**Supplementary Figure Legends**

**Figure S1**. Negative control targeting mouse *Gapdh* for FISH in the fly brain.

(A) Nuclear stain using DAPI. (B) Mouse *Gapdh* FISH. (C) Brightfield image. (D) Visualization of DAPI and mouse *Gapdh*. DAPI staining is in cyan, mouse *Gapdh* staining is in magenta. All images were taken under 40X objective.

**Figure S2**. *Achl* loss-of-function decreases the number of rhythmic genes in male and female flies. Heatmap of median-normalized expression of genes rhythmically expressed in female control (A), female *Achl* RNAi (B), male control (C) and male *Achl* RNAi flies (D). The top gray and black bars represent subjective day and night, respectively. Yellow indicates higher expression, and blue indicates lower expression (intensity scale at the bottom).

**Figure S3**. Overlap between three methods used to determine differential rhythmicity in control and *Achl* RNAi flies.

Venn diagram showing overlap between three methods used to determine differential expression in female (A) and male (B) flies. For DODR, p-values obtained from robustDODR method and lowest p-value obtained after multiple testing correction were chosen for analysis. For LimoRhyde, the p-value threshold corresponds to a q-value threshold of 0.19 in males and 0.35 in females.

**Figure S4**. *Jhl-21* is a CCG regulated by *Achl*.

*Jhl-21* is rhythmically expressed in both male and female flies (A), and it loses rhythmicity in *Achl* RNAi flies (B). Dark blue line with solid squares: Male control; dark orange line with solid triangles: Female control; light blue line with open squares: Male *Achl* RNAi; light orange line with open triangles: Female *Achl* RNAi.

**Figure S5**. Phase distribution of genes losing rhythmicity in *Achl* RNAi.

(A) Phase distribution of all genes losing rhythmicity in *Achl* RNAi in either male (solid blue bar) or female (hollow orange bar) flies. (B) Pair-wise comparison of twelve genes losing rhythmicity common to both male (blue squares) and female (orange dots) flies. Although there is a difference in overall phase distribution of genes losing rhythmicity in either sex, the phases of genes losing rhythmicity common to both sexes are the same.

**Figure S6**. Comparison of genes rhythmic in both male and female flies.

(A) Simplified GO enrichment of genes rhythmic in both male and female flies. A complete GO table is available as **Table S9**. (B) Pairwise comparison of all genes rhythmic in both male (blue squares) and female (orange dots) flies. Genes are sorted by average phase. Each position on x-axis represents a gene. There is no absolute phase difference for genes rhythmic in both sexes.

**Supplementary Table Legends**

**Table S1**. STAR statistics.

This table shows alignment statistics against *Drosophila melanogaster* chromosomal & mitochondrial genome and transcriptome after the first STAR run.

**Table S2**. Detailed JTK results for core clock genes displayed in **Figure 2B**.

**Table S3**. Genes differentially expressed in control and *Achl* RNAi flies.

**Table S4**. Full Gene Ontology enrichment table for simplified table shown in **Figure 2E**.

Differential expression is detected using LimoRhyde followed by Limma.

**Table S5**. DODR statistics of differential rhythmicity in control and Achl RNAi flies.

This table shows differential rhythmicity in control and *Achl* RNAi flies using DODR all methods.

**Table S6**. LimoRhyde statistics of differential rhythmicity in control and Achl RNAi flies.

This table shows differential rhythmicity in control and *Achl* RNAi flies using LimoRhyde followed by Limma.

**Table S7**. JTK\_CYCLE statistics of genes lose rhythmicity in *Achl* RNAi flies in both male and female.

**Table S8**. Detailed gene names and JTK\_CYCLE p-value for **Figure 4A.**

This table includes genes rhythmic in at least one sex and their corresponding JTK\_CYCLE p-values.

**Table S9**. Full Gene Ontology enrichment table for simplified table shown in **Figure S6**.

This is a full Gene Ontology enrichment table generated by DAVID 6.8 online software.

**Table S10**. Differential rhythmicity in male and female flies detected by DODR and LimoRhyde.

This table shows differential rhythmicity in male and female control flies using DODR and LimoRhyde followed by Limma.

**Table S11**. Full Gene Ontology enrichment table for simplified table shown in **Figure 4B**.

This is a full Gene Ontology enrichment table generated by DAVID 6.8 online software.