amplify inflammation in humans as well as mice (fig. S15).

Mortality from sepsis ranges between 30 and 50% and is rising because of drug-resistant organisms, a growing elderly population, and an increased incidence of immunosuppression (*25*–*28*). The failures of anti–Toll-like receptor 4, recombinant activated protein C, and anti–TNF-α therapies in clinical trials necessitate a rethinking of sepsis' pathophysiology (*6*, *29*–*33*). Because many early-phase inflammatory cytokines operate concurrently and redundantly, identifying upstream triggers may generate therapies with broad downstream benefits. Altogether, the evidence shown here supports the hypothesis that IL-3 mediates experimental and human sepsis, is a major upstream orchestrator of the septic inflammatory phase, and can be harnessed for therapeutic intervention.

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SUPPLEMENTARY MATERIALS

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CIRCADIAN RHYTHMS

Time-restricted feeding attenuates age-related cardiac decline in *Drosophila*

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Circadian clocks orchestrate periods of rest or activity and feeding or fasting over the course of a 24-hour day and maintain homeostasis. To assess whether a consolidated 24-hour cycle of feeding and fasting can sustain health, we explored the effect of time-restricted feeding (TRF; food access limited to daytime 12 hours every day) on neural, peripheral, and cardiovascular physiology in *Drosophila melanogaster*. We detected improved sleep, prevention of body weight gain, and deceleration of cardiac aging under TRF, even when caloric intake and activity were unchanged. We used temporal gene expression profiling and validation through classical genetics to identify the TCP-1 ring complex (TRiC) chaperonin, the mitochondrial electron transport chain complexes, and the circadian clock as pathways mediating the benefits of TRF.

To determine whether a daily rhythm of feeding and fasting without reducing caloric intake can improve health metrics, we subjected a 2-week-old wild-type (WT) Oregon-R strain (table S1) of *Drosophila melanogaster* adults to ad libitum feeding (ALF) or 12-hour time-restricted feeding (TRF) of a standard cornmeal diet exclusively during daytime. At nighttime, the TRF cohorts were placed in vials with 1.1% agar to prevent desiccation (fig. S1A). The daily food intake was equivalent in both groups, although ALF flies consumed some of their food during nighttime (Fig. 1A). Unlike ALF flies, the TRF group did not gain body weight at 5 and 7 weeks of age (Fig. 1B). The ability to fly (flight index) was slightly improved in the TRF group (Fig. 1C). Although the total daily activity was equivalent between both groups of flies (Fig. 1D), the TRF flies were more active during daytime. Sleep (defined as five consecutive minutes of inactivity) (*1*) assessment revealed that flies on TRF had less daytime sleep, but more nighttime and more total sleep, than the ALF flies (Fig. 1E and fig. S1).

Increase in sleep duration correlates with improved cardiac function (*2*). Therefore, by high-speed video imaging of ex vivo denervated hearts bathed in artificial hemolymph (*3*), we measured the diameter of the beating *Drosophila* heart at full relaxation and contraction and the time interval between successive contractions to calculate cardiac function parameters (Fig. 2A). At 3 weeks of age, the performance of both ALF and TRF hearts was indistinguishable with equivalent heart period (HP), systolic diameter (SD), systolic interval (SI), diastolic diameter (DD), diastolic interval (DI), arrhythmia index (AI), and heart contractility, measured as fractional shortening (FS) (Fig. 2, B to F; fig. S2; and movie S1). In the next 2 weeks, the cardiac performance in ALF flies exhibited characteristic age-dependent deterioration (*4*), with increased SI, DI, HP, and AI and reduced DD, SD, and FS. TRF flies showed smaller changes in these cardiac performance parameters in both genders (fig. S2).

We investigated whether a limited period of TRF early or late in life could attenuate age-dependent

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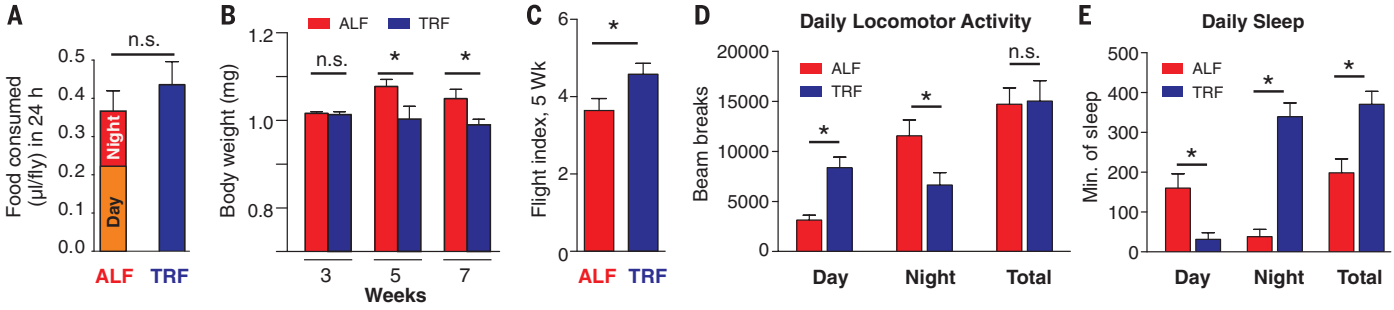

Fig. 1. TRF improves sleep and prevents body weight gain without reducing caloric intake. (**A**) Food consumption (Capillary Feeder or CAFÉ assay) over a 24-hour period in 5-week-old WT Oregon-R flies. (**B**) Body weight of 3-, 5-, and 7-week-old flies. (**C**) Flight index of 5-week-old flies ($n > 30$ flies). (**D**) Activity counts and (**E**) sleep duration of 5-week-old flies averaged from at least 7 days of recording. Values are mean + SEM, $*P < 0.05$, not significant (n.s.): $P > 0.05$, Student's t test.

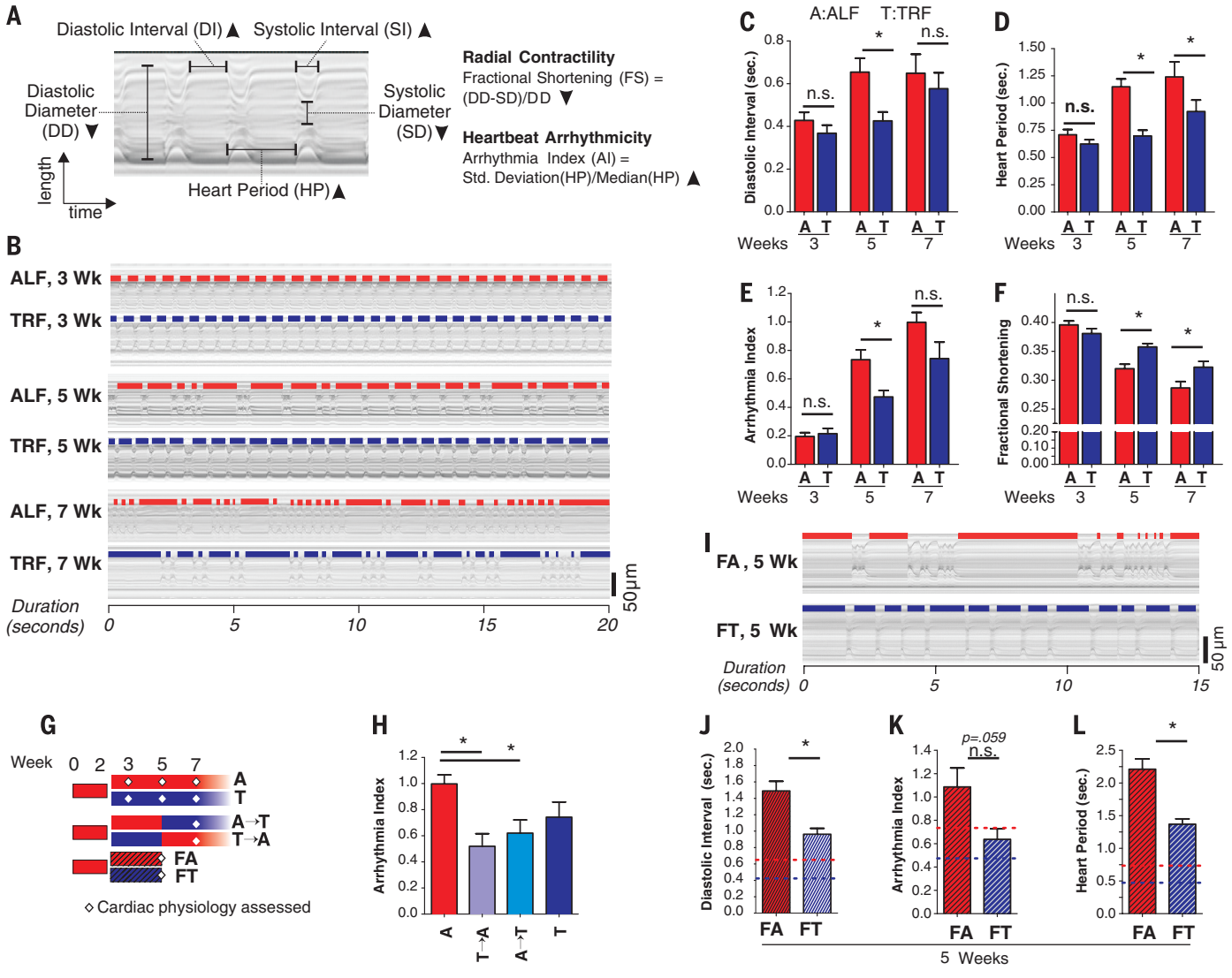

Fig. 2. TRF protects against age- and diet-induced decline in cardiac function. (**A**) M-mode (mechanical mode) traces showing the movement of the heart tube edge (y axis) over time (x axis) were generated from videos of the heart beneath the third thoracic segment by digitally excising and aligning a 1-pixel-wide vertical strip spanning the heart tube from a fixed location in successive frames. From the M-mode, cardiac parameters are calculated. Arrowheads indicate the direction of age- or high-fat diet–induced changes. (**B**) Example 20-s M-mode traces of ALF and TRF flies with superimposed orange (ALF) or blue (TRF) bar indicating diastolic intervals. Average (**C**) DI, (**D**) HP, (**E**) AI, and (**F**) FS show protection from age-dependent deterioration in the TRF flies. (**G**) Feeding regimens used to test the effect of TRF at an early or late age revealed improvement in (**H**) AI. (**I**) Representative M-modes of 5-week-old flies subjected to fat diet ALF (FA) or TRF (FT). Average (**J**) DI, (**K**) AI, and (**L**) HP improved under TRF. Average values for ALF and TRF flies fed normal cornmeal diets are shown as broken lines for reference. Averages ($n > 30$) are shown. $*P < 0.05$, Mann Whitney test. Error bars: SEM.

decline in cardiac performance. Flies on ALF or TRF at 5 weeks of age were switched to the TRF or ALF condition, respectively (Fig. 2G). In both groups, 7-week-old flies showed improvement in some (but not all; see fig. S3) parameters, including reduced HP and AI, as well as increased FS relative to that of flies maintained in ALF for 7 weeks (Fig. 2H).

Because fat-containing diets deteriorate cardiac performance (*5*, *6*), we tested the effect of TRF on flies fed a standard cornmeal diet supplemented with 2% w/v coconut oil. Flies on this fat diet ad libitum (FA) for 3 weeks showed severe deterioration of cardiac performance relative to standard cornmeal-fed counterparts, including long HP, increased AI, and reduced FS. Yet flies fed the same fat-supplemented diet under TRF condition (FT; access to fat diet for 12 hours of daytime) showed smaller declines in cardiac performance (Fig. 2, I to L; fig. S4; and movie S2) relative to the FA cohort.

The improved cardiac function under TRF could result from systemic changes, local changes in the heart, or both. We measured RNA concentrations in the head and periphery (i.e., entire fly except the head) of 3-, 5-, and 7-week-old ALF and TRF (standard diet) flies collected every 6 hours over 24 hours (ZT or zeitgeber time 0, 6, 12, and 18). In TRF flies, the gene expression signature had no resemblance to that of flies exposed to starvation (*7*) or dietary restriction (DR) (*8*) (figs. S5 and S6 and tables S2 and S3). No transcript showed a large change (fold change > 2 between ALF and TRF group, $P < 0.05$) at both 5 and 7 weeks of age, indicating that either the diurnal expression pattern or a small but concerted change in the expression level of multiple, functionally related genes might account for the health benefits of TRF.

To assess diurnal gene expression, we examined transcripts from the head and the periphery of 5-week-old flies (on normal diet; ALF or TRF) at eight different time points spanning 24 hours. A total of 868 transcripts in the head and 1233 transcripts in the periphery were defined as rhythmic under both ALF and TRF conditions ($P < 0.05$, 22 hours < period < 26 hours) (Fig. 3, A and B, and tables S4 and S5). There were differences in the amplitude and synchrony of these oscillating transcripts in ALF and TRF flies. The amplitude (peak-to-trough difference)

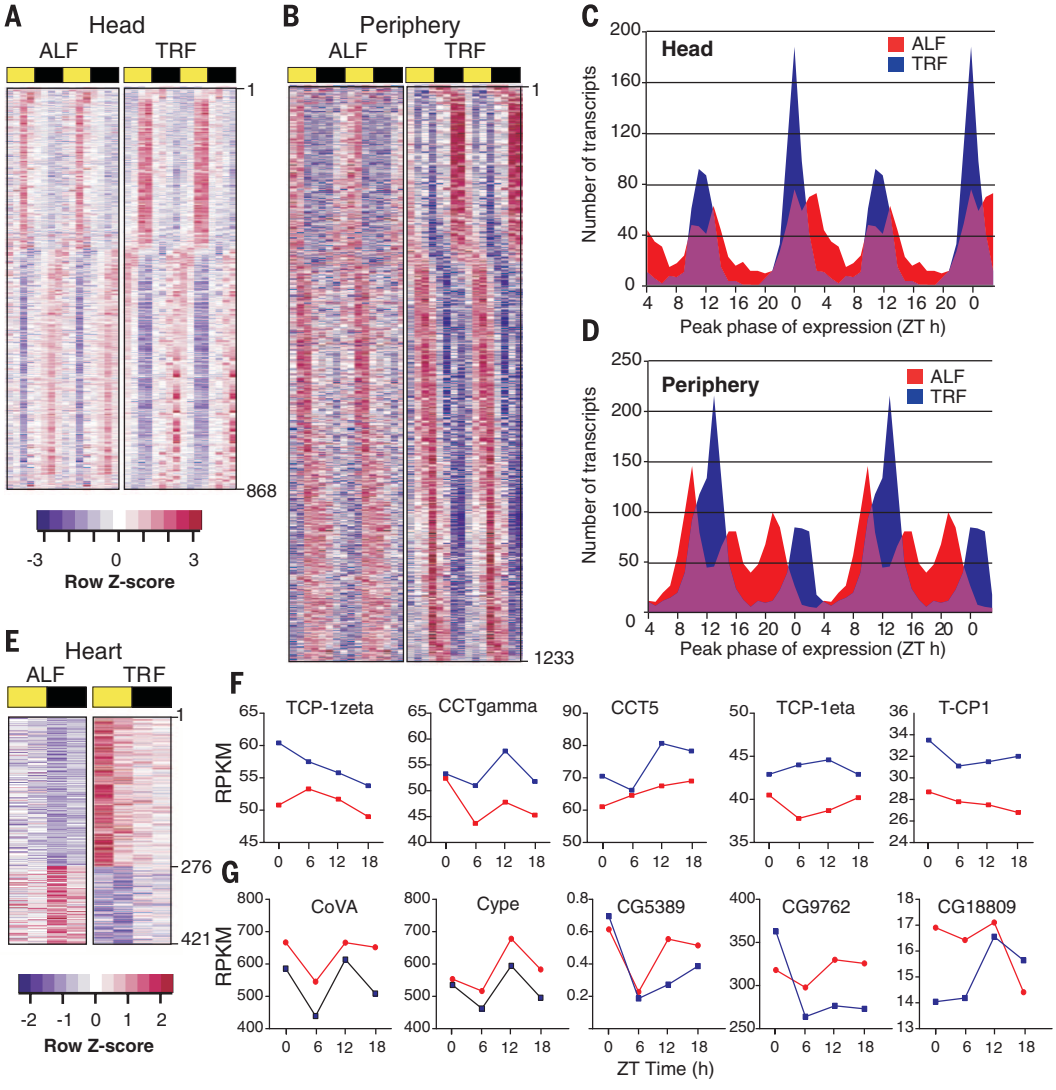

Fig. 3. Transcriptional correlates of improved health in TRF. Heat-map representation of transcripts scored rhythmic under both ALF and TRF conditions in (**A**) head and (**B**) periphery of 5-week-old flies. Normalized and color-coded transcript levels at eight different Zeitgeber times (ZT) spanning day (yellow bar) and night (dark bar) are shown. Area plots showing the peak phase of expression of rhythmic transcripts in ALF (red) or TRF (blue) fly (**C**) head or (**D**) periphery binned into 1-hour intervals. For clarity, data in (A) to (D) are double plotted over two 24-hour periods. (**E**) Heat-map representation of transcripts that are up- (purple) or down- (blue) regulated in TRF flies. Transcript levels in fly hearts collected at 6-hour intervals over 24 hours are shown. (**F**) Expression level (RPKMs; reads per kilobase of gene model per million reads uniquely aligned to the genome) of example TRiC chaperonin subunits and (**G**) ETC components in ALF and TRF hearts at 5 weeks age.

of oscillation of 876 transcripts (71%) from the periphery and 516 transcripts from the head (59%) increased under the TRF condition. Furthermore, the peak phases of expression of rhythmic transcripts in ALF flies were distributed over 24 hours, whereas TRF consolidated the peak phases to two principal times of the day corresponding to the ends of the feeding and fasting periods (Fig. 3, C and D). These synchronous transcript oscillations may coordinate fasting- or feeding-related metabolism to the appropriate time. As seen in mice (*9*), the combined action of the molecular circadian clock and the imposed rhythm of feeding and fasting may improve gene expression rhythms under TRF and offer systemic metabolic benefit.

To identify the transcriptomic correlates of improved cardiac physiology, we measured cardiac gene expression in 5-week-old ALF and TRF flies every 6 hours over a 24-hour period. Heart-enriched transcripts including *Hand*, *Tinman*, *He*, and *H15* (*10*) were confirmed to be more abundant in the heart than in the head and the periphery. Comparisons between ALF and TRF yielded 145 and 276 transcripts that showed decreased or increased expression, respectively, at all four time points in the TRF hearts (Fig. 3, E to G, and table S6). Functional annotation of these transcripts identified the adenosine 5′-triphosphate (ATP)–dependent TCP-1 ring complex (TRiC) (also known as chaperonin-containing TCP-1; CCT) chaperonin (*11*) and mitochondrial electron transport chain (ETC) as the top functional clusters with increased or decreased expression in the TRF heart, respectively. Seven out of eight TRiC subunit RNAs were more abundant at all four time points (fig. S7 and table S8). Concurrently, mRNAs encoding 52 components of the ETC were decreased in abundance at three out of four time points and 27 at all four time points in TRF hearts (fig. S8 and table S7). Thus, we considered the circadian clock, TRiC chaperonin, and mitochondrial ETC as potentially mediating the beneficial cardioprotective effects of TRF.

The *Drosophila* circadian oscillator is based on a transcriptional negative feedback loop generated by the activators clock (CLK) and cycle (CYC) and the repressors period (PER) and timeless (TIM) (*12*). To test the role of the molecular clock in TRF-dependent improvement of cardiac physiology, we examined flies carrying loss-of-function mutations in oscillator components: *clk*, *cyc*, *per*, or *tim*. These fly strains lacking both molecular and behavioral circadian rhythms are born without major morphological defects of the heart. Although these mutants all lacked a functional circadian oscillator, cardiac performance in 5-week-old flies was variably affected under ALF (Fig. 4A and fig. S9). For example, the heart period of per^{01} and clk^{ar} mutants was comparable to that of WT, whereas cyc^{01} and tim^{01} mutants showed slower heart rate (Fig. 4A). The WT flies on TRF showed improved relaxation-contraction function relative to their ALF counterparts, as reflected in decreased heart period and arrhythmicity. However, the heart period increased in clk^{ar} mutant flies and did not change significantly in per^{01} and cyc^{01} mutants. TRF increased arrhythmia in cyc^{01} flies and had smaller or no significant effect in per^{01}, tim^{01}, or clk^{ar} flies (Fig. 4, A and B, and movie S3). Thus, imposition of a diurnal feeding rhythm was insufficient for protecting against cardiac aging unless endogenous circadian oscillations were intact (figs. S9 and S10).

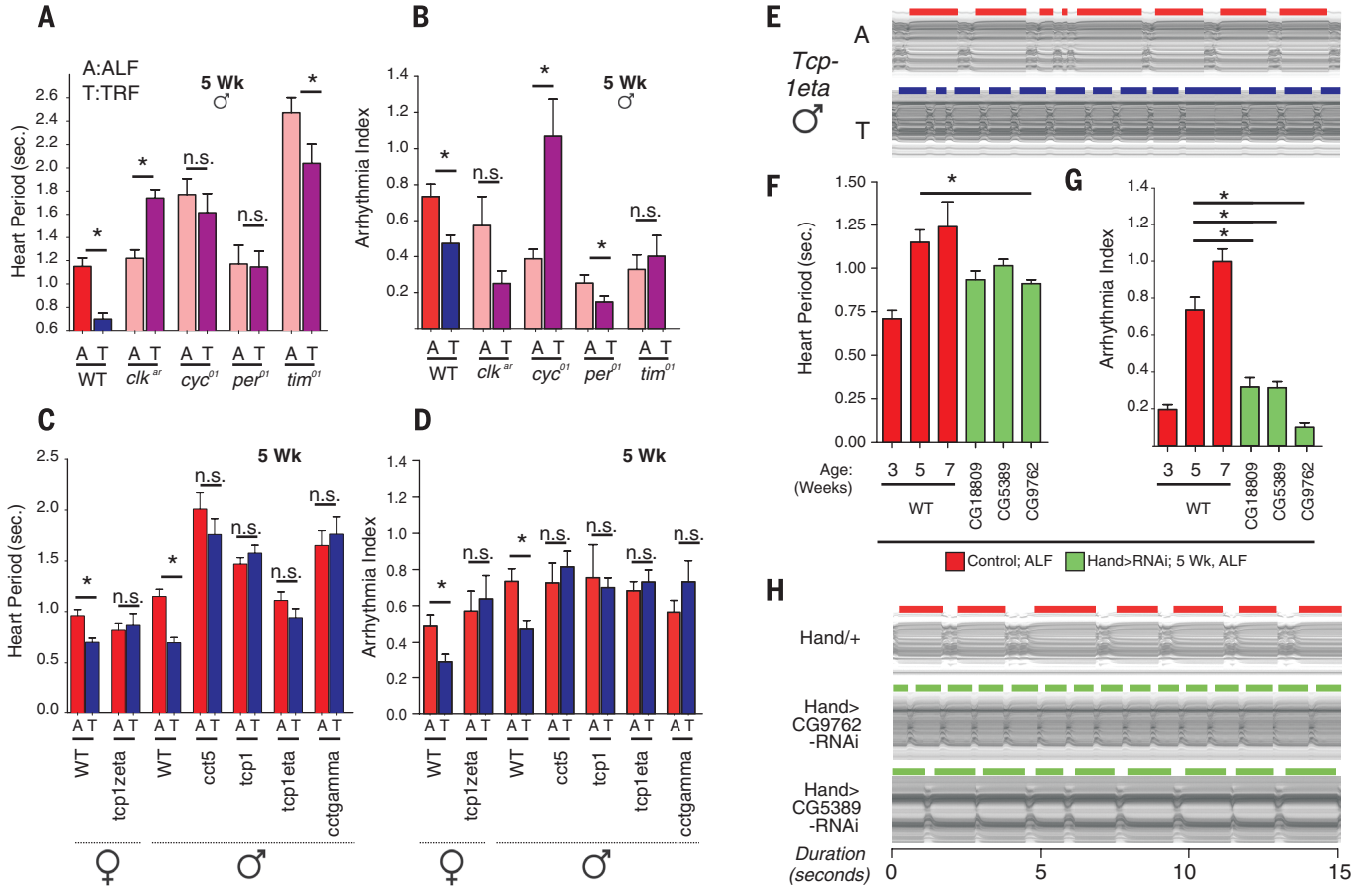

Fig. 4. Genetic basis for the beneficial effects of TRF. Five-week-old flies carrying loss-of-function mutations in (**A** and **B**) clock components or heterozygous for P-element insertions in (**C** and **D**) TRiC chaperonin components fail to improve (A and C) HP and (B and D) AI under TRF ($n \geq 12$ circadian mutants, $n \geq 17$ TRiC mutants). WT (Oregon-R) data are included for reference. (**E**) Representative M-modes of *Tcp-1eta* mutant flies exhibiting lack of TRF-driven cardioprotection. (**F**) HP, (**G**) AI, and (**H**) representative M-modes show improved cardiac function in 5-week-old ALF flies with heart-specific knockdown of genes encoding mitochondrial ETC proteins relative to 5-week-old male WT flies ($n \geq 24$). Three- and 7-week-old male WT data are included for reference. *$P < 0.05$, Mann Whitney test. Error bars: SEM.

We tested whether the TRiC chaperonin complex contributes to the beneficial cardioprotective effect of TRF. We investigated heterozygous P-element insertional mutants for five TRiC chaperonin subunits (*cct5*, *cct-gamma*, *tcp1*, *tcp-1eta*, *tcp-1zeta*) under ALF and TRF. No gross morphological heart defect was seen in ALF TRiC flies (fig. S11). For the TRiC mutants, TRF failed to improve cardiac contractility relative to genotype- and age-matched ALF flies as measured by heart period and arrhythmicity (Fig. 4, C to E; figs. S11 and S12; and movie S4). A potential dominant-negative effect of the P-element insertion or the reduced expression of some TRiC components (fig. S12) might affect normal function of the TRiC complex in these mutants. The lack of cardioprotective benefits of TRF by multiple mutants for different TRiC subunits provides genetic evidence that the integrity of the entire TRiC complex supports TRF-driven deceleration of cardiac aging.

To determine whether the cardiac tissue–restricted reduction of mitochondrial ETC transcripts contributes to TRF-dependent cardioprotection, we tested flies with heart-specific RNA interference (RNAi)–mediated reduction of ETC complex components. Heart-specific RNAi of complex I component CG9762 led to improved cardiac physiology in 5-week-old ALF flies (Fig. 4, F to H, and movie S5), reminiscent of TRF benefits in WT flies. Heart-specific RNAi of two additional components, CG5389 and CG18809, also led to reduced arrhythmia in 5-week-old ALF flies, although improvement in HP was not significant (Fig. 4, F to H, and fig. S13). Thus, lowering of ETC function may account for at least a part of the beneficial effect of TRF.

Genetic, dietary, and lifestyle (shiftwork) perturbation of circadian rhythms predisposes organisms to chronic diseases, including cardiovascular diseases. In rodents, the daily cycle of feeding-fasting under TRF reinforces diurnal rhythms in multiple organs and prevents metabolic diseases when the animals are administered a high-fat diet (*13*). Here we show that TRF protects against cardiac tissue aging in flies on either a normal or a fat-supplemented diet. This benefit appears to be mediated by the circadian clock, the TRiC chaperonin, and mitochondrial ETC components.

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SUPPLEMENTARY MATERIALS

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