

Table S1. Primer sequences used in the study.

Gene	Forward sequence (5' → 3')	Reverse sequence (5' → 3')	Product size (bp)
<i>SdhA</i> [1]	CATGCTGCTGTGTTCCGCGA	ACCATCCAGGGGCTTGCTGA	133
<i>Act5C</i> [1]	GGCGCAGAGCAAGCGTGGTA	GGGTGCCACACGCAGCTCAT	124
<i>RpL32</i> [2]	ATGCTAAGCTGTCGCACAAATG	GTTTCGATCCGTAACCGATGT	107
<i>eEF1α2</i> [2]	GCGTGGGTTTGTGATCAGTT	GATCTTCTCCTTGCCCATCC	125
<i>Clk</i>	TCGCTGGTCAACGATCTCAG	GCGATCGGTGGCCTCATTAT	117
<i>cyc</i> [3]	TGGACAATCACCCGAACATAC	CTGAGGCAGGAAACCAATCA	113
<i>per</i> [4]	TGATGGGCGACTACAACCTCC	GTCGCTATTCCCATTGCTGT	89
<i>tim</i> [4]	GGTGGCATCTGTGTACGAAA	GATCTCGGTTGCTCAAGTC	118
<i>Pdp1</i>	GGCCACATAACCACAAAGCG	GGAGGCGAACGAAAATGTTGA	108
<i>vri</i> [4]	ATGAACAACGTCCGGCTATC	CTGCGGACTTATGGATCCTC	114
<i>cry</i>	AGGAATTTGTTACGAGCCCT	GCTCCGATAATGGACTCCG	84
<i>Hsp83</i>	CATACAAGATGCCAGAAGAAGCA	AGATCAACTCGCGCAGGAAA	120
<i>Fst</i>	ATCGTGCAGGAACAGAGGTG	TGACCATTTTCGCCATCCCA	86
<i>smp-30</i> [5]	GAAGGACCATTGTTCCCTGA	TGGGTGGTTGGAATTTTGTAT	140

References

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Table S2. Three-way ANOVAs for the mesor, amplitude, and phase of gene expression in *Drosophila* heads.

	Gene	Main effects			Interaction			
		Age	Sex	Temperature	Age x Sex	Age x Temperature	Sex x Temperature	Age x Sex x Temperature
Mesor	<i>Clk</i>	<0.0001	0.000	<0.0001	0.951	<0.0001	0.794	0.312
	<i>cyc</i>	<0.0001	<0.0001	0.528	0.014	<0.0001	0.162	0.446
	<i>per</i>	<0.0001	0.004	0.036	0.224	0.000	0.911	0.042
	<i>tim</i>	0.013	<0.0001	<0.0001	0.290	<0.0001	0.118	0.003
	<i>Pdp1</i>	<0.0001	<0.0001	<0.0001	0.345	<0.0001	0.005	<0.0001
	<i>vri</i>	<0.0001	0.005	<0.0001	0.044	0.000	0.012	0.950
	<i>cry</i>	0.024	0.022	0.901	0.093	0.076	0.038	0.094
	<i>Hsp83</i>	<0.0001	0.507	0.276	0.306	<0.0001	<0.0001	0.177
	<i>Fst</i>	0.030	0.341	0.004	0.197	0.114	0.847	0.215
	<i>smp-30</i>	<0.0001	<0.0001	0.006	0.000	0.187	0.165	0.031
Amplitude	<i>Clk</i> [^]	<0.0001	-	<0.0001	-	0.001	-	-
	<i>Clk</i> [†]	-	0.008	0.208	-	-	0.546	-
	<i>cyc</i> [*]	0.188	0.032	-	0.977	-	-	-
	<i>per</i>	0.001	0.089	0.001	0.572	0.143	0.940	0.248
	<i>tim</i>	0.790	<0.0001	<0.0001	0.510	<0.0001	0.115	0.015
	<i>Pdp1</i>	0.001	0.020	0.016	0.371	<0.0001	0.025	0.047
	<i>vri</i>	0.646	0.101	0.089	0.218	0.055	0.004	0.875
	<i>cry</i> [‡]	0.571	-	0.068	-	0.061	-	-
	<i>cry</i> [†]	-	0.306	0.001	-	-	0.019	-
	<i>Hsp83</i> [*]	<0.0001	<0.0001	-	0.838	-	-	-
	<i>Fst</i>	NA	NA	NA	NA	NA	NA	NA
<i>smp-30</i>	NA	NA	NA	NA	NA	NA	NA	
Phase	<i>Clk</i> [^]	0.206	-	0.078	-	0.151	-	-
	<i>Clk</i>	-	0.078	<0.0001	-	-	0.360	-
	<i>cyc</i> [*]	0.394	0.637	-	0.111	-	-	-
	<i>per</i>	0.372	0.795	<0.0001	0.777	0.903	0.973	0.499
	<i>tim</i>	0.903	0.458	0.396	0.110	0.898	0.042	0.785
	<i>Pdp1</i>	0.393	0.831	0.050	0.773	0.872	0.041	0.028
	<i>vri</i>	0.636	0.211	0.004	0.092	0.017	0.648	0.431
	<i>cry</i> [‡]	0.426	-	0.002	-	0.001	-	-
	<i>cry</i> [†]	-	0.547	0.049	-	-	0.063	-
	<i>Hsp83</i> [*]	0.001	0.858	-	0.006	-	-	-
	<i>Fst</i>	NA	NA	NA	NA	NA	NA	NA
<i>smp-30</i>	NA	NA	NA	NA	NA	NA	NA	

Bold text show significant values ($p < 0.05$). CircaCompare was used to make pairwise comparisons of simple effects where main effects or interactions were significant. Two-way ANOVAs were performed when one or more profiles being compared were non-rhythmic ($p > 0.05$) and a three-way ANOVA could not be performed. Missing values denoted by 'NA' are shown when neither a three-way or a two-way ANOVA could be computed from the available rhythmic profiles. In these cases, pairwise comparisons were made using CircaCompare. Rhythmicity was not taken into account in the calculation for mesor, so three-way ANOVAs were performed. The following annotations indicate the configurations of the two-way ANOVAs: *cyc*^{*}, a two-way ANOVA was performed (2 levels sex, 2 levels age) for the T_{cyc} profiles; *Clk*[^], a two-way ANOVA was performed (2 levels age, 2 levels temperature) for the male profiles; *Clk*[†], a two-way ANOVA was performed (2 levels sex, 2 levels temperature) for the aged profiles; *cry*[‡], a two-way ANOVA was performed (2 levels age, 2 levels temperature) for the female profiles; *cry*[†], a two-way ANOVA was performed (2 levels sex, 2 levels temperature) for the aged profiles; *Hsp83*^{*} a two-way ANOVA was performed (2 levels sex, 2 levels age) for the T_{cyc} profiles.

Table S3. Table of p-values of pairwise comparisons made in CircaCompare for gene expression in *Drosophila* heads.

	Heads			<i>Clk</i>	<i>cyc</i>	<i>per</i>	<i>tim</i>	<i>Pdp1</i>	<i>vri</i>	<i>cry</i>	<i>Hsp83</i>	<i>Fst</i>	<i>smp-30</i>	
Mesor	Effect of age	Male	T _{CON}	0.239	0.000	0.014	0.000	0.083	0.158	0.000	0.000	0.130	0.000	
			T _{CYC}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.878	0.000	0.398	0.013
		Female	T _{CON}	0.011	0.000	0.000	0.000	0.020	0.414	0.369	0.000	0.000	0.185	0.000
			T _{CYC}	0.000	0.000	0.003	0.000	0.000	0.001	0.697	0.000	0.000	0.104	0.000
	Effect of temperature	Male	Young	0.000	0.183	0.148	0.000	0.000	0.002	0.216	0.758	0.798	0.645	
			Aged	0.465	0.005	0.000	0.370	0.006	0.016	0.046	0.000	0.000	0.067	0.000
		Female	Young	0.000	0.009	0.849	0.000	0.000	0.002	0.194	0.001	0.022	0.000	
			Aged	0.545	0.222	0.039	0.170	0.052	0.426	0.000	0.414	0.000	0.000	
	Effect of gender	T _{CON}	Young	0.017	0.004	0.002	0.000	0.000	0.080	0.000	0.015	0.176	0.000	
			Aged	0.003	0.000	0.055	0.000	0.000	0.000	0.121	0.000	0.252	0.075	
		T _{CYC}	Young	0.308	0.031	0.927	0.488	0.223	0.601	0.959	0.178	0.479	0.188	
			Aged	0.001	0.000	0.009	0.000	0.000	0.081	0.756	0.001	0.005	0.000	
Amplitude	Effect of age	Male	T _{CON}	0.676	n.r.	0.342	0.000	0.043	0.838	0.009	#	0.246	#	
			T _{CYC}	0.000	0.435	0.017	0.014	0.001	0.154	#	0.000	#	n.r.	
		Female	T _{CON}	0.639	n.r.	0.005	0.005	0.149	0.079	0.023	#	0.690	#	
			T _{CYC}	#	0.147	0.245	0.005	0.004	0.564	0.438	0.000	0.604	#	
	Effect of temperature	Male	Young	0.000	#	0.749	0.000	0.000	0.051	#	#	0.547	#	
			Aged	0.253	#	0.003	0.171	0.385	0.109	0.487	0.000	#	n.r.	
		Female	Young	#	#	0.141	0.000	0.450	0.771	0.981	0.000	0.435	#	
			Aged	0.581	#	0.041	0.677	0.036	0.025	0.000	#	0.278	#	
	Effect of gender	T _{CON}	Young	0.522	n.r.	0.043	0.000	0.007	0.076	0.133	#	0.544	0.361	
			Aged	0.009	n.r.	0.292	0.000	0.032	0.000	0.338	#	0.159	n.r.	
		T _{CYC}	Young	#	0.261	0.983	0.434	0.742	0.429	#	0.004	0.437	n.r.	
			Aged	0.193	0.035	0.132	0.000	0.479	0.933	0.022	0.000	#	#	
Phase	Effect of age	Male	T _{CON}	0.008	n.r.	0.252	0.093	0.133	0.520	0.445	n.r.	n.r.	n.r.	
			T _{CYC}	0.927	0.091	0.805	0.708	0.330	0.054	n.r.	0.001	n.r.	n.r.	
		Female	T _{CON}	0.216	n.r.	0.863	0.094	0.012	0.045	0.031	n.r.	n.r.	n.r.	
			T _{CYC}	n.r.	0.579	0.603	0.337	0.595	0.806	0.013	0.711	n.r.	n.r.	
	Effect of temperature	Male	Young	0.863	n.r.	0.016	0.607	0.438	0.830	n.r.	n.r.	n.r.	n.r.	
			Aged	0.001	n.r.	0.002	0.530	0.063	0.000	0.017	0.607	n.r.	n.r.	
		Female	Young	n.r.	n.r.	0.029	0.083	0.068	0.320	0.004	0.586	n.r.	n.r.	
			Aged	0.007	n.r.	0.000	0.014	0.011	0.000	0.932	n.r.	n.r.	n.r.	
	Effect of gender	T _{CON}	Young	0.100	n.r.	0.637	0.006	0.031	0.304	0.171	n.r.	n.r.	0.023	
			Aged	0.028	n.r.	0.271	0.622	0.225	0.937	0.401	n.r.	n.r.	n.r.	
		T _{CYC}	Young	n.r.	0.281	0.843	0.737	0.309	0.233	n.r.	0.277	n.r.	n.r.	
			Aged	0.599	0.471	0.933	0.002	0.734	0.536	0.061	0.015	n.r.	n.r.	

Bold text show significant values ($p < 0.05$). 'n.r.' indicate comparisons where one or both profiles being compared were non-rhythmic ($p > 0.05$). The annotation '#' indicate significant changes in rhythmicity, where a rhythmic profile was being compared with a non-rhythmic profile, and was considered a significant change in amplitude.

Table S4. Three-way ANOVAs for the mesor, amplitude, and phase of gene expression in *Drosophila* bodies.

		Main effects			Interaction			
		Age	Sex	Temperature	Age x Sex	Age x Temperature	Sex x Temperature	Age x Sex x Temperature
Mesor	<i>Clk</i>	0.278	<0.0001	0.017	0.019	0.787	0.004	0.112
	<i>cyc</i>	<0.0001	<0.0001	0.025	0.004	0.482	0.020	0.230
	<i>per</i>	0.409	<0.0001	0.185	0.288	0.547	0.495	0.250
	<i>tim</i>	<0.0001	<0.0001	0.134	<0.0001	0.001	0.040	0.001
	<i>Pdp1</i>	<0.0001	<0.0001	0.053	<0.0001	0.038	<0.0001	0.011
	<i>vri</i>	0.352	<0.0001	0.004	0.332	0.343	0.796	0.233
	<i>cry</i>	<0.0001	<0.0001	0.017	<0.0001	0.524	0.109	0.404
	<i>Hsp83</i>	<0.0001	<0.0001	0.101	<0.0001	0.006	0.575	0.044
	<i>Fst</i>	0.148	0.015	0.004	0.745	0.012	0.427	0.658
<i>smp-30</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Amplitude	<i>Clk</i>	0.039	<0.0001	0.132	0.511	0.298	0.106	0.667
	<i>cyc</i>	NA	NA	NA	NA	NA	NA	NA
	<i>per</i>	0.646	<0.0001	<0.0001	0.713	0.086	0.053	0.076
	<i>tim</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.005	0.000
	<i>Pdp1</i>	0.011	<0.0001	0.017	<0.0001	0.002	0.001	0.028
	<i>vri</i>	0.145	<0.0001	0.026	0.016	0.174	0.906	0.146
	<i>cry</i>	<0.0001	<0.0001	0.182	<0.0001	0.251	0.598	0.432
	<i>Hsp83*</i>	0.005	0.032	-	0.271	-	-	-
	<i>Fst</i>	NA	NA	NA	NA	NA	NA	NA
<i>smp-30</i>	NA	NA	NA	NA	NA	NA	NA	
Phase	<i>Clk</i>	0.696	0.159	<0.0001	0.421	0.506	0.897	0.274
	<i>cyc</i>	NA	NA	NA	NA	NA	NA	NA
	<i>per</i>	0.518	0.161	0.007	0.735	0.207	0.637	0.727
	<i>tim</i>	0.007	0.002	0.029	0.113	0.215	0.300	0.521
	<i>Pdp1</i>	0.703	0.071	0.028	0.527	0.471	0.391	0.582
	<i>vri</i>	0.280	0.374	0.069	0.417	0.002	0.912	0.730
	<i>cry</i>	0.872	0.702	0.106	0.625	0.948	0.276	0.657
	<i>Hsp83*</i>	0.065	0.007	-	0.046	-	-	-
	<i>Fst</i>	NA	NA	NA	NA	NA	NA	NA
<i>smp-30</i>	NA	NA	NA	NA	NA	NA	NA	

Bold text show significant values ($p < 0.05$). CircaCompare was used to make pairwise comparisons of simple effects where main effects or interactions were significant. Two-way ANOVAs were performed when one or more profiles being compared were non-rhythmic ($p > 0.05$) and a three-way ANOVA could not be performed. Missing values denoted by 'NA' are shown when neither a three-way or a two-way ANOVA could be computed from the available rhythmic profiles. In these cases, pairwise comparisons were made using CircaCompare. Rhythmicity was not taken into account in the calculation for mesor, so three-way ANOVAs were performed. *hsp** indicates a two-way ANOVA (2 levels age, 2 levels sex) was performed for the T_{CVC} profiles for *Hsp83*.

Table S5. Table of p-values of pairwise comparisons made in CircaCompare for gene expression in *Drosophila* bodies.

		Bodies		<i>Clk</i>	<i>cyc</i>	<i>per</i>	<i>tim</i>	<i>Pdp1</i>	<i>vri</i>	<i>cry</i>	<i>Hsp83</i>	<i>Fst</i>	<i>smp-30</i>	
Mesor	Effect of age	Male	T _{CON}	0.005	0.166	0.270	0.000	0.000	0.414	0.000	0.000	0.197	0.000	
			T _{CYC}	0.620	0.126	0.586	0.001	0.000	0.539	0.000	0.001	0.013	0.000	
		Female	T _{CON}	0.043	0.211	0.022	0.281	0.023	0.090	0.000	0.000	0.088	0.000	
			T _{CYC}	0.969	0.003	0.070	0.348	0.002	0.092	0.000	0.000	0.006	0.165	
	Effect of temperature	Male	Young	0.213	0.281	0.922	0.055	0.224	0.077	0.592	0.839	0.364	0.685	
			Aged	0.003	0.050	0.150	0.001	0.000	0.271	0.000	0.006	0.021	0.000	
		Female	Young	0.491	0.356	0.289	0.007	0.039	0.590	0.549	0.422	0.980	0.003	
			Aged	0.158	0.012	0.912	0.003	0.009	0.000	0.268	0.001	0.007	0.000	
	Effect of gender	T _{CON}	Young	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	
			Aged	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.211	0.000	
		T _{CYC}	Young	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000
			Aged	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Amplitude	Effect of age	Male	T _{CON}	0.023	n.r.	0.003	0.000	0.000	0.015	0.000	#	n.r.	n.r.	
			T _{CYC}	0.696	0.514	0.476	0.045	0.225	0.695	0.000	0.000	0.174	#	
		Female	T _{CON}	0.104	n.r.	0.947	0.184	0.262	0.406	0.000	n.r.	n.r.	#	
			T _{CYC}	0.562	n.r.	0.913	0.634	0.025	0.484	0.010	0.294	#	n.r.	
	Effect of temperature	Male	Young	0.551	#	0.319	0.924	0.839	0.807	0.988	0.000	#	n.r.	
			Aged	0.049	#	0.000	0.000	0.000	0.058	0.000	#	#	#	
		Female	Young	0.553	n.r.	0.039	0.147	0.267	0.041	0.780	#	n.r.	#	
			Aged	0.691	n.r.	0.010	0.401	0.940	0.105	0.027	#	#	n.r.	
	Effect of gender	T _{CON}	Young	0.000	n.r.	0.000	0.000	0.000	0.000	0.000	#	n.r.	n.r.	
			Aged	0.000	n.r.	0.000	0.000	0.049	0.000	0.211	n.r.	n.r.	#	
		T _{CYC}	Young	0.000	#	0.002	0.000	0.000	0.000	0.000	0.000	0.093	#	#
			Aged	0.000	#	0.000	0.000	0.000	0.000	0.000	0.000	0.321	0.985	n.r.
Phase	Effect of age	Male	T _{CON}	0.299	n.r.	0.402	0.226	0.405	0.130	0.857	n.r.	n.r.	n.r.	
			T _{CYC}	0.245	0.025	0.783	0.775	0.440	0.165	0.948	0.000	0.915	n.r.	
		Female	T _{CON}	0.705	n.r.	0.181	0.012	0.691	0.024	0.638	n.r.	n.r.	n.r.	
			T _{CYC}	0.467	n.r.	0.791	0.309	0.584	0.362	0.914	0.929	n.r.	n.r.	
	Effect of temperature	Male	Young	0.040	n.r.	0.234	0.292	0.210	0.469	0.348	0.452	n.r.	n.r.	
			Aged	0.000	n.r.	0.131	0.758	0.383	0.035	0.054	n.r.	n.r.	n.r.	
		Female	Young	0.003	n.r.	0.824	0.045	0.139	0.310	0.729	n.r.	n.r.	n.r.	
			Aged	0.031	n.r.	0.113	0.518	0.373	0.009	0.930	n.r.	n.r.	n.r.	
	Effect of gender	T _{CON}	Young	0.414	n.r.	0.421	0.048	0.176	0.384	0.952	n.r.	n.r.	n.r.	
			Aged	0.486	n.r.	0.555	0.242	0.189	0.953	0.405	n.r.	n.r.	n.r.	
		T _{CYC}	Young	0.271	n.r.	0.778	0.379	0.949	0.331	0.717	0.598	n.r.	n.r.	
			Aged	0.893	n.r.	0.785	0.668	0.141	0.893	0.313	0.000	0.002	n.r.	

Bold text show significant values ($p < 0.05$). 'n.r.' indicate comparisons where one or both profiles being compared were non-rhythmic ($p > 0.05$). The annotation '#' indicate significant changes in rhythmicity, where a rhythmic profile was being compared with a non-rhythmic profile, and was considered a significant change in amplitude.