Aus dem Zentralinstitut für Klinische Chemie und Laboratoriumsdiagnostik

der Heinrich-Heine-Universität Düsseldorf

Direktor: Univ.-Prof. Dr. med. Fritz Boege

Revealing compounds with protective effects on the age-associated features in *Caenorhabditis elegans*

Dissertation

zur Erlangung des Grades eines Doktors der Medizin

der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Shiwa Afshar

Jahr der Promotion: 2025

Als Inauguraldissertation gedruckt mit Genehmigung der

Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

gez.:

Dekan: Prof. Dr. med. Nikolaj Klöcker

Erstgutachter/in: Dr. med. Dr. rer. nat. Natascia Ventura

Zweitgutachter/in: Prof. Dr. rer. nat. Andreas Reichert

Dedication

I dedicate my thesis to my dear family,

especially to my mother, my father and my younger brother.

Teile dieser Arbeit wurden veröffentlicht:

Maglioni, S., Arsalan N., Hamacher A., Afshar S., Schiavi A., Beller, M., Ventura N., (2021), High-Content C. elegans Screen identifies natural compounds impacting mitochondria-lipid homeostasis and promoting healthspan. *Cells*, (11), Issue 1, 1-19 [1].

Zusammenfassung

Umweltfaktoren und Ernährung sind in der Medizin in den letzten Jahrzehnten aufgrund ihres Einflusses auf Gesundheit und Altern sehr bedeutend geworden. Mitochondrien spielen eine grundlegende Rolle beim Altern und bei altersassoziierten Erkrankungen. Während schwerwiegender mitochondrialer Stress eine kritische Rolle in der Entwicklung einer Vielzahl von neurodegenerativen Erkrankungen spielt, hat milder mitochondrialer Stress begünstigende Auswirkungen auf die Gesundheit und resultiert in einer Lebensverlängerung. Dieser Dosis-Wirkungs-Effekt wird mitochondriale Hormesis oder *Mitohormesis* genannt und wird zumindest teilweise durch reaktive Sauerstoffspezies vermittelt. In der Tat: es rekapituliert die Hormesis-Antwort, welches durch verschiedene Dosierungen von Chemikalien, Hitze oder oxidativem Stress induziert wird. Chemikalien, Hitze oder oxidativer Stress können positive Effekte in niedriger Dosierung oder schädliche Effekte in höherer Dosierung haben. Das Labor von Dr. Ventura nutzt den Nematoden *C. elegans* als überzeugendes Modellorganismus, um die Rolle von Mitochondrien im umweltmoduliertem und ernährungsmoduliertem Altern zu verstehen. Dank einer kürzlich entwickelten Phänotyp-basierten Screening-Plattform, haben sie verschiedene in der Natur vorkommende Verbindungen identifiziert, wie Kahalalide F und Lutein, welche die Gesundheitsspanne durch mitochondriale Hormesis fördern können.

Um die Befunde der Screening-Plattform zu validieren, war das Ziel meines Projekts zweierlei: 1) die Effekte (begünstigend oder toxisch) der zwei Verbindungen an altersassoziierten Parametern des *C. elegans* zu untersuchen; 2) um deren Auswirkung auf Mitochondrien-bedingte Eigenschaften zuzuordnen.

Um das erste Ziel zu behandeln, habe ich die Nematoden mit den jeweiligen Verbindungen gefüttert und den Effekt auf Resistenz gegenüber oxidativem Stress untersucht. Zusätzlich habe ich den Effekt von Kahalalide F auf die Motilität während des Alterns untersucht.
Um das zweite Ziel zu behandeln, habe ich die verschiedenen Nematoden-Stämme mit den Verbindungen gefüttert. Es waren Nematoden-Stämme, die transgen-fluoreszierende Reporter für Gene exprimieren; und zwar solche Gene, welche normalerweise durch Langlebigkeits-begünstigende Interventionen assoziiert mit mildem mitochondrialem Stress induziert werden, nämlich *cyp-14a4, gst-4, hsp-6, daf-16* and *nlg-1*.

Die Ergebnisse meines Projekts weisen darauf hin, dass Kahalalide F und Lutein eine begünstigende Wirkung auf das Altern haben: Beide Verbindungen verbesserten die Resistenz der Nematoden gegenüber oxidativem Stress und Kahalalide F führte zu einer besseren Motilität während des Alterns. Von den verschiedenen untersuchten *Mitochondrial Stress Response Genes* in diesem Projekt, erhöhten beide Verbindungen außerdem die Expression von *nlg-1* unter Ausgangsbedingungen. NLG-1 ist ein Adhäsionsmolekül, welches in der synaptischen Funktionalität involviert ist. Beide Verbindungen erhöhen oxidative Stress-Resistenz in der Anwesenheit eines intakten *nlg-1*. Schließlich wurde beobachtet, dass Kahalalide F auch eine Art *nlg-1* - abhängige Wirkung auf die Motilität des Nematoden hat.

Wir schlussfolgern, dass Kahalalide F und Lutein die Gesundheit und Langlebigkeit in *C. elegans* fördern, und dass sie auf ein intaktes *nlg-1* angewiesen sind. Daher schlagen wir vor diese Verbindungen aufgrund ihrer begünstigenden Wirkungen in altersassoziierten neurodegenerativen Erkrankungen zu untersuchen.

Summary

Environmental and dietary factors have become very important in the medical field during the last decades for their impact on health and ageing. Mitochondria play a fundamental role in ageing and age-associated diseases. Interestingly, while severe mitochondrial dysfunction plays a critical role in the development of a variety of neurodegenerative disorders, mild mitochondrial stress has beneficial effects on healthspan resulting in lifespan extension. This dose-response effect has been termed mitochondrial hormesis or *mitohormesis* and is mediated at least in part by reactive oxygen species (ROS). Indeed, it very nicely recapitulates the hormesis response induced by different doses of chemicals, heat or oxidative stress, which can elicit positive effects at low doses while begin detrimental at higher doses. The laboratory of Dr. Ventura exploits the nematode *C. elegans* as a powerful model organism to investigate the role of mitochondria in environmentally- and dietary-modulated ageing. Thanks to a recently developed phenotype-based screening platform, they have identified different natural compounds, such as Kahalalide F and Lutein, which may promote healthspan acting through mitochondrial hormesis.

To validate the screening findings, the aim of my study was twofold: 1) to investigate the effects (beneficial or toxic) of these two compounds on *C. elegans* age-associated parameters; 2) to address their impact on mitochondria-related features.

To address the first aim, I fed either one of the two compounds to the animals and addressed the effect on animals' resistance to oxidative stress. Kahalalide F was also examined due to effects on motility during ageing of *C. elegans*.
To address the second aim, I fed the compounds to different strains expressing transgenic fluorescent reporters for genes normally induced by pro-longevity interventions associated with mild mitochondrial stress, namely *cyp-14a4, gst-4, hsp-6, daf-16* and *nlg-1*.

Results from my study indicate that Kahalalide F and Lutein have beneficial effects on ageing: both significantly improved animals' resistance to oxidative stress and Kahalalide F also contributed to a better locomotion ability during ageing. Moreover, within the different investigated mitochondrial stress response genes, in this project the two compounds increase the expression of *nlg-1* in basal conditions. NLG-1 is an adhesion molecule involved in synaptic functionality. Both compounds increase oxidative stress resistance in the presence of a functioning *nlg-1*. Finally, I could observe that Kahalalide F also impacts animals' motility in part in a *nlg-1*-dependent manner.

In conclusion, Kahalalide F and Lutein promote health and longevity in *C. elegans* and appear to rely on an intact *nlg-1*, suggesting they could be investigated for their beneficial effects in age-associated neurodegenerative diseases.

List of abbreviations

AChe	Acetylcholinesterase
AD	Alzheimer's Disease
Aβ	Amyloid β; amyloid-beta
C. elegans	*Caenorhabditis elegans*
corr. P	Corrected P-value
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleic Acid
E. coli	*Escherichia coli*
ETC	Electron chain transport
GFP	*Green fluorescent protein*
HMAD	Human mitochondrial associated disease
IIS	*Insulin/Insulin-like growth factor 1 (IGF-1) signalling*
IPTG	*Isopropyl-β-D-thiogalactopyranosid*
LB	Lysogeny broth
MERS-CoV	Middle East Respiratory Syndrome- Corona Virus
NGM	*Nematode Growth Medium*
PD	Parkinson's Disease
RNAi	RNA interference
ROS	Reactive oxygen species
UPR^{mt}	*The mitochondrial unfolded protein response*
UV	Ultraviolet

Content

1 Introduction

1.1 Ageing

1.1.1 Features of ageing

Ageing is a process observed in organisms and leads to increasing biological and psychological changes during life [2, 3]. The ageing process is also said to be universal, physiological and irreversible leading to functional deficits. The functional deficits develop, because ageing affects organs and tissues of the body, which undergo physiological changes. For example, in the circulator system the left ventricle becomes hypertroph, in the respiratory system the trachea and bronchi size reduce, in the musculoskeletal-locomotor system there is a decline in bone density, in the nervous system the brain is affected by an accumulation of amyloid plaque and many other systems and tissues are concerned. And these changes cause functional deficits and impairment [3]. As a consequence, the accumulation of functional deficits leads to a higher risk for diseases such as heart disease, cancer, Alzheimer's disease, etc. [4].

The biological mechanisms of ageing can be divided in three main categories: first, damage-inducing mechanisms, second, the antagonistic responses to damage and third, the culprits causing phenotypical changes [4]. First, damage-inducing mechanisms are those mechanisms such as chemicals which cause genetic damage. The older organisms become, the higher is the risk that accumulated genetic damage leads to diseases. Second, antagonistic responses to damage, are responses such as mitochondrial stress [4], which can be beneficial at low doses leading to longevity and detrimental at higher doses (see subchapter 1.1.4.2 mitohormesis) leading to diseases and shorter lifespan [5]. Third, the culprits causing phenotypical changes, are for example changes in intercellular communication. When intercellular communication is affected and changed signalling such as neuronal or inflammatory signalling cannot work properly, this can result in higher secretion of cytokines. And a high secretion of cytokines can lead to chronic inflammation causing diseases and death. So, the biological mechanisms during ageing contribute to morbidity and mortality in elder organisms [4].

1.1.2 Protective dietary factors influencing ageing

Environmental factors such as dietary factors influence lifespan and health. The lifespan of an individual is mentioned to be inherited at approximately 25%, which is indicated in twin studies. This means that factors other than genetic, such as environmental factors, play a fundamental role in lifespan determination [6]. Such environmental factors include dietary habits and physical activity [7]. Researchers have realized that people from several regions in Asia, Africa, Latin America and Middle East have similar diets and similar appearance of diseases, which is caused through the similarity in dietary change between these regions. In that case, dietary change means eating higher amounts of products containing fat, sugar and animal products. This is also termed as the "Western Diet", since these regions have adopted it from Western countries, where nutrient deficiencies have led to metabolic diseases such as type 2 diabetes, cardiovascular illnesses and cancers [8, 9]. Besides, changing dietary factors in patients at risk of diabetes can have positive effects on their health similar to the effect of medication. So, dietary factors can have positive or negative effects on ageing and health [6].

In this chapter, I will focus on those diets which have protective effects on human health, starting with the main dietary pattern such as Mediterranean diet, and also explaining the less-well known Okinawan diet. Afterwards important ingredients of both diets are mentioned.

Diets such as the Mediterranean diet [10] and the Okinawan diet increase lifespan and promote health [8]. People reaching very high ages are said to live in a part of Sardinia in Italy and in Okinawa in Japan and few other regions in the world [6]. The Mediterranean diet consists of high amounts of fruits, vegetables, whereas meat and dairy are consumed less. Olive oil is used as fat in Mediterranean food and a moderate coffee consumption is also present in this dietary pattern [11]. The Okinawan diet is almost near to those of vegetarians, since Okinawans consume mainly green-yellow vegetables and legumes, such as soybeans. The Okinawan population belong to the healthiest population, probably because they also eat one of the healthiest vegetables, namely the staple sweet potato. The Okinawan diet also contains fish in proper limits, but less meat, less dairy products and less sodium [8]. So, the relevant similar dietary properties of these two healthy diets (Mediterranean and Okinawan Diet) is said to be a large percentage of fruits and vegetables with high amounts of unrefined low glycemic carbohydrates, healthy fats and lean proteins, moderate eating of

marine food and use of herbs and spices and less meat. Both diets have similarities and promote health and increase lifespan [8, 12, 13].

The ingredients of these healthy diets especially the phytochemicals promote health and extend lifespan [8, 14, 15]. Okinawans are aware of the potential ability in promoting health, since they consider food as medicine. Indeed, research studies show that the ingredients, especially phytochemicals, in those vegetables, spices and herbs are one of the reasons for the longevity of the Okinawan population. The production of these phytochemicals is reinforced by a higher UV light exposure in that region. So, climate is relevant [8, 14]. The effect of phytochemicals as cellular stress response is also called "phytohormetic stress resistance", in which an oxidative stress response regulating pathway is induced and protects against stressors [8]. That means phytochemicals promote health and longevity, because they have influence on the oxidative stress response, which is a relevant aspect concerning ageing [8, 15].

The group of phytochemicals classifies different groups, for example carotenoids and polyphenols, that have beneficial effects on health [15, 16]. First, carotenoids particularly marine-based carotenoids are found in algae and seaweed that are found in the Okinawan diet [8]. Carotenoids are involved in regulations of the immune system and inhibit inflammation by decreasing molecules such as IL-2 and TNF-α [17]. Fucoxanthin is a carotenoid which is found in brown algae and decreases insulin level and blood glucose. Fucoxanthin in seaweed extract combined with pomegranate seed oil leads also to weight loss in obese women [8]. Another carotenoid such as the trans-crocin-4 is found in saffron (*Crocus sativus* L.), which is a typical spice in Traditional Persian Medicine. In former times saffron has already been used against diseases of the central nervous system and mental diseases such as Alzheimer's disease. Indeed, researchers found out that trans-crocin-4 suppressed amyloid aggregation, that means it inhibits the pathophysiologic process and has neuroprotective effects [18]. So, carotenoids have beneficial effects on metabolism and reduce inflammation [16].

Second, polyphenols are healthy phytochemicals, because they also have anti-oxidant and anti-inflammatory effects [12]. Polyphenols are divided into two main classes: the so-called flavonoids and non-flavonoids [19]. Flavonoids are found in soy, for example [8]. Soy is utilized in preparation of Okinawan tofu and contains high amounts of flavonoids. Flavonoids are reported to have anti-oxidant effects and are involved in hormesis via an activation of *Sirtuin-FOXO* pathway promoting longevity. Hormesis is a cellular stress

response contributing to stress resistance which can be caused through phytochemicals (further explained in a separate chapter) [8]. Concerning the Sirtuin-FOXO pathway: Sirtuins are a group of protein deacetylases and have an impact on the FoxO family (forkhead protein) of transcription factors, promoting longevity and the FoxOs have influence on the cell adaptation towards stress [20]. Quercetin, a flavonoid, induces SIRT1 (Sirtuin 1) and decreases reactive oxygen species (ROS) formation [21]. Quercetin glycoside intake by human is suggested to inhibit amyloid β (Aβ) accumulation in a randomized, double-blind, and placebo-controlled study by Nakamura et al. [22]. So, flavonoids have beneficial effects, because they also decrease Aβ pathology resulting in retarding the progression of the Alzheimer's disease. But further investigations are needed to understand the clear signalling pathways [11]. There are also other flavonoids such as isoflavones in soy, that have been proved to protect against cancer and to retard postmenopausal-associated osteoporosis [8]. The other group of polyphenols, the non-flavonoids include curcumin and resveratrol [23]. Curcumin is found in turmeric, which is used in Okinawan diet for tea or soups. Curcumin is a phenolic compound [23] and suppresses inflammation, because it suppresses pro-inflammatory cytokines such as interleukins (*IL-1β, IL-6)* and tumor-necrosis factor-alpha (*TNF-α).* Curcumin has also an anti-oxidant impact by neutralizing reactive oxygen species (ROS). In addition to that, researchers claim that curcumin would be the leading compound that causes partially the lower Alzheimer' disease (AD) rate in India. The reduced AD rate could be probably achieved through curcumin, because it reduces the accumulation of amyloid-beta (Aβ) peptide [8]. Another relevant polyphenol for lifespan extension is resveratrol [8], which is found in grapes [18] and cranberries [24]. Resveratrol is trans-3,4',5-trihydroxystilbene [18] and is also a phenolic compound such as curcumin [23], but resveratrol belongs to a special group as the name implies: the stilbenes [25]. And stilbenes belong to the phenolic phytoestrogens such as the isoflavones [26]. Resveratrol has anti-inflammatory and antiviral effects [17]. It is mentioned to inhibit a variant of the coronavirus, the so-called Middle East Respiratory Syndrome (MERS-CoV), because it suppresses the cell death and the RNA expression of MERS-CoV [24]. Resveratrol has also positive impacts on cognitive impairment, because it decreases secreted Aß peptides and amyloid plaque pathology in several brain regions and leads to higher levels of mitochondrial complex IV protein. These beneficial effects are dependent on *SIRT1* (Sirtuin 1) and *AMPK* pathways, which are activated through resveratrol [18]. AMPK is abbreviation for 5'adenosine-monophosphate-activated protein kinase [27]. The AMPK pathway belongs to the nutrient-sensing pathways like the sirtuins [28]. Additionally, polyphenols such as

curcumin and resveratrol have caloric restriction mimetic effects resulting in healthy ageing. So, polyphenols have protective effects on health [12].

Dietetic interventions such as caloric restriction (CR) or different types of fasting might lead to longevity and health [29]. Caloric restriction is mentioned to reduce blood pressure [30], glucose and insulin [31]. But genetic factors and the time when caloric restriction is started are discussed to be relevant aspects, otherwise caloric restriction could lead to earlier mortality than to longevity [12]. In the CALERIE Study Group, a randomized controlled trial, Fontana *et al.* showed that caloric restriction did not lead to reduction of insulin-like growth factor (IGF) in serum in humans who had a long-term (24 months) caloric restriction. Possible factors such as a high-normal protein intake could be the reason [32], because other studies have mentioned that reducing protein intake are important to decrease IGF-1 serum concentrations in humans that had caloric restriction [32, 33]. Time restricted feeding can have beneficial effects on health in humans following an 8-hour window during light time [12]. It is also described that not only daytime, but even meal frequency are factors influencing health. For instance, if humans eat in a five-hour or seven-hour time window they cause a better health status than those eating three to five times in a day. So, dietary restriction itself has been revealed to have beneficial impact on health in ageing humans [34].

Gut microbiota is also important for healthy ageing and is even affected by dietary compounds (such as polyphenols) and dietary restriction. For example, caloric restriction changes gut microbiota and results in better health status [35]. The beneficial impact of compounds such as polyphenols depends on the composition of gut microbiota, because the bioavailability of such compounds is mediated through microbiota. And also vice versa: polyphenols such as curcumin can also modulate the composition of gut microbiota [36]. It is suggested that probiotic bacteria should be included in diet in order to achieve an improved metabolism through an appropriate composition of the gut microbiome [10]. So, human gut microbiota composition is another important factor during the ageing process. The human gut microbiota consists of approximately 10^4 bacteria, fungi, viruses, archaea and protozoa and possesses a 150 times greater gene pool than the human being. This gut microbiota composition can be altered through both genetic components as well as environmental interactions, such as diets, antibiotic intake and geographical factors. The resilience of gut microbiota is depicted to decline approximately in older individuals later than the age of 65 years. Healthy centenarian and supercentenarian individuals possess an extensive range of

particular microbiomes, including *Bilophila*, *Butyricimonas* and *Eggerthella*, which would have advantageous effects on metabolism and have been shown by investigations of the centenarians' faecal microbiota composition [37]. Physical activity is reported to modulate the composition of gut microbiota as well and is even described to lead to a higher diversity of gut microbiota in young humans, but further research is necessary to evaluate its impact on older people [37]. A healthy composition of gut microbiota especially maintaining the diversity of microbiota is important during ageing, since older people have difficulties in chewing their food and have also a lower absorption [36]. So, the composition of gut microbiota is a relevant aspect in healthy ageing [10].

1.1.3 C. elegans as a genetic model for ageing studies

The nematode *C. elegans* is used as a favourable model organism for ageing studies because of its short lifespan [38]. These nematodes feed on bacteria and fungi [39], exist in soil and decomposing fruits and have been used for laboratory investigations for more than 50 years [40]. *C. elegans* has a mean lifespan of 15 to 18 days and nematodes emerging from embryo, reach the state of a reproductive organism in nearly 3 days (see **Fig. 1.1)**. The egg production lasts during the first 4 days of adulthood and vanishes in older nematodes, who attain the post-reproductive phase [41]. Besides, the nematodes enter a particular phase as dauer larvae, which occurs under certain circumstances such as the presence of stressors, caloric restriction, etc.. The population of nematodes consists mainly of hermaphrodites, but male nematodes can also develop through non-disjunction concerning the X-chromosome [39].

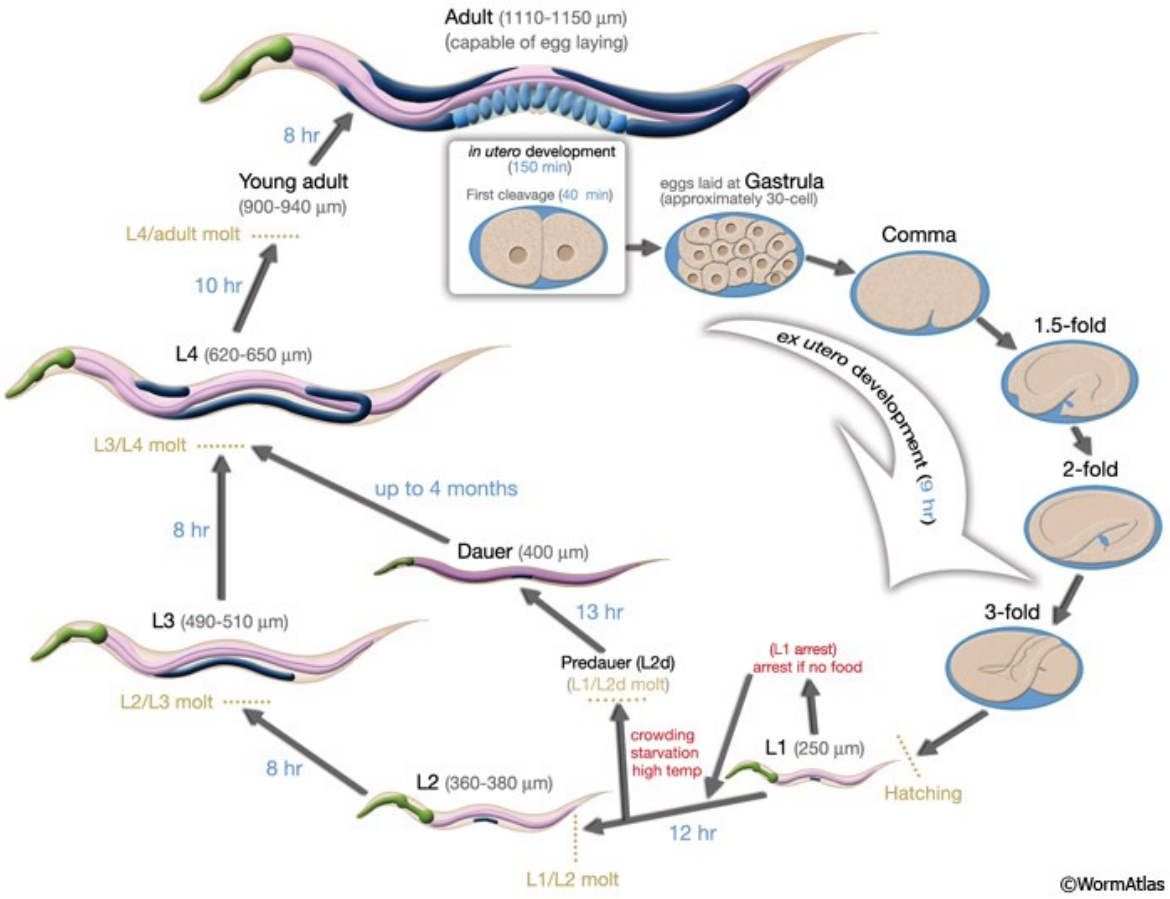

Fig. 1.1 Development of *C. elegans* from embryo to adult nematode
The figure shows the lifecycle of *C. elegans* from embryo to adult worm, which is divided into several phases: egg, L1, L2, L3, L4, adult worm. If L1 is present under food absence, it transforms into the Predauer (L2d) and later into the Dauer form, which can reach the L4-phase under certain circumstances. The figure is adapted from the *wormatlas* website [42].

C. elegans has many other strengths as a model organism. Advantages for using *C. elegans* is the completely sequenced genome of the nematode [39]. A "genetically tractable organism" like *C. elegans* provides the possibility to elucidate pathways regulating biological processes including ageing [38]. *C. elegans* can be used to generate transgenic as well as knock-out strain for every gene of interest [43]. Other strengths of this model include conservation of several signalling pathways and neuronal function, possessing motor, neuronal, digestive and reproductive properties. Its behavioural reactions to cues can be investigated as well through many different assays, for example developmental toxicity [39], fertility and survival to stress [43]. Besides, several experimental methods exist in order to study ageing in *C. elegans*, such as lifespan assays or abiotic stress resistance assays to understand the connection between environmental stresses and physiological features [44]. Concerning the administration of compounds, this nematode is a very useful model, since absorption, distribution, metabolism, excretion and toxicity (AMDET) can be observed at the same time [45]. In laboratory conditions, its maintenance is at temperatures between 15

to 25°C and it can be easily tested in high numbers in parallel experiments [39]. The storage of the nematodes at extreme temperature of -80°C is possible to maintain them available for many years [44].

The use of *C. elegans* for experiments has also limitations. Limitations concerning the use of this model organism include the absence of several mammalian organs such as lungs, kidney, heart, adaptive immunity. Besides, results can change through little alterations in salt concentrations but also through thermal alterations, for instance [39]. Interestingly, another point to consider while utilizing these nematodes in assays is the transgenerational epigenetic inheritance. Transgenerational epigenetic inheritance means gene expressions might be altered when the generations before were exhibited under certain extreme conditions such as food deprivation [46].

C. elegans is an important model organism to investigate and understand the ageing process on tissues [41], physiology [47, 48] and behaviour [38]. Concerning tissues, the body phenotype during ageing of *C. elegans* is characterized by a deposit of lipofuscin and dark pigment. Electron microscopic observation reveals intestinal, muscular, and hypodermal conglomeration of lipid formation. In muscles a progressive disorganization of sarcomeres and a decreasing amount of myosin thick filaments in each sarcomere unit are observed during ageing. Concerning physiology, structural changes in muscles such as sarcopenia or muscle deterioration might reduce motility in ageing nematodes like *C. elegans* [41]. In the intestine of *C. elegans*, increasing concentration of the bacteria *Escherichia coli (E. coli)*, which is used as diet for *C. elegans*, have been discovered during ageing. This accumulation of bacteria in the nematode's intestine is related as an ageing biomarker. Ageing animals appear to become more sensitive to bacterial infection and the morphology of intestinal cells presents earlier deteriorated alterations during ageing than neurons [49]. Interestingly, assays using *C. elegans* revealed that the regulation of longevity is one of the main fields the network of neurons is responsible for and that it is coordinated through interaction between neuroendocrine signals and the intestine [50]. Moreover, this means that the detection and the processing of environmental signals by the brain are followed by signalling the intestine to regulate lifespan [51]. Cold temperatures extend lifespan and are mediated by glutamate and serotonin which influence *DAF-16/FOXO* regulation in the intestine through their receptors [51]. Concerning behaviour, behavioural impairments during ageing might be related to locomotory deficits [38]. Furthermore, different abilities appear to decline at different life stages. For instance, *C. elegans* show a deterioration of higher-level cognitive

skills such as long-term memory, massed learning and spaced learning much earlier than a reduced chemotaxis and locomotory ability [52].

C. elegans is useful in order to examine neurodegenerative diseases and the use of natural compounds for prevention or therapy. Several natural compounds and extracts have already been investigated and indeed compounds such as epigallocatechin, curcumin, resveratrol, etc. revealed to possess favourable properties. For instance, beta-amyloid toxicity was decreased in a transgenic *C. elegans* AD model through an extract of tea seed pomace (*Camellia tenuifolia*) [53]. The details how dietary factors influence ageing in *C. elegans* will be described in subchapter 1.1.4.3.

1.1.4 Main C. elegans longevity pathways

1.1.4.1 Genetic pathways regulating ageing in *C. elegans*

Certain genes regulate ageing and are connected with signalling pathways [54]. A well-known genetic pathway is insulin/insulin-like growth factor-1 (IGF-1) signalling (IIS) pathway [54]. One component of IIS is DAF-2, which is a receptor tyrosine kinase induced by ligands such as insulin-like peptides (ILPs) [55]. So, when DAF-2 is activated by ligands, AGE-1/PI3K is recruited to the cell membrane [56] and causes biological ageing (senescence) [57]. AGE-1 is the *C. elegans* homolog of the downstream phosphatidylinositol 3-kinase [54]. And when AGE-1/PI3K is recruited, AGE-1/PI3K leads to activation of serine/threonine kinases AKT-1, AKT-2 and SGK-1 [56] and lead to phosphorylation of DAF-16, which is a transcription factor [55]. When DAF-16 is phosphorylated, then DAF-16 is inhibited from translocating into the nucleus and cannot induce stress response and longevity [56]. In contrast to that: if there is a reduction or inhibition of insulin/Insulin-like growth factor 1 (IGF-1) signalling (IIS), then there is lifespan extension [54]. For example, if DAF-2 activity is reduced, then lifespan extends and this can be caused through mutations [54]. DAF-2, AGE-1 and DAF-16 are the main factors of IIS pathway [54, 58] (see **Fig. 1.2)**.

Reduced IIS activates three main transcription factors DAF-16, HSF-1 and SKN-1 that concur to lifespan extension. These transcriptions factors act as downstream mediators and impact genes for lifespan extension [59]. First, DAF-16 (FOXO) is important for regulating homeostasis in stress conditions. Decreased activity of insulin/IGF-1-like receptor causes a

failing phosphorylation of DAF-16, which results in its nuclear translocation and longevity [59]. But increased IIS, that means activated DAF-2 induces AKT-1, AKT-2 and SGK-1 and then DAF-16 is phosphorylated and remains in the cytoplasm [55]. Second, the heat shock transcription factor HSF-1 (HSF1) is responsible for the expression of chaperones under certain stress conditions such as heat. If HSF-1 is activated because of intermittent heat shock, nematodes live longer. When there is no heat, but decreased IIS, then HSF-1 is also activated, but in this case its activation is ascribed to phosphorylation of DDL-1 (CCDC53) by a kinase. This phosphorylation destabilizes a complex (DDL-1/DDL-2 (WASH2)/HSB-1 (HSBP1)-complex, which otherwise would inhibit HSF-1 [59]. So, decreasing IIS leads to decreasing formation of this complex also described as the DDL-1 containing HSF-1 inhibitory complex (DHIC) [60]. Third, the SKN-1 (skinhead-1) transcription factor is an orthologue of NRF2 [61] and is activated under stress conditions such as oxidative stress [59]. Because of oxidative stress SKN-1 accumulates in the intestinal nuclei and induces expression of genes that are important for detoxification [61]. SKN-1 is reported to be involved in lifespan extension. When IIS is decreased, then *skn-1* is mentioned to lead to lifespan extension [62]. For instance, both *hsf-1* and *skn-1* are decisive factors for the pro-longevity effect of compounds which bind to amyloid protein aggregates, but whether this lifespan extension is caused through an impact on stress signalling or direct impact on protein aggregates has not been found out yet [59].

AKT-1/2, SGK-1 and GSK-3 inhibit SKN-1 by phosphorylation under basal conditions, but in cases of oxidative stress SKN-1 is activated by PMK-1/p38 MAP-kinase dependent phosphorylation [59]. It has been reported that a reduced activity of PMK-1 p38 MAPK (mitogen-activated protein kinase) pathway occurs with advancing age, which plays a role in defence of pathogens. Ageing nematodes lose their defence towards bacterial infection (see 1.1.3). A higher accumulation of *E. coli* is a factor causing death in nematodes and indeed *pmk-1* mutants show a higher intestinal accumulation of *E. coli* during ageing compared to wild-type nematodes [49].

Except of IIS pathway, there are three other pathways/lifespan-regulating genes such as TOR signalling, sirtuins and AMPK [54] (see **Fig.1.2**). When TOR signalling (mechanistic/ mammalian target of rapamycin) is suppressed, longevity is promoted [54]. And TOR is mentioned to contribute to longevity, when it inhibits IIS pathway during fasting. So, it is suggested that TOR might have different effects on lifespan, which depends on environmental conditions [54]. Both IIS and TOR signalling regulate activity of SKN-1 and

DAF-16 [63]. Sirtuins belong to the group of nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases and are suggested to extend lifespan under special fasting conditions [54]. In *C. elegans* Sir-2.1, homologue of human Sirtuin-1, induces DAF-16 resulting in longevity [27]. When Sir-2.1 is suppressed, lifespan is shortened [58]. AMPK (AMP-activated protein kinase) is an energy sensor in cells and DAF-16 is an important factor for lifespan extension via AMPK [54]. AMPK, TOR and sirtuins are all pathways that are affected by caloric restriction [27].

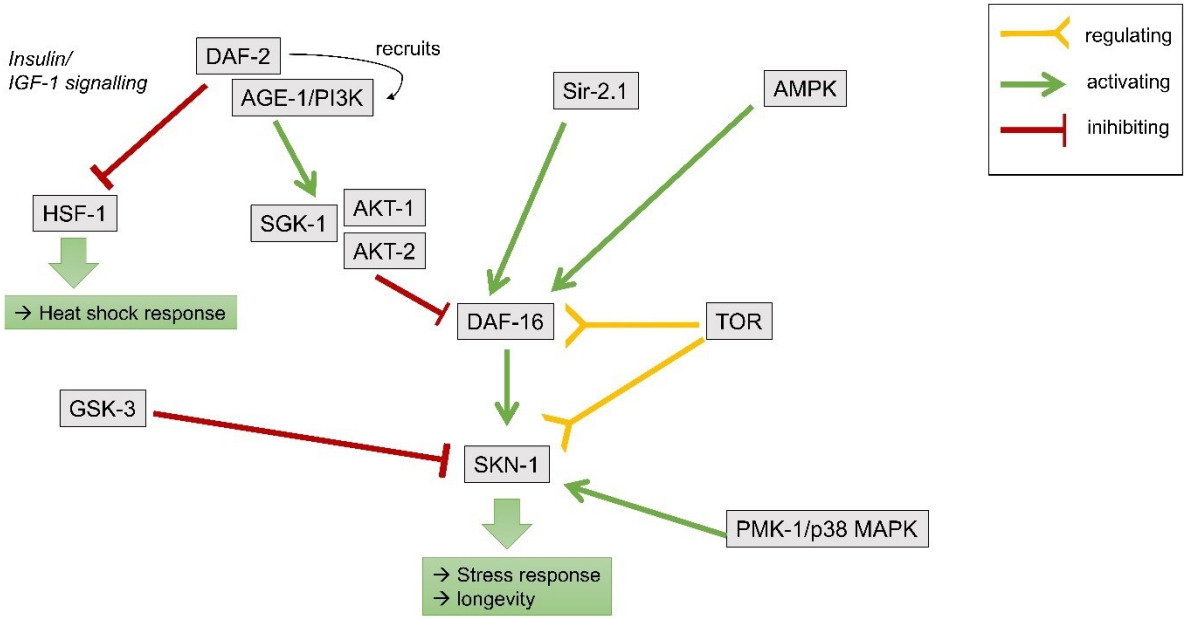

Fig. 1.2 Simplified scheme about genetic pathways regulating ageing in *C. elegans* A schematic and summarizing overview of the pathways regulating (directly or indirectly) ageing and longevity, that are described in the text. Figure has been prepared in *Microsoft Powerpoint* using information from different sources [27, 54, 55, 57-59, 62, 63].

1.1.4.2 Mitohormesis

In eukaryotic cells mitochondria are important organelles with different functions. They regulate cell death by releasing *cytochrome c*, and they deliver ATP and metabolites for survival of cells and provide differentiation. Mitochondria act as cytosolic calcium regulators, function as scaffolds to arrange signalling complexes and are involved in signalling pathways through protein delivery and release of metabolites and reactive oxygen species (ROS) [64]. Mitochondria generate the most amount of ROS in a cell [65].

Depending on the concentration of reactive oxygen species (ROS) either deteriorating or beneficial effects occur. Higher concentrations of ROS contribute to cellular damage, also

called oxidative stress [5]. The rise in ROS levels is caused by altered electron transport chain, that has occurred through mitochondrial dysfunction. What follow are damaging effects emerging in the genome [64] and proteins, etc. [66]. Low concentrations of ROS are described to have signalling functions and to cause beneficial effects by activating defence mechanisms. The beneficial effect by a non-linear response to substances that are potentially toxic, is called "hormesis". Concerning this phenomenon in mitochondria, it is also termed as mitochondrial hormesis or *mitohormesis* [5]. When mitochondria are only mildly damaged, they promote stress response signals ultimately improving cellular function and promoting lifespan extension [67]. While it is known that mitochondrial dysfunction causes oxidative stress, it is suggested that mitochondrial dysfunction plays a vital role in the development of illnesses like neurodegenerative diseases, cancer or diabetes [5]. So, it has been suggested that *mitohormesis* means that ROS regulate longevity [65] and that it includes non-linear signalling processes, that can be mediated by mitochondrial ROS [5].

However, two other main criteria have to be taken into consideration in order to understand in which form mitochondrial stress affects longevity. The two criteria are the timepoint when it is caused and the length of the stress period [65, 68]. Because depending on these factors, it could lead to opposite results: Expanded lifespan has been investigated in worms treated with high glucose during development, but those worms who received a high dose of dietary glucose at the age of adulthood had a shortened lifespan [65]. The mitochondrial unfolded protein response (UPR^{mt}) is a pathway for adaptation to mitochondrial proteotoxic stress [67]. The UPR^{mt} is activated via glucose in both cases but results in two different phenomena. One relevant aspect is, that the induction of UPR^{mt} decreases during ageing: HSP-6 is a part of UPR^{mt} and its expression has been shown in 1 to 3-day-old adult nematodes exposed to high dietary glucose, but not in older ones [65]. Glucose is a stressor for cells and mitochondria, and it is suggested that activated cellular pathways during development might cause faster ageing, when their activity still remains during adulthood. This theory is also called the hyperfunction theory [65].

Opposite effects on lifespan of *C. elegans* can also be caused by mitochondrial mutations [69]. Mutations that disrupt electron chain transport (ETC) can have life-shortening or life-expanding effects. Those mutations that have life-expanding effects by disrupting ETC are called *Mit* (mitochondrial) mutants [70]. It is suggested that in *Mit* mutants mitochondrial dysfunction is mild resulting in a longer lifespan. *Mit* mutants can be distinguished into 3 groups: first, gene inactivation by RNA interference (RNAi), second, classical gene

mutations and third, external interventions. The first group: RNAi-induced *Mit* mutants can have an extended lifespan [71], and it depends in which degree RNAi inhibits the expression of those genes that affect mitochondrial proteins. This can be observed through RNAi dilution: a moderate degree of inhibition of those genes results in moderate ETC disruption and leads to longevity. Low inhibition by RNAi causes no response and too high inhibition leads to earlier death [70]. There are still scientific gaps about the longevity pathways in *Mit* mutants and it is assumed that there might be more than one pathway that is responsible for longevity [72]. The second group of *Mit* mutants, namely classical gene mutations, includes *clk-1* [71]. *Clk-1* encodes a hydroxylase which plays a role in biosynthesis of ubiquinone [73] and ubiquinone is a component of the ETC [69]. Mutations in *clk-1* lead to increased ROS and lifespan extension [73]. The third group comprises external interventions such as exposure to chemicals, which have an impact on the ETC. Antioxidants such as ubiquinone belong to this group. So, the largest group of *Mit* mutants is the gene inactivation by RNAi [71].

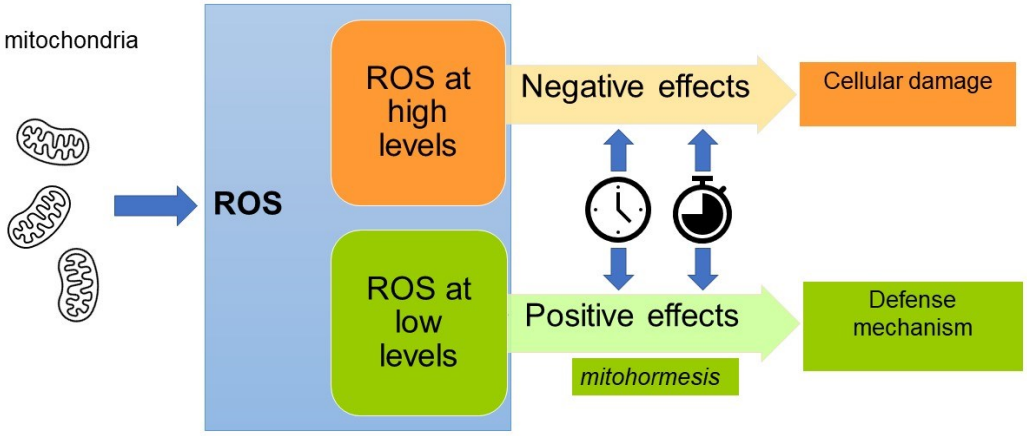

Fig. 1.1.1 Positive and negative effects of ROS (reactive oxygen species) levels
The figure depicts two different conditions: ROS at low levels has positive effects on defense mechanism (*mitohormesis*), whereas at high levels it contributes to negative effects resulting in cellular damage. Mitochondria generate most of the ROS. The effects are also determinated by the timepoint of the stress and the length of the stress period. Figure has been prepared in *Microsoft Powerpoint* using information from different sources [5, 65, 68].

Different natural compounds can have beneficial effects on longevity via mitochondrial hormesis [27]. For screening particular compounds and small molecules that could affect longevity through mitochondria in *C. elegans*, an *in vivo* high-content automated microscopy

strategy has been developed among different screening platforms (Maglioni, Arsalan, Ventura et al. 2015). Discovering the phenotypic alterations which are dependent on different levels of mitochondrial stress, makes it possible to use them for automated microscopy quantification such as fluorescent transgenes [74]. This screening platform is described to identify prolongevity interventions through mild mitochondrial stress [45].

1.1.4.3 Dietary factors influencing ageing in *C. elegans*

Many natural compounds used in the diets are shown also from my hosting laboratory, to promote longevity in *C. elegans*, such as caffeic acid, curcumin [27, 75], quercetin [76], lutein and kahalalide [1].

Dietary factors can regulate cellular pathways which lead to longevity in *C. elegans*. Cellular pathways are the TOR pathway and the insulin pathway [27], for example. More precisely: the LET-363/mTOR (mechanistic target of rapamycin) and the IIS (insulin/insulin like growth factor 1) signalling are the main two nutrient-sensing pathways for longevity by dietary restriction. Under dietary restriction LET-363/mTOR is prevented from phosphorylating the transcription factors DAF-16/FOXO and HLH-30/TFEB. In that case, DAF-16/FOXO and HLH-30/TFEB pass into the nucleus and lead to transcription of target genes that encode proteins that are necessary for autophagy [77]. Autophagy is a pathway that delivers damaged organelles or proteins to the lysosomes [78]. The other pathway, the IIS, is not activated under low nutrient levels. That means processes for longevity are stimulated, because mTOR is not activated by IIS [77]. IIS also regulates the transcription factor SKN-1/Nrf2. When IIS is reduced, SKN-1/Nrf2 is activated and causes stress resistance and dietary-induced lifespan extension [77]. Metformin is a dimethylbiguanid, derives from herbs and is used for lowering blood-glucose in patients with diabetes [79]. Metformin acts via AMPK and SKN-1 and leads to lifespan extension, but independent of insulin pathway [80] (see **Fig. 1.3**). Dietary restriction is also described to be a low-intensity stressor that promotes longevity through hormesis [81].

There are three observations suggesting that natural compounds used for diet can extend lifespan via hormesis [27]. First, several compounds have dose-dependent effects typical for hormesis: Siberian ginseng and the tea polyphenol epigallocatechin gallate (EGCG) extend lifespan at low dose. But if higher doses are used, lifespan is reduced [27]. EGCG has impact on activity of transcription factors such as DAF-16 and SKN-1 resulting in longevity of *C. elegans* [82] (see **Fig. 1.3**). Second, natural compounds can activate stress resistance

pathways such as autophagy [27]. Autophagy is suggested to cause cell death and to provide energy and nutrients, when there are stress conditions like formation of misfolded proteins or nutrient shortage [78]. So, autophagy is a process that occurs when cells respond to stress and resveratrol activates stress resistance pathways such as autophagy [27]. Both caloric restriction and resveratrol activate Sir-2.1 and then autophagy follows [78]. Third, natural compounds can cause *mitohormesis*, that means they cause low ROS concentrations that trigger stress resistance. EGCG and glucosamine are examples for compounds causing *mitohormesis* [27]. Previously, researchers believed that natural compounds would scavenge reactive oxygen species and therefore lead to longevity, but newer research shows evidence that longevity is achieved by contributing stress resistance through these natural compounds [27]. Furthermore, it is suggested that oxidative stress is necessary for longevity and that antioxidants even prevent the beneficial effect of compounds that cause hormesis [83]. Results have already revealed that anti-oxidants such as ascorbic acid (vitamin C) and α-tocopherol derivative Trolox (vitamin E) could have inverse effects on longevity, which means that consumption of anti-oxidants might result in negative outcomes on health [84].

However, nutrients are capable of affecting the expression of several genes, that are involved in longevity [48]. SKN-1 is involved in healthy lifespan extension and is important for the longevity effects through vitamin D3. Moreover, the expression of SKN-1 target genes such as *gst-4* (glutathione-transferase 4) playing a role in oxidative stress response, is increased by vitamins like vitamin D3 [85]. Vitamin B6, which can be synthesized by different bacteria, is also an important factor for lifespan extension in *C. elegans*, because it induces the *cysl* (cysteine synthases) genes that are responsible for cellular detoxification [86]. Thus, certain genes and nutrients can be further examined to better understand which and how compounds would be efficient to promote longevity [48].

Furthermore, it has been shown that the intestinal microbiome of *C. elegans* plays an important role for its health and lifespan as well. Researchers have realized that those nematodes, which live freely in their natural environment, possess several diverse microbiota in their intestine, which protect *C. elegans* from pathogens and have beneficial effects on their stress resistance and their growth [40]. Particular types of bacterial diet are decisive for neuroprotection in *C. elegans*. For instance, *E. coli* HT115 presents the most advantageous neuroprotective impact and relies on DAF-16. Moreover, bacterial metabolites such as GABA produced by HT115 are considered to be involved in neuroprotection [87]. Additionally, bacteria appear to have more advantageous effects for nematodes, namely even

in cases of toxicity. For instance, researchers ascertained that live OP50 used as bacterial diet for nematodes improved the resistance towards methylmercury (MeHg), which belongs to the category of neurotoxicants. Nematodes fed with dead dehydrated bacteria presented a lower resistance and fasted worms were even the most sensitive towards toxicity of MeHg. Possible reasons for these observations might be that MeHg not only reaches live bacteria through active transport triggered by cysteine transport mechanism, but it might be also transported via passive diffusion in these microbes, resulting in decreasing MeHg concentrations. So, living or dead bacterial diet have different impacts on the nematodes stress resistance [88]. Furthermore, research studies have realized that the variation of metabolism existing in different bacteria like OP50 and HT115 should be taken into consideration when experimental results are discussed [89]. For example, molecules such as ROS that can be differentially produced by different bacteria, in turn activate *gst* and *cyp* expression in order to induce cellular detoxification in the nematode and these can differentially impact in nematode resistance to stressors [86]. It is thus recommended that a standard should be defined for bacterial diet and media composition for all studies working with *C. elegans* [89].

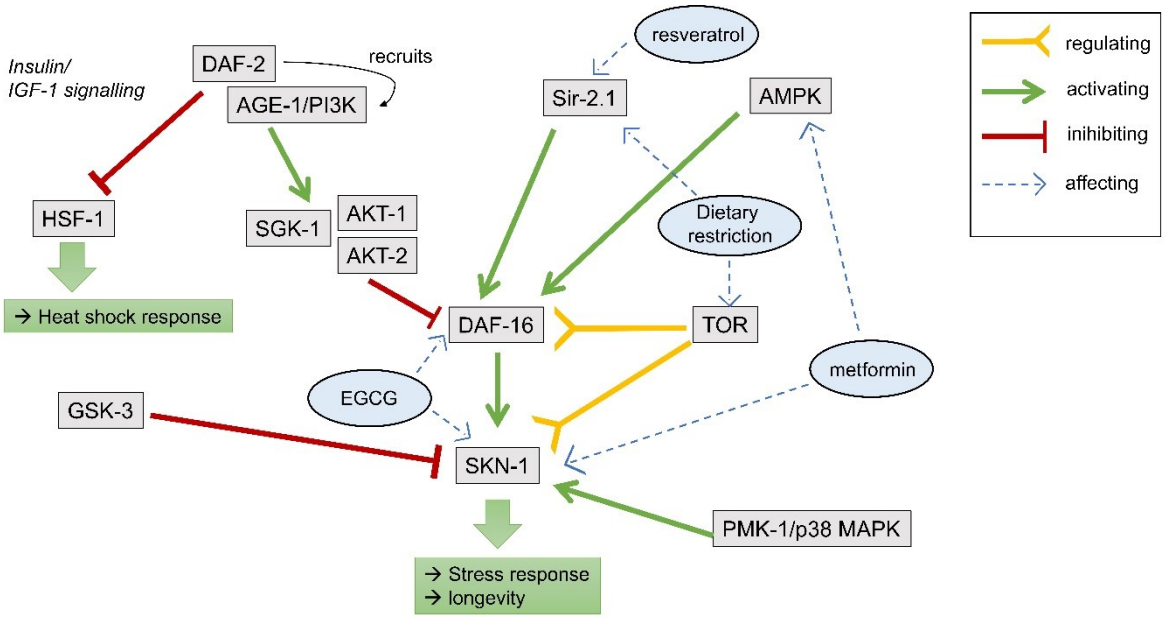

Fig. 1.3 Simplified scheme about dietary factors affecting genetic pathways in *C. elegans*
A schematic and summarizing overview of few dietary factors affecting genetic pathways, which regulate (directly or indirectly) ageing and longevity in *C. elegans*. For a better understanding the same scheme with the information from the subchapter 1.1.4.1 has been used [27, 54, 55, 57-59, 62, 63]. Here, only few dietary factors have been included additionally, that are mentioned in the text of this subchapter. Figure has been prepared in *Microsoft Powerpoint* using information from different sources [77, 78, 80, 82]. The IIS pathway is also affected by dietary restriction, because it is not activated [77] (not shown in scheme).

1.2 Aims of thesis

Dietary factors like natural compounds can impact on ageing and neurodegenerative diseases such as the Alzheimer's disease (AD). However, the underlying molecular mechanisms of their protective effect, besides their antioxidant ability are still unknown.

A preliminary study in the laboratory of Dr. Ventura identified different natural compounds promoting healthspan in the nematode *C. elegans*, such as Kahalalide F [1] (see **Fig. 1.4**) and Lutein [90] (see **Fig. 1.5**). The depsipeptide Kahalalide F ($C_{75}H_{124}N_{14}O_{16}$), which exists in the mollusk *Elysia rufescens* [91] and in alga *Bryopsis pennata*, which is its food source, is considered as a cytotoxic therapeutical compound against several carcinogen diseases [92]. Lutein is found in vegetables, belongs to the group of carotenoids and is mostly known as an antioxidative compound often used in the ophthalmological field for prevention of age-related macular degeneration [93] but has been discussed to be also a potential therapeutical candidate for Alzheimer's disease [94].

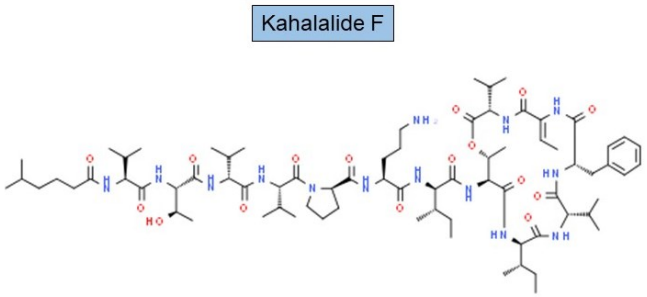

Fig. 1.4 Chemical structure of Kahalalide F
(adapted from the website ChemSpider, ChemSpider ID 32818457) [95]

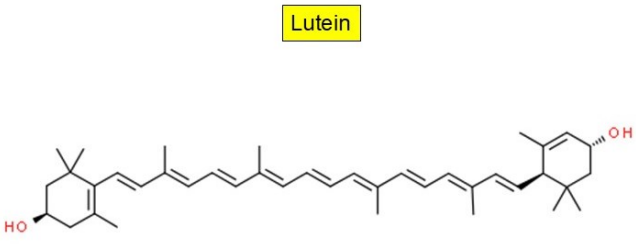

Fig. 1.5 Chemical structure of Lutein
(adapted from the website ChemSpider, ChemSpider ID 4444655) [96]

In my thesis work the two compounds, Kahalalide F and Lutein, were further investigated for their effects on specific age-associated features in *C. elegans*, namely expression of mitochondrial stress reporter genes associated with ageing and animals' resistance to stress and movement.

2 Materials and methods

2.1 *C. elegans* strains and maintenance

All *C. elegans* strains were maintained at standard conditions on OP50-plates (see 2.2 and 2.3) and kept in an 20°C-incubator [97]. In this work the following strains were employed: N2 (wild-type) (from Caenorhabiditis Genetic Center CGC)), TJ356 (zls356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)] (from CGC) [73], (cyp-14A4p::gfp::cyp-14A4 3'UTR) (from Gary Ruvkun Lab) [98], CL2166(dvIs19[pAF15(gst-4::GFP::NLS)) III) (from CGC), SJ4100 (zcIs13[hsp-6::GFP]) (from CGC), vjls48[Pnlg-1::gfp]I (from Derek Sieburth Lab) [99], CRR100 nlg-1 (ok259) X; crrEx4 [pPD95.77;pDD04 Neo^R (Pmyo-2::GFP)] [100] and VC228 nlg-1(ok259)X (from CGC).

Strains that were going to be used for experiments were transferred to HT115(pL4440)-plates with each compound (DMSO (dimethyl sulfoxide), Kahalalide F, Lutein) for egg-laying and were removed afterwards, to achieve a synchronization of the future worms' population for experiments. Through this synchronization method, 3 days after the egg-laying, the larvae reached the age of 1-day-old adult nematodes, which were mainly employed for all assays. In the locomotion assay (see 2.8), not only 1-day-old adult but also 5-day-old and 8-day-old adult nematodes were examined.

2.2 Preparation of NGM-plates

The *Nematode Growth Medium* (NGM) was prepared in a bottle with the following components mentioned in **Table 1** and sterilized for about 20 minutes at 120°C in the autoclave. After this sterilization procedure, the NGM-bottles were placed in the water bath to keep the temperature stable (55°C). After reaching the desired temperature the next components $CaCl_2$, Cholesterol, $MgSO_4$ and KPO_4 were added to the NGM-medium (see **Table 2**). Finally, each medium petri plate (diameter 6 cm) was spotted with 7 ml NGM and 250µl bacteria one to two days later, which depended on the dryness of NGM-plates. This preparation of NGM-plates was used throughout the project for maintaining the strains on NGM-plates seeded with OP50-bacteria before using for the further experiments.

Besides, HT115(pL4440)-plates were prepared as well, but here IPTG, and two antibiotics (Ampicillin and Tetracycline) were added to NGM additionally, shortly before spotting on

plates (see **Table 3**). So, these agar plates were utilized as HT115(pL4440)-plates later for the assays, where strains developed from egg until adulthood.

Table 1: Preparation of NGM for autoclaving

	Amount for 600ml NGM
NaCl (Sigma Aldrich)	1,8g
Agar (Sigma Aldrich)	12g
Bacterial Peptone (Sigma Aldrich)	1,5g
Distilled water	600ml

Table 2: Components added after autoclaving NGM

Adding following compounds after autoclave	**Amount for 600ml NGM**
1M $CaCl_2$	600µl
5µg/ml Cholesterol (Sigma Aldrich)	600µl
1M $MgSO_4$ (Sigma Aldrich)	600µl
1M KPO_4 (Sigma Aldrich)	600µl

Table 3: Additional components used for HT115(pL4440)-plates

Components added to NGM-plates for experiments (later HT115(pL4440)-plates)	**Amount for 600ml NGM**
1M IPTG (Sigma Aldrich)	600µl
100 mg/ml Ampicillin (Sigma Aldrich)	600µl
5 mg/ml Tetracycline (Sigma Aldrich)	600µl

2.3 Preparation of bacteria

As mentioned in 2.2, two different kinds of plates were prepared: OP50-plates for maintaining the strains and HT115(pL4440)-plates were utilized for the worm generation, that was going to be investigated for the assays.

For standard NGM-plates OP50-bacteria were employed, whereas HT115(pL4440)-bacteria were used on NGM-plates containing IPTG and antibiotics. However, LB-medium was poured into a sterile flask or falcon and 1-2µl of bacteria were added via a pipette afterwards. Consequently, it was placed in an incubator at 37°C and rotating for nearly 15 hours. In the

case of HT115(pL4440)-bacteria, Ampicillin and Tetracycline were added in a 1:1000 dilution before incubation. Accordingly, OP50-bacteria were spotted on medium plates after the incubation, but before adding to the plates, the medium with HT115(pL4440)-bacteria had to be diluted to a concentration of 0,9 (OD600=0,9).

The measurement of the optical density through a common spectrophotometer helped to calculate the volume of the bacteria and the LB-medium for a 0,9-dilution. Both Ampicillin and Tetracycline were added for the dilution again (each 1:1000 to the LB-medium). Subsequently, the medium NGM-plates were spotted with 250µl bacteria.

2.4 Preparation of compounds

The HT115(pL4440)-plates were spotted with the compounds Kahalalide F and Lutein (both dissolved in DMSO which was used as a control), in the following concentrations as presented in **Table 4**. The stock solutions of Kahalalide F and Lutein were prepared in DMSO and diluted with S-Basal for spotting 150µl of these dilutions on HT115(pL4440)-plates (see **Table 4**).

Table 4: Dilution of compounds for HT115(pL4440)-plates

compounds	C_f (µM)	Volume to spot (µl)	S-Basal to add(µl)	C_i (µM)	V_f (µl)	V_i (µl) compounds to spot)	n (plate)	For 3 plates	
								Comp. to spot	S-Basal
Kahalalide F	**0,5**	**150**	146,3	1000	7400	3,7	1	11,1	438,9
	0,5	150	149,26	5000	7400	0,74	1	2,22	447,78
Lutein	**100**	**150**	113	20000	7400	37	1	111	339

The HT115(pL4440)-plates were spotted with 150µl of either:

0,5µM Kahalalide F,
100µM Lutein or
0,25% DMSO.

After the plates were spotted with the diluted compounds, they were located on a rotator for one hour to dry. Finally, they were stored in a box at 4°C for later use (no longer than one week).

2.5 Juglone resistance assay

Juglone was used as an oxidative stressor for the stress resistance assay [101] to find out whether the compounds Kahalalide F and Lutein would protect the nematodes from oxidative stress. Both wild-type strain and *nlg-1* knock-out strain were used for this assay (see **Table 5**).

The juglone resistance assay was established similar to previous studies [99, 102]. In order to prepare juglone plates one day before starting the experiment, 200ml NGM were prepared in a bottle, autoclaved (121°C) and put in the warm bath (55°C). About 5ml LB-medium were pipetted in a falcon with 1µl OP50-bacteria and incubated at 37°C until the morning of the next day, when the juglone resistance assay was going to be carried out. Thus, on the day of the experiment 50mM juglone (Calbiochem) stock solution was prepared: the calculated and weighted juglone in the Eppendorf was diluted in DMSO and vortexed. Afterwards, the bottle with 200ml NGM was taken out of the water incubator and 50ml were pipetted in a new falcon, where 200µl of the juglone stock solution were added as well. The falcon which contained 200µM juglone in NGM medium finally, was vortexed and spotted in each new medium plate. Consequently, the juglone plates were covered with an aluminium foil and left at room temperature to dry for about one hour. After drying, the plates were spotted with 25µl OP50-bacteria as food source, which had been prepared before. The lids were left half open around the Bunsen burner, so that it could dry faster. After almost 30 minutes, the juglone plates were set to be utilized for the resistance assay. The reason why juglone plates were prepared on the same day was due to the fact that juglone has a propensity to lose its toxic potential clearly fast [102].

25 alive worms, which were grown up from egg to 1-day-old adult nematodes and were present on each HT115(pL4440)-plates with different compounds DMSO (control), Kahalalide F and Lutein (see 2.4), were transferred on the juglone plates (timepoint 0). After each hour, the number of alive, dead and censored worms were noted down on a sheet and later analysed via *OASIS 2* (https://sbi.postech.ac.kr/oasis2/) and *Microsoft Excel*. Censored

worms were defined as those worms that were crawling on the walls of the agar plates. Censored and dead worms were removed directly after each observation.

Table 5: Strains used for juglone resistance assay

Strains/Genotype	Source
N2 (wild-type)	Caenorhabditis Genetic Center (CGC)
VC228 ***nlg-1* (ok259) X**	Caenorhabditis Genetic Center (CGC)

2.6 Heat shock

Heat shock assays are further methods to examine stress resistance [101]. DAF-16/FOXO is a Forkhead transcription factor and suggested to be an important factor in stress resistance and longevity [103]. Because *daf-16* is mentioned to translocate to the nucleus under stress conditions [73], the *daf-16*::GFP strain (see **Table 6**) was examined upon heat shock to observe the effects of Kahalalide F and Lutein on localization of DAF-16.

Table 6: Reporter strain *daf-16::*GFP used for heat shock assay

Reporter strain/Genotype	Name	Source
***daf-16*::GFP** **TJ356 (zIs356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]**	FOXO family of transcription factors	Caenorhabditis Genetics Center (CGC) [73]

About 30 to 40 worms were grown on HT115(pL4440)-plates, containing either DMSO, Kahalalide F or Lutein for each heat treatment on different plates, as described previously (see 2.4). For each heat shock duration (0min, 15min, 30min, 60min) the 1-day-old adult nematodes on the plates, each containing different compounds, were checked directly after heat treatment and after 2 hours-recovery (20°C). The plates were sealed with Parafilm during the heat shock treatment and placed in the incubator at 35°C without any rotation. At the defined timepoints, the particular plates were examined under a microscope. Accordingly, the slides were prepared before: on each slide 13µl of 15mM NaN_3 were spotted and used as anaesthetics for the nematodes [104]. Between 15 to 20 worms from each petri plate were transferred into the anaesthetics on each slide and covered with a glass

cover cautiously. Using a fluorescence microscope with 25x-magnification the images of the nematodes expressing GFP were taken immediately. In addition to that, representative images of worms treated for one hour with heat treatment were captured with 100x magnification as well. Through the programme *ImageJ*, the microscopic images were used to evaluate the GFP localization. This type of categorization distinguishes between cytosolic, intermediate and nuclear localization of GFP [105, 106]. In this heat shock experiment, worms which revealed only fluorescent dots, indicating totally nuclear localization, were classified as "nuclear". Those which showed no nuclear fluorescence were defined as "cytosolic". Finally, those nematodes showing both GFP-signal in the body line and nuclei were assigned to the category "intermediate". The measured numbers were expressed in percentage, also because the total number of examined worms varied minimally among conditions. The statistical analysis and the graphs were prepared via *GraphPad Prism 9.*

2.7 Examination of GFP-expression in reporter strains (in basal conditions)

The examination of GFP-expression of three mitochondrial stress reporter genes (*cyp14a4*, *gst-4*, *hsp-6*) and of *nlg-1* were established in basal conditions first in order to test an effect of the two compounds (see **Table 7**). Three replicates were used for each strain. Only for *cyp14a4* four replicates were used.

Table 7: reporter strains marked with GFP

Reporter strains /Genotype	Name	Source
cyp14a4 (cyp-14A4p::gfp::cyp-14A4 3'UTR)	Cytochrome P450 family	Gary Ruvkun Lab [98]
gst-4 CL2166(dvIs19[pAF15(gst-4::GFP::NLS)) III)	Glutathione S-transferase 4	Caenorhabditis Genetic Center (CGC)
hsp-6 SJ4100 (zcIs13[hsp-6::GFP])	Heat shock protein	Caenorhabditis Genetic Center (CGC)
nlg-1 vjIs48[Pnlg-1::gfp]I	Neuroligin 1	Derek Sieburth Lab [99]

The 1-day-old adult worms grown on plates that contained either DMSO (control), Kahalalide F or Lutein, were investigated: 20 nematodes of each condition were relocated on a slide with 13µl sodium azide (15mM NaN_3), covered with a glass cover and imaged under a Zeiss Imager M2 fluorescence microscope (Carl Zeiss AG, Oberkochen, GER) microscope. Images of *cyp14a4-, gst-4-* and *hsp-6* strain were taken in 25x magnification, images of *nlg-1::GFP* strain in 400x magnification. Damaged worms were excluded for the further analysis. Then the GFP-intensity was scored through *CellProfiler* programme and all calculations were copied in a *Microsoft Excel* file. Finally, the values of the integrated intensity of GFP were copied in *GraphPad Prism 9* and were later normalized for the analysis of the pool of each tested strain. The analysis of *nlg-1* differed, because instead of analysing the GFP-expression in the whole worm, only a part of the ventral nerve cord in the worm's tail was considered. The images were opened in *ImageJ*: the contrast was enhanced (saturated pixels 0,3%), a rectangle was placed among the area of the ventral cord. The empty ("black") background was also scored in order to measure the *CTCF (Corrected total cell fluorescence)* for each worm in *Microsoft Excel:*

CTCF= Integrated Density – (area of selection * mean fluorescence of background)

2.8 Locomotion assay

Previous investigations have reported a decline in movement, expressed in a reduction of body bends per time (f. ex. 20 seconds) in ageing worms [38]. In order to examine whether a compound such as Kahalalide F had effects on motility during ageing and to find out whether *nlg-1* played a role in motility during ageing, both the wild-type strain N2 and the *nlg-1* knock-out strain were used for the locomotion assay. In contrast to the *nlg-1* knock-out strain utilized for the juglone resistance assay, a different *nlg-1* knock-out strain was used here as a control line which was marked with GFP (see **Table 8**).

Table 8: *nlg-1* knock-out strain marked with GFP

Strain	Properties	Source
nlg-1 (ok259) X; crrEx4 [pPD95.77;pDD04 Neo^R (Pmyo-2::GFP)]	*nlg-1* knock-out marked with GFP	[100]

As mentioned in the previous experiments, nematodes laid eggs on HT115(pL4440)-plates, which contained either DMSO (control) or Kahalalide F. Consequently, they were removed after egg-laying. For this assay, one aspect was considered to be very important, namely, to have an adequate number of worms for each condition. Therefore, each condition was prepared on two petri plates. For counting the body bends, normal standard NGM-plates were prepared without any bacteria, because food source would have led to altered locomotory behaviour, as mentioned in previous studies [107]. Moreover, 10 worms at the age of 1-day-old adulthood of each condition were picked through a platinum wire cautiously and transferred on those NGM-plates without bacterial lawn. After one minute, the number of body bends of each worm was counted during 20 seconds by eye via a LeicaMZ10F modular stereomicroscope. To avoid scoring the locomotion of one worm more than once, each worm was removed from the plate after counting its body bends. The same method of counting the number of body bends was carried out at the next time points (5-day-old adult and 8-day-old adult worms). In this experiment 1-day-old adult nematodes were transferred on new HTT15(pL4440)-plates with their previously chosen compounds (DMSO and Kahalalide F) and this method was carried out every day, to avoid starvation and to examine the effect of treatment during ageing. Besides, one further reason was also to avoid contamination with progeny. The number of body bends per 20 seconds was multiplied by factor 3 for each worm, to obtain the value per minute.

2.9 Statistical analysis

For the statistical analysis of the juglone resistance assay *OASIS 2* (https://sbi.postech.ac.kr/oasis2/) and *Microsoft Excel* were used. The other experiments were analysed via the software *GraphPad Prism 9, One-way ANOVA* and Bonferroni's *post hoc* test. For the examination of GFP-expression of *nlg-1* (see 2.7) the values of the *CTCF* of each worm (ventral nerve cord) were inserted in *GraphPad Prism 9* and after normalizing the values, they were pooled and analysed via *One-way ANOVA* and Bonferroni's *post hoc* test. The Locomotion assay was repeated three times. Afterwards the values were normalized via *GraphPad Prism 9* and analysed through *One-way ANOVA* followed by Bonferroni's *post hoc* test.

The statistical significance was classified as *p =0,01 to 0,05, **p= 0,001 to 0,01, ***p= 0,0001 to 0,001 and ****p<0,0001. The data were from at least 3 replicas for each experiment and were shown as mean ±SEM.

3 Results

3.1 Kahalalide F and Lutein promote resistance against oxidative stress

Previous experiments in the lab have identified natural compounds extending lifespan possibly working through mitochondrial hormesis [90].

Here, the first question we wanted to answer was whether Kahalalide F and Lutein would have protective effects against oxidative stress on wild-type worms. A better oxidative stress resistance is indeed associated with lifespan extension [62], also promoted by mild mitochondrial stress [67].

To examine the oxidative stress resistance, wild-type worms (N2) were grown from egg to 1-day-old adult worms on agar plates, spotted with either DMSO (control), Kahalalide F or Lutein and were finally transferred on plates containing the pro-oxidant juglone. The number of survived and dead worms was recorded. Those worms moving or lying on the wall of the plates were censored, that means they were removed directly from the juglone plates. I found out that N2 worms grown on Kahalalide F and Lutein show a higher survival to oxidative stress than those on control condition (DMSO) (**Fig. 3.1**) and animals grown on Kahalalide F presented the highest survival rate. After 5 hours treatment with juglone, less than 10% of DMSO-treated animals were still alive while around 20% of Lutein-treated and 40% of Kahalalide F-treated worms were still alive.

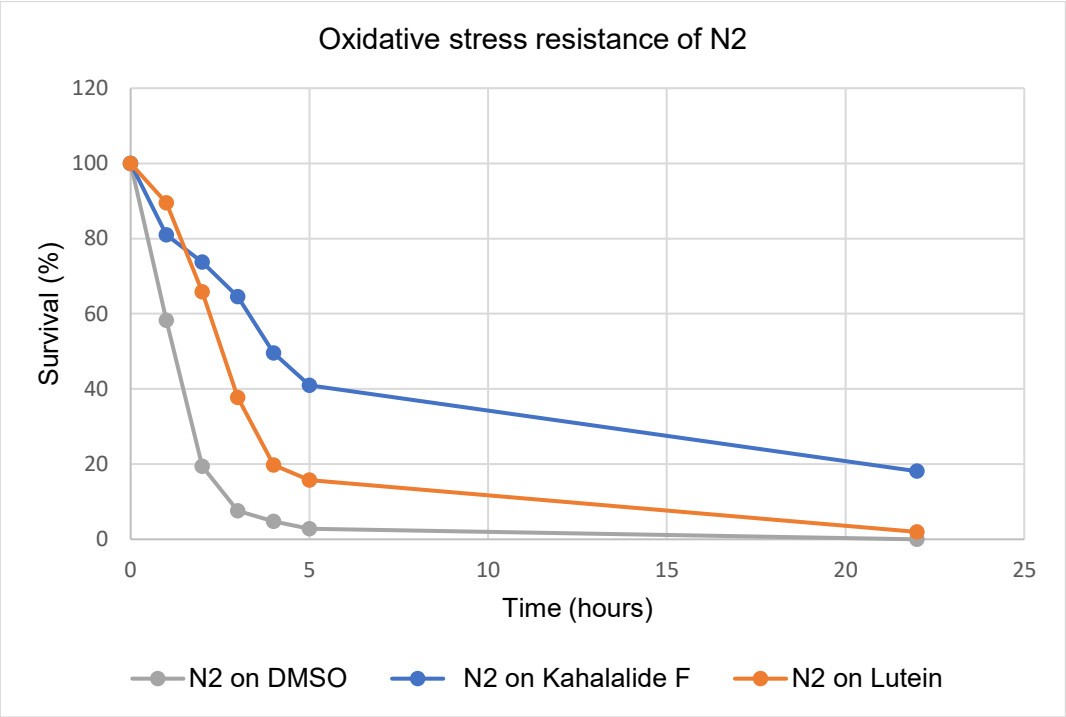

Fig. 3.1 Survival of N2 due to oxidative stress and pre-treatment with compounds
The results show a pool of three replicates. The lines depict the survival (%) of N2 strain at the age of 1-day-old adulthood with the particular compound treatment (DMSO, Kahalalide F, Lutein) during juglone exposure time (hours). Those worms grown on control condition (DMSO) shows the lowest survival in contrast to those treated with Lutein (****, corr.P=0) and Kahalalide F (****, corr.P=0). N2 raised on Kahalalide F reveals the highest survival on juglone compared to worms on Lutein (****, corr.P=0,000033). Statistical analysis of results via https://sbi.postech.ac.kr/oasis2/ and *Microsoft Excel* (2021).

The results are statistically significant and highlight that Kahalalide F and Lutein increase oxidative stress resistance of wild-type worms. Here, the increased oxidative stress resistance showed a protective impact on the survival of worms exposed to oxidative stress. So, since certain genes, such as *daf-16*, are known to play a role in stress resistance and longevity [73], the next aim was to examine whether the compounds affect the expression of genes such as *daf-16*.

3.2 Kahalalide F and Lutein do not alter *daf-16* nuclear localization upon heat shock

Resistance to thermal stress (between 30-35°C) often correlate with lifespan extension in *C. elegans* [101]. The Forkhead transcription factor DAF-16/FOXO is involved in stress resistance and ageing and is described to translocate to the nucleus under stress conditions, thus modulating the expression of a variety of downstream factors [73]. Translocation of DAF-16 to nucleus causes increased resistance to stress and extended lifespan [73]. Then I

asked, whether Kahalalide F and Lutein impact on *daf-16* nuclear translocation in basal conditions or after heat shock.

In order to answer this question, *daf-16*::GFP strain was treated from egg to 1-day-old adult nematodes with DMSO, Kahalalide F or Lutein and were exposed to heat shock (35°C) for different durations (0, 15, 30, 60 minutes). All conditions were observed directly via microscope and after a recovery of two hours. Then *daf-16*::GFP induction was quantified. In basal conditions (without heat shock) Kahalalide F and Lutein did not have any effect on the subcellular localization of *daf-16*, which remained cytosolic in all conditions, also two hours after the first observation (**Fig. 3.2** and **Fig. 3.3**).

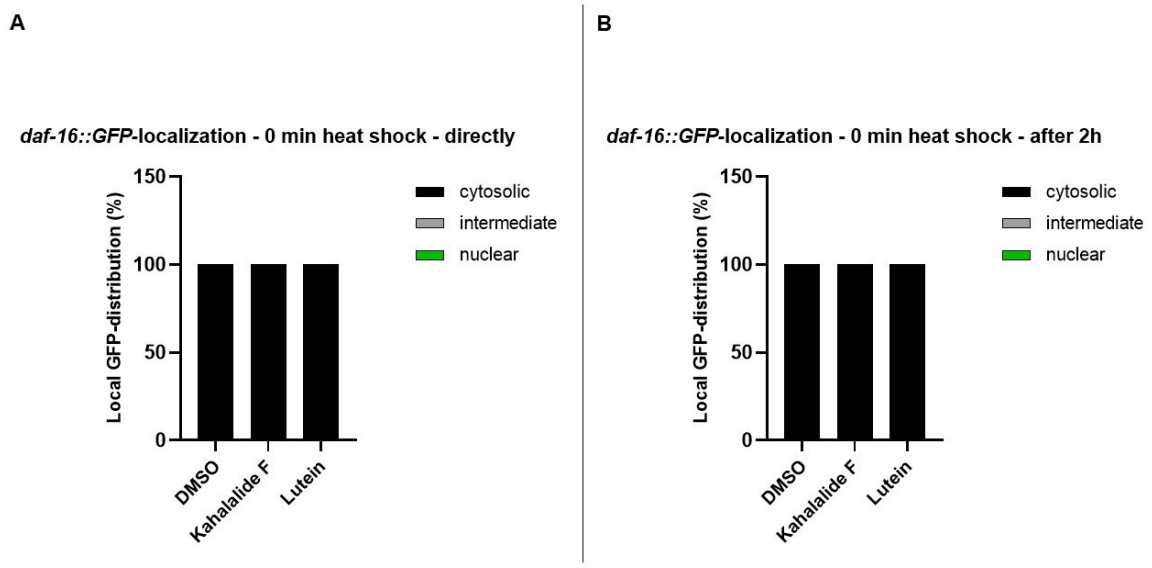

Fig. 3.2 Direct and 2h-later observation of *daf-16::GFP*-localization without heat shock. Graphs show *daf-16-GFP*-localization of worms after direct observation (A) and after 2h-later observation (B). The colours of the bars present the local *daf-16::GFP*-distribution (%) due to the whole worm's body, which is 100% cytosolic in all tested dietary conditions without heat shock. Data were analysed via *Graphpad Prism 9 (2021).*

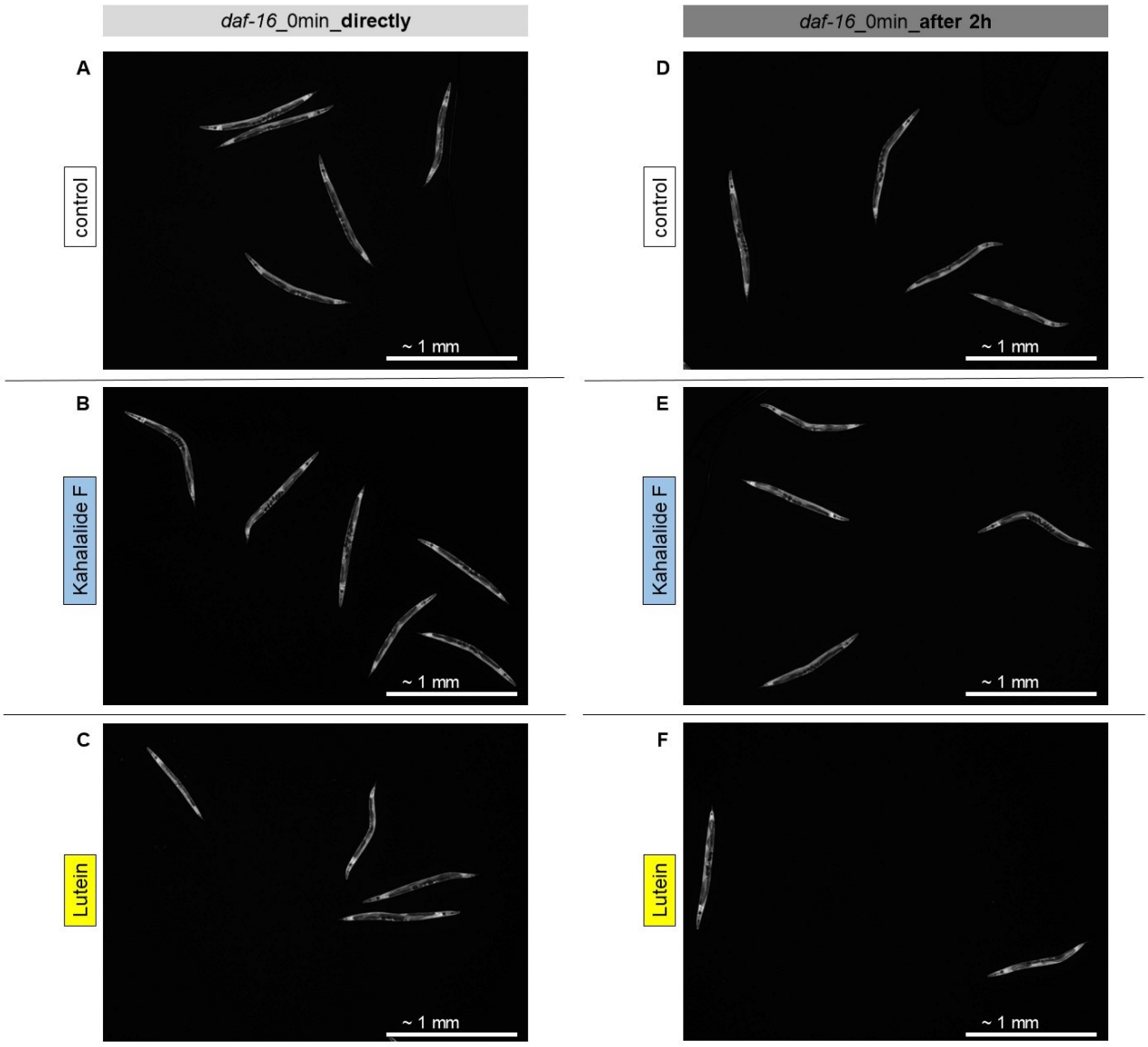

Fig. 3.3 Representative images of direct and 2h-later observation of *daf-16::GFP*-localization without heat shock.
(A)-(C) Representative microscopic images directly taken of *daf-16::GFP* strain treated with either DMSO (A), Kahalalide F (B) or Lutein (C). (D)-(F) Representative microscopic images of *daf-16::GFP* strain taken 2h later of worms treated with either DMSO (D), Kahalalide F (E) and Lutein (F). The white signal reveals the *daf-16::GFP* expression in worms and depicts cytosolic localization in all three conditions

After 15 minutes heat shock, all conditions presented worms with a beginning shift in *daf-16::GFP* localization from cytosolic towards intermediate right after heat shock. But there were no statistically significant differences between the conditions. Instead, after two hours recovery as expected, animals treated with F and Lutein showed complete cytosolic localization and the same result was observed in the control condition (see **Fig. 3.4** and **Fig. 3.5**).

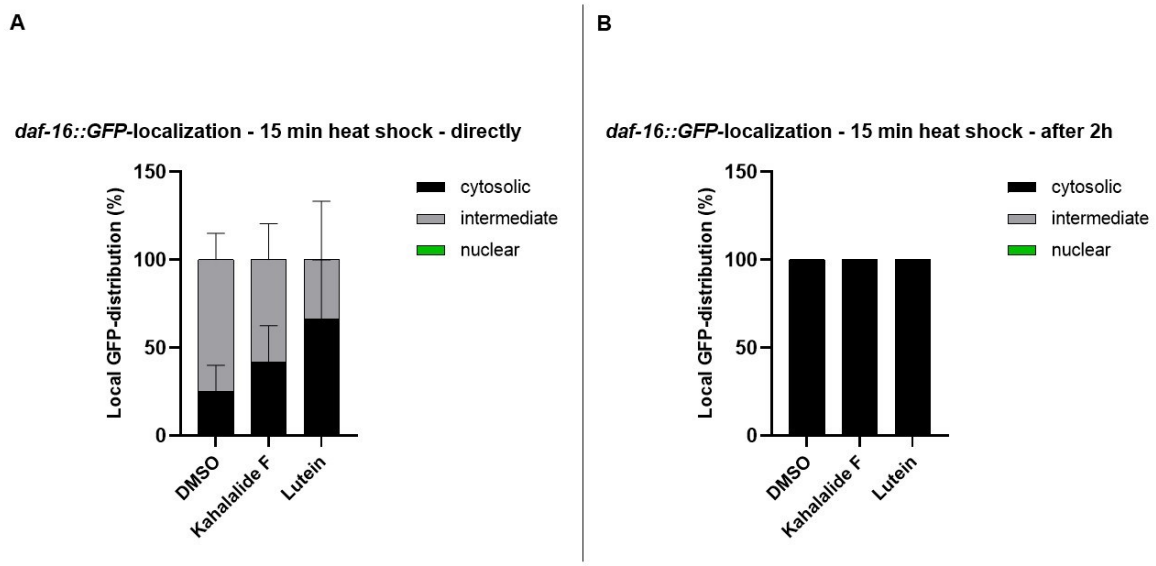

Fig. 3.4 Direct and 2h-later observation of *daf-16::GFP*-localization with 15 min heat shock. The colours of the bars present the local *daf-16::GFP*-distribution (%) ±SEM due to the whole worm's body, which is both cytosolic and intermediate in all conditions after direct observation of worms treated with 15min-long heat shock (A), but with different distributions, which are not significant (ns, P>0,05). (B) After 2 hours recovery, the local *daf-16::GFP*-distribution (%) due to the whole worm's body depicts a 100% cytosolic localization of *daf-16::GFP* in all dietary conditions. Data were analysed via *Graphpad Prism 9 (2021).*

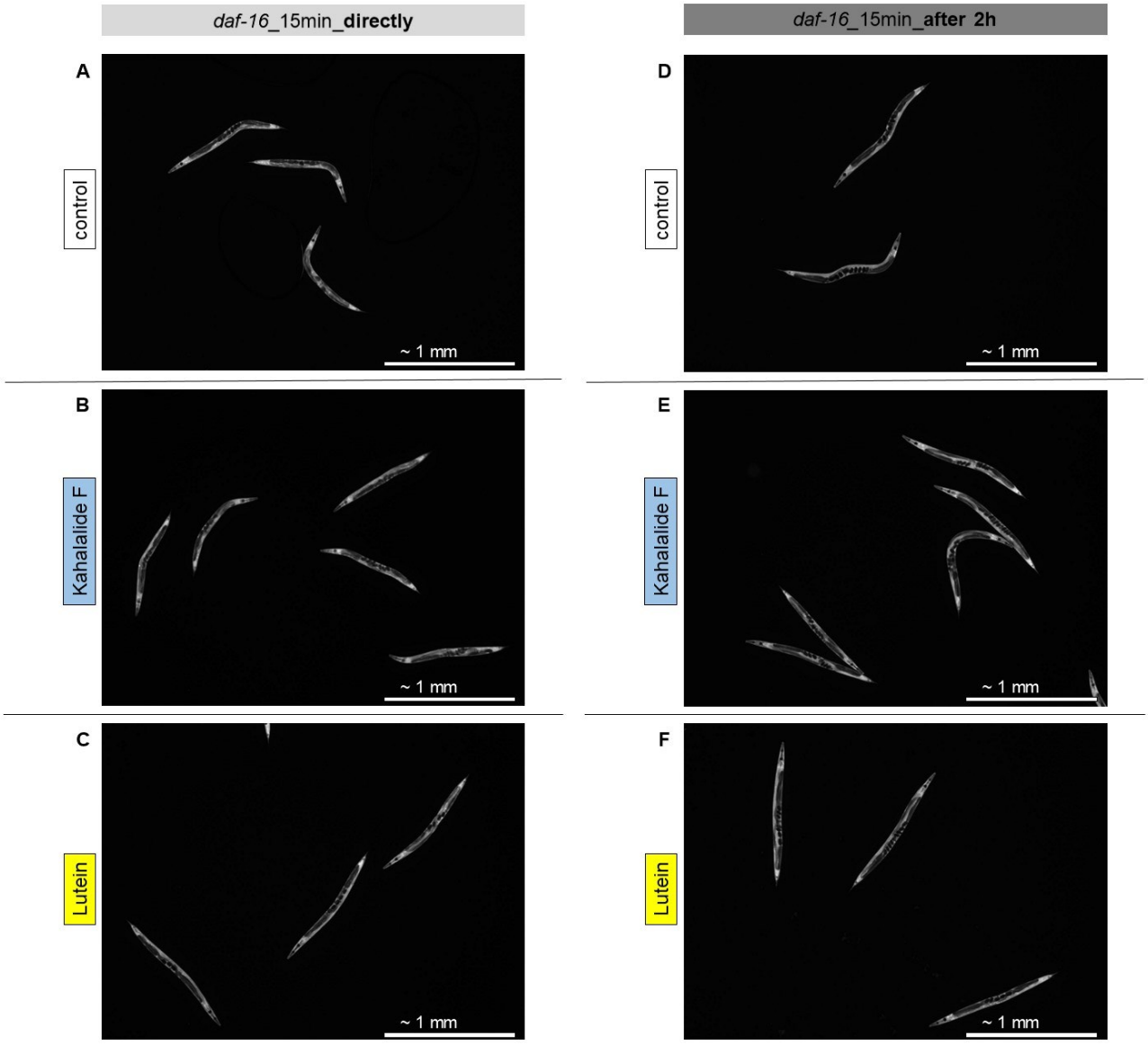

Fig. 3.5 Representative images of direct and 2h-later observation of *daf-16::GFP*-localization with 15 min heat shock.
(A)-(C) Representative microscopic images of *daf-16::GFP* strain taken directly after 15min heat shock, which have been treated with either DMSO (A), Kahalalide F (B) or Lutein (C). The white signal reveals the *daf-16::GFP* expression in worms and depicts cytosolic and intermediate localization in all three conditions (A)-(C). (D)-(F) Representative microscopic images after 2 hours recovery of *daf-16::GFP* strain treated with either DMSO (D), Kahalalide F (E) or Lutein (F). The white signal reveals the *daf-16::GFP* expression in worms and depicts 100% cytosolic localization in all three conditions after 2 hours recovery at 20°C (D)-(F).

After 30 minutes heat shock (the double time of thermal stress) contributed to a complete shift in intermediate localization of *daf-16*::GFP expression in all conditions (**Fig. 3.6** and **Fig. 3.7**). After 2 hours recovery, again cytosolic localization was observed, but the percentage of intermediate localization still predominated in all conditions, without any statistically significant differences.

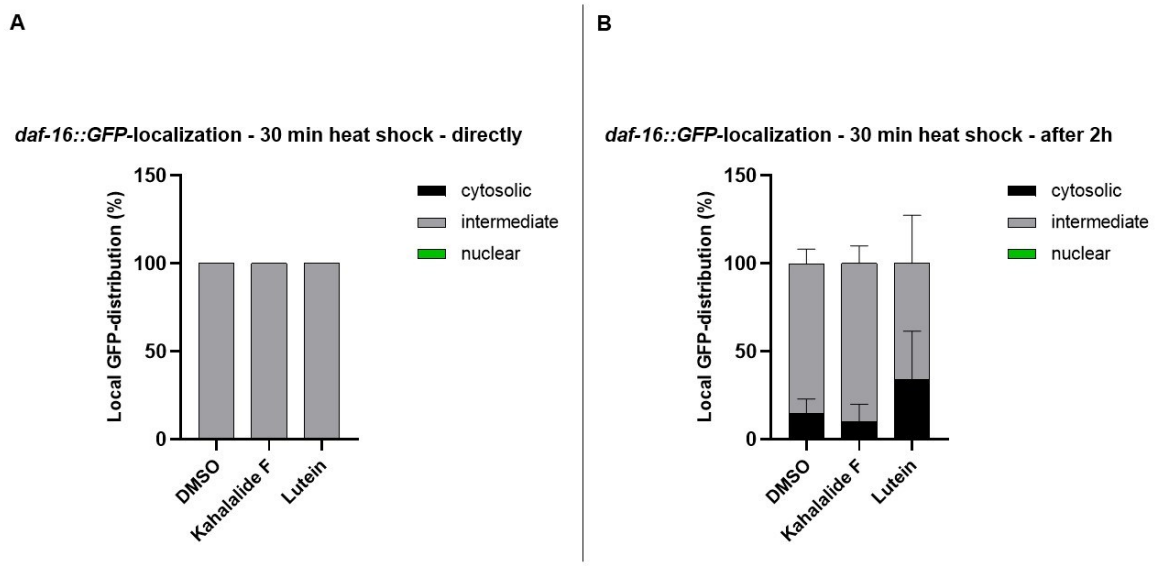

Fig. 3.6 Direct and 2h-later observation of *daf-16::GFP*-localization with 30 min heat shock.
(A)-(B) The colours of the bars present the local *daf-16::GFP*-distribution (%) due to the whole worm's body.
(A) A 100% intermediate localization of *daf-16::GFP* is shown in all dietary conditions after direct observation of 30min-long heat shock.
(B) After 2h recovery different local *daf-16::GFP*-distribution (%) ±SEM due to the whole worm's body. The local *daf-16::GFP* distribution is between 10-15% cytosolic and 85-90% intermediate in 1-day-old adult worms grown on either control or on Kahalalide F. Worms treated with Lutein show different local *daf-16::GFP* distribution: almost 34% cytosolic and approximately 66% intermediate *daf-16::GFP* distribution. Whereas worms grown on DMSO or Kahalalide F depicted less percentage of cytosolic distribution, but without significant differences between the three effects of compounds (ns, P>0,05). Data were analysed via *Graphpad Prism 9 (2021).*

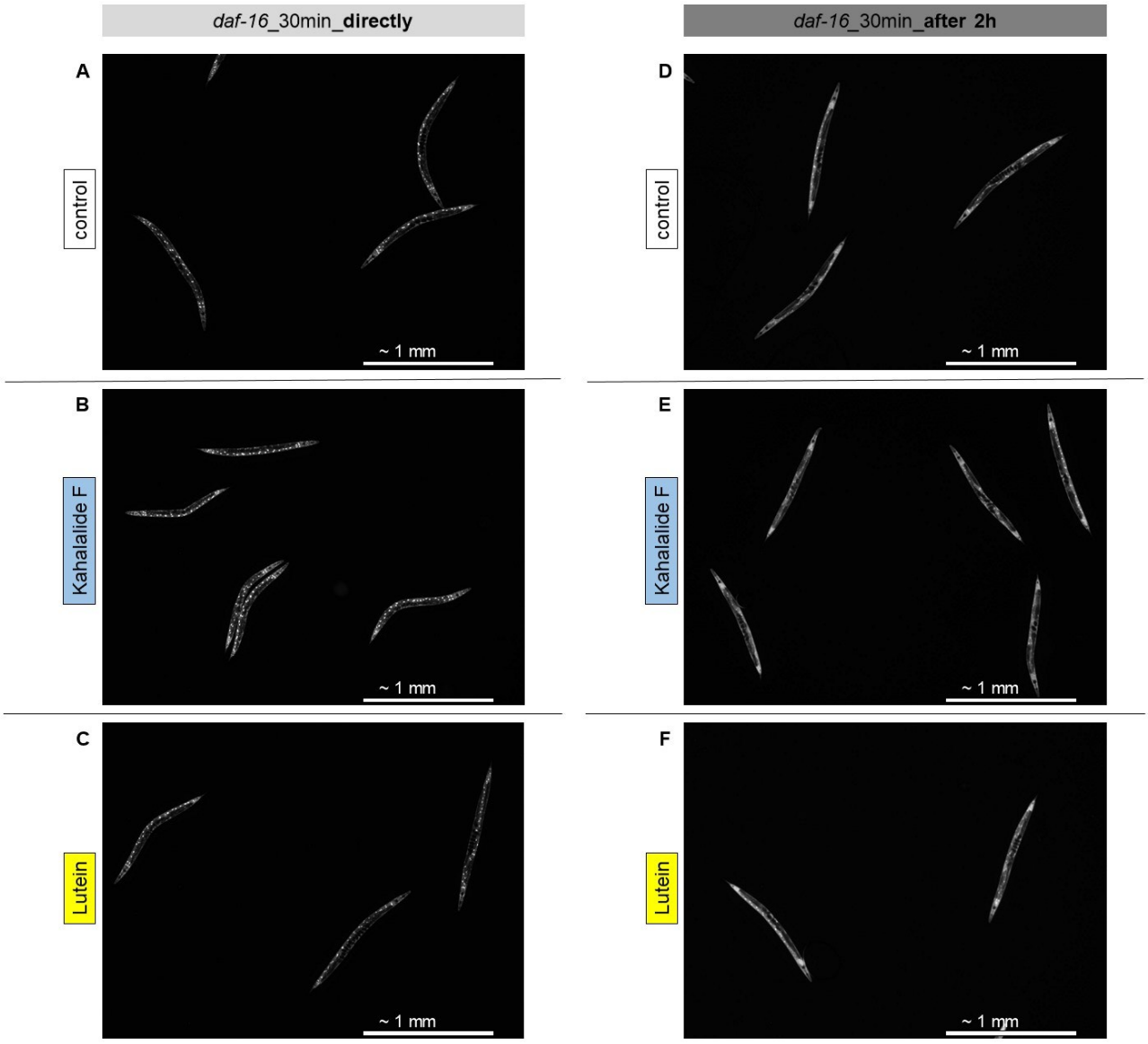

Fig. 3.7 Representative microscopic images of direct and 2h-later observation of *daf-16::GFP*-localization with 30 min heat shock.
(A)-(C) Representative microscopic images taken directly after heat shock of *daf-16::GFP* strain treated with either DMSO (A), Kahalalide F (B) or Lutein (C). The white signal reveals the *daf-16::GFP* expression in worms and depicts 100% intermediate localization in all three conditions treated at 35°C for 30min. (D)-(F) Representative microscopic images taken 2 hours after heat shock of *daf-16::GFP* strain treated with either DMSO (D), Kahalalide F (E) or Lutein (F). The white signal reveals the *daf-16::GFP* expression in worms and depicts both cytosolic and intermediate localization in all three conditions treated at 35°C for 30min and recovered after 2 hours.

All nematodes that received a 60 minutes-long heat shock treatment, showed complete nuclear *daf-16::GFP* localization and revealed complete intermediate localization of *daf-16* after two hours (see **Fig. 3.8** and **Fig. 3.9**). According to **Fig. 3.10**, images at 100x magnification are presented, depicting the distal part of the nematodes' body. The distal part of the nematodes' body reveals a complete nuclear localization of *daf-16::GFP* in all three

dietary conditions. Whereas images taken after the two hours-long recovery time show intermediate local shift of *daf-16::GFP* distribution in all worms.

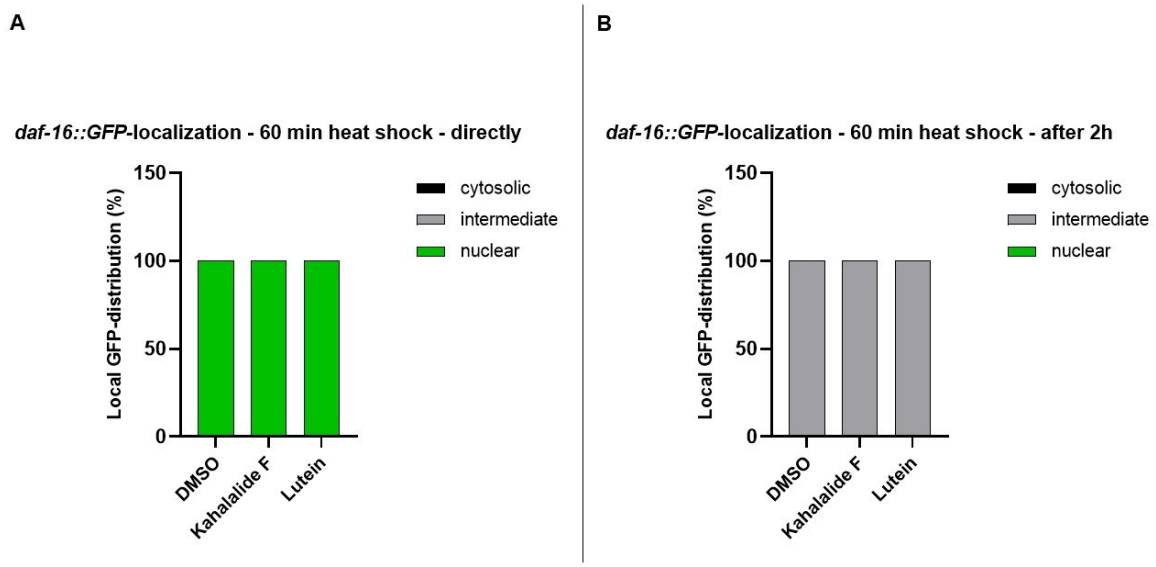

Fig. 3.8 Direct and 2h-later observation of *daf-16::GFP*-localization with 60 min heat shock.
(A)-(B) The colours of the bars present the different local *daf-16::GFP*-distribution (%) due to the whole worm's body.
(A) The local *daf-16::GFP* distribution is 100% nuclear in worms grown on DMSO (control), Kahalalide F as well as those worms treated with Lutein.
(B) The local *daf-16::GFP* distribution is 100% intermediate in worms grown on DMSO (control), Kahalalide F as well as those worms treated with Lutein. Data were analysed via *Graphpad Prism 9 (2021).*

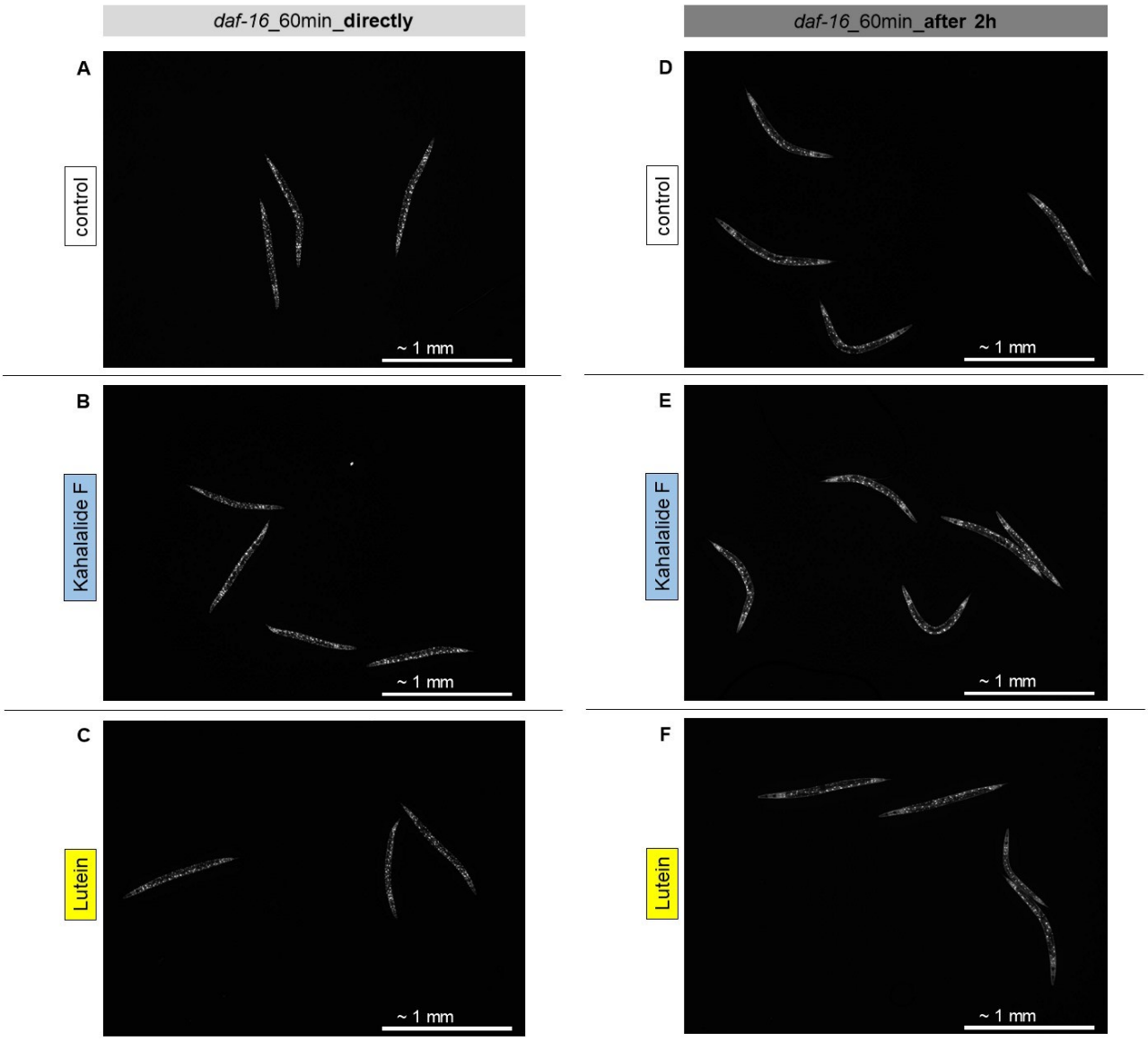

Fig. 3.9 Microscopic images of direct observation of *daf-16::GFP*-localization with 60 min heat shock .
(A)-(C) Representative microscopic images taken directly after heat shock (35°C for 60min) of *daf-16::GFP* strain treated with either DMSO (A), Kahalalide F (B) or Lutein (C). The white signal reveals the *daf-16::GFP* expression in worms and depicts 100% nuclear localization in all three conditions treated at 35°C for 60min and observed directly. (D)-(F) Representative microscopic images taken 2 hours after heat shock (35°C for 60min) of *daf-16::GFP* strain, treated with either DMSO (D), Kahalalide F (E) or Lutein (F). The white signal reveals the *daf-16::GFP* expression in worms and depicts intermediate localization in all three conditions after 2h recovery.

As shown in **Fig. 3.10**, the characteristic criteria manifesting intermediate *daf-16::GFP* localization after 2-hours-recovery are observed more clearly: on the one hand, the body line is depicted and reveals cytosolic distribution and on the other hand, the nuclear signals are shown, which are presented as white dots in the worms' body.

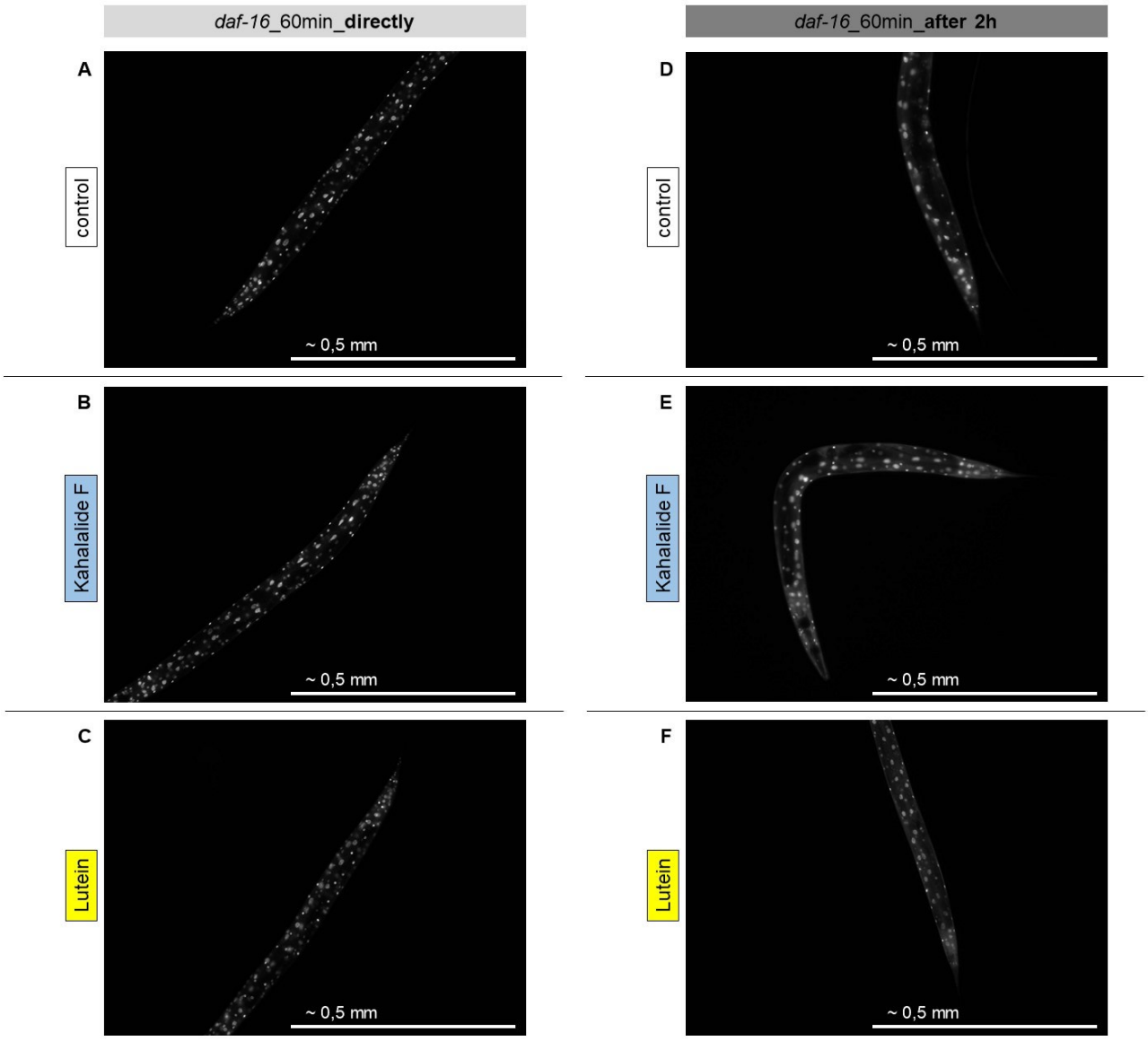

Fig. 3.10 Direct and 2h-later observation of *daf-16:GFP* with 60min heat shock in 100xmagnification.
(A)-(C) Representative microscopic images taken directly after heat shock (35°C for 60min) of distal body part of *daf-16::GFP* strain. The nematodes were treated with either DMSO (A), Kahalalide F (B) or Lutein (C). The white signal reveals the *daf-16::GFP* expression in worms and depicts 100% nuclear localization in all three conditions. (D)-(F) Representative microscopic images taken 2 hours after heat shock (35°C for 60min) of *daf-16::GFP* strain, treated with either DMSO (D), Kahalalide F (E) or Lutein (F). The white signal reveals the *daf-16::GFP* expression in worms and depicts intermediate localization in all three conditions. Both the body line revealing cytosolic distribution and the white dots distributed in the worms body with focus on the distal body part, indicate the criteria of an intermediate localization of *daf-16::GFP*.

To sum up, the results reveal no statistically significant impact of Kahalalide F and Lutein on nuclear translocation of *daf-16.* So, *daf-16* appears to be not involved in the impact of those compounds, since its activation is probably not affected by them. The only factor seen here that influences *daf-16* nuclear, intermediate or cytosolic translocation, is the heat shock duration without any major differences by dietary conditions such as Kahalalide F or Lutein.

3.3 Kahalalide F and Lutein do not affect the expression of different mitochondrial stress response genes (*cyp-14a4*, *gst-4*, *hsp-6*)

In order to find out whether Kahalalide F and Lutein activate certain mitochondrial stress reporter genes, three genes were chosen. The chosen genes showed increased expression under different treatments in previous works and these are: *cyp14a4* [98], *gst-4* and *hsp-6* [108]. The CYP-14A subfamily of cytochrome P450 genes has been reported to be involved in metabolism of particular xenobiotic compounds [109] and to be strongly activated in response to mitochondrial stress [98]. The GST-4 is an enzyme interacting in the detoxification metabolism [110] and it is expressed in a SKN-1-dependent manner [111]. SKN-1 (skinhead-1) [61] is a transcription factor activated in response to mitochondria [62] and oxidative stress [108]. The *hsp-6* belongs to the group of mitochondrial chaperone genes [112] normally activated in response to mitochondrial stress [108] and plays an important role in larval development [112]. These mitochondrial stress reporter genes *cyp-14a4*, *gst-4* and *hsp-6* were therefore used to examine the impact of Kahalalide F and Lutein on them.

Similar to the assays before, the *cyp-14a4::GFP*, *gst-4::GFP* and *hsp-6::GFP*-strains were grown from egg until 1-day-old adulthood on plates containing either DMSO, Kahalalide F or Lutein. Then these nematodes were examined due to their GFP-expression under the microscope. The images were analysed via *Cell Profiler* and *GraphPad Prism* through the *Bonferroni's post hoc test*.

The analysis shows that *cyp14a4::GFP* expression is almost identical in all worms treated with either DMSO, Kahalalide F and Lutein. According to the images, the gene expression can mainly be seen in the head of the nematodes (see **Fig. 3.11**). So, both compounds Kahalalide F and Lutein show no effect on *cyp-14a4* expression compared to the control condition.

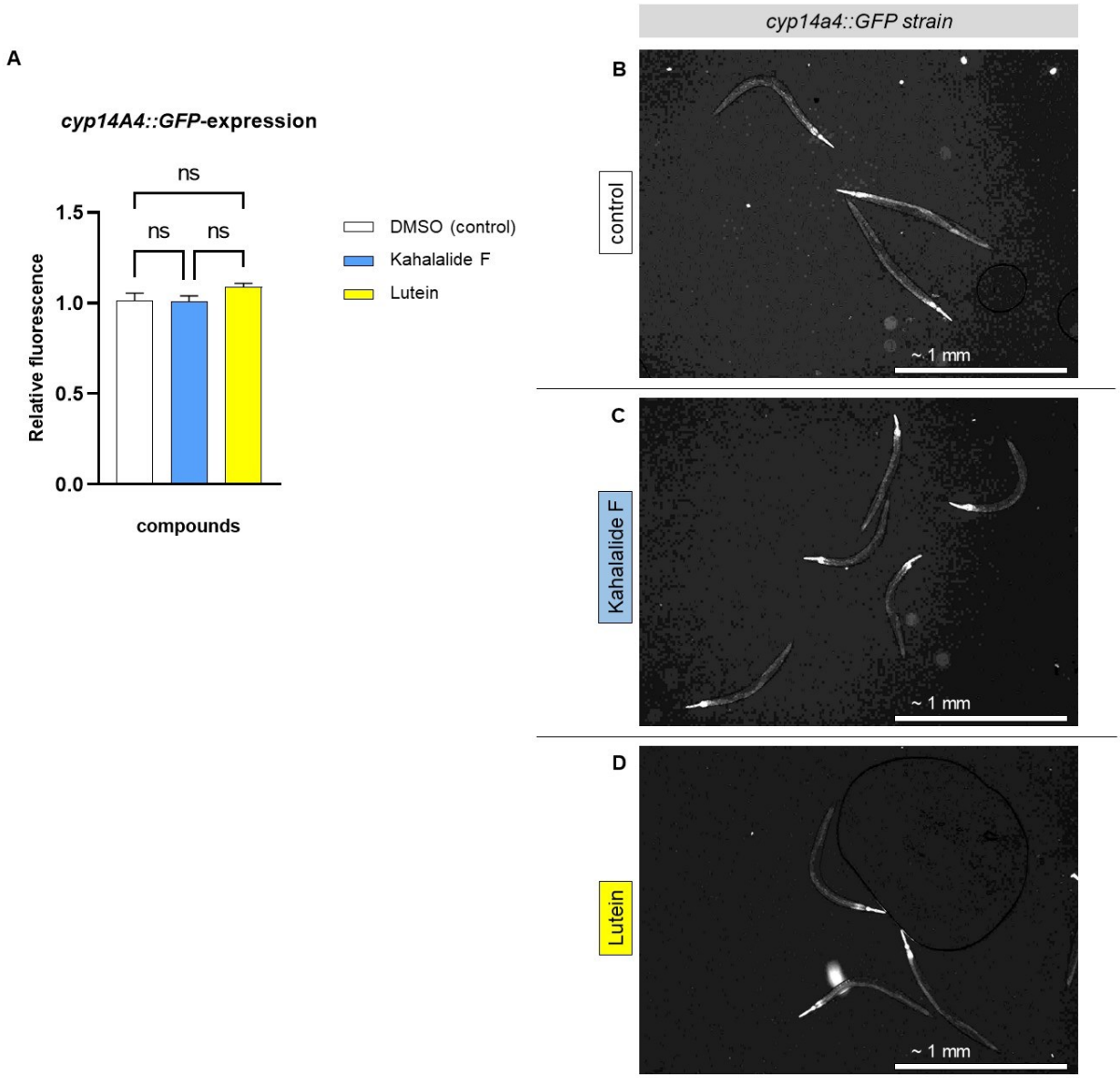

Fig. 3.11 *cyp14A4::GFP*-expression due to pre-treatment with compounds.
The bars depict the normalized GFP-intensity ±SEM in *cyp14A4*. There were no significant differences of GFP-intensity due to pre-treatment with DMSO (control), Kahalalide F or Lutein (ns, P>0,05). The values were calculated from four replicates and analysed through One-way ANOVA and *Bonferroni's post hoc test*. (B)-(D) Representative microscopic images taken of 1-day-old adult worms of *cyp14a4::GFP* strain, treated with either DMSO (B), Kahalalide F (C) or Lutein (D). The white signal reveals the *cyp14a4::GFP* expression in worms, which is localized mainly in the part of the worms' heads. These images shown here are depicted with an enhanced contrast in order to present the worms more clearly in this figure. Images were analysed via *CellProfiler, Microsoft Excel* and *Graphpad Prism 9 (2021).*

Similar to the results for *cyp14a4*::GFP expression, Kahalalide F and Lutein have no significant statistical impact on *gst-4*::GFP expression (see **Fig. 3.12**). There is a little higher *gst-4* induction demonstrated for worms treated with Kahalalide F and a little lower *gst-4*

expression in nematodes treated with Lutein, but none of these results are statistically significant. Therefore *gst-4* expression is also not affected by those compounds.

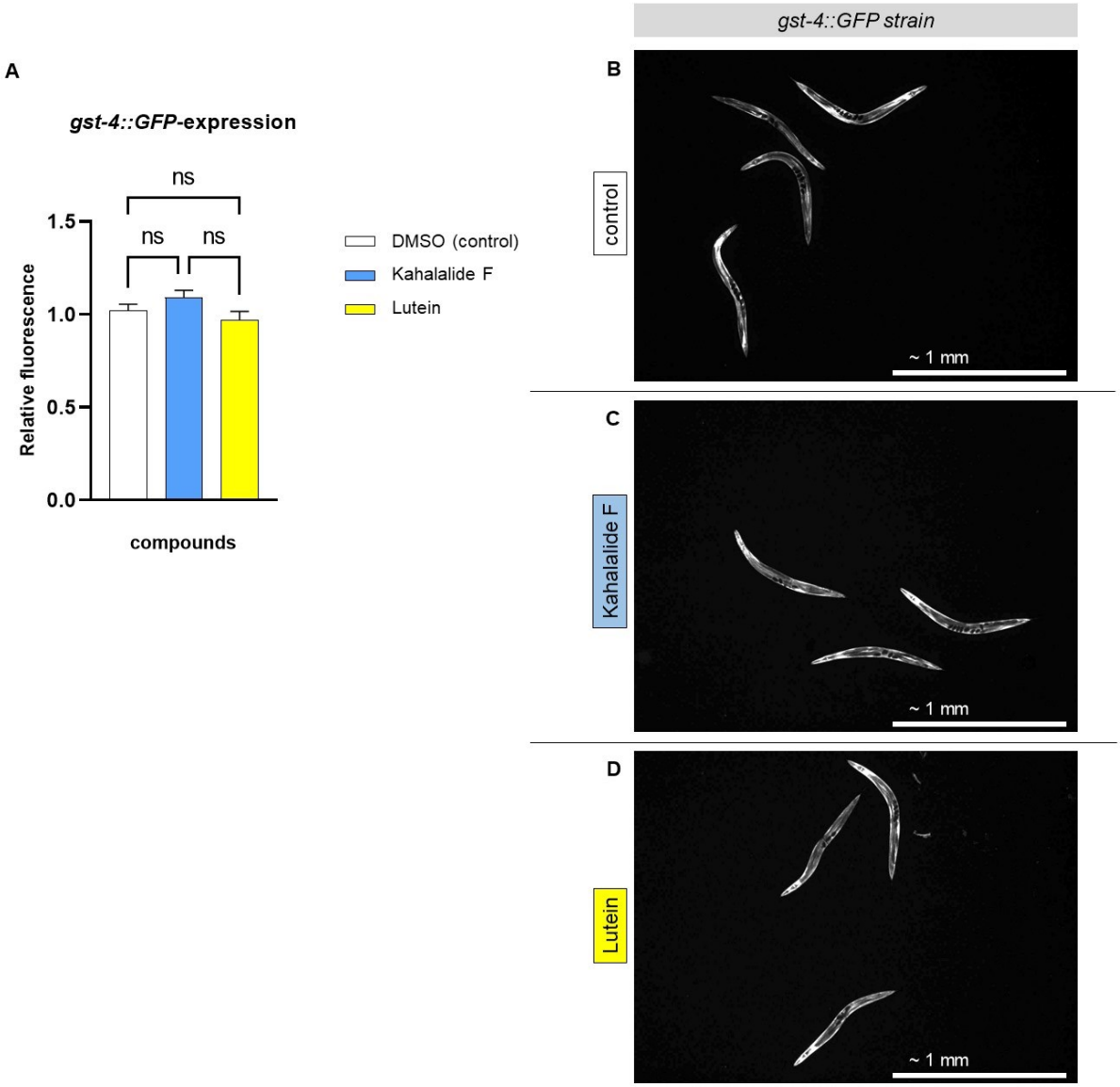

Fig. 3.12 *gst-4::GFP*- expression due to pre-treatment with compounds.
The bars depict the normalized GFP-intensity ±SEM in *gst-4*. There were no significant differences of GFP-intensity due to pre-treatment with DMSO (control), Kahalalide F or Lutein (ns, P>0,05). The values were calculated from three replicates and analysed through One-way ANOVA and *Bonferroni's post hoc test*. (B)-(D) Representative microscopic images taken of 1-day-old adult worms of *gst-4::GFP* strain, treated with either DMSO (B), Kahalalide F (C) or Lutein (D). The white signal reveals the *gst-4::GFP* expression in worms. These images shown here are depicted with an enhanced contrast in order to present the worms more clearly in this figure. Images were analysed via *CellProfiler, Microsoft Excel* and *Graphpad Prism 9 (2021).*

The third mitochondrial stress response gene, namely *hsp-6* which was examined due to its expression, also appears to be not affected in its induction through the compounds (see **Fig.**

3.13). Again Kahalalide F contributed to a little higher expression, but still without statistical significance.

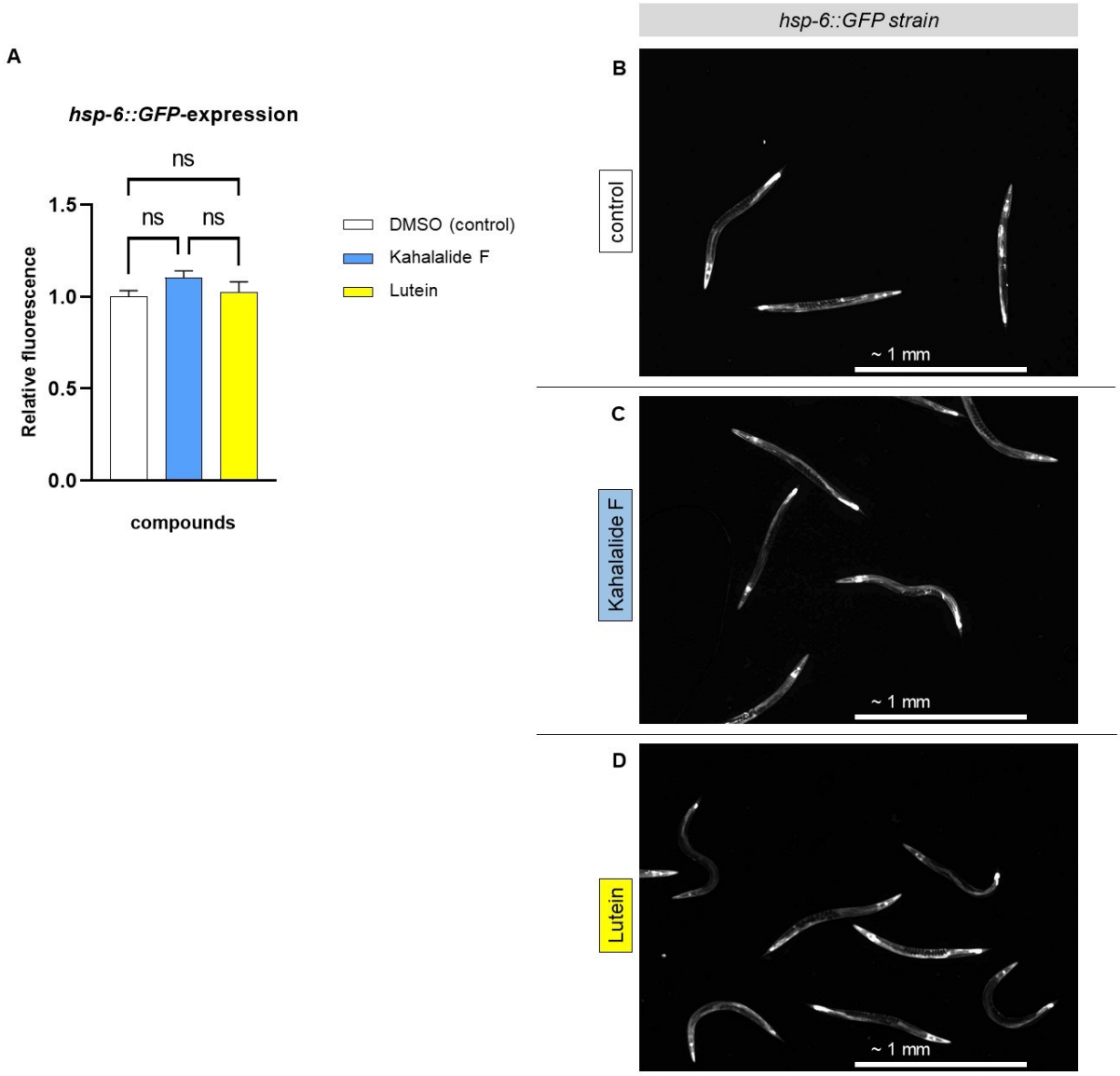

Fig. 3.13 *hsp-6::GFP*-expression due to pre-treatment with compounds
The bars depict the normalized GFP-intensity ±SEM in *hsp-6*. There were no significant differences of GFP-intensity due to pre-treatment with DMSO (control), Kahalalide F or Lutein (ns, P>0,05). The values were calculated from three replicates and analysed through One-way ANOVA and *Bonferroni's post hoc test*. (B)-(D) Representative microscopic images taken of 1-day-old adult worms of *hsp-6::GFP* strain, treated with either DMSO (B), Kahalalide F (C) or Lutein (D). The white signal reveals the *hsp-6::GFP* expression in worms. These images shown here are depicted with an enhanced contrast in order to present the worms more clearly in this figure. Images were analysed via *CellProfiler, Microsoft Excel* and *Graphpad Prism 9 (2021).*

According to these results gained by statistical analysis with *Bonferroni's post hoc test*, neither Kahalalide F nor Lutein had statistically significant impacts on the expression of the

mitochondrial stress reporter genes *cyp-14a4, gst-4* and *hsp-6.* Thus, other genes might be involved in the protective impacts of these two compounds.

3.4 *nlg-1* induction is causally involved in Kahalalide F promoted motility

The oxidative stress resistance assay showed that Kahalalide F and Lutein have an advantageous effect on wild-type worms. In search of genes which could mediate the beneficial effects of the two compounds we turned to a previous study [90] of the lab, where one gene has already been shown to be a target of Lutein, namely *nlg-1*. In that study *nlg-1* was overexpressed in a mitochondrial complex I-linked disease model and Lutein decreased *nlg-1* overexpression. When Lutein decreased *nlg-1* overexpression, the synaptic defect was reduced and this phenomenon protected from several neuronal deficits [90]. *nlg-1* has been also reported to show a higher expression in presence of mitochondrial stressors such as juglone [99]. In healthy worms Lutein did not alter *nlg-1* expression when used at 1µM [90]. We therefore wondered whether, according to a hormetic effect, pro-longevity doses (100µM) of Lutein can actually affect *nlg-1* expression.

*Nlg-1::*GFP worms received different diets (DMSO, Kahalalide F and Lutein) from egg to 1-day-old adulthood and were examined via fluorescence microscope. As shown in **Fig. 3.14**, Kahalalide F has a great impact on the expression of *nlg-1* and causes an increased *nlg-1*-induction. Additionally, Lutein induces the *nlg-1* expression as well, but it is statistically not significant according to *Bonferroni's post hoc test*.

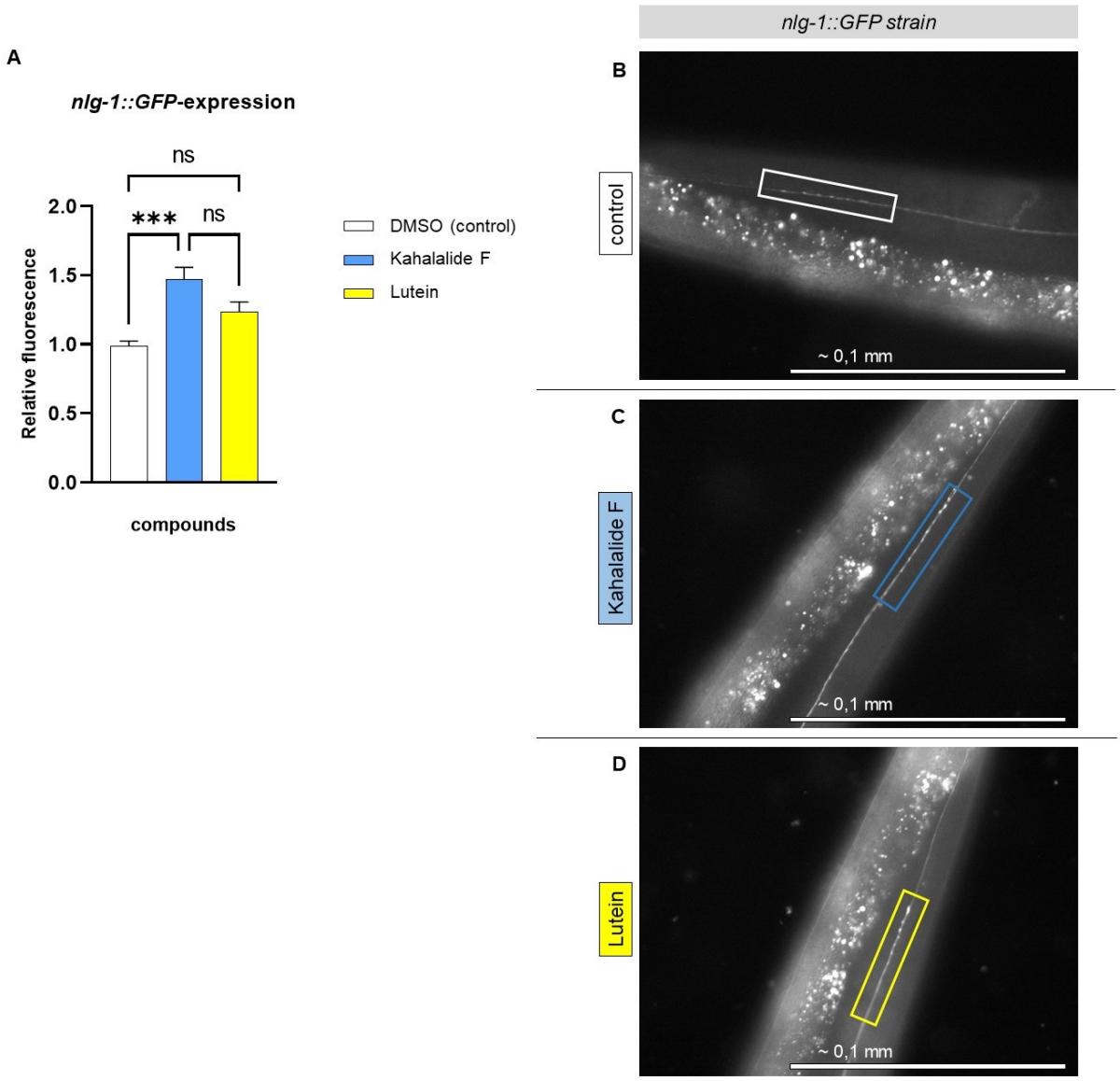

Fig. 3.14 *nlg-1::GFP*-expression due to pre-treatment with compounds.
A) The bars depict the normalized GFP-intensity ±SEM in *nlg-1*. The *nlg-1*::GFP expression was significantly higher in worms grown on Kahalalide F (***P=0,0003). GFP-intensity measured in worms treated with Lutein were not significantly different than the control (ns, P>0,05) The values were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test. (B-D) Representative microscopic images of the distal body part (tail region) of the 1-day-old adult nematodes of *nlg-1::GFP* strain, treated with either DMSO (B), Kahalalide F (C) or Lutein (D). The white signal reveals the *nlg-1* expression. The rectangles indicate the parts of the ventral nerve cord that have been analysed. These images shown here are depicted with an enhanced contrast in order to present the worms more clearly. Images were analysed via *ImageJ, Microsoft Excel* and *Graphpad Prism 9 (2021).*

Afterwards, another oxidative stress assay was established in order to find out whether *nlg-1* is necessary for those compounds to promote stress resistance in *C. elegans*. Therefore, this time *nlg-1* knock-out worms and wild-type worms were treated with the compounds

(from egg to 1-day-old adulthood) and their survival on oxidative stress was examined (similar to previous experiment).

Surprisingly, after 5 hours, less than 10% of N2 and more than 60% of *nlg-1* knock-out worms on control condition survived, which means that *nlg-1* knock-out strain has an overall higher stress resistance ability compared to the wild-type worms in this experiment (see **Fig. 3.15**). A previous study with the same *nlg-1* knock-out strain showed a different phenomenon: in that study *nlg-1* mutants were shown to have a higher sensitivity towards juglone but OP50 was used as a bacterial food source instead of HT115(pl4440) [99].

Both Kahalalide F and Lutein promote the survival of the wild-type and *nlg-1* knock-out strain upon juglone exposure, but the impact of the compounds on oxidative stress resistance of the wild-type worms has a higher statistical significance than the impact on *nlg-1* knock-out mutants. In addition to that, Kahalalide F appears to improve oxidative stress resistance in wild-type nematodes more than Lutein. My results indicate that while *nlg-1* deficiency per se promote resistance to juglone (at least on this bacterial diet), its presence and correct functioning might in part favour Kahalalide F and Lutein protection against oxidative stress resistance.

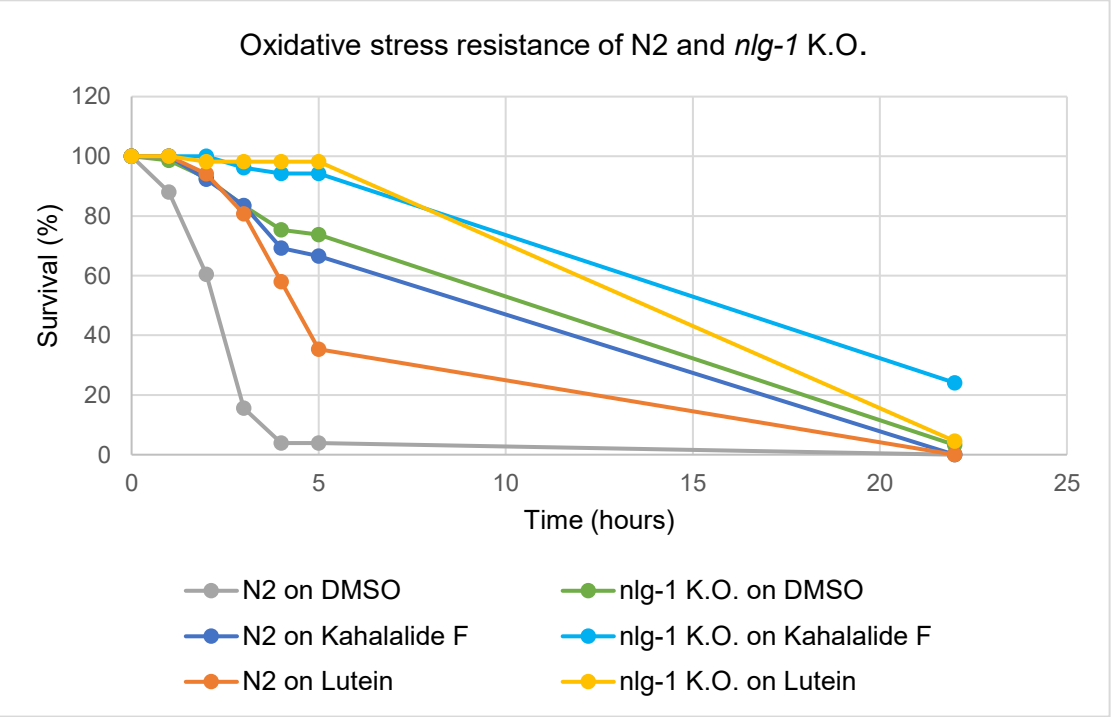

Fig. 3.15 Survival of N2 and *nlg-1* knock-out strain upon oxidative stress and pre-treatment with compounds.
The results show a pool of three replicates. The lines depict the survival (%) of each strain at the age of 1-day-old adulthood (N2 and *nlg-1* knock-out) with the particular compound pre-treatment (DMSO, Kahalalide F, Lutein) during juglone exposure time (hours). Oxidative stress resistance of

N2 is significantly lower than that of *nlg-1* knock-out due to DMSO as the control condition (****, corr.P=0). The higher oxidative stress resistance of *nlg-1* knock-out is mirrored in all conditions compared to N2. But N2 is affected with a higher significance by Kahalalide F (****, corr.P=0) and Lutein (****, corr.P=0) due to DMSO than *nlg-1* knock-out, in which Kahalalide F (***,corr.P=0,0003) and Lutein (**,corr.P=0,0056) cause a high oxidative stress resistance in comparison to *nlg-1* knock-out, but not as much as they affect N2. Statistical analysis of results via https://sbi.postech.ac.kr/oasis2/ and *Microsoft Excel* (2021).

The next question we wanted to answer was whether Kahalalide F might have beneficial effects on motility at different ages, since it is known that locomotion deteriorates during ageing [38]. Another aim was to examine how far its impact on locomotion might depend on *nlg-1*. In order to find this out, 1-day-old, 5-day-old and 8-day-old adult worms from both wild-type strain and *nlg-1* knock-out strain, treated with DMSO or Kahalalide F from egg until the age of 8-day-old adult nematodes, were investigated.

As shown in **Fig. 3.16**, the motility/locomotion in 1-day-old wild-type strain treated with Kahalalide F is statistically significantly higher compared to *nlg-1* knock-out strain treated with Kahalalide F. Kahalalide shows a slightly greater impact on wild-type worms that on the *nlg-1* knock-out worms. However, the compounds do not have a significant effect within each strain at the age of 1-day-old adulthood. So, at the age of 1-day-old adulthood there is no clear effect seen.

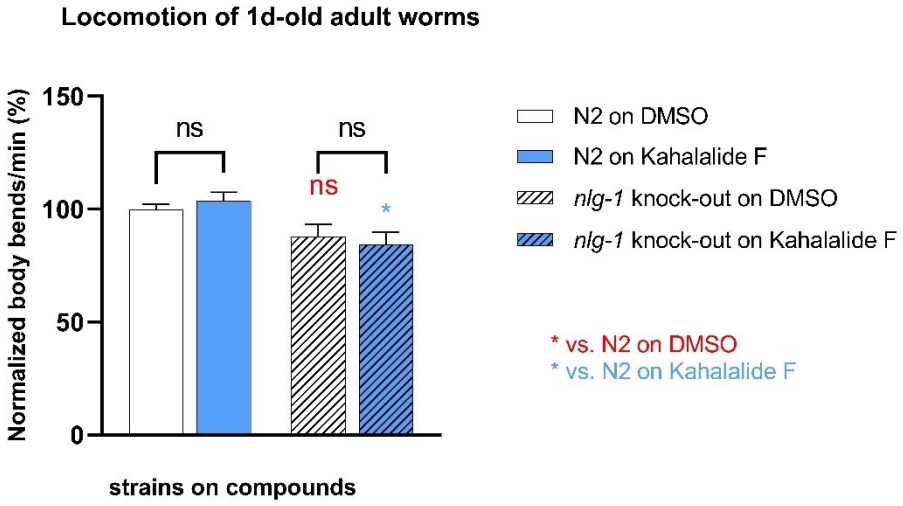

Fig. 3.16 Locomotion of 1-day-old adult N2 and *nlg-1* knock-out worms.
The bars depict the normalized number of body bends/min ±SEM in 1-day-old adult wild-type (N2) and *nlg-1* knock-out worms each in two different dietary treatment. The locomotory speed of N2 raised on Kahalalide F is significantly higher (*P<0,1). Other conditions do not reveal any remarkable differences (ns, P>0,05). The data were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test (2021).

So, is there a different or major impact at a later age? According to **Fig. 3.17**, those 5-day-old adult N2 worms that were treated with Kahalalide F, showed a statistically significant higher locomotion, namely 35% faster than those N2 worms grown on DMSO. The Kahalalide F-treated N2 had a 50% rise in motility in contrast to *nlg-1* knock-out mutants grown on Kahalalide F. Within the *nlg-1* knock-out strain there is no effect seen: *nlg-1* knock-out worms grown either on DMSO or Kahalalide F reveal to have almost the same locomotory speed. Indeed, there is a slightly higher motility observed in *nlg-1* knock-out mutants treated with Kahalalide F than those raised on DMSO, but it is not statistically significant.

The higher age of the worms also reveals another aspect: The 5-day-old adult *nlg-1* knock-out mutants on DMSO were very slower than 5-day-old adult wild-type worms on DMSO.

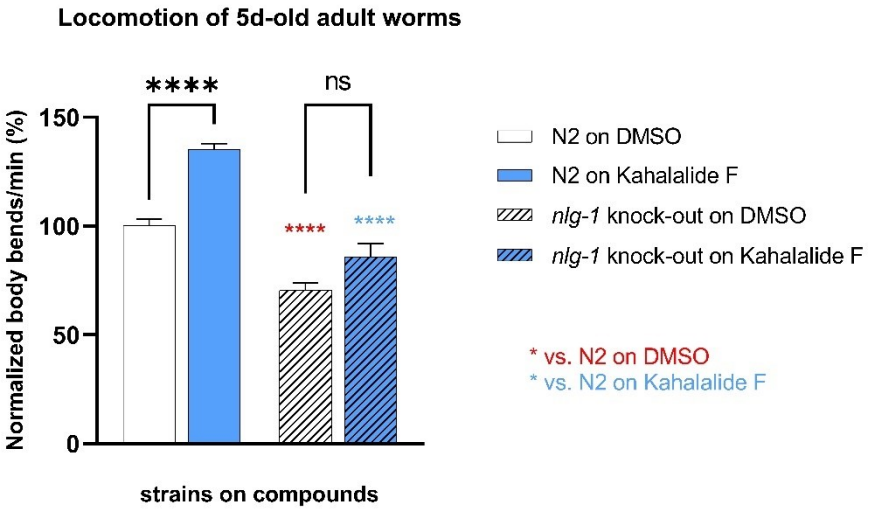

Fig. 3.17 Locomotion of 5-day-old adult N2 and *nlg-1* knock-out worms.
The bars depict the normalized number of body bends/min ±SEM in 5-day-old adult wild-type (N2) and *nlg-1* knock-out worms each in two different dietary pre-treatment. Significant difference was found between N2 worms grown on DMSO and Kahalalide F (****P<0,0001). There were no significant differences between *nlg-1* knock-out worms on the compounds (ns, P<0,05). *Nlg-1* knock-out worms pre-treated with Kahalalide F were significantly slower than N2 grown on Kahalalide F(****P<0,0001). The data were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test (2021).

So, Kahalalide F increases motility with statistical significance in wild-type worms (N2), but not in nematodes lacking *nlg-1*. Moreover, we wanted to examine the locomotory behaviour at a later age (8-day-old adult worms) and these are the following results:

Both in wild-type and in *nlg-1* knock-out strain, worms grown on plates with Kahalalide F presented a much higher number of body bends than on the control condition (**Fig. 3.18)**. Again, the *nlg-1* knock-out mutants on DMSO were slower than the wild-type worms on the same control condition. The same phenomenon is observed between both strains, when the locomotion of Kahalalide F-treated worms is compared: the wild-type strain on Kahalalide F is almost 41% faster than *nlg-1* knock-out strain on Kahalalide F.

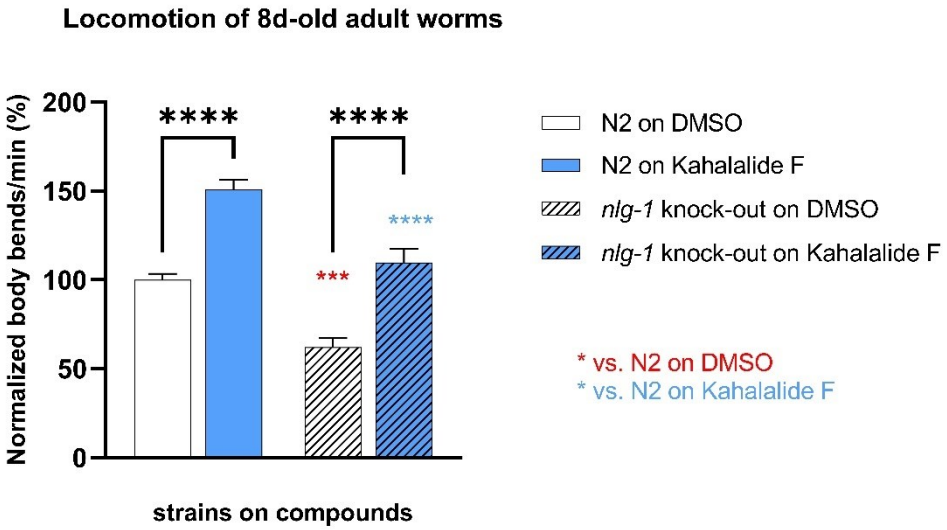

Fig. 3.18 Locomotion of 8-day-old adult N2 and *nlg-1* knock-out worms.
The bars depict the normalized number of body bends/min ±SEM in 8-day-old adult wild-type (N2) and *nlg-1* knock-out worms each in two different dietary treatments. Significant locomotory differences were found within both strains when comparing locomotion on DMSO and Kahalaide F: Both N2 and *nlg-1* knock-out strain showed a significant higher locomotion with Kahalalide F (****,P<0,0001). *nlg-1* knock-out treated with Kahalalide F has a significantly lower motility than N2 on Kahalalide F (****,P<0,0001). *nlg-1* knock-out raised on the control condition was significantly slowlier than N2 on control (***,P=0,0002). The data were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test (2021).

Fig. 3.19 summarizes the results of locomotion rate at different ages within the wild-type strain: the beneficial effect of Kahalalide F is observed from the age of 5-day-old to 8-day-old adult worms. Kahalalide F increases the locomotion in 5-day-old adult worms to a level that is almost near to that speed of 1-day-old adult worms on Kahalalide F. In contrast to that, the decline in number of body bends is greater within the condition without Kahalalide F: the 5-day-old adult worms on DMSO have about 35% lower speed than 1-day-old adult worms on DMSO.

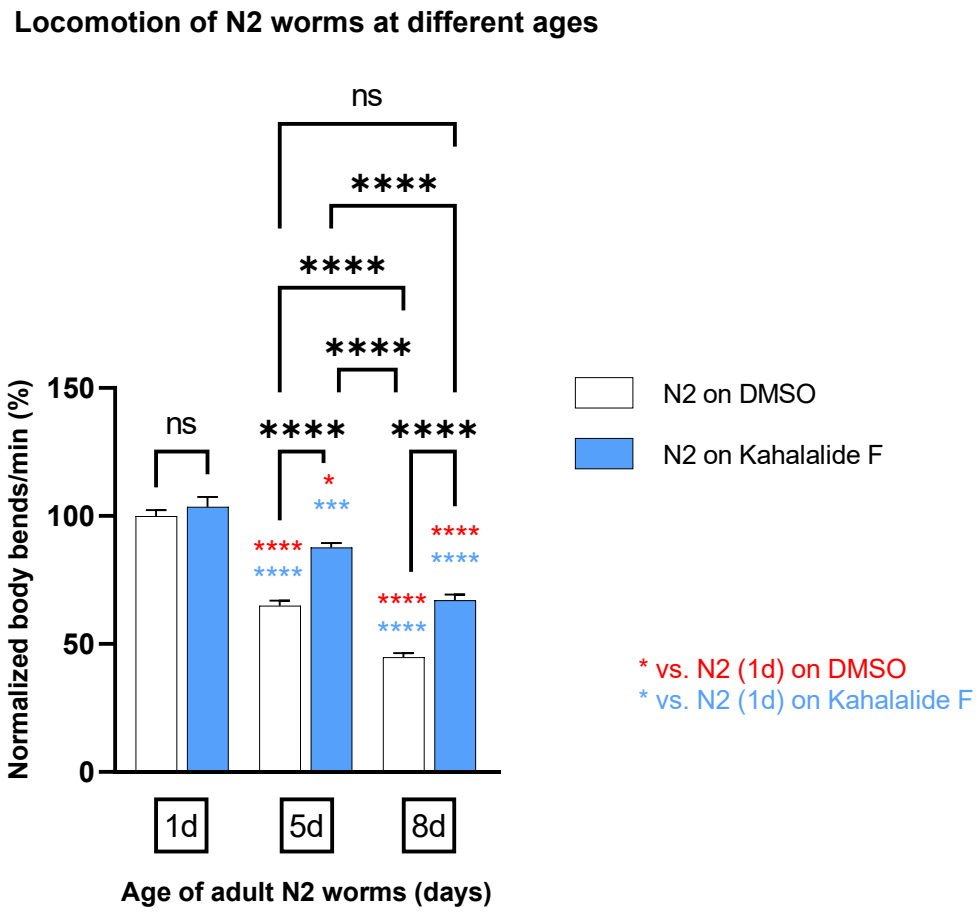

Fig. 3.19 Locomotion of N2 worms at different ages.
The bars depict the normalized number of body bends/min ±SEM in wild-type (N2) strain at the age of 1-, 5- and 8-day-old adult worms. Significant differences between the dietary conditions within each age are present at the age of 5-day- and 8-day-adulthood (****,P<0,0001) and not in 1-day-old adult worms (ns, P<0,9999). N2 worms from both dietary conditions at the age of 5-day and 8-day show highly significant deficits in motility compared to those 1-day-old adult worms (****,P<0,0001 and (***P=0,0003), except of the 5-day-old adult worms grown on Kahalalide F in comparison to 1-day-old adult worms raised on control condition, which had a lower significance (*,P=0,0103). 8-day-old adult worms continually treated with Kahalalide F achieved almost the same locomotory speed as 5-day-old adult worms grown on DMSO (ns, P>0,9999).
The data were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test (2021).

Comparing the locomotion of *nlg-1* knock-out worms at different ages and the different dietary treatments, the locomotion declined with ageing similar to the phenomenon observed in N2, but there was a remarkable difference (see **Fig. 3.20**): Kahalalide F did not have any impact on locomotion until the age of 5-day-old adulthood. Only at the age of 8-day-old adult *nlg-1* knock-out mutants, Kahalalide F increased the motility with statistical significance, but the effect is still weaker than in the wild-type at this age.

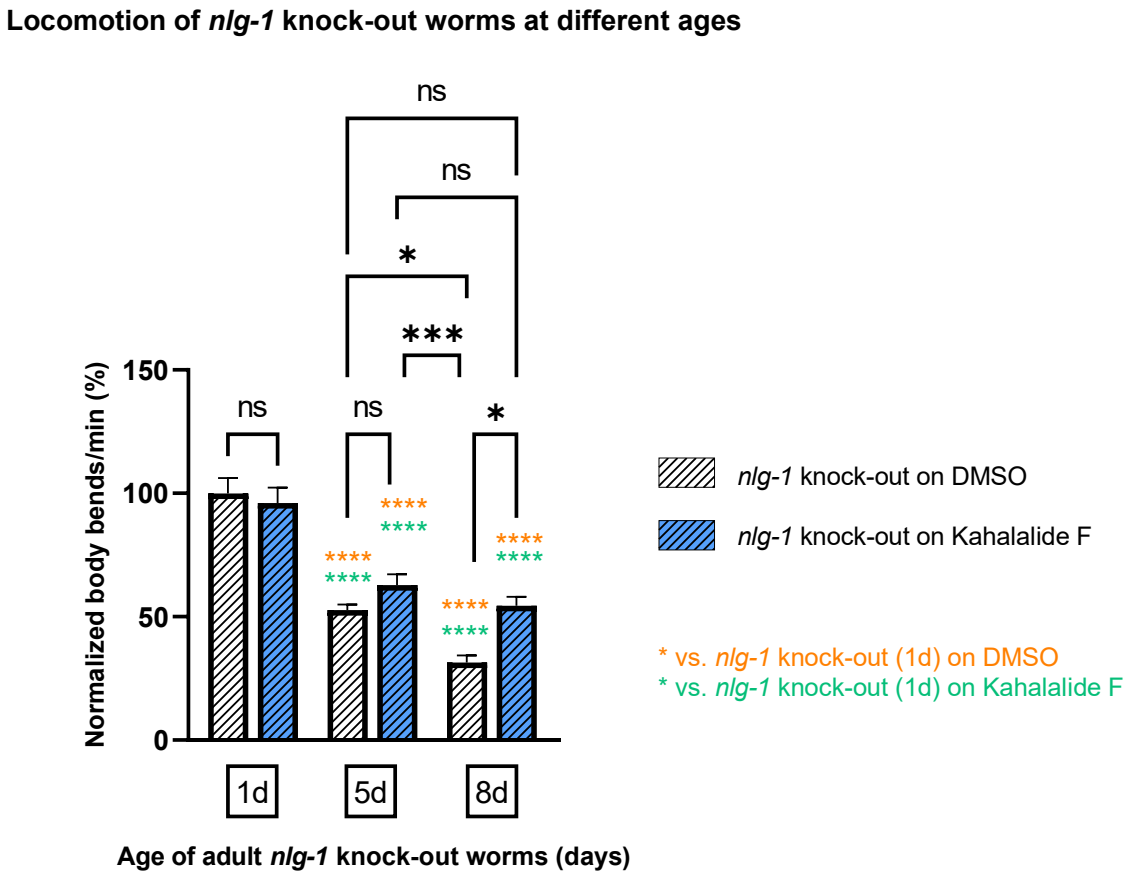

Fig. 3.20 Locomotion of *nlg-1* knock-out at different ages.
The bars depict the normalized number of body bends/min ±SEM in wild-type (N2) strain at the age of 1-, 5- and 8-day-old adult worms. Significant differences in motility through DMSO (control condition) and Kahalalide F were only measured in 8-day-old adult worms (*, P=0,0113). All conditions of 5-day-old and 8-day-old adult *nlg-1* knock-out worms had a significant lower number of body bends/min than 1-day-old worms (****, P<0,0001). 8-day-old adult *nlg-1* knock-out worms treated on DMSO had significant locomotory deficits compared to 5-day-old grown on DMSO (*, P<0,05) and Kahalalide F (***, P=0,0001). 8-day-old adult *nlg-1* knock-out worms treated with Kahalalide F reached nearly the same locomotory speed as 5-day-old worms on each dietary condition (ns, P>0,9999).
The data were calculated from three replicates and analysed through One-way ANOVA and Bonferroni's *post hoc* test (2021).

The results of the locomotion assay, in which DMSO (control) and Kahalalide F have been used as dietary treatments, show that Kahalalide F has a stronger impact on locomotion in the wild-type strain (N2) (see **Fig. 3.19**) than in *nlg-1* knock-out strain (see **Fig. 3.20**). Though *nlg-1* is important for Kahalalide's beneficial impact on locomotion, it is not the only factor for a higher locomotion, because Kahalalide F improves the locomotion at a later age in *nlg-1* knock-out worms.

4 Discussion

4.1 Conclusions

The main aims of my thesis were the investigation of possible protective effects of two compounds, namely Kahalalide F and Lutein, on age-associated features and revealing pathways mediating their beneficial impacts. Indeed, the results show, that Kahalalide F and Lutein have protective effects on age-associated features (resistance to oxidative stress and motility), and *nlg-1* is shown to be a factor at least in part mediating their beneficial effects.

Concerning the first question, whether Kahalalide F and Lutein protect wild-type worms from oxidative stress, we concluded that indeed both compounds have protective effects against oxidative stress since they increase stress resistance against juglone which was used as an oxidative stressor. This is consistent with the other work from the lab showing that both compounds also increase heat shock resistance [1]. Thus, Kahalalide F and Lutein can affect ageing, since it is known that improved stress resistance is associated with longevity [59].

The second question was whether age-regulatory genes such as *daf-16* would be modulated by Kahalalide F or Lutein. Since FOXO transcription factors such as *daf-16* have been shown to be involved in lifespan extension [54, 106], both Kahalalide F and Lutein were examined concerning their influence on *daf-16* nuclear translocation upon heat stress. Neither Kahalalide F nor Lutein showed any impact on the nuclear translocation of *daf-16* during thermal resistance compared to the control condition (see 3.2), which indicate that these compounds might not be involved in DAF-16 regulation. However, at least three reasons might be possible for the observed phenomenon. First: probably, these compounds might be dependent on DAF-16 but might not have any effect on the subcellular localization of DAF-16, such as the serine-threonine kinase SGK-1, which does not alter the localization of DAF-16, but is dependent on DAF-16 [103] in order to promote longevity [105]. It is suggested, that SGK-1 regulates certain proteins such as SKN-1 that interact with DAF-16/FoxO, which then results in longevity and stress resistance [105]. Second, another reason could be, that Kahalalide F and Lutein have beneficial effects on longevity, but act independently of *daf-16*, maybe similar to rapamycin which prolongs lifespan in *daf-16* mutants, but dependent on *skn-1* [63]. Third, as mentioned in a previous study, bacterial diet might have effects on *daf-16* expression in stress conditions, so different bacterial diets could be established in

these experiments as well to discover any bacterial interaction in *C. elegans* [88]. The slight differences in intermediate and cytosolic distribution could be probably explained through the fact that the examination of each condition took 10 to 15 minutes. Therefore, always starting with the control condition first led to a 15 minutes (Kahalalide F) and 30 minutes (Lutein) observation delay of the *daf-16*::GFP-expression.

The third question was whether mitochondrial stress reporter genes are affected by Kahalalide F or Lutein, since the compounds were identified in a screen looking at mitochondrial parameters associated with lifespan extension. As mitochondria play a fundamental role in stress response and longevity [59], we investigated the effect of compounds on mitochondrial stress reporter genes by quantifying the gene expression and using *C. elegans* fluorescent reporter strains, namely *cyp-14a4, gst-4* and *hsp-6*. Neither of these genes appeared to be affected by Kahalalide F and/or Lutein with any statistical significance. Activation of *hsp-6* might not be needed for longevity, as it has been suggested in a previous study [113]. Furthermore, the activation of such genes might depend on specific doses to be expressed significantly [70] and it is known that doses of compounds (such as metformin) can have different impacts in wild-type and mutant strains [80].

Fourth question was whether Kahalalide F and Lutein have similar or different effects on *nlg-1* expression in wild-type nematodes at basal conditions. The reason why *nlg-1* was used for the next experiments was, that the *nlg-1* expression has already been revealed to be affected by Lutein in a mitochondrial complex I-disease model. And *nlg-1* has been shown to play an important role in Lutein's beneficial impact [90]. Neuroligin/*nlg-1* belongs to the synaptic cell adhesion molecules and it is described to extend lifespan as a response to oxidative stress [99]. Together with neurexins they are involved in regulation of retrograde synaptic signals modulating neurotransmitter release at neuromuscular junctions [114]. Indeed, Kahalalide F affected the induction of *nlg-1* expression by increasing it. In contrast to Lutein, the impact of Kahalalide F was highly statistically significant (Bonferroni's post hoc test). In our publication, again the *unpaired t-test* was used and there the expression of *nlg-1* is not only statistically significant in Kahalalide F but also statistically significant in Lutein condition (see [1]). However, to find out whether the protective impact of the compounds might rely on a functioning *nlg-1*, we examined stress resistance of wild-type and *nlg-1* knock-out nematodes. Paradoxically, the results showed that *nlg-1* knock-out mutants had an overall better survival compared to wild-type worms (N2). But wild-type worms were protected with a much higher statistical significance by the compounds than

nlg-1 knock-out mutants, which means *nlg-1* plays an important role in the beneficial effect of the compounds. In contrast to my experiment, a previous study showed that *nlg-1* knock-out worms would actually be more sensitive to juglone [99]. The possible reason for the different sensitivity of *nlg-1* mutants to juglone in my thesis compared to the other study, might be the fact that the other study used different bacteria, namely OP50 without antibiotics and not HT115(pL4440) with antibiotics and IPTG. Specially, different bacteria are considered to affect toxicological results differently [88, 115].

The fifth question was to examine whether Kahalalide F would also have beneficial effects on motility of wild-type worms during ageing and whether its effect on motility would be dependent on *nlg-1*. Indeed, Kahalalide F increases motility via *nlg-1*, because the results of the locomotion assay show that Kahalalide F has a greater effect in the wild-type strain with a functioning *nlg-1* gene than on *nlg-1* knock-out worms. The less but still existing locomotory increase in 8-day-old adult *nlg-1* knock-out worms treated with Kahalalide F, compared to those on the control condition, implies that other genes than *nlg-1* might also contribute to an increased motility via Kahalalide F, but probably play a role in higher ages. For instance, a similar phenomenon has already been reported before, namely that two aspects concerning mitochondrial stress play a role: the fact at which timepoint it occurs and for how long it takes, since activation of several genes depending on these factors might cause inverse outcomes for life expectancy of the nematodes [65].

Compounds such as Lutein seem to have beneficial effects through opposite mechanisms that depend on dose and context [1]. As already mentioned, a previous study of the host laboratory revealed that *nlg-1* is the target of Lutein, which suppresses the overexpression of *nlg-1* in the case of mitochondrial disruption caused by mitochondrial complex I-linked disease model [90]. In that study, it is assumed, that in the case of impaired synaptic signalling, which is caused by mitochondrial disruption, neurons try to compensate this impaired synaptic signalling through overexpression of *nlg-1*. But *nlg-1* overexpression would lead to pathological synaptic excitation. So, when Lutein suppressed *nlg-1* overexpression, Lutein had beneficial effects in that disease model [90]. It is important to consider that Lutein was used at a very lower concentration in the previous study, namely 1µM [90] compared to here (100µM). In this thesis, we observed an increased *nlg-1* expression through Kahalalide F (0,5µM) and Lutein (100µM) that mediates their beneficial effect, probably through a mild mitochondrial stress [1]. It would be interesting to test whether Lutein would still suppress *nlg-1* overexpression in the disease model, if it was

higher concentrated like 100μM and not lower concentrated. However, through these observations we can speculate that Lutein shows its beneficial effects through different phenomena. In the case of severe mitochondrial stress (mitochondrial disruption caused by disease model) Lutein at lower dose suppresses *nlg-1* overexpression [90]. Instead, Lutein at a higher dose increases *nlg-1* expression through mild mitochondrial stress. It is suggested that carotenoids like Lutein and β-carotenes have prooxidant effects at high doses [116]. Another important conclusion concerning *nlg-1* is that compensatory pathways such as the *nlg-1* pathway might be beneficial in cases of mild mitochondrial stress, but when it exceeds to mitochondrial disruption, these pathways cause detrimental deficits [90].

Moreover, Kahalalide F and Lutein might have an impact on the SKN-1 (Nrf2) pathways, since it is involved in the regulation of *nlg-1.* SKN-1 is a neuronal gene and positively regulates *nlg-1* expression. Oxidative stress leads to an activation of SKN-1. When SKN-1 is activated, then the abundance of NLG-1 in synapses rises. SKN-1 is also suggested to be a mitochondrial stress sensor. And in SKN-1-dependent response to mitochondrial stress, NLG-1 is mentioned to promote survival [99].

Besides, we tested the role of *nlg-1* in thermal resistance through heat shock treatments as well (see **Fig. 4.1**). The *nlg-1::GFP* expression after heat shock on DMSO (control) was examined in one replicate, in order to observe changes directly after heat shock and after 2 hours recovery, but no significant differences have been revealed in *nlg-1*::GFP expression. So, it might be not affected at a temperature of 35°C for those durations.

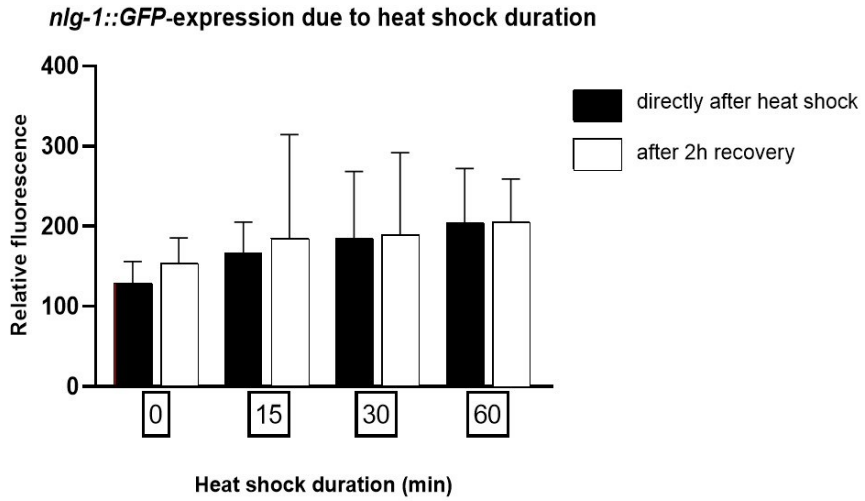

Fig. 4.1 *nlg-1::GFP*- expression directly after heat shock and after 2h-recovery.
The data was not normalized, but shows the average *nlg-1::GFP*-intensity (±SD) in worms grown on DMSO (control) due to different heat shock durations (0 min, 15 min, 30 min, 60 min) and each after 2 hours recovery. Again, only a defined region of the ventral nerve cord (in the distal body part of the nematodes) was analysed due to its *nlg-1::GFP* expression. There were no significant differences between the conditions (ns, P>0,05). The data of one replicate was analysed through one-way ANOVA and followed by Bonferroni's *post hoc* test.

Comparing the *nlg-1* expression between those worms treated without heat shock and those exposed at 35°C for one hour, the observation directly after the thermal resistance assay depicted a statistically significant higher expression of *nlg-1* in those worms treated with one hour heat shock (see **Fig. 4.2**), which could be further investigated concerning the impact of compounds such as Kahalalide F on *nlg-1* expression after longer heat shock durations.

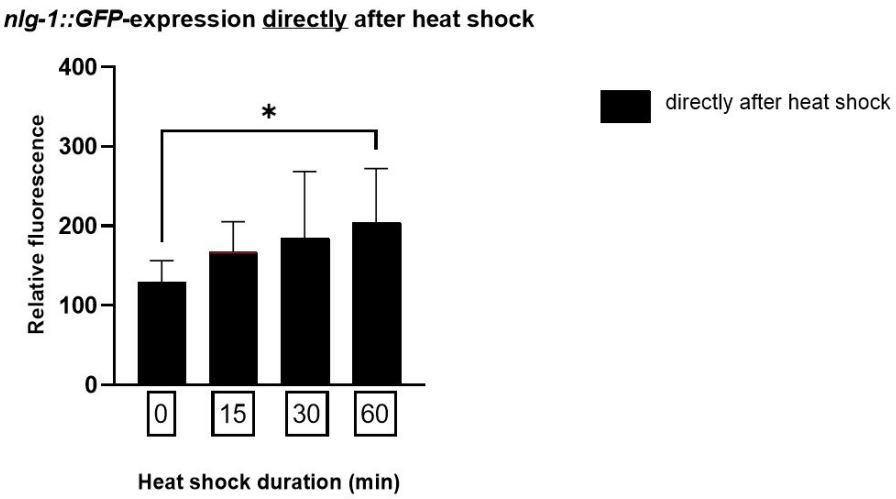

Fig. 4.2 Direct observation of *nlg-1::GFP*- expression after heat shock.
The bars depict the average GFP-intensity due to different heat shock durations (0 min, 15 min, 30 min, 60 min) directly after the heat shock treatment. Again, only a defined region of the ventral nerve cord (in the distal body part of the nematodes) was analysed due to its *nlg-1::GFP* expression. There is one significant difference between the GFP-intensity in worms treated 60 min with heat shock and those worms not treated with heat (*, P=0,0161). The intensity rises proportionally to the heat shock duration, but without statistical significance. The data of one replicate was analysed through One-way ANOVA and followed by Bonferroni's *post hoc* test.

To sum up, both Lutein and Kahalalide F reveal protective effects on age-associated features. and *nlg-1* plays a main target in mediating their ameliorating impact, but it is probably not the only target, since Kahalalide F and Lutein have remaining beneficial effect in *nlg-1* knock-out mutants. Although the chosen mitochondrial stress reporter genes appeared to be not affected by these compounds (via Bonferroni's *post hoc* test), other mitochondrial stress reporter genes might be involved.

4.2 Perspectives

There are few aspects which could be suggested for future studies.

Bacterial diet and antibiotics should be taken into consideration when performing assays. Concerning the better survival of *nlg-1* knock-out worms than wild-type worms against oxidative stress, future examinations should concentrate on possible variables such as bacterial diet and the role of antibiotics and IPTG. It is possible that they might interact in the oxidative stress response, which could result in different effects for wild-type and *nlg-1*

knock-out strains, similar to suggestions of other studies [88, 115, 117]. Another question to investigate is whether Kahalalide F or Lutein might be metabolized by bacteria. This could be examined through the establishment of growth-arrested bacteria in experiments [84].

The effect of Kahalalide F on locomotion/motility should be tested also in higher ages on *C. elegans*. In the locomotion assay, the wild-type had a remarkable advantage in motility than *nlg-1* knock-out and Kahalalide F had a beneficial effect at 5-day-old and 8-day-old adult worms. Therefore, future experiments could include worms at even older ages, to observe the duration of the beneficial effect. In this way, the effect of Kahalalide F not only in wild-type but also in *nlg-1* knock-out could be analysed more precisely.

Kahalalide F and Lutein should be further tested, not only on *nlg-1* but also on other genes affected in neurodegenerative diseases. It is known that Neuroligin is one of the affected in neurodegenerative diseases such as the Alzheimer's disease (AD) and that it is reduced in the hippocampus by Aβ oligomers, resulting in cognitive deficits in patients [118]. The expression of *nlg-1* and various genes in *C. elegans* are regulated by SKN-1, which is the homolog of Nrf being the regulatory factor of response towards oxidative stress. Reactive oxygen species contribute to neurodegenerative diseases such as the Alzheimer's disease (AD) [99]. It is known that mutation in the human neuroligin 4 is also linked to the neurological disorders such as autism spectrum disorders (ASD) [119]. So, understanding the molecular impact of Kahalalide F on *nlg-1* might help to find further therapeutical targets treating AD and ASD. Lutein, a xantophyll carotenoid, has also been suggested to be used in the form of a nutritional strategy to treat AD [120]. To gain a better understanding of neurodegenerative diseases such as AD, it might also be advantageous to test these and other natural compounds and their role on neurexins and presenilins. Neurexins interact with neurologins at synapses. Presenilins are responsible for the proteolytical processing of neurexins [121] and presenilin genes are affected in AD [122]. Concerning neurodegenerative diseases, the role of Kahalalide F and Lutein due to DNA methylation would also be an interesting aspect to investigate in order to understand and evaluate their further role as potential therapeutical compounds for AD, because it has been previously shown that certain genes would be hypo- or hypermethylated in AD [123]. So, Kahalalide F and Lutein could be tested on several genes to understand these neurodegenerative processes.

Kahalalide F and Lutein could be tested on locomotory behavioural responses such as BSR and ESR to understand the underlying pathways. The basal slowing response (BSR) is a slow

movement of nematodes in bacterial presence, whereas enhanced slowing response (ESR) is slower than BSR and is observed in fasted nematodes. The locomotory rate of BSR and ESR is defective in nematodes carrying *nlg-1* mutations [124]. Thus, another informative assay would be to investigate compounds such as Kahalalide F on BSR and ESR behaviours of *nlg-1* mutants, in order to elucidate the molecular mechanism of Kahalalide F due to special pathways, because it is known that BSR depends on the dopaminergic and ESR on the serotonergic pathway. Such experiments have been already done with fluoxetine, which caused a restoration of ESR and also with methylphenidate, that was able to restore the BSR in nematodes with *nlg-1* deficiency [124]. Lutein is mentioned to affect the dopaminergic and cholinergic system in *Drosophila melanogaster* [125]. In addition to that, *nlg-1* mutants reveal an overexpression of *comt-4* which has been depicted to be the factor for BSR and ESR impairments [126]. Therefore, experiments considering the expression of *comt-4* in nematodes treated with Kahalalide F, would also be supportive in clarifying molecular impacts of such compounds.

So, these two compounds should be investigated further in order to elucidate their impact in detail. Kahalalide F, a marine-derived compound [91] is used as a novel antitumor agent [127]. Kahalalide F is suggested to cause a necrosis-like cell death process, and it is shown to suppress the phosphatidylinositol 3-kinase (PI3K)-Akt signalling pathway in sensitive cell lines. Yet ErbB3 (HER3) has also been suggested to be involved in the cytotoxic activity of Kahalalide F [127]. The exact cytotoxic mechanism has not been clarified yet. But it is mentioned that mitochondria and lysosomes are factors affecting cytotoxicity of Kahalalide F [128]. Lutein, the xantophyll carotenoid found in vegetables [93], is already known for its antioxidative effect. It protects the retina from hypoxic damage, reduces risk of skin cancer and atherosclerosis [129]. Lutein's anti-inflammatory activity seems to be based on a suppression of NF-κB activation, which was investigated on uveitis in rats. When NF-κB is supressed, then proinflammatory mediators are produced less [93]. Interestingly, Lutein shows its beneficial effects on acetylcholinesterase (ACh) in different cases: it can reduce AChE activity [130] or it can restore AChE activity [125]. That means: first, Lutein reduces AChE activity, when ACh activity is increased by ethanol. In this way Lutein reduced the ethanol-induced memory deficits in rats [130]. Second, Lutein restores AChE activity, when ACh activity is decreased by rotenone (oxidative stressor) in *Drosophila melanogaster*. Rotenone suppresses mitochondrial complex I (MCI), which leads to oxidative stress [125]. So, here Lutein is suggested to protect neurodegenerative diseases such as Parkinson Disease (PD), because decreased ACh activity is associated with neurodegenerative diseases and

Lutein restores it [125]. So, both Lutein and Kahalalide F could be tested for their future therapeutical targets.

Mitochondrial stress plays a fundamental role in ageing and depending on the degree of mitochondrial stress, it can either lead to healthy ageing or development of complex diseases such as neurodegenerative diseases [67]. Neurodegenerative ocular diseases such as macular degeneration and glaucoma are associated with oxidative stress as well. Glaucoma belongs to the optic neuropathies and is associated with the damage and death of retinal ganglion cells, resulting in peripheral visual loss. An increased intraocular pressure is one of the main factors for glaucoma. Glaucoma is another cause for irreversible blindness [131], similar to macular degeneration [132]. Normal tension glaucoma is a glaucoma subtype and it shows a progressive damage of retinal ganglion cells, although the intraocular pressure is within the normal range. Now, research shows a link between normal tension glaucoma and the Alzheimer's disease (AD), because they appear to have a similar pathogenesis, including same biomarkers such as Amyloid b peptide (Aβ) and brain atrophy. So, finding therapeutical targets could address both diseases [131]. Kahalalide F has an impact on the phosphatidylinositol 3-kinase (PI3K)-Akt signalling pathway [127], which is a relevant pathway in glaucoma [133] and AD [134]. Lutein targets the PI3K/Akt signalling pathway as well [135]. Since PI3K/Akt signalling pathway has a key role in neuroprotection [134], it would be very interesting to examine and compare the impact of Kahalalide F and Lutein in disease models such as AD and glaucoma and to observe effects on relevant biomarkers.

There are many aspects that might be further investigated in order to understand the underlying protective mechanisms of Kahalalide F and Lutein. As we see, they have potential role for elucidating the pathways affected in neurodegenerative diseases such as *nlg-1*. And *nlg-1* is probably involved in releasing "*mitokines"* as a neuronal signal in response to mitochondrial dysfunction [99] in order to contribute to survival [136].

5 Bibliography

1. Maglioni, S., et al., *High-Content C. elegans Screen Identifies Natural Compounds Impacting Mitochondria-Lipid Homeostasis and Promoting Healthspan.* Cells, 2021. **11**(1).
2. Kritsilis, M., et al., *Ageing, Cellular Senescence and Neurodegenerative Disease.* Int J Mol Sci, 2018. **19**(10).
3. Dziechciaż, M. and R. Filip, *Biological psychological and social determinants of old age: bio-psycho-social aspects of human aging.* Ann Agric Environ Med, 2014. **21**(4): p. 835-8.
4. Aunan, J.R., et al., *Molecular and biological hallmarks of ageing.* Br J Surg, 2016. **103**(2): p. e29-46.
5. Ristow, M. and K. Schmeisser, *Mitohormesis: Promoting Health and Lifespan by Increased Levels of Reactive Oxygen Species (ROS).* Dose Response, 2014. **12**(2): p. 288-341.
6. Partridge, L., J. Deelen, and P.E. Slagboom, *Facing up to the global challenges of ageing.* Nature, 2018. **561**(7721): p. 45-56.
7. Steves, C.J., T.D. Spector, and S.H. Jackson, *Ageing, genes, environment and epigenetics: what twin studies tell us now, and in the future.* Age Ageing, 2012. **41**(5): p. 581-6.
8. Willcox, D.C., G. Scapagnini, and B.J. Willcox, *Healthy aging diets other than the Mediterranean: a focus on the Okinawan diet.* Mech Ageing Dev, 2014. **136-137**: p. 148-62.
9. Popkin, B.M., L.S. Adair, and S.W. Ng, *Global nutrition transition and the pandemic of obesity in developing countries.* Nutr Rev, 2012. **70**(1): p. 3-21.
10. Bektas, A., et al., *Aging, inflammation and the environment.* Exp Gerontol, 2018. **105**: p. 10-18.
11. Vauzour, D., et al., *Nutrition for the ageing brain: Towards evidence for an optimal diet.* Ageing Res Rev, 2017. **35**: p. 222-240.
12. Ros, M. and J.M. Carrascosa, *Current nutritional and pharmacological anti-aging interventions.* Biochim Biophys Acta Mol Basis Dis, 2020. **1866**(3): p. 165612.
13. Willcox, D., et al., *The Okinawan Diet: Health Implications of a Low-Calorie, Nutrient-Dense, Antioxidant-Rich Dietary Pattern Low in Glycemic Load.* Journal of the American College of Nutrition, 2009. **28 Suppl**: p. 500S-516S.
14. Shao, A., et al., *Optimal nutrition and the ever-changing dietary landscape: a conference report.* European Journal of Nutrition, 2017. **56**(1): p. 1-21.
15. Martucci, M., et al., *Mediterranean diet and inflammaging within the hormesis paradigm.* Nutr Rev, 2017. **75**(6): p. 442-455.
16. Pérez-Torres, I., et al., *Oxidative Stress, Plant Natural Antioxidants, and Obesity.* Int J Mol Sci, 2021. **22**(4).
17. Detopoulou, P., C.A. Demopoulos, and S. Antonopoulou, *Micronutrients, Phytochemicals and Mediterranean Diet: A Potential Protective Role against COVID-19 through Modulation of PAF Actions and Metabolism.* Nutrients, 2021. **13**(2).
18. Iranshahy, M. and B. Javadi, *Diet therapy for the treatment of Alzheimer's disease in view of traditional Persian medicine: A review.* Iran J Basic Med Sci, 2019. **22**(10): p. 1102-1117.
19. Di Lorenzo, C., et al., *Polyphenols and Human Health: The Role of Bioavailability.* Nutrients, 2021. **13**(1).
20. Almeida, M. and R.M. Porter, *Sirtuins and FoxOs in osteoporosis and osteoarthritis.* Bone, 2019. **121**: p. 284-292.
21. Cui, Z., et al., *Therapeutic application of quercetin in aging-related diseases: SIRT1 as a potential mechanism.* Front Immunol, 2022. **13**: p. 943321.
22. Nakamura, Y., et al., *Effect of quercetin glycosides on cognitive functions and cerebral blood flow: a randomized, double-blind, and placebo-controlled study.* Eur Rev Med Pharmacol Sci, 2022. **26**(23): p. 8700-8712.

23. Costea, T., et al., *Alleviation of Multidrug Resistance by Flavonoid and Non-Flavonoid Compounds in Breast, Lung, Colorectal and Prostate Cancer.* Int J Mol Sci, 2020. **21**(2).
24. Lin, S.C., et al., *Effective inhibition of MERS-CoV infection by resveratrol.* BMC Infect Dis, 2017. **17**(1): p. 144.
25. Manach, C., et al., *Polyphenols: food sources and bioavailability.* Am J Clin Nutr, 2004. **79**(5): p. 727-47.
26. Cassidy, A., B. Hanley, and R.M. Lamuela-Raventos, *Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health.* Journal of the Science of Food and Agriculture, 2000. **80**(7): p. 1044-1062.
27. Martel, J., et al., *Plant and fungal products that extend lifespan in Caenorhabditis elegans.* Microb Cell, 2020. **7**(10): p. 255-269.
28. Wahl, D., et al., *Nutritional strategies to optimise cognitive function in the aging brain.* Ageing Res Rev, 2016. **31**: p. 80-92.
29. Duregon, E., et al., *Intermittent fasting: from calories to time restriction.* Geroscience, 2021. **43**(3): p. 1083-1092.
30. Al Attar, A.A., et al., *Mechanisms underlying the effects of caloric restriction on hypertension.* Biochemical Pharmacology, 2022. **200**: p. 115035.
31. Hammer, S., et al., *Prolonged Caloric Restriction in Obese Patients With Type 2 Diabetes Mellitus Decreases Myocardial Triglyceride Content and Improves Myocardial Function.* Journal of the American College of Cardiology, 2008. **52**(12): p. 1006-1012.
32. Fontana, L., et al., *Effects of 2-year calorie restriction on circulating levels of IGF-1, IGF-binding proteins and cortisol in nonobese men and women: a randomized clinical trial.* Aging Cell, 2016. **15**(1): p. 22-7.
33. Fontana, L., et al., *Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans.* Aging Cell, 2008. **7**(5): p. 681-7.
34. Fontana, L. and L. Partridge, *Promoting health and longevity through diet: from model organisms to humans.* Cell, 2015. **161**(1): p. 106-118.
35. Muralidharan, J., et al., *Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study.* Am J Clin Nutr, 2021. **114**(3): p. 1148-1158.
36. Gadecka, A. and A. Bielak-Zmijewska, *Slowing Down Ageing: The Role of Nutrients and Microbiota in Modulation of the Epigenome.* Nutrients, 2019. **11**(6).
37. Ticinesi, A., et al., *Aging Gut Microbiota at the Cross-Road between Nutrition, Physical Frailty, and Sarcopenia: Is There a Gut-Muscle Axis?* Nutrients, 2017. **9**(12).
38. Glenn, C.F., et al., *Behavioral deficits during early stages of aging in Caenorhabditis elegans result from locomotory deficits possibly linked to muscle frailty.* J Gerontol A Biol Sci Med Sci, 2004. **59**(12): p. 1251-60.
39. Hunt, P.R., *The C. elegans model in toxicity testing.* J Appl Toxicol, 2017. **37**(1): p. 50-59.
40. Jiang, H. and D. Wang, *The Microbial Zoo in the C. elegans Intestine: Bacteria, Fungi and Viruses.* Viruses, 2018. **10**(2).
41. Herndon, L.A., et al., *Stochastic and genetic factors influence tissue-specific decline in ageing C. elegans.* Nature, 2002. **419**(6909): p. 808-14.
42. Altun, Z.F.a.H., D.H. *Handbook of C. elegans Anatomy. In WormAtlas*. 2021; Available from: https://www.wormatlas.org/hermaphrodite/introduction/Introframeset.html.
43. Weinhouse, C., et al., *Caenorhabditis elegans as an emerging model system in environmental epigenetics.* Environ Mol Mutagen, 2018. **59**(7): p. 560-575.
44. Park, H.H., Y. Jung, and S.V. Lee, *Survival assays using Caenorhabditis elegans.* Mol Cells, 2017. **40**(2): p. 90-99.
45. Maglioni, S., N. Arsalan, and N. Ventura, *C. elegans screening strategies to identify pro-longevity interventions.* Mech Ageing Dev, 2016. **157**: p. 60-9.
46. Dimov, I. and M.F. Maduro, *The C. elegans intestine: organogenesis, digestion, and physiology.* Cell Tissue Res, 2019. **377**(3): p. 383-396.

47. Torgovnick, A., et al., *Healthy aging: what can we learn from Caenorhabditis elegans?* Z Gerontol Geriatr, 2013. **46**(7): p. 623-8.
48. Kenyon, C.J., *The genetics of ageing.* Nature, 2010. **464**(7288): p. 504-12.
49. Youngman, M.J., Z.N. Rogers, and D.H. Kim, *A decline in p38 MAPK signaling underlies immunosenescence in Caenorhabditis elegans.* PLoS Genet, 2011. **7**(5): p. e1002082.
50. da Silva, P.F.L. and B. Schumacher, *DNA damage responses in ageing.* Open Biol, 2019. **9**(11): p. 190168.
51. Zhang, B., et al., *Brain-gut communications via distinct neuroendocrine signals bidirectionally regulate longevity in C. elegans.* Genes Dev, 2018. **32**(3-4): p. 258-270.
52. Kauffman, A.L., et al., *Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age.* PLoS Biol, 2010. **8**(5): p. e1000372.
53. Pohl, F. and P. Kong Thoo Lin, *The Potential Use of Plant Natural Products and Plant Extracts with Antioxidant Properties for the Prevention/Treatment of Neurodegenerative Diseases: In Vitro, In Vivo and Clinical Trials.* Molecules, 2018. **23**(12).
54. Uno, M. and E. Nishida, *Lifespan-regulating genes in C. elegans.* NPJ Aging Mech Dis, 2016. **2**: p. 16010.
55. Ayuda-Durán, B., et al., *Epicatechin modulates stress-resistance in C. elegans via insulin/IGF-1 signaling pathway.* PLoS One, 2019. **14**(1): p. e0199483.
56. Gami, M.S. and C.A. Wolkow, *Studies of Caenorhabditis elegans DAF-2/insulin signaling reveal targets for pharmacological manipulation of lifespan.* Aging Cell, 2006. **5**(1): p. 31-7.
57. Kimura, K., et al., *daf-2, an Insulin Receptor-Like Gene That Regulates Longevity and Diapause in Caenorhabditis elegans.* Science (New York, N.Y.), 1997. **277**: p. 942-6.
58. Zhang, S., et al., *Caenorhabditis elegans as a Useful Model for Studying Aging Mutations.* Front Endocrinol (Lausanne), 2020. **11**: p. 554994.
59. Denzel, M.S., L.R. Lapierre, and H.I.D. Mack, *Emerging topics in C. elegans aging research: Transcriptional regulation, stress response and epigenetics.* Mech Ageing Dev, 2019. **177**: p. 4-21.
60. Chiang, W.-C., et al., *HSF-1 Regulators DDL-1/2 Link Insulin-like Signaling to Heat-Shock Responses and Modulation of Longevity.* Cell, 2012. **148**(1): p. 322-334.
61. Loboda, A., et al., *Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism.* Cell Mol Life Sci, 2016. **73**(17): p. 3221-47.
62. Blackwell, T.K., et al., *SKN-1/Nrf, stress responses, and aging in Caenorhabditis elegans.* Free Radic Biol Med, 2015. **88**(Pt B): p. 290-301.
63. Robida-Stubbs, S., et al., *TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO.* Cell Metab, 2012. **15**(5): p. 713-24.
64. Zole, E. and R. Ranka, *Mitochondria, its DNA and telomeres in ageing and human population.* Biogerontology, 2018. **19**(3-4): p. 189-208.
65. Tauffenberger, A., A. Vaccaro, and J.A. Parker, *Fragile lifespan expansion by dietary mitohormesis in C. elegans.* Aging (Albany NY), 2016. **8**(1): p. 50-61.
66. Mukherjee, A., et al., *Mitochondrial Reactive Oxygen Species in Infection and Immunity.* Biomolecules, 2024. **14**(6).
67. Barbour, J.A. and N. Turner, *Mitochondrial stress signaling promotes cellular adaptations.* Int J Cell Biol, 2014. **2014**: p. 156020.
68. Lima, T., et al., *Pleiotropic effects of mitochondria in aging.* Nature Aging, 2022. **2**(3): p. 199-213.
69. Rea, S.L., *Metabolism in the Caenorhabditis elegans Mit mutants.* Experimental Gerontology, 2005. **40**(11): p. 841-849.
70. Rea, S.L., N. Ventura, and T.E. Johnson, *Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans.* PLoS Biol, 2007. **5**(10): p. e259.

71. Ventura, N., S.L. Rea, and R. Testi, *Long-lived C. elegans mitochondrial mutants as a model for human mitochondrial-associated diseases.* Exp Gerontol, 2006. **41**(10): p. 974-91.
72. Munkácsy, E. and S.L. Rea, *The paradox of mitochondrial dysfunction and extended longevity.* Exp Gerontol, 2014. **56**: p. 221-33.
73. Senchuk, M.M., et al., *Activation of DAF-16/FOXO by reactive oxygen species contributes to longevity in long-lived mitochondrial mutants in Caenorhabditis elegans.* PLoS Genet, 2018. **14**(3): p. e1007268.
74. Maglioni, S., et al., *An automated phenotype-based microscopy screen to identify pro-longevity interventions acting through mitochondria in C. elegans.* Biochim Biophys Acta, 2015. **1847**(11): p. 1469-78.
75. Brinkmann, V., et al. *Aryl Hydrocarbon Receptor-Dependent and -Independent Pathways Mediate Curcumin Anti-Aging Effects*. Antioxidants, 2022. **11**, DOI: 10.3390/antiox11040613.
76. Schiavi, A., et al., *Abl depletion via autophagy mediates the beneficial effects of quercetin against Alzheimer pathology across species.* Cell Death Discov, 2023. **9**(1): p. 376.
77. Dall, K.B. and N.J. Færgeman, *Metabolic regulation of lifespan from a C. elegans perspective.* Genes Nutr, 2019. **14**: p. 25.
78. Morselli, E., et al., *Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy.* Cell Death Dis, 2010. **1**(1): p. e10.
79. Bailey, C.J., *Metformin: historical overview.* Diabetologia, 2017. **60**(9): p. 1566-1576.
80. Onken, B. and M. Driscoll, *Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1.* PLoS One, 2010. **5**(1): p. e8758.
81. Rattan, S.I., *Hormesis in aging.* Ageing Res Rev, 2008. **7**(1): p. 63-78.
82. Song, Y., et al., *Protective effects of EGCG on acrolein-induced Caenorhabditis elegans and its mechanism of life extension.* Food & Function, 2024. **15**(11): p. 5855-5867.
83. Carretero, M., R.L. Gomez-Amaro, and M. Petrascheck, *Pharmacological classes that extend lifespan of Caenorhabditis elegans.* Front Genet, 2015. **6**: p. 77.
84. Schulz, T.J., et al., *Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress.* Cell Metab, 2007. **6**(4): p. 280-93.
85. Mark, K.A., et al., *Vitamin D Promotes Protein Homeostasis and Longevity via the Stress Response Pathway Genes skn-1, ire-1, and xbp-1.* Cell Rep, 2016. **17**(5): p. 1227-1237.
86. Haçariz, O., et al., *The symbiotic relationship between Caenorhabditis elegans and members of its microbiome contributes to worm fitness and lifespan extension.* BMC Genomics, 2021. **22**(1): p. 364.
87. Urrutia, A., et al., *Bacterially produced metabolites protect C. elegans neurons from degeneration.* PLoS Biol, 2020. **18**(3): p. e3000638.
88. Ke, T. and M. Aschner, *Bacteria affect Caenorhabditis elegans responses to MeHg toxicity.* Neurotoxicology, 2019. **75**: p. 129-135.
89. Revtovich, A.V., R. Lee, and N.V. Kirienko, *Interplay between mitochondria and diet mediates pathogen and stress resistance in Caenorhabditis elegans.* PLoS Genet, 2019. **15**(3): p. e1008011.
90. Maglioni, S., et al., *Neuroligin-mediated neurodevelopmental defects are induced by mitochondrial dysfunction and prevented by lutein in C. elegans.* Nat Commun, 2022. **13**(1): p. 2620.
91. Suárez, Y., et al., *Kahalalide F, a new marine-derived compound, induces oncosis in human prostate and breast cancer cells.* Mol Cancer Ther, 2003. **2**(9): p. 863-72.
92. Wang, B., et al., *An efficient and cost-effective approach to kahalalide F N-terminal modifications using a nuisance algal bloom of Bryopsis pennata.* Biochim Biophys Acta, 2015. **1850**(9): p. 1849-54.

93. Jin, X.H., et al., *Inhibitory effects of lutein on endotoxin-induced uveitis in Lewis rats.* Invest Ophthalmol Vis Sci, 2006. **47**(6): p. 2562-8.
94. Kiko, T., et al., *Significance of lutein in red blood cells of Alzheimer's disease patients.* J Alzheimers Dis, 2012. **28**(3): p. 593-600.
95. *Kahalalide F, CSID:32818457*. [cited 2024 July 14, 2024]; Available from: http://www.chemspider.com/Chemical-Structure.32818457.html.
96. *Lutein, CSID:4444655*. [cited 2024 July 14, 2024]; Available from: http://www.chemspider.com/Chemical-Structure.4444655.html.
97. Stiernagle, T., *Maintenance of C. elegans.* WormBook, 2006: p. 1-11.
98. Mao, K., et al., *Mitochondrial Dysfunction in C. elegans Activates Mitochondrial Relocalization and Nuclear Hormone Receptor-Dependent Detoxification Genes.* Cell Metab, 2019. **29**(5): p. 1182-1191.e4.
99. Staab, T.A., et al., *Regulation of synaptic nlg-1/neuroligin abundance by the skn-1/Nrf stress response pathway protects against oxidative stress.* PLoS Genet, 2014. **10**(1): p. e1004100.
100. Calahorro, F. and M. Ruiz-Rubio, *Functional phenotypic rescue of Caenorhabditis elegans neuroligin-deficient mutants by the human and rat NLGN1 genes.* PLoS One, 2012. **7**(6): p. e39277.
101. Zhou, K.I., Z. Pincus, and F.J. Slack, *Longevity and stress in Caenorhabditis elegans.* Aging (Albany NY), 2011. **3**(8): p. 733-53.
102. Senchuk, M.M., D.J. Dues, and J.M. Van Raamsdonk, *Measuring Oxidative Stress in Caenorhabditis elegans: Paraquat and Juglone Sensitivity Assays.* Bio Protoc, 2017. **7**(1).
103. Sun, X., W.D. Chen, and Y.D. Wang, *DAF-16/FOXO Transcription Factor in Aging and Longevity.* Front Pharmacol, 2017. **8**: p. 548.
104. Massie, M.R., et al., *Exposure to the metabolic inhibitor sodium azide induces stress protein expression and thermotolerance in the nematode Caenorhabditis elegans.* Cell Stress Chaperones, 2003. **8**(1): p. 1-7.
105. Chen, A.T., et al., *Effects of Caenorhabditis elegans sgk-1 mutations on lifespan, stress resistance, and DAF-16/FoxO regulation.* Aging Cell, 2013. **12**(5): p. 932-40.
106. Somogyvári, M., E. Gecse, and C. Sőti, *DAF-21/Hsp90 is required for C. elegans longevity by ensuring DAF-16/FOXO isoform A function.* Sci Rep, 2018. **8**(1): p. 12048.
107. Bargmann, C.I., *Chemosensation in C. elegans.* WormBook, 2006: p. 1-29.
108. Ventura, N., et al., *p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress.* Aging Cell, 2009. **8**(4): p. 380-93.
109. Schafer, P., et al., *Cytochrome P450-dependent metabolism of PCB52 in the nematode Caenorhabditis elegans.* Arch Biochem Biophys, 2009. **488**(1): p. 60-8.
110. Alcántar-Fernández, J., et al., *High-glucose diets induce mitochondrial dysfunction in Caenorhabditis elegans.* PLoS One, 2019. **14**(12): p. e0226652.
111. Hu, Q., et al., *The Caenorhabditis elegans Oxidative Stress Response Requires the NHR-49 Transcription Factor.* G3 (Bethesda), 2018. **8**(12): p. 3857-3863.
112. Al-Amin, M., et al., *Caffeine Induces the Stress Response and Up-Regulates Heat Shock Proteins in Caenorhabditis elegans.* Mol Cells, 2016. **39**(2): p. 163-8.
113. Bennett, C.F., et al., *Activation of the mitochondrial unfolded protein response does not predict longevity in Caenorhabditis elegans.* Nat Commun, 2014. **5**: p. 3483.
114. Hu, Z., et al., *Neurexin and neuroligin mediate retrograde synaptic inhibition in C. elegans.* Science, 2012. **337**(6097): p. 980-4.
115. Mahesh, R., et al., *Probiotics Interactions and the Modulation of Major Signalling Pathways in Host Model Organism Caenorhabditis elegans.* Indian J Microbiol, 2021. **61**(4): p. 404-416.
116. Eghbaliferiz, S. and M. Iranshahi, *Prooxidant Activity of Polyphenols, Flavonoids, Anthocyanins and Carotenoids: Updated Review of Mechanisms and Catalyzing Metals.* Phytother Res, 2016. **30**(9): p. 1379-91.

117. Dvorak, P., et al., *Exacerbation of substrate toxicity by IPTG in Escherichia coli BL21(DE3) carrying a synthetic metabolic pathway.* Microb Cell Fact, 2015. **14**: p. 201.
118. Dufort-Gervais, J., et al., *Neuroligin-1 is altered in the hippocampus of Alzheimer's disease patients and mouse models, and modulates the toxicity of amyloid-beta oligomers.* Sci Rep, 2020. **10**(1): p. 6956.
119. Hunter, J.W., et al., *Neuroligin-deficient mutants of C. elegans have sensory processing deficits and are hypersensitive to oxidative stress and mercury toxicity.* Dis Model Mech, 2010. **3**(5-6): p. 366-76.
120. Nolan, J.M., et al., *Nutritional Intervention to Prevent Alzheimer's Disease: Potential Benefits of Xanthophyll Carotenoids and Omega-3 Fatty Acids Combined.* J Alzheimers Dis, 2018. **64**(2): p. 367-378.
121. Calahorro, F., *Conserved and divergent processing of neuroligin and neurexin genes: from the nematode C. elegans to human.* Invert Neurosci, 2014. **14**(2): p. 79-90.
122. Manzine, P.R., et al., *ADAM10 in Alzheimer's disease: Pharmacological modulation by natural compounds and its role as a peripheral marker.* Biomed Pharmacother, 2019. **113**: p. 108661.
123. Qazi, T.J., et al., *Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation.* Mol Neurobiol, 2018. **55**(2): p. 1026-1044.
124. Izquierdo, P.G., F. Calahorro, and M. Ruiz-Rubio, *Neuroligin modulates the locomotory dopaminergic and serotonergic neuronal pathways of C. elegans.* Neurogenetics, 2013. **14**(3-4): p. 233-42.
125. Fernandes, E.J., et al., *Exposure to lutein-loaded nanoparticles attenuates Parkinson's model-induced damage in Drosophila melanogaster: Restoration of dopaminergic and cholinergic system and oxidative stress indicators.* Chem Biol Interact, 2021. **340**: p. 109431.
126. Rodríguez-Ramos, Á., et al., *Impaired Dopamine-Dependent Locomotory Behavior of C. elegans Neuroligin Mutants Depends on the Catechol-O-Methyltransferase COMT-4.* Behav Genet, 2017. **47**(6): p. 596-608.
127. Janmaat, M.L., et al., *Kahalalide F induces necrosis-like cell death that involves depletion of ErbB3 and inhibition of Akt signaling.* Mol Pharmacol, 2005. **68**(2): p. 502-10.
128. von Schwarzenberg, K. and A.M. Vollmar, *Targeting apoptosis pathways by natural compounds in cancer: marine compounds as lead structures and chemical tools for cancer therapy.* Cancer Lett, 2013. **332**(2): p. 295-303.
129. Milani, A., et al., *Carotenoids: biochemistry, pharmacology and treatment.* Br J Pharmacol, 2017. **174**(11): p. 1290-1324.
130. Geiss, J.M.T., et al., *Oral administration of lutein attenuates ethanol-induced memory deficit in rats by restoration of acetylcholinesterase activity.* Physiol Behav, 2019. **204**: p. 121-128.
131. Ho, K., N.E. Bodi, and T.P. Sharma, *Normal-Tension Glaucoma and Potential Clinical Links to Alzheimer's Disease.* J Clin Med, 2024. **13**(7).
132. Xue, C., et al., *Management of Ocular Diseases Using Lutein and Zeaxanthin: What Have We Learned from Experimental Animal Studies?* J Ophthalmol, 2015. **2015**: p. 523027.
133. Basavarajappa, D., et al., *Signalling pathways and cell death mechanisms in glaucoma: Insights into the molecular pathophysiology.* Mol Aspects Med, 2023. **94**: p. 101216.
134. Pan, J., et al., *The role of PI3K signaling pathway in Alzheimer's disease.* Front Aging Neurosci, 2024. **16**: p. 1459025.
135. Nurul Iman Nurul, F., et al., *Lutein: A Comprehensive Review on its Chemical, Biological Activities and Therapeutic Potentials.* Pharmacognosy Journal, 2020. **12**(6s).
136. Durieux, J., S. Wolff, and A. Dillin, *The cell-non-autonomous nature of electron transport chain-mediated longevity.* Cell, 2011. **144**(1): p. 79-91.

6 Appendix

6.1 Data of Fig. 3.1: Oxidative stress resistance of N2

Data for OASIS2: summary of the pool for N2. The tables show number of dead and censored worms for each condition (DMSO, Kahalalide F, Lutein).
(the term “#days” means hours and not days. It has to be written in this mode, so that the OASIS2 programme works.)

% Kahalalide F		
#days	dead	censored
0	0	1
1	28	18
2	9	12
3	10	1
4	16	1
5	9	0
22	24	19

% Lutein		
#days	dead	censored
0	0	0
1	16	16
2	32	7
3	35	3
4	21	3
5	4	0
22	14	2

% control (DMSO)		
#days	dead	censored
0	0	0
1	63	4
2	56	5
3	14	1
4	3	0
5	2	0
22		0

Data from OASIS2:

(„days“ mentioned in the tables are hours, as it is explained before)

% Kahalalide F	
Time (days)	Percent survival
0.000000	100.000000
1.000000	80.952381
2.000000	73.738802
3.000000	64.521452
4.000000	49.559956
5.000000	40.982271
22.000000	18.108445

% Lutein	
Time (days)	**Percent survival**
0.000000	100.000000
1.000000	89.542484
2.000000	65.861827
3.000000	37.750071
4.000000	19.732992
5.000000	15.786394
22.000000	1.973299

% control (DMSO)	
Time (days)	**Percent survival**
0.000000	100.000000
1.000000	58.278146
2.000000	19.426049
3.000000	7.601497
4.000000	4.750936
5.000000	2.850561
22.000000	0.000000

condition	**chi**	**p-value**	**corrected_p-value**	
Kahalalide F v.s. Lutein	18.54	0.000017	0.000033	****
Kahalalide F v.s. control (DMSO)	94.58	0	0	****
Lutein v.s. Kahalalide F	18.54	0.000017	0.000033	****
Lutein v.s. control (DMSO)	65.84	0	0	****
control (DMSO) v.s. Kahalalide F	94.58	0	0	****
control (DMSO) v.s. Lutein	65.84	0	0	****

Data used for Fig. 3.1 in Microsoft Excel:

Here the table shows each timepoint, starting with timepoint 0, when 100% of N2 strain from each condition were alive.

Time (hours)	N2 on Kahalalide F	N2 on Lutein	N2 on DMSO
0	100	100	100
1	81	90	58
2	74	66	19
3	65	38	8
4	50	20	5
5	41	16	3
22	18	2	0

6.2 Data of *daf-16*::*GFP*-localization: Direct and 2h-later observation of *daf-16::GFP*-localization without/with heat shock (for figures shown in results (see subchapter 3.2)):

Two replicates were analysed, namely replicate 2 and replicate 3. Replicate 1 was left out. The numbers in the tables depict the percentage of worms with the particular *daf-16::GFP*- localization (cytosolic, intermediate, nuclear). Without heat shock all worms showed cytosolic *daf-16::GFP*-localization. The conditions were tested without heat shock and with heat shock (duration: 15min, 30min, 60min) and were observed directly after heat shock and 2h hours after heat shock. Data were then analysed by *GraphPad Prism 9* (see figures in subchapter 3.2).

***daf-16*-pool-0min-directly**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	100	100	0	0	0	0
Kahalalide F	100	100	0	0	0	0
Lutein	100	100	0	0	0	0

***daf-16*-pool-0min-after 2h**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	100	100	0	0	0	0
Kahalalide F	100	100	0	0	0	0
Lutein	100	100	0	0	0	0

***daf-16*-pool-15min-directly**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	10	40	90	60	0	0
Kahalalide F	21.43	62.5	78.57	37.5	0	0
Lutein	33.33	100	66.67	0	0	0

***daf-16*-pool-15min-after 2h**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	100	100	0	0	0	0
Kahalalide F	100	100	0	0	0	0
Lutein	100	100	0	0	0	0

***daf-16*-pool-30min-directly**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	0	0	100	100	0	0
Kahalalide F	0	0	100	100	0	0
Lutein	0	0	100	100	0	0

***daf-16*-pool-30min-after 2h**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	23.07	6.67	76.92	93.3	0	0
Kahalalide F	20	0	80	100	0	0
Lutein	6.67	61.54	93.33	38.46	0	0

***daf-16*-pool-60min-directly**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	0	0	0	0	100	100
Kahalalide F	0	0	0	0	100	100
Lutein	0	0	0	0	100	100

***daf-16*-pool-60min-after 2h**						
	cytosolic		intermediate		nuclear	
	replicate2	replicate3	replicate2	replicate3	replicate2	replicate3
DMSO	0	0	100	100	0	0
Kahalalide F	0	0	100	100	0	0
Lutein	0	0	100	100	0	0

6.3 Data of *cyp14A4::GFP-* expression due to pre-treatment with compounds (see Fig. 3.11):

Here, four replicates were used. The following tables show the pool of replicates.

DMSO (control)_*cyp14a4*			
1.19782094	1.31160762	0.8879316	1.06885948
1.21359897	1.13084888	0.89501383	0.87056658
0.75963629	1.1674039	0.99458863	1.00216631
0.56134851	0.30497013	0.97206662	0.91706513
0.45673061	1.08805536	1.1145057	0.95328063
1.35130849	1.14751272	1.13156736	0.93278996
1.25731338	0.25138365	0.98069566	1.03224253
1.32275593	1.05877271	1.07766188	0.93358255
0.87949655	0.97487345	1.11502445	0.75602625
	1.13250884	1.15482569	1.00393558
	1.08157926	0.6761059	0.97047306
	1.16279004		1.71452289
	1.02782635		0.9986807
	1.15984256		0.80590032
			0.96578733
			1.07410499

Kahalalide F_*cyp14a4*			
0.87362712	0.96737901	0.84334698	0.71865746
1.06683838	0.98263171	1.00477932	0.74433837
1.07923718	1.01838993	0.88992478	0.73990793
1.03269349	0.95567312	0.87640807	0.68123809
0.98369711	1.01419186	0.94374081	0.75110911
1.15437789	1.04029693	1.03755946	0.90068589
1.09915363	1.03107564	0.64600357	0.85068253
1.10826097	2.63766739	0.58564564	0.87668291
1.28352957	1.0925242	0.92249859	0.74436133
1.01444125	1.12001097	0.96295208	1.16321175
0.92802891	1.07850817	0.93789026	0.8418624
1.08358501		0.96145562	0.82134503
0.99319202		1.04659982	0.85260639
1.09681222		0.88749646	0.93884914
1.01282325			0.76594532
0.82280341			
1.28988303			
1.20083876			
1.12590287			
1.15938797			

Lutein_*cyp14a4*			
1.14072404	1.12466871	1.00499015	0.90472426
1.01864815	1.27828797	1.012254	1.0097554
1.20493863	1.19313117	1.07237144	0.97058455
1.27984859	0.98643325	0.9069752	1.01265968
1.18654315	1.13196141	0.94785619	1.01391415
1.26451685	1.21986983	0.95790708	0.8702279
1.20728234	1.44190126	1.10640934	1.00622622
1.21758061	1.18088427	0.85271639	0.90027274
1.22821108	1.04814242	1.01573106	1.17092544
1.07807931	0.98213298	1.00315969	1.0488438
0.85739188	1.06803628		0.98267996
0.98445678	1.28670819		1.03850501
0.92073721	0.99914143		1.08272838
1.02547613	1.11516379		0.89033118
1.13268544			
1.19178399			
1.310741			

pool-*cyp14a4*-ANOVA results	
Table Analyzed	cyp14A4-pool
Data sets analyzed	A-C
ANOVA summary	
F	2.005
P value	0.1453
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.07425

pool-*cyp14a4*-ANOVA multiple comparisons								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
DMSO (control)_cyp14a4 vs. Kahalalide F_cyp14a4	0.006127	-0,1030 to 0,1153	No	ns	>0,9999	A-B		
DMSO (control)_cyp14a4 vs. Lutein_cyp14a4	-0.07383	-0,1872 to 0,03952	No	ns	0.3388	A-C		

Kahalalide F_cyp14a4 vs. Lutein_cyp14a4	-0.07996	-0,1873 to 0,02740	No	ns	0.2129	B-C		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF
DMSO (control)_cyp14a4 vs. Kahalalide F_cyp14a4	1.016	1.009	0.006127	0.04406	16	20	0.139	50
DMSO (control)_cyp14a4 vs. Lutein_cyp14a4	1.016	1.089	-0.07383	0.04576	16	17	1.613	50
Kahalalide F_cyp14a4 vs. Lutein_cyp14a4	1.009	1.089	-0.07996	0.04334	20	17	1.845	50

pool-*cyp14a4*-ANOVA-descriptive statistics			
	DMSO (control)_*cyp14a4*	**Kahalalide F_*cyp14a4***	**Lutein_*cyp14a4***
Number of values	16	20	17
Minimum	0.6889	0.8228	0.9694
25% Percentile	0.9149	0.9108	1.033
Median	0.9981	0.9598	1.078
75% Percentile	1.098	1.111	1.124
Maximum	1.439	1.302	1.311
Mean	1.016	1.009	1.089
Std. Deviation	0.1597	0.1392	0.08378
Std. Error of Mean	0.03993	0.03112	0.02032
Lower 95% CI	0.9304	0.9443	1.046
Upper 95% CI	1.101	1.075	1.132

6.4 Data of *gst-4::GFP*- expression due to pre-treatment with compounds:

Here, three replicates were used. The following tables show the pool of replicates.

DMSO (control) _*gst-4*		
0.95512001	0.99977079	0.80616439
1.07447627	0.93824557	1.0354454
0.57965763	1.18837055	0.995424
0.94661494	0.63425145	1.29435588
1.53312601	1.08609263	0.75083102
0.65447354	1.11612128	1.12601655
0.82883245	0.71824354	0.92835242
0.51777041	0.78646124	0.95100234
1.62840135	1.01580413	1.16645287
0.93140945	0.93633008	1.13678615

0.7135353	1.12552448	1.04979054
1.1508743	1.36177298	0.76758089
1.36079046	0.74737199	0.99179948
1.12492034	0.90054006	
	1.18947296	
	1.25561293	

Kahalalide F_*gst-4*		
0.60659833	1.01846476	1.11814982
1.037821	0.88273322	1.106481
0.78668265	0.93822559	1.02408664
0.70875712	1.00537818	1.29335902
1.26733693	1.12209472	1.33815833
0.85992412	2.24678826	1.09816871
0.6880316	0.88892831	1.19578489
0.8256321	1.08027211	0.97666208
0.86319533	1.11619428	1.09395533
0.65600804	1.16222617	1.17345133
0.79532178	1.0480938	1.07435277
0.91001542	1.55793266	0.83692741
0.66976705	2.15643404	0.98842163
0.66967722	2.08923509	0.84817291
0.62720514	1.24947192	1.0182662
0.78703248	1.28855024	0.87606462
		1.17237125
		0.92414414
		1.39014268
		1.43338901

Lutein_*gst-4*		
1.00994435	1.09827875	1.29495795
0.82403802	1.02942353	1.00273372
0.80932116	0.75842693	1.19739585
0.96650133	0.99873818	0.59907212
1.21621555	1.07531396	1.38644966
1.178458	0.8405006	0.97503068
0.68288802	0.79992	0.77077127
0.80209645	0.98299922	0.8785706
1.14477151	0.60464106	1.22890818
0.76419761	0.63971498	1.20505379
1.07574264	1.22222752	0.86665482
0.74496822	0.59313456	1.88209537
0.8447518	0.39761932	0.18977967
	1.03353077	0.73564705

	0.65050777	1.36971211
	0.58595881	2.06531432
	0.77237002	
	1.24516993	

pool-*gst-4*-ANOVA results	
Table Analyzed	gst-4_pool
Data sets analyzed	A-C
ANOVA summary	
F	2.329
P value	0.1077
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.08368

pool-*gst-4*-ANOVA-multiple comparisons								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
DMSO (control) _gst-4 vs. Kahalalide F_gst-4	-0.0717	-0,2174 to 0,07396	No	ns	0.6859	A-B		
DMSO (control) _gst-4 vs. Lutein_gst-4	0.05037	-0,09885 to 0,1996	No	ns	>0,9999	A-C		
Kahalalide F_gst-4 vs. Lutein_gst-4	0.1221	-0,01903 to 0,2632	No	ns	0.1111	B-C		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF
DMSO (control) _gst-4 vs. Kahalalide F_gst-4	1.019	1.091	-0.0717	0.05884	16	20	1.219	51
DMSO (control) _gst-4 vs. Lutein_gst-4	1.019	0.9684	0.05037	0.06028	16	18	0.8356	51
Kahalalide F_gst-4 vs. Lutein_gst-4	1.091	0.9684	0.1221	0.057	20	18	2.142	51

pool-*gst-4*-ANOVA-descriptive statistics			
	DMSO (control) _*gst-4*	Kahalalide F_*gst-4*	Lutein_*gst-4*
Number of values	16	20	18
Minimum	0.7517	0.9144	0.4774
25% Percentile	0.9305	0.9619	0.8659
Median	1.007	1.006	0.9724
75% Percentile	1.116	1.232	1.089
Maximum	1.27	1.433	1.326
Mean	1.019	1.091	0.9684
Std. Deviation	0.1422	0.1749	0.2008
Std. Error of Mean	0.03554	0.03911	0.04732
Lower 95% CI	0.9431	1.009	0.8686
Upper 95% CI	1.095	1.172	1.068

6.5 Data of *hsp-6::GFP*-expression due to pre-treatment with compounds:

Here, three replicates were used. The following tables show the pool of replicates.

DMSO (control)_*hsp-6*		
0.9003585	0.87893252	0.86073078
1.13896578	1.1926786	0.95496999
0.86511021	0.95624187	1.18318144
1.04269735	0.68327951	0.84109653
0.92097721	0.79521993	0.82875328
1.20681716	0.75813493	0.80772301
0.98831887	1.01028265	1.24595675
1.25484111	2.17364004	1.07088995
0.91597683	0.95123334	1.00002719
1.00497648	0.97729301	0.92404403
0.77665211	0.99886535	1.15836272
1.07615481	0.8069279	1.14286608
0.90814401	0.80025917	1.09087213
	1.03249263	0.89052167
	1.09856659	
	0.93079873	
	0.95744314	
	0.66709167	
	1.1517584	
	1.12686033	
	0.86451726	

	1.18749307	

Kahalalide F_*hsp-6*		
1.19041981	0.89951407	1.12993339
0.91955296	1.05186032	0.88135654
0.9265203	0.97185264	1.10054255
1.5674098	0.91787776	0.79304533
1.14463097	0.88454008	0.96343863
1.45435875	1.00906709	0.91843172
1.38591589	0.84596037	0.84625485
0.88288625	0.99695756	0.87128505
1.65187759	0.65440614	0.81650532
0.85159906	1.17523042	0.93377183
1.29093238	1.01190212	0.83137867
1.48720193	0.80415089	
1.15955674	1.08836677	
1.357474	1.24411574	
	1.25524002	
	1.16751652	
	1.59911256	
	1.00911359	

Lutein_*hsp-6*		
0.70066853	0.98376725	0.98995671
1.18644761	0.74941318	0.84515445
2.28062936	0.77633164	0.94484051
0.93872988	0.90695244	1.23476277
1.02383764	1.37204994	1.91693088
1.56059931	0.90048054	0.90612679
2.67319124	0.69345822	1.39685937
0.94497078	0.75488341	1.41317173
1.07809578	0.96739623	1.01287066
1.26366296	0.70799865	1.24910548
0.92351493	0.88145896	1.14617641
1.6295253	0.84020736	1.31212228
1.87668542	1.11611841	1.44897396
1.13024616	0.86142782	1.07588078
1.92233873	0.98585738	1.26816144
1.72930004	0.08680629	1.39124909
	0.97575992	
	1.03513466	
	0.08389641	
	0.91563324	
	0.76483053	

	0.82384892	
	0.89392186	
	0.69549161	
	0.85324519	
	0.86694902	

***hsp-6*-pool-ANOVA results**	
Table Analyzed	hsp6-pool
Data sets analyzed	A-C
ANOVA summary	
F	1.147
P value	0.3241
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.03513

pool-*hsp-6*-ANOVA-multiple comparisons								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
DMSO (control)_hsp-6 vs. Kahalalide F_hsp-6	-0.1043	-0,2805 to 0,07193	No	ns	0.4513	A-B		
DMSO (control)_hsp-6 vs. Lutein_hsp-6	-0.02317	-0,1838 to 0,1375	No	ns	>0,9999	A-C		
Kahalalide F_hsp-6 vs. Lutein_hsp-6	0.08113	-0,08889 to 0,2511	No	ns	0.7349	B-C		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF
DMSO (control)_hsp-6 vs. Kahalalide F_hsp-6	0.9989	1.103	-0.1043	0.07165	22	18	1.456	63
DMSO (control)_hsp-6 vs. Lutein_hsp-6	0.9989	1.022	-0.02317	0.06531	22	26	0.3549	63
Kahalalide F_hsp-6 vs. Lutein_hsp-6	1.103	1.022	0.08113	0.06913	18	26	1.174	63

pool-*hsp-6*-ANOVA-descriptive statistics			
	DMSO (control)_*hsp-6*	**Kahalalide F_*hsp-6***	**Lutein_*hsp-6***
Number of values	22	18	26

Minimum	0.6671	0.917	0.0839
25% Percentile	0.9132	0.9991	0.8853
Median	0.9651	1.059	1.021
75% Percentile	1.096	1.151	1.157
Maximum	1.5	1.599	1.588
Mean	0.9989	1.103	1.022
Std. Deviation	0.1631	0.1593	0.2974
Std. Error of Mean	0.03478	0.03755	0.05833
Lower 95% CI	0.9266	1.024	0.902
Upper 95% CI	1.071	1.182	1.142

6.6 Data of *nlg-1::GFP*-expression due to pre-treatment with compounds:

Here, three replicates were used. The following tables show the pool of replicates.

Control (DMSO)_*nlg-1*		
0.97093521	0.82752242	0.83037112
1.62760053	0.76262499	0.91704939
0.95679087	1.20665871	0.9737203
1.32100873	0.85785055	1.24822626
0.87035378	0.97935989	1.25020515
0.90827579	0.68131022	1.26515936
0.77217775	1.09947916	0.60858663
1.03474687	1.25172161	1.16463784
0.91017736	1.3913103	0.67889191
0.81002106	0.94212869	1.06316925
0.81788407		

Kahalalide F_*nlg-1*		
1.00539262	1.32209074	0.69752036
1.03621174	2.89756687	1.48964847
0.88979039	1.69965307	1.52224689
1.33839024	1.35364283	2.37339396
1.6099003	1.13654053	1.99238401
1.29935283	0.92701335	1.58430472
1.49279317	0.94946784	0.85200102
1.12183066	0.91322168	1.44130017
1.23349848	1.55389778	2.31906036

1.34916015	1.34134586	1.5987157
0.85454514	0.76772453	1.83070994
1.46286189	0.6435848	
	1.76599253	
	2.43350486	
	1.84935403	
	0.9394383	
	1.54071497	
	1.5672334	
	1.55475017	

Lutein_*nlg-1*		
1.18914376	0.71393926	1.27604855
1.00854382	0.53619888	1.17110765
0.96846127	1.28086916	1.21974625
0.99556214	0.90236706	0.77346912
1.13722377	1.29203	1.17307113
1.14911111	2.48464197	0.7379921
1.92195884	2.14739314	1.19983507
1.22365332	1.75954998	1.0540618
1.22655802	1.11696012	1.06258705
0.81182295	1.13102589	0.82431558
	1.55741732	1.09819351
	1.96286539	1.52000988
	0.87649868	1.68387545
	1.10309946	

pool-*nlg-1*-gfp-ANOVA results	
Table Analyzed	nlg-1-GFP_pool
Data sets analyzed	A-C
ANOVA summary	
F	9.465
P value	0.0004
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R squared	0.3159

pool-*nlg-1-gfp*-ANOVA-multiple comparisons								
Number of families	1							

Number of comparisons per family	3							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
Control (DMSO)_nlg-1 vs. Kahalalide F_nlg-1	-0.4831	-0,7632 to -0,2030	Yes	***	0.0003	A-B		
Control (DMSO)_nlg-1 vs. Lutein_nlg-1	-0.2455	-0,5434 to 0,05241	No	ns	0.1382	A-C		
Kahalalide F_nlg-1 vs. Lutein_nlg-1	0.2376	-0,02281 to 0,4980	No	ns	0.0841	B-C		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF
Control (DMSO)_nlg-1 vs. Kahalalide F_nlg-1	0.989	1.472	-0.4831	0.1122	11	19	4.305	41
Control (DMSO)_nlg-1 vs. Lutein_nlg-1	0.989	1.234	-0.2455	0.1193	11	14	2.057	41
Kahalalide F_nlg-1 vs. Lutein_nlg-1	1.472	1.234	0.2376	0.1043	19	14	2.278	41

pool-*nlg-1-gfp*-ANOVA-descriptive statistics			
	Control (DMSO)_*nlg-1*	**Kahalalide F_*nlg-1***	**Lutein_*nlg-1***
Number of values	11	19	14
Minimum	0.8179	0.9394	0.8905
25% Percentile	0.8763	1.151	1.025
Median	0.9935	1.541	1.179
75% Percentile	1.102	1.702	1.374
Maximum	1.15	2.434	1.756
Mean	0.989	1.472	1.234
Std. Deviation	0.1183	0.3701	0.2761
Std. Error of Mean	0.03566	0.08491	0.0738
Lower 95% CI	0.9095	1.294	1.075
Upper 95% CI	1.068	1.65	1.394

6.7 Data of locomotion of N2 and *nlg-1* knock-out strain:

Pool of N2 strain:

N2_1d-adult_DMSO		
replicate 1	replicate 2	replicate 3
92.3076923	114.285714	112.676056
92.3076923	85.7142857	84.5070423
107.692308	100	98.5915493
92.3076923	114.285714	112.676056
107.692308	85.7142857	98.5915493
123.076923	85.7142857	98.5915493
92.3076923	85.7142857	112.676056
92.3076923	128.571429	112.676056
123.076923	100	70.4225352
76.9230769	100	98.5915493

N2_5d-adult_DMSO		
replicate 1	replicate 2	replicate 3
46.1538462	57.1428571	56.3380282
61.5384615	71.4285714	56.3380282
76.9230769	71.4285714	56.3380282
61.5384615	71.4285714	84.5070423
76.9230769	71.4285714	70.4225352
61.5384615	57.1428571	70.4225352
61.5384615	71.4285714	70.4225352
61.5384615	71.4285714	70.4225352
61.5384615	57.1428571	70.4225352
46.1538462	71.4285714	56.3380282

N2_8d-adult_DMSO		
replicate 1	replicate 2	replicate 3
92.3076923	28.5714286	42.2535211
61.5384615	14.2857143	56.3380282
46.1538462	57.1428571	28.1690141
46.1538462	57.1428571	28.1690141
30.7692308	42.8571429	56.3380282
61.5384615	57.1428571	42.2535211
46.1538462	42.8571429	28.1690141
61.5384615	28.5714286	42.2535211
30.7692308	57.1428571	42.2535211
30.7692308	42.8571429	42.2535211

N2_1d-adult_Kahalalide F		
replicate 1	replicate 2	replicate 3
76.9230769	85.7142857	126.760563
123.076923	85.7142857	112.676056
123.076923	100	126.760563
76.9230769	100	126.760563
107.692308	114.285714	126.760563
107.692308	114.285714	140.84507
92.3076923	85.7142857	126.760563
76.9230769	71.4285714	126.760563
92.3076923	100	112.676056
76.9230769	57.1428571	112.676056

N2_5d-adult_Kahalalide F		
replicate 1	replicate 2	replicate 3
92.3076923	57.1428571	98.5915493
92.3076923	71.4285714	112.676056
76.9230769	85.7142857	126.760563
76.9230769	71.4285714	126.760563
76.9230769	71.4285714	126.760563
92.3076923	71.4285714	112.676056
76.9230769	71.4285714	98.5915493
92.3076923	71.4285714	84.5070423
92.3076923	71.4285714	84.5070423
92.3076923	71.4285714	84.5070423

N2_8d-adult_Kahalalide F		
replicate 1	replicate 2	replicate 3
92.3076923	57.1428571	70.4225352
46.1538462	42.8571429	112.676056
61.5384615	42.8571429	84.5070423
92.3076923	42.8571429	84.5070423
61.5384615	42.8571429	112.676056
76.9230769	42.8571429	70.4225352
76.9230769	42.8571429	112.676056
76.9230769	42.8571429	84.5070423
61.5384615	42.8571429	70.4225352
76.9230769	42.8571429	42.2535211

locomotion-pool-N2_1d,5d,8d	
Table Analyzed	N2-1d,5d,8d-pool
Data sets analyzed	A-F
ANOVA summary	
F	90.63
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.8935

locomotion-pool-multiple comparisons-N2_1d,5d,8d								
Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
N2_1d-adult_DMSO vs. N2_5d-adult_DMSO	35.04	24,59 to 45,49	Yes	****	<0,0001	A-B		
N2_1d-adult_DMSO vs. N2_8d-adult_DMSO	55.18	44,72 to 65,63	Yes	****	<0,0001	A-C		
N2_1d-adult_DMSO vs. N2_1d-adult_Kahalalide F	-3.586	-14,04 to 6,868	No	ns	>0,9999	A-D		
N2_1d-adult_DMSO vs. N2_5d-adult_Kahalalide F	12.26	1,808 to 22,71	Yes	*	0.0103	A-E		
N2_1d-adult_DMSO vs. N2_8d-adult_Kahalalide F	32.97	22,51 to 43,42	Yes	****	<0,0001	A-F		
N2_5d-adult_DMSO vs. N2_8d-adult_DMSO	20.14	9,682 to 30,59	Yes	****	<0,0001	B-C		
N2_5d-adult_DMSO vs. N2_1d-adult_Kahalalide F	-38.63	-49,08 to -28,17	Yes	****	<0,0001	B-D		
N2_5d-adult_DMSO vs. N2_5d-adult_Kahalalide F	-22.78	-33,23 to -12,33	Yes	****	<0,0001	B-E		
N2_5d-adult_DMSO vs. N2_8d-adult_Kahalalide F	-2.074	-12,53 to 8,379	No	ns	>0,9999	B-F		
N2_8d-adult_DMSO vs. N2_1d-adult_Kahalalide F	-58.76	-69,22 to -48,31	Yes	****	<0,0001	C-D		
N2_8d-adult_DMSO vs. N2_5d-adult_Kahalalide F	-42.91	-53,37 to -32,46	Yes	****	<0,0001	C-E		
N2_8d-adult_DMSO vs. N2_8d-adult_Kahalalide F	-22.21	-32,66 to -11,76	Yes	****	<0,0001	C-F		
N2_1d-adult_Kahalalide F vs. N2_5d-adult_Kahalalide F	15.85	5,393 to 26,30	Yes	***	0.0003	D-E		
N2_1d-adult_Kahalalide F vs. N2_8d-adult_Kahalalide F	36.55	26,10 to 47,01	Yes	****	<0,0001	D-F		
N2_5d-adult_Kahalalide F vs. N2_8d-adult_Kahalalide F	20.71	10,25 to 31,16	Yes	****	<0,0001	E-F		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF

N2_1d-adult_DMSO vs. N2_5d-adult_DMSO	100	64.96	35.04	3.403	10	10	10.3	54
N2_1d-adult_DMSO vs. N2_8d-adult_DMSO	100	44.82	55.18	3.403	10	10	16.21	54
N2_1d-adult_DMSO vs. N2_1d-adult_Kahalalide F	100	103.6	-3.586	3.403	10	10	1.054	54
N2_1d-adult_DMSO vs. N2_5d-adult_Kahalalide F	100	87.74	12.26	3.403	10	10	3.603	54
N2_1d-adult_DMSO vs. N2_8d-adult_Kahalalide F	100	67.03	32.97	3.403	10	10	9.686	54
N2_5d-adult_DMSO vs. N2_8d-adult_DMSO	64.96	44.82	20.14	3.403	10	10	5.916	54
N2_5d-adult_DMSO vs. N2_1d-adult_Kahalalide F	64.96	103.6	-38.63	3.403	10	10	11.35	54
N2_5d-adult_DMSO vs. N2_5d-adult_Kahalalide F	64.96	87.74	-22.78	3.403	10	10	6.693	54
N2_5d-adult_DMSO vs. N2_8d-adult_Kahalalide F	64.96	67.03	-2.074	3.403	10	10	0.6094	54
N2_8d-adult_DMSO vs. N2_1d-adult_Kahalalide F	44.82	103.6	-58.76	3.403	10	10	17.27	54
N2_8d-adult_DMSO vs. N2_5d-adult_Kahalalide F	44.82	87.74	-42.91	3.403	10	10	12.61	54
N2_8d-adult_DMSO vs. N2_8d-adult_Kahalalide F	44.82	67.03	-22.21	3.403	10	10	6.526	54
N2_1d-adult_Kahalalide F vs. N2_5d-adult_Kahalalide F	103.6	87.74	15.85	3.403	10	10	4.656	54
N2_1d-adult_Kahalalide F vs. N2_8d-adult_Kahalalide F	103.6	67.03	36.55	3.403	10	10	10.74	54
N2_5d-adult_Kahalalide F vs. N2_8d-adult_Kahalalide F	87.74	67.03	20.71	3.403	10	10	6.084	54

locomotion-pool-descriptive statistics_N2_1d,5d,8d						
	N2_1d-adult_DMSO	N2_5d-adult_DMSO	N2_8d-adult_DMSO	N2_1d-adult_Kahalalide F	N2_5d-adult_Kahalalide F	N2_8d-adult_Kahalalide F
Number of values	10	10	10	10	10	10
Minimum	87.51	53.21	38.63	82.25	82.31	54.01
25% Percentile	95.63	61.77	42.26	95.28	82.73	61.79
Median	99.96	65.45	43.82	101.6	87.23	67.66
75% Percentile	106.4	69.3	46.5	116.3	92.14	73.24
Maximum	111.2	72.92	54.38	120.9	96.47	77.49
Mean	100	64.96	44.82	103.6	87.74	67.03
Std. Deviation	7.166	6.188	5.251	12.04	5.538	7.396
Std. Error of Mean	2.266	1.957	1.66	3.807	1.751	2.339
Lower 95% CI	94.87	60.53	41.07	94.97	83.78	61.74

Upper 95% CI	105.1	69.39	48.58	112.2	91.7	72.32

Pool of *nlg-1* knock-out strain:

***nlg-1*-k.o._1d_DMSO**		
replicate 1	replicate 2	replicate 3
100	125	107.692308
83.3333333	107.142857	107.692308
100	89.2857143	107.692308
133.333333	125	92.3076923
133.333333	125	76.9230769
100	160.714286	123.076923
83.3333333	107.142857	123.076923
133.333333	35.7142857	76.9230769
66.6666667	107.142857	92.3076923
66.6666667	17.8571429	92.3076923

***nlg-1*-k.o._5d_DMSO**		
replicate 1	replicate 2	replicate 3
50	71.4285714	46.1538462
50	53.5714286	61.5384615
50	71.4285714	46.1538462
33.3333333	35.7142857	61.5384615
66.6666667	35.7142857	92.3076923
50	71.4285714	46.1538462
33.3333333	89.2857143	30.7692308
33.3333333	71.4285714	46.1538462
33.3333333	53.5714286	30.7692308
33.3333333	71.4285714	61.5384615

***nlg-1*-k.o._8d_DMSO**		
replicate 1	replicate 2	replicate 3
33.3333333	53.5714286	30.7692308
16.6666667	17.8571429	30.7692308
16.6666667	17.8571429	15.3846154
50	17.8571429	30.7692308
33.3333333	71.4285714	30.7692308
33.3333333	17.8571429	46.1538462
33.3333333	17.8571429	46.1538462
50	17.8571429	30.7692308
50	35.7142857	30.7692308
16.6666667	35.7142857	15.3846154

***nlg-1*-k.o._1d_Kahalalide F**		
replicate 1	replicate 2	replicate 3
66.6666667	107.142857	107.692308
100	89.2857143	92.3076923
100	107.142857	92.3076923
100	125	123.076923
83.3333333	89.2857143	107.692308
116.666667	125	92.3076923
100	107.142857	92.3076923
116.666667	89.2857143	107.692308
100	89.2857143	123.076923
33.3333333	53.5714286	46.1538462

***nlg-1*-k.o._5d_Kahalalide F**		
replicate 1	replicate 2	replicate 3
83.3333333	53.5714286	92.3076923
66.6666667	53.5714286	107.692308
50	71.4285714	46.1538462
66.6666667	89.2857143	107.692308
100	35.7142857	76.9230769
50	53.5714286	46.1538462
33.3333333	35.7142857	92.3076923
66.6666667	35.7142857	76.9230769
33.3333333	35.7142857	76.9230769
33.3333333	35.7142857	76.9230769

***nlg-1*-k.o._8d_Kahalalide F**		
replicate 1	replicate 2	replicate 3
50	35.7142857	46.1538462
66.6666667	53.5714286	76.9230769
66.6666667	35.7142857	76.9230769
50	17.8571429	107.692308
33.3333333	35.7142857	46.1538462
50	35.7142857	76.9230769
83.3333333	35.7142857	107.692308
50	35.7142857	46.1538462
33.3333333	53.5714286	92.3076923
33.3333333	53.5714286	46.1538462

pool-*nlg-1*-knock-out_1d,5d,8d_ANOVA results

Table Analyzed	nlg-1-knock out-1d,5d,8d-pool
Data sets analyzed	A-F
ANOVA summary	
F	34.72
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.7627

pool-*nlg-1*-knock-out_1d,5d,8d-ANOVA-multiple comparisons								
Number of families	1							
Number of comparisons per family	15							
Alpha	0.05							
Bonferroni's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value			
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._5d_DMSO	47.29	27,58 to 67,00	Yes	****	<0,0001	A-B		
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._8d_DMSO	68.51	48,80 to 88,22	Yes	****	<0,0001	A-C		
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	3.886	-15,82 to 23,60	No	ns	>0,9999	A-D		
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	37.22	17,51 to 56,93	Yes	****	<0,0001	A-E		
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	45.58	25,87 to 65,29	Yes	****	<0,0001	A-F		
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._8d_DMSO	21.23	1,517 to 40,94	Yes	*	0.0252	B-C		
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	-43.4	-63,11 to -23,69	Yes	****	<0,0001	B-D		
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	-10.06	-29,77 to 9,646	No	ns	>0,9999	B-E		
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	-1.706	-21,42 to 18,00	No	ns	>0,9999	B-F		
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	-64.63	-84,34 to -44,92	Yes	****	<0,0001	C-D		
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	-31.29	-51,00 to -11,58	Yes	***	0.0001	C-E		
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	-22.93	-42,64 to -3,223	Yes	*	0.0113	C-F		
nlg-1-k.o._1d_Kahalalide F vs. nlg-1-k.o._5d_Kahalalide F	33.34	13,63 to 53,05	Yes	****	<0,0001	D-E		

nlg-1-k.o._1d_Kahalalide F vs. nlg-1-k.o._8d_Kahalalide F	41.69	21,98 to 61,40	Yes	****	<0,0001	D-F		
nlg-1-k.o._5d_Kahalalide F vs. nlg-1-k.o._8d_Kahalalide F	8.358	-11,35 to 28,07	No	ns	>0,9999	E-F		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	t	DF
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._5d_DMSO	100	52.71	47.29	6.417	10	10	7.369	54
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._8d_DMSO	100	31.49	68.51	6.417	10	10	10.68	54
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	100	96.11	3.886	6.417	10	10	0.6055	54
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	100	62.78	37.22	6.417	10	10	5.8	54
nlg-1-k.o._1d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	100	54.42	45.58	6.417	10	10	7.103	54
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._8d_DMSO	52.71	31.49	21.23	6.417	10	10	3.308	54
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	52.71	96.11	-43.4	6.417	10	10	6.763	54
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	52.71	62.78	-10.06	6.417	10	10	1.568	54
nlg-1-k.o._5d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	52.71	54.42	-1.706	6.417	10	10	0.2659	54
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._1d_Kahalalide F	31.49	96.11	-64.63	6.417	10	10	10.07	54
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._5d_Kahalalide F	31.49	62.78	-31.29	6.417	10	10	4.876	54
nlg-1-k.o._8d_DMSO vs. nlg-1-k.o._8d_Kahalalide F	31.49	54.42	-22.93	6.417	10	10	3.574	54
nlg-1-k.o._1d_Kahalalide F vs. nlg-1-k.o._5d_Kahalalide F	96.11	62.78	33.34	6.417	10	10	5.195	54
nlg-1-k.o._1d_Kahalalide F vs. nlg-1-k.o._8d_Kahalalide F	96.11	54.42	41.69	6.417	10	10	6.497	54
nlg-1-k.o._5d_Kahalalide F vs. nlg-1-k.o._8d_Kahalalide F	62.78	54.42	8.358	6.417	10	10	1.302	54

pool-nlg-1-knock out_1d,5d,8d_descriptive statistics						
	nlg-1-k.o._1d_ DMSO	nlg-1-k.o._5d_ DMSO	nlg-1-k.o._8d_ DMSO	nlg-1-k.o._ 1d_ Kahalalide F	nlg-1-k.o._ 5d_ Kahalalide F	nlg-1-k.o._ 8d_ Kahalalide F
Number of values	10	10	10	10	10	10
Minimum	58.94	39.22	16.64	44.35	48.66	38.4
25% Percentile	87.03	48.61	22.38	93.73	49.6	43.96
Median	102	55.24	32.66	99.82	57.81	56.36
75% Percentile	113	55.86	38.93	106.2	76.08	61.26
Maximum	127.9	64.9	45.18	116	87.88	75.58
Mean	100	52.71	31.49	96.11	62.78	54.42
Std. Deviation	19.67	7.192	8.826	19.68	13.98	11.67
Std. Error of Mean	6.219	2.274	2.791	6.225	4.42	3.691
Lower 95% CI	85.93	47.57	25.17	82.03	52.78	46.07
Upper 95% CI	114.1	57.86	37.8	110.2	72.78	62.77

Acknowledgements

During this doctoral dissertation project I gained skills and support and I would like to thank all. First, I thank Mrs. Dr. med. Dr. rer. nat. Natascia Ventura and Mr. Prof. Dr. med. Boege for giving me the opportunity to carry out my project at the IUF Duesseldorf in cooperation with the Heinrich-Heine University and I also thank Mr. Prof. Dr. rer. nat. Reichert for his acceptance to be the second supervisor for my doctoral thesis. Although I had no sufficient laboratory experience in general, the whole research group (AG Ventura) supported me in learning the basic lab skills in a relative short time in order to be able to establish my project. Therefore, I am very thankful towards Mrs. Dr. med. Dr. rer. Natascia Ventura, for being a supporting competent group leader in coordinating journal clubs, discussing and consulting project progressions in lab meetings and during lab work. Accordingly, I would like to acknowledge the whole research group for creating such a peaceful and instructive project phase, including Mrs. Dr. Silvia Maglioni (Postdoc), Mrs. Dr. Margherita Romeo (Postdoc) and Mr. Dr. Alfonso Schiavi (Postdoc) and the two students Mrs. Laura Schröter (PhD-student) and Mrs. Jadranka Skorput (Master student). I am very thankful, especially towards Mrs. Dr. Silvia Maglioni, who I thank for her advice anytime when I had questions due to lab methods and statistical programmes and also Mrs. Dr. Margherita Romeo for her time showing and explaining me laboratory techniques apart from my project.

Of course, I would have not been carrying out my medical study and doctoral thesis without the support of my family: I thank my dear mother, who always supported me in good and difficult times during my whole life, my father for his financial support during my medical study and especially, I thank my dear younger brother Shaian, who supported me anytime he could.

Furthermore, I would like to thank all of my family, my friends and all people who believed in me and motivated me to achieve my goals.

Above all, I thank God.