

WHITE PAPER

Linking functional phenotypic profiling with molecular aging signatures

An integrated workflow illustrating how SydLab™ One can support a healthy aging study end-to-end.





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Healthy aging: a multifaceted process

Aging is a multifactorial process involving interconnected mechanisms, including genomic instability, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, dysbiosis, and others (López-Otín C et al., *Cell*, 2023). The expanded hallmarks framework underscores the complexity of aging and the importance of assessment through complementary biological layers. Accordingly, interventions targeted to support healthy aging are increasingly evaluated across functional and molecular signatures associated with the biological state.

***C. elegans*: a relevant model for scalable screening in healthy aging and nutrition**

Caenorhabditis elegans (*C. elegans*) has paved the way for the effective study of aging and its pathways, with major discoveries revealing that mutations in highly conserved genes can double lifespan (1). Orthologs of those genes and others, including the insulin/insulin-like growth factor (IGF-I) and FOXO3, also seem to affect longevity in mammals (2), (3). These studies exemplify how *C. elegans* has advanced the understanding of aging genetics and helped establish aging as a biologically regulated process rather than a passive decline (4).

Importantly, lifespan and healthspan in *C. elegans* are not only regulated by mutations but also by dietary interventions (5). Across model organisms, dietary restriction is one of the most established interventions known to extend lifespan, including *C. elegans* (6). Conversely, excess glucose has been shown to produce a measurable decline in lifespan and physiological function, including altered reproduction, increased oxidative stress, and disrupted lipid metabolism (7). This connection makes the model particularly relevant for studying interventions that aim to support healthy aging through nutritional or dietary mechanisms and helps explain why many natural compounds and functional ingredients have been investigated for their effects in this model (4).

Beyond its well-characterized genome and conserved aging and nutrient-sensing pathways, *C. elegans* is also a practical *in vivo* model for early screening. Its short life cycle and small size enable high-throughput studies while preserving the integrated physiology of a whole organism that undergoes progressive changes while aging. Finally, as a non-vertebrate, *C. elegans* can support the generation of functional, system-level data in alignment with legal, regulatory, and ethical efforts to refine, reduce, or replace animal testing where appropriate.

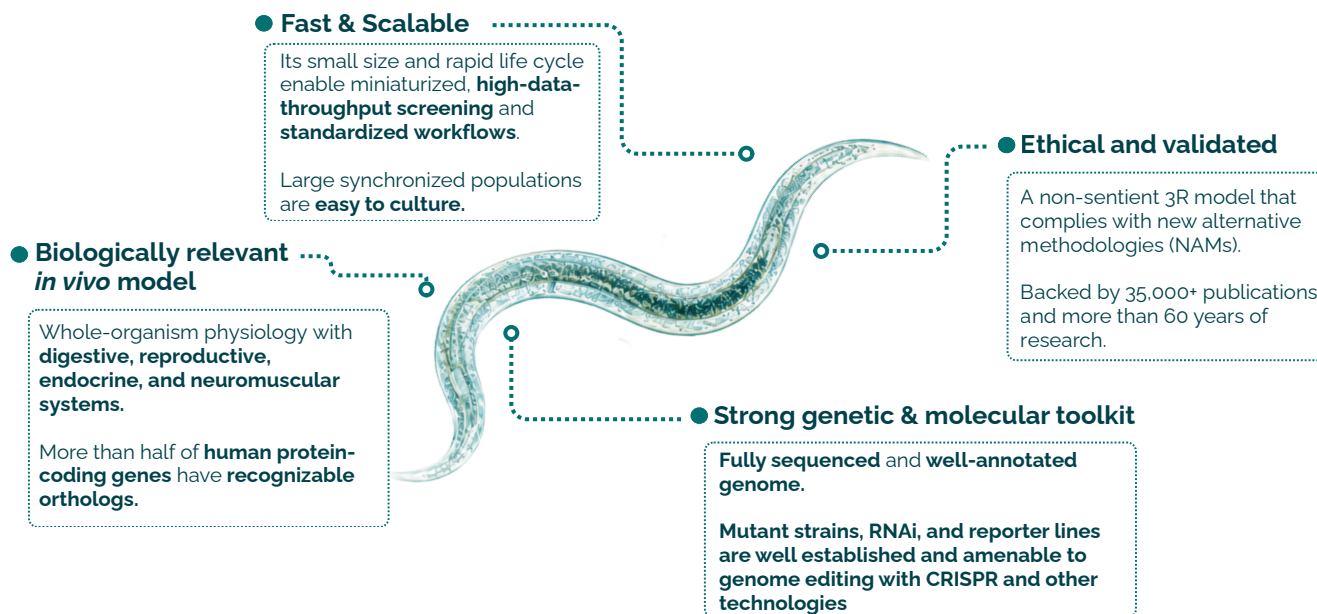


Figure 1. **Key advantages of *C. elegans* as model for aging research.**

From automated phenotyping to molecular aging signatures

SydLab™ One: an automated platform for functional phenotypic profiling

Phenotypic profiling is a central component of healthy aging research since it captures the functional impact of an intervention in a living organism. In *C. elegans*, many age-related changes at the molecular and tissue level become apparent physiologically and can be easily assayed. Readouts such as survival, movement, morphology, development, and reproduction can provide direct evidence of how a natural compound or a functional ingredient affects organism-level biology (4).

SydLab™ One is an automated microfluidic platform that supports controlled exposure and longitudinal monitoring of *C. elegans*, enabling standardized phenotypic characterization across screening conditions (8). By capturing reproducible functional readouts over time, the platform provides a robust foundation for evaluating interventions based on measurable biological outcomes.

Nagi™ Biological Age: a phenotypic clock for quantifying biological aging dynamics

Longitudinal phenotypic profiling enabled by SydLab™ One, combined with machine learning, led to the introduction of Nagi™ Biological Age, a non-invasive phenotypic clock that estimates biological age at single-organism resolution. Built from approximately 230,000 observations across 60+ phenotypic features in about 3,750 animals, the phenotypic clock predicted biological age with high accuracy and resolved organism-wide aging trajectories over time (9). This additional layer of phenotypic interpretation not only corroborates the characterization of functional outcomes but also demonstrates the capability to quantify biological aging dynamics.

Transcriptomics: the integrated workflow to connect function with molecular mechanism

The integration of transcriptomics extends the phenotypic foundation of SydLab™ One by adding a molecular characterization layer to biological age profiling. By incorporating worm retrieval into the workflow, worms monitored for phenotypic outcomes can be recovered for RNA extraction, RNA-seq, and downstream transcriptomic analysis using tools such as BiT age (10). In the context of natural compounds and functional ingredients, RNA-seq can help identify molecular pathways associated with nutrient sensing, metabolism, mitochondrial function, and stress responses relevant to longevity. This workflow connects functional outcomes to gene expression profiling and aging-related pathways, providing an integrated framework for healthy aging applications.

Application case study

Phenotypic and transcriptomic age profiling of natural compounds

To demonstrate the integrated SydLab™ One workflow, spermidine, a natural compound relevant to healthy aging, was tested across three independent experiments in collaboration with the research and development division at Nestlé. Caloric restriction (CR) was included as a reference intervention because of its well-studied benefits in aging (12). The primary objective was to assess whether Nagi™ Biological Age phenotypic quantification, RNA-seq-derived transcriptomic age estimation, and downstream gene-expression analysis could be generated and interpreted within a coordinated workflow. **The study produced reproducible phenotypic outputs, captured expected transcriptomic age-related structure through day 1, day 5, and CR reference conditions, and enabled pathway-level interpretation of molecular responses related to CR and spermidine.**

Study design with the integrated SydLab™ One phenotypic-to-transcriptomic workflow

The study was designed around a controlled SydLab™ One exposure workflow with phenotypic and molecular branches (Figure 2). L4-stage worms were loaded onto the platform and maintained under controlled feeding and compound exposure. A day 1 retrieval branch provided an early baseline RNA reference, a Day 5 retrieval branch provided RNA-seq material after on-chip exposure, and the Day 12 branch preserved the same exposure context for extended phenotyping and Nagi™ Biological Age analysis.

SydLab™ One exposure workflow with phenotypic and molecular branches

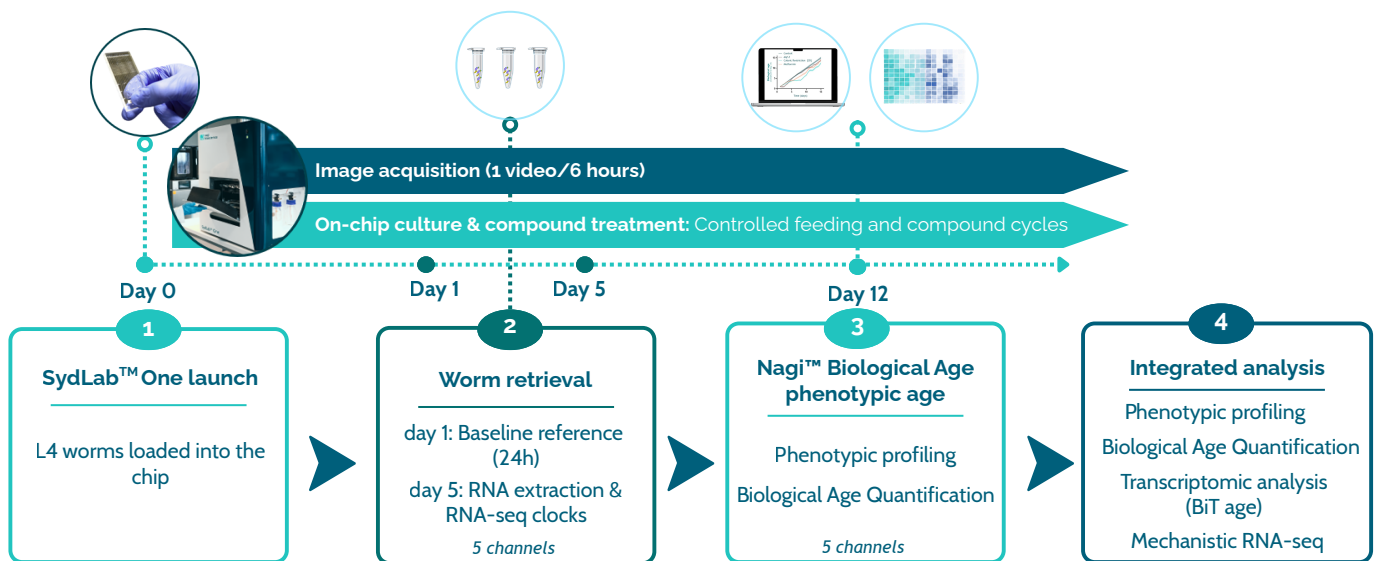


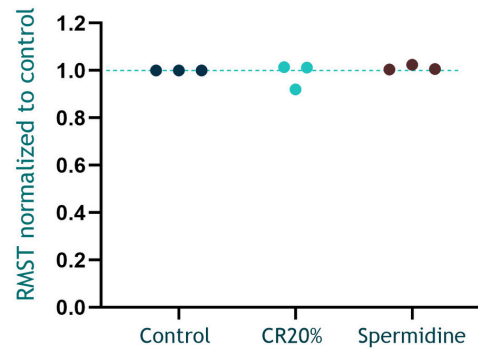
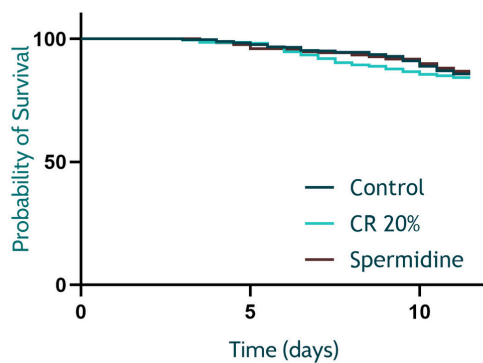
Figure 2. Integrated SydLab™ One workflow combining longitudinal phenotyping with time-resolved molecular analysis. L4-stage worms are loaded into the chip at day 0 and undergo controlled feeding and compound cycles. Worms are retrieved at day 1 and day 5 for transcriptomic analysis. The remaining on-chip population is monitored longitudinally until day 12 for phenotypic profiling and biological age quantification with Nagi™ Biological Age. Phenotypic and transcriptomic readouts, including BiT age and mechanistic RNA-seq analysis, are subsequently integrated to characterize treatment effects across molecular and organism-level aging trajectories.

Modest trend across functional responses

As outlined at the beginning, healthy aging studies should assess multiple functional parameters to provide a more comprehensive view of organismal health. Although survival remains a central endpoint, it should not be interpreted as the sole proxy for healthspan. Motility, for example, is a critical indicator of healthspan across species, including *C. elegans*, because coordinated movement requires functional neuromuscular signaling, body-wall muscle integrity, and sufficient energy production (12) (13). Reproduction and growth also provide important biological context by reporting on fitness, developmental progression, and treatment tolerance, helping to distinguish healthy aging effects from delayed development or nonspecific stress (14).

In this study, spermidine 50 μ M and CR 20% were evaluated for all these parameters. Spermidine did not show any phenotypic shifts until day 12, while CR 20% induced improved motility (Figure 3). These longitudinal phenotypic data provided the foundation for biological age quantification using Nagi™ Biological Age and enabled downstream comparison with molecular aging readouts.

A) Survival



B) Motility

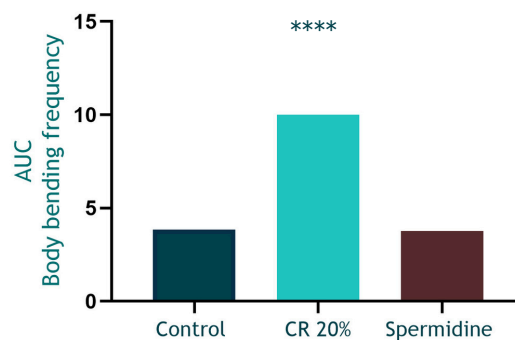
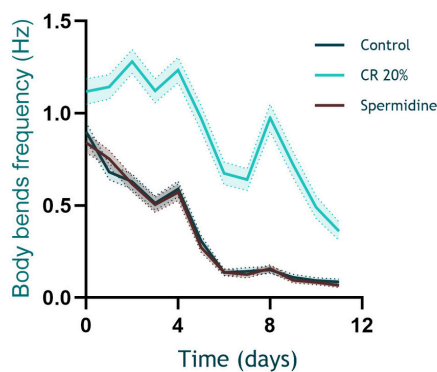


Figure 3. Graphs of pooled data from 3 independent experiments of phenotypic profiling, including survival curves and body bend frequency. (A) Kaplan–Meier survival curves generated from pooled survival data from three independent experiments. Censored observations were included in the Kaplan–Meier estimates. Restricted mean survival time (RMST) was calculated independently for each experiment and normalized to the control. Individual points represent independent experiments ($n=3$), with the dashed line representing the normalized control. No significant differences were detected. (B) Motility was assessed over time using head amplitude, tail amplitude, body-bend frequency, and displacement speed. Body-bending frequency is shown as a representative of motility. Pooled means were calculated as N-weighted means based on the number of worms contributing to each experiment and time point. Pooled SD incorporated both within-experiment variability and differences between experiment means. Statistical analysis was performed using two-way ANOVA to assess overall differences across curves, followed by Bonferroni's multiple comparisons test. **** $p<0.0001$

CR 20% and spermidine improve the phenotypic biological age score

CR 20% showed a significantly reduced biological age score (27%) compared to the control, consistent with the broad literature on its benefits for aging and longevity (12), providing a positive reference. Spermidine also reduced phenotypic biological age (6%) starting at day 8 (Figure 4). Importantly, the analysis generated a quantitative phenotypic age that could be directly compared with transcriptomic age estimates and support an interpretation of biological age from both organismal function and molecular data perspectives.

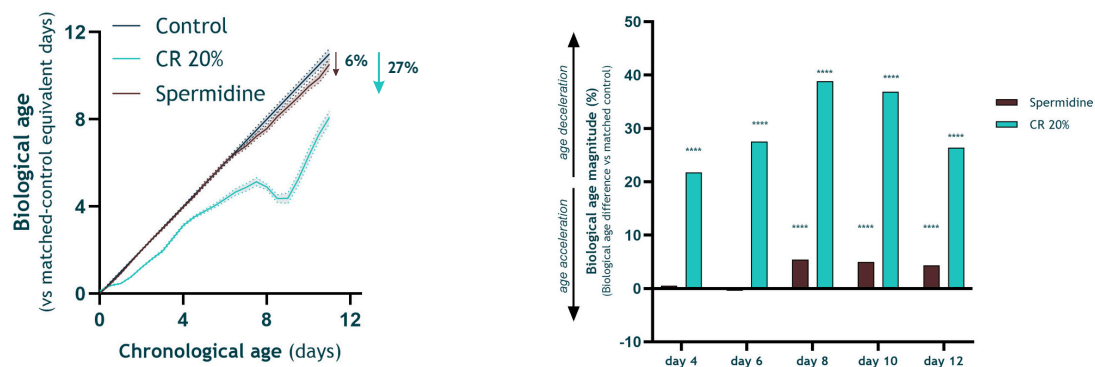


Figure 4. Nagi™ Biological Age phenotypic age evolution normalized to the control for selected Day 12 branch conditions. A bar plot illustrates the variation in predicted biological age compared to the negative control at different time points. Statistical significance was assessed with two-way ANOVA followed by Bonferroni's multiple comparisons test. **** $p < 0.0001$

Day 1 and CR as transcriptomic validation anchors

Gene-expression profiles were first analyzed using BiT age to obtain transcriptome-based biological age estimates (10). As expected, day 1 samples were estimated as biologically younger than day 5 samples, confirming that the transcriptomic age estimation captured the expected chronological difference between the two time points. CR 20% showed a similar younger-associated transcriptomic profile, consistent with and comparable to Nagi™ Biological Age phenotypic age (Figure 5). These results provide a validation anchor for the integrated SydLab™ One workflow, linking time-resolved worm retrieval with RNA-seq-based biological age estimation.

Spermidine produced a limited younger-associated transcriptomic profile. Therefore, the analysis was extended beyond biological age estimation to include differential gene expression, comparison with age-associated differentially expressed genes, and pathway enrichment.

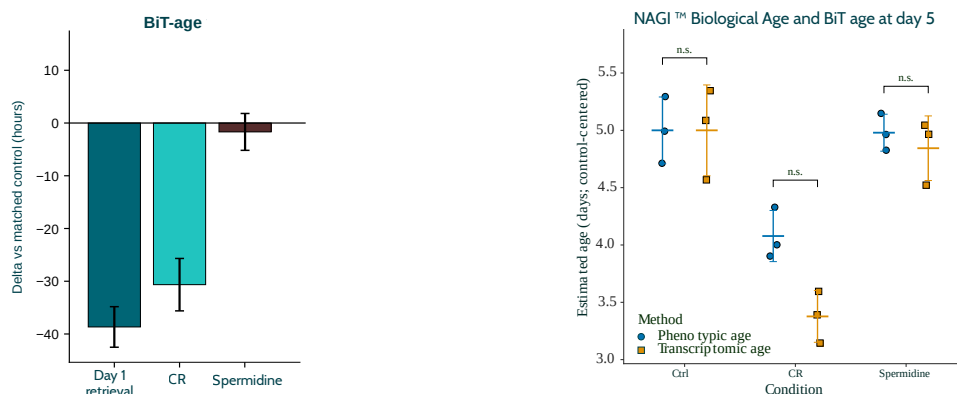


Figure 5. Transcriptomic biological age estimation using BiT age and comparison with Nagi™ Biological Age phenotypic age. Worms recovered from the SydLab™ One workflow were processed for RNA-seq and analyzed using BiT age (left). Day 1 samples served as early-age transcriptomic references, while day 5 samples were used to evaluate transcriptomic age estimates for CR 20% and spermidine. Individual points at NAGI™ Biological Age represent pooled data from independent experiments. Statistical significance was assessed using a paired Wilcoxon test (right).

CR 20% differentially regulated a set of age-associated genes

Additional gene-expression-level analysis provided important biological context. CR 20% affected a substantial subset of age-associated genes, with changes occurring predominantly in the opposite direction to aging (Figure 6), suggesting that CR 20% partially counteracted age-associated transcriptional patterns under the conditions tested.

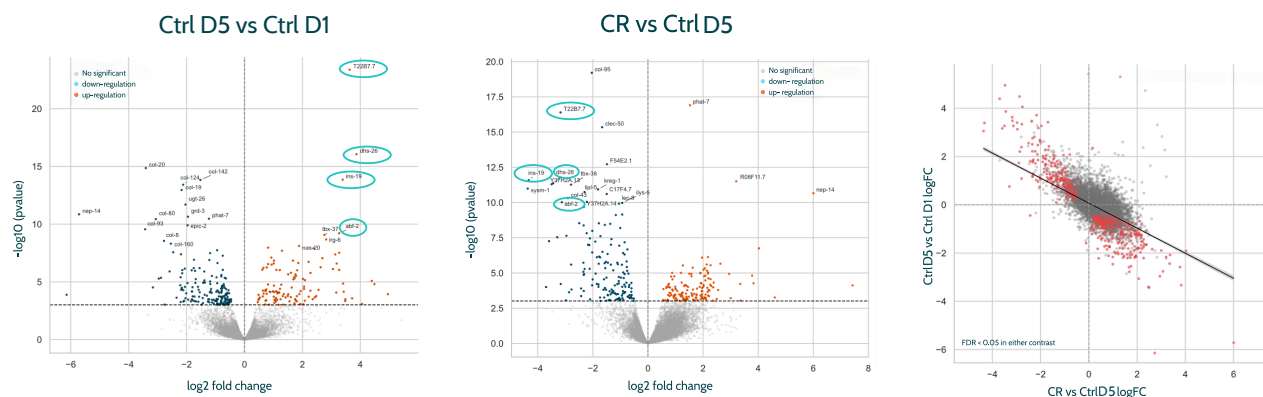


Figure 6. Comparison of age-associated and CR-associated gene-expression patterns. Volcano plots show differentially expressed genes for the age comparison between day 5 control worms and day 1 control worms (left graph) and for CR 20% compared to control (middle graph). Genes are plotted according to log₂ fold change and statistical significance, with upregulated genes shown in red, downregulated genes shown in blue, and non-significant genes shown in grey. Selected genes are highlighted as examples of age-associated genes whose expression changes in the opposite direction under CR 20%. The correlation plot on the right compares gene-expression changes associated with aging against those induced by CR 20%. The negative correlation (right graph) indicates that many genes changing with age are shifted in the opposite direction under CR 20%, supporting its use as an aging-relevant transcriptomic reference condition in this workflow.

Spermidine-associated gene-expression and metabolic pathway response

As spermidine showed a younger phenotypic biological age, differential gene-expression analysis was used to investigate whether spermidine also induced a detectable molecular response. Although transcriptomic age-clock shifts were limited under the tested conditions, pathway-level RNA-seq analysis provided additional biological context by identifying molecular processes modulated in spermidine-treated worms. KEGG enrichment highlighted metabolic pathways, fatty acid metabolism, fatty acid elongation, and amino-acid/one-carbon-related metabolism, suggesting that spermidine induced a metabolism-associated transcriptional response under the conditions tested (Figure 7).

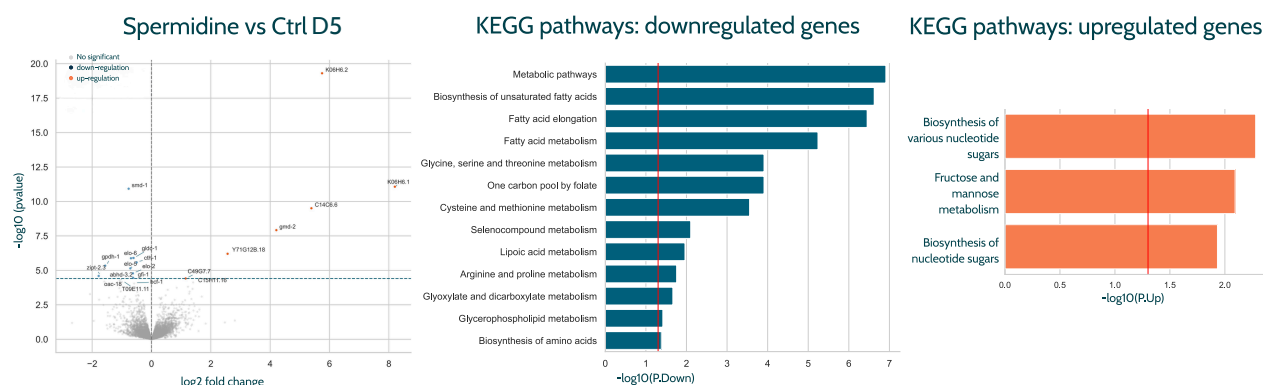


Figure 7. Spermidine-associated transcriptomic response highlights metabolic pathway regulation.

The volcano plot (left graph) shows gene expression changes between spermidine and the control. Genes were plotted according to log₂ fold change and statistical significance. Upregulated genes are shown in orange, downregulated genes in blue, and non-significant genes in grey. KEGG enrichment analysis groups genes into curated biological pathways. Downregulated genes (middle graph) were enriched for metabolic pathways, biosynthesis of unsaturated fatty acids, fatty acid elongation, fatty acid metabolism, and one-carbon/amino acid-related metabolism. Upregulated genes (right graph) were enriched for pathways related to nucleotide-sugar biosynthesis and fructose/mannose metabolism.

Because RNA-seq was performed on Day 5 and Nagi™ Biological Age was assessed in the later phenotypic branch, the transcriptomic results should be interpreted as molecular context for the spermidine response rather than as a direct explanation of the phenotypic biological age shift.

Conclusions and Perspectives

This application study demonstrates the feasibility of extending SydLab™ One from automated longitudinal phenotypic profiling toward an integrated phenotypic-transcriptomic workflow for healthy aging research. Longitudinal organism-level readouts provided the basis for Nagi™ Biological Age phenotypic quantification, while worm retrieval enabled RNA-seq-based transcriptomic analysis, including biological age estimation with BiT age, differential gene expression, and pathway enrichment.

Transcriptomic age estimation showed the expected age-related structure between day 1 samples and day 5 samples, while CR 20% supported the interpretation of a younger-associated molecular profile. At the gene-expression level, CR 20% affected age-associated transcriptional patterns in the opposite direction of aging, reinforcing its value as a positive transcriptomic reference for the workflow.

Differential gene expression and pathway analyses revealed a spermidine-associated molecular response. This included regulation of genes linked to polyamine and lipid metabolism, such as *smd-1*, *elo-5*, and *elo-6* (15, 16), together with pathway-level enrichment related to metabolic regulation. These results complement the biological age analysis by identifying molecular programs associated with the spermidine response, without implying a direct causal relationship between pathway changes and biological age estimates.

Overall, the study validates that an integrated phenotypic-transcriptomic workflow is feasible. Phenotypic profiling and Nagi™ Biological Age provide functional and organism-level interpretation, transcriptomic clocks summarize molecular aging state, and KEGG pathway analyses reveal candidate molecular programs associated with intervention responses. Together, these readouts provide a multi-layer framework to evaluate healthy-aging interventions across multiple biological layers.

Future studies should incorporate multiple doses, additional sampling timepoints, and closer alignment between phenotypic and transcriptomic collection windows. This will help determine whether molecular responses emerge before, alongside, or after measurable phenotypic biological age changes.

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