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Research Article

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Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11344557/v1

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Additional Declarations: No competing interests reported.

Effects of low-dose sildenafil on survival and age-associated phenotypes in the African turquoise killifish

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Abstract

Sildenafil, a phosphodiesterase type 5 inhibitor, is clinically used for vascular disorders, but its potential role in vertebrate aging remains incompletely understood. In this study, we evaluated the effects of sildenafil on lifespan-associated phenotypes using the African turquoise killifish, a short-lived vertebrate model suitable for aging research. Sildenafil was orally administered once weekly at doses of 5 or 30 mg/kg to sex-balanced GRZ killifish after sexual maturation. Survival was monitored daily, while body length, body weight, and locomotor activity were assessed longitudinally. In the result, 5 mg/kg sildenafil delayed the initiation of death from 63 days in controls to 93 days and modestly increased median lifespan from 121.5 to 126 days. Maximum lifespan was also increased from 186 to 204 days. In contrast, 30 mg/kg sildenafil delayed the first death but did not improve median or maximum lifespan. Growth changes were broadly comparable among groups, suggesting that sildenafil did not markedly impair somatic growth. Locomotor activity declined with age across groups, while treatment-associated differences require further validation. These findings suggest that low-dose sildenafil may delay early mortality and modestly improve lifespan parameters in African turquoise killifish, supporting further investigation of phosphodiesterase signaling as a potential modulator of vertebrate aging.

Keywords: Sildenafil, Viagra, African turquoise killifish, lifespan, phosphodiesterase 5, vertebrate aging model

Introduction

Aging is a complex biological process characterized by the progressive decline of physiological integrity, leading to functional impairment, increased susceptibility to age-associated diseases, and ultimately mortality (Lopez-Otin et al., 2023). Aging is driven by interconnected molecular and cellular processes, including genomic instability, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, chronic inflammation, and altered intercellular communication (Lopez-Otin et al., 2023). Because these mechanisms are biologically modifiable, pharmacological interventions targeting aging-related pathways have become an important area of translational aging research. In particular, drug repurposing has emerged as a practical strategy for identifying anti-aging candidate compounds, whereby clinically used or well-characterized drugs are reevaluated for their potential to modulate aging biology, lifespan, or healthspan-related phenotypes (Kulkarni et al., 2022). Compared with de novo drug development, this approach may reduce translational barriers because pharmacological properties, toxicity profiles, and clinical experience are often at least partially established. Several clinically used drugs, including rapamycin, metformin, and acarbose, have provided proof-of-principle that pharmacological modulation of conserved aging-associated pathways can alter lifespan or health-related outcomes in preclinical models (Harrison et al., 2019; Harrison et al., 2009; Martin-Montalvo et al., 2013).

Sildenafil, widely known by the trade name Viagra, is a phosphodiesterase type 5 (PDE5) inhibitor that was originally developed for cardiovascular indications and is now widely used for erectile dysfunction and pulmonary arterial hypertension (Ghofrani et al., 2006). Mechanistically, sildenafil inhibits PDE5-mediated degradation of cyclic guanosine monophosphate, thereby augmenting nitric oxide–cGMP signaling (Andersson, 2018; Corum et al., 2020). In cellular and preclinical contexts, PDE5 inhibition has been linked to mitochondrial regulation, angiogenic responses, and protection against tissue injury, suggesting that sildenafil may influence biological processes that intersect with aging-related physiology. However, direct evidence that sildenafil modulates organismal aging or lifespan remains limited. Supportive evidence from a parallel *Drosophila melanogaster* study suggests that sildenafil may influence aging-related phenotypes in a dose- and sex-dependent manner (Min et al., 2026). In that study, wild-type Canton-S flies were exposed to dietary sildenafil at 20, 100, or 200 μM, and lifespan, fecundity, body weight, food intake, and locomotor activity were evaluated. Low-dose sildenafil at 20 μM significantly extended lifespans in both males and females, whereas 100 μM extended lifespan only in males and 200 μM showed no significant lifespan benefit. The study further reported increased fecundity and body weight without detectable changes in food intake, suggesting that the lifespan effect was unlikely to be explained solely by caloric restriction or reduced nutrient intake. In contrast, locomotor activity was not substantially improved by sildenafil treatment. These findings raise the possibility that sildenafil may modulate longevity and physiological aging through a dose-sensitive mechanism, while also highlighting the need to validate its effects in vertebrate aging models.

The African turquoise killifish (*Nothobranchius furzeri*) has emerged as a powerful vertebrate model for aging research because of its naturally compressed lifespan and rapid development of age-associated phenotypes (Harel et al., 2015; Kim et al., 2016). Unlike classical vertebrate models such as mice, which require years for lifespan studies, the African turquoise killifish enables longitudinal assessment of survival, growth,

physiological decline, and behavioral changes within a few months. At the same time, as a vertebrate, it retains organ-level complexity and physiological features that are absent in invertebrate models. Previous studies have established genetic and genomic tools for this species and have shown that killifish aging is accompanied by multiple phenotypes relevant to vertebrate aging, including functional decline, reduced regenerative capacity, neurodegenerative changes, and increased incidence of age-associated pathologies (Cui et al., 2020; Ma et al., 2025). The GRZ strain is particularly useful for rapid aging studies because its lifespan can be completed within approximately 4–6 months under laboratory conditions.

In this study, we evaluated whether sildenafil modulates lifespan-associated phenotypes in the African turquoise killifish. Using a longitudinal experimental design, we examined survival, somatic growth, and locomotor activity following weekly oral administration of low-dose (5 mg/kg) and high-dose (30 mg/kg) sildenafil. This study was designed as an exploratory preclinical evaluation of sildenafil as a potential lifespan-modulating compound in a short-lived African turquoise killifish. We found that low-dose sildenafil delayed the initiation of death and modestly improved lifespan parameters, whereas high-dose sildenafil did not confer consistent survival benefits. In addition, sildenafil did not markedly impair somatic growth, and treatment-associated differences in locomotor activity were limited and variable. These findings suggest that low-dose sildenafil may delay early mortality and modestly improve lifespan-associated outcomes in African turquoise killifish, supporting further investigation of phosphodiesterase signaling as a potential modulator of vertebrate aging.

Materials and Methods

Animals and experimental design

African turquoise killifish (*Nothobranchius furzeri*) GRZ strain was used in this study. The sample size was selected a priori for an exploratory preclinical screening study rather than for definitive efficacy testing. Each group included 12 fish, with six males and six females, to maintain sex balance while minimizing animal use. This design was intended to identify relatively large treatment-associated effects on survival and age-associated phenotypes, but it may have limited sensitivity for detecting small or sex-specific effects (Fig. 1). All procedures were conducted under the approved animal protocol UNISTIACUC-25-009. During the lifespan assessment, fish were fed twice daily with a mixture of thawed bloodworms and baby brine shrimp, and individual feeding status was monitored at regular intervals.

Sildenafil preparation and administration

Sildenafil (Sildenafil citrate, SML3033, Sigma Aldrich, Sigma-Aldrich, St. Louis, MO) was prepared as an aqueous solution using distilled water as the solvent. Fish in the sildenafil-treated groups received either 5 mg/kg or 30 mg/kg sildenafil, while the control group was assigned to 0 mg/kg treatment. Sildenafil administration began at 5 weeks after hatching and was performed by oral administration once per week (Fig. 1). The administered amount of sildenafil was calculated based on body weight-associated growth indices of the killifish.

Survival analysis

Survival was monitored daily throughout the experimental period (Fig. 1). Mortality was recorded for each individual fish and used for survival curve analysis. Lifespan was calculated as days after hatching. When an individual fish died, body length and body weight were measured immediately and recorded. The initiation of death was defined as the age at which the first death occurred in each experimental group. Median lifespan was defined as the age at which 50% of individuals in a group had died, and maximum lifespan was defined as the age at death of the longest-lived individual in each group. The coefficient of variation (CV) was calculated as SD divided by mean lifespan and expressed as a percentage to describe lifespan variability within each group. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank (Mantel–Cox) test. Hazard ratios were estimated using Cox proportional hazards models with treatment and sex as covariates.

Growth measurements

Somatic growth was assessed by longitudinal measurement of body length and body weight (Fig. 1). Baseline growth measurements were performed at 5 weeks after hatching before the initiation of sildenafil treatment. Body length was measured every 2 weeks beginning at 5 weeks after hatching, and body weight was measured every 5 weeks during the experimental period. For body length measurement, fish were imaged with a scale included in the image to enable standardized measurement. The collected images were analyzed using image analysis software, and individual body length values were recorded as longitudinal growth data. Body weight was

measured at the designated time points and recorded for each fish.

Locomotor activity assay

Locomotor activity was assessed at 5, 10, 15, and 17 weeks after hatching (Fig. 1). The 5-week measurement was as a baseline measurement before the first sildenafil administration, whereas the 10-, 15-, and 17-week measurements were performed after treatment initiation. For each assay, fish were recorded simultaneously for 20 min, and the videos were analyzed using EthoVision XT. Total distance traveled, mean velocity, cumulative duration of moving, and cumulative duration of not-moving were quantified as locomotor parameters. Missing data at later time points reflected individuals that had died before behavioral assessment.

Relative exploration area analysis

To quantitatively assess the spatial extent of exploration during the locomotor assay, x–y center-coordinate data exported from EthoVision XT were analyzed for each individual fish. Tracking coordinates were sampled at 1-s intervals, and the convex hull encompassing the valid x–y positions during the 20-min recording period was calculated. This area was defined as an approximation of the exploration area traversed by the individual. Convex hull area was expressed in cm^2 and normalized to the total arena area (20 × 10 cm = 200 cm^2) to obtain relative exploration area (%).

$$\text{Relative exploration area (\%)} = \frac{\text{Convex hull area } (cm^2)}{200\ cm^2} \times 100$$

Measurements were performed at 5, 10, 15, and 17 weeks after hatching, with the 5-week assessment representing the pretreatment baseline. Animals that had died before a scheduled behavioral assessment were not included at the corresponding time point. Data are presented as mean ± standard deviation.

Preliminary* in silico *comparison of PDE5A-like phosphodiesterase sequences

To preliminarily assess the conservation of sildenafil-responsive phosphodiesterase signaling components, PDE5A protein sequences from human (*Homo sapiens*) and mouse (*Mus musculus*) were compared with an annotated PDE5A-like phosphodiesterase sequence from African turquoise killifish (*Nothobranchius furzeri*) using Clustal Omega. Because the available *N. furzeri* annotation appeared to be N-terminally truncated relative to mammalian PDE5A, the alignment was interpreted only as evidence of sequence conservation within the aligned region and not as confirmation of a full-length PDE5A ortholog.

Statistical analysis

The JMP statistical package (SAS, NC, USA) was used for the analyses. The test for normality (Shapiro-Wilk test) and the statistical probabilities (F-test, *t*-test, Wilcoxon rank sum test, and Kruskal–Wallis test) of data in this study were performed using R studio software.

Results

Low-dose sildenafil tended to delay early mortality and modestly improve survival parameters

To investigate whether sildenafil modulates lifespan-associated phenotypes in African turquoise killifish, we monitored the survival of fish treated with vehicle, 5 mg/kg sildenafil, or 30 mg/kg sildenafil. In the overall population, low-dose sildenafil delayed the initiation of death and showed favorable, non-significant trends in lifespan parameters. The first death occurred at 63 days after hatching in the control group, whereas it was delayed to 93 days in the 5 mg/kg sildenafil group (47.62% increase) and to 82 days in the 30 mg/kg sildenafil group (30.16% increase). The mean lifespan increased from 123.83 ± 10.03 days in controls to 131.92 ± 8.97 days in the 5 mg/kg sildenafil group (Fig. 2a and Table 1, 6.5% increase, Log-rank test, $p = 0.4297$). Median lifespan increased from 121.5 to 126 days (Table 1, 3.7% increase), and maximum lifespan increased from 186 to 204 days (Table 1, 9.7% increase). In contrast, 30 mg/kg sildenafil did not improve overall lifespan parameters, with mean, median, and maximum lifespan values of 121.42 ± 7.31 (Fig. 2a and Table 1, 1.9% decrease, Log-rank test, $p = 0.6919$), 118.5 (Table 1, 2.5% decrease), and 183 days (Table 1, 1.6% decrease), respectively. To further describe the distribution of lifespan within each group, we calculated the SD and coefficient of variation (CV) from individual age-at-death values (Table 1). In the overall population, the 5 mg/kg sildenafil group showed a modestly lower SD and CV than the control group, suggesting reduced variability in lifespan distribution. However, this pattern was not consistent in sex-stratified analyses, particularly in females, where lifespan variability was higher in the 5 mg/kg sildenafil group. Therefore, these distributional indices should be interpreted descriptively and do not provide definitive evidence of survival-curve steepening. Sex-stratified analysis showed that the effect of 5 mg/kg sildenafil differed between males and females. In males, 5 mg/kg sildenafil increased mean lifespan by 8.6% and median lifespan by 11.0%, although maximum lifespan was reduced compared with control males (Fig. 2b and Table 1, Log-rank test, $p = 0.8647$). In females, 5 mg/kg sildenafil increased mean, median, and maximum lifespan by 4.8%, 7.2%, and 12.7%, respectively (Fig. 2c and Table 1, Log-rank test, $p = 0.5076$). By contrast, 30 mg/kg sildenafil did not produce consistent lifespan benefits in either sex (Log-rank test, Male, $p = 0.9225$; Female, $p = 0.8874$). Although no statistically significant differences were observed, low-dose sildenafil showed a tendency to modestly improve lifespan parameters in African turquoise killifish, whereas high-dose sildenafil did not confer additional survival benefits.

We further assessed age-associated mortality patterns in African turquoise killifish treated with vehicle, 5 mg/kg sildenafil, or 30 mg/kg sildenafil using ln-transformed mortality rates (Fig. 2d–2f and Table 1). In the overall population, sildenafil-treated groups showed altered mortality pattern compared with the vehicle-treated control group, suggesting that sildenafil may influence the age-dependent increase in mortality (Fig. 2d and Table 1). When analyzed by sex, male fish treated with sildenafil showed a tendency toward reduced mortality pattern in both the 5 mg/kg and 30 mg/kg groups compared with vehicle-treated males. This pattern suggests that sildenafil treatment may attenuate age-associated mortality progression in males, although the effect did not clearly translate into an extension of maximum lifespan (Fig. 2e and Table 1). In females, the mortality pattern appeared to differ by dose (Fig. 2f and Table 1). The 5 mg/kg sildenafil group showed a tendency toward reduced late-life mortality compared with vehicle-treated females, consistent with the observed increase in female mean, median, and maximum lifespan. In contrast, the 30 mg/kg sildenafil group exhibited a relatively higher mortality

pattern across the observation period, suggesting that high-dose sildenafil did not confer a mortality-lowering effect in females. Taken together, these results suggest that sildenafil may modulate age-associated mortality in a sex- and dose-dependent manner. Overall, the 5 mg/kg survival curve was characterized by a delayed initial decline and a modest rightward shift during the early-to-middle lifespan, although it did not remain significantly separated from the control curve across the full lifespan. In contrast, the 30 mg/kg curve showed only a limited early shift without a sustained late-life advantage, and the curve profiles varied between sexes.

Table 1 Lifespan of killifish with sildenafil supplementation

Sex	Treat	n	Initiation of death (days)	Mean lifespan (days)	SD (days)	CV (%)	Change in mean lifespan (%)	Median lifespan (days)	Maximum lifespan (days)	χ^2	*p*-value	Mortality slope	R^2
All	Mock	12	63	123.83 ± 10.03	34.74	28.06		121.5	186			0.0198	0.8347
	5 mg/kg	12	93	131.92 ± 8.97	31.07	23.55	6.5	126	204	0.6236	0.4297	0.0311	0.7978
	30 mg/kg	12	82	121.42 ± 7.31	25.33	20.86	-1.9	118.5	183	0.1571	0.6919	0.0354	0.8627
Male	Mock	6	63	114.83 ± 17.42	42.68	37.17		113.5	186			0.0178	0.8629
	5 mg/kg	6	93	124.67 ± 11.01	26.96	21.63	8.6	126	162	0.029	0.8647	0.0153	0.8267
	30 mg/kg	6	82	116.17 ± 9.21	22.55	19.41	1.2	118.5	143	0.0095	0.9225	0.0238	0.858
Female	Mock	6	110	132.83 ± 10.33	25.29	19.04		124.5	181			0.05	0.9315
	5 mg/kg	6	101	139.17 ± 14.55	35.64	25.61	4.8	133.5	204	0.4389	0.5076	0.0272	0.8695
	30 mg/kg	6	101	126.67 ± 11.81	28.93	22.84	-4.6	118.5	183	0.02	0.8874	0.0509	0.8806

Sildenafil did not markedly alter somatic growth pattern

To determine whether sildenafil-associated changes in lifespan were accompanied by alterations in somatic growth, we longitudinally measured body length and body weight in control and sildenafil-treated African turquoise killifish. Overall, body length and body weight showed broadly comparable age-dependent growth change across the control, 5 mg/kg sildenafil, and 30 mg/kg sildenafil groups (Fig. 3). No obvious growth retardation was observed in sildenafil-treated fish, suggesting that the modest lifespan benefit observed in the 5 mg/kg sildenafil group was unlikely to be a secondary consequence of delayed development or generalized suppression of somatic growth. Sex-stratified analysis revealed modest, non-significant differences in growth patterns between males and females. In males, both body length and body weight tended to be higher in the 5 mg/kg sildenafil group compared with the control group, whereas the 30 mg/kg sildenafil group showed a tendency toward reduced growth change (Fig. 3b and 3e). In females, 5 mg/kg sildenafil produced little apparent change in either body length or body weight, while 30 mg/kg sildenafil was associated with a slight downward trend in growth parameters (Fig. 3c and 3f). These observations suggest that low-dose sildenafil did not negatively affect somatic growth, whereas high-dose sildenafil may have exerted mild growth-suppressive effects in a sex-dependent manner.

We next compared body length and body weight at the time of death to determine whether sildenafil altered terminal somatic status (Fig. 4). Terminal body length was broadly similar among groups when analyzed

in the overall population, in males alone, or in females alone. In contrast, terminal body weight showed a non-significant tendency to increase in the 5 mg/kg sildenafil group in both males and females. Because terminal body length was not markedly altered, the tendency toward higher terminal body weight in low-dose sildenafil-treated fish may reflect better maintenance of body condition rather than accelerated linear growth. However, this interpretation should be made cautiously because terminal measurements are influenced by age at death and the small number of animals in each sex-stratified group. Collectively, these findings indicate that low-dose sildenafil-associated lifespan improvement was not driven by growth retardation or developmental delay and may instead be accompanied by partial preservation of somatic body condition at late life.

Sildenafil did not robustly prevent age-associated locomotor decline

To assess whether sildenafil treatment affected functional aging-associated phenotypes, we analyzed locomotor activity in African turquoise killifish during aging. Locomotor activity is a useful behavioral readout of age-associated functional decline because reductions in spontaneous movement, swimming velocity, and spatial exploration can reflect impaired physiological performance during aging. Previous longitudinal behavioral studies in African turquoise killifish have shown that locomotor activity, exploration-related behavior, immobility, and spatial occupancy change with age, supporting the use of video-based behavioral tracking as a functional aging-associated readout in this model (Bedbrook et al., 2026; Marien et al., 2023). Fish were recorded for 20 min at 5, 10, 15, and 17 weeks after hatching, and video-based behavioral tracking was performed using EthoVision XT. Total distance traveled, mean velocity, cumulative duration of moving, and cumulative duration of not-moving were quantified as locomotor parameters. Across all groups, total distance traveled and mean velocity generally declined with age, indicating an age-associated reduction in locomotor performance (Fig. 5a and 5d). This decline was observed in the overall population as well as in sex-stratified analyses. Although sildenafil-treated fish showed variable changes in locomotor parameters at later time points, neither 5 mg/kg nor 30 mg/kg sildenafil produced a consistent improvement in total distance or mean velocity across all ages and sexes. In males, 5 mg/kg sildenafil tended to maintain higher locomotor activity at some later time points compared with controls, whereas 30 mg/kg sildenafil did not show a consistent beneficial pattern (Fig. 5b and 5e). In females, locomotor responses to sildenafil were less apparent and varied across time points (Fig. 5c and 5f). These findings suggest that sildenafil did not robustly prevent the age-associated decline in locomotor activity under the present experimental conditions.

We next examined cumulative moving and non-moving durations to further assess behavioral activity patterns (Fig. 6). Overall, the duration of movement and rest did not show clear or statistically significant treatment-dependent differences when analyzed in all fish (Fig. 6a and 6d), males (Fig. 6b and 6e), or females (Fig. 6c and 6f). Although fluctuations were observed at individual time points, particularly at later ages, these changes were not consistent across sex or dose. Thus, sildenafil treatment did not markedly alter the overall balance between active and inactive behavioral states. Representative heatmaps provided qualitative information on spatial occupancy during the locomotor assay (Figs. S1–S3). At 10 weeks after hatching, control and sildenafil-treated fish showed broadly similar occupancy patterns, with heat signals distributed along the longitudinal axis of the tank and frequent localization near peripheral or terminal regions (Fig. S1). Such peripheral or terminal localization is consistent with previous observations that killifish can exhibit edge-associated homebase behavior

in open-field-like behavioral assays (Marien et al., 2023). At 15 weeks after hatching, spatial occupancy patterns became more heterogeneous across groups (Fig. S2). In the control group, several individuals showed localized hotspots. In the 5 mg/kg sildenafil group, some fish displayed broader heatmap distributions along the longitudinal axis of the tank, suggesting a possible tendency toward broader spatial occupancy in a subset of animals. In contrast, the 30 mg/kg sildenafil group showed variable patterns, with some individuals exhibiting broader occupancy and others showing restricted localization to specific tank regions. Because heatmaps were used as qualitative visualizations rather than primary quantitative endpoints, these observations should be interpreted cautiously and in conjunction with total distance, mean velocity, and moving/non-moving duration. At 17 weeks after hatching, locomotor heatmaps showed more restricted spatial occupancy patterns across groups, consistent with late-life functional decline (Fig. S3). Several individuals in the 5 mg/kg group visually appeared to occupy broader regions of the tank, whereas other fish showed restricted or edge-associated localization. To quantitatively complement the heatmap-based observations, relative exploration area was calculated from individual x–y tracking coordinates using the convex hull of the recorded positions and expressed as a percentage of the total arena area (Fig. S4). At the pretreatment 5-week assessment, relative exploration area already differed among the experimental groups (Kruskal–Wallis test, $p = 0.0157$), with the 30 mg/kg group showing a greater baseline exploration area than the control group. At 10 weeks, a group difference was again detected ($p = 0.0037$), primarily reflecting a greater exploration area in the 30 mg/kg group than in the control and 5 mg/kg groups. Because the 30 mg/kg group already showed a greater exploration area before treatment initiation, the 10-week difference cannot be attributed specifically to sildenafil exposure. At 15 and 17 weeks, relative exploration area did not differ significantly among groups ($p = 0.9880$ and $p = 0.8013$, respectively). Notably, the visually broader spatial distributions observed in some 5 mg/kg-treated fish at later ages were not accompanied by a significant group-level increase in relative exploration area.

Taken together, these results indicate that aging was accompanied by a general decline in locomotor performance in African turquoise killifish, whereas sildenafil did not produce a robust or consistent improvement in the quantitative behavioral parameters examined. Although representative heatmaps revealed broader spatial distributions in some individual low-dose sildenafil-treated fish, quantitative analysis of relative exploration area did not support a treatment-dependent preservation of spatial exploration at the group level. Because later behavioral measurements included only surviving individuals, particularly at 15 and 17 weeks, these late-life measurements should be interpreted cautiously due to progressive mortality-related reductions in sample size and potential survivor bias. In addition, because circadian rhythmicity and locomotor behavior can change with aging in turquoise killifish, future studies using repeated behavioral assays should standardize the timing of behavioral measurements and consider circadian state as a potential contributor to locomotor variability (Lee et al., 2021).

Discussion

The present study evaluated the effects of sildenafil on lifespan-associated phenotypes in the African turquoise killifish, a short-lived vertebrate model of aging. The major finding was that weekly oral administration of low-dose sildenafil delayed the initiation of death and showed favorable, non-significant changes in lifespan (Fig. 2 and Table 1) without overtly impairing somatic growth or producing robust changes in locomotion (Figs.

3–7). In contrast, high-dose sildenafil did not extend median or maximum lifespan, suggesting that sildenafil does not exert a simple dose-dependent pro-longevity effect in this model. Instead, the observed pattern is compatible with the possibility that sildenafil acts within a relatively narrow beneficial dose range. The most prominent effect of low-dose sildenafil was the delay in early mortality. In the 5 mg/kg sildenafil group, the first death occurred later than in the control group, and mean, median, and maximum lifespan showed modest increases. Although overall survival curves of the control and 5 mg/kg sildenafil groups were not statistically significantly different by the log-rank test ($p = 0.4297$), the data suggest a favorable trend toward delayed early mortality rather than a robust or uniform extension of survival across the entire population. Consistent with this interpretation, lifespan distribution analysis showed modestly lower SD and CV values in the overall 5 mg/kg sildenafil group compared with controls. However, this pattern was not consistent across sex-stratified analyses. Therefore, these distributional indices do not provide definitive evidence of survival-curve steepening and should be interpreted descriptively. In contrast, 30 mg/kg sildenafil delayed the first death but failed to improve median or maximum lifespan, indicating that high-dose sildenafil did not provide a sustained survival advantage. These findings are consistent with a dose-sensitive, and possibly hormetic-like response (Caruso et al., 2024). In this context, moderate modulation of sildenafil-responsive pathways may promote beneficial physiological effects, whereas higher exposure may fail to provide additional benefit or may disrupt physiological homeostasis.

The dose-sensitive effect observed in killifish is supported by findings from a parallel *Drosophila melanogaster* study (Min et al., 2026). In that study, sildenafil supplementation showed dose-dependent and sex-specific effects on lifespan. Low-dose sildenafil (20 μM) significantly extended lifespan in both male and female flies, an intermediate dose (100 μM) extended lifespan only in males, and the highest dose (200 μM) showed no significant benefit. Similarly, in the present killifish study, 5 mg/kg sildenafil showed favorable, non-significant trends in lifespan, whereas 30 mg/kg sildenafil did not show comparable survival-related benefits. However, direct dose translation between the *Drosophila* and killifish experiments is limited because the two models differed in route, frequency, and mode of exposure. In *Drosophila*, sildenafil was provided continuously through the diet, whereas killifish received sildenafil by once-weekly oral administration on a body weight-adjusted basis. Therefore, the dose-sensitive effects observed across the two systems should not be interpreted as equivalent pharmacological exposures, but rather as parallel evidence that sildenafil responses may depend on exposure level, biological context, and species-specific pharmacokinetics. Together, these observations suggest that sildenafil may modulate aging-associated physiology across evolutionarily distant organisms, but that its beneficial effects may depend strongly on dose, sex, and biological context. Importantly, the phenotypic outcomes were not identical between the two models. In *Drosophila*, sildenafil increased fecundity and body weight without substantially improving locomotor activity. In the present killifish study, low-dose sildenafil did not markedly alter somatic growth and showed visually broader spatial occupancy in some late-life locomotor heatmaps. However, quantitative analysis of relative exploration area did not demonstrate a significant group-level increase at later ages. Quantitative locomotor parameters, including total distance, mean velocity, moving duration, and non-moving duration, generally declined with age across groups and did not show a robust or consistent sildenafil-dependent improvement. Thus, in both models, the lifespan-associated effects of sildenafil were not clearly accompanied by broad enhancement of locomotor performance. The comparison between *Drosophila* and killifish is also important from a translational perspective. Although *Drosophila* is a powerful genetic model for aging

research (He & Jasper, 2014), it does not appear to possess a clear one-to-one ortholog of mammalian PDE5A. However, *Drosophila* encodes cGMP-hydrolyzing phosphodiesterases, including DmPDE5/6/Pde6, that are related to vertebrate PDE5/6 enzymes. Therefore, sildenafil may act in *Drosophila* through PDE5/6-like phosphodiesterases or other conserved cyclic nucleotide signaling components rather than through a canonical mammalian PDE5 target (Day et al., 2008; Day et al., 2005). In contrast, the African turquoise killifish is a vertebrate model and therefore provides a more physiologically relevant context for evaluating sildenafil as a candidate lifespan-modulating compound. As a preliminary in silico observation, an annotated PDE5A-like phosphodiesterase sequence was identified in *N. furzeri* (Fig. S5 and Table S1). However, this annotation appears incomplete, with an apparent N-terminal truncation relative to mammalian PDE5A. Sequence alignment showed conservation between the annotated *N. furzeri* PDE5A-like sequence and human or mouse PDE5A within the aligned region, but these data do not establish the presence of a full-length PDE5A ortholog. Therefore, full-length PDE5A annotation, PDE5A enzymatic activity, and sildenafil sensitivity should be validated in future studies. The partial agreement between the invertebrate and vertebrate datasets strengthens the possibility that sildenafil-sensitive pathways may influence aging-related physiology, while also emphasizing that the underlying mechanisms may differ across species.

Sex-stratified analyses suggested possible sex-related differences in the response to sildenafil, although the small number of animals per sex limits definitive interpretation. In males, low-dose sildenafil was associated with modest increases in mean and median lifespan, but maximum lifespan was not extended. In females, low-dose sildenafil showed modest favorable trends across mean, median, and maximum lifespan (Fig. 1 and Table1). These results suggest that the beneficial effect of low-dose sildenafil was not restricted to a single sex, but the small number of animals in the sex-stratified analysis limits definitive interpretation. Sex differences in sildenafil response may reflect differences in drug exposure, metabolism, reproductive physiology, tissue sensitivity to cyclic nucleotide signaling, or baseline aging. Future studies should therefore incorporate larger sex-balanced cohorts and pharmacokinetic measurements to determine whether sex-specific responses are driven by differential exposure or by downstream biological sensitivity. Somatic growth analysis further supports the interpretation that the lifespan benefit of low-dose sildenafil was not merely a consequence of growth suppression or developmental delay. Across the experimental period, body length and body weight change were broadly comparable among control and sildenafil-treated groups. In addition, terminal body length did not markedly differ among groups, while terminal body weight showed a non-significant tendency to increase in low-dose sildenafil-treated fish. These findings suggest that low-dose sildenafil did not impose overt somatic impairment and may be compatible with maintenance of body condition during aging. However, because terminal measurements are influenced by age at death, these data should be interpreted cautiously. Locomotor activity was included as a functional aging-related phenotype. In the present study, locomotor parameters generally declined with age, consistent with age-associated reduction in swimming performance. This interpretation is supported by previous studies showing age-related alterations in killifish locomotion, exploration-related behavior, immobility, and lifelong behavioral organization (Bedbrook et al., 2026; Marien et al., 2023). However, sildenafil did not robustly prevent this decline. Representative heatmaps suggested that some low-dose sildenafil-treated fish showed broader spatial occupancy at later ages. However, convex hull-based quantification of relative exploration area did not reveal significant group differences at 15 or 17 weeks, indicating that the visually broader distributions observed in some individuals

were not supported by a group-level increase in spatial exploration (Fig. S4). Significant group differences were observed at 5 and 10 weeks, primarily reflecting greater exploration area in the 30 mg/kg group. However, because this difference was already present at the pretreatment baseline, the 10-week difference cannot be attributed specifically to sildenafil exposure. Moreover, behavioral measurements at later time points included only surviving individuals, introducing potential survivor bias. Therefore, the locomotor data do not provide evidence that sildenafil consistently increased spatial exploration or broadly improved locomotor function during aging. Although the present study did not directly investigate molecular mechanisms, several candidate pathways may be considered. Sildenafil inhibits PDE5-mediated cGMP degradation and thereby enhances nitric oxide–cGMP signaling (Ghofrani et al., 2006), a pathway linked to vascular function, tissue perfusion, mitochondrial regulation, and cellular stress responses (Ghofrani et al., 2006; Nisoli et al., 2003; Zhu et al., 2022). Moderate activation of these pathways could potentially improve physiological resilience during aging. However, excessive pathway activation or high systemic exposure may disrupt homeostatic balance (Calabrese, 2021; Rattan, 2008), which could explain the absence of lifespan benefit at 30 mg/kg. Future studies should examine whether low-dose sildenafil alters cGMP levels, PDE5 activity, mitochondrial function, oxidative stress resistance, inflammatory signaling, or tissue-specific aging markers in killifish.

This study has several limitations. First, the cohort size was appropriate for an initial exploratory screening design but remained relatively small, particularly for sex-stratified analyses. As a result, the study may have had limited statistical power to detect modest lifespan effects or subtle sex-specific responses. In addition, the experiment was not independently replicated. Second, molecular, biochemical, and histological analyses were not performed, limiting mechanistic interpretation. Third, pharmacokinetic validation of sildenafil exposure was not conducted, making it difficult to determine whether dose- and sex-dependent effects reflect differences in systemic drug levels or downstream sensitivity. Fourth, locomotor analyses at later ages may be affected by survivor bias because only surviving individuals were available for behavioral assessment. These limitations indicate that the present findings should be interpreted as exploratory and require validation in larger, independently replicated cohorts. In conclusion, low-dose sildenafil delayed early mortality and modestly increased lifespan in African turquoise killifish without overtly impairing somatic growth, whereas high-dose sildenafil did not confer consistent survival benefits. Together with supportive evidence from *Drosophila*, these findings suggest that sildenafil may act as a dose-sensitive modulator of aging-associated physiology across model organisms. Further studies are warranted to determine whether the observed effects are mediated by PDE5–NO–cGMP signaling, mitochondrial homeostasis, vascular function, oxidative stress resistance, or other downstream mechanisms, and to establish whether sildenafil can reproducibly influence vertebrate lifespan and healthspan under controlled experimental conditions.

Author contributions

H.Y. Lee, Y. Kim and K.J. Min conceived and designed the study. S. Lee, N. Gwak and Y. Choi performed the killifish experiments as a commissioned service. H.Y. Lee analyzed the data and drafted the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. RS-2026-25524693).

Declarations of interest statement

The authors report there are no competing interests to declare.

Fig. 1

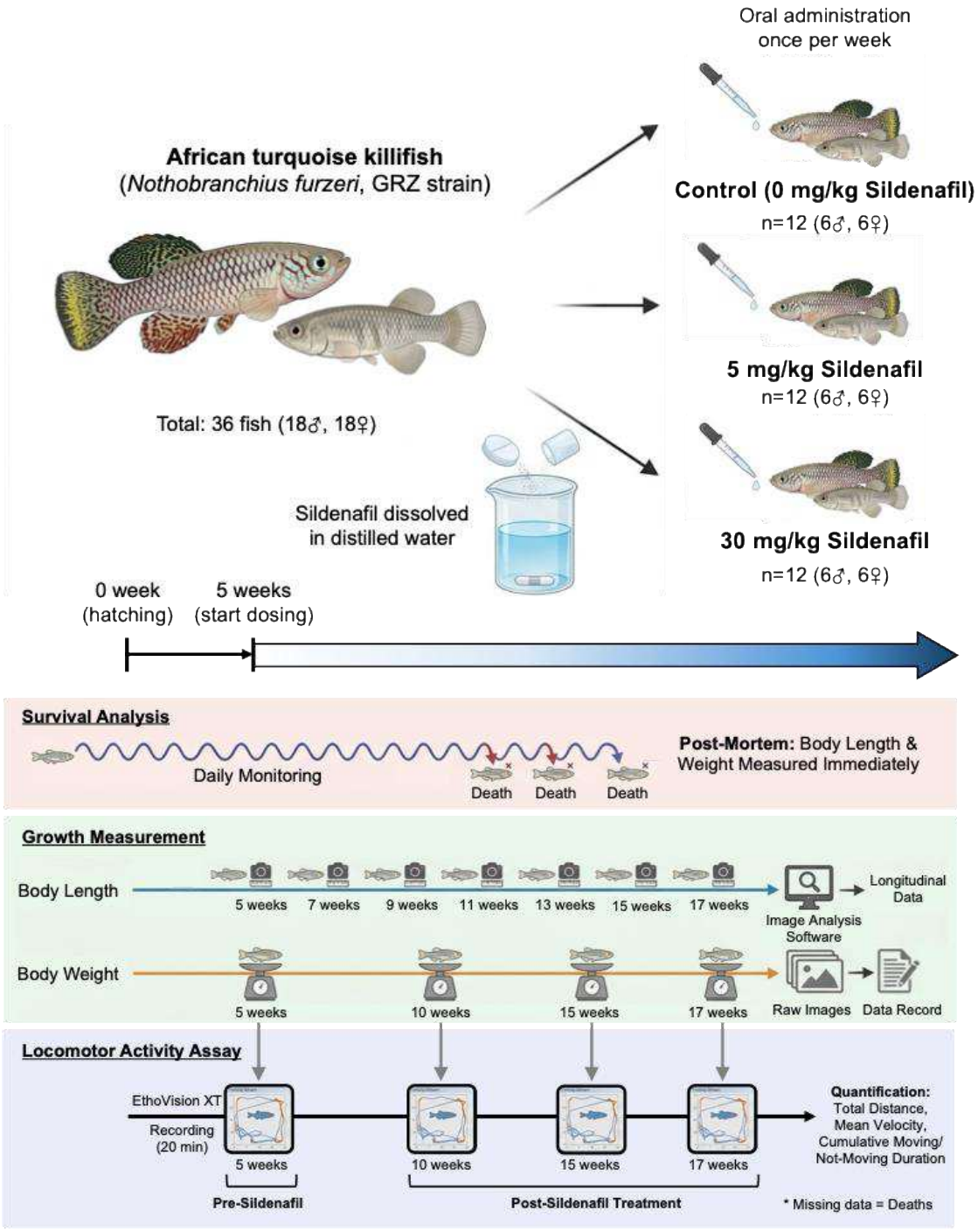
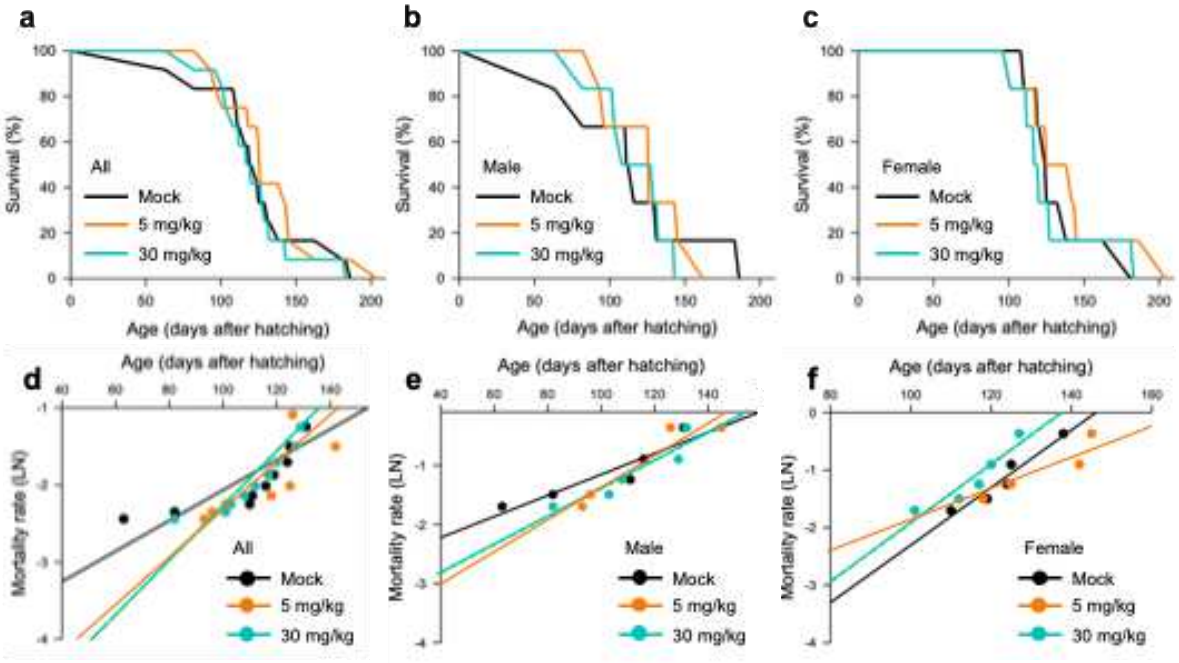


Fig. 1 Experimental design for evaluating the effects of sildenafil in African turquoise killifish

African turquoise killifish (Nothobranchius furzeri, GRZ strain) were assigned to three experimental groups (Mock control, 5 mg/kg sildenafil, and 30 mg/kg sildenafil). Each group consisted of 12 fish, including six males and six females. Sildenafil was administered orally once per week beginning at 5 weeks after hatching. Survival was monitored daily, while somatic growth and locomotor activity were assessed longitudinally. Body length was measured every 2 weeks beginning at 5 weeks after hatching, body weight was measured every 5 weeks, and locomotor activity was evaluated at 5, 10, 15, and 17 weeks after hatching using video-based behavioral tracking.

422 **Fig. 2**



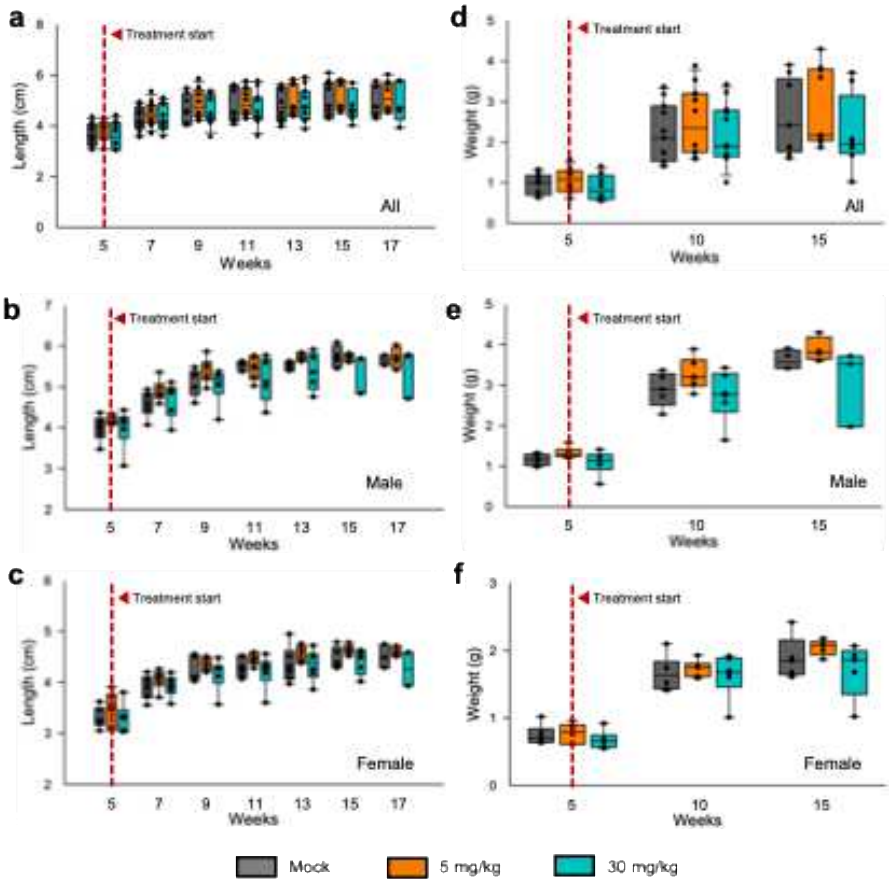
423

424 **Fig. 2 Low-dose sildenafil delayed early mortality and modestly improved lifespan parameters in African**
425 **turquoise killifish**

426 (a–c) Survival curves of Mock-, 5 mg/kg sildenafil-, and 30 mg/kg sildenafil-treated killifish in the overall
427 population (a), males (c), and females (c). (d–f) Ln-transformed mortality rate plots in all fish (d), males (e), and
428 females (f). Black: Mock control group; Orange: 5 mg/kg sildenafil group; Cyan: 30 mg/kg sildenafil group.

429

430 **Fig. 3**



431

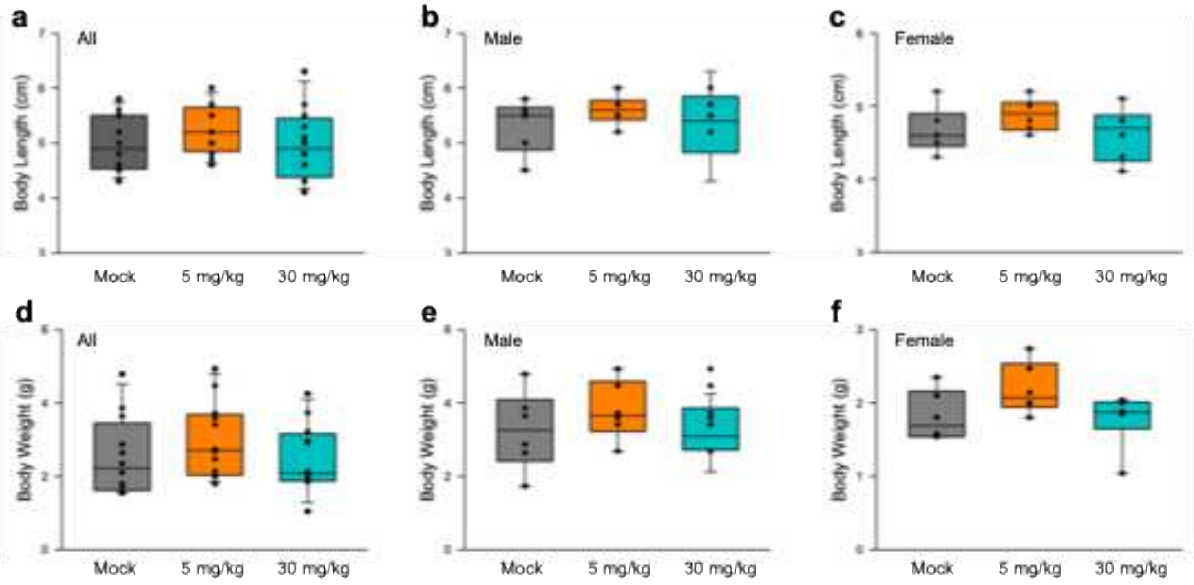
432 **Fig. 3 Sildenafil did not markedly affect somatic growth patterns during aging**

433 Longitudinal changes in body length and body weight were assessed in African turquoise killifish treated with
434 Mock, 5 mg/kg sildenafil, or 30 mg/kg sildenafil. (a, d) Body length and body weight in the overall population.
435 (b, e) Body length and body weight in male fish. (c, f) Body length and body weight in female fish. Gray: Mock
436 control group; Orange: 5 mg/kg sildenafil group; Cyan: 30 mg/kg sildenafil group.

437

438

439 **Fig. 4**



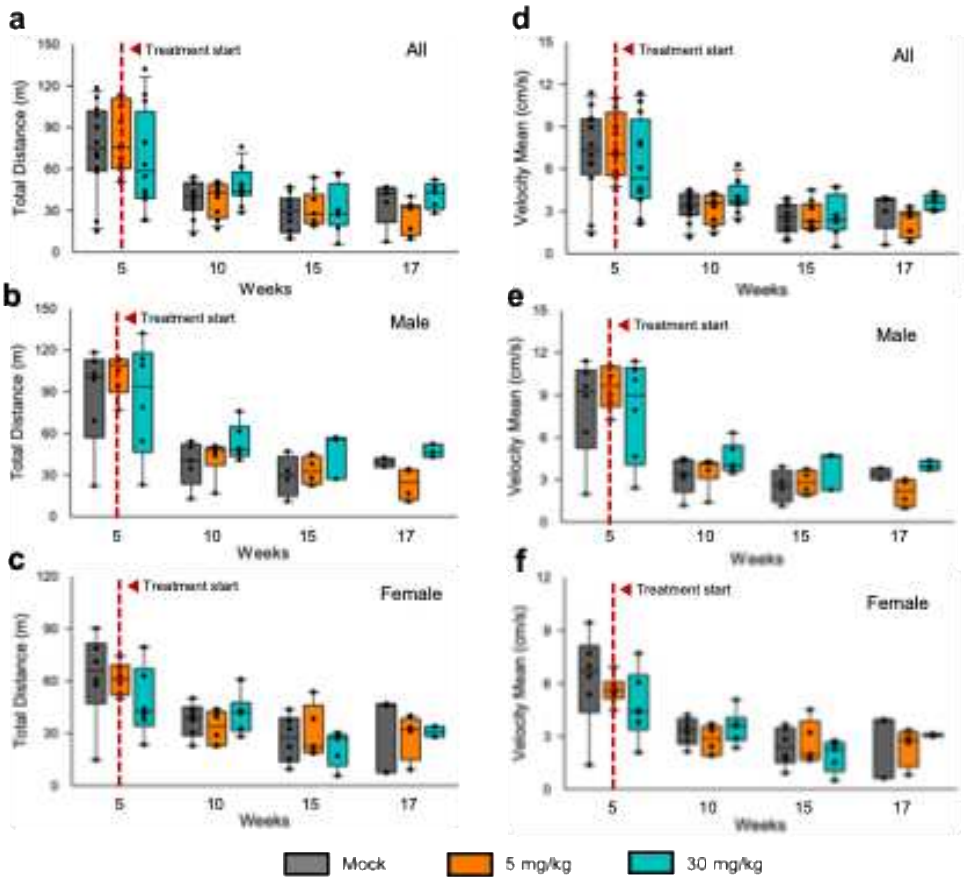
440

441 **Fig. 4 Low-dose sildenafil did not reduce terminal body size or body weight at death**

442 Body length and body weight were measured at the time of death to evaluate terminal somatic status in Mock-, 5
443 mg/kg sildenafil-, and 30 mg/kg sildenafil-treated killifish. (a, d) Terminal body length and body weight in all fish.
444 (b, e) Terminal body length and body weight in males. (c, f) Terminal body length and body weight in females.
445 Data are presented as box-and-whisker plots with individual data points. Gray: Mock control group; Orange: 5
446 mg/kg sildenafil group; Cyan: 30 mg/kg sildenafil group.

447

448 **Fig. 5**



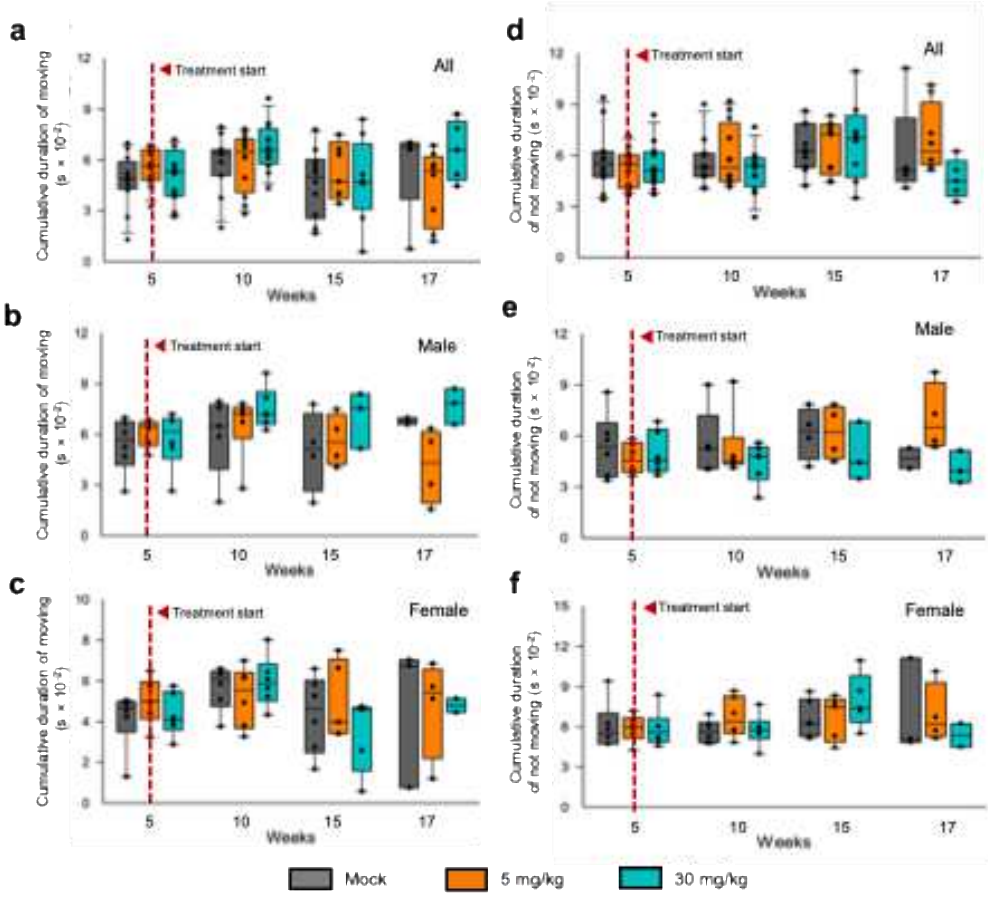
449

450 **Fig. 5 Sildenafil did not robustly prevent age-associated decline in locomotor activity**

451 Locomotor activity was assessed in African turquoise killifish treated with Mock, 5 mg/kg sildenafil, or 30 mg/kg
452 sildenafil. Total distance traveled and mean velocity were quantified as representative locomotor parameters. The
453 red dashed line indicates the start of sildenafil treatment at 5 weeks after hatching. (a–c) Total distance traveled in
454 all fish (a), males (b), and females (c). (d–f) Mean velocity in all fish (d), males (e), and females (f). Data are
455 presented as box-and-whisker plots with individual data points. Gray: Mock control group; Orange: 5 mg/kg
456 sildenafil group; Cyan: 30 mg/kg sildenafil group.

457

458 **Fig. 6**



459

460 **Fig.6 Sildenafil did not consistently alter moving or non-moving duration during aging**

461 Cumulative duration of moving and not-moving was assessed in African turquoise killifish treated with Mock, 5
462 mg/kg sildenafil, or 30 mg/kg sildenafil. The red dashed line indicates the start of sildenafil treatment at 5 weeks
463 after hatching. (a–c) Cumulative duration of moving in all fish (a), males (b), and females (c). (d–f) Cumulative
464 duration of not-moving in all fish (d), males (e), and females (f). Data are presented as box-and-whisker plots with
465 individual data points. Gray: Mock control group; Orange: 5 mg/kg sildenafil group; Cyan: 30 mg/kg sildenafil
466 group.

467

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