Automated, Transparent, and Scalable Longevity Research Across Model Organisms: Introducing the pump.science Protocol

[3,4]Leibowitz, B., [2]Lee, M.B., [2]Blue, B., [1]Petr, M., [3,4]Casalini, J.

Affiliations

[1]Tracked Biotechnologies LLC, 11100 Endeavor Ct., Suite 137, 20109, Manassas, VA, USA

[2]Ora Biomedical, Inc., 12101 Tukwila International Blvd., Ste. 210, 98168, Tukwila, WA, USA

[3]Pump.Science, C/O MJP PARTNERS AG, Bahnhofstrasse 20, 6300 Zug, Switzerland

[4]Molecule AG, Schwanenfelsstrasse 10a, 8212 Neuhausen am Rheinfall, Switzerland

Abstract

Aging research has produced numerous candidate interventions, yet most remain untested across phylogenetically diverse models. Scalable and reproducible pipelines for evaluating geroprotective compounds remain a critical, unmet need for translational geroscience. This study introduces pump.science, a decentralized, cross-species platform for open-access, high-throughput screening of longevity candidates. Using standardized protocols, the effects of Urolithin A and rifampicin were assessed in *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus*, incorporating both lifespan and functional healthspan metrics. Rifampicin significantly extended the lifespan of nematodes, but failed to confer benefits in flies and mice. Urolithin A modestly preserved midlife activity in flies without affecting longevity or neuromuscular performance. While fly and mouse studies were limited by a smaller number of animals tested, these proof-of-concept studies allowed validation of the Pump.Science decentralized science protocol. These findings emphasize the importance of comparative multi-species validation. The pump.science framework provides a proof-of-concept for

transparent decentralized intervention testing. Future efforts should focus on compound diversification, integration of multi-omic endpoints, and adaptive protocol refinement to enhance mechanistic insights and translational relevance.

Keywords: Longevity; Rifampicin; Urolithin A; Aging; In vivo; Real-time monitoring

Corresponding author: B. Leibowitz, C/O MJP PARTNERS AG, Bahnhofstrasse 20, 6300 Zug, Switzerland. Email: benji@pump.science. Tel: +41 041 711 13 66.

1. Introduction

Aging is a systemic, multifactorial process characterized by progressive loss of physiological integrity, ultimately leading to increased susceptibility to chronic diseases and functional decline. The Hallmarks of Aging framework categorizes this complexity into twelve conserved biological processes, including genomic instability, mitochondrial dysfunction, deregulated nutrient sensing, and altered intercellular communication, which collectively drive age-related decline [1,2]. These hallmarks are not merely descriptive but mechanistically actionable targets, forming the foundation for the Geroscience Hypothesis, which proposes that interventions targeting fundamental aging processes can delay or prevent the onset of multiple chronic diseases simultaneously [2,3].

Despite substantial progress in elucidating the molecular mechanisms of aging, the translation of promising interventions such as caloric restriction mimetics, NAD^+ boosters, and mTOR inhibitors into broadly effective gerotherapies for humans remains elusive. This translational bottleneck arises not from a lack of mechanistic insight, but from methodological fragmentation: longevity research often depends on isolated, species-specific results derived under non-standardized conditions, with limited reproducibility and restricted access to data and protocols [4,5,6]. Even well studied compounds such as rapamycin or metformin frequently yield conflicting results across taxa, highlighting the need for systematic cross-species pipelines that combine scalability with mechanistic rigor and transparency [2,3].

Cross-species experimentation has emerged as a critical strategy in longevity studies, allowing researchers to distinguish between evolutionarily conserved aging pathways and species-specific responses to interventions. While human aging remains the ultimate target of geroscience research, ethical and practical constraints necessitate the use of model organisms as proxies, each offering distinct advantages and limitations for interrogating aging processes. Translational geroscience has emerged as a critical strategy in longevity studies, allowing researchers to distinguish between evolutionarily conserved aging pathways and species-specific responses to interventions [7]. While human aging remains the ultimate target of geroscience research, ethical and practical constraints necessitate the use of model organisms as proxies, each offering distinct advantages and limitations for interrogating aging processes [8].

Model organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, have long served as efficient platforms for high-throughput lifespan screening [9,10]. These invertebrate systems offer genetic tractability and conserved pathways including those linked to mitochondrial function, proteostasis, and stress resistance [5,11,12]. Meanwhile, mammalian models such as *Mus musculus* remain indispensable for translational validation, where short-term functional phenotypes, such as endurance, motor coordination, and molecular biomarkers can serve as early surrogates for health span [13,14]. However, existing research infrastructure typically assesses these species in isolation, lacks transparency in results, and operates within centralized, high-cost laboratory systems that are inaccessible to early stage experimentation.

In response to these challenges, we have developed pump.science, a decentralized, community-driven research platform designed to overcome the current limitations of longevity pipelines. By integrating citizen funding, open protocols, and data transparency, pump.science enables a tiered testing protocol from *C. elegans* to *D. melanogaster* to *M. musculus*, advancing only those compounds that demonstrated non-toxic and non-detrimental effects on lifespan or surrogate phenotypes in the preceding model. All experimental data, including raw images, metadata, and null results, were streamed to a public repository and logged for verification ensuring transparency and reproducibility. This approach supports systematic cross-species comparisons, facilitates hypothesis testing and impedes falsification of data at scale through transparent reporting, and lowers barriers to entry for innovative, mechanistically grounded interventions.

To validate this platform and provide a proof-of-concept for its use in geroscience research, we tested two mechanistically distinct compounds, previously shown to extend lifespan and health measures, Urolithin A and rifampicin, in *C. elegans*, *D. melanogaster*, and *M. musculus*. Urolithin A is a gut-derived metabolite produced by the microbiome from ellagitannins and has been shown to induce mitophagy and improve mitochondrial function, with evidence for both lifespan extension in invertebrates and improved muscle endurance in rodents [6,14]. Additionally, in a randomized, placebo-controlled trial in middle-aged adults, Urolithin A has been shown to exert clinically meaningful improvements on aerobic endurance and physical performance [15]. Rifampicin, on the other hand, is a broad-spectrum antibiotic with proposed geroprotective properties in invertebrates, potentially via hormetic mechanisms or modulation of bacterial metabolites in live feed systems [12,16]. Beyond its antimicrobial activity, rifampicin exhibits anti-inflammatory effects and proteostasis support, though it has never been tested for lifespan extension in humans due to the risk of potential hepatotoxicity [17, 18, 19]. While both compounds have shown beneficial effects in individual models, a standardized evaluation of phylogenetically diverse organisms has not yet been conducted.

In summary, this study introduces and validates pump.science as a decentralized, high-throughput protocol for the systematic evaluation of longevity interventions. This work represents a proof-of-concept pilot with acknowledged limitations in statistical power. Our aims are twofold: (1) to demonstrate a staged, automated, and community-funded workflow for cross-species assessment of candidate compounds with real-time data transparency; and (2) to generate preliminary data on compound efficacy across phylogenetically diverse organisms.

The modest sample sizes, particularly in the fly and mouse experiments, limit definitive conclusions about intervention efficacy but provide valuable insights for protocol refinement and future study design.

2. Methodology

Standardized, high-throughput workflows were implemented to assess the cross-species efficacy of Urolithin A and rifampicin, encompassing automated lifespan and activity monitoring in *C. elegans* and *D. melanogaste*r as well as functional rotarod performance testing in *M. musculus.* An overview of the staged species-escalation protocol guiding compound progression is presented in **Figure 1**.

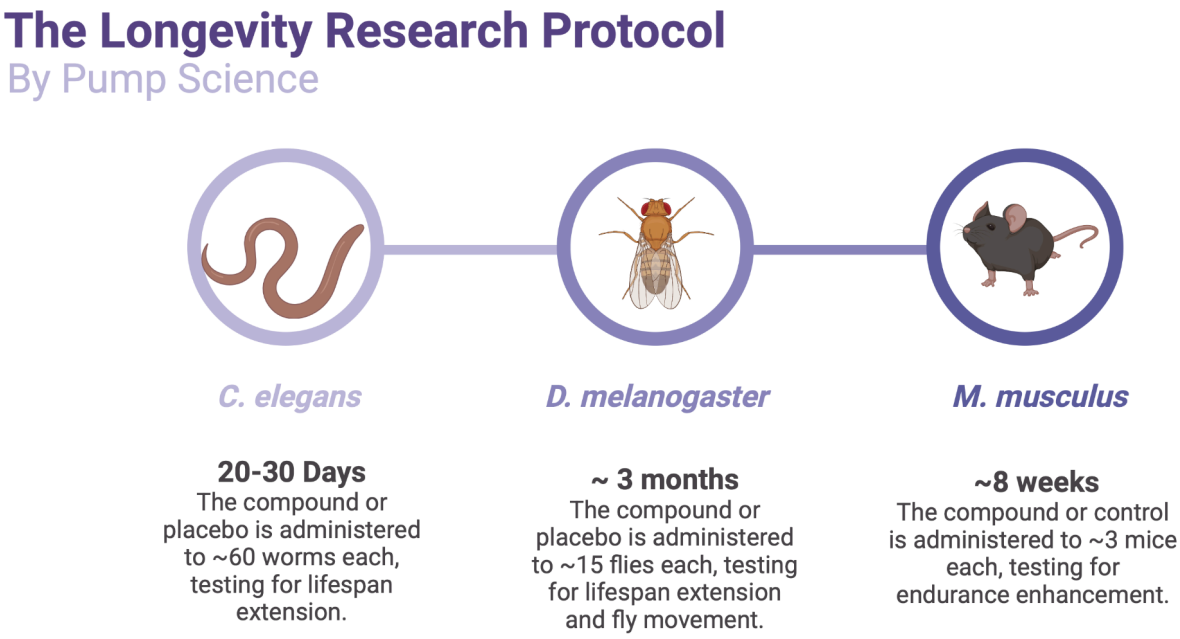


Figure 1. *The pump.science Longevity research protocol. To determine whether a compound has longevity potential it follows the research protocol in the illustration (C. elegans -> D. melanogaster -> M. musculus). If a compound does not reduce maximum lifespan (or endurance), it progresses to the next animal model.*

2.1 C. elegans Lifespan Assays:

Lifespan experiments were conducted by Ora Biomedical as part of the Million Molecule Challenge [4]. In brief the experiment was run using the wild-type *C. elegans* strain N2 (Bristol) at 25 °C. Synchronized *C. elegans* populations were obtained by allowing eggs to hatch overnight and grow to late L4 larval stage on nematode growth medium (NGM) agar seeded with live *E. coli*. At L4, *C. elegans* were transferred to NGM plates containing either Urolithin A (80 µM; Sigma, cat#SML1791), rifampicin (30 µM; Sigma, cat#R3501), or 1% DMSO as control immediately before recording, typically at 1 day old. Test compounds were all dissolved in

DMSO. Each treatment was tested in five replicates (n=5) per group with populations of ~18–30 *C. elegans* per plate. To prevent confounding by progeny, plates included 50 µM floxuridine (FUdR). *C. elegans* survival was monitored for 27 days, with images being captured multiple times a day (75-98 per condition) using the WormBot automated imaging platform [16]. The WormBot-AI pipeline automatically distinguished live vs dead worms in captured images, generating high-frequency survival data. Lifespan data from replicates per condition were pooled for analysis. Survival curves were analyzed by the log-rank test (Mantel–Cox) to assess statistical significance of differences between each treatment and the control. A high-contrast WormBot snapshot of synchronized L4-stage *C. elegans* on NGM agar containing 30 µM rifampicin, captured under custom illumination to enable AI-based live/dead classification, is shown in **Figure 2**.

Figure 2. *Representative WormBot image of C. elegans grown on an NGM agar plate treated with 30 µM rifampicin. The illuminated and clear acrylic well enabled high-contrast imaging, allowing the WormBot-AI pipeline to automatically distinguish live from dead C. elegans during daily survival monitoring. Each treatment was tested in five replicates (n=5) per group with populations of ~18–30 C. elegans per plate.*

2.2 Drosophila Lifespan and Activity Monitoring:

Cohorts of adult *Drosophila melanogaster* (strain w1118, only males) were reared and tested at 25 °C with 12-hour light/12-hour dark cycles. *D. melanogaster* were maintained in groups (each n≈15–20 per vial, 1 vial per group) on a standard sugar-yeast diet. Urolithin A (80 µM in 0.8% DMSO; Sigma, cat#SML1791) and rifampicin (30 µM in 0.3% DMSO; Sigma, cat#R3501) were mixed into the food. Control groups received DMSO at 0.3% or 0.8%. Flies were transferred to fresh food vials 1–2 times per week to maintain drug exposure and food quality. Survival was recorded daily: TrackedFlyBox™, an automated imaging system, captured video recordings and

an algorithm detected mortality events. In addition to lifespan, locomotor activity was continuously tracked using an optical motion tracking system from TrackedFlyBox™. Each vial was placed in the TrackedFlyBox™that recorded movement (via video tracking) in 1-day time bins. This provided measures of total distance traveled and average speed per day for each group. The high temporal resolution also enabled circadian analysis: activity data were binned by time-of-day (hour 0–23) and by age group (young 0–20 days, mid-age 21–40 days, old 41+ days) to examine how diurnal activity patterns changed with age and treatment. Additionally, *D. melanogaster* position within the vial (vertical distribution) was analyzed by dividing the space into zones (Z1 bottom, Z2 middle, Z3 top). The percentage of time *D. melanogaster* spent in each zone at different ages was computed as an indicator of climbing ability. Statistical comparisons were made between treatment and control for locomotor metrics: t-tests or ANOVA on mean distance/speed, and chi-square tests were used for zone occupancy distributions, with Bonferroni corrections for multiple time points. A representative frame from the TrackedFlyBox™ showing AI-detected bounding boxes around individual *w1118* flies in treatment vials for simultaneous survival and activity tracking is shown in **Figure 3**.

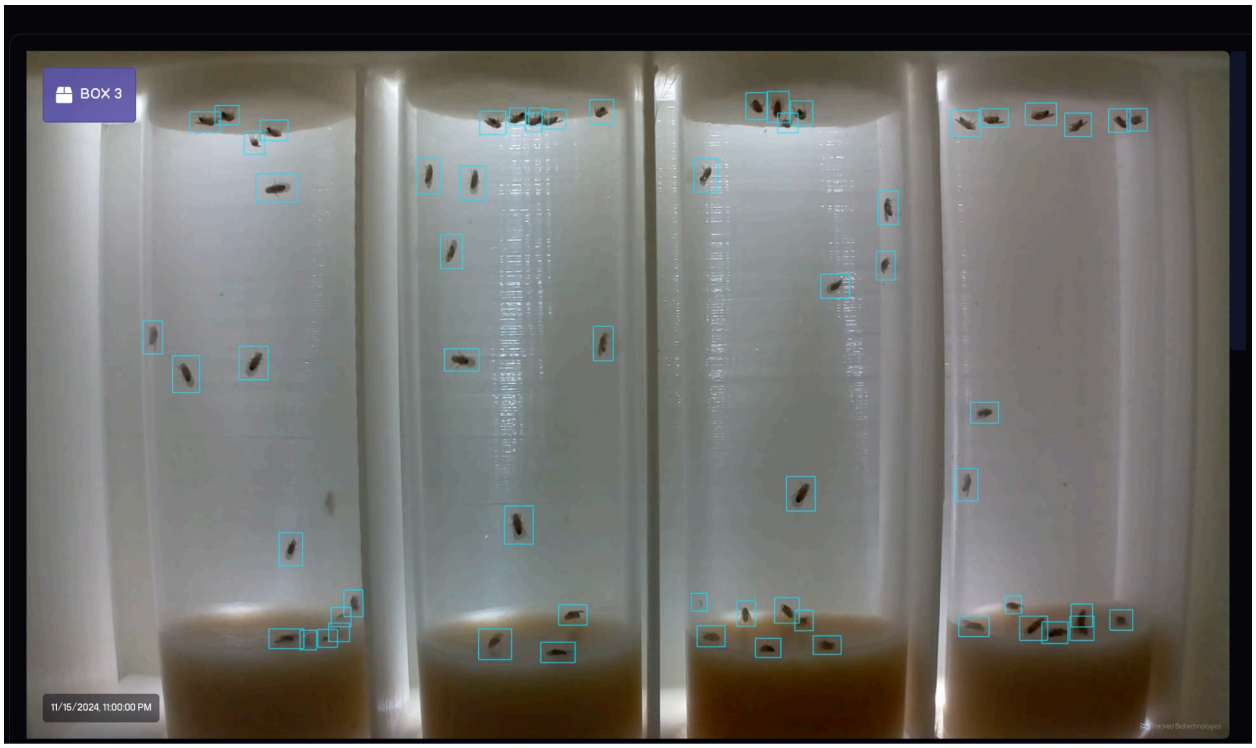


Figure 3. *Representative frame from TrackedFlyBox™ showing male w1118 flies maintained on food containing 30 µM rifampicin (0.3% DMSO). Cyan bounding boxes denote each D. melanogaster detected by the optical motion-tracking algorithm, enabling automated measurement of survival and daily locomotor activity under 12:12 light:dark conditions. Each treatment was tested in one replicate (n=1) per group with populations of 15 flies per vial.*

2.3 Mouse Rotarod Performance Test:

To probe potential functional effects in a mammalian model, a rotarod test was conducted in adult mice *(M. musculus)* from The Jackson Laboratory, by Tracked Biotechnologies LLC. Groups of C57BL/6 mice (18 months old, both sexes, n=3 per group) were treated with either Urolithin A dietary supplement, rifampicin, or vehicle. Urolithin A (Neurogan, 99% Urolithin A) was administered via daily oral gavage (roughly 50 mg/kg/day, based on prior rodent studies) [20], and rifampicin (Sigma, cat#R3501) via daily intraperitoneal injection (approximately 10 mg/kg/day) [14,21]. Vehicle conditions per group: Urolithin A: 25% DMSO for females, 42% DMSO for males; Rifampicin: 3% DMSO for females, 5% DMSO for males. Treatment lasted 8 weeks. Mice were tested on an accelerating rotarod (a rotating rod apparatus) once per week to assess motor coordination and endurance. Prior to the first test, all subjects underwent a pre-training session on the rotarod (10 minutes at a constant low speed of 4 rpm) to familiarize them with the apparatus. For each weekly test, mice were placed on the rod (with sufficient spacing to avoid interference) and the rotarod was started at 0 rpm, then gradually accelerated at 7.2 rpm^2 with an acceleration interval of 5 seconds. The maximum speed and duration were set such that if a subject remained on the rod for 10 minutes, the trial would end to avoid exhaustion (in practice, the accelerating protocol caused all subjects to fall before the full 10 minutes). Each subject underwent 3 trials per session, with 15-minute rest intervals between trials. The primary outcome measured was latency to fall (in seconds) for each trial, and secondary outcomes were the rotational speed at fall and distance traveled on the rod (which correlates with latency and rod speed). Weekly performance across the 8-week period was recorded. A representative view of the rotarod apparatus is shown in **Figure 4**, where six mice were simultaneously tested in numbered lanes beneath a clear safety shield, allowing the automated capture of each animal's fall latency under the accelerating protocol.

Table 1. Summary of *M. musculus* experimental treatments.

Subject	Treatment	Sex
Mouse 1	Rifampicin: 10mg/kg/day in 5% DMSO	Male
Mouse 2	Urolithin A: 50mg/kg/day in 42% DMSO	Male
Mouse 3	Rifampicin Control: 5% DMSO	Male
Mouse 4	Rifampicin: 10mg/kg/day in 5% DMSO	Male
Mouse 5	Urolithin A: 50mg/kg/day in 42% DMSO	Male
Mouse 6	Urolithin A Control: 42% DMSO	Male
Mouse 7	Rifampicin: 10mg/kg/day in 3% DMSO	Female
Mouse 8	Urolithin A: 50mg/kg/day in 25% DMSO	Female

Mouse 9	Urolithin A Control: 25% DMSO	Female

Figure 4. *Representative rotarod setup for endurance testing in C57BL/6 mice. Six mice (18 months old, both sexes) were positioned on the rotating rod apparatus, with individual lanes numbered 1–6. The acrylic safety shield prevents falls from escaping the capture area. The rotarod accelerates from 0 rpm at 40 rpm² (5 s intervals) until each animal falls (or 600 s elapses), and the overhead video records each run for automated latency-to-fall analysis. After testing the male mice on the rotarod, the apparatus was wiped clean with isopropyl alcohol before further use.*

2.4 Funding and Execution Mechanics

Experiments in this study were initiated through the pump.science platform, which operates using a staged funding model denominated in USD target funding amounts, based on the cost to run each experiment. Each candidate intervention was launched and tokenized, where fees from token trading accumulated a research treasury. *C. elegans* data was provided free of charge to facilitate developing the pump.science protocol. Critical experimental stages were triggered only after reaching predefined funding thresholds: *D. melanogaster* lifespan assays initiated at approximately $6,000 USD, and mouse endurance studies at approximately $46,000 USD (pump.science). The value reached corresponds to the cost of running the respective studies. Importantly, a toxicity and feasibility review was conducted at each transition point before experiments proceeded to the next organism, serving as a go/no-go filter. If initial results indicated unacceptable toxicity or impracticality, subsequent stages could be halted to prevent

unnecessary use of animals and resources. This staged, conditional execution model allowed for rapid yet responsible progression through species, while dynamically aligning scientific priorities with funding and early experimental outcomes.

2.5 Ethical Considerations and Approvals

All experiments were conducted under strict ethical supervision and in compliance with species-specific regulatory requirements.

Invertebrate studies (*C. elegans* and *D. melanogaster*)

Under Directive 2010/63/EU, which by Article 1(3) explicitly applies only to live non-human vertebrates (including cephalopods) and excludes all other invertebrates, research conducted with the nematode *C. elegans* and the fruit fly *D. melanogaster* did not require vertebrate-use protocol approval. Nevertheless, to ensure maximal rigor and reproducibility, all invertebrate assays were conducted at accredited partner laboratories (Ora Biomedical and Tracked Biotechnologies LLC) following the standardized, high-throughput pipelines detailed in Sections 2.1–2.2 of this manuscript.

Vertebrate studies (*M. Musculus*)

M. musculus experiments were conducted under the Institutional Animal Care and Use Committee (IACUC) protocol held by the contract facility, following its submission to, review by, and approval from the facility's IACUC prior to initiation and in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (US DHHS, 2002) and the National Research Council's Guide for the Care and Use of Laboratory Animals (ILAR, 1996).

3. Results

Lifespan extension and healthspan outcomes for rifampicin and Urolithin A were assessed in *C. elegans, D. melanogaster*, and *M. musculus* using the pump.science platform for automated high-throughput assays.

3.1 Lifespan Extension in *C. elegans*

Aging research frequently utilizes *C. elegans* as a model organism due to its short lifespan, genetic tractability, and the conservation of many aging-related pathways with higher organisms [5]. *C. elegans* has proven valuable for high-throughput screening of compounds with potential pro-longevity effects, enabling rapid assessment of interventions that may modulate aging and extend lifespan [5]. Among the compounds tested in recent studies, both rifampicin and Urolithin A have emerged as candidates capable of promoting longevity in *C. elegans*, though their mechanisms and efficacy differ [6,16].

Lifespan assays revealed that both rifampicin and Urolithin A significantly extended the survival of *C. elegans* compared to the DMSO control, as illustrated in **Figure 5**. Rifampicin treatment resulted in a pronounced increase in longevity, with a 32.9% extension in lifespan ($p<0.001$), as indicated by the substantial rightward shift of the survival curve relative to the control. In contrast, Urolithin A produced a more modest but still statistically significant extension of 6.4% ($p<0.001$). These findings indicate that while both compounds possess pro-longevity effects in *C. elegans*, rifampicin exhibits a markedly greater efficacy under the conditions tested. The data support rifampicin as a potent lifespan-extending intervention, consistent with previous reports of its ability to modulate aging-related pathways in this model organism [16].

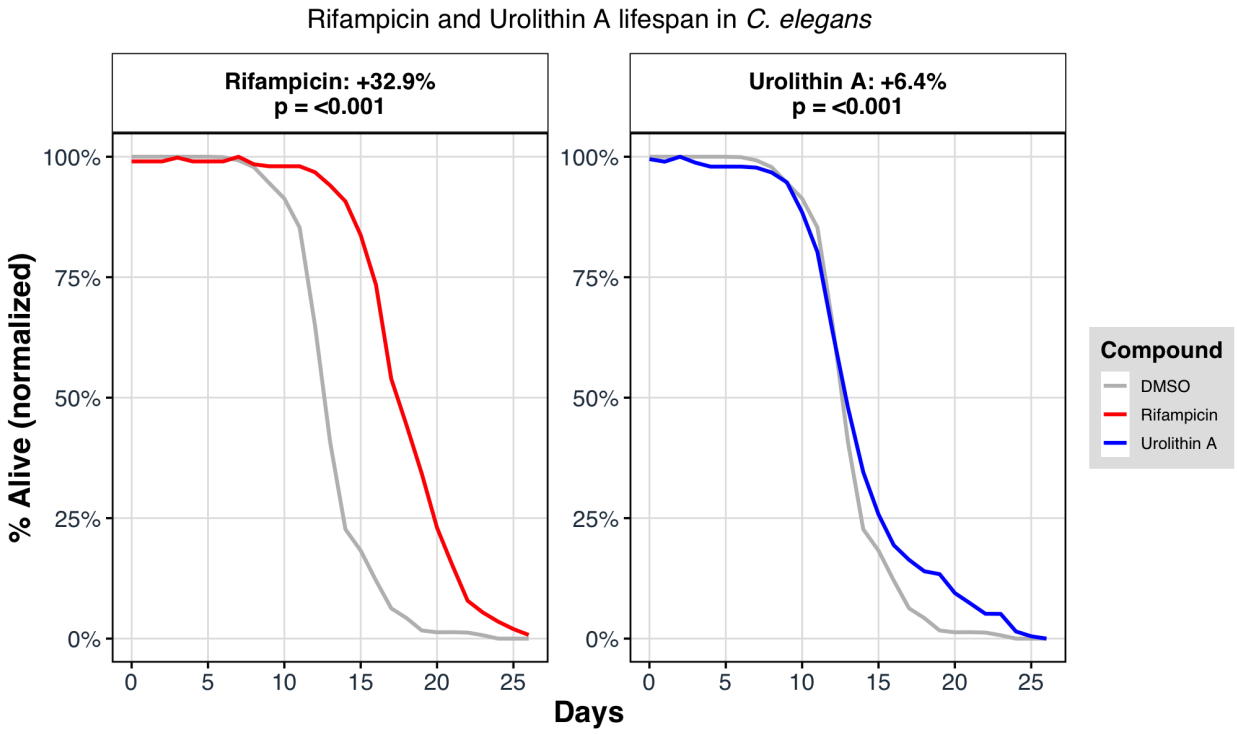


Figure 5. *C. elegans survival data revealed effects of Urolithin A and rifampicin on longevity. Percentage of alive individuals was calculated for each treatment versus control. Legend: 1% DMSO control; grey, 80 µM Urolithin A; blue, 30 µM rifampicin; red. Five replicate populations of 15-25 animals per group (N=5).*

3.1.1 Comparison of pump.science longevity studies with existing rifampicin and Urolithin A lifespan extension data in *C. elegans.*

To contextualize the longevity findings in *C. elegans*, the newly generated median lifespan extension data was benchmarked against existing studies recorded on the DrugAge database - a manually curated repository of 1,046 longevity-affecting compounds tested across 27 model organisms. This comparison leveraged DrugAge's standardized records of lifespan experiments (3,423 assays from 663 studies), including key parameters like dosage and statistical significance thresholds [22]. In regards to rifampicin, 11 combined data points of varying

concentrations (including 1μM, 5μM, 10μM, 20μM, 30μM, 50μM, 100μM, 200μM) exhibited a 30% median lifespan extension [16]. The result of the previously performed studies aligns with what was observed in these experiments (32.9% median lifespan extension), further indicating that rifampicin treatment extends the lifespan of *C. elegans* (**Figure 6A**). Likewise, for Urolithin A, the DrugAge database lists 6 total data points (concentrations: 10μM, 30μM, 50μM), which exhibited at minimum a 24% median lifespan extension [6]. Interestingly, this study observed a moderate median lifespan extension of 6.4% when treating *C. elegans* with 80μM of Urolithin A. Though a statistically significant extension of lifespan in *C. elegans* was achieved, it must be noted that a median extension of 6.4% is lower than what was exhibited in other studies (**Figure 6B**). Of note, this study used a higher concentration of Urolithin A than was previously tested. Together, this indicates that although both Urolithin A and rifampicin extend lifespan in *C. elegans*, which is supported by previously existing experiments [6,16], there may be a maximum effective dose in Urolithin A that was exceeded in the current experiments.

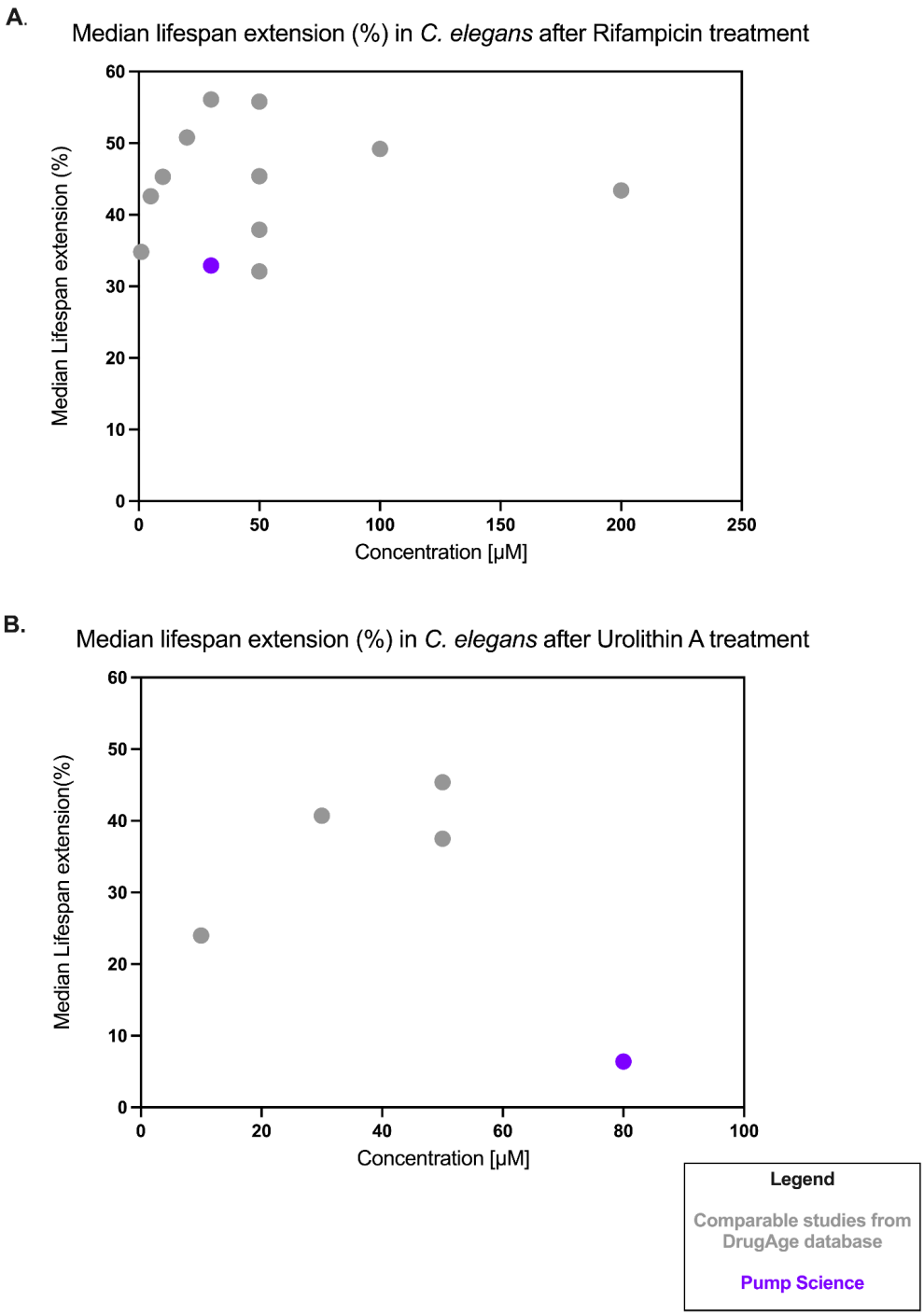


Figure 6. *Comparison of median lifespan extension from longevity studies on DrugAge database with pump.science experiments in C. elegans treated with rifampicin (A) and Urolithin A (B). Legend: historical data; gray, pump.science results; purple.*

3.2 Lifespan and Healthspan Extension in *D. Melanogaster*

To assess the effects of Urolithin A and rifampicin on lifespan and healthspan, a series of survival and behavioral assays were conducted in *D. melanogaster*, conducted by Tracked Biotechnologies LLC. These experiments were designed to determine whether compounds known to modulate aging in other model organisms, such as *C. elegans*, could similarly influence longevity and functional decline in flies. In addition to lifespan measurements, locomotor activity, circadian rhythm integrity, and climbing ability were systematically evaluated to provide a comprehensive overview of how each intervention impacts both survival and age-associated behavioral phenotypes.

All groups had similar early survival, but differences emerged in mid and late life (after 40 days; **Figure 7**). By the end of the experiment at 80 days, all animals in each group had died (as expected given the species' natural lifespan). Urolithin A extended *D. melanogaster* lifespan modestly: median survival was 68.0 days compared to 59.0 days in the corresponding control (DMSO 0.8%). The mean lifespan was also highest for Urolithin A at 65.3 days, with a maximum lifespan of 79 days observed (versus 72 days max in controls). In contrast, rifampicin reduced *D. melanogaster* longevity: median survival was 45.0 days, compared to 60.0 days in the control (DMSO 0.3%), and mean lifespan was slightly higher for rifampicin (49.1 days) than its median, indicating an early mortality effect.

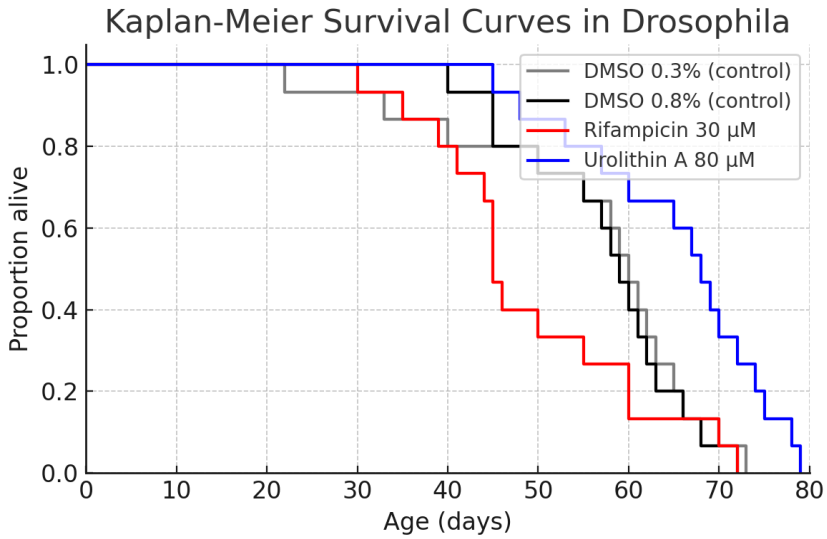


Figure 7. *Kaplan–Meier survival curves for D. melanogaster. Urolithin A (blue line) slightly extends median lifespan relative to solvent control (black lines), whereas rifampicin (red line) shortens lifespan. Shaded gray and black lines indicate control groups (DMSO 0.3% and 0.8% respectively). Groups were n=15 flies per vial, 1 vial per group.*

Statistical analysis showed that neither compound's effect on *D. melanogaster* survival was significant at $p<0.05$. The survival advantage of Urolithin A approached significance: log-rank $p=0.0508$ for Urolithin A vs control, with a hazard ratio HR=0.47 (Cox $p=0.16$) indicating a ~53% reduction in risk of death relative to control. Meanwhile, rifampicin's lifespan-shortening trend did

not reach significance (log-rank p=0.128, HR=1.67 for rifampicin vs control). The concurrent inclusion of two control groups (0.3% and 0.8% DMSO) confirmed that the solvent itself had no significant impact on survival; the two control medians (60.0 vs 59.0 days) were nearly identical. In summary, while neither compound tested produced a significant result, Urolithin A trended towards conferring a mild longevity benefit in flies, whereas rifampicin trended towards a mild detrimental effect on lifespan. Urolithin A-treated flies on average lived about 9 days (15%) longer than controls, whereas rifampicin-treated *D. melanogaster* lived ~15 days (25%) shorter than controls, but variability prevents further conclusions.

3.2.1 Locomotor Activity and Circadian Rhythm in Flies

Beyond survival, continuous monitoring of *D. melanogaster* locomotor activity provided insight into how each intervention impacted healthspan-related metrics such as daily movement and maintenance of circadian rhythms. **Figure 8** illustrates the age-related trajectory of average daily locomotor activity for flies under each condition.

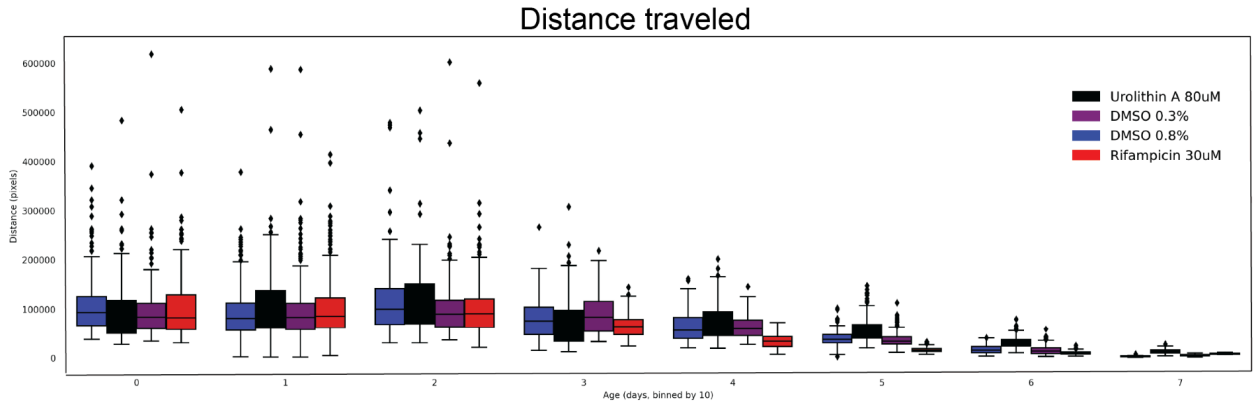


Figure 8. *Age-related decline in average daily locomotor activity (distance moved per day) in Drosophila across treatment groups. Urolithin A (black); rifampicin (red); purple and blue control groups (DMSO 0.3% and 0.8% respectively).*

All groups showed a decline in activity with age: young flies (<20 days) were highly active, while old flies (>60–70 days) were mostly sedentary. However, clear treatment differences emerged. Rifampicin consistently reduced locomotor activity across all ages. Rifampicin-treated flies traveled shorter daily distances than both controls and Urolithin A groups, with the gap widening in mid-life. By old age, activity was low in all groups, but rifampicin flies were the least active. Statistically, mean lifetime activity was significantly lower in the rifampicin group ($p<0.05$) compared to both DMSO controls ($p<0.05$), indicating a consistent negative effect. In contrast, Urolithin A preserved locomotor activity over time. Although activity in old age converged with controls, flies receiving Urolithin A had the highest total distance traveled over their lifespan. While the mean daily distance of flies receiving Urolithin A was slightly higher than DMSO 0.3% controls (not significant, $p=0.294$), it was significantly greater than rifampicin-treated flies ($p<0.05$).

Average speed (distance per unit time while moving) is shown in **Figure 9**. While Urolithin A flies traveled more distance overall, the DMSO 0.8% group had slightly higher peak speeds during bursts of activity, however both observations were not significant. The combination of shorter distance and lower speed in rifampicin flies indicates possible reduced willingness to move but or neuromuscular or metabolic impairment adversely affecting their speed.

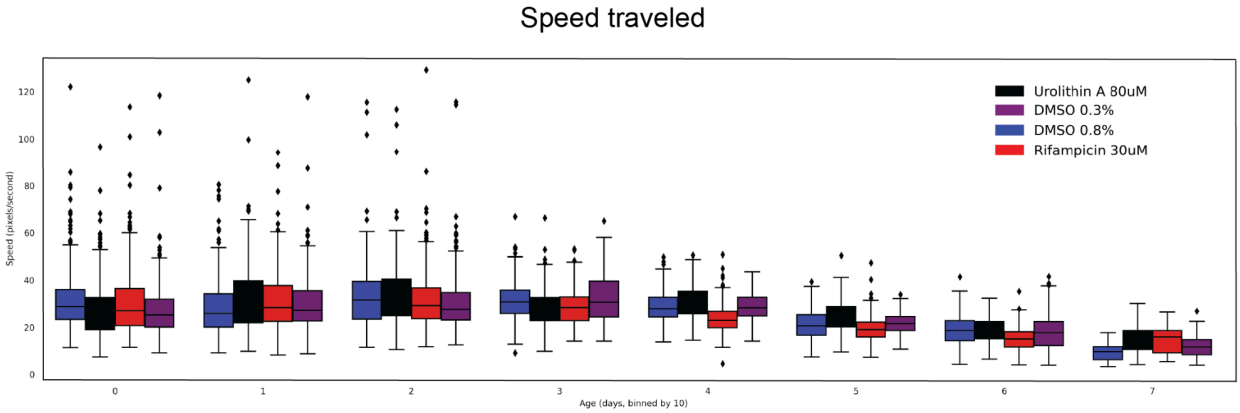


Figure 9. *Activity measurements of speed traveled by Drosophila represented as box and whisker plots, plotted with the mean. Age (days) on x-axis is binned by 10 days; speed on y-axis is measured by pixels/second. Legend: Urolithin A; black, DMSO 0.3%; purple, DMSO 0.8%; blue, rifampicin; red.*

3.2.2 Circadian Activity Patterns

Analysis of activity by time-of-day revealed robust diurnal rhythms in young flies of all groups. **Figure 10** shows that during the daytime (lights on), activity was high, with periodic peaks in the morning and afternoon, while at night (lights off), activity dropped to low baseline levels, consistent with normal *D. melanogaster* circadian behavior. This rhythmic pattern was evident in the 0–20 day age group for each treatment, with only minor differences between groups. For instance, in young control flies, the average distance traveled in the first hour of the day was ~1.0×10^5 units, dropping to ~2.5×10^4 units in the middle of the night – a clear day-night contrast. Urolithin A and rifampicin groups showed similar circadian modulation early in life, indicating neither drug grossly disrupted the central clock or sleep-wake cycles initially.

With aging, however, overall activity amplitude declined and some circadian dysregulation appeared. As fruit flies are crepuscular, they are more active during dawn and dusk periods, and this activity reduces with age [23]. By the 41+ day age group, flies were much less active overall, and the diurnal difference flattened (**Figure 10** lower panels). All treatments showed this age-related dampening of rhythms: daytime activity in old flies might reach only ~5×10^4 units/hour at peak, and nighttime activity ~$1–2\times10^4$ units/hour. There was no strong evidence that Urolithin A preserved circadian rhythmicity better than controls; rather, all groups lost rhythmic vigor similarly in old age. Rifampicin did not accelerate circadian rhythm loss beyond what was seen in controls. In summary, neither compound altered the fundamental circadian activity pattern of the flies; age was the dominant factor in circadian rhythm attenuation.

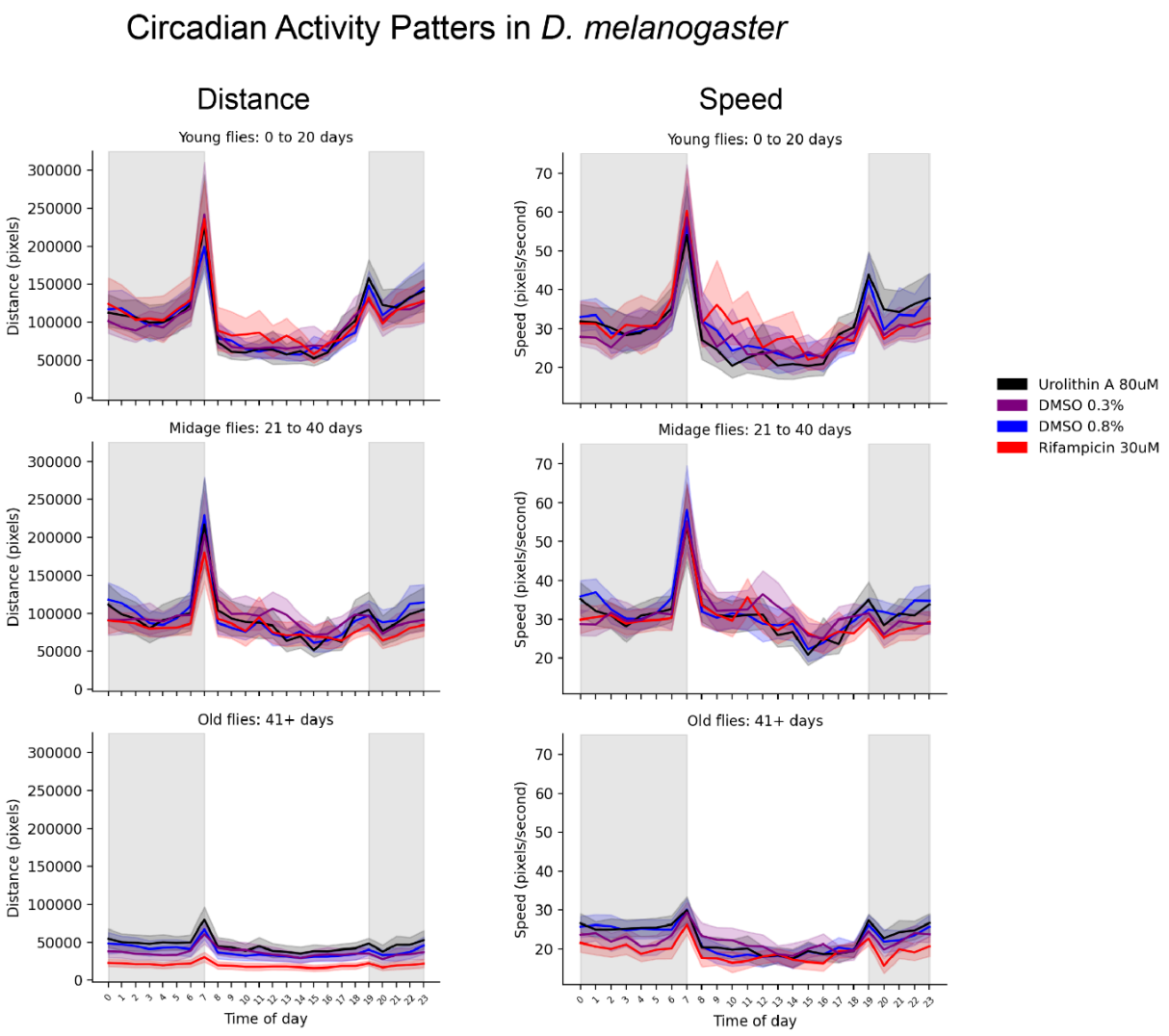


Figure 10. *Circadian activity patterns in Drosophila melanogaster across age groups and treatment conditions. Panels show average distance traveled (left column) and average speed (right column) over the 24-hour cycle for young (0–20 days), mid-age (21–40 days), and old (41+ days) flies. Activity was measured in flies treated with Urolithin A (80 μM, black), DMSO 0.3% (purple), DMSO 0.8% (blue), or rifampicin (30 μM, red). Shaded areas represent the standard error of the mean. Gray backgrounds indicate dark phases of the light/dark cycle. Activity peaks correspond to transitions between light and dark phases. Data illustrate age-dependent declines in activity and speed, as well as treatment-specific effects on circadian locomotor behavior.*

3.3.3 Home-vial (Climbing) Zone Analysis

An additional metric for assessing healthspan involved zonal analysis, utilizing Tracked Biotechnologies' LLC TrackedFlyBox™ platform. This analysis quantified the distribution of time spent by flies in upper, middle and lower zones of the vial (**Figure 11A**). The percentage of time spent in each zone was calculated by summing the number of flies in each zone at specific

timepoints i.e. 7am, and determining the proportion of flies in each zone relative to the total number of flies. This measurement was taken hourly throughout the day. For young flies (days 0 to 20), averages were calculated for each timepoint along with the standard error for this age group. This process was repeated for each time of day, age group, zone, and condition.

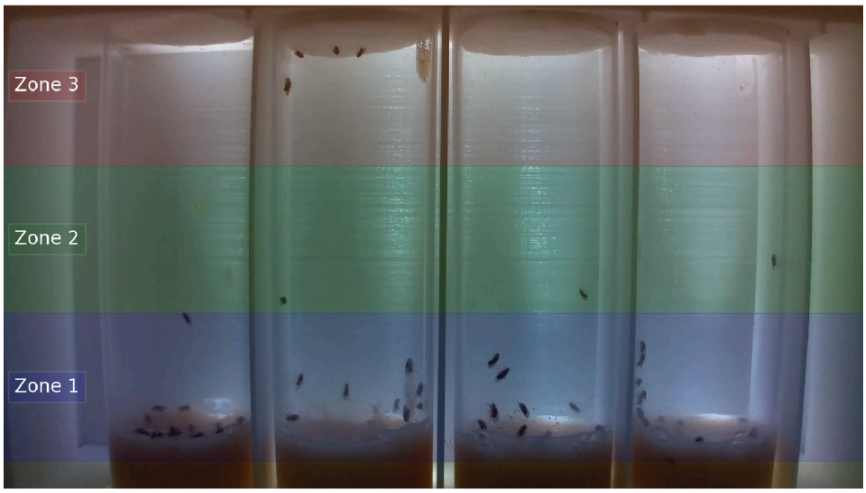
Across all experimental groups, young flies tended to spend more time in zones 2 (Z2) and zone 3 (Z3). As the flies aged, they increasingly remained in the bottom zone 1 (Z1), which corresponded to a reciprocal decrease in time spent in Z2 and Z3, indicating a decline in vigor(**Figure 11B**). The climbing patterns of the treated flies mirrored those of their respective controls, suggesting a concentration-dependent effect of the solvent. Among young flies, no significant difference was observed between DMSO 0.3% and DMSO 0.8% regarding time spent in Z1 ($p=0.7516$). However, in midage flies, a significant increase in time spent in Z1 was observed in DMSO 0.3% treated flies compared to DMSO 0.8% ($p=<0.05$). By old age, the DMSO 0.8% treated flies spent more time in Z3 ($p=<0.05$), DMSO 0.3%.

Young flies treated with Urolithin A spent significantly less time in Z1 ($p<0.05$) and more time in Z2 ($p<0.05$) and Z3 ($p=0.0074$) compared to DMSO 0.8% controls. In mid-age, no significant difference was noted between Urolithin A and DMSO 0.8% groups in Z1, although DMSO 0.8% flies spent more time in Z3 ($p<0.05$) compared to those treated with Urolithin A. When comparing flies treated with Urolithin A and rifampicin, the latter spent more time in Z1 during mid-age ($p<0.05$), while the former occupied Z3 ($p=0.0003$). In old flies, rifampicin-treated flies spent more time in both Z1 and Z3 compared to those treated with Urolithin A ($p<0.05$).

Overall, the activity and behavioral analyses in D. melanogaster suggest that solvent concentration may influence climbing behavior, and the use of zones Z1 and Z3 may be more informative than using all three zones (Z1, Z2, and Z3). Despite observing significant differences, drawing definitive conclusions about the impact on climbing ability is challenging, as neither treatment increased the duration of time spent by the flies in Z3 compared to their controls.

Vertical Zone Analysis in *D. melanogaster*

A.



B.

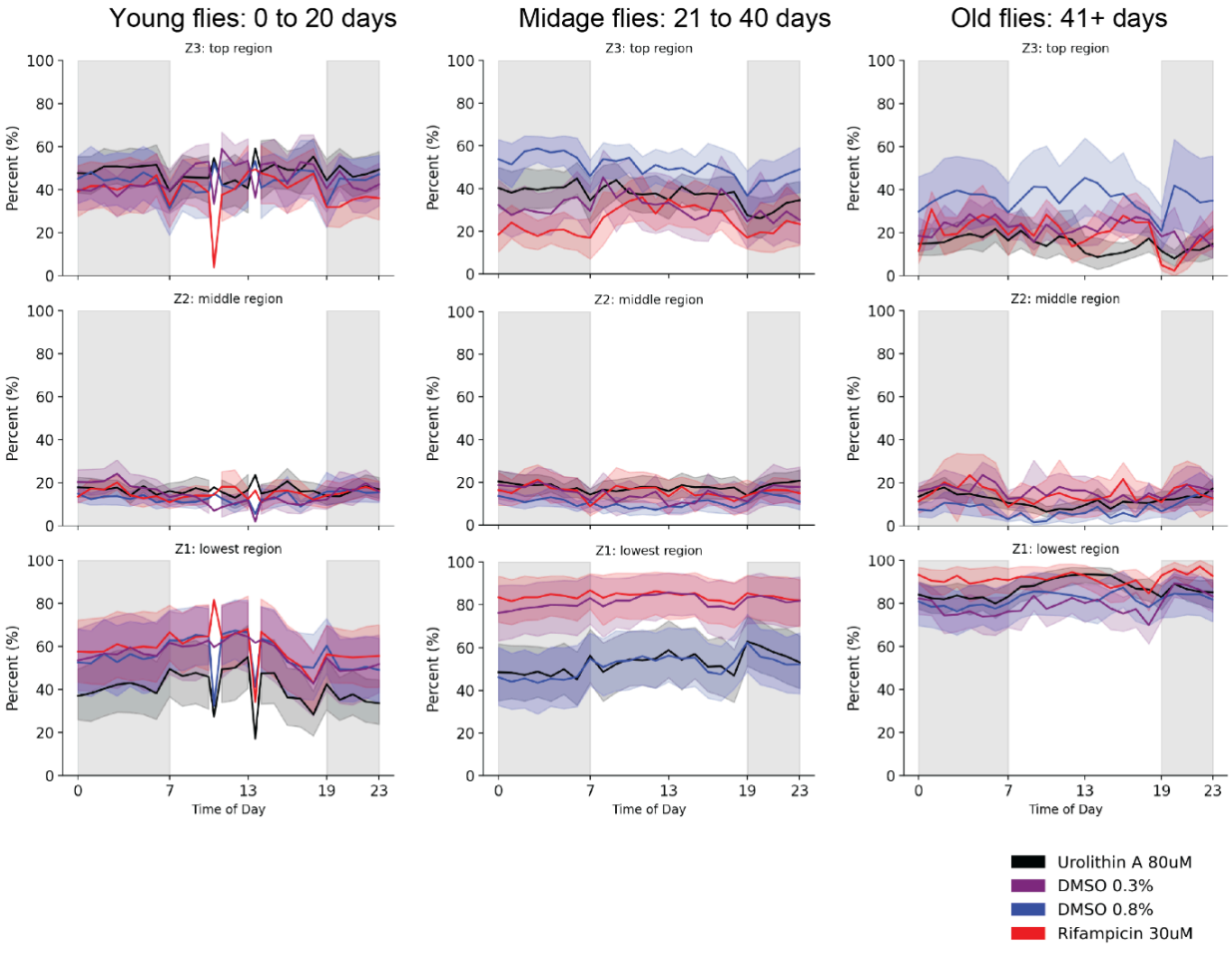


Figure 11. *Vertical Zone Analysis in Drosophila melanogaster. (A) Schematic representation of the vertical zone assay. Flies were housed in vertical tubes divided into three equal regions: Zone 1 (bottom), Zone 2 (middle), and Zone 3 (top), as indicated by colored overlays. The distribution of flies within each zone was recorded to assess vertical positioning. (B) Age- and treatment-dependent distribution of flies across vertical zones. Line plots show the percentage of flies present in each zone (top: Z3, middle: Z2, bottom: Z1) throughout the 24-hour cycle for young (0–20 days), mid-age (21–40 days), and old (41+ days) flies. Flies were treated with Urolithin A (80 μM, black), DMSO 0.3% (purple), DMSO 0.8% (blue), or rifampicin (30 μM, red). Shaded areas represent the standard error of the mean, and gray backgrounds indicate dark phases of the light/dark cycle.*

3.3 *M. Musculus* Functional Endurance Outcomes

To continue with the cross-species assessment of candidate compounds, and to assess whether the effects on longevity benefits or detriments observed in invertebrates translated into mammalian physiology, the Urolithin A dietary supplement and rifampicin were evaluated for their impacts on mouse (*M. musculus*) motor performance using the rotarod assay, conducted by Tracked Biotechnologies LLC. Mice were tested weekly over 8 weeks of treatment to see if either intervention could improve or preserve motor coordination and endurance - traits that tend to decline with age. Concentrations used for both compounds were based on prior published data [24, 25].

3.3.1 Training and Baseline evaluation

The training and baseline sessions took place during the first two weeks of the study. During the initial rotarod sessions, all subjects, regardless of group, showed rapid improvement in latency to fall as they learned the task. By the second week, each subject could remain on the accelerating rod for an extended duration (on average around 180–220 seconds before falling, starting from ~120 seconds in the first trial of week 1). This improvement reflects the learning/acclimation phase and was similar across controls and treated groups, indicating that neither Urolithin A nor rifampicin affected short-term motor learning or coordination in a detectable way.

3.3.2 Performance Over 8 Weeks

Over the subsequent weeks (weeks 3–8 of the trial), performance gains plateaued and slight declines were observed in some subjects. Each subject's best performance (longest latency) per session as well as average latency were tracked across the trials. In the control group (vehicle-treated), rotarod latency gradually declined by roughly 19% from the peak achieved at week 5 compared to the end of week 8. Possible age-related or fatigue-related decline in motor endurance over the 2-month period in the middle-aged mice was observed.

There was no significant difference in average latency to fall between any of the groups at any week. For instance, at week 4, Urolithin A mice averaged 275.1s versus 229.7s for matched

controls. By Week 8 Urolithin A mice recorded average latency of 287.4s against 213.9s for controls. No statistics could be performed due to the small group size.

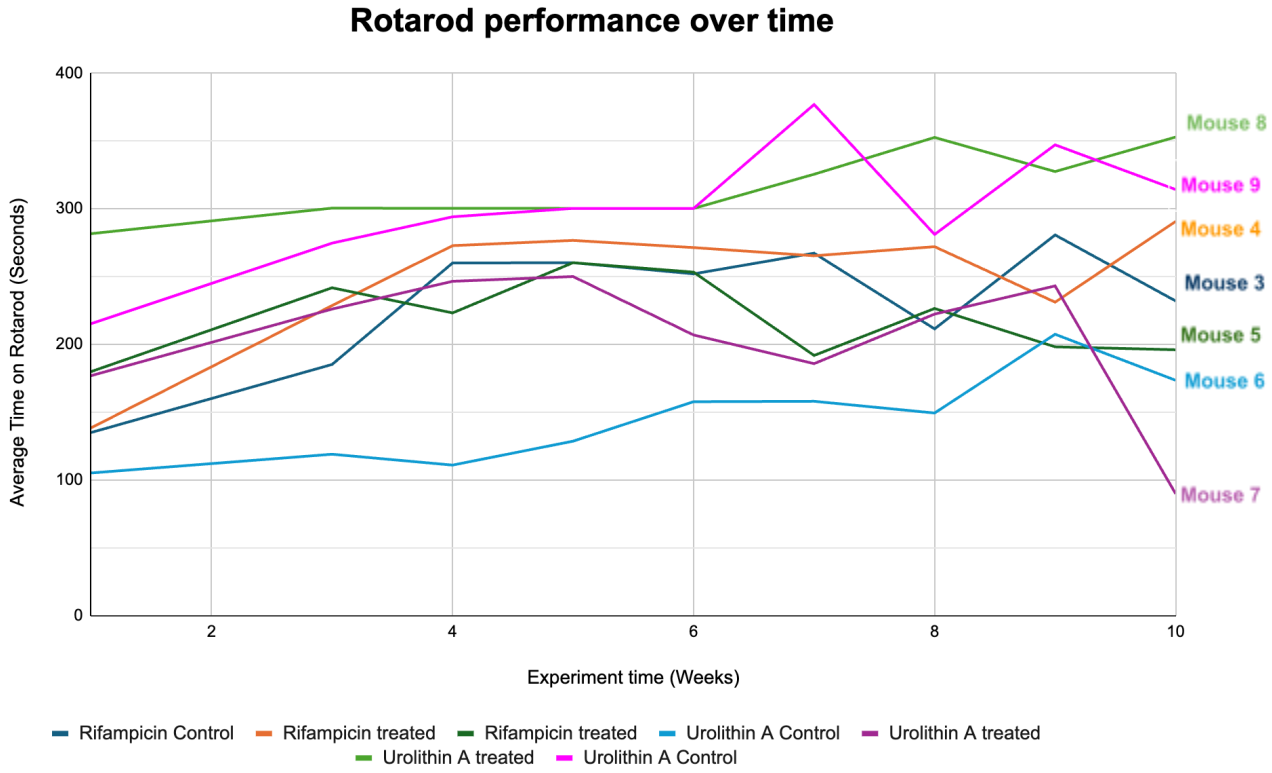


***Figure 12**. Rotarod performance over time for individual mice in different treatment groups. The graph shows the average time (seconds) each mouse remained on the rotarod during weekly testing across a 10-week experimental period. Each colored line represents a single mouse: Rifampicin Control (blue, Mouse 3), Rifampicin Treated (orange, Mouse 4), Urolithin A Control (green = Mouse 8 and pink = Mouse 9), and Urolithin A Treated (purple, Mouse 7; olive green, Mouse 5; light blue, Mouse 6). Mice 1 and 2 were euthanized prior to the end of experiment due to illness. The y-axis indicates average time on the rotarod (seconds), and the x-axis shows experiment duration in weeks.*

3.3.3 Reduced Group Sizes Due to Early Mouse Deaths

Of note, two mice were euthanized during the course of the experiment. The IACUC guidelines on euthanization were met for both mice (i.e. more than 20% weight loss, not eating, self-mutilation, etc.) and cause of death was listed as severe dermatitis, a common occurrence in C57BL/6 mice. The first afflicted mouse was part of the rifampicin-treated group. The second afflicted mouse was part of the Urolithin-A treated group, and was euthanized 8 days after the first mouse. Therefore, after 8 weeks, there were only 2 mice remaining per treatment group, and 3 in the control groups.

3.4 pump.science Usage Metrics

In addition to experimental data, in order to determine the viability of the platform as a host for transparent community-funded experiments, we also collected data on the usage and engagement of the pump.science platform. The pump.science platform has attracted 659,449 unique visitors as of the writing of this article, and over that time, experienced a peak of 28,000 daily active users (DAUs). Additionally, user feedback across multiple social media channels including X and Telegram, further demonstrated exceptional adoption patterns, with users particularly valuing the live experimental interaction, which enables real-time observation of ongoing experiments. Notably, the platform fostered proactive user engagement, evidenced by unsolicited update requests and active discussion of results, a dynamic not often observed in traditional research paradigms. Since the launch of the pump.science protocol, over $2.4B USD in token trading volume has been achieved.

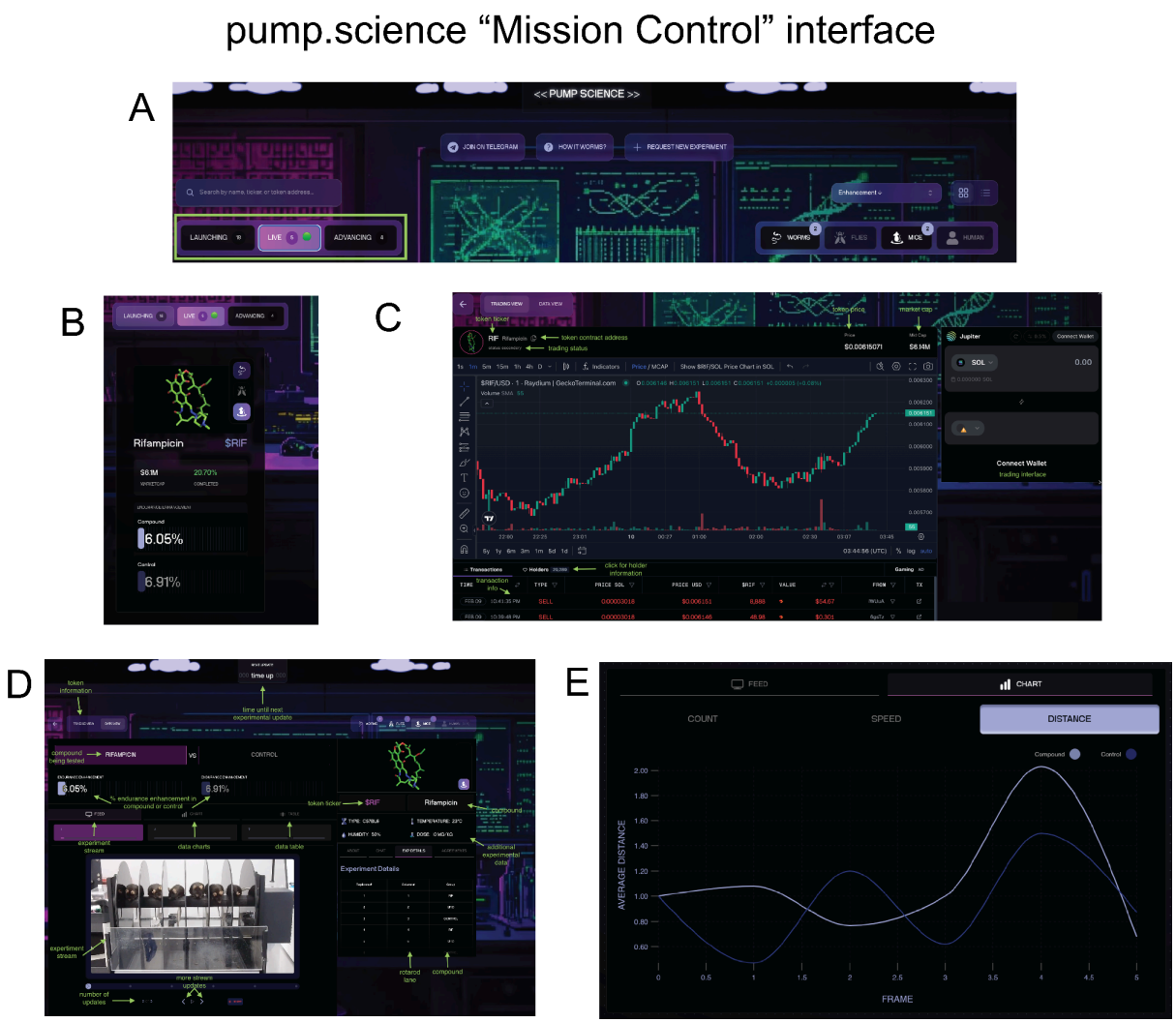


Figure 13. pump.science “Mission Control” interface: *A) pump science homepage tabs. B) Compound card in the live tab indicating RIF is currently in mice experiments that are ~20% complete. C) Trading view tab for a compound token. D)The different components of the pump.science experiment view. E) An example graph of the AI output, which shows the average distance covered by the flies in a particular experimental condition.*

A crucial element in the accessibility and user engagement of the pump.science platform is the platform's intuitive "Mission Control" interface. The homepage of pump.science is strategically organized into three clear sections: 1) Launching, which displays new compound tokens actively fundraising via bonding curves, 2) Live, which shows compounds with active experiments in *C. elegans*, *D. melanogaster*, or *M. musculus*, and 3) Advancing, which features compounds ready for next phase testing (Fig. 13A). Each compound card provides information on funding progress, experiment completion percentage, and key metrics, which allow for assessment of status at a glance (Fig. 13B). Clicking any compound card opens a detailed lab page with a trading view detailing real-time token transactions with dynamic fee alerts (Fig. 13C), or data view which integrates live/replayed video feeds, survival graphs, and structured tables to deliver transparent experimental insights (Fig. 13D). Additionally, the Chart tab makes use of AI-driven analytics so that users may leverage the data of key metrics such as count, speed distance, latency to interpret complex longevity data (Fig. 13E). By presenting real-time science alongside token economics in a unified interface, pump.science empowers users to actively participate in research evaluation and decision-making without the need for specialized expertise.

4. Discussion

This study was designed to assess and validate pump.science as a decentralized, high-throughput protocol for the systematic evaluation of longevity interventions. The aims of the study were (1) to demonstrate a staged, automated, and community-funded workflow for cross-species assessment of candidate compounds with real-time data transparency; and (2) to generate preliminary data on compound efficacy across phylogenetically diverse organisms.

The experiments were performed by contract research organization (CRO) partners, each having their own expertise. Aligning study designs and data specifications that can be generalized across different types of experiments was a requirement for interpretation of the data. The results presented in this study represent a first attempt at integrating common readouts for longevity and healthspan measurements across different species, with the aim of drawing conclusions about efficacy in one species, in order to justify testing an intervention in a subsequent model organism.

While the interpretation of results across groups is important, the primary challenge has been in experimental design- specifically, balancing the need for cost efficiency with the requirement for adequate statistical power to ensure meaningful findings.

4.1 Lifespan Extension in *C. elegans*

The nematode *Caenorhabditis elegans* has long been established as a powerful model in aging research due to its short lifespan, genetic tractability, and the conservation of key longevity pathways, such as insulin/IGF-1 signaling, mitochondrial homeostasis, and stress resistance mechanisms [5,11]. In this study, both rifampicin and Urolithin A extended *C. elegans* lifespan;

however, the magnitude and interpretability of these effects differ substantially and highlight important mechanistic considerations.

Rifampicin extended the median lifespan by over 30%, corroborating prior findings, which hypothesize that its benefits extend beyond antimicrobial action to the modulation of host aging pathway. Golegaonkar et al. (2015) previously showed that rifampicin extends lifespan in *C. elegans* through the activation of the stress-responsive transcription factor DAF-16, a homolog of mammalian FOXO3, as well as its upstream kinase JNK-1. The necessity of both components for lifespan extension, which is demonstrated by the loss of effect in *daf-16* and *jnk-1* mutants, firmly places rifampicin's mechanism within canonical longevity pathways. This distinguishes it from other compounds that act indirectly on bacterial food sources. Previous reports show that rifampicin retains its life-extending capacity even when worms are cultured on heat-killed bacteria, ruling out indirect effects via the microbiome and supporting a direct pharmacological action on the host [16].

At the cellular level, rifampicin treatment has been previously shown to reduce the accumulation of advanced glycation end products (AGEs) and protein aggregates, both hallmarks of proteostatic collapse in aging [16]. This effect is accompanied by the upregulation of cytoprotective genes associated with the stress response and protein homeostasis, suggesting that rifampicin may function as a mitohormetic agent that bolsters the cell's intrinsic defense systems against age-related damage. The activation of DAF-16 and suppression of AGEs places rifampicin among a select group of compounds with the potential to address multiple facets of the aging process.

In contrast, Urolithin A produced only a modest increase in the lifespan of *C. elegans* under the same experimental conditions. Previous reports have documented substantial lifespan extensions, ranging from 10% to 24% at lower doses, particularly in the 10–50 μM range [6]. In this study, the use of a higher dose (80 μM) did not produce lifespan extending effects. A future study should be performed to define the optimal concentration for Urolithin A to extend the lifespan of *C. elegans*. Moreover, the use of live *E. coli* as a food source could impact interpretation as other studies use UV-killed bacteria for feeding worms during treatment [26]. Both feeding methods have been used before with different concentrations of Urolithin A, therefore it is unclear if there are preferences for using one over the other. The metabolism of ellagitannins into Urolithin A is microbially mediated in mammals; although the compound itself was directly administered here, interactions between host and bacterial metabolism remain a potential modifier of bioavailability and efficacy [6]. These nuances highlight the importance of optimizing dosing regimens and considering microbial context in future studies of Urolithin A and related compounds. It is plausible that microbial metabolism, redox state, or nutrient-sensing cues alter the physiological context of Urolithin A action [27].

This high-throughput assay, enabled by automated WormBot imaging, confirmed rifampicin's efficacy while highlighting its direct pharmacological action on host aging pathways rather than indirect microbiome effects [6]. Urolithin A showed a smaller but significant lifespan extension (+6.4%, $p<0.001$), though weaker than historical data, potentially due to dose-dependent biphasic effects or interactions with live E. coli food sources

4.2 Lifespan and Healthspan Extension in *D. Melanogaster*

Findings from *Drosophila melanogaster* highlight both translational opportunities and species-specific pitfalls in aging pharmacology. Despite the well-established utility of *D. melanogaster* in medium-throughput aging studies, differences in drug metabolism, stress response, and neuroendocrine regulation often yield results that diverge from those in nematodes or mammals [10]. The study on *D. melanogaster* provides critical insights into aging through multiple readouts, each offering unique perspectives on longevity and healthspan. *Lifespan measurement* serves as the foundational metric, directly quantifying survival duration. In this experiment, Urolithin A showed a non-significant trend toward extending median lifespan ($p=0.0508$), while rifampicin showed a non-significant trend towards reduced lifespan ($p=0.128$). In order to confirm any species-specific responses to treatment, results would need to be confirmed in a larger cohort.

Locomotor activity reflects the overall level of movement, and is a key indicator of its health and well-being, which can be used to assess both spontaneous activity (movement without external stimuli) and its activity in response to specific cues or stimuli [28]. In these experiments, locomotor activity was measured via total distance traveled and average speed. Rifampicin consistently reduced locomotor activity across the lifespan, resulting in significantly lower lifetime movement compared to both control and Urolithin A groups ($p < 0.05$). While Urolithin A preserved activity with age and significantly outperformed rifampicin ($p < 0.05$), it did not lead to a statistically significant increase in activity compared to DMSO controls ($p = 0.294$). Urolithin A was shown to induce mitophagy and prolong lifespan in *C. elegans* and improve muscle function in rodents [26]. Removal of the microbiome in *Drosophila* by antibiotic treatment has been shown to alter locomotor activity, in a sex-dependent manner, which may reduce the production of beneficial metabolites or alter nutrient absorption and impact systemic health and motor function [29].

Circadian rhythm analysis tracked systemic timing robustness. Circadian integrity is a biomarker of systemic aging, correlating with neurodegeneration and sleep disorders [30]. Young flies exhibited strong diurnal activity patterns, but age-related dampening occurred across all groups, unaffected by interventions.

Climbing ability, assessed through vertical zone occupancy, revealed age-related motor decline. All groups spent increasing time in the bottom zone (Z1) with age. Tracked Biotechnologies LLC has established the first zonal analysis with age utilizing TrackedFlyBox™. Typically, with age, they have observed that a higher percentage of flies accumulate in zone 1 and respectively, lower amounts in zone 3. Zone 2 is typically static. The zonal accumulation to zone 1, may imply worse healthspan and motor function, as the flies utilize less energy to stay on the ground versus climbing.

These readouts collectively map distinct facets of aging. Measuring lifespan screens for gross longevity effects, while locomotor and climbing metrics assess healthspan.

The single-vial Drosophila design, though statistically underpowered, provides multiple measurements in a single system in contrast to traditionally multiple users or systems for multiple measurements. Additionally, the high-resolution automated tracking (TrackedFlyBox™) analyzes all the flies in their home vials, reducing vias and health concerns of taking flies from their vials as researchers do in traditional methods. Prior work demonstrates that 15–20 flies/vial yield reproducible data when density and food quality are tightly controlled [31]. However, replicating across vials would reduce positional variability in incubators-a recommended refinement for subsequent studies [32].

4.3 *M. Musculus* Endurance Function Assessment

Although invertebrate models offer high-throughput screening potential for candidate geroprotectors, rodent studies remain essential for evaluating translational relevance. However, unlike longevity-focused endpoints in *C. elegans* and *Drosophila*, murine models often prioritize functional outputs as early indicators of intervention efficacy. This is due to the fact that full murine lifespan studies typically extend over two years resulting in substantial cohort sizes and resources, so using functional readouts can disclose treatment effects in weeks to months before committing to a definitive survival study [33, 34] In this study, endurance and neuromuscular coordination in *M. musculus* were assessed using the accelerating rotarod test over an 8-week period, a standard test of integrated motor performance and cerebellar function. The results from these experiments were inconclusive, given the small group sizes. However, many important learnings can be derived from this study, and can be used to improve future experiments.

The rotarod assay, while sensitive to age-related motor decline [16], is inherently confounded by factors such as trial-to-trial learning effects, inter-individual variability in motivation, and fatigue during accelerating protocols. In longitudinal paradigms, ceiling effects can obscure treatment effects unless animals are challenged at multiple fixed speeds or stratified by age and performance baseline. These considerations emphasize the need for complementary context-specific sensitivity when using behavioral readouts in middle-aged mice.

An additional confounder arose in the C57BL/6 murine model, which is prone to ulcerative dermatitis (UD), a condition linked to compulsive grooming behaviors analogous to trichotillomania in humans [35]. This raises the possibility that discomfort or nociceptive hypersensitivity, rather than purely neuromuscular decline, may have influenced rotarod performance. The presence of UD-associated discomfort or nociceptive hypersensitivity in treated mice may have indirectly impaired rotarod performance, independent of neuromuscular function. Currently, there is no direct evidence linking UD in mice to impaired rotarod performance. A systematic review of UD in C57BL/6 mice did not identify any studies that assessed motor function or rotarod outcomes in the context of UD [36]. However, it's important to consider that UD can cause significant discomfort and distress in affected mice, potentially

influencing behavioral and physiological parameters. While no studies have specifically evaluated rotarod performance in mice with UD, the condition's impact on overall health could feasibly affect motor coordination and endurance. This highlights the importance of strain selection and close monitoring of animals during testing.

Future studies should consider integrating multiple behavioral paradigms, such as grip strength, voluntary wheel running, or frailty indices, along with rotarod testing, as well as incorporating biochemical and histological markers to detect subclinical benefits. Ultimately, *M. musculus* findings reinforce the importance of dose optimization, strain selection, and endpoint diversification in preclinical longevity research. Negative or null outcomes at the functional level should not be taken to invalidate a compound's potential but rather as a call for more comprehensive, multimodal evaluation strategies that acknowledge the complexity of aging in mammalian systems. Given that costs are a barrier to performing longevity studies in mice, pump.science could investigate a model in the future in which this could be the next step for promising compounds.

4.4 Limitations

This study provides a proof-of-concept for a decentralized, cross-species platform to evaluate longevity interventions; however, several limitations should be acknowledged.

First, the sample sizes were modest and limited the statistical power and resulting conclusions. In flies, only one replicate per condition was used, and only male flies were studied. In the murine arm, there were three animals per condition, and the numbers were further reduced by the euthanasia of one animal per treatment group. In addition, the control animals received varying amounts of DMSO depending on sex or treatment group, ultimately resulting in underpowering across control groups for both rifampicin and Urolithin A. This limited statistical power and increased susceptibility to individual variability resulted in an inability to draw conclusions about efficacy of treatment, especially in behavioral assays such as the rotarod, where adequate replication is essential to distinguish true effects from noise. While some of the mice received relatively high concentrations of DMSO due to solubility of the compounds, there were no body weight changes in any group of the mice (data not shown).

Second, only a single dose per compound was assessed, which precludes the evaluation of dose–response relationships or identification of optimal therapeutic windows. This is particularly relevant for compounds like rifampicin, which can display concentration-dependent shifts from beneficial to toxic effects [16]. Without pharmacokinetic or tissue-level distribution data, it remains unclear whether the selected dosages achieved target engagement in the relevant tissues. No untreated groups were included in these pilot experiments, therefore solvent-dependent effects cannot be ruled out.

Third, the use of high throughput screening offers the benefit of using a standard protocol to test a large number of conditions in a resource efficient manner. However, there may be compounds which would benefit from an adjusted protocol. A broader dose response can be utilized to

better understand optimal dosage in *C. elegans* studies, along with different mutant strains that can shed light on mechanisms that contribute to drug activity. In addition, *C. elegans* can be fed either live or dead *E. coli* as a food source. Compounds that are able to exert an effect on bacteria may therefore add a confounding factor to study designs which employ the use of live *E. coli*, such as the setup used in these experiments. Given that rifampicin is a potent antibiotic, and that activity of Urolithin A may be impacted by microbial metabolism, redox state, or nutrient-sensing cues [27], it may be reasonable to consider the use of dead *E. coli* as an alternative food source in future studies.

Fourth, the treatment duration was relatively short, and phenotypic assessments were limited in scope in the murine experiments. Although motor coordination via rotarod testing provides a functional readout, broader profiling, such as metabolic assays, cognitive tasks, molecular biomarkers, or transcriptomic analysis, would have provided a more comprehensive picture of the healthspan. The choice of using supplement versus pure Urolithin A compound in rodent studies should also be further considered in future research,

Additionally, the intervention in rodents began in old age (18 months), which may be suboptimal for certain geroprotective strategies. Timing is a critical dimension in geroscience, and interventions applied too late may have diminished efficacy owing to the reduced plasticity of aging systems or pre-existing pathology. Preclinical studies have suggested that earlier administration of Urolithin A may yield more pronounced benefits, potentially due to its greater capacity for mitophagy activation and metabolic reprogramming [37]. Conversely, lifelong administration may induce desensitization or adaptive resistance, underscoring the importance of temporally stratified design.

The choice of mouse strain also introduces potential confounding factors: C57BL/6 mice are prone to ulcerative dermatitis (UD), a condition linked to compulsive grooming behaviors [35]. The presence of UD lesions may have influenced behavioral performance independently of neuromuscular function, complicating interpretation of rotarod results.

Furthermore, while the pump.science platform offers advantages in decentralization, open data access, and auditability, it is not immune to procedural challenges. Stage gating based on community funding milestones may introduce variability in compound progression timelines or bias selection toward interventions perceived as more popular or profitable rather than those supported by the strongest preliminary evidence. Additionally, although all data were streamed in real-time and registered immutably, formal blinding, randomization protocols, and replication were not uniformly implemented across all arms of the study, which are elements that are critical to the institutional acceptance of decentralized research models.

4.5 Conclusions and Future studies

This study introduces a novel cross-species research infrastructure that combines automated phenotyping, open-access data transparency, and decentralized funding to accelerate early

stage evaluation of candidate geroprotectors. Systematic assessment of Urolithin A and rifampicin across *C. elegans, D. melanogaster*, and *M. musculus* highlights the importance and necessity of multi-organism pipelines for resolving mechanistic ambiguity and uncovering context-specific effects. Rifampicin and Urolithin A both showed lifespan-extending effects in C. elegans, with rifampicin producing robust benefits and Urolithin A yielding more modest gains. However, lifespan outcomes in flies and healthspan outcomes in mice remain inconclusive due to limitations in study design.

To maximize the translational potential of cross-species longevity studies, future work should adopt a multi-layered approach that addresses both methodological rigor and mechanistic depth. First, increasing sample sizes across models is critical to ensure statistical robustness. In Drosophila, expanding from 1 to 10–15 vials per group would mitigate microenvironmental variability and provide ≥80% power to detect 15–20% lifespan changes, as demonstrated in the NIA Interventions Testing Program [30]. For murine studies, cohorts of 10–12 animals per group are recommended to account for individual heterogeneity in aging trajectories. Power calculations using tools like G*Power should guide these adjustments, particularly for detecting subtle healthspan effects (e.g., grip strength, cognitive decline) that may precede lifespan changes.

Moreover, the decentralized execution model prototyped here enables real-time hypothesis testing with public traceability, an approach that may help mitigate long-standing concerns regarding reproducibility and transparency in biomedical research. Although methodological limitations exist, this study offers a scalable proof-of-concept for rethinking how aging interventions are tested, prioritized, and disseminated. Future work should focus on expanding the compound repertoire, incorporating expanded phenotyping, and developing protocols that support iterative refinement through community-driven experimentation.

Conflict of Interest

All the authors disclose the following affiliations and potential conflicts of interest related to this work. Lee, M.B. and Blue, B. are associated with Ora Biomedical, and Petr, M. is affiliated with Tracked Biotechnologies LLC; both organizations contributed directly to the experiments reported. Additionally, several authors are co-founders of the pump.science platform. Other co-authors are affiliated with Molecule AG and BIO.XYZ, which maintain collaborative relationships with pump.science. These affiliations may be perceived as potential conflicts of interest. The authors declare that these relationships did not influence the study design, data collection, interpretation, or decision to publish.

Funding Statement

Petr, M. (Tracked Biotechnologies LLC) received funding to conduct the experiments presented in this publication. The co-founders of pump.science hold a financial interest in the usage and potential success of the pump.science platform. All other authors are compensated for their ongoing contributions to Molecule AG and BIO.XYZ but did not receive any additional remuneration specifically for the preparation of this publication.

Acknowledgments

The drafting and editing of the manuscript was performed by Kishore Kumar, Fabio Laredo, Suyen Espinoza, Karlin Compton, Lukas Weidener, and Logan Bishop-Currey. The authors are solely responsible for the content and its accuracy. Benji Leibowitz and Jillian Cassalini designed and funded the research, and manage the pump.science platform. Mitchell Lee, Ben Blue, and Michael Petr are valued contract research partners, and contributed to the design and managed the execution of the experiments.

Declaration of generative AI in scientific writing

During the preparation of this manuscript, the authors used OpenAI's ChatGPT (versions 4o and 4.5) to assist with grammar correction, spelling, formatting, and reformulation of selected passages for clarity and style. All content generated through this tool was critically reviewed, edited, and approved by the authors. The authors take full responsibility for the integrity and accuracy of the final manuscript.

References

1. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2023). Hallmarks of aging: An expanding universe. Cell, 186(2), 243–278.
2. Kennedy, B.K., Berger, S.L. and Brunet, A., 2014. Geroscience: linking aging to chronic disease. Cell, 159(4), pp.709–713.
3. Mouchiroud, L., Houtkooper, R.H. and Auwerx, J., 2013. NAD^+ metabolism: a therapeutic target for age-related metabolic disease. Critical Reviews in Biochemistry and Molecular Biology, 48(4), pp.397–408.
4. Lee, M.B., Blue, B. and Muir, M., 2023. The million-molecule challenge: a moonshot project to rapidly advance longevity intervention discovery. Geroscience, 45(6), pp.3103–3113.
5. Zhang, S., Li, F. and Zhou, T., 2020. Caenorhabditis elegans as a useful model for studying aging mutations. Frontiers in Endocrinology, 11, p.554994.
6. Ryu, D., Mouchiroud, L. and Andreux, P.A., 2016. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and improves muscle function in rodents. Nature Medicine, 22(8), pp.879–888.
7. Lee MB, Kaeberlein M. Translational Geroscience: From invertebrate models to companion animal and human interventions. Transl Med Aging. 2018 Jan;2:15-29.
8. Sierra, F., Caspi, A., Fortinsky, R. H., Haynes, L., Lithgow, G. J., Moffitt, T. E., Olshansky, S. J., Perry, D., Verdin, E., & Kuchel, G. A. (2021). Moving geroscience from the bench to clinical care and health policy. Journal of the American Geriatrics Society, 69(9), 2455–2463. https://doi.org/10.1111/jgs.17301

9. Petrascheck, M., Ye, X. and Buck, L.B., 2009. A high-throughput screen for chemicals that increase the lifespan of C. elegans. Aging Cell, 8(3), pp.258–269.
10. Linford, N.J., Bilgir, C. and Ro, J., 2013. Measurement of lifespan in Drosophila melanogaster. Journal of Visualized Experiments, (71), p.e50068.
11. Kim, D.K., Kim, T.H. and Lee, S.J., 2016. Mechanisms of aging-related proteinopathies in Caenorhabditis elegans. Experimental and Molecular Medicine, 48(10), p.e263.
12. Zhao, Y., Gilliat, A.F. and Ziehm, M., 2017. Two forms of death in ageing Caenorhabditis elegans. Nature Communications, 8, p.15458.
13. Brooks, S.P. and Dunnett, S.B., 2009. Tests to assess motor phenotype in mice: a user's guide. Nature Reviews Neuroscience, 10(7), pp.519–529.
14. Chen, P., Lei, J. and Cheng, F., 2020. Ameliorative effect of Urolithin A on D-gal-induced liver and kidney damage in aging mice via its antioxidative, anti-inflammatory and antiapoptotic properties. RSC Advances. doi:10.1039/D0RA00774A.
15. Singh, A., D'Amico, D., Andreux, P. A., Fouassier, A. M., Blanco-Bose, W., Evans, M., Aebischer, P., Auwerx, J., & Rinsch, C. (2022). Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults. Cell reports. Medicine, 3(5), 100633. https://doi.org/10.1016/j.xcrm.2022.100633
16. Golegaonkar, S., Tabrez, S.S. and Pandit, A., 2015. Rifampicin reduces advanced glycation end products and activates DAF-16 to increase lifespan in Caenorhabditis elegans. Aging Cell, 14(3), pp.463–473.
17. Pitt, J.N. et al., 2019. WormBot, an open-source robotics platform for survival and behavior analysis in C. elegans. GeroScience, 41, pp.961–973.
18. Saukkonen, J. J., Cohn, D. L., Jasmer, R. M., Schenker, S., Jereb, J. A., Nolan, C. M., Peloquin, C. A., Gordin, F. M., Nunes, D., Strader, D. B., Bernardo, J., Venkataramanan, R., Sterling, T. R., & ATS (American Thoracic Society) Hepatotoxicity of Antituberculosis Therapy Subcommittee (2006). An official ATS statement: hepatotoxicity of antituberculosis therapy. American journal of respiratory and critical care medicine, 174(8), 935–952. https://doi.org/10.1164/rccm.200510-1666ST
19. Diallo, D., Somboro, A. M., Diabate, S., Baya, B., Kone, A., Sarro, Y. S., Kone, B., Diarra, B., Diallo, S., Diakite, M., Doumbia, S., Toloba, Y., Murphy, R. L., & Maiga, M. (2021). Antituberculosis Therapy and Gut Microbiota: Review of Potential Host Microbiota Directed-Therapies. Frontiers in cellular and infection microbiology, 11, 673100. https://doi.org/10.3389/fcimb.2021.673100
20. Xu, M.Y. et al., 2024. Urolithin A promotes atherosclerotic plaque stability by limiting inflammation and hypercholesteremia in Apolipoprotein E-deficient mice. Acta Pharmacologica Sinica, 45(11), pp.2277–2289.
21. de Steenwinkel, J.E. et al., 2013. Optimization of the rifampin dosage to improve the therapeutic efficacy in tuberculosis treatment using a murine model. American Journal of Respiratory and Critical Care Medicine, 187(10), pp.1127–1134.
22. Barardo, D.G. et al., 2017. Machine learning for predicting lifespan-extending chemical compounds. Aging (Albany NY), 9(7), pp.1721–1737.
23. Rieger D, Fraunholz C, Popp J, Bichler D, Dittmann R, Helfrich-Förster C. The fruit fly Drosophila melanogaster favors dim light and times its activity peaks to early dawn and

late dusk. J Biol Rhythms. 2007 Oct;22(5):387-99.
24. Andreux, P.A. et al., 2019. The mitophagy activator Urolithin A is neuroprotective against amyloid-β pathology. Aging Cell, 18(6), p.e13033.
25. Huang, J.-R. et al., 2023. Urolithin A ameliorates obesity-induced metabolic cardiomyopathy in mice via mitophagy activation. Acta Pharmacologica Sinica, 44, pp.321–331.
26. Ryu, D., Mouchiroud, L., Andreux, P. et al. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat Med 22, 879–888 (2016).
27. Tomella, C., 2024. Urolithin A: a novel geroprotectant capable of stimulating mitophagy. Journal of Stem Cell Research, 5(2), pp.1–13.
28. Martin, Jean. (2003). Locomotor activity: A complex behavioural trait to unravel. Behavioural processes. 64. 145-160. 10.1016/S0376-6357(03)00132-3.
29. Heys C, Lizé A, Blow F, White L, Darby A, Lewis ZJ. The effect of gut microbiota elimination in Drosophila melanogaster: A how-to guide for host-microbiota studies. Ecol Evol. 2018 Mar 26;8(8):4150-4161. doi: 10.1002/ece3.3991. PMID: 29721287; PMCID: PMC5916298.
30. Fonseca Costa, S. S., & Ripperger, J. A. (2015). Impact of the circadian clock on the aging process. Frontiers in neurology, 6, 43.
31. Bellantuono I, de Cabo R, Ehninger D, Di Germanio C, Lawrie A, Miller J, Mitchell SJ, Navas-Enamorado I, Potter PK, Tchkonia T, Trejo JL, Lamming DW. A toolbox for the longitudinal assessment of healthspan in aging mice. Nat Protoc. 2020 Feb;15(2):540-574. doi: 10.1038/s41596-019-0256-1.
32. Nadon, N. L., Strong, R., Miller, R. A., & Harrison, D. E. (2017). NIA Interventions Testing Program: Investigating Putative Aging Intervention Agents in a Genetically Heterogeneous Mouse Model. EBioMedicine, 21, 3–4. https://doi.org/10.1016/j.ebiom.2016.11.038
33. Singh, A., D'Amico, D., Andreux, P. A., Fouassier, A. M., Blanco-Bose, W., Evans, M., Aebischer, P., Auwerx, J., & Rinsch, C. (2022). Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults. Cell reports. Medicine, 3(5), 100633. https://doi.org/10.1016/j.xcrm.2022.100633
34. Saukkonen, J. J., Cohn, D. L., Jasmer, R. M., Schenker, S., Jereb, J. A., Nolan, C. M., Peloquin, C. A., Gordin, F. M., Nunes, D., Strader, D. B., Bernardo, J., Venkataramanan, R., Sterling, T. R., & ATS (American Thoracic Society) Hepatotoxicity of Antituberculosis Therapy Subcommittee (2006). An official ATS statement: hepatotoxicity of antituberculosis therapy. American journal of respiratory and critical care medicine, 174(8), 935–952. https://doi.org/10.1164/rccm.200510-1666ST
35. Williams, L.K. et al., 2012. Ulcerative dermatitis in C57BL/6 mice exhibits an oxidative stress response consistent with normal wound healing. Comparative Medicine, 62(3), pp.166–171.
36. Sargent, J.L., Koewler, N.J. and Diggs, H.E., 2015. Systematic literature review of risk factors and treatments for ulcerative dermatitis in C57BL/6 mice. Comparative Medicine, 65(6), pp.465–472.

37. Liu, S. et al., 2025. Urolithin A provides cardioprotection and mitochondrial quality enhancement preclinically and improves human cardiovascular health biomarkers. iScience, 28(2), p.111814.
38. Ora Biomedical, 2025. pump.science – Project Documentation and Protocol Overview. Available at: https://pump.science/ (Accessed: April 2025).
39. Pump Science Project Website, 2025. pump.science – Protocol Overview and Tokenomics Documentation. Available at: https://pump.science/ (Accessed: April 2025).
40. Pump.science, 2025. Experiment documentation and compound lifecycle explorer. Available at: https://pump.science/data-explorer (Accessed: April 2025).