

Supplemental Information

**Circadian Regulation of Cochlear Sensitivity
to Noise by Circulating Glucocorticoids**

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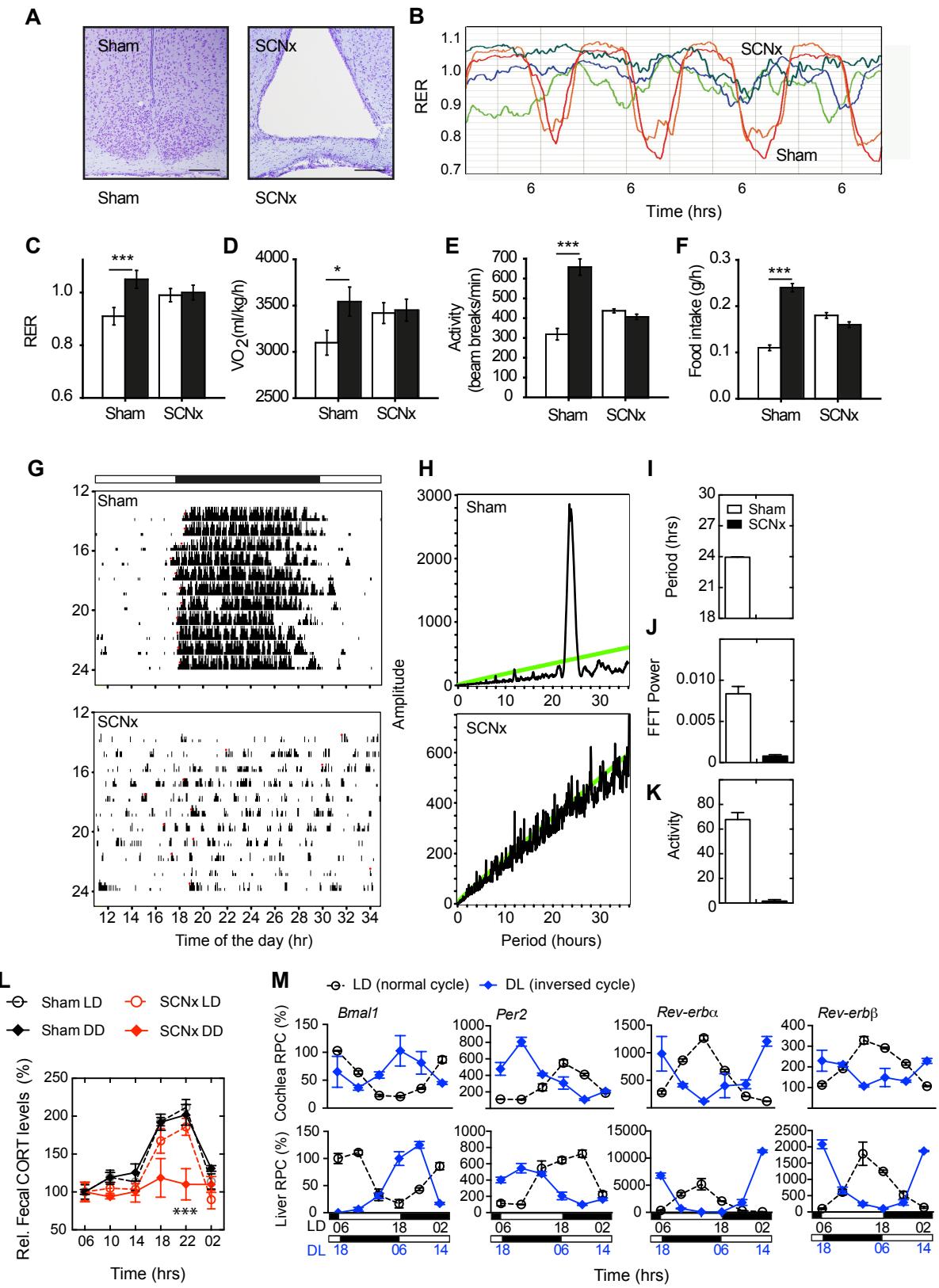


Figure S1. Validation of SCN ablations. Related to Figure 1. Histological examples of the SCN from sham animal and electrolytic lesioned SCN (SCNx) stained with cresyl violet. (B) Representative examples of Respiratory Exchange Rate (RER) determined by CLAMS over 4 days for sham (red) and SCNx (blue/green). Quantified data of (C) RER, (D) VO₂, (E) ambulatory movement counts, and (F) food intake during 12 h period according to active phase (6 p.m. – 6 a.m., black) and inactive phase (6 a.m. – 6 p.m., white) comparing sham animals with SCNx animals. (G) Representative actograms and (H) periodograms showing normal behavior in light/dark (LD) cycles in sham animals (above), and complete disruption of activity in SCNx mice (below). Quantification of (I) the period, (J) FFT power and (K) overall activity showing normal patterns in sham mice (white bar), and disrupted activity in SCNx mice (black bar). (L) Fecal CORT values obtained every 4th hour around the clock after being acclimatized for 3 days to the metabolic cages. (M) Nanostring nCounter analysis of cochlear and liver mRNA samples collected every 4th hour around the clock in normal light/dark cycles (LD, open circles) and inverted dark/light cycles (DL, blue diamonds). Values are expressed in relative percentage change (RPC). The horizontal axis shows the sampling time of the day, dark horizontal bars represent the active phase and the white horizontal bar the inactive phase. Data represent mean \pm SEM (A-F, n = 16 - 28 mice per group; G-K, n = 3 – 5; L, M, n = 3); * p < 0.05, ** p < 0.01, *** p < 0.001, two-way ANOVA with Bonferroni post-hoc analysis. Scale bar = 200 μ m.

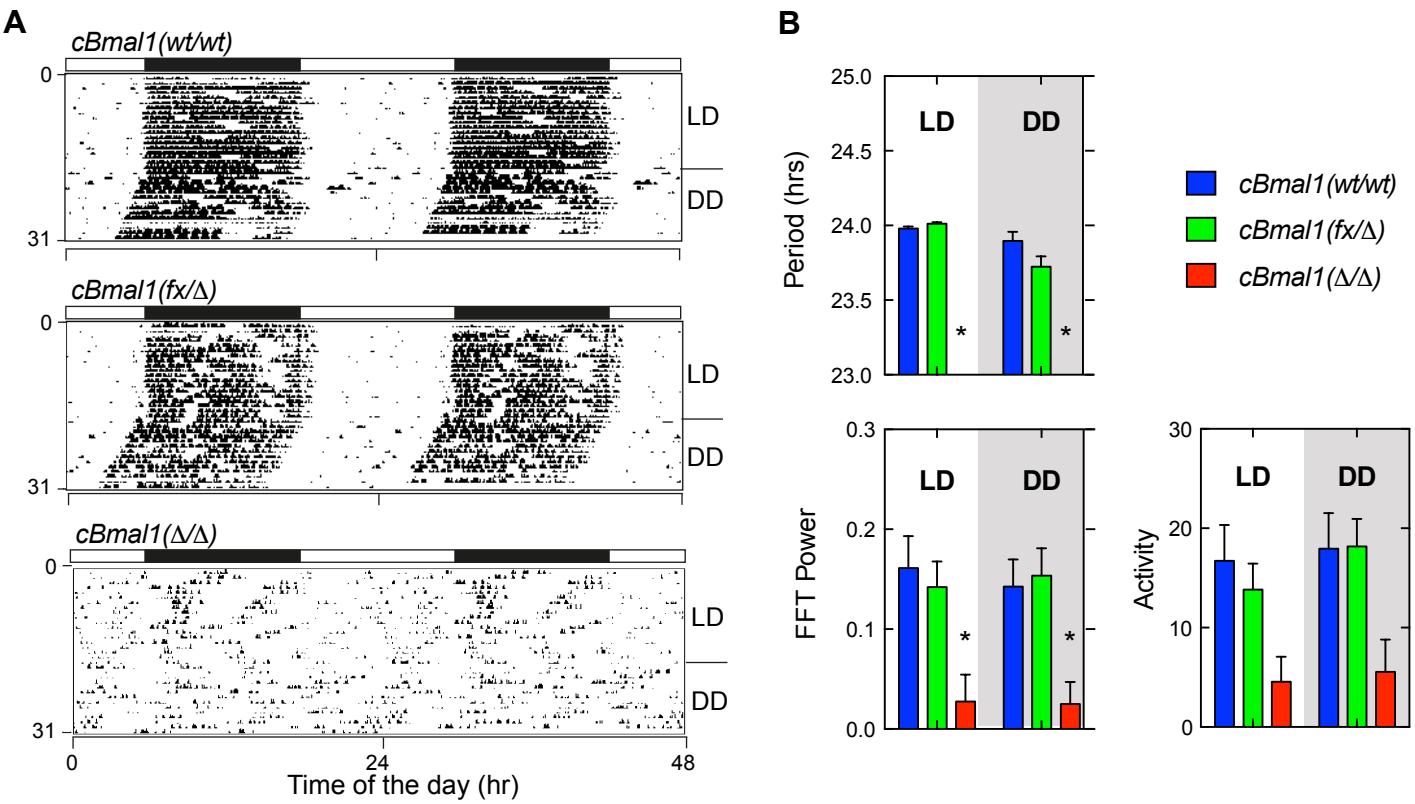


Figure S2. Lack of systemic effects of the cochlear-specific *Bmal1* deletion on locomotor activity. Related to Figure 2. (A) Representative actograms showing normal behavior in light/dark (LD) cycles or in dark/dark (DD) cycles in both *cBmal1(wt/wt)* and *(fx/D)* animals, and complete disruption of activity in *cBmal1(D/D)*. (B) Quantification of the period, FFT power and overall activity showing normal patterns between the *cBmal1(wt/wt)* and *(fx/D)* mice, but disrupted period and FFT power in *(D/D)* mice, but not overall activity. Results are mean values \pm SEM, $n = 2-8$, $*p < 0.05$; one-way ANOVA with Newman-Keuls multiple comparison test.

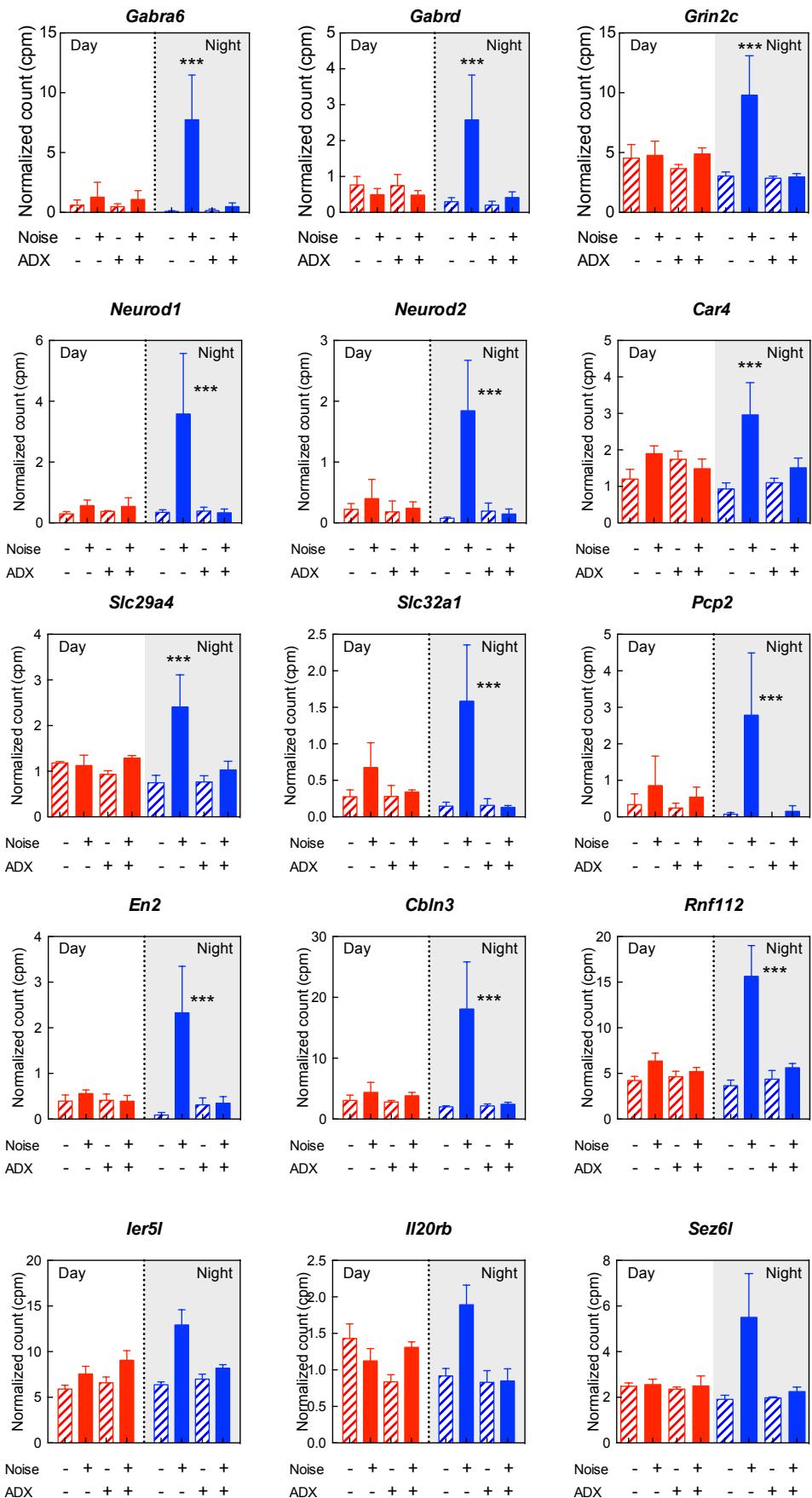


Figure S3. GC-dependent genes induced by night noise exposure and rescued in ADX mice. Related to Figure 3. Gene induction upon night noise trauma in sham animals but rescued by ADX. The differentially expressed genes were identified with a GLM (general linear model) with a negative binomial distribution (see Methods). The differentially expressed genes p-values are corrected for multiple testing error with a 5% FDR (false discovery rate) and corrected with Benjamin-Hochberg. All genes that were differentially expressed between night noise exposure (NN) and sham night noise (ShNN) exposure in sham-ADX (ShADX) mice [NNShADX vs ShNNShADX], and for which no differential expression was found in the ADX mice after night noise (NNADX sv ShNNShADX) are shown. Results are mean values \pm SEM; *** p < 0.001 using ShNNShADX as a reference.

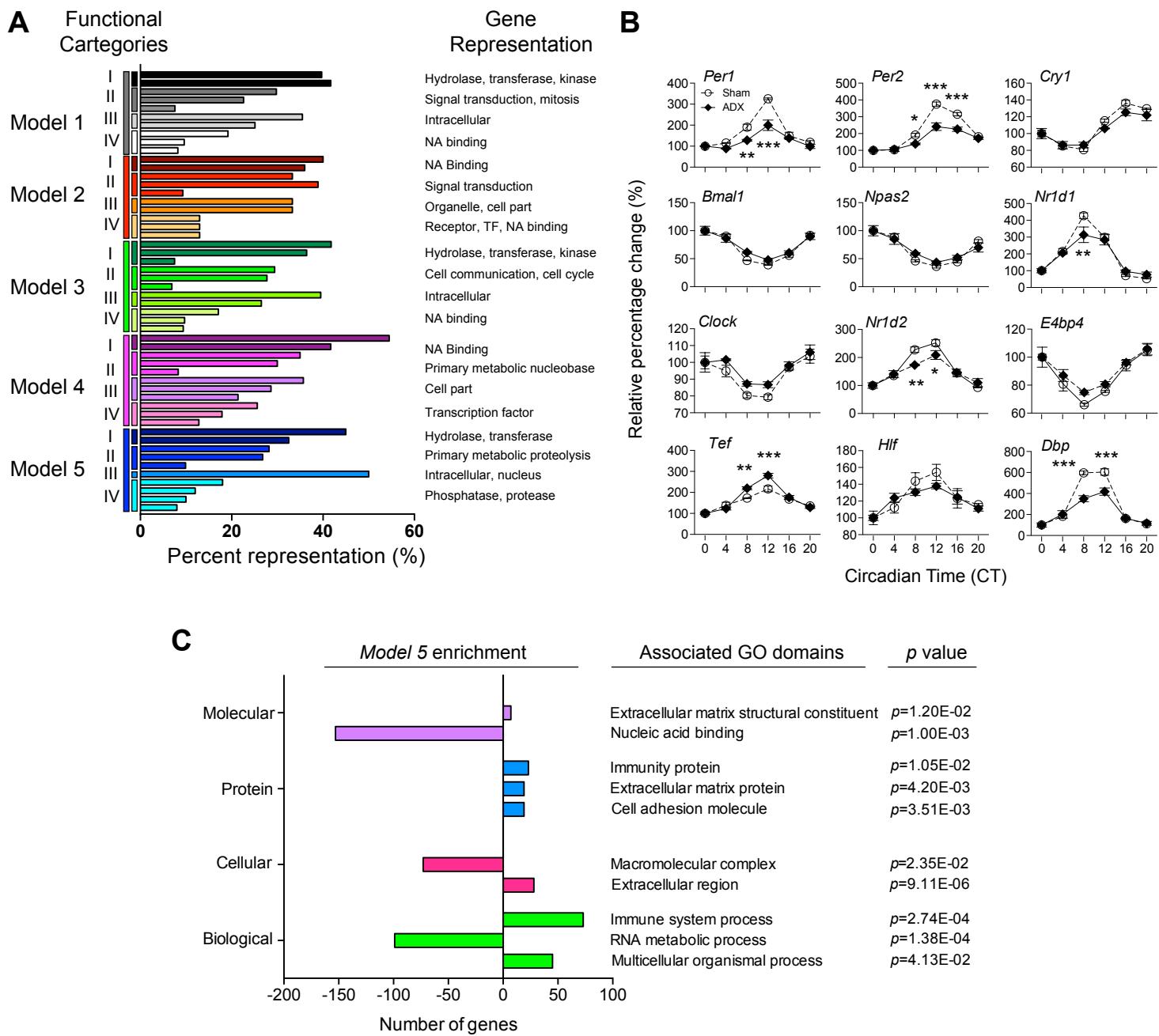


Figure S4. GO analysis and evaluation of ADX on core clock genes in the cochlea. Related to Figure 4. (A) Gene ontology (GO) analysis revealed a differential representation of GO terms for models 1 to 5 shown in Figure 4. Model 1 is in dark/grey, Model 2 is in orange/red, Model 3 is green, Model 4 is purple and Model 5 is blue. Bars represent the percentage of genes found in the most significant GO terms in four functional categories: I) Molecular processes, II) Biological processes, III) Cell component and IV) Protein class. The individual bars are representing subclassifications of the GO terms listed under Gene Representation. (B) Transcriptional profiles of twelve key core clock and clock-controlled cochlear genes in sham and ADX conditions and belonging to Model 4 (slightly affected in phase or in amplitude). Open circles/dashed lines indicate sham operated samples and solid circles/full lines represent ADX samples. The horizontal axis shows the sampling time as CT. All conditions were plotted as relative percentage change (RPC) using CT 0 as baseline value. Values are mean \pm SEM; p values obtained from a two-way ANOVA with Sidak's post hoc analysis, * p < 0.05, ** p < 0.01, *** p < 0.001. n = 3. Note that the ANOVA does not detect amplitude changes from paired analysis on all genes as does the multiple linear regression. (C) Statistically significant gene ontology enrichment for genes in Model 5 (loss of rhythmicity in ADX cochleae) only (p < 0.05) obtained using the Mann–Whitney rank-sum test (U test) algorithm, which identifies GO domains in the ADX group from model 5 that differ from the total reference input (sham-ADX model 5). This figure shows that the immune system is overrepresented in Model 5 (see Table S3, for a list of immune-system related genes).

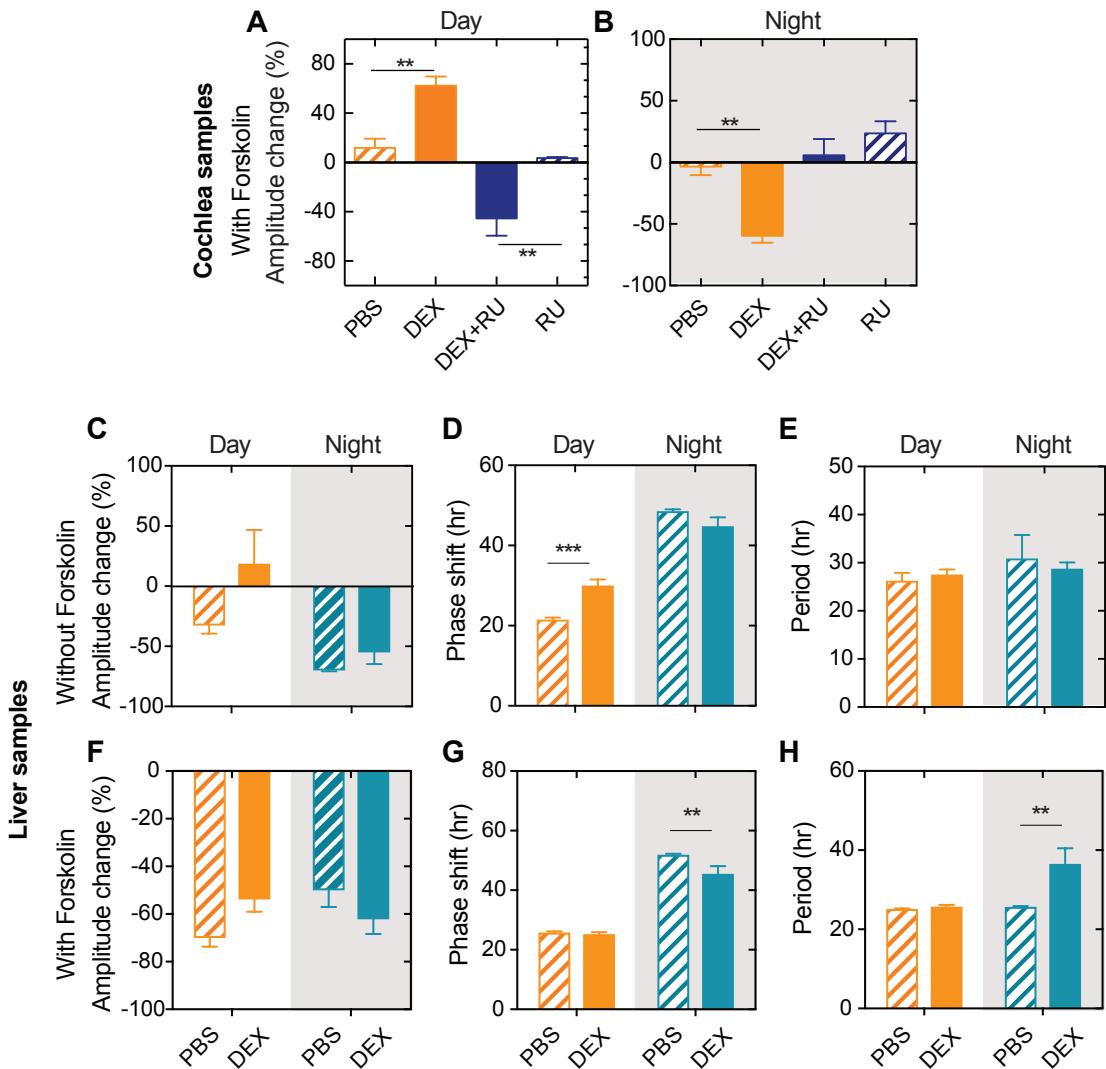


Figure S5. DEX treatment in the cochlea and the liver. Related to Figure 5. Quantification of the PER2::LUC amplitude change after DEX and RU486 (RU) treatment during the day (A) or during the night (B) in presence of forskolin pre-synchronization. Quantification of PER2::LUC amplitude, phase and period change after DEX treatment of liver tissue in absence (C-E) or presence (F-H) of forskolin pre-treatment (day, orange filled bars; night, green filled bars). PBS treatment is shown with hatched bars. Grey shaded areas indicate the nighttime. Results are mean values \pm SEM, n = 5-11 (A-B), n = 3-5 (C-E), n = 6-7 (F-H). ** p < 0.01, *** p < 0.001; two-way ANOVA with Bonferroni post-hoc analysis.

	Significantly differentially expressed genes with FDR 5%					
	down regulated	no change	up regulated	# with FC2	< 2	> 2
Group 2 vs group 1	16	15459	105	32	0	32
Group 3 vs group 1	72	15393	115	33	22	11
Group 4 vs group 1	187	15210	183	67	21	46
Group 5 vs group 1	67	15386	127	38	30	8
Group 6 vs group 1	99	15208	273	78	9	69
Group 7 vs group 1	162	15120	298	67	52	15
Group 8 vs group 1	96	15273	211	82	27	55
Group 6 vs group 5	21	15400	159	60	2	58
Group 7 vs group 5	188	15081	311	65	38	27
Group 8 vs group 5	34	15412	134	56	7	49

Table S1. Statistics of differentially expressed genes after day or night exposure (FDR, false discovery rate < 5%) and the number of genes that have a greater than 2-fold change (FC2) in expression. Related to Figure 3.

PANTHER Pathways	Mus musculus - REFLIST (22322)	Model 4 (111)	Model 4 (expected)	Model 4 (over/under)	Model 4 (fold Enrichment)	Model 4 (P-value)
Circadian clock system (P00015)	9	4	00.4	+	89.38	2.42E-05
GO biological process complete	Mus musculus - REFLIST (22322)	Model 4 (111)	Model 4 (expected)	Model 4 (over/under)	Model 4 (fold Enrichment)	Model 4 (P-value)
Circadian regulation of gene expression (GO:0032922)	59	9	0.29	+	30.68	1.95E-07
Regulation of circadian rhythm (GO:0042752)	106	10	0.53	+	18.97	1.52E-06
Circadian rhythm (GO:0007623)	121	10	0.6	+	16.62	5.37E-06
Rhythmic process (GO:0048511)	266	13	1.32	+	9.83	7.75E-06

Table S2 PANTHER statistical overrepresentation results for Model 4 (enriched with clock genes). Related to Figure 4.

PANTHER family	Gene name	NCBI Gene ID	Amplitude Sham	Amplitude ADX	Delta amplitude	BICW
<i>Antibacterial response</i> <i>(PC00051)</i>	<i>Adgrfa</i>	224792	0.0587	0.0161	3.6538	0.4756
	<i>Adgrg5</i>	382045	0.0746	0.0215	3.4693	0.4264
	<i>Pth1r</i>	19228	0.1031	0.0315	3.2686	0.6377
	<i>Col1a2</i>	12843	0.2371	0.0210	11.2685	0.7536
	<i>Col5a2</i>	12832	0.3061	0.0328	9.3229	0.7130
	<i>Col11a1</i>	12814	0.3431	0.0629	5.4543	0.7514
	<i>Col1a1</i>	12842	0.3660	0.0694	5.2754	0.8656
	<i>Col13a1</i>	12817	0.1337	0.0336	3.9769	0.6259
	<i>Col5a1</i>	12831	0.1769	0.0455	3.8918	0.8819
<i>Cytokine/cytokine receptor</i> <i>(PC00083)</i>	<i>Loxl2</i>	94352	0.1589	0.0664	2.3926	0.7571
	<i>Cd109</i>	235505	0.1962	0.0098	19.9880	0.9700*
	<i>Il2rg</i>	16186	0.1044	0.0401	2.6012	0.6656
	<i>Gm20489</i>	6608568	0.0799	0.0315	2.5346	0.5438
<i>Defense/immunity</i> <i>(PC00090)</i>	<i>Ifngr2</i>	15980	0.0627	0.0265	2.3622	0.7501
	<i>Cfh</i>	12628	0.0935	0.0051	18.4409	0.9403*
	<i>Fas</i>	14102	0.0679	0.0079	8.6189	0.6107
	<i>Fap</i>	14089	0.1284	0.0186	6.8998	0.8873
	<i>Numb1</i>	18223	0.0609	0.0093	6.5167	0.5501
	<i>Bcl6b</i>	12029	0.0943	0.0158	5.9593	0.9291*
	<i>Fbln2</i>	14115	0.0748	0.0126	5.9557	0.5573
	<i>Itpr3</i>	16440	0.0555	0.0096	5.7858	0.8464
	<i>Zcchc7</i>	319885	0.0358	0.0085	4.2031	0.7857
	<i>Tns1</i>	21961	0.1045	0.0261	3.9989	0.7270
	<i>Hspa13</i>	110920	0.0742	0.0208	3.5745	0.8566
	<i>Wif1</i>	24117	0.0853	0.0240	3.5504	0.8618
	<i>Irak2</i>	108960	0.0405	0.0126	3.2060	0.8214
	<i>Gapt</i>	238875	0.0926	0.0305	3.0340	0.5981
	<i>Vav3</i>	57257	0.0616	0.0206	2.9879	0.5221
	<i>Ilf2</i>	67781	0.0534	0.0185	2.8911	0.6844
	<i>Limk2</i>	16886	0.0680	0.0237	2.8641	0.6556
	<i>Runx2</i>	12393	0.1174	0.0433	2.7104	0.7979
	<i>Lhx3</i>	16871	0.1226	0.0473	2.5920	0.4635
<i>Immunoglobulin receptor superfamily</i> <i>(PC00125)</i>	<i>Adat1</i>	30947	0.0655	0.0270	2.4284	0.4424
	<i>Slfn1</i>	20555	0.1320	0.0604	2.1831	0.4427
	<i>Ccr2</i>	12772	0.1922	0.0187	10.2951	0.9480*
	<i>Ighg3</i>	380795	0.1726	0.0216	8.0090	0.4587
	<i>Trem1</i>	71326	0.1289	0.0206	6.2482	0.6530
	<i>Ighg2c</i>	40411	0.1922	0.0310	6.1982	0.5046
	<i>Ccr9</i>	12769	0.0861	0.0147	5.8655	0.9335*
	<i>Clec4e</i>	56619	0.1193	0.0211	5.6485	0.7203
	<i>Igha</i>	238447	0.2000	0.0443	4.5166	0.4528
	<i>Igdcc3</i>	19289	0.0930	0.0287	3.2405	0.8326

	<i>Cntn2</i>	21367	0.1333	0.0614	2.1713	0.4579
	<i>Fcer1g</i>	14127	0.0964	0.0482	1.9986	0.4357
	<i>Ccr7</i>	12775	0.1108	0.0580	1.9111	0.4726
Interferon superfamily (PC00127)	<i>Oas3</i>	246727	0.1286	0.0156	8.2693	0.5263
	<i>Oas12</i>	23962	0.2543	0.0859	2.9621	0.5675
	<i>Irf5</i>	27056	0.0867	0.0301	2.8805	0.5302
Response to stress (GO: 0006950)	<i>Sprint1</i>	20732	0.1316	0.0514	2.5587	0.5088
	<i>Grap2</i>	17444	0.1118	0.0456	2.4525	0.4505
	<i>Map3k5</i>	26408	0.0564	0.0232	2.4318	0.5104
	<i>Rela</i>	19697	0.0370	0.0175	2.1177	0.4406

Table S3: Overrepresented immune system related genes in Model 5 (ADX-dependent circadian genes). Related to Figure 4.