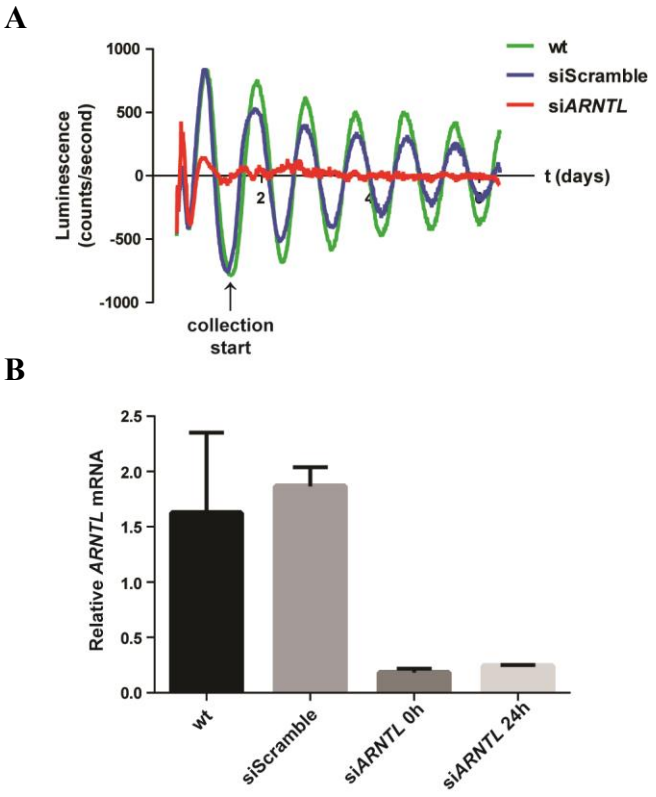


Figure S1. *PER2*-luciferase oscillations and *Arntl* levels in siRNA treated U2OS cells. (A) Luminescence measurements from dexamethasone synchronized U2OS cells expressing luciferase under control of the *PER2* promoter. Treatments with siRNAs are indicated. (B) Quantitative PCR measurements of *Arntl* mRNA in si*ARNTL* cells through the course of the experimental collection.

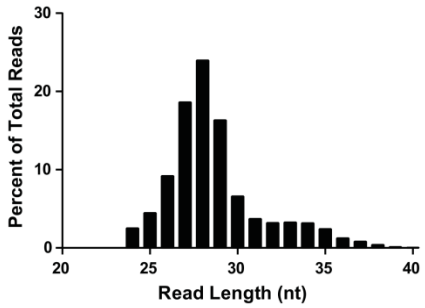
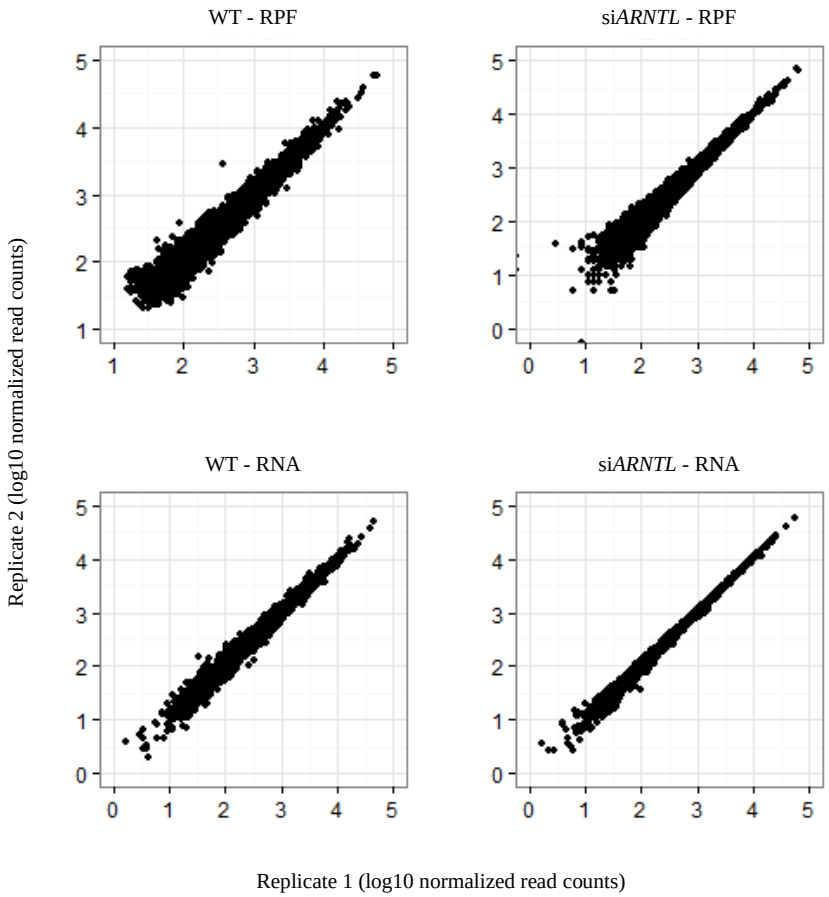


Figure S2. Histogram of the sequenced RPF read lengths across all datasets.

A



B

Spearman correlation coefficients for replicate experiments

CT	WT RPF	si*ARNTL* RPF	WT RNA	si*ARNTL* RNA
0	0.9768	0.9834	0.9926	0.9978
2	0.9652	0.9895	0.9925	0.9959
4	0.9836	0.9876	0.9919	0.9977
6	0.9864	0.9912	0.9921	0.9980
8	0.9832	0.9849	0.9925	0.9915
10	0.9831	0.9885	0.9930	0.9973
12	0.9682	0.9894	0.9927	0.9972
14	0.9858	0.9921	0.9936	0.9805
16	0.9863	0.9907	0.9931	0.9962
18	0.9860	0.9872	0.9918	0.9751
20	0.9866	0.9904	0.9909	0.9969
22	0.9885	0.9907	0.9925	0.9968

Figure S3. Correlation between replicate data. (A) Representative correlation plots between replicates s for CT00, across all experimental conditions. Plots display between log10-transformed, normalized read counts. (B) Table displaying correlation coefficients (Spearman's rho) between replicates, across all experimental conditions and time points.

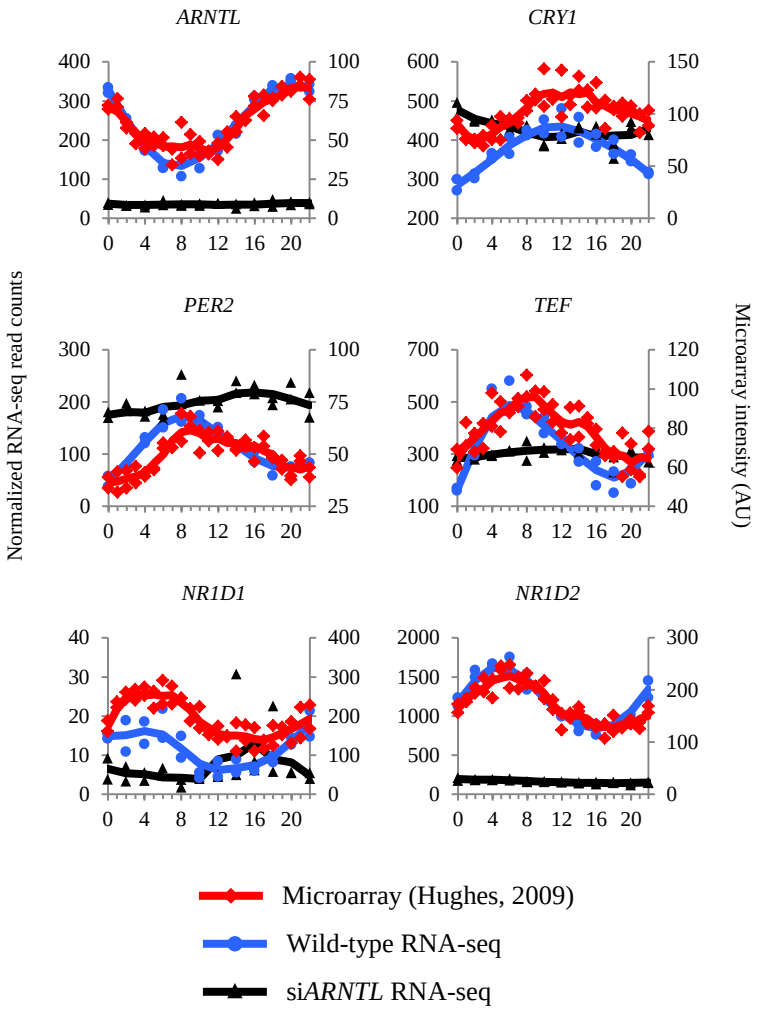
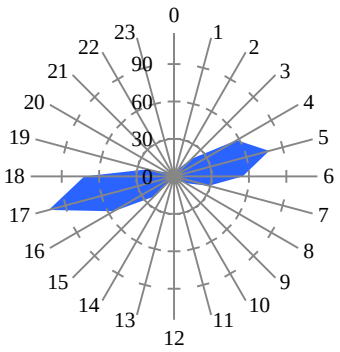


Figure S4. Analysis of mRNA abundance for the canonical clock genes. Normalized read counts from total RNA-seq data obtained in parallel for the core clock genes are plotted alongside mRNA abundance measurements from U2OS cells publically available online at CircaDB (Hughes et al. 2009).

A

All RNA cyclers

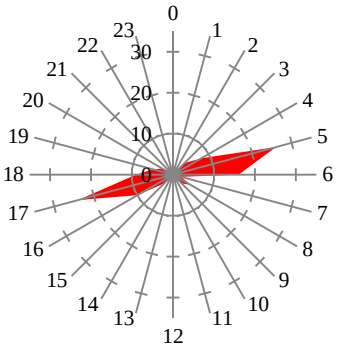


n = 563

B

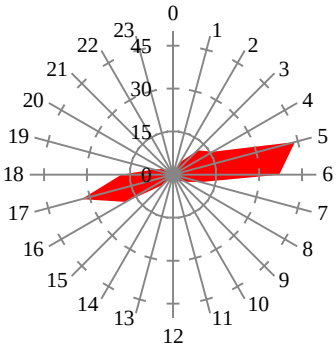
All RPF cyclers

FDR < 0.1



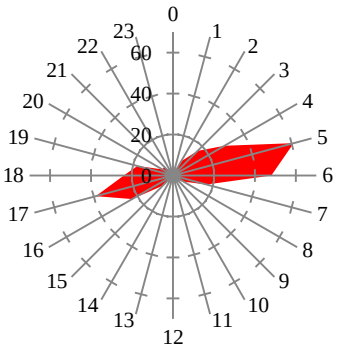
n = 122

FDR < 0.15



n = 224

FDR < 0.2



n = 321

Figure S5. Peak timing for all RNA and RPF oscillators. (A) Radial diagram displaying the number of genes that cycle with a given circadian phase in mRNA accumulation, for all genes that oscillate in the RNA data. (B) Radial diagrams displaying the number of genes that cycle with a given circadian phase in RPF accumulation, for all genes that oscillate in the RPF data at various statistical cutoffs. The number of genes in each set is displayed below the corresponding radial diagrams. The blue and red radial diagrams display phase information for RNA and RPF data, respectively. All phases were calculated using JTK_CYCLE.

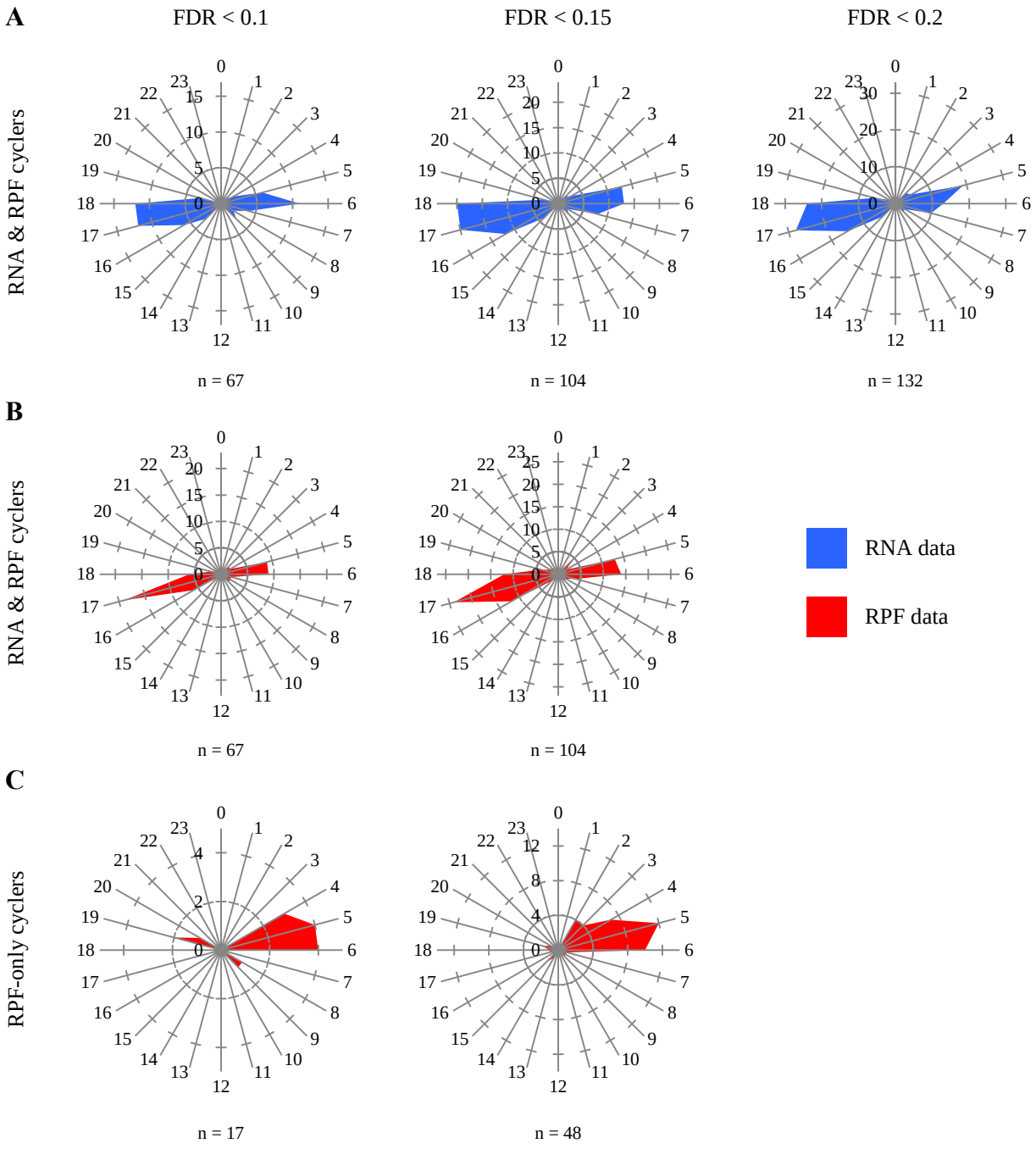
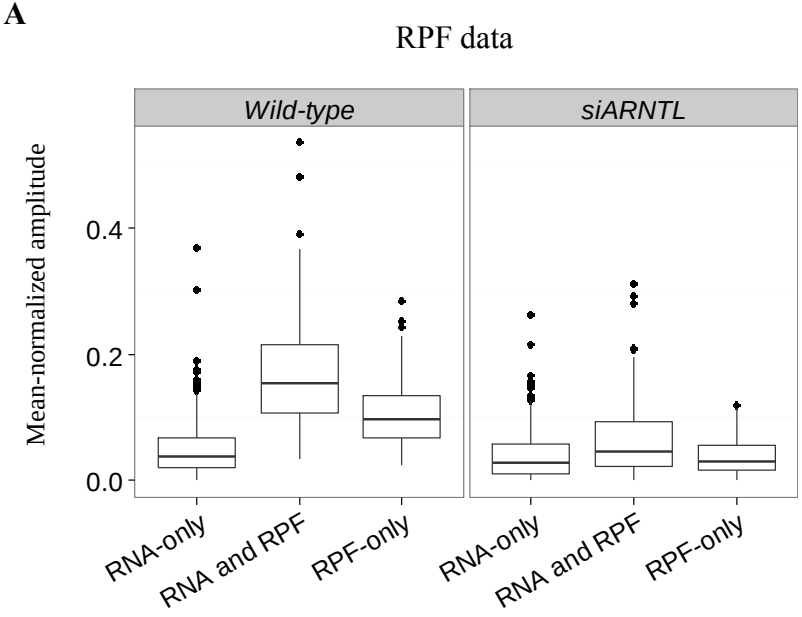

A
FDR < 0.1
FDR < 0.15
FDR < 0.2
RNA & RPF cyclers
n = 67
n = 104
n = 132
B
RNA & RPF cyclers
n = 67
n = 104
RNA data
RPF data
C
RPF-only cyclers
n = 17
n = 48

Figure S6. Peak RNA and RPF timing in cycling genes across various statistical cutoffs. Radial diagrams displaying the number of genes that cycle at the level of mRNA or RPF accumulation with a given circadian phase, at varying statistical cutoffs. Phase information is displayed for (A) RNA and (B) RPF data from genes that oscillate in both the RNA and RP data, as well as (C) RPF data from genes that oscillate in the RPF data only. Phases from RPF data for those genes identified at a statistical cutoff false discovery rate (FDR) < 0.2 are omitted as they are already displayed in Figure 3B and 3C. The blue and red radial diagrams display phase information for RNA and RPF data, respectively. All phases were calculated using JTK_CYCLE.

A


RPF data
Wild-type
siARNTL
Mean-normalized amplitude
0.0
0.2
0.4
RNA-only
RNA and RPF
RPF-only
RNA-only
RNA and RPF
RPF-only

B

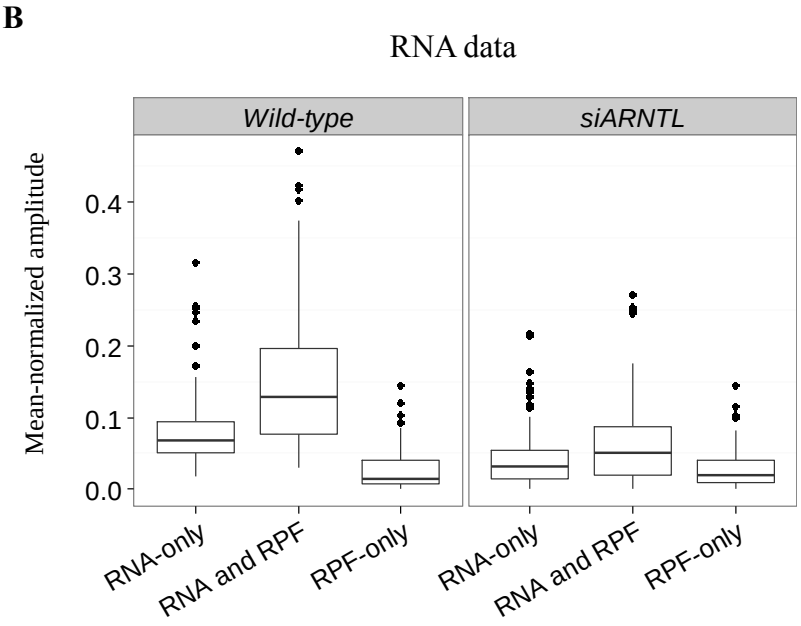

RNA data
Wild-type
siARNTL
Mean-normalized amplitude
0.0
0.1
0.2
0.3
0.4
RNA-only
RNA and RPF
RPF-only
RNA-only
RNA and RPF
RPF-only

Figure S7. Distribution of amplitudes across groups of cycling genes. Box plots depicting the distribution of amplitude values are displayed for genes in each of the three gene groups (RNA-only cyclers, RNA and RPF cyclers, RPF-only cyclers). Since the amplitude values calculated by JTK_CYCLE are proportional to a gene's level of expression, amplitudes were mean-normalized. These normalized amplitudes from (A) RPF data and (B) RNA data are plotted for both the wild-type and si*ARNTL* conditions.

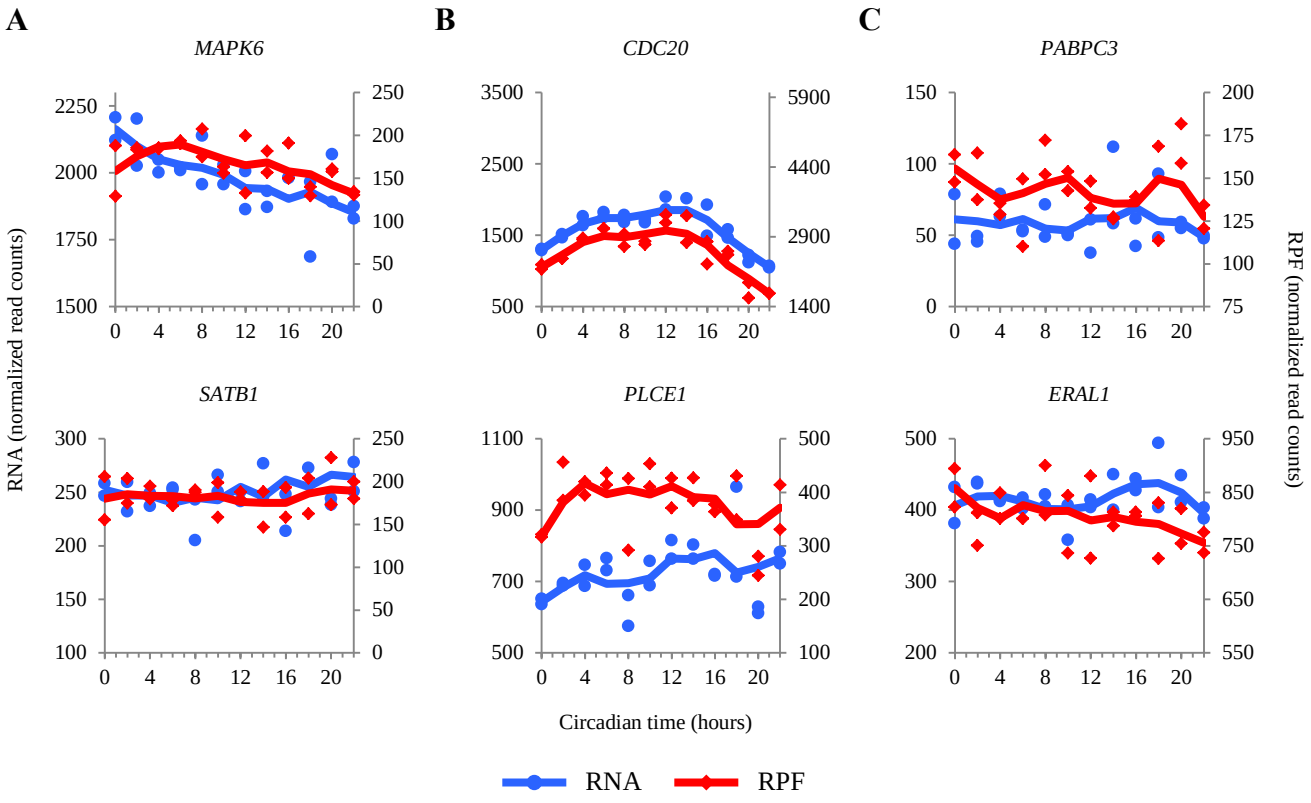


Figure S8. si*ARNTL* data for genes cycling in RNA and RPF data. si*ARNTL* RNA and RPF data for the corresponding traces displayed in Figure 2 D-F. (A) *MAPK6* and *SATB1* oscillate in the RNA data only. (B) *CDC20* and *PLCE1* oscillate in both the RNA and RPF data. (C) *PABPC3* and *ERAL1* oscillate in the RPF data only. Blue traces for the RNA data use the axes on the left. Red traces for the RPF data use the axes on the right. Points from both replicates are displayed and the lines are plotted using a moving average (see *Supplementary Methods* for further detail).

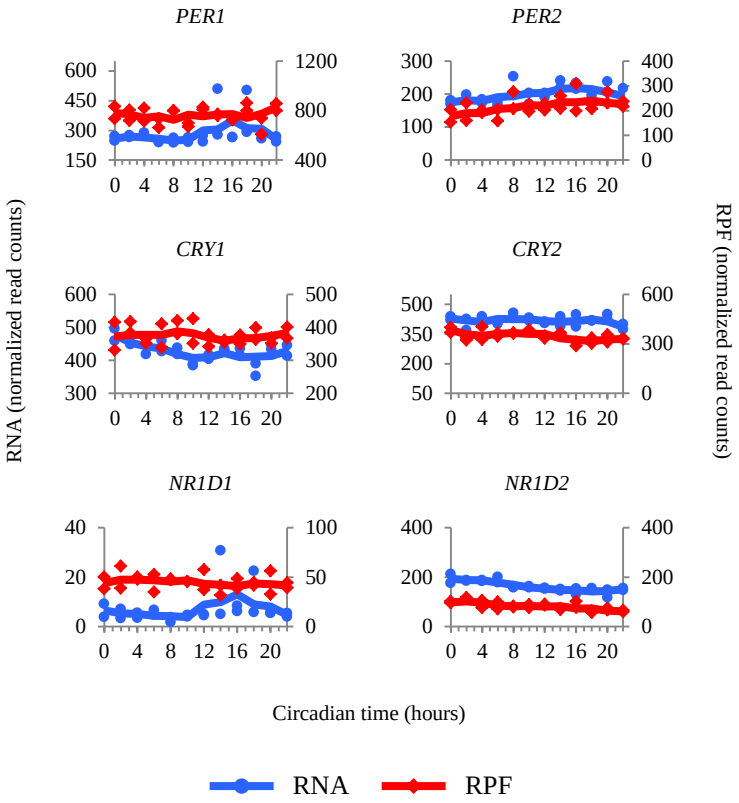


Figure S9. si*ARNTL* data for transcriptional repressors of the core circadian clock. si*ARNTL* RNA and RPF data for the corresponding traces displayed in Figure 3C. Blue traces for the RNA data use the axes on the left. Red traces for the RPF data use the axes on the right. Points from both replicates are displayed and the lines are plotted using a moving average (see *Supplementary Methods* for further detail).

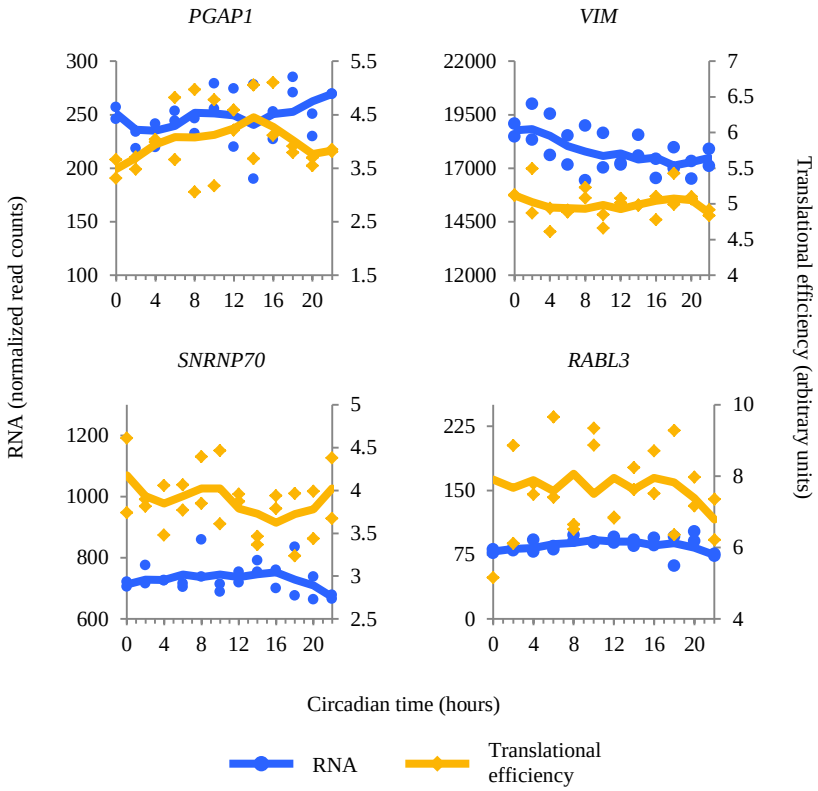


Figure S10. si*ARNTL* data for genes with cycling translational efficiency. si*ARNTL* traces for RNA and translational efficiency (TE) from *PGAP1*, *VIM*, *SNRNP70*, and *RABL3* are plotted as a function of time. These data correspond to those displayed in Figure 4A. Blue traces for the RNA data use the axes on the left. Yellow traces for the TE data use the axes on the right. Points from both replicates are displayed and the lines are plotted using a moving average (see *Supplementary Methods* for further detail).