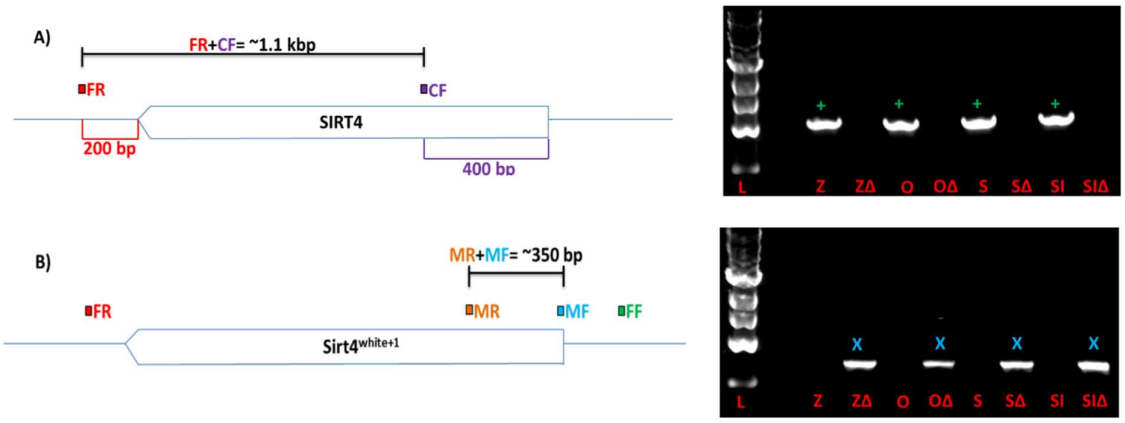

Figure S1. The genotype of w^{1118} and *SIRT4*$^{white+1}$ were confirmed through PCR. (A) Partial PCR products of the *SIRT4* gene were generated using the primers *SIRT4*-coding F (CF in purple) and *SIRT4*-flank R (FR in red), which yielded a band of approximately 1.1 kb. (B) Samples containing the Sirt4$^{white+1}$ marker were *SIRT4*-knockouts. Mini-w F (MF in blue) and Mini-w R (MR in orange) were specific to *SIRT4*$^{white+1}$ and resulted in the partial amplification of the construct around 350 bp. Lane identification: L=ladder, *Z=Zim*^{53}*;OreR*, O=*OreR;OreR*, S=*sm21;OreR*, SI=*si1;OreR*. *SIRT4Δ* is indicated by Δ next to the strain, whereas w^{1118} lacks any symbols besides the strain. PCR amplification of the *SIRT4* gene is identified by the green plus sign (+), whereas amplification of *Sirt4*$^{white+1}$ is identified by the blue X (X)**.**

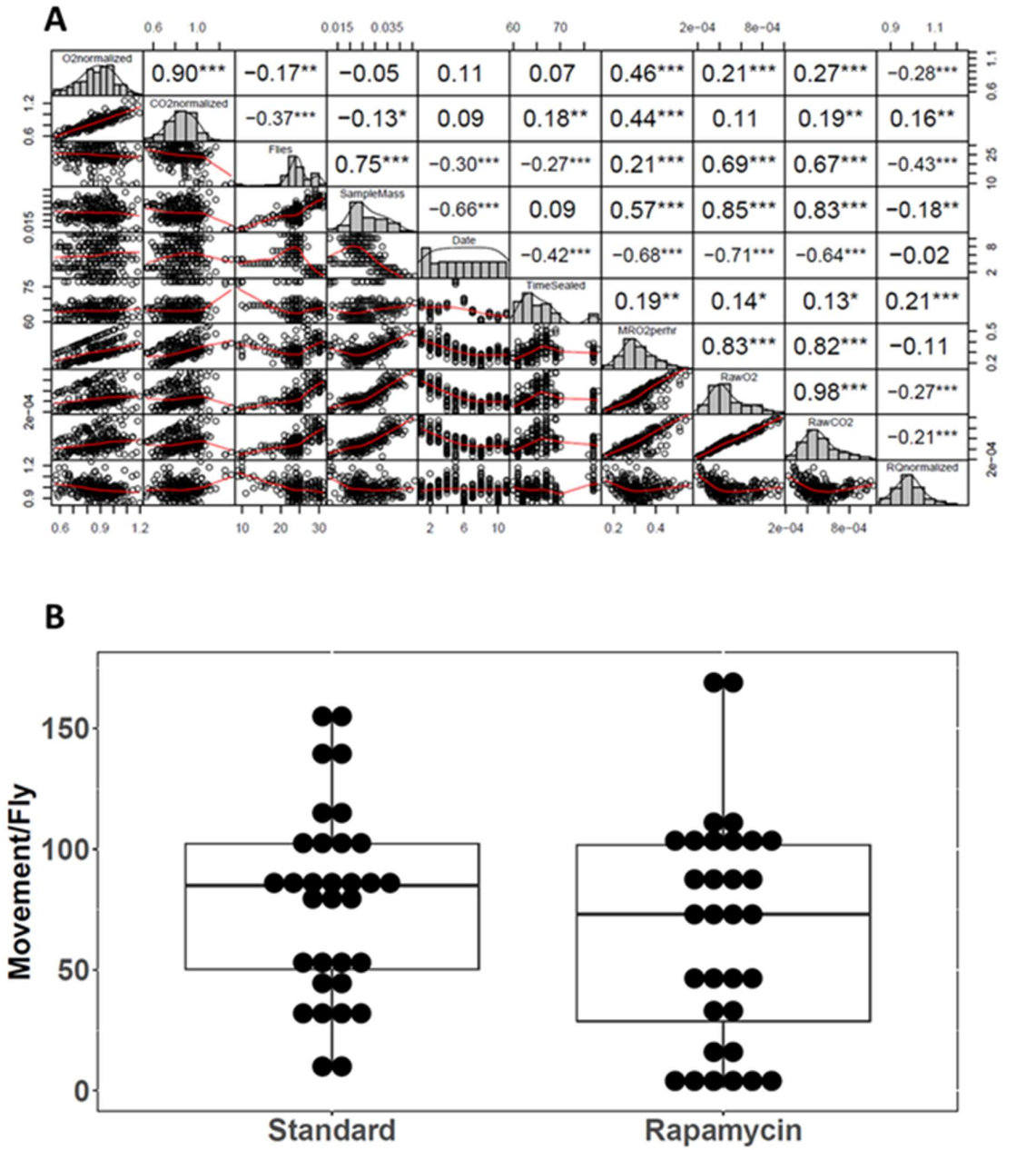

Figure S2. (A) Correlation matrix to determine if there were any relationships between the continuous variables. Oxygen consumption normalized to the reference sample (w^{1118} under standard treatment) negligibly correlated with the number of flies, mass of the flies, date, and time sealed (incubation period in the syringes). However, the raw oxygen consumption (RawO2) and metabolic rate per hour (MRO2perhr) were profoundly correlated with the number of flies, date, and mass. Thus, normalizing the data to the reference strain was the optimal way to eliminate any correlation with the other values, and no covariate was necessary. The following R packages and relevant functions were used to produce Figure. S2A: PerformanceAnalytics::chart.Correlation (Peterson and Carl 2019). (B) The total number of movements of wild type *OreR;OreR* under standard and rapamycin treatments**.** Movement was not statistically different between the treatments (Type 2 ANOVA p=0.2605) [Levene Test p=0.2324; Shapiro test p= 0.0482]. The following R packages and relevant functions were used to produce Fig. S2B: ggplot2::ggplot, ggplot2::geom_boxplot, and ggplot2::geom_dotplot (Wickham 2016).

Supplementary methods:

Verification of *Drosophila* mtDNA haplotype and SIRT4 mutants

From each mtDNA haplotype, 10 female flies from each stock were homogenized, and the respective DNA were extracted and purified using the Invitrogen: PureLink^{TM} Genomic DNA Mini Kit (ThermoFisher Scientific, n.d.). PCR verified that all stocks were from the genus *Drosophila* by amplifying *cytochrome c oxidase subunit I* (*COX1*), which is a highly conserved mitochondrial locus (Montooth *et al.* 2010). The *COX1* forward primer (3593F) had the oligonucleotide sequence of 5'-GAACAGTTCCCGCTTTAGGAG-3' and the sequence of the reverse primer (4528R) was 5'-GCAGTTAATCGGACAGCTAATGTTCCC-3'. Then, Restriction Fragment Length Polymorphism (RFLP) was utilized. The endonuclease, RsaI, had specificity for the palindromic 5′-GT^AC-3′ sequence present in *D. melanogaster COX1*, thereby digesting the *D. melanogaster COX1* into smaller fragments (Lynn *et al.* 1980) (Data not shown). PCR was also applied to verify the absence of the *SIRT4* gene in the *SIRT4Δ* stocks. DNA were isolated and purified using the Invitrogen: PureLinkTM Genomic DNA Mini Kit (ThermoFisher Scientific, n.d.). Several combinations of primers were used to amplify different regions of the *SIRT4* locus and *Sirt4$^{white+1}$* construct (Fig. S1). Initially, a pair of primers flanking the *SIRT4* loci (5'-GATGAGCGAATCCTTTTCCCG-3' and 5'-GTAACTTCTGGGTTCACGGCG-3') were annealed to assess the complete size of the gene/construct (*SIRT4* or *Sirt4$^{white+1}$)* integrated in that region. *SIRT4*-flank F corresponded to region 1879-1899 on Chromosome X, and annealed around 100 bp upstream the 5' end of *SIRT4*. *SIRT4*-flank R was homologous to region 3531-3511 on Chromosome X, and annealed around 200 bp upstream the 3' end of the *SIRT4* gene. The flanking primers were calculated to produce the complete amplification of *SIRT4* around 1.6 kbp, whereas total amplification of *Sirt4$^{white+1}$* was estimated to be greater than 2.8 kbp (Fig. S1). Other combinations of primers yielded partial products. A separate primer annealed 400 bp downstream the 5'end of the *SIRT4* open reading frame (region 2416-2436 on Chromosome X); this primer was referred to as *SIRT4*-coding F and its sequence was 5'-GTATCCGGAAGTTCTCGATGT-3'. Mini-w F had homology with the start codon of the *Sirt4$^{white+1}$* open reading frame, and Mini-w R annealed to the second exon of *Sirt4$^{white+1}$*, thereby yielding a partial product of *Sirt4$^{white+1}$* around 350 bp. Their respective oligonucleotides were 5'-ATGGGCCAAGAGGATCAGGAG-3' and 5'-GTTCTTGAGCAAATGTTTCCTG-3'. PCR was applied using a combination of these primers in order to verify that the genotypes of each stock were not contaminated (Fig. S1).

Movement measurement

Flies were fed standard food or 200 μM rapamycin food for 10 days prior to the measurement of movement under constant temperature, light and humidity conditions. The locomotive activity of the fruit flies were observed and recorded using the TriKinetics Drosophila Activity Monitor (DAM) (TriKinetics Inc, Waltham, Massachusetts). In total, 32 wild type *OreR;OreR* wildtype flies, from each treatment, were used in this assay. Individual flies were placed into 32 separate tubes, sealed and introduced into the activity monitor. Fly

movements were detected by infrared beams that were aimed at the midpoint of the tube; fly activity was recorded each instance that a fly crossed the beam (Pfeiffenberger *et al.* 2010).