Disease	5.83E-07 - 2.05E-20	218
Organismal Injury and Abnormalities	6.05E-07 - 2.05E-20	545
Endocrine System Disorders	5.93E-07 - 3.58E-20	188
Gastrointestinal Idisease	3.49E-07 - 3.58E-20	505

Top Physiological System Development and Function		
Name	P-value	# Molecules
Cardiovascular System Development and Function	3.63E-07 - 2.11E-30	201
Organismal Development	5.52E-07 - 7.86E-28	301
Organ Morphology	4.08E-07 - 6.32E-24	133
Skeletal and Muscular System Development and Function	3.50E-07 - 2.83E-20	136
Organismal Survival	7.20E-12 - 4.19E-19	198

**Figure S9. Pathway analysis confirms Dex induction of cardiac hypertrophy –related genes. A.** Genes involved in transcription network and differentially regulated after 1hr of Dex treatment vs. control cardiomyocytes are presented, as generated by IPA software, and with respect to their subcellular localization. **B.** Screenshot of IPA-Tox list from IPA software showing the genes that may be involved in toxicity function from genes regulated at 24hrs Dex vs. control. Top 20 of the list are shown in graph, where X axis represents functional list, while y axis is  $-\log(p\text{-value})$ . Higher the  $-\log(p\text{-value})$ , more significant the association. Threshold p-value is 0.05 and ratio shown is extent of overlap of data with the Tox list. Top 5 from the graph are shown in table below, with p value. **C.** Genes differentially regulated at 24hrs of Dex treatment vs. control and identified by IPA as involved in enlargement of heart is presented as a network generated by IPA software. Enlargement of heart (cardiac enlargement) was identified as the first category in cardiac hypertrophy list. **D.** Tables showing the top Tox cardiotoxicity related function, Top disease and disorders, Top physiological system development and function. The tables include the names, p-value and number of genes (#molecules) associated. Genes differentially regulated only at 24hr time point with Dex treatment vs. control were used for these analyses.