

**Supplemental Information**

**Cistronic Reprogramming of the Diurnal**

**Glucocorticoid Hormone Response by High-Fat Diet**

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## **Supplemental Information**

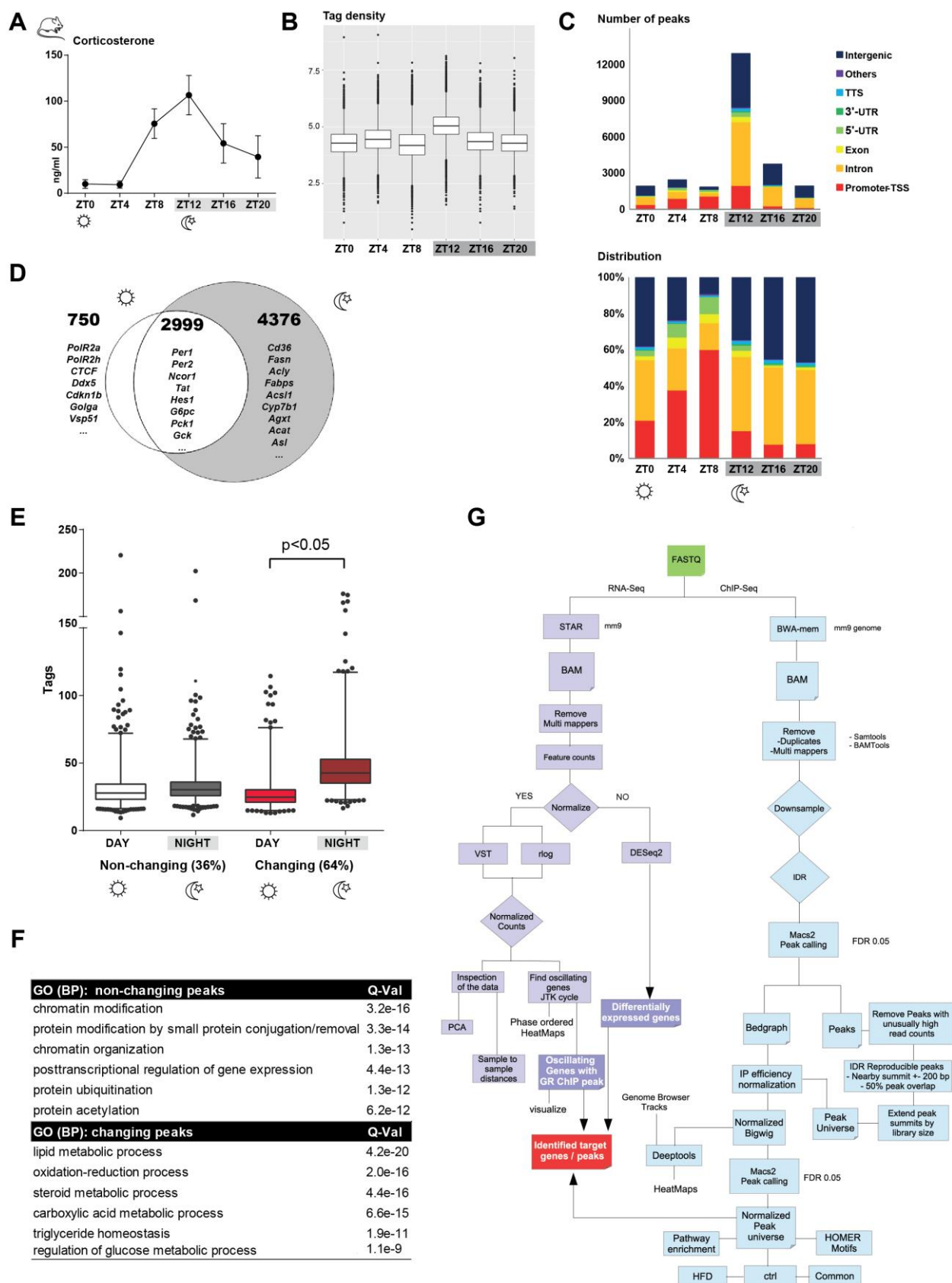
**Cistronic reprogramming of the diurnal glucocorticoid hormone response by high fat diet**

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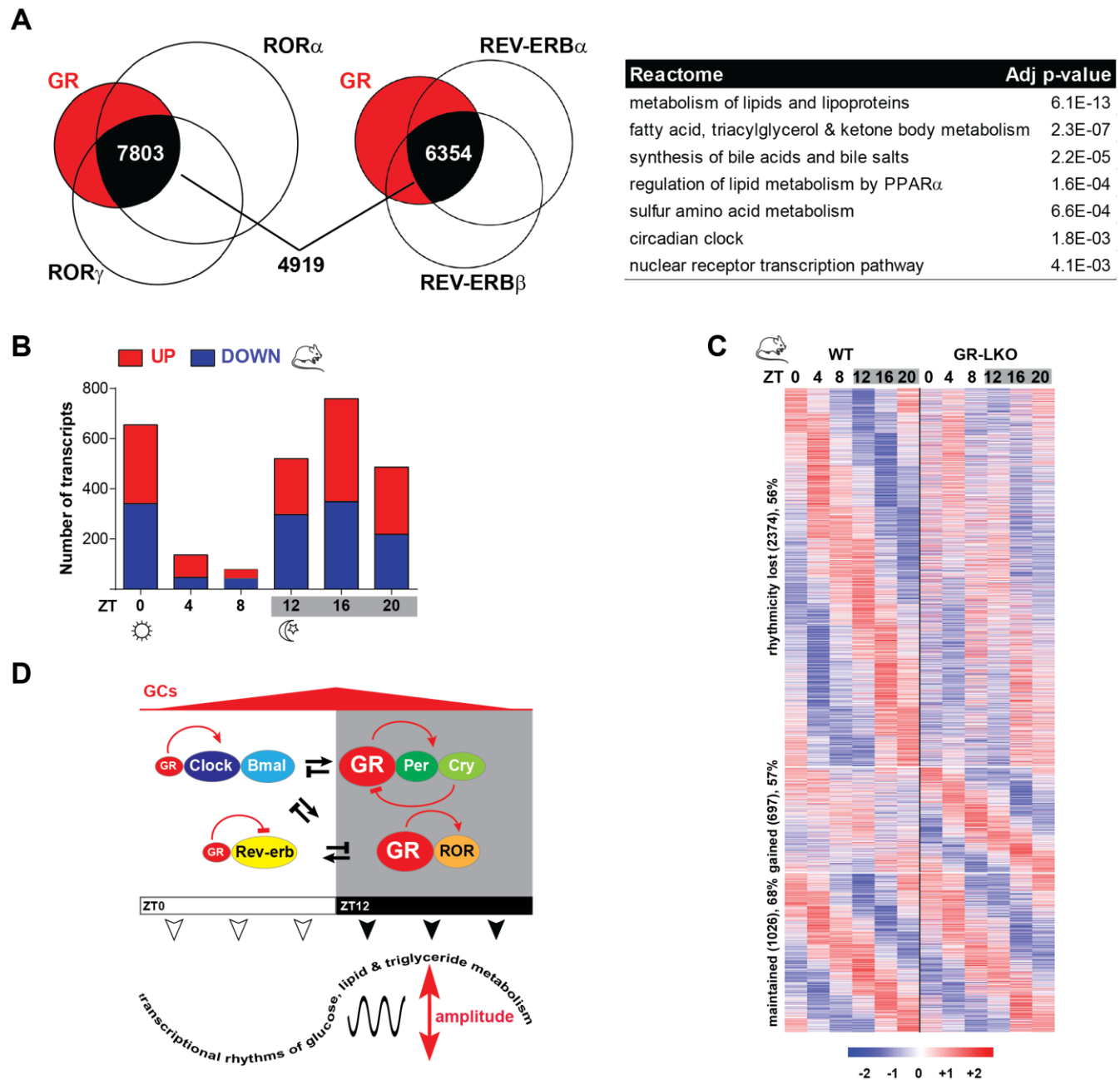
Figure S1, related to Figure 1.



**Supplementary Figure 1, related to Figure 1. Differential genomic binding of GR during the day and night cycle in mouse livers**

(A) Serum corticosterone levels, measured from euthanized mice every 4h (ZT0-20) by ELISA. Values are represented as mean $\pm$ SD; n=3. (B) Box plot of log2-transformed read densities of GR ChIP-Seq in mouse livers from ZT0-20. (C) The total number of reproducible peaks and the genomic peak distribution for ZT0-20. (D) Venn diagram showing the number and examples of genes associated with GR binding during the Day (ZT0,4,8) and the Night (ZT12,16,20). (E) The tag density of the 2,999 'common' peaks in panel S1D was averaged separately for the Day (ZT0,4,8, n=6), and for the Night (ZT12,16,20, n=6), and statistically sub-grouped into non-changing ( $p>0,05$ ; 36%) and changing ( $p<0,05$ ; 64%) (significance by multiple t-test). (F) Enriched GO terms for peaks near genes with either constant or differential binding, see S1E. (G) Overview of the bioinformatic analysis pipelines used for ChIP- and RNA-Seq data.

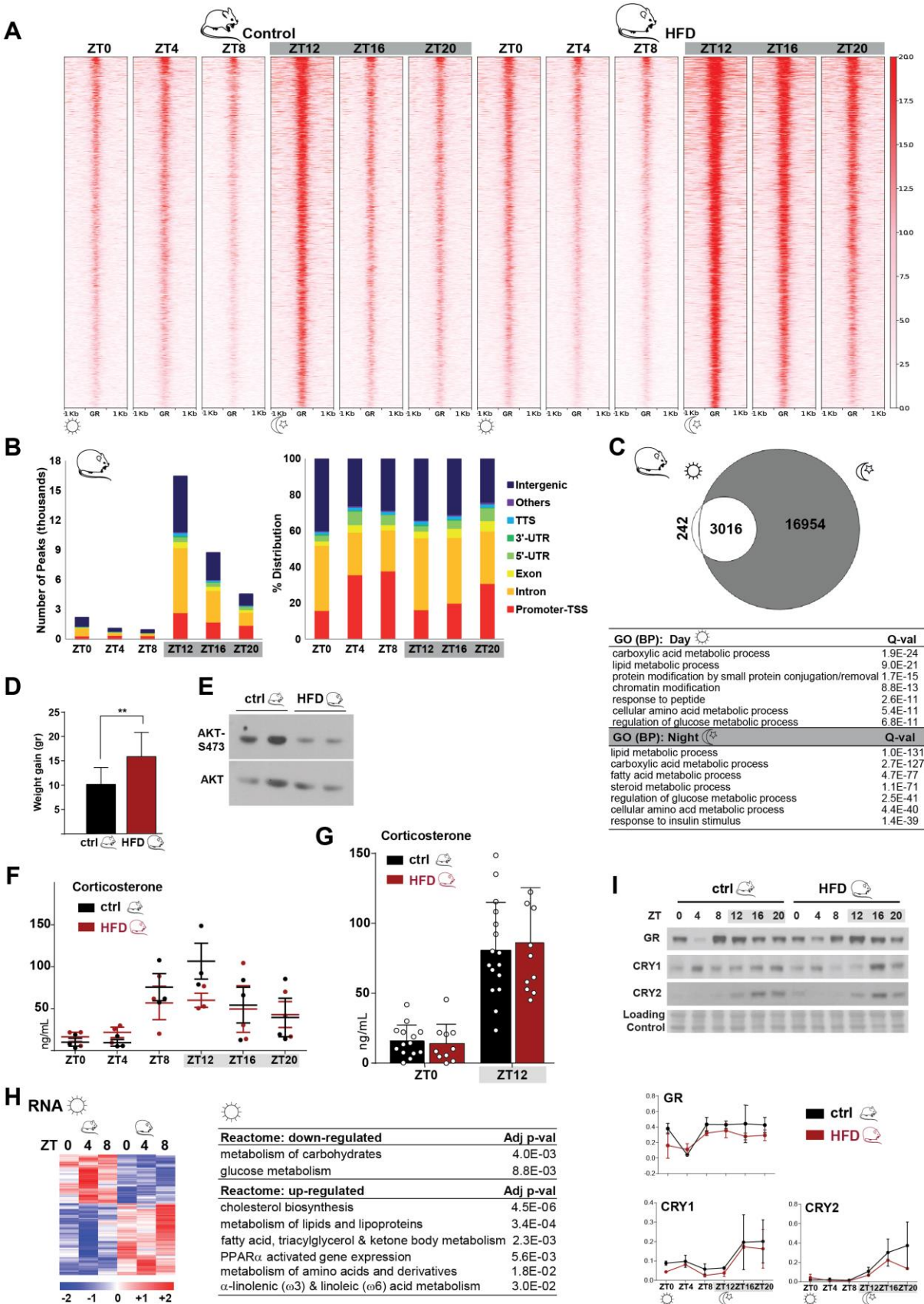
Figure S2, related to Figure 2.



**Supplementary Figure 2, related to Figure 2. GR binding overlaps with core clock factors to generate transcriptional rhythms**

(A) Overlap of GR with ROR $\alpha/\gamma$  and REV-ERB $\alpha/\beta$  cistromes. GR universe (sum of all GR ChIP-Seq peaks from all time points) intersection with ROR $\alpha/\gamma$  universes (ZT10 and ZT22; Zhang Y et al., Fang B et al.) and REV-ERB $\alpha/\beta$  universes (ZT8; Cho H et al). 4,919 sites are bound altogether by GR-RORs-REV-ERBs. Functional annotation (Enrichr) of genes near overlapping sites. (B) Number of deregulated transcripts in GR-LKO (*Alb-Cre* x *GR<sup>fl/fl</sup>*) livers at ZT0, 4, 8, 12, 16 and 20. Differential gene expression between GR-LKO and floxed littermates was calculated per time point using DESeq2 (n=3, adj p<0.05). (C) Phase sorted heatmap of all oscillating transcripts in livers from control and GR-LKO mice, for ZT0-20. Rhythmicity was determined using JTK Cycle (period 24, adj p<0.05), n=3. (D) Schematic depicting the interactions of GR with both the core and the ancillary clock machinery to generate rhythmic output.

Figure S3, related to Figure 3.



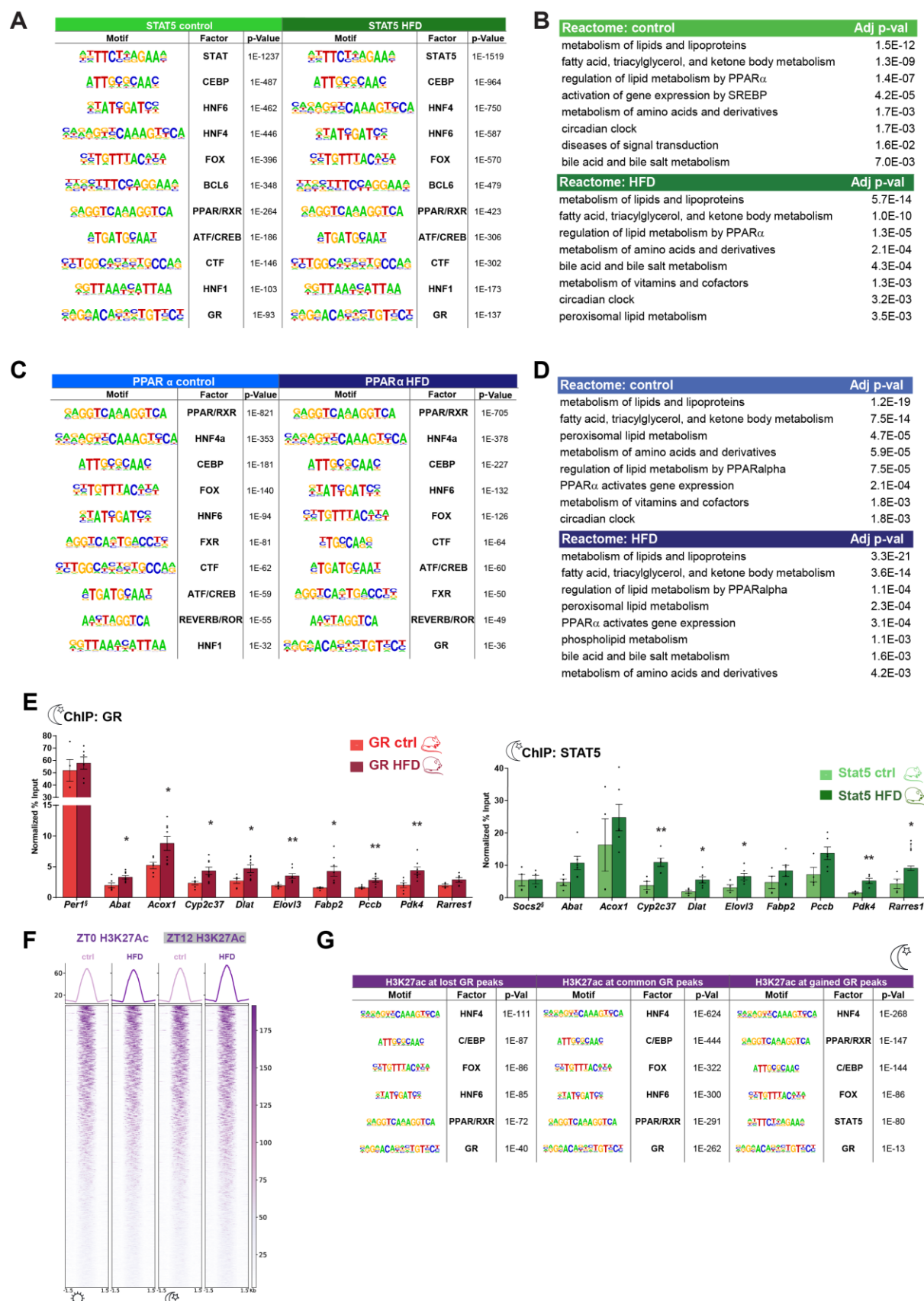
### **Supplementary Figure 3, related to Figure 3. GR cistromes are reprogrammed by HFD**

(A) Heatmap of GR genome-wide binding in control and HFD livers for ZT0-20, analogous to Figures 1B and 3A. (B) The total number of reproducible GR peaks and their genomic distribution for ZT0-20 on HFD. (C) Venn diagram showing called GR peaks during the Day (ZT0,4,8) and Night (ZT12,16,20). GO annotation (GREAT) of the genes nearest to the 3,258 'Day' binding sites and the 16,954 'Night-only' binding sites. (D) Weight gain from C57BL/J mice after 12 weeks of HFD feeding (58% fat) compared to controls fed LFD (11% fat). Values are represented as mean $\pm$ SD. (E) Western Blots of AKT in total liver extracts from control and HFD mice at ZT12. (F) Serum corticosterone levels of control and HFD mice measured by ELISA. Blood was taken every 4h. Values are mean $\pm$ SD, n=3. (G) Serum corticosterone levels from HFD and control mice either at ZT0 (GC trough) or at ZT12 (GC peak). Values are mean $\pm$ SD, n=10-15. (H) Heatmap of transcripts deregulated by HFD during the day (ZT0,4,8) and associated with 'gained' GR binding from Figure 3E (9,354 peaks). Differential gene expression in livers from HFD fed and control wildtype mice was calculated by using DESeq2 (n=3, adj p<0.05) as described. Pathway annotation was performed for transcripts either up- or down-regulated in HFD livers. (I) Temporal protein accumulation of GR, CRY1, and CRY2 in HFD and control livers. Western blots on nuclear extracts; naphtol blue black staining was used as loading control. Corresponding mean densitometry values, normalized to the loading control. Data for each time point are mean  $\pm$  SD, n=2.

**\*\*** $P$ <0.01 (two tailed  $t$  test).



Figure S4, related to Figure 4.



**Supplementary Figure 4, related to Figure 4. GR, PPAR $\alpha$  and STAT5 signaling pathways intersect at functional enhancers**

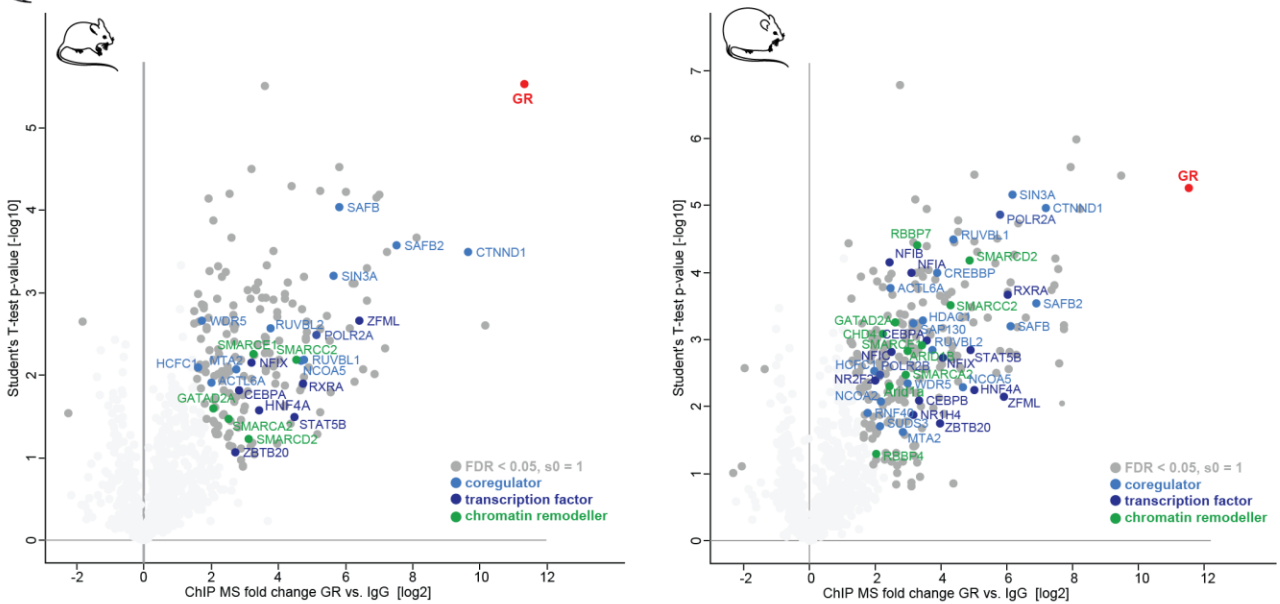
(A) Motif analyses of the control and HFD liver cistromes for STAT5 (A) and PPAR $\alpha$  (C) at ZT12. Functional annotation of the genes near STAT5 (B) and PPAR $\alpha$  (D) binding sites in HFD and control livers. (E) GR and STAT5 ChIP-qPCR data from control and HFD livers. § The *Per1* and *Socs2* loci are non-changing sites, the other loci showed increased occupancy for both factors with ChIP-seq. Enrichment was calculated over a negative locus. Values are mean $\pm$ SEM (n=4-8). (F) Total H3K27ac profiles from control and HFD livers at ZT0 and ZT12. Each row shows the normalized unique ChIP-Seq tag counts for H3K27ac ordered by signal strength (n=2 mice per group). (G) Motif analysis for H3K27ac peaks overlapping with 'lost' (3,031 out of 3,076), 'common' (10,520 out of 10,616), or 'gained' (9,291 out of 9,354) GR peaks as described in Figure 3E.

\* $P < 0.05$ , \*\* $P < 0.01$  (two tailed  $t$  test).

Figure S5, related to Figure 5.

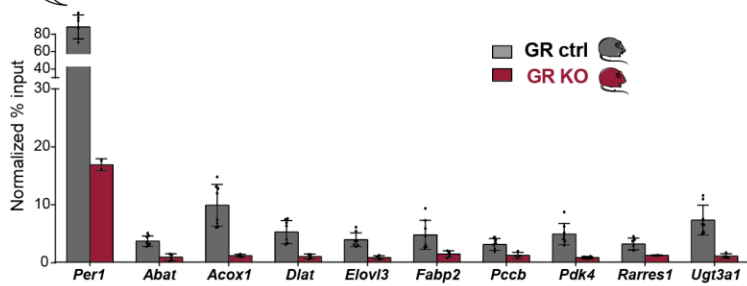
A

ChIP: GR



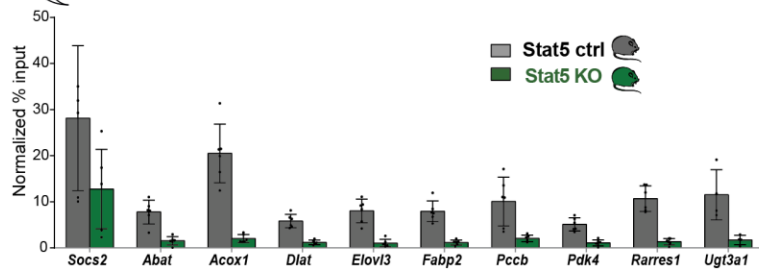
B

ChIP: GR in GR-LKO



C

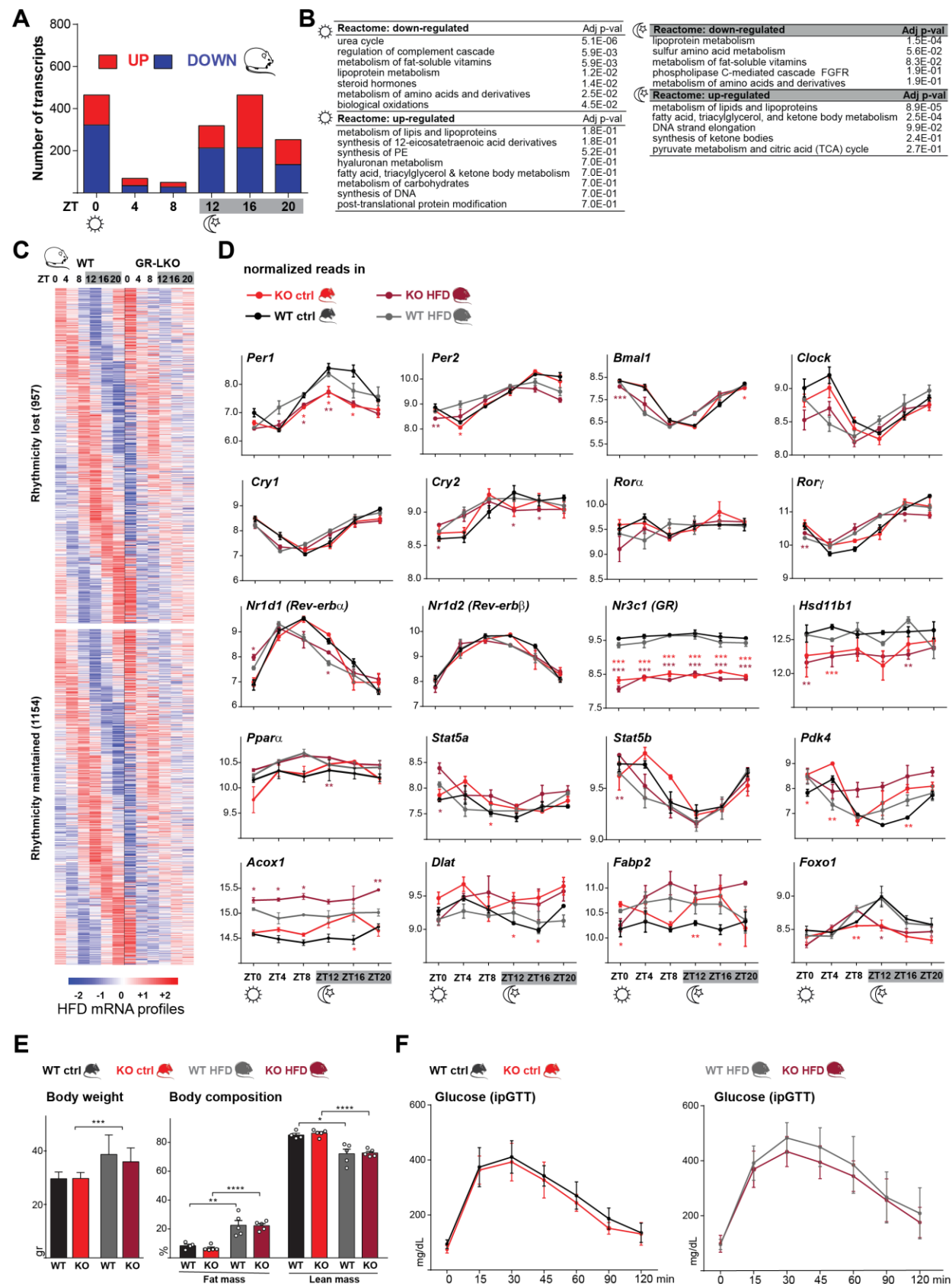
ChIP: STAT5 in Stat5-LKO



**Supplementary Figure 5, related to Figure 5. Cistronic reprogramming by HFD depends on STAT5 occupancy**

(A) Volcano plot of significantly enriched proteins in GR IP samples versus IgG, in control and HFD livers. Proteins of special interest are colored, dark grey: Fisher's exact test ( $FDR < 0.05$ ,  $s_0 = 1$ );  $n = 3$ . (B) GR ChIP-qPCR analysis in GR mutant ( $Alb-Cre \times GR^{f/f}$ ) and control livers ( $GR^{f/f}$ ) after 12 weeks of HFD. Enrichment was calculated over a negative locus. Values are mean  $\pm$  SEM ( $n = 2-8$  per group; all loci are significantly different between the two groups, multiple t-test). (C) STAT5(a/b) ChIP-qPCR analysis in Stat5 knockout ( $Alb-Cre \times Stat5^{f/f}$ ) and control ( $Stat5^{f/f}$ ) livers after 12 weeks of HFD. Enrichment was calculated over a negative locus. Values are mean  $\pm$  SEM ( $n = 6$  per group; all loci are significantly different between the two groups).

Figure S6, related to Figure 6.



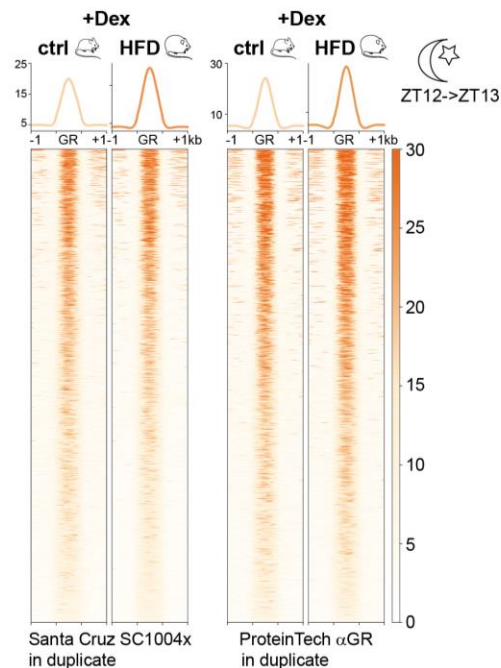
**Supplementary Figure 6, related to Figure 6. GR liver specific deletion leads to deregulation of glucose and triglyceride metabolism**

(A) Number of deregulated transcripts in GR-LKO (*Alb-Cre* x *GR<sup>fl/fl</sup>*) livers at ZT0, 4, 8, 12, 16 and 20 after 12 weeks of HFD. Differential gene expression between GR-LKO and floxed littermates was calculated per time point using DESeq2 (n=3, adj p<0.05). (B) Pathway annotation for transcripts either up- or down-regulated in GR-LKO during the 'Day' (ZT0, 4, 8) and during the 'Night' (ZT12, 16, 20) as defined in Figure 6A. (C) Phase sorted heatmap of all oscillating transcripts around the clock in livers from control and GR-LKO mice on HFD. Rhythmicity was determined using JTK Cycle (period 24, adj p<0.05), n=3. (D) Rlog transformed counts from normalized RNA-Seq data of selected transcripts in livers from GR-LKO and floxed littermates, fed either high fat or control diet. Values are mean±SEM (n=3). (E) Body weight (n=20 per group) and body composition analysis of 20-22 week old GR-LKO male mice and littermate controls (*GR<sup>fl/fl</sup>*), values are mean ± SD (n=20). (F) i.p. glucose tolerance tests (GTT) in 18-20 week old male mice, after 10 weeks of HFD or control diet. Values are mean±SD (n=4-15).

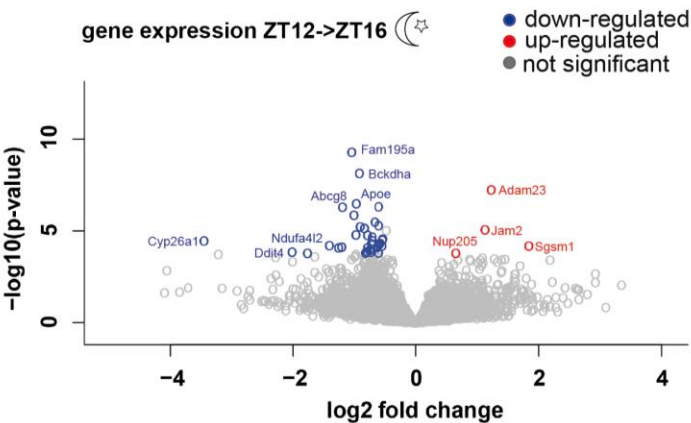
\**P*<0.05, \*\**P*<0.01, \*\*\* *P*<0.001, \*\*\*\* *P*<0.0001 (two tailed *t* test).

Figure S7, related to Figure 7.

A



B



**Supplementary Figure 7, related to Figure 7. Ligand independent genomic responses on HFD**

(A) Heatmaps showing GR genomic binding in Dex treated livers on control and HFD. Dex was injected at ZT12 and mice sacrificed at ZT13. Two different GR antibodies (Santa Cruz, SC-1004x and Protein Tech, 24050-1-AP) were used. (B) Volcano plot showing transcripts differentially responding to Dex treatment in HFD versus control diet (n=2-3, adj p<0.05). Mice were injected with Dex at ZT12 and livers were processed at ZT16. Genes significantly associated with diet-dependent differential responses are shown in blue (down-regulated) and red (up-regulated).



**Table S1, related to Figure 2.**

Gene Name	Peak			Time Point	Position	+DEX (Log2FC)
<b><i>Clock</i></b>	chr5	76732589	76734428	ZT0;4;8;12	Promoter	0,78
	chr5	76734709	76735615	ZT12	Intergenic	
<b><i>Arntl</i></b>	chr7	120350700	120351665	ZT0;4;8;12;16	Intron 1	nc
	chr7	120403794	120404700	ZT12;16	Intron 2	
<b><i>Cry1</i></b>	chr10	84647390	84648345	ZT4	Promoter	nc
	chr10	84652487	84653585	ZT0;12	Intergenic	
<b><i>Cry2</i></b>	chr2	92261760	92262673	ZT12	Intron 2	1,2
	chr2	92272274	92273180	ZT12	TTS	
<b><i>Per1</i></b>	chr11	68910121	68911027	ZT12	Promoter	1,97
	chr11	68912491	68913397	ZT0;4;8;12;16;20	Intergenic	
<b><i>Per2</i></b>	chr1	93332654	93333565	ZT12	Intron 1	-1,58
	chr1	93336185	93337113	ZT0;12	Intron 4	
	chr1	93342379	93343285	ZT12	Intron 7	
	chr1	93352188	93353154	ZT0;4;12;16;20	intron 9	
	chr1	93359681	93360587	ZT12	Intergenic	
<b><i>Rora</i></b>	chr9	68957620	68958540	ZT8;12	Promoter	1,41
	chr9	69031405	69032335	ZT12;16	Intron 1	
	chr9	69132473	69133470	ZT0;12;16	Intron 1	
	chr9	69135683	69136589	ZT12;16	Intron 1	
	chr9	69137114	69138020	ZT12	Intron 1	
	chr9	69154162	69155177	ZT12	Intron 1	
	chr9	69181649	69182582	ZT12;20	Intron 1	
	chr9	69186770	69187676	ZT12	Intron 1	
	chr9	69160803	69161831	ZT12	Intron 1	
	chr9	69179267	69180274	ZT12	Intron 2	
	chr9	69180284	69181190	ZT12	Intron 2	
<b><i>Rorc</i></b>	chr3	94171203	94172228	ZT12;16	Intron 1	nc
	chr3	94177426	94178332	ZT12	Intergenic	
<b><i>Nr1d1</i></b>	chr11	98644175	98645081	ZT12	Intergenic	-1,97
<b><i>Nr1d2</i></b>	chr14	19071427	19072196	ZT8	Promoter	nc

**Table S2, related to STAR Methods.** Primer list for ChIP-qPCR.

<b>Name</b>	<b>Strand</b>	<b>Sequence</b>
<i>Per1</i>	Forward	GTAGGTCCCGCAAAGAGAACC
	Reverse	GACAGCGGTCCTGTACAAAAG
<i>Negative ctrl</i>	Forward	GCTGGCAGAATAGCATCCG
	Reverse	TGATGAAGCACTCGTTGAGGC
<i>Socs2</i>	Forward	CTCGGGAACAAGGAGAAGTC
	Reverse	GAGAAGAGGGGCTGCTATTC
<i>Rarres1</i>	Forward	GTGGGTTCTAAGACCTGAGGG
	Reverse	CACACTTTCCCTGTTTCCAGC
<i>Abat</i>	Forward	TGGCACACAACCAATCCCAG
	Reverse	TGACACACTACCCATTCCAGC
<i>Dlat</i>	Forward	AACTATGGAGTAACAATGTGGG
	Reverse	TAGGCAGCAGTAGTGAGAAAC
<i>Elovl3</i>	Forward	CCAAACTCAGAGAGAAAGCAG
	Reverse	CAGCCAGTGTCTAGAAATCC
<i>Pdk4</i>	Forward	CATCAGGTCAAGGTTTGTACTC
	Reverse	ATGTGTGTGGACTCTAGTTTCC
<i>Acox1</i>	Forward	CTGTTGATTTTACTGGAACCC
	Reverse	TAGCCAACGACAATGAACC
<i>Cyp2c37</i>	Forward	GGGCAATGTGCATCACAACA
	Reverse	TGTTGAACTTTGTGATACGGGC
<i>Fabp2</i>	Forward	ATGTGAGGCGGTTAGGTTATC
	Reverse	TCCAGTCCTGTCCACTAGAGAG
<i>Pccb</i>	Forward	AAGCTGGTAGCAGTCACAGG
	Reverse	AAGTCCTTGTTCTGTGTGGC
<i>Ugt3a1</i>	Forward	TCTCTACTCTGAGTTTCCTGGTC
	Reverse	CCTGGACTCCTCCTTTCAGAG

**Table S3, related to STAR Methods.** Summary of the results from our ChIP-Seq bioinformatics pipeline.

Type	Code	Diet	Sample	Type of sequence	# of mapped reads	% of mapped reads	# of mapped reads w/out duplicates	% of duplicates	# of uniquely mapped reads (>24)	% of reads used	# macs2peaks (FDR-0.05)	Scaling factor
GR	ZT0	CTRL	Rep1	pair-end	49821035	96.77	36882891	25.97	33071118	66.38	5890	0.1856
GR	ZT0	CTRL	Rep2	pair-end	23545907	98.11	20088577	14.68	18008935	76.48	4971	0.1739
GR	ZT4	CTRL	Rep1	pair-end	27970302	97.91	23730832	15.16	21247124	75.96	3619	0.1681
GR	ZT4	CTRL	Rep2	pair-end	47762630	93.18	26104183	45.35	22607447	47.33	18101	0.1261
GR	ZT8	CTRL	Rep1	single-end	29989335	94.24	21327340	28.88	18261739	60.89	8360	0.1289
GR	ZT8	CTRL	Rep2	pair-end	29090717	98.23	22264367	23.47	19774145	67.97	2752	0.2454
GR	ZT12	CTRL	Rep1	single-end	29182848	95.68	20801305	28.72	17938845	61.47	23534	0.1464
GR	ZT12	CTRL	Rep2	pair-end	39561957	97.19	25370018	35.87	22355227	56.51	24403	0.1872
GR	ZT16	CTRL	Rep1	pair-end	28002841	97.36	22791872	18.61	20501937	73.21	9567	0.1815
GR	ZT16	CTRL	Rep2	pair-end	45136520	99.22	38494278	14.72	34886926	77.29	6512	0.1588
GR	ZT20	CTRL	Rep1	pair-end	38074861	98.74	32039673	15.85	29057486	76.32	7484	0.1328
GR	ZT20	CTRL	Rep2	pair-end	17373101	98.22	15423999	11.22	13896001	79.99	3133	0.1618
GR	ZT0	HFD	Rep1	pair-end	19392586	98.47	16113698	16.91	14503304	74.79	5862	0.1845
GR	ZT0	HFD	Rep2	pair-end	68835259	97.43	54958388	20.16	50463935	73.31	23878	0.0874
GR	ZT4	HFD	Rep1	pair-end	28712926	95.21	22306944	22.31	19946682	69.47	1998	0.1788
GR	ZT4	HFD	Rep2	pair-end	25059275	97.53	21487565	14.25	19355293	77.24	12197	0.1173
GR	ZT8	HFD	Rep1	pair-end	39387334	98.68	31754704	19.38	28638566	72.71	2556	0.2454
GR	ZT8	HFD	Rep2	pair-end	35021832	97.99	28614984	18.29	26078073	74.46	25666	0.0827
GR	ZT12	HFD	Rep1	pair-end	41336379	98.6	30518017	26.17	23356466	56.5	29045	0.1549
GR	ZT12	HFD	Rep2	pair-end	76748986	96.67	54282974	29.27	49718202	64.78	40934	0.1067
GR	ZT16	HFD	Rep1	pair-end	38262648	98.45	28189873	26.33	21704393	56.72	38972	0.1002
GR	ZT16	HFD	Rep2	pair-end	56862314	98.66	34383965	39.53	30136554	53	12811	0.1610
GR	ZT20	HFD	Rep1	pair-end	61272128	99.07	46963210	23.35	42225476	68.91	8956	0.1407
GR	ZT20	HFD	Rep2	pair-end	69727647	98.59	49583131	28.89	45489201	65.24	10140	0.1392
GR	.	mixed	input	pair-end	192581321	99.35	124787275	35.2	111042277	n/a	.	.
STAT5	ZT12	HFD	Rep1	pair-end	25110524	94.47	15219321	39.4	13349164	53.16	30559	0.0834
STAT5	ZT12	HFD	Rep2	pair-end	64374196	97.81	44882152	30.2	40388712	62.74	33465	0.0787
STAT5	ZT12	CTRL	Rep1	pair-end	32526033	96.27	20636095	36.5	18002967	55.35	11396	0.1452

STAT5	ZT12	CTRL	Rep2	pair-end	42774150	98.30	25855654	39.5	22747958	53.18	19765	0.1188
PPARA	ZT12	CTRL	Rep1	pair-end	48125505	98.67	28087183	41.6	11724029	24.36	12701	0.1030
PPARA	ZT12	CTRL	Rep2	single-end	.	.	16352720	.	13539989	.	6093	0.1030
PPARA	ZT12	HFD	Rep1	pair-end	43035390	98.49	25248145	41.3	11005152	25.57	17079	0.1036
PPARA	ZT12	HFD	Rep2	single-end	.	.	12388782	.	10192046	.	6545	0.0915
DEX	ZT0	CTRL	Rep1	pair-end	.	98.72	35021253	.	31254219	.	1605	0.2530
DEX	ZT0	CTRL	Rep2	pair-end	.	97.41	33974624	.	30296455	.	12568	0.1271
DEX	ZT0	HFD	Rep1	pair-end	.	98.35	34834975	.	31120695	.	7485	0.1721
DEX	ZT0	HFD	Rep2	pair-end	.	97.89	35232304	.	31235195	.	2031	0.1570
DEX (PT)	ZT12	CTRL	Rep1	pair-end	31535073	99.33	24005225	23.9	21327120	67.6	15954	0.2933
DEX (PT)	ZT12	CTRL	Rep2	pair-end	44756966	99.41	33313453	25.6	30076082	67.2	37083	0.1293
DEX (PT)	ZT12	HFD	Rep1	pair-end	31491633	99.35	25802125	18.1	23126212	73.4	21829	0.1718
DEX (PT)	ZT12	HFD	Rep2	pair-end	32608848	99.42	24545669	24.7	22269509	68.3	28192	0.1421
DEX	ZT12	CTRL	Rep1	pair-end	37362882	97.86	27401154	26.7	24273134	65	14404	0.1393
DEX	ZT12	CTRL	Rep2	pair-end	29785772	98.33	24066597	19.2	21682700	72.8	34023	0.0675
DEX	ZT12	HFD	Rep1	pair-end	31928484	94.16	25681532	19.6	22524924	70.5	21246	0.1176
DEX	ZT12	HFD	Rep2	pair-end	28495535	98.77	22581987	20.8	20291786	71.2	32357	0.1288
H3K27ac	ZT0	CTRL	Rep1	pair-end	86001548	99.09	41521207	51.7	38241993	44.5	46594	0.1738
H3K27ac	ZT0	CTRL	Rep2	pair-end	81914623	99.40	49961499	39	46244532	56.5	41501	0.1640
H3K27ac	ZT0	HFD	Rep1	pair-end	73353936	99.22	41732804	43.1	38653402	52.7	47273	0.1485
H3K27ac	ZT0	HFD	Rep2	pair-end	91379344	99.31	58779485	35.7	54636101	59.8	45622	0.1602
H3K27ac	ZT12	CTRL	Rep1	pair-end	83933293	99.25	56562839	32.6	52843661	63	43439	0.1682
H3K27ac	ZT12	CTRL	Rep2	pair-end	79206302	99.07	58423838	26.2	54837431	69.2	44402	0.1664
H3K27ac	ZT12	HFD	Rep1	pair-end	84552836	99.30	49329740	41.7	46049057	54.5	49453	0.1647
H3K27ac	ZT12	HFD	Rep2	pair-end	94871058	99.37	64956552	31.5	60929164	64.2	48979	0.1669

**Table S4, related to STAR Methods.** Static peak list used for IP efficiency normalization.

<b>GR, Stat5, PPAR<math>\alpha</math> Chip-seq data</b>				
<b>Chromosome</b>	<b>Start</b>	<b>Stop</b>	<b>Gene</b>	<b>Location</b>
chr1	74767896	74768942	<i>Cyp27a1</i>	intron-1
chr5	90878666	90879685	<i>Alb</i>	promoter
chr8	112527807	112528871	<i>Tat</i>	promoter
chr10	11000311	11001369	<i>Fbxo30</i>	promoter
chr10	41922139	41923197	<i>Foxo3</i>	intron-2
chr10	76553985	76555035	<i>Col18a1</i>	intron-2
chr15	31155792	31156844	<i>Dap</i>	intron-1
chr17	85092692	85093762	<i>Abcg8</i>	intron-5
chr18	64485085	64486104	<i>Onecut2</i>	promoter
chr18	64509730	64510838	<i>Onecut2</i>	intron-1
<b>H3K27ac Chip-seq data</b>				
<b>Chromosome</b>	<b>Start</b>	<b>Stop</b>	<b>Gene</b>	<b>Location</b>
chr12	105101829	105102829	<i>Serpina1b</i>	promoter
chr17	12570475	12571475	<i>Plg</i>	promoter
chr3	94690880	94691880	<i>Psmc4</i>	promoter
chr5	90888915	90889915	<i>Alb</i>	promoter
chr7	13620126	13621126	<i>Chmp2a</i>	promoter
chr8	112513336	112514336	<i>Tat</i>	promoter