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InVivo Biosystems

Healthy Aging Assessment

Sample Data Report

FIRST OF ALL

THANK YOU

FOR CONSIDERING US!

At InVivo Biosystems, our mission is to help our clients develop and deliver solutions that improve the quality of human health.

Our technology and expertise benefit early-stage investigations the most by focusing on proof-of-principle experiments that provide the preliminary data needed to make a go/no-go decision.

We provide the infrastructure and experienced personnel that give your project a strong start to help you accelerate the development of compounds and move them down the pipeline.

Our scientists work one-on-one with our clients to provide the consultation and research needed to detect early risks and move new projects forward quickly.

Starting with a thorough understanding of your needs, we will work with you closely to bring your project to successful closure. We will always ensure timely communication and the most efficient solutions to your problems.

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INTRODUCTION

In the last decade, more and more companies are looking to simple, more cost- and time-effective ways to move forward in the discovery and development of new compounds that will improve human health.

NemaMetrix has developed a healthy aging assessment platform for researchers who need to determine as early as possible which compounds or formulation to focus on for future development, and understand their mode of action. For our clients, time and scientific accuracy are the most important. Leveraging our in-vivo technology early-on during the R&D process can considerably accelerate the process of identifying the most promising compounds or formulation for commercialization or clinical trial.

Although test on rodents or mammalian cells are the gold standard, many are concerned about the cost and time of such approaches at an early stage due to time and cost constraints.

NemaMetrix is a market leader in early in-vivo testing with small animal models. Our technology can perform compound efficacy testing within 6 months and help researchers answer preliminary questions quickly.

Q: How relevant are small animal models for testing compounds destined for humans?

> **A: C. elegans *is well-established as an aging research model and has enabled the identification of pathways influencing aging, such as the insulin/IGF-1 (IIS) and mTOR pathways, which are evolutionary conserved in mammals. [...] A number of recent studies have identified microbial pathways affecting aging and longevity in* C. elegans*, introducing this model as a viable way to understand the role of the gut microbiota in health and aging.***
>
> ~ Ezcurra M., (2018) [1]

C. elegans, a nematode originally used to establish the genetic and molecular basis of development, has become one of the leading model organisms for research on aging over the last decade. This invertebrate has spearheaded the identification of a connection between cellular signaling pathways and longevity. The heterologous expression of human proteins in the worm allows the analysis of their involvement in the control of signaling pathways modulating aging. Different proteins have already been successfully expressed in

the worm, maintaining their functions, or integrating into the signaling pathways of this organism. Human agonists or antagonists of signaling pathways have also been successfully tested in the worm. *C. elegans* is therefore relevant for the identification of receptors, ligands and transduction factors, present in humans, and capable of controlling aging without the influence of inflammation and immunosenescence, which could mask some signaling pathways that are modulated into more complex models. *C. elegans* provides insight into the biological mechanisms that accelerate or decelerate aging. Such biomarkers may be useful to track the effectiveness of interventions aimed at slowing down the rate of aging and preventing its consequences such as multi-morbidity and disability.

The nematode *C. elegans*, with its short lifespan of ~3 weeks, ease of culture and genetic manipulation, and well-characterized aging biology, represents a very attractive model system to identify compounds that moduate lifespan and age-related phenotypes. Several studies have identified a number of candidate anti-aging compounds using *C. elegans* as a model organism.[2, 3, 4] The National Institute of Aging has recently sponsored a pharmacological intervention program called the Caenorhabditis Ontervention Testing Program (CITP) using this organism as a model system, analogous to similar ongoing efforts in the mouse. We utilize the same methods to test our clients' compounds.[5]

PROJECT AIMS

In this project, we sought to answer the following questions:

- Whether compounds A and B have an effect on lifespan
- Whether compounds A and B impact the mitochondrial pathway

OUR APPROACH

Our team of scientific experts work with you to design an experimental plan that will provide the most efficient solutions to your problems.

We provide five classes of tests using *C. elegans* to test your formulation:

Library of offerings to test your formulation

	Approach	See if:
LIFESPAN ASSAY Can your formulation extend the lifespan of live animals?	Survival rate over time	- Worms exposed to your formulation live to be older than healthy, unexposed animals and how much longer they live. - Your formulation increases the likelihood of individuals being alive after a certain age
HEALTHSPAN ASSAYS Can your formulation promote healthy aging?	Behavior	Your formulation affects neuromuscular activity
	Morphology	Your formulation affects tissue organization
	Reproduction	Your formulation has the potential to affect reproduction
MODE OF ACTION What pathways does your formulation target to promote healthy aging?	Genetic	Your formulation affects aging-related pathways such as mitochondrial health and response to stress.
	Biochemical	Your formulation affect critical aging-related mechanisms such as mitochondrial and DNA repair pathways.

CONCLUSIONS

Both compounds are promising anti-aging agents

- Compound A and B produced a significant increase in the lifespan of *C. elegans*.
- Both positive controls used significantly increased lifespan.

Compounds A and B do not impact the expression of mitochondrial markers *mev-1* and *atp-1* under the tested conditions

- We detected no significant change in *atp-1* and *mev-1* expression in either A- or B-treated worms in either young (day 1) or old (day 8) adults.

RESULTS / ANALYZED DATA

Lifespan assay and survival analysis showed both compounds significantly increased the lifespan of *C. elegans.*

In this study, we first tested the ability of compounds A and B to improve the longevity of live animals using a lifespan assay and survival analysis. In order to detect a a difference of at least 10% in survival prospects with high confidence, 200-300 worms were tested for each condition.[6] The positive controls were carefully selected based on the nature and hypothesized activity of the compounds to be tested.

A survival assay was conducted with measurements of live, dead, and censored worms at time points every 2-3 days from the start of adulthood, until no living worms remained. Each condition started with a pool of ~200 worms and scoring began on day 5 after separating worms onto assay plates.

Using Whole Transcriptome Sequencing (WTS) analysis to identify which pathways the genes are involved in.

To understand the mode of action of compounds A and B, we ran a Whole Transcriptome Analysis (WTS) on the individuals that were exposed to the compounds of interest. This method allowed us to identify the genes whose expression is modified in the presence of the compounds. We then ran a gene ontology analysis to identify which pathways those genes are involved in.

In this example, the customer wanted to test the specific hypothesis that genes *mev-1* and *atp-1* were impacted by compounds A and B. In order to do this, we performed a quantitative PCR (qPCR), a highly sensitive method to quantify the expression level of those two genes. We then used the expression of mitochondrial housekeeping genes to normalize the data.

The data invalidated the original hypothesis that genes *mev-1* and *atp-1* are impacted by compounds A and B. Instead, the data showed that *mev-1* and *atp-1* expression does not change upon short or long exposure to compounds A and B.

Based on these findings, we then proceeded to a broader approach using whole transcriptome analysis via RNAseq, the most advanced transcriptomic approach where the total complement of RNAs from a given sample is isolated and sequenced using high-throughput technologies (often called Next-Generation Sequencing).

Table 1. Lifespan assay summary

	A	B	Control 1	Thioflavin T	25% DMSO	1% DMSO
Death events	**179**	**184**	**178**	**150**	**168**	**151**
Censored subjects (#worms)	19	19	16	27	22	34
Median lifespan (days)	24	21	21	21	21	21
Maximum lifespan (days)	31	33	35	33	26	26

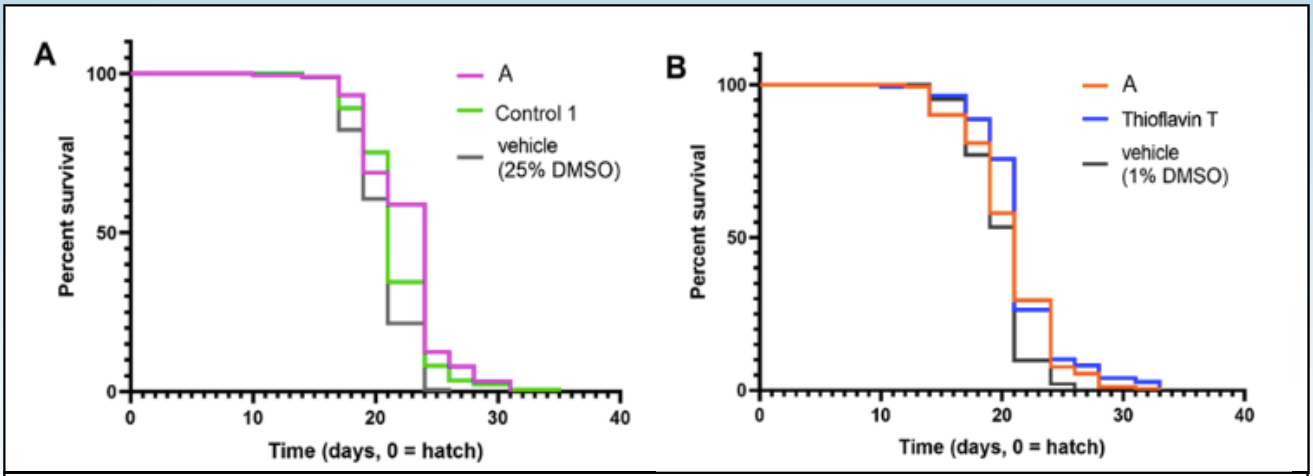

Figure 3. Survival curves from lifespan assay. Worms were scored three days per week, starting at day 5 post hatch. To maintain chemical exposure, worms were transferred to new plates every other scoring day. (A) 50µM compound A, 50µM Control 1, or vehicle control (25% DMSO). (B) 50µM Compound B, 50µM Thioflavin T, or vehicle control (1% DMSO).

Table 2. Pairwise statistical analysis of survival curves.

Curve comparison vs. vehicle	Hazard Ratio	Log-rank (Mantel-Cox) test†	Gehan-Breslow-Wilcoxon test‡
Compound A	0.3587	<0.0001 ****	<0.0001 ****
Compound B	0.6520	0.0048 **	0.0416 *
Control 1	0.5413	<0.0001 ****	0.0004 ***
Thioflavin T	0.4411	<0.0001 ****	<0.0001 ****
25% DMSO vs 1%	0.7151	0.0394 *	0.0292 *

The hazard ratio is the relative probability of death at a given time relative to control.
†Mantel-Cox test compares groups across the duration of the lifespan.
‡Gehan-Breslow-Wilcoxon test applies more weight to earlier deaths.
Numbers and asterisks represent P-value and significance, respectively.

To determine whether A and B affected mitochondrial function, we measured expression of genes encoding mitochondrial proteins in both young and aged adult worms.

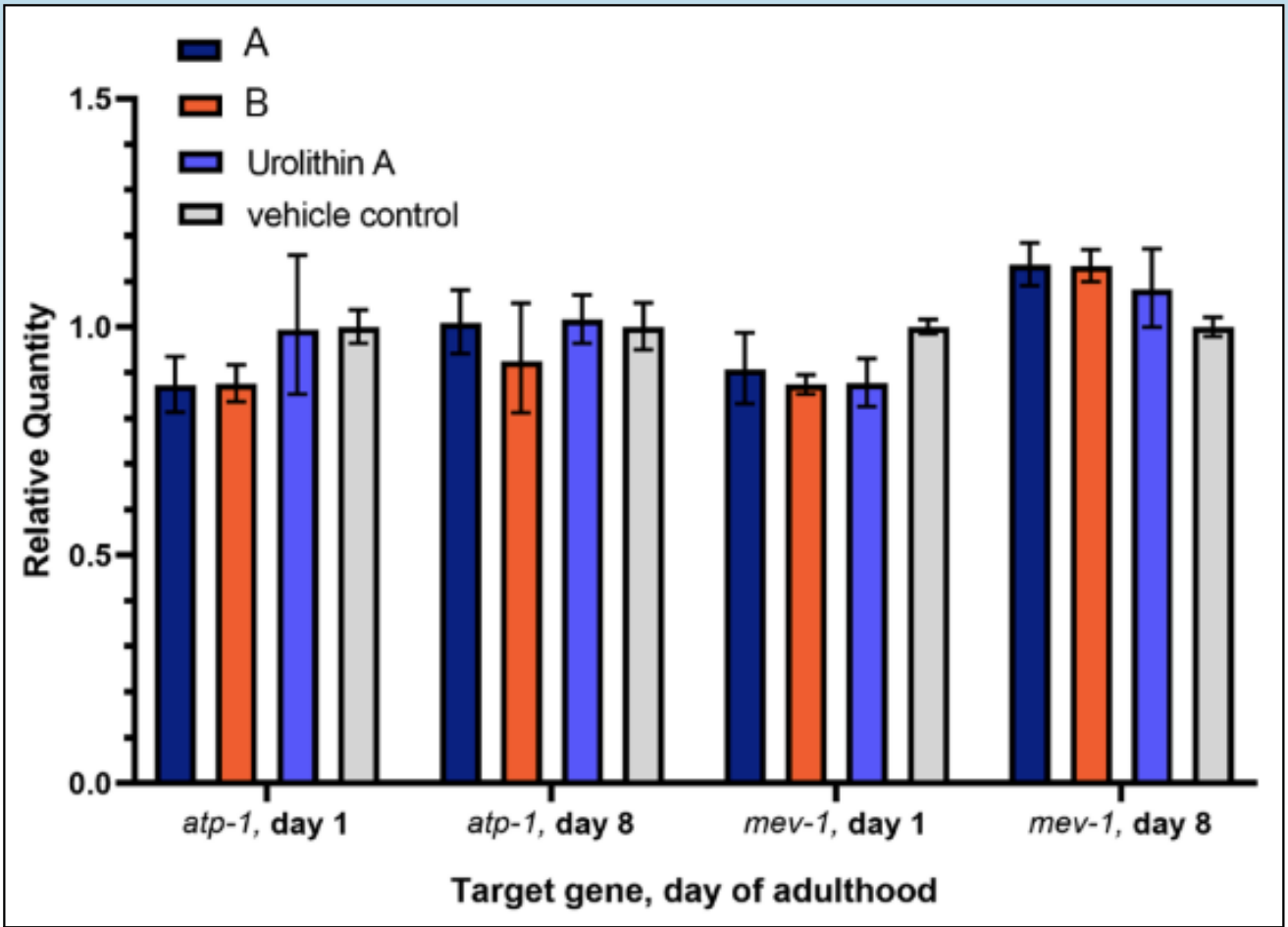

Figure 4. Expression of genes encoding mitochondrial respiratory proteins at day 1 of adulthood and day 8 of adulthood. Columns represent average quantity of two independent biological replicates relative to corresponding vehicle control. Error bars = 95% confidence interval of a Student's t-test. All P-values for comparisons with vehicle control were greater than 0.1.

Compounds A and B may act via mitochondrial pathway to promote healthy aging, however, a whole transcriptome approach will be the most efficient way to identify the mitochondrial genes and pathways affected.

SUPPLEMENTAL DATA

qPCR Quality Control

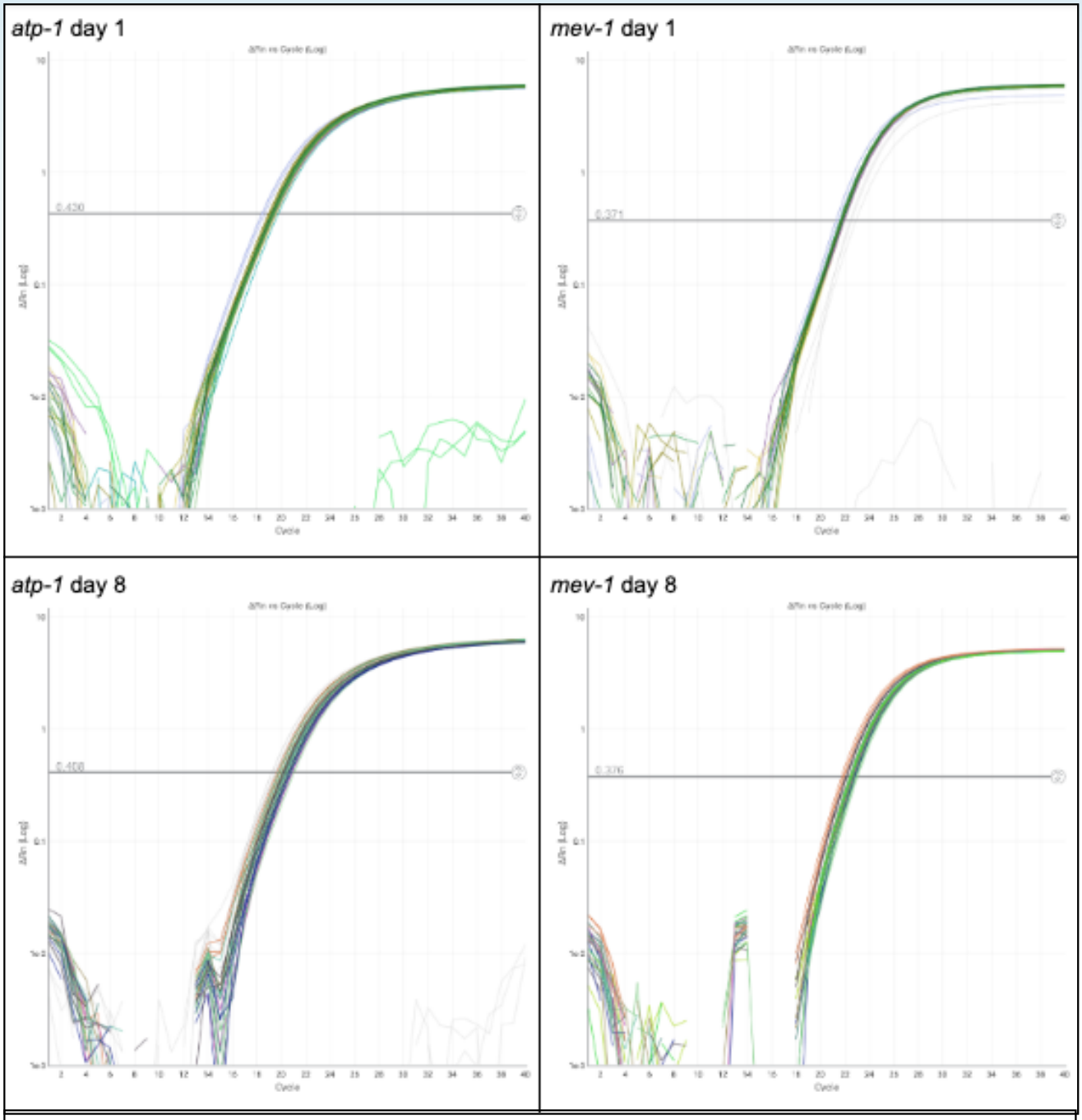

Figure S1. Snapshots of amplification curves for atp-1 and mev-1 in day 1 (left) and day 8 (right) worms.

Table S1. Quality control summary for qPCR analysis.	
Number of independent biological replicates.	2
RNA yields	>2µg/sample
RNA quality	All Qubit IQ scores >8.0
RNA to cDNA	100ng/reaction
qPCR technical replicates	3
qPCR reaction flags	none
qPCR reactions excluded	none

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