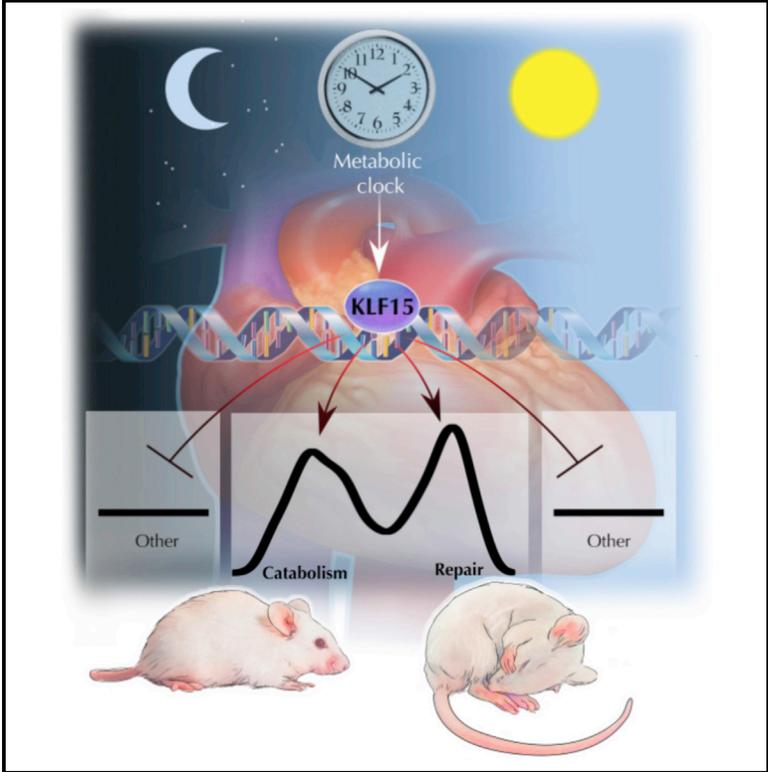


## KLF15 Establishes the Landscape of Diurnal Expression in the Heart

### Graphical Abstract



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### In Brief

Zhang et al. identify the transcription factor KLF15 as a regulator of the bimodal circadian gene expression landscape in the heart, with a distinct catabolic phase and a remodeling and repair phase. They further find that KLF15 promotes and represses oscillation in a different subset of genes.

### Highlights

- KLF15 directly regulates metabolic genes in the active phase
- KLF15 is required for repression of oscillation by REV-ERB $\alpha$  and NCOR

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# KLF15 Establishes the Landscape of Diurnal Expression in the Heart

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## SUMMARY

Circadian rhythms offer temporal control of anticipatory physiologic adaptations in animals. In the mammalian cardiovascular system, the importance of these rhythms is underscored by increased cardiovascular disease in shift workers, findings recapitulated in experimental animal models. However, a nodal regulator that allows integration of central and peripheral information and coordinates cardiac rhythmic output has been elusive. Here, we show that kruppel-like factor 15 (KLF15) governs a biphasic transcriptomic oscillation in the heart with a maximum ATP production phase and a remodeling and repair phase corresponding to the active and resting phase of a rodent. Depletion of KLF15 in cardiomyocytes leads to a disorganized oscillatory behavior without phasic partition despite an intact core clock. Thus, KLF15 is a nodal connection between the clock and meaningful rhythmicity in the heart.

## INTRODUCTION

A large number of physiological processes in animals exhibit circadian rhythmicity. These oscillations are under the control of the molecular clock, an auto-regulatory transcriptional network that exists in all cells. It is estimated that ~50% of the genome has the potential to oscillate, although only 3%–10% do in any given tissue context (Akhtar et al., 2002; Panda et al., 2002; Storch et al., 2002; Ueda et al., 2002; Zhang et al., 2014). Further, even genes oscillating in more than one tissue often vary in phases among different organs (Panda et al., 2002; Zhang et al., 2014). Thus, the optimum physiological benefit at an organ level is achieved by a coordinated gene expression integrating signals from the central clock, local clock, and environmental cues (zeitgebers). However, our understanding of how a universally oscillating clock achieves this sophisticated regulation of gene expression in each peripheral organ remains limited.

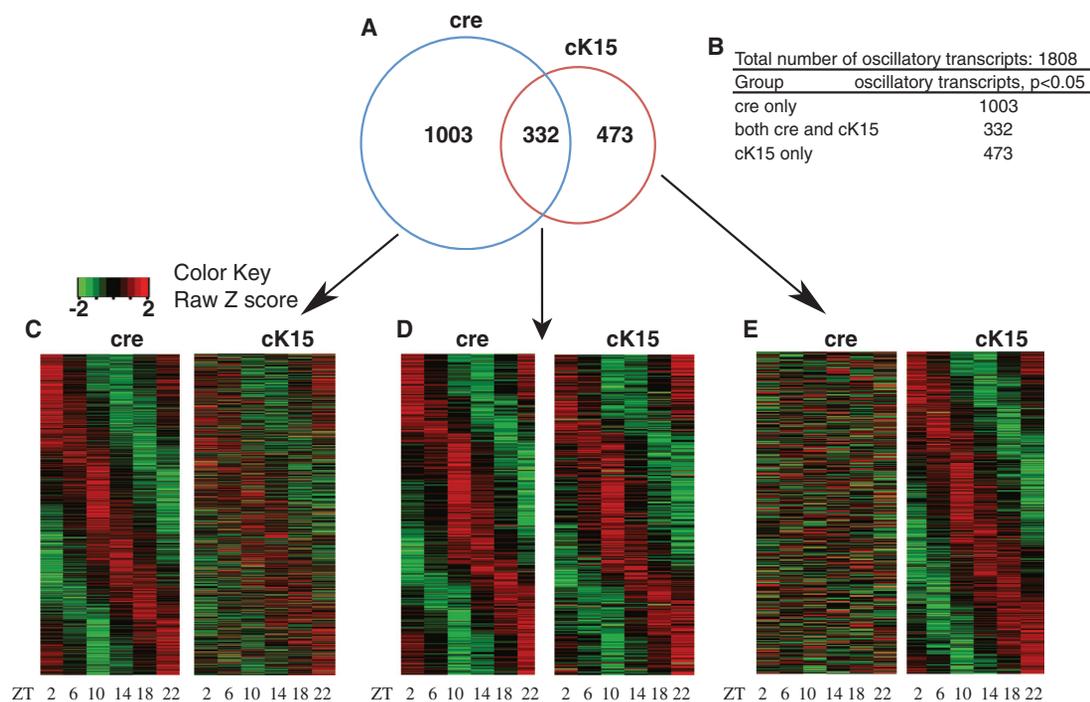
Clinical and experimental evidence supports a critical role for circadian rhythms and the molecular clock in cardiovascular physiology and pathobiology. A number of physiologic parameters such as heart rate and blood pressure have a 24-hr rhythm (Millar-Craig et al., 1978). There is a clear predilection for major cardiac events (e.g., heart attack or sudden death) to occur at a certain time of the day, specifically the early morning period (Cohen et al., 1997; Muller et al., 1985). Night shift workers are at increased risk for cardiovascular and metabolic diseases (Knutsson et al., 1986; Viitasalo et al., 2012). Finally, animals bearing a disruption in the molecular clock components are predisposed to cardiovascular disease (e.g., coronary artery disease and cardiomyopathy) along with a number of other problems such as metabolic syndrome and accelerated aging (Durgan et al., 2011; Lefta et al., 2012).

The heart must anticipate and adapt to an unrelenting energy demand over a wide spectrum of physiologic states throughout a 24-hr day. Recent studies have identified the transcription factor KLF15 as a circadian metabolic regulator of nutrient flux in the context of day/night cycles, fasting, and exercise adaptation (Gray et al., 2007; Haldar et al., 2012; Jeyaraj et al., 2012b; Prosdocimo et al., 2014). We sought to investigate the role of KLF15 in the diurnal rhythms in the heart.

## RESULTS

### KLF15 Is a Nodal Regulator of Cardiac Diurnal Gene Expression

To assess the role of KLF15 in cardiac circadian regulation, we generated cardiomyocyte-specific KLF15 knockout mice (cK15) by crossing the  $\alpha$ -MHC *cre* mice (*cre*) with floxed *Klf15* mice (Figure S1A). Although phenotypically indistinguishable at baseline, the cK15 mice demonstrated decompensated cardiac function following pressure overload stress (Figures S1B–S1F), similar to what has been observed in the systemic knockout (Fisch et al., 2007). For cardiac diurnal transcriptomic studies, unstressed cK15 and *cre* mice were kept in a 12-hr light-dark cycle with ad lib food access, and hearts were sampled every 4 hr during a continuous 24-hr period. Analysis of the diurnal cardiac transcriptome was performed by RNA sequencing analysis (RNaseq) followed by JTK\_CYCLE (Hughes et al., 2010) to predict genes that display a 24-hr rhythmicity.



**Figure 1. The Circadian Transcriptome Is Altered by KLF15 Deficiency in the Heart**

(A and B) The number of all oscillating transcripts identified by RNaseq using JTK\_CYCLE,  $p < 0.05$ . (A) Venn diagram. (B) Summary. (C–E) Expression heat map organized by phase of genes oscillating only in cre (C), common in both cre and cK15, (D), and only in cK15 (E), respectively.

We identified 1,335 (1,003 + 332) oscillating genes in the control mice (cre) using a cutoff of  $p < 0.05$  (Figures 1A and 1B; also, see Table S1 for all differentially expressed genes). This is consistent with the previous transcriptomic studies in different tissues/organs that 3%–10% of the transcriptome oscillate (Akhtar et al., 2002; Panda et al., 2002; Storch et al., 2002; Ueda et al., 2002). In these 1,335 genes, 1,003 (75%, 1,003/1,335) oscillates in a KLF15-dependent fashion, and 332 (25%, 332/1,335) genes are rhythmic in both genotypes (Figures 1A–1D). In addition, there are a surprisingly large number of genes (473) that oscillate exclusively in cK15 (Figure 1E). The mean expression of either the 1,003 genes or the 473 genes is not significantly different between cre and cK15, although there is a trend toward reduced expression in cK15 in the 1,003 genes (Figure S1G). This result suggested a significant alteration in the diurnal expression gating in the KLF15-deficient heart.

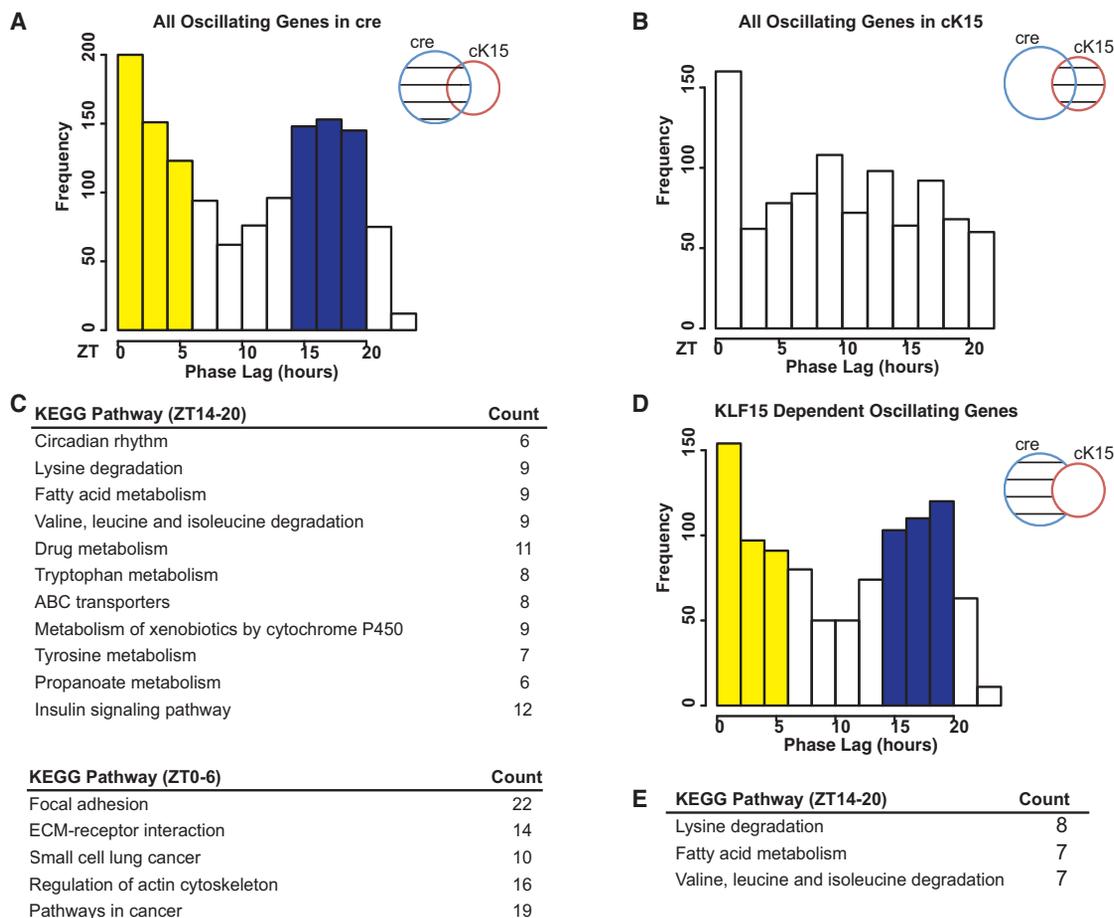
### KLF15 Regulates a Biphasic Temporal and Functional Clustering of Cardiac Genes

To deduce the clock control on cardiac physiology, we plotted the phase distribution of oscillating genes. Interestingly, in cre controls, the phase lag shows a bimodal distribution with one peak during the day (ZT0–ZT6) and the other during the night (ZT14–ZT20) (Figure 2A). We confirmed this bimodal distribution in a previously published independent dataset from the heart (Tsimakouridze et al., 2012) (Figure S2A). A similar distribution was not seen in datasets from several other tissues, including the liver (Figure S2B) (Hoogerwerf et al., 2008; Masri et al., 2014; Rudic et al., 2005). Remarkably, the biphasic distribution

of phase lag observed in cre is abolished in cK15 mice despite still having a significant total number of oscillating genes (805, 332 + 473) (Figure 2B), indicating that the biphasic phase distribution is dependent on the presence of KLF15 in the cardiomyocytes.

We hypothesized that the genes clustered under each of these two peaks might be informative of the specific aspects of cardiac physiology governed by the peripheral clock. In cre, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the oscillating genes with peak expression during the night (ZT14–ZT20) showed enrichment for the core clock machinery (circadian rhythm) and key catabolic pathways, including fatty acid metabolism and several amino acid degradation pathways (lysine, branch chain amino acids, tryptophan, and tyrosine) (Figure 2C, top). This is consistent with the peak energy demand and food intake during active phase for nocturnal rodents. On the other hand, oscillating genes with peak expression during the day (ZT0–ZT6) showed a distinct enrichment for focal adhesion, extracellular matrix receptor interaction, and regulation of actin cytoskeleton (Figure 2C, bottom). Several cancer-related pathways also have peak expression at daytime, consistent with previously proposed resting phase DNA repair in neurons (Kang et al., 2009). This suggests a function priority shift of the heart from providing energy to sustain activity phase to a remodeling and repair phase.

Importantly, the known core clock genes oscillate in both cre and cK15, including *Arntl* (BMAL1) and *Per2* (Figures 1D and S2C). The undisturbed core clock machinery confirmed our previous observation that *Klf15* functions downstream of the clock



**Figure 2. KLF15 Is Required for the Biphasic Transcriptomic Oscillation in the Heart**

(A and B) Histogram of phase distribution for all oscillating genes in cre (A) and in cK15 (B); yellow shows the peak during daytime (ZT0-6), and blue shows the peak during nighttime (ZT14-20).

(C) KEGG pathway analysis of all oscillating genes in cre with phase lag between ZT14-20 (upper) and ZT0-6 (lower).

(D) Histogram of phase distribution for KLF15 dependent oscillating genes.

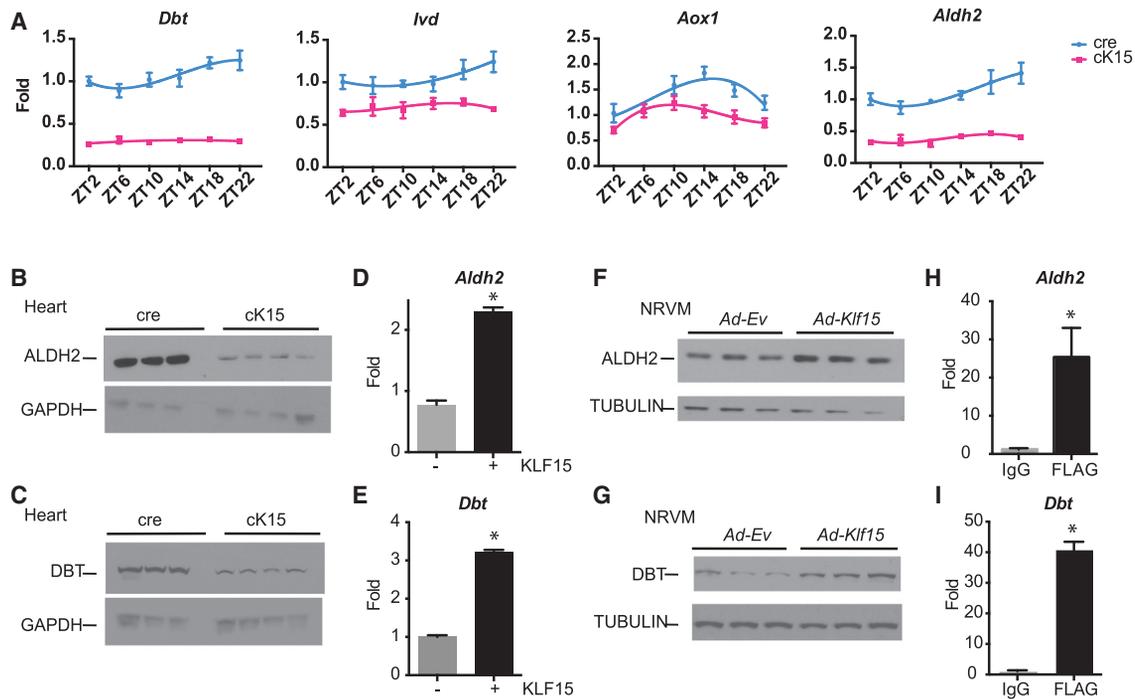
(E) KEGG pathway analysis of KLF15-dependent oscillating genes with phase lag between ZT14-20.

machinery (Jeyaraj et al., 2012a). Furthermore, known nuclear receptors PPARs that may contribute to diurnal rhythmic gene expression are not affected (Figure S2D). The common oscillating genes are also enriched for ABC transporter, glutathione metabolism, and drug metabolism during the night (ZT14–ZT20) and focal adhesion and ECM receptor signaling during the day (ZT0–ZT6) (Figures S2E and S2F), suggesting these are KLF15-independent oscillatory processes. Interestingly, a small but discernible phase advance in cK15 is observed in these 332 genes that oscillate in both genotypes (Figure S2G).

### KLF15 Directly Regulates Genes for Catabolism in the Active Phase

Given the large number of genes that lost rhythmic expression in cK15, we then focused on these 1,003 genes (Figure 1C). Similar to the oscillatory transcriptome in cre, most genes have a peak expression clustered either at the night-day transition or the day-night transition (Figure 2D), further confirming that the biphasic phase lag is driven by KLF15. KEGG analysis of

the 407 KLF15-dependent oscillatory genes with peak expression between ZT14–ZT20 showed enrichment for lysine degradation, fatty acid metabolism, and branch chain amino acid degradation (Figure 2E), a subset of enriched pathways in the control mice, all of which are critical for energy conversion from food source. This indicates a central role of KLF15 in optimizing the ATP production during the night when the energy demand is at peak in the heart. We confirmed the expression of several key components of these metabolic pathways by qRT-PCR (Figure 3A). ALDH2 (mitochondrial aldehyde dehydrogenase 2) and DBT (dihydrolipoamide branched chain transacylase E2) were selected for further verification and showed reduced protein expression in the heart of the cK15 mice (Figures 3B and 3C). In vitro, NRVMs (neonatal rat ventricular myocytes) overexpressing KLF15 showed induced expression of both RNA and protein of ALDH2 and DBT, consistent with a cell autonomous effect (Figures 3D–3G). Furthermore, chromatin immunoprecipitation confirmed the enrichment of KLF15 on *Aldh2* and *Dbt* promoter, indicating a direct transcriptional



**Figure 3. KLF15 Directly Regulates Multiple Target Genes in the Fatty Acid and Amino Acid Metabolic Pathways**

(A) Circadian qRT-PCR using heart samples collected every 4 hr during a 24-hr period,  $n = 4$  for each time point. Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*. *Dbt*, \*time, \*\*\*\*-genotype; *Ivd*, \*\*\*\*-genotype; *Aox1*, \*\*\*time, \*\*\*\*-genotype; *Aldh2*, \*\*time, \*\*\*\*-genotype, two-way ANOVA, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

(B and C) Immunoblot showing decreased ALDH2 and DBT protein expression in cK15; GAPDH is used as loading control.

(D and E) qRT-PCR using NRVM infected with adenovirus carrying empty vector (-) or *Klf15* cDNA (+),  $n = 3$ . Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*. \* $p < 0.0001$ .

(F and G) Immunoblot of NRVM overexpressing KLF15 (*Ad-Klf15*) versus empty vector (*Ad-Ev*). Tubulin is used as loading control.

(H and I) NRVM is infected with adenovirus carrying FLAG-tagged mouse *Klf15* cDNA. Recruitment of KLF15 on the *Aldh2* and *Dbt* promoter is confirmed using chromatin immunoprecipitation with anti-FLAG antibody followed by qPCR. Result is normalized to IgG.  $n = 5$ . Values are mean  $\pm$  SEM,  $p = 0.05$  (*Aldh2*),  $p < 0.0001$  (*Dbt*). Primers can be found in Table S3.

regulation (Figures 3H and 3I). KLF15 likely regulates multiple targets directly in these catabolic pathways enriched during the active phase, thereby enabling a coordinated effort for ATP production. This metabolic signature is consistent with previous microarray studies on the liver and the skeletal muscle of the systemic KLF15 knockout mice (Table S2).

There are 342 KLF15-dependent oscillatory genes with peak expression during the early daytime (ZT0–ZT6). The top three pathways are MAPK signaling, regulation of actin cytoskeleton, and small cell lung cancer, also representing a significant subset of that of control mice. However, KEGG pathway analysis with a cutoff of  $q < 0.05$  after Benjamini correction did not reach statistical significance. It is likely that KLF15 is also involved in the regulation of cellular signaling pathways for cardiac remodeling and repair.

### KLF15 Is Required for REV-ERB $\alpha$ and NCOR-Dependent Repression of Gene Oscillation

Most surprisingly, we identified 473 genes that gain oscillatory behavior in the absence of KLF15 (Figure 1E). To further delineate the possible mechanisms that may account for this gain in oscillation, we then performed an unbiased, de novo motif anal-

ysis using MEME (Multiple EM for Motif Elicitation) (Bailey et al., 2009) on the promoter of the 473 genes that gained oscillation to determine what motifs might be enriched. The top 15 motifs were matched to database of known transcription factors. Six motifs yielded cognate sites for transcription factor, and two of them pointed to nuclear receptors (Figure 4A). Indeed, we found cAMP-sensitive nuclear receptor, *Nr4a3* (NOR1), among genes oscillating only in cK15. NR4A subgroup includes three members; *Nr4a1* (NUR77) and *Nr4a3* have significant functional overlap, and both showed de novo diurnal oscillation in cK15, with peak expression at ZT10 (Figure 4B). *Nr4a2* (Nurr1) is also cAMP sensitive; however, it remained non-oscillatory (Figure S3A), suggesting cAMP is unlikely the direct reason for *Nr4a1* or *Nr4a3* to gain oscillation. Transient knockdown of *Klf15* in NRVM resulted in increased expression of both *Nr4a3* and *Nr4a1* mRNA and protein, suggesting that the gain of expression and oscillation observed in the cK15 heart is directly related to the loss of KLF15 (Figures 4C and 4D). In addition, overexpression of *Nr4a3* in NRVM leads to upregulation of several targets identified by RNASeq (Figures 4E and 4F), further supporting that these nuclear receptors at least account for a subset of the genes that gains oscillation in the cK15 heart.

Because REV-ERB $\alpha$  (*Nr1d1*) is a circadian repressor with a peak expression time that coincides with that of KLF15, we asked whether REV-ERB $\alpha$  is critical for dampening the expression of *Nr4a1* and *Nr4a3* and whether this inhibition requires KLF15. There is no significant difference in the expression of *Nr1d1* or *Ncor* (encodes its co-repressor NCOR) between cK15 and cre (Figure S3B). However, the binding of REV-ERB $\alpha$  and NCOR on the promoter of *Nr4a1* and *Nr4a3* is strongly attenuated in the absence of KLF15 (Figure 4G). Also, ChIP-seq of REV-ERB $\alpha$  in the heart of cK15 mice showed a significantly reduced number of binding sites compared to the floxed mice, including those associated with key regulatory elements on the promoter of *Nr4a1* and *Nr4a3*, while the binding on the *Bmal1* promoter is unchanged as predicted (Figures S4A–S4D). To further substantiate our hypothesis, we tested whether loss of KLF15 leads to increased responsiveness of *Nr4a1* and *Nr4a3* to other cellular signals. We chose to test glucocorticoid (GC), as it has a well-characterized diurnal rhythmicity reaching peak serum level between ZT9–12, during which time the expression of *Nr4a1* and *Nr4a3* also peaked. As predicted, dexamethasone administration was only able to induce *Nr4a1* and *Nr4a3* in the absence of KLF15 (Figures 4H and 4I). As GC induces KLF15, this complex relationship exemplifies a recently identified mode of GC action, namely, incoherent feed forward loop, (Sasse et al., 2013) and allows integration of complex input signals.

In contrast to the genes that oscillate in the WT hearts or those that oscillate in a KLF15-dependent fashion, the phase lag of the genes that gain oscillation in cK15 does not reveal a distinctive clustering around dawn or dusk (Figure 4J). Additionally, KEGG pathway or Gene Ontology (GO) analysis also did not reveal any significant clustering of annotation. This lack of both an organized temporal distribution and coordinated functional pathway suggests that these genes were inhibited from oscillation by KLF15 under physiological condition to ensure efficiency toward the desired function of the heart. Conversely, KLF15 deficiency may lead to futile or even harmful fluctuation in the system.

## DISCUSSION

The circadian clock is conserved through evolution and is critical for organismal homeostasis, yet it is highly adaptive, with different output in different tissue and cell types. It is poorly understood how this is achieved. Our findings demonstrate that Kruppel-like factor, KLF15, establishes the diurnal landscape of gene expression in the heart. It directs 75% of the oscillatory transcriptome in a biphasic fashion and prevents genes potentially oscillates in other tissue context from creating noise, thus allowing a temporal and functional specific partition in the heart (Figure 4K).

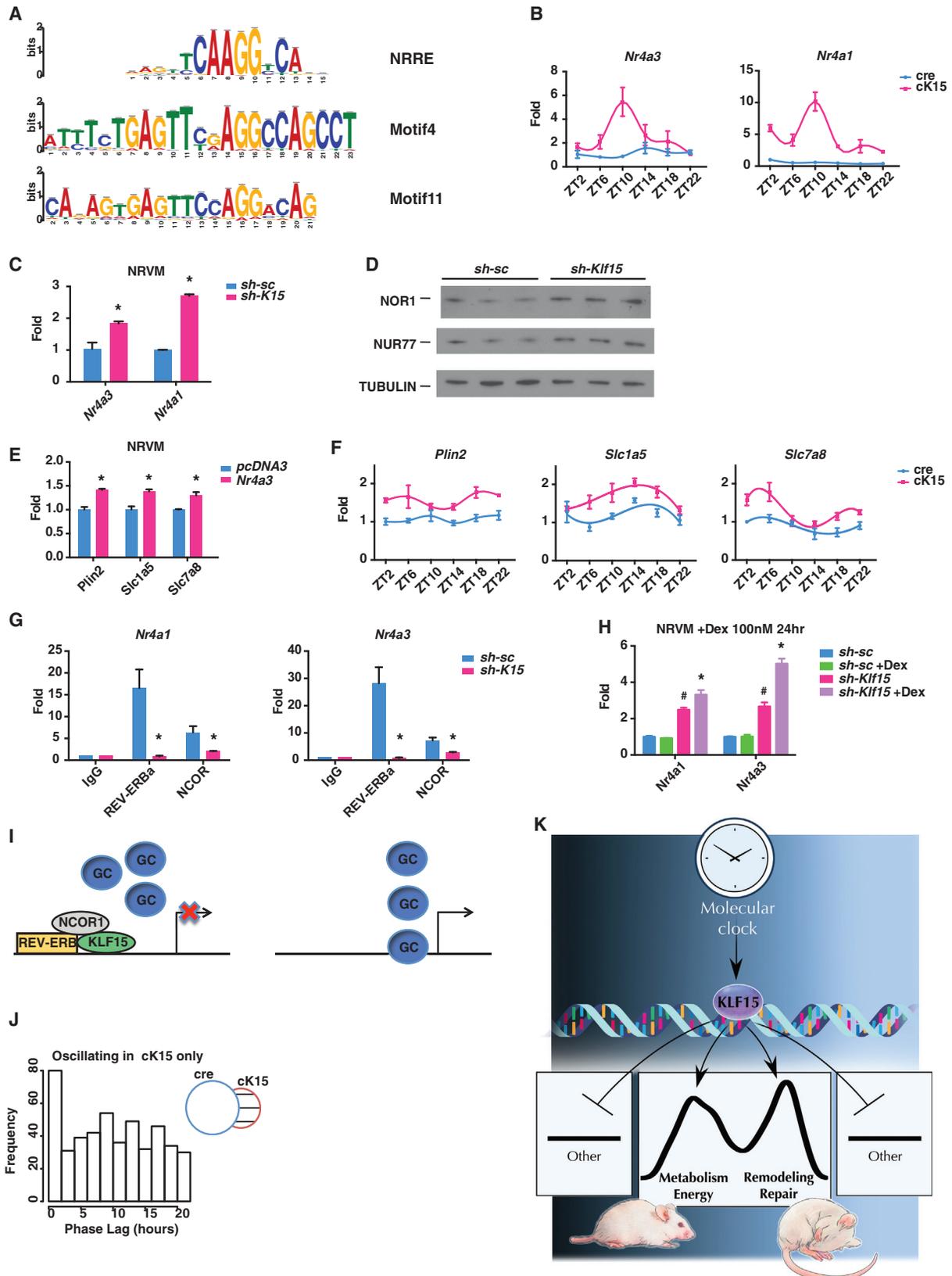
Our work adds important insights to the previous understanding of circadian gene expression in the heart. Among the two other published dataset, one is the pioneer work by Storch et al. (2002), which identified a single cluster around CT4 (circadian time). The other one is the recently published comprehensive study by Zhang et al. (2014), which identified another single cluster around CT16. As both studies sampled during

“free-run,” it is unlikely the testing condition can account for the difference. However, a closer examination of the dataset reveals that the earlier versions of oligoarray represent only ~30% of the mouse genome, which may lead to decreased detection. The more recent study used RNaseq, but sampled more sparsely (every 6 hr) and had more extensive pooling (six samples per lane) features that may reduce sensitivity. As each of these datasets represents a different subset of our result, which is also replicated using a completely independent dataset obtained on a different platform (oligoarray) (Tsimakouridze et al., 2012), we believe the results provided here offer an improved depiction.

Most work in circadian biology to date has focused on mechanisms promoting oscillation. However, mechanisms that suppress oscillation are just as important to orchestrate a specific gene expression landscape. Our unexpected observation that a significant number of genes gain oscillatory behavior in the absence of KLF15 offered a novel mechanism of diurnal gene regulation. We show that KLF15 is required for binding of circadian repressors, REV-ERB $\alpha$  and NCOR to maintain quiescent expression of a subset of genes in the heart, and lack of KLF15 leads to aberrant oscillation of gene expression responding to cellular signals including the GC. The binding of REV-ERB $\alpha$  on the *Arntl* promoter is not KLF15 dependent. In fact, the observation that core clock genes, such as *Arntl*, did not change expression pattern, despite being a REV-ERB $\alpha$  target further strengthened our notion that the core clock gene defines “time” and KLF15 defines “locale”; together, they achieve a perfectly orchestrated “temporal-spatial” expression.

It was recently noted that changes in metabolic state may drive rhythmic gene expression in metabolic organs through the rhythmic ligand availability of nuclear receptors such as PPAR (Eckel-Mahan et al., 2013). We did not detect a rhythmic expression of PPAR $\alpha$ ,  $\beta$ , or  $\gamma$ , among which PPAR $\alpha$  has long been known for its critical role in cardiac energy metabolism. More importantly, we have recently reported that the ligand-induced PPAR $\alpha$  activity in the heart is dependent on the presence of KLF15 (Prosdocimo et al., 2015); thus, it is unlikely that the fluctuation of ligand or metabolic states will account for the gain of oscillatory genes in cK15.

The unique biphasic physiology of the heart allows the heart time to repair for the damage as a direct result of cellular metabolism each day. Multiple lines of evidence have suggested that the heart is more vulnerable to hypertrophy yet receives greater cardiac remodeling benefit from angiotensin-converting enzyme inhibitors during the resting phase in both animal and human studies (Durgan et al., 2011; Flack and Nasser, 2011; Hermida and Ayala, 2009; Hermida et al., 2010; Martino et al., 2011; Sleight et al., 2001). The rationale behind this was not understood. We discovered that the transcriptome of the heart is programmed to perform cardiac remodeling and repair during the resting phase, and KLF15 deficiency, as seen in multiple chronic cardiovascular disorders, such as heart failure and aortic aneurysm, leads to loss of this critical window for “routine maintenance.” Importantly, we have identified a list of potential targets (peak expression at ZT0–6), which opens the window for chronotherapeutic options for the prevention and treatment of cardiovascular disease.



(legend on next page)

## EXPERIMENTAL PROCEDURES

Detailed methods are provided in the [Supplemental Experimental Procedures](#). See [Table S3](#) for primers.

### Animals

Male mice on C57BL/6J background at 11 to 12 weeks of age were housed in 12-hr light/12-hr dark conditions. Heart samples (n = 4 mice) were collected every 4 hr. All animal experiments were in accordance with guidelines of the Case Western Reserve University Animal Care and Use Committee.

### Whole Transcriptome Sequencing

RNA was isolated from apex of the heart using Trizol (Life Technologies) followed with miRNeasy Kit (QIAGEN). Ribo-depleted RNA was used to generate library using the Illumina TruSeq Stranded Total RNA kit; 100-bp paired-end sequencing was performed on pooled libraries in groups of three using an Illumina HiSeq 2500 instrument at IIGB Genomics Core at the University of California, Riverside.

### RNaseq Data Analysis

Illumina reads were mapped to mouse genome release mm10 using GSNAP program. Reads mapped to single location in mm10 genome were retained for further analysis. The raw read counts for each gene in mm10 RefSeq annotation were generated using HTSeq program (Anders et al., 2014). The expression levels of genes were presented as FPKM (fragments per kilo bases of exon per million of mapped reads) values.

### Time Series Analysis for Circadian Rhythmicity

RNA cycling was assessed by JTK\_cycle (Hughes et al., 2010) with a period of 24 hr and a cutoff of  $p \leq 0.05$ .

### ACCESSION NUMBERS

The data discussed in this manuscript have been deposited in NCBI GEO and are accessible under accession number GEO: GSE63243 and GEO: GSE73741.

### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.11.038>.

## AUTHOR CONTRIBUTIONS

L.Z. and M.K.J. designed the study. L.Z., X.B., and C.F. performed the computational analyses. F.C. prepared the libraries for sequencing. L.Z. and D.A.P. performed the animal experiment. L.Z. performed the cellular and molecular biology experiment. L.Z. wrote the manuscript with contributions from D.A.P., X.L., and J.C. M.K.J. supervised the research.

## ACKNOWLEDGMENTS

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## REFERENCES

- Akhtar, R.A., Reddy, A.B., Maywood, E.S., Clayton, J.D., King, V.M., Smith, A.G., Gant, T.W., Hastings, M.H., and Kyriacou, C.P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr. Biol.* 12, 540–550.
- Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* 15, 166–169.
- Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., and Noble, W.S. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208.
- Cohen, M.C., Rohtla, K.M., Lavery, C.E., Muller, J.E., and Mittleman, M.A. (1997). Meta-analysis of the morning excess of acute myocardial infarction and sudden cardiac death. *Am. J. Cardiol.* 79, 1512–1516.
- Durgan, D.J., Tsai, J.Y., Grenett, M.H., Pat, B.M., Ratcliffe, W.F., Villegas-Montoya, C., Garvey, M.E., Nagendran, J., Dyck, J.R., Bray, M.S., et al. (2011). Evidence suggesting that the cardiomyocyte circadian clock modulates responsiveness of the heart to hypertrophic stimuli in mice. *Chronobiol. Int.* 28, 187–203.
- Eckel-Mahan, K.L., Patel, V.R., de Mateo, S., Orozco-Solis, R., Ceglia, N.J., Sahar, S., Dilag-Penilla, S.A., Dyar, K.A., Baldi, P., and Sassone-Corsi, P.

## Figure 4. De-repression of REV-ERB $\alpha$ on Nuclear Receptors Nor1 (*Nr4a3*) and NUR77 (*Nr4a1*) Is Partially Responsible for Genes Gained Oscillation in cK15

- (A) Motif alignment of nuclear receptor responsive element (NRRE) and two promoter motifs common to genes gained oscillation in cK15 generated by MEME.
- (B) Circadian qRT-PCR, using heart samples collected every 4 hr during a 24-hr period, n = 4 for each time point. Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*. *Nr4a3*, \*time, \*\*\*-genotype, \*\*-interaction; *Nr4a1*, \*\*\*\*-time, \*\*\*\*-genotype, \*\*\*\*-interaction, two-way-ANOVA, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.
- (C) qRT-PCR of NRVM knockdown with short hairpin RNA against scrambled target (*sh-sc*) or KLF15 (*sh-K15*), n = 3, p = 0.02 (*Nr4a3*), p < 0.0001 (*Nr4a1*). Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*.
- (D) Immunoblot of NRVM knockdown with *sh-sc* or *sh-Klf15*. Tubulin is used as loading control.
- (E) qRT-PCR of the indicated genes in NRVM transfected with control vector or a plasmid containing *Nr4a3* cDNA. n = 3, p = 0.002 (*Plin2*), p = 0.008 (*Slc1a5*), p = 0.013 (*Slc7a8*). Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*.
- (F) Circadian qRT-PCR, using heart samples collected every 4 hr during a 24-hr period, n = 4, for each time point. Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*. *Plin2*, \*\*\*\*-genotype; *Slc1a5*, \*\*\*-time, \*\*\*\*-genotype; *Slc7a8*, \*\*\*\*-time, \*\*\*\*-genotype, two-way-ANOVA, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.
- (G) Chromatin immunoprecipitation in NRVM knockdown with short hairpin RNA against scrambled target (*sh-sc*) or KLF15 (*sh-K15*). Recruitment of REV-ERB $\alpha$  and NCOR is shown on the *Nr4a1* and *Nr4a3* promoter using chromatin immunoprecipitation followed by qPCR; the result is normalized to input and IgG. n = 3, values are mean  $\pm$  SEM, p = 0.02 (*Nr4a1*, REV-ERB $\alpha$ ), p = 0.04 (*Nr4a1*, NCOR), p = 0.009 (*Nr4a3*, REV-ERB $\alpha$ ), p = 0.015 (*Nr4a3*, NCOR). Primers can be found in [Table S3](#).
- (H) qRT-PCR of NRVM knockdown with short hairpin RNA against scrambled target (*sh-sc*) or KLF15 (*sh-K15*), with or without dexamethasone, n = 3, \**sh-sc* versus *sh-K15*, p = 0.0002 (*Nr4a1*), p < 0.001 (*Nr4a3*); #*sh-K15* versus *sh-K15*+Dex, p = 0.037 (*Nr4a1*), p = 0.002 (*Nr4a3*). Values are mean  $\pm$  SEM. Expression is normalized to *Ppib*.
- (I) Schematic diagram of KLF15 recruiting REV-ERB $\alpha$  and NCOR to repress target genes and desensitize to GC.
- (J) Histogram of phase distribution for genes only oscillated in cK15.
- (K) Schematic diagram of KLF15 in the circadian gene expression in the heart.

- (2013). Reprogramming of the circadian clock by nutritional challenge. *Cell* 155, 1464–1478.
- Fisch, S., Gray, S., Heymans, S., Haldar, S.M., Wang, B., Pfister, O., Cui, L., Kumar, A., Lin, Z., Sen-Banerjee, S., et al. (2007). Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. *Proc. Natl. Acad. Sci. USA* 104, 7074–7079.
- Flack, J.M., and Nasser, S.A. (2011). Benefits of once-daily therapies in the treatment of hypertension. *Vasc. Health Risk Manag.* 7, 777–787.
- Gray, S., Wang, B., Orihuela, Y., Hong, E.G., Fisch, S., Haldar, S., Cline, G.W., Kim, J.K., Peroni, O.D., Kahn, B.B., and Jain, M.K. (2007). Regulation of gluconeogenesis by Krüppel-like factor 15. *Cell Metab.* 5, 305–312.
- Haldar, S.M., Jeyaraj, D., Anand, P., Zhu, H., Lu, Y., Prosdocimo, D.A., Eapen, B., Kawanami, D., Okutsu, M., Brotto, L., et al. (2012). Kruppel-like factor 15 regulates skeletal muscle lipid flux and exercise adaptation. *Proc. Natl. Acad. Sci. USA* 109, 6739–6744.
- Hermida, R.C., and Ayala, D.E. (2009). Chronotherapy with the angiotensin-converting enzyme inhibitor ramipril in essential hypertension: improved blood pressure control with bedtime dosing. *Hypertension* 54, 40–46.
- Hermida, R.C., Ayala, D.E., Mojón, A., and Fernández, J.R. (2010). Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiol. Int.* 27, 1629–1651.
- Hoogerwerf, W.A., Sinha, M., Conesa, A., Luxon, B.A., Shahinian, V.B., Cornelissen, G., Halberg, F., Bostwick, J., Timm, J., and Cassone, V.M. (2008). Transcriptional profiling of mRNA expression in the mouse distal colon. *Gastroenterology* 135, 2019–2029.
- Hughes, M.E., Hogenesch, J.B., and Kornacker, K. (2010). JTK\_CYCLE: an efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *J. Biol. Rhythms* 25, 372–380.
- Jeyaraj, D., Haldar, S.M., Wan, X., McCauley, M.D., Ripperger, J.A., Hu, K., Lu, Y., Eapen, B.L., Sharma, N., Ficker, E., et al. (2012a). Circadian rhythms govern cardiac repolarization and arrhythmogenesis. *Nature* 483, 96–99.
- Jeyaraj, D., Scheer, F.A., Ripperger, J.A., Haldar, S.M., Lu, Y., Prosdocimo, D.A., Eapen, S.J., Eapen, B.L., Cui, Y., Mahabeshwar, G.H., et al. (2012b). Klf15 orchestrates circadian nitrogen homeostasis. *Cell Metab.* 15, 311–323.
- Kang, T.H., Reardon, J.T., Kemp, M., and Sancar, A. (2009). Circadian oscillation of nucleotide excision repair in mammalian brain. *Proc. Natl. Acad. Sci. USA* 106, 2864–2867.
- Knutsson, A., Akerstedt, T., Jonsson, B.G., and Orth-Gomer, K. (1986). Increased risk of ischaemic heart disease in shift workers. *Lancet* 2, 89–92.
- Lefta, M., Campbell, K.S., Feng, H.Z., Jin, J.P., and Esser, K.A. (2012). Development of dilated cardiomyopathy in Bmal1-deficient mice. *Am. J. Physiol. Heart Circ. Physiol.* 303, H475–H485.
- Martino, T.A., Tata, N., Simpson, J.A., Vanderlaan, R., Dawood, F., Kabir, M.G., Khaper, N., Cifelli, C., Podobed, P., Liu, P.P., et al. (2011). The primary benefits of angiotensin-converting enzyme inhibition on cardiac remodeling occur during sleep time in murine pressure overload hypertrophy. *J. Am. Coll. Cardiol.* 57, 2020–2028.
- Masri, S., Rigor, P., Cervantes, M., Ceglia, N., Sebastian, C., Xiao, C., Roqueta-Rivera, M., Deng, C., Osborne, T.F., Mostoslavsky, R., et al. (2014). Partitioning circadian transcription by SIRT6 leads to segregated control of cellular metabolism. *Cell* 158, 659–672.
- Millar-Craig, M.W., Bishop, C.N., and Raftery, E.B. (1978). Circadian variation of blood-pressure. *Lancet* 1, 795–797.
- Muller, J.E., Stone, P.H., Turi, Z.G., Rutherford, J.D., Czeisler, C.A., Parker, C., Poole, W.K., Passamani, E., Roberts, R., Robertson, T., et al. (1985). Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med.* 313, 1315–1322.
- Panda, S., Antoch, M.P., Miller, B.H., Su, A.I., Schook, A.B., Straume, M., Schultz, P.G., Kay, S.A., Takahashi, J.S., and Hogenesch, J.B. (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109, 307–320.
- Prosdocimo, D.A., Anand, P., Liao, X., Zhu, H., Shelkay, S., Artero-Calderon, P., Zhang, L., Kirsh, J., Moore, D., Rosca, M.G., et al. (2014). Kruppel-like factor 15 is a critical regulator of cardiac lipid metabolism. *J. Biol. Chem.* 289, 5914–5924.
- Prosdocimo, D.A., John, J.E., Zhang, L., Efraim, E.S., Zhang, R., Liao, X., and Jain, M.K. (2015). KLF15 and PPAR $\alpha$  Cooperate to Regulate Cardiomyocyte Lipid Gene Expression and Oxidation. *PPAR Res.* 2015, 201625.
- Rudic, R.D., McNamara, P., Reilly, D., Grosser, T., Curtis, A.M., Price, T.S., Panda, S., Hogenesch, J.B., and FitzGerald, G.A. (2005). Bioinformatic analysis of circadian gene oscillation in mouse aorta. *Circulation* 112, 2716–2724.
- Sasse, S.K., Mailloux, C.M., Barczak, A.J., Wang, Q., Altonsy, M.O., Jain, M.K., Haldar, S.M., and Gerber, A.N. (2013). The glucocorticoid receptor and KLF15 regulate gene expression dynamics and integrate signals through feed-forward circuitry. *Mol. Cell. Biol.* 33, 2104–2115.
- Sleight, P., Yusuf, S., Pogue, J., Tsuyuki, R., Diaz, R., and Probstfield, J.; Heart Outcomes Prevention Evaluation (HOPE) Study (2001). Blood-pressure reduction and cardiovascular risk in HOPE study. *Lancet* 358, 2130–2131.
- Storch, K.F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F.C., Wong, W.H., and Weitz, C.J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417, 78–83.
- Tsimakouridze, E.V., Straume, M., Podobed, P.S., Chin, H., LaMarre, J., Johnson, R., Antenos, M., Kirby, G.M., Mackay, A., Huether, P., et al. (2012). Chronomics of pressure overload-induced cardiac hypertrophy in mice reveals altered day/night gene expression and biomarkers of heart disease. *Chronobiol. Int.* 29, 810–821.
- Ueda, H.R., Chen, W., Adachi, A., Wakamatsu, H., Hayashi, S., Takasugi, T., Nagano, M., Nakahama, K., Suzuki, Y., Sugano, S., et al. (2002). A transcription factor response element for gene expression during circadian night. *Nature* 418, 534–539.
- Viitasalo, K., Lindström, J., Hemiö, K., Puttonen, S., Koho, A., Härmä, M., and Peltonen, M. (2012). Occupational health care identifies risk for type 2 diabetes and cardiovascular disease. *Prim. Care Diabetes* 6, 95–102.
- Zhang, R., Lahens, N.F., Ballance, H.I., Hughes, M.E., and Hogenesch, J.B. (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. USA* 111, 16219–16224.