Multilayered Reprogramming in Response to Persistent DNA Damage in *C. elegans*

**Graphical Abstract**

**Highlights**
- Proteome responses to persistent DNA damage correlate with starvation and aging
- Proteostatic shift reduces ubiquitin proteasome and chaperones and relies on autophagy
- Metabolic adaptations to DNA damage reduce fatty acid synthesis
- Insulin-, EGF-, and AMPK-like signaling pathways respond to UV-induced DNA damage

**Authors**
Diletta Edifizi, Hendrik Nolte, Vipin Babu, ..., Susanne Brodesser, Marcus Krüger, Björn Schumacher

**Correspondence**
bjoern.schumacher@uni-koeln.de

**In Brief**
Edifizi et al. provide a comprehensive proteomics, phosphoproteomics, and lipidomics analysis of the response to persistent DNA damage in a metazoan organism. Proteostasis shifts toward autophagy, fatty acid metabolism is attenuated, and the insulin-, EGF-, and AMPK-like signaling pathways form the center of the response network.

**Accession Numbers**
PXD005649

Edifizi et al., 2017, *Cell Reports* 20, 2026–2043
August 29, 2017 © 2017 The Author(s).
http://dx.doi.org/10.1016/j.celrep.2017.08.028
Multilayered Reprogramming in Response to Persistent DNA Damage in C. elegans

Diletta Edifizi, Hendrik Nolte, Vipin Babu, Laia Castells-Roca, Michael M. Mueller, Susanne Brodesser, Marcus Krüger, and Björn Schumacher

1Institute for Genome Stability in Aging and Disease, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 26, Cologne 50931, Germany
2Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases and Systems Biology of Aging Cologne, University of Cologne, Joseph-Stelzmann-Str. 26, Cologne 50931, Germany
3Center for Molecular Medicine, University of Cologne, Robert-Koch-Str. 21, 50931 Cologne, Germany
4Present address: Centre for Cancer Research and Cell Biology, Queen’s University Belfast, 97 Lisburn Road, Belfast BT9 7AE, UK
5Present address: Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain
6Lead Contact
*Correspondence: bjoern.schumacher@uni-koeln.de
http://dx.doi.org/10.1016/j.celrep.2017.08.028

SUMMARY

DNA damage causally contributes to aging and age-related diseases. Mutations in nucleotide excision repair (NER) genes cause highly complex congenital syndromes characterized by growth retardation, cancer susceptibility, and accelerated aging in humans. Orthologous mutations in Caenorhabditis elegans lead to growth delay, genome instability, and accelerated functional decline, thus allowing investigation of the consequences of persistent DNA damage during development and aging in a simple metazoan model. Here, we conducted proteome, lipidome, and phosphoproteome analysis of NER-deficient animals in response to UV treatment to gain comprehensive insights into the full range of physiological adaptations to unrepair DNA damage. We derive metabolic changes indicative of a tissue maintenance program and implicate an autophagy-mediated proteostatic response. We assign central roles for the insulin-, EGF-, and AMPK-like signaling pathways in orchestrating the adaptive response to DNA damage. Our results provide insights into the DNA damage responses in the organismal context.

INTRODUCTION

DNA damage accumulation is a driving factor for the aging process. Congenital syndromes that are caused by mutations in genome maintenance pathways are characterized by accelerated aging and premature onset of aging-associated diseases (Vijg and Suh, 2013). The role of unrepaired DNA lesions in cancer development and (premature) aging is particularly well exemplified in nucleotide excision repair (NER) deficiency syndromes (Edifizi and Schumacher, 2015). NER removes bulky lesions such as UV-induced cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs). Two distinct NER sub-pathways recognize the lesions: global-genome (GG) NER scans throughout the entire genome, and transcription-coupled (TC) NER initiates repair when RNA polymerase II stalls at a lesion. Although mutations in GG-NER lead to highly elevated skin cancer susceptibility in xeroderma pigmentosum (XP) patients, TC-NER-deficient Cockayne syndrome (CS) patients suffer from growth and mental retardation and premature aging (Ribezzo et al., 2016).

Given the highly complex NER phenotypes in human patients and respective mouse models, we have employed the nematode C. elegans as a metazoan model to better understand the consequences of unrepaired DNA damage. Mutations in the GG-NER gene xpc-1 lead to genome instability in proliferating cells, which in adult nematodes are restricted to the germline, whereas TC-NER-deficient csb-1 mutants cease developmental growth when exposed to UV irradiation (Lans et al., 2010; Mueller et al., 2014). Thus, GG-NER defects are linked to genome instability in proliferating cells, a hallmark of cancer development in humans, whereas TC-NER defects mirror the growth defects and accelerated decline in tissue functionality associated with CS (Edifizi and Schumacher, 2015). We have previously employed the nematode NER mutants to gain insights into the response mechanisms to persistent DNA damage during development and aging. We established that the insulin/insulin-like signaling (IIS) effector DAF-16 counteracts DNA damage-driven aging by elevating tolerance to persistent DNA damage (Mueller et al., 2014). Strikingly, NER-deficient csb-1;xpa-1 double mutant and ercc-1 mutant mice that display growth defects and accelerated aging show dampening of the IIS-equivalent somatotropic axis (Niedernhofer et al., 2006; van der Pluijm et al., 2007). The main signaling components of the somatotropic axis, the growth hormone receptor (GHR) and insulin-like growth factor-1 receptor (IGF-1R), are downregulated in response to persistent transcription-blocking lesions (Garinis et al., 2009), suggesting highly conserved DNA damage response mechanisms during nematode and mammalian development and aging.

Given the role of unrepaired DNA lesions in progeroid syndromes and the contribution of accumulating DNA damage to...
the aging process, we devised a study aimed to gain a more comprehensive understanding of the response mechanisms to persistent DNA lesions on the organismal level. We used xpc-1;csb-1 mutant worms that are defective in both GG-NER and TC-NER, thus leading to complete inability to remove UV-induced DNA lesions resulting in persistent DNA damage after UV treatment (Mueller et al., 2014). We used the UVB irradiation in order to induce helix-distorting CPDs and 6-4 PPs throughout the tissues of the animal. We analyzed proteome, phosphoproteome, and lipidome alterations in response to UV irradiation of NER-deficient animals. On the proteome level, we found similarities between the response to UV irradiation in NER-deficient animals and proteome alterations during aging (Walther et al., 2015) and in the response to starvation (Larance et al., 2015), both of which are regulated through the IIS pathway (Depuydt et al., 2014). Next, we devised a comprehensive signaling response network to DNA damage by integrating proteome and phosphoproteome changes upon persistent DNA damage. Our analysis thus provides insights into the animals’ physiological adaptations to unrepaired DNA damage. Furthermore, analyzing the lipidome, we identified metabolic alterations that indicate a shift to somatic preservation in response to DNA damage. Consistent with the metabolic adjustments, we observed a reduction in proteins functioning in carbohydrate, amino acid, and lipid metabolism that resemble metabolic changes observed upon starvation and during aging. Mechanistically, we determined an important role of autophagy and AMPK signaling in the maintenance of tissue functioning amid persistent DNA damage.

RESULTS

We applied mass-spectrometry-based quantitative proteomics to completely NER-deficient xpc-1(tm3886);csb-1(ok2335) double-mutant C. elegans. We analyzed synchronized worms at the first larvae stage (L1), 6 hr after UVB or mock treatment. Proteins were digested in solution followed by peptide identification and quantification by liquid chromatography and tandem mass spectrometry (LC-MS/MS) (Figure 1A). In total, more than 7,500 proteins were quantified at a false discovery rate (FDR) of less than 1% at the protein and peptide spectrum match level, of which more than 5,000 proteins were quantified between UV and untreated conditions at least in two out of three biological replicates. Excellent reproducibility (r > 0.95 for biological replicates) was determined with the Pearson correlation coefficient (r). Hierarchical clustering revealed strong segregation of different conditions indicating distinct proteomic changes and high data quality that allows for systematic analysis (see also Figure S1).
To systematically analyze protein changes upon persistent UV lesions, we used Gene Ontology (GO) classification as well as UniProt (January 2016 release) and the C. elegans portal WormBase (version WS246). Abundance changes of significantly regulated proteins were observed in most of the subcellular compartments (Figure 1B). In the volcano plot, the log<sub>2</sub> ratio of UV-treated worms to untreated worms for each protein group is plotted against the respective –log<sub>10</sub> p value (Figure 1C). In order to improve the annotations of C. elegans proteins and obtain insights into potential functions, we used BLAST search results (e < 10<sup>-5</sup>) of well-annotated human and mouse proteins. We used GO, Kyoto Encyclopedia of Genes and Genomes (KEGG), and gene set enrichment analysis (GSEA) annotations provided by the UniProt database for C. elegans protein entries and the corresponding human orthologs raising protein annotations from ~35% to ~62% (Figure S2).

We used 1D enrichment to identify groups of proteins that are involved in identical pathways, carry similar PFAM domains, or localize in the same compartment (e.g., categorical annotations). We visualized significantly regulated groups (Benjamini-Hochberg FDR < 0.02) by plotting the mean log<sub>2</sub> ratio of UV-treated to mock-treated worms for all proteins with the particular categorical annotation against the enrichment score (Figure 1D). Categories grouping proteins related to nuclear mechanisms and synaptic machinery showed a positive enrichment score, whereas categories related to protein synthesis and cellular metabolic processes showed a significant negative score (Table S2). Overall, the systematic analysis indicates widespread changes of protein levels upon UV-induced DNA damage in C. elegans.

**Upregulated Protein Clusters upon Genotoxic Stress**

Proteins belonging to the categories related to nuclear mechanisms such as chromatin remodelers, regulator of transcription, protein-DNA complex, and structures of the nuclear pore showed clear upregulation, consistent with chromatin remodeling modulating replication and transcription in response to DNA damage. In addition, the increased expression of members of the synaptic machinery and G protein signaling partners, belonging to plasma membrane and extracellular space categories, suggests that signals are released from genotoxically compromised cells that mediate the adaptation to the damage.

The significantly enriched upregulated proteins belonging to the nuclear GO category (Figure 1B; Table 1) includes chromatin remodelers (CHD-7, BAF-1, SWSN-4, SNFC-5, and LMN-1), transcription regulators (HMG-1.2, RTFO-1, STA-1, NONO-1, EMB-5, SPT-4, HCF-1, and SMK-1), and histone post-translational modifiers (SPR-5, HIL-2, HTZ-1, and HDA-3) associated with the epigenetic control of gene expression. The chromatin-associated proteins BAF-1, SWSN-4, and HCF-1 were previously shown to interact with the IIS effector DAF-16 to remodel chromatin and activate transcription (Li et al., 2008; Riedel et al., 2013). Other transcription factors mediate specifically the response to DNA damage and oxidative stress (SMK-1) (Wolff et al., 2006) or play a role in the UV-induced DNA damage response in mammalian cells (NONO-1) (Alfano et al., 2016).

The upregulated proteins include transcription elongation, pre-mRNA processing proteins, and ribonucleoprotein (RNP) (Table 1), in line with changes in spliceosome organization and the post-translational modifications of splicing factors, recently implicated in the DNA-damage response (Tresini et al., 2015).

**Nuclear Import and Export Transport Is Enhanced upon DNA Damage**

Increased proteins regulating translation, spliceosome assembly, and nuclear-cytoplasmic transport suggest an involvement of RNA biogenesis and translocation in the DNA damage response (Table 1). The nuclear pore complex proteins (nucleoporins [NPPs]), together with Ran-GTPases, play an important role not only in nuclear import and export and nuclear envelope (NE) assembly dynamics but also in the localization of MEL-28 (Fernandez and Piano, 2006), a structural NE component that regulates the distribution of the integral nuclear-envelope proteins EMR-1, LMN-1, LEM-2, and BAF-1 (Table 1) (Galy et al., 2006). These nuclear proteins provide an anchor attaching chromosomes to the nuclear membrane, are required for proper chromosome segregation (Liu et al., 2003), and promote the reorganization of damaged chromatin upon UV and ionizing radiation (IR)-induced DNA damage (Dittrich et al., 2012). When responding to stress, BAF-1 is immobilized at the nuclear lamina, stabilizes the chromatin structure, and influences the gene expression via histone post-translational modifications (Montes de Oca et al., 2011). Exposure of human cells to UV-induced DNA damage causes BAF-1 to dynamically interact with the histone H3/H4 ubiquitin ligase complex (CUL4–DDB-ROC1), facilitating the recruitment of repair proteins to the damaged DNA (Montes de Oca et al., 2009). BAF-1 expression is regulated by transcription factors that modulate lifespan, including SKN-1, PHA-4, DAF-16, and ELT-3 (Bar et al., 2014).

Similar to aged IIS mutant worms (Halaschek-Wiener et al., 2005) and cells responding to DNA damage (Matsuoka et al., 2007), proteins belonging to the nuclear category implicated in DNA replication and cell-cycle progression (CDK-1, MCM-2, MCM-7, and RFC-4) were decreased in abundance upon UV treatment (Table S1).

**Differences in Ion Transport and Synaptic Transmission in Worms Treated with UV Irradiation**

We also observed upregulation of proteins belonging to plasma membrane and extracellular space, suggesting possible intracellular trafficking of signals from genotoxically compromised cells (Figures 1B and 1D). The plasma membrane category contains transmembrane channel proteins, ATPases, amino acid, ion and ATP transporters, and heterotrimeric G proteins (key regulators of G protein-coupled receptor [GPCR] signaling) (Table 1). GPCR signaling has been implicated in fundamental aspects of development and behavior, including the synaptic transmission in the ventral cord motor neurons (Nurrish et al., 1999). Excitable cells display the highest expression of heterotrimeric G proteins together with components of the endocytic pathway involved in the initial vesicles assembly (ARF-6, ARL-8, and DYN-1), vesicle fusion (SNAP-29 and AEX-3), and vesicles recycling through the endo-lysosomal system (ITSN-1 and SQST-1). The upregulation of those proteins might indicate neuronal signals responding to DNA damage (Table 1). DNA repair defects have been linked to the impaired neuronal development in various human congenital progeroid syndromes.
### Table 1. Most Significantly Overrepresented Cluster of Proteins (FDR < 5%) Increased (Upper Section) or Decreased (Lower Section) in Abundance in xpc-1;csb-1 Double Mutants upon UV Irradiation

| Protein Name | Biological Function | Fold Change |
|--------------|---------------------|-------------|
| **Proteins Significantly (FDR < 5%) Increased in Abundance in UV-Treated versus Untreated xpc-1;csb-1 Double Mutants** |
| **Nuclear** |
| spr-5 | lysine-specific histone demethylase 1 | 1.7 |
| rfp-1 | E3 ubiquitin-protein ligase mediating monoubiquitination of histone H2B | 1.99 |
| htz-1 | histone H2A | 2.08 |
| hda-3 | histone deacetylase | 2.3 |
| hil-2 | histone H1.2 | 2.56 |
| htz-1 | histone H2A | 2.08 |
| rfp-1 | E3 ubiquitin-protein ligase mediating monoubiquitination of histone H2B | 1.99 |
| spr-5 | lysine-specific histone demethylase 1 | 1.7 |
| **Chromatin organizers** |
| swn-4 | SWI/SNF nucleosome remodeling complex component | 1.61 |
| chd-7 | chromodomain and helicase domain protein | 1.65 |
| vrk-1 | Ser/Thr kinase regulating the association of baf-1 with chromatin and nuclear membrane proteins | 1.66 |
| lmm-1 | lamin-1, major component of the nuclear lamina | 1.67 |
| emr-1 | emerin homolog, involved in chromosome segregation and cell division | 1.85 |
| baf-1 | barrier-to-autointegration factor, essential role in NE formation | 2.26 |
| lem-2 | LEM protein, involved in chromosome segregation and cell division | 2.5 |
| snf-5 | SNF chromatin remodeling complex component | 2.53 |
| **Chromosome cohesion** |
| smc-3 | structural maintenance of chromosomes protein 3 | 1.94 |
| coh-1 | cohesin complex subunit | 2.52 |
| scc-3 | cohesin complex subunit | 3.1 |
| **Regulators of transcription from RNA polymerase II promoter** |
| math-33 | ubiquitin carboxyl-terminal hydrolase | 1.62 |
| smk-1 | suppressor of MEK null proteins; affects the transcription of DAF-16 target genes | 1.64 |
| emb-5 | regulator of transcriptional elongation by RNA polymerase II | 1.71 |
| nono-1 | conserved nuclear protein, forms a complex with the mRNA export factor NFX-1 | 1.85 |
| ceh-38 | homeobox protein, DNA-binding regulatory protein | 1.92 |
| hmg-1,2 | positive regulation of transcription from RNA polymerase II promoter | 2.24 |
| sta-1 | signal transducer and activator of transcription 1 | 2.46 |
| hcf-1 | transcriptional regulator that associates with histone modification enzymes | 2.5 |
| rito-1 | RNA polymerase-associated protein, component of the PAF1 complex | 2.56 |
| spt-4 | transcription elongation factor | 2.63 |
| **Synthetic multivulva class B** |
| lin-53 | synthetic multivulva class B (synMuvB) protein, transcription factor member of the (DRM) complex | 1.78 |
| lin-37 | synthetic multivulva class B (synMuvB) protein, transcription factor member of the (DRM) complex | 1.89 |
| lin-35 | synthetic multivulva class B (synMuvB) protein, transcription factor member of the (DRM) complex | 2.83 |
| dpl-1 | synthetic multivulva class B (synMuvB) protein, transcription factor member of the (DRM) complex | 3.02 |

(Continued on next page)
| Table 1. Continued |
|-------------------|
| **Protein Name** | **Biological Function** | **Fold Change** |
| mRNA processing | teg-4 | pre-mRNA splicing factor, tumorous enhancer of Glp-1 | 1.51 |
|                  | uaf-2 | splicing factor | 1.76 |
|                  | pap-1 | poly(A) polymerase | 1.83 |
|                  | rmp-4 | core component of the splicing-dependent multiprotein exon junction complex (EJC) | 1.83 |
|                  | prp-65 | pre-mRNA processing factor 6 | 1.95 |
|                  | rsp-4 | splicing factor | 2.33 |
|                  | lsm-7 | mRNA splicing factor, via spliceosome | 2.9 |
| Ribonucleoproteins (RNP) | snr-3/-6/-7 | heptameric complex required for biogenesis and function of the snRNPs | 1.88/2.62/2.91 |
|                  | fust-1 | FUS/TLS RNA binding protein homolog | 1.95 |
|                  | rmp-2 | small nuclear ribonucleoprotein (snRNP)-associated protein RNP-2/U1A | 2.48 |
|                  | rop-1 | protein component of the Ro ribonucleoprotein (RNP) complex | 4.06 |
|                  | hrpf-1/-2 | orthologous to human hnRNP F and hnRNP H, act as pre-mRNA splicing factors | 4.12/2.71 |
| Transport | pgl-1/-3 | P granule abnormality protein | 1.52/3.72 |
|                | npp-2/4/4/-7/-9 | nuclear pore complex proteins | 1.57–4.04 |
|                | xpo-1 | nuclear export receptor | 1.61 |
|                | ran-1/2 | GTP-binding nuclear protein | 1.64/1.63 |
|                | hel-1 | spliceosome RNA helicase DDX39B homolog | 1.79 |
|                | thec-3 | THO complex (transcription factor/nuclear export) subunit | 1.94 |
|                | iff-1 | eukaryotic translation initiation factor 5A-1 | 2.01 |
|                | nxf-1 | nuclear RNA export factor 1 | 2.14 |
|                | nxt-1 | NTF2-related export protein | 2.15 |
|                | imb-1 | importin beta family | 2.15 |
|                | aly-1/-3 | Ref/ALY RNA export adaptor family | 3.45/1.58 |
| Extracellular | ttr-5/-6/-15/-17 | transthyretin-like protein | 1.8–4.17 |
| Transthyretins | nrf-5 | lipid-binding protein | 1.9 |
| FA binding proteins/transporters | lbp-1 | FA-binding protein | 2.5 |
| Others | mec-5 | collagen unique in the number of Gly-X-Y repeats | 1.58 |
|                | egl-3 | prohormone convertase | 1.92 |
|                | sod-4 | extracellular superoxide dismutase (Cu-Zn) | 8.32 |
| Plasma Membrane | inx-3/-6/-12/-16 | innexin | 1.7–3.26 |
| Transmembrane channel proteins | gpb-1 | guanine nucleotide-binding protein subunit beta-1 | 1.65 |
| Heterotrimeric G proteins | goa-1 | heterotrimeric G protein alpha subunit Go (Go/Gi class) | 1.93 |
|                | egl-30 | heterotrimeric G protein alpha subunit Gq (Gq/G11 class) | 1.95 |
|                | eat-16 | RGS protein, interacts with the egl-30 and goa-1 signaling pathways | 2.42 |
| ATPases | eat-6 | alpha subunit of a sodium/potassium ATPase | 1.7 |
|                | nkb-1 | sodium/potassium-transporting ATPase subunit beta-1 | 1.79 |
|                | vha-5 | V-type proton ATPase subunit a | 1.97 |
|                | mca-3 | calcium-transporting ATPase | 3.32 |
|                | catp-3 | cation transporting ATPase | 3.46 |

(Continued on next page)
| Protein Name | Biological Function | Fold Change |
|--------------|---------------------|-------------|
| Amino acid, ion, and ATP transporters | mrp-2/-7 ATP-binding cassette transporter, member of the ABCC subfamily | 1.51/1.58 |
| | abts-1/3 sodium-driven chloride-bicarbonate transporter | 1.77/1.75 |
| | atgp-1/-2 amino acid transporter glycoprotein subunit | 2.16/1.51 |
| | haf-2/-7 transmembrane protein of the ATP-binding cassette transporter superfamily | 2.75/2.16 |
| Endocytosis and vesicle trafficking | aex-3 MAP kinase protein required for intracellular vesicle trafficking as well as synaptic vesicle release | 1.83 |
| | arf-6 ADP-ribosylation factor | 1.83 |
| | itsn-1 endocytic adaptor protein to regulate cargo sorting through the endolysosomal system | 1.84 |
| | rab-3 involved in exocytosis by regulating a late step in synaptic vesicle fusion | 1.9 |
| | snap-29 SNARE, soluble essential protein for fusion of cellular membrane | 2.12 |
| | dyn-1 dynamin GTPase, its activity is required for endocytosis, synaptic vesicle recycling | 2.13 |
| | arl-8 Arf-like small GTPase, regulates transport of axonal presynaptic vesicle protein cargo | 3.05 |
| | sqst-1 ATP-binding cassette transporter, member of the ABCC subfamily | 30.27 |

Proteins Significantly (FDR < 5%) Decreased in Abundance in UV-Treated versus Untreated xpc-1;csb-1 Double Mutants

| Ribosomes | rpl-4/-5/-6/-7/1(6/-0S | 60S ribosomal proteins | 0.42–0.070 |
| Small subunit | ubl-1 ubiquitin-like protein 1-40S ribosomal protein | 0.4 |
| | rps-1/-4/-6/-8/- | 40S ribosomal proteins | 0.44–0.69 |
| Translation initiation factors | ifb-1 eukaryotic translation initiation factor eIF5B | 0.56 |
| | elf-3.D eukaryotic translation initiation factor 3 subunit D | 0.62 |
| | elf-3.E eukaryotic translation initiation factor 3 subunit E | 0.65 |
| | egl-45 eukaryotic translation initiation factor 3 subunit A | 0.66 |
| Others | rrbs-1 ribosome biogenesis regulatory protein homolog | 0.31 |
| | mrps-2/5/-22/-30 mitochondrial ribosomal protein, small | 0.46–0.69 |
| | mrpl-15/-22/-35/-38/-40/-50 mitochondrial ribosomal protein, large | 0.49–0.69 |

UPS Machinery and Chaperones

| Ubiquitous proteasome system | sao-1 suppressor of aph-1, regulates the notch receptor signaling pathway | 0.26 |
| | ubc-26 ubiquitin conjugating enzyme | 0.31 |
| | hecd-1 E3 ubiquitin protein ligase 1 homolog, involved in ubiquitin-dependent protein catabolic process | 0.50 |
| | cul-3 RING-finger protein, form the catalytic core of an SCF-type E3-ubiquitin ligase complex | 0.58 |
| | rpt-4 ATPase subunit of the 19S regulatory complex of the proteasome | 0.68 |

Chaperones

| drn-2/-11/-13/-27/-29 ribosome-associated molecular chaperones | 0.09–0.69 |
| | D2030.2 orthologous to human atP-dependent Clp protease atP-binding subunit clpX-like, hsp100 family | 0.48 |
| | cct-5/-6 T-complex protein 1 subunit epsilon and zeta | 0.67–0.67 |

(Continued on next page)
including CS, and to age-related neurodegenerative disorders such as Alzheimer’s disease (AD) and amyotrophic lateral sclerosis (ALS) (Martin, 2008). Consistent with neuronal developmental processes being affected by unrepaired DNA damage, we found also elevated levels of proteins implicated in axonal outgrowth (EAT-6, CAM-1, UNC-44, and TBB-4) and neuronal positioning during development (SAX-7, WRK-1, UNC-33, and UNC-37) (Table S1).

| Table 1. Continued |
|---------------------|
| **Protein Name** | **Biological Function** | **Fold Change** |
| **Mitochondria** | | |
| Mitochondria machinery | mspn-1 | mitochondrial sorting homolog | 0.42 |
| | timm-23 | translocase, inner mitochondrial membrane | 0.47 |
| | W02B12.9 | mitochondrial iron transporter that mediates iron uptake enzymes | 0.49 |
| | K11H3.3 | putative tricarboxylate transport protein, mitochondrial | 0.5 |
| | acdh-13 | acyl-coenzyme A (CoA) dehydrogenase involved in FA beta-oxidation | 0.53 |
| | F53F10.3 | mitochondrial pyruvate carrier 2 | 0.58 |
| **Peroxisomes** | | |
| Peroxisome machinery | acox-1 | acyl-coenzyme A oxidase | 0.3 |
| | C48B4.1 | peroxisomal acyl-coenzyme A oxidase 5 | 0.34 |
| | gstk-15 | glutathione S-transferase kappa 1 | 0.35 |
| | daf-22 | ortholog of human sterol carrier protein SCP2, which catalyzes the final step in peroxisomal fatty acid beta-oxidation | 0.6 |
| | cti-2 | peroxisomal catalase 1 | 0.7 |
| **Endoplasmic Reticulum** | | |
| ER chaperones | hsp-3 | heat shock 70 kDa protein C | 0.6 |
| | phy-2 | prolyl 4-hydroxylase subunit alpha-2 | 0.11 |
| Others | dpy-185 | prolyl 4-hydroxylase subunit alpha-1 | 0.11 |
| | C14B9.2 | protein disulfide-isomerase A4 | 0.28 |
| | enpl-1 | endoplasmic homolog | 0.58 |
| | Fkb-3/-4/-5 | peptidyl-prolyl cis-trans isomerase | 0.35/0.32/0.20 |
| | srp-68 | signal recognition particle subunit SRP68, has 7S RNA binding activity | 0.45 |
| | pdi-2 | protein disulfide-isomerase 2 | 0.55 |
| **FA Metabolic Processes** | | |
| Fatty acid biosynthesis | 5/fat-1/-2/-4/-6 | omega-3 FA desaturases | 0.02/0.24 |
| | D1/acs-1/-5/-7/-16/ | FA CoA synthetase family | 0.06–0.57 |
| | fasn-1 | FA synthase | 0.20 |
| | pod-2 | acetyl-CoA carboxylase, catalyzes the first step in de novo FA biosynthesis | 0.30 |
| | elo-1/2/-5/-6 | elongation of very long chain FA proteins | 0.31/0.45 |
| | ech-6/-7 | enoyl-CoA hydratase | 0.45/0.31 |
| | bcat-1 | branched-chain-amino-acid aminotransferase | 0.53 |
| **Glycerolipid and glycerophospholipid metabolism** | | |
| sams-1 | S-adenosylmethionine synthase 1 | 0.42 |
| mboa-3 | membrane-bound O-acyl transferase | 0.48 |
| acl-6/-7 | glycerol-3-phosphate acyltransferase predicted to play a role in triacylglycerol biosynthesis | 0.51/0.32 |
| ckb-4 | choline/ethanolamine kinase | 0.51 |
| ckc-1 | choline/ethanolamine kinase | 0.55 |
| **SL metabolism** | | |
| sptl-2/-3 | glycerol-3-phosphate acyltransferase, plays a role in triacylglycerol biosynthesis | 0.25–0.28 |
### A

#### Protein Synthesis
Ribosomal subunits and Translation initiation factors

- rpl-4/5/6/7/10/12/13/18/20/30
- ubi-1
- rps-1/4/6/8/12/13/16/22/24
- rfb-1
- efl-3,D
- efl-3,E
- egf-45

Fold change
- 0.42–0.70
- 0.4
- 0.44–0.69
- 0.56
- 0.62
- 0.65
- 0.66

#### Protein refolding and degradation
UPS machinery and Chaperones

- sao-1
- ubc-26
- hedl-1
- cul-3
- ria-1
- dnp-11/13/27/29
- D2030.2
- oot-6

Fold change
- 0.26
- 0.31
- 0.50
- 0.58
- 0.88
- 0.59–0.69
- 0.48
- 0.67–0.67

#### Autophagy
Macroautophagy sub-pathway

- atg-3
- atg-18
- sqd-1

Fold change
- 2.30
- 2.53
- 3.27

---

### B

#### C. elegans developmental assay

![Graph showing developmental assay results for different conditions](image)

Legend:
- L4
- L3
- L2
- L1

---

(legend on next page)
Increased extracellular proteins were mainly hormone carrier transthyretin (TTR)-related factors, also reported as elevated in aged C. elegans (Liang et al., 2014) and associated with neuroprotection in a murine AD model (Buxbaum et al., 2006). The extracellular Cu²⁺/Zn²⁺ superoxide dismutase SOD-4 and some lipid binding proteins and transporters (NRF-5, LBP-1, and EGL-3), which sequester respectively potentially toxic peroxidation products and toxic fatty acids (FAs), were also upregulated (Table 1).

**Downregulated Protein Clusters upon Genotoxic Stress**

A large number of ribosomal proteins, including components of the small (40S), large (60S), and mitochondrial ribosome subunits, together with components of the translation machinery, were downregulated upon UV treatment (Table 1). A similar drop was observed also for factors involved in protein homeostasis, localized between cytoplasm, mitochondria, endoplasmic reticulum (ER), peroxisomes, and for regulators of FA metabolism (Figures 1B and 1D; Table 1). This general decline in protein synthesis and dampening of metabolic processes upon UV treatment is consistent with previous reports from proteomic studies of aged animals (Ben-Zvi et al., 2009; Narayan et al., 2016), supporting parallels between the DNA damage response and aging.

**Protein Targeting for Degradation**

Impaired protein homeostasis has been suggested as a hallmark of aging (Powers et al., 2009). Protein-fold stabilization restores the structure of misfolded polypeptides, or remove and degrade, via the proteasome or the lysosome, aberrant proteins. Upon UV treatment, many components of the proteostasis network, as chaperones, ubiquitin ligases, and members of the ubiquitin-proteasome system (UPS) machinery, together with ER, peroxisomal and mitochondrial homeostasis-related proteins were downregulated (Table 1). This included the E3 ubiquitin ligase Y54E10A.11 that contains a RING-type-domain homologous to the small (40S), large (60S), and mitochondrial ribosome subunits, together with components of the translation machinery, were downregulated upon UV treatment (Table 1). A similar drop was observed also for factors involved in protein homeostasis, localized between cytoplasm, mitochondria, endoplasmic reticulum (ER), peroxisomes, and for regulators of FA metabolism (Figures 1B and 1D; Table 1). This general decline in protein synthesis and dampening of metabolic processes upon UV treatment is consistent with previous reports from proteomic studies of aged animals (Ben-Zvi et al., 2009; Narayan et al., 2016), supporting parallels between the DNA damage response and aging.

**Metabolic Alterations in Worms Treated with UV Irradiation**

Translation and autophagy are regulated, in parallel to IIS signaling, by the target of rapamycin (CeTOR) LET-363 in complex with the raptor protein DAF-15 to influence cell growth and longevity (Wullschleger et al., 2006). Upon UV treatment, autophagy-related proteins as well as CeTOR and IIS pathway members were elevated (Table S1), reminiscent of the increase of the same members of those pathways during aging (Narayan et al., 2016). Autophagy has been reported to mobilize lipids via the breakdown of lipid droplets (lipophagy) (Singh et al., 2009). Upon UV treatment, we observed a decrease of proteins involved in lipid metabolism and localized between the cytoplasm, ER, peroxisomes and mitochondria (Table 1), similar to their decrease during C. elegans aging (Narayan et al., 2016).

Taken together, these observations suggest that amid persistent DNA damage worms reduce DNA replication and translation, thus potentially avoiding the production of aberrant proteins. Moreover, protein-refolding mechanisms are reduced, chaperone-mediated autophagy, or sequestered in autophagosomes during macroautophagy (Megalou and Tavernarakis, 2009). Upon DNA damage, we found an upregulation of macroautophagy sub-pathway members: ATG-3, ATG-18, and SQST-1, the p62 homologous. Autophagy, via the elimination of SQST-1, was recently implicated in the regulation of the DNA damage response via chromatin ubiquitination (Wang et al., 2016). The decrease in protein synthesis, together with the impairment of protein refolding and degradation mechanisms and the decreased mitochondrial homeostasis, suggests general organismal energy depletion upon UV-induced DNA damage. Given the role of autophagy in degrading aberrant proteins and in recycling nutrients and energy, we hypothesized a proteostatic shift toward autophagy to allow the organism tolerating the consequences of impaired protein homeostasis (Figure 2A).

To monitor autophagy, we used a GFP-fusion transgene of the ubiquitin-like, microtubule-associated Atg8/LC3 ortholog LGG-1 required for autophagic vesicle growth (Levine and Klionsky, 2004). Within 4–10 hr after UV treatment, we observed significantly increase of the lipoidated LGG-1(Il) form indicative of autophagy (Figures 2B and S3). To assess whether autophagy was required for withstanding DNA damage, we tested the UV sensitivity of two autophagy mutants, atg-3(bp412) and atg-9(bp564). We observed a significantly higher sensitivity of the autophagy mutants than of wild-type (WT) worms (Figures 2C and S4), suggesting that proteins involved in the formation of autophagosomes are essential for enduring DNA damage. The impaired UPS machinery and the increased autophagy activity are discussed below in the network analysis (Figure 5).
whereas autophagy is elevated, suggesting a rerouting of protein recycling as part of metabolic shift in response to the DNA damage.

**Correlation between Proteome and Transcriptome**

To address the role of transcriptional responses to UV-induced DNA damage, we compared the proteomes with previously published transcriptome data of \textit{xpa-1} mutants as NER deficient as the \textit{xpc-1;csb-1} mutants (and phenotypically identical in response to UV irradiation) (Mueller et al., 2014). We found a significant moderate positive correlation ($r = 0.347$) between the significantly changed transcripts and proteins upon UV treatment (Figure 3A; Table S3), suggesting that the expression of only a part of proteins can be explained by transcription, while a large fraction is subject to post-transcriptional regulation (see later discussion).

**Correlation between Proteome upon UV Treatment and Aging**

To address whether proteome changes in response to DNA damage might bear similarities to those occurring during aging, we conducted a correlation analysis between proteomes of UV-treated \textit{xpc-1;csb-1} double mutants, unable to repair the UV-induced DNA damage, and WT worms during aging (Walther et al., 2015). Indeed, the proteomes of UV-treated NER deficient animals and WT worms during aging were positively correlated (day 12, $r = 0.26$; day 27, $r = 0.34$; Figure 3B), suggesting that the regulation at the protein level upon persistent DNA damage bears similarities to proteome alterations during aging. The significant positive correlations were more striking when we compared the DNA damage responses of L1 larvae with those of aging adult animals. The similarly regulated processes revealed a general enrichment of factors.

---

*Figure 3. Response to Persistent DNA Damage Correlates with Starvation Stress and Aging Proteomes*

(A–C) Correlation analyses (A) between proteome of \textit{xpc-1;csb-1} double mutants (FDR $< 5\%$) and transcriptome of \textit{xpa-1} mutants after UV treatment (similarly regulated proteins and genes in red and green; specific protein clusters are detailed in Table S3), (B) between proteins detected in \textit{xpc-1;csb-1} double mutants upon UV treatment versus aging in WT worms ($p < 2.2 \times 10^{-16}$ for the three Pearson correlation coefficients, $r$), and (C) between proteins changed in abundance of at least 2-fold (FDR $< 5\%$) in \textit{xpc-1;csb-1} double mutants upon UV treatment versus starvation.
involved in FA metabolism, oxidative stress response, UPR, and IIS.

**Correlation between Proteome upon UV and Starvation Treatment**

L1 larvae arrest their growth not only upon genotoxic treatment but also for extended periods of time in the absence of food and resume developmental growth only when food becomes available. We have previously found similar and contrasting transcription responses between starvation conditions and UV-induced DNA damage (Mueller et al., 2014). In parallel to UV treatment, we also performed starvation experiment in xpc-1;csb-1 double mutants: three independent biological replicates were analyzed, with excellent reproducibility (r > 0.95 for biological replicates) (see also Figure S1). We obtained a positive Pearson correlation between the proteomes of UV-irradiated and starved animals (r = 0.77) (Figure 3C). The similarities were composed of proteins associated with chromatin, vesicle/neurotransmitter trafficking including heterotrimeric G proteins implicated in the starvation-induced activation of the Ras-MAP kinase pathway (You et al., 2006), and metabolic pathways involved in the synthesis and use of carbohydrate, amino acid, and lipids (Tables 1 and S1). Key enzymes involved in FA biosynthesis (Figure 4A) and playing important roles in FA accumulation and consumption during lifespan (Horikawa et al., 2008) were downregulated (Tables 1 and S1). The expression of the same class of genes related to lipid metabolism has been found significantly decreased in the UV-irradiated and photoaged human skin, suggesting that inhibition of de novo lipid synthesis could have a detrimental effect, leading also to collagen destruction (Kim et al., 2010) (Table S1).

**Lipidomics Analysis upon DNA Damage and Starvation**

Prompted by the alterations in FA biosynthesis enzymes, we next traced lipid profiles of xpc-1;csb-1 double mutants upon UV treatment and starvation by using thin-layer chromatography (TLC) and MS. We observed a decrease in triacylglycerols, the storage form of FAs (Figures 4B and S4) that is consistent with the worms’ deriving energy from degradation of fat stored to survive stress such as food deprivation (Elle et al., 2012).

The downstream products of these FA biosynthetic pathways are normally used to synthesize more complex lipids: saturated FAs (SFAs) serve as building blocks for the sphingolipids (SLs), whereas both SFAs and unsaturated FAs (UFAs) are incorporated into glycerophospholipids (Figure 4A). SLs are highly conserved components of cell membranes having regulatory roles in growth control and aging in a wide range of organisms (Cutler et al., 2014). SL works as an intermediate for the production of ceramide (Cer), a key product for the synthesis of glucosylceramide and sphingomyelin (SM) (Zhang et al., 2015) (Figure 4A). Cer is produced from SM in UV- and IR-treated mammalian cells (Zeidan et al., 2008), whereas an increased synthesis of SM from Cer is associated to accelerated development and aging (Cutler et al., 2014). Similarly to aging studies, MS-based quantitative SL profiling showed a general increase in SM and decrease in Cer upon both treatments (Figures 4C and S4), potentially as a consequence of the impaired SFAs biosynthesis. elo-5 mutants, deficient for monomethyl branched chain FA (mmBCFA) synthesis, arrest development similar to starved L1 larvae (Kniazeva et al., 2008) and could be rescued by SFA-derived SLs, d17iso-glucosylceramides (d17iso-GlcCer), together with downstream factors of the CeTOR pathway (Zhu et al., 2013). Intriguingly, upon UV treatment, we observed an increased abundance of members of the CeTOR pathway (Table S1) and of d17iso-GlcCer (Figures 4C and S4). In line with a previous study (Kniazeva et al., 2008) reporting stable mmBCFA levels in starved L1 larvae, we also observed stable levels of d17iso-GlcCer species upon starvation (Figures 4C and S4). The role of the GlcCer/TOR pathway in promoting development independently from the IIS and DAF-7/TGFβ-signaling (Zhu et al., 2013) suggests it regulates the developmental response to UV-induced DNA damage.

Another major component of cellular membranes is the lipid class of glycerophospholipids, synthesized from the intermediate phosphatidic acid, through a series of reduction and acylation reactions (Figure 4A). Phosphatidic acid is dephosphorylated to yield DAG, which is converted into phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which can be both intermediates for the formation of phosphatidylserine (PS). PS and phosphatidylinositol (PI) are generally synthesized from cytidine diphosphatiacylglycerol (CDP-DAG), substrate for the synthesis of phosphatidylglycerol (PG) and cardiolipin (Zhang et al., 2015). Quantitative glycerophospholipids MS profiling, upon starvation and UV treatment, showed a change of the DAG downstream products, indicating a preferential direction in the phospholipid synthesis (Figures 4D and S4). Upon UV, the PC and the PC-derived PS were increased, whereas PE was reduced. In contrast, upon starvation, the PE and the PE-derived PS were elevated, whereas PC was decreased. Other CDP-DAG-derived phospholipids (PI and PG) were not changed, except for a significant reduction of PG in response to starvation (Figures 4D and S4). Taken together, these observations suggest that the worms respond to persistent DNA damage by a metabolic shift reminiscent of adaptations during starvation (Larance et al., 2015) and aging (Liang et al., 2014).

**Proteome and Phosphoproteome-Coupled Analysis to Build a Regulatory Network**

In order to also follow the dynamics of PTMs, we extended our label-free quantitative MS analysis by performing phosphopeptide...
enrichment with the titanium bead (TiO₂) method. The correlation plot of the phosphoproteome dataset upon each treatment (untreated, UV treated, and starvation) shows how the biological replicates cluster together, in a correlation range from 0.7 to 1, as reported in the color key map (Figure S5A). The distributions of the individual phosphorylated residues (Ser/Thr/Tyr) (Figure S5B) and the number of phospho-groups per peptide we detected were similar to those obtained in previous studies (Lundby et al., 2012). Among the 7,430 detected phosphosites, we identified 3,276 significantly modulated in response to UV treatment, with 1,571 more than 1.5-fold downregulated and 1,705 more than 1.5-fold upregulated (p < 0.05) (Table S4). We used Cytoscape and the C. elegans data repository (WormBase) interaction data to generate a protein-protein interaction map (Cytoscape), including only significantly regulated proteins and phosphosites normalized to the proteome (Figure 5). The central node of the network is the DAF-2 protein, a component of IIS signaling, a pathway that has been implicated in the regulation of both the DNA damage response and longevity (McElwee et al., 2004; Mueller et al., 2014). Among the upregulated proteins arising from the DAF-2 central node encompass chromatin organizers, the synthetic multivulva class B family of proteins, the CeTOR, and proteins involved in nuclear-cytoplasmic transport (Figure 5; Table 1). Nuclear transport proteins as PGL-1 and PGL-3 are intermediary nodes between DAF-2 and some autophagy proteins, in particular with the highly upregulated SQST-1, which together with other upregulated components of the endocytic pathway is involved in the neuronal synaptic machinery (Table 1). The upregulated synaptic machinery for the hormone and neurotransmitter release, like heterotrimeric G proteins, or for the mechanosensation, like the MEC proteins, were indirectly linked to DAF-2. As mentioned earlier, factors belonging to the UPS machinery, as well as some chaperones, and members of the ER proteostasis network were downregulated (Figure 5; Table 1). FA metabolic enzymes (Table 1) and proteins involved in amino-acid biosynthesis (SAMS-1), development (DAO-2, DNJ-25, CALU-1, and CUA-1), and stress response (NSY-1 and LYS-7) were also decreased. Using the BiNGO tool, we determined a significant overrepresentation of the GO biological processes larval development, cellular biosynthesis modulating translation, and organic acid biosynthesis, in particular the FA biosynthetic processes within this interaction map (Figure S6).

We next derived signaling pathways that respond to persistent DNA damage (Figure 6A) on the basis of the combining proteins that showed significant alterations at the proteome and/or phosphoproteome level. As central signaling platform appears EGF
A

Chromosome segregation

Histone modifications and chromatin organization

Synthetic multivulva class I

Cell Reports 20, 2026–2043, August 29, 2017

G-protein signaling

Dopaminergic and Serotoninergic synapses

Acetylcholine release from motorneurons

Glucose and lipid metabolism

Vesicle docking and trafficking

B

C. elegans developmental assay

| Strains | 0 mJ/cm² | 45 mJ/cm² | 55 mJ/cm² |
|---------|----------|-----------|-----------|
| Wild type | L1 | L2 | L3 |
| ask-2(2g23) | L1 | L2 | L3 |
| ask-2(2k24) | L1 | L2 | L3 |

(strains and stages legend on next page)
signaling, which has been linked to development, metabolism, and longevity in C. elegans (Iwasa et al., 2010). The EGF signaling cascade is transduced through the phospholipase C\(\gamma\) (PLC)/protein kinase C (PKC), the PI 3-kinase (PI3)/Akt, and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways (Jorissen et al., 2003) as well as through the RAS/extracellular signal-regulated kinase (ERK) to regulate protein homeostasis via the expression of antioxidant genes and the stimulation of the UPS activity via the activation of SKR-5 protein (Liu et al., 2011). EGF signaling also regulates cell growth and survival via the PI3K/Akt kinase cascade that impacts the activity of CeTOR and the IIS effector DAF-16 (Hay, 2011).

EGF and G protein signaling regulate the PLC-mediated hydrolysis of PI 4,5 bisphosphate (PIP2) into the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) (Rhee, 2001). Although IP3 promotes calcium release (Ca\(^{2+}\)), DAG is an intermediate of the glycerolphospholipids synthesis (Figure 4) and functions as a cofactor for the activation of PKC. The cluster of proteins downstream of PKC have been implicated in the regulation of daf-2 IIS-dependent control of dauer formation (Monje et al., 2011) and in the secretion of synaptic vesicles at motor neurons (Sieburth et al., 2007). Consistently, we detected a high abundance of the downstream components of the G protein signaling, involved in the neuronal synaptic machinery, and mediating the initial vesicles assembly (Figures 1D, 5, and 6A; Table 1). The heterotrimeric G proteins \(\alpha\) subunits, EGL-30 and GOA-1, not only mediate serotonin signaling, promoting intracellular vesicle trafficking and synaptic transmission (Nurrish et al., 1999) but also regulate the expression of DAF-7, a member of the TGF\(\beta\)-signaling pathway that during larval development regulates DAF-16 and STA-1 nuclear localization (Myers, 2012; Shaw et al., 2007; Wang and Levy, 2006). The activity of the two G protein subunits EGL-30 and GOA-1 in regulating neurotransmitter secretion is itself regulated by the guanine nucleotide exchange factor RIC-8 (Miller et al., 2000). RIC-8 is able to activate another \(\alpha\) subunit of the heterotrimeric G proteins protein, GSA-1, which in turn activates the adenyl cyclase ACY-1 to produce cyclic AMP (cAMP). This signaling cascade leads to the activation of the regulatory subunits (KIN-1 and KIN-2) of the AMP-dependent protein kinase A (PKA), to modulate growth and locomotion (Schade et al., 2005). Once activated, PKA acts on the cAMP-responsive element (CRE)-binding protein (CREB; CRH-1 in C. elegans), modifying its phosphorylation status and thus altering its subcellular localization. This stimulates the association of CRH-1 with its cAMP-regulated transcriptional co-activator (CRTC; CRTC-1 in C. elegans). Together these two factors target CREs on promoter genes, which regulate the glucose and lipid metabolism (Altarejos and Montminy, 2011). The downregulation of CRH-1 and CRTC-1 in an AMP-activated protein kinase (AMPK; AAK-2 in C. elegans)- and calcineurin-dependent manner, induces transcriptional responses that modulates longevity (Mair et al., 2011). Given that AAK-2 plays a central role in controlling energy metabolism and regulating longevity through the CeTOR and the daf-2-mediated IIS pathways (Curtis et al., 2006), we wondered whether the AMPK homolog might be involved in the response to persistent DNA damage. Indeed, two independent aak-2 mutant alleles showed a significantly more sensitivity to UV treatment than WT worms (Figures 6B and S4). Another factor involved in the determination of adult lifespan via the negative regulation of the IIS downstream target, DAF-16, is the enzyme HCF-1 (Li et al., 2008). This factor works also as transcriptional regulator of chromatin modification and histone phosphorylation (Lee et al., 2007). Consistently, we also found a number of histone modifiers and chromatin organizers highly upregulated in response to UV treatment (Figures 1D and 6A; Table 1). Other increased proteins belong to the cohesion complexes that require the activity of the four upregulated proteins SMCA-1, SMCA-3, COH-1, and SCC-3 for chromosome segregation and the repair of double-strand breaks (Baumann et al., 2011) (Table 1). AIR-1 and PLK-1 kinases or the cleavage by separase (SEP-1) are required for the dissociation of this cohesion complex allowing the segregation of sister chromatids during mitosis (Tsou et al., 2009). Taken together, the network analysis reveals an intricate network of differentially regulated signaling nodes and centrally places IIS, EGF, and AMPK signaling in the DNA damage response.

**DISCUSSION**

The similarities between the proteome changes we observe upon acute DNA damage and those occurring during aging are consistent with the causal role of DNA damage accumulation in the physiological adaptations in aged animals. Previous experiments based primarily on transcriptome analyses of NER-deficient mice suggested an adaptive “survival response” to accumulating DNA damage during aging that preserves tissue functionality by attenuating the somatic growth axis (Garinis et al., 2009; Niedernhofer et al., 2006; van der Puij et al., 2007). The proteomics, phosphoproteomics, and lipidomics alterations we observed in response to persistent DNA damage further support such a shift of the organism’s resources to preservation of somatic functioning. Our analysis places DAF-2 as a central hub, consistent with the role of the IIS effector transcription factor DAF-16 in countering the detrimental consequences of DNA damage (Mueller et al., 2014). Similarly to previous studies of aged IIS mutant worms (Halaschek-Wiener et al., 2005) and cells challenged with DNA damage (Matsuoka et al., 2007), we detected a reduction in DNA replication-associated processes and induction of proteins involved in nuclear

---

**Figure 6. Network Analysis of Proteome and Phosphoproteome Alterations in Response to Persistent DNA Damage**

(A) Network of interactions between proteins regulated in xpc-1;csb-1 double mutants upon UV treatment. Symbols are as follows: full circles, proteins detected by MS as downregulated (blue) or upregulated (red) or not significantly regulated (white); dotted circles, proteins that are not quantified by MS; and stars, phosphopeptides detected by MS as decreased (blue) or increased (red) \(p < 0.05\). (B) WT, aak-2(gt33), and aak-2(ok524) L1 larvae were irradiated or mock treated, and developmental stages were evaluated 48 hr later. An average of three independent experiments per strain and dose is shown; >540 individuals were analyzed per experiment. Error bars show standard deviation; \(p < 0.05\), \({}^{**}p < 0.01\), and \({}^{***}p < 0.001\) (two-tailed t test compared with WT).
mechanisms, consistently with chromatin remodeling modulating repair, replication, and transcription upon DNA damage. As in proteome studies of aged animals (Liang et al., 2014), levels of ribosomal proteins and the translation machinery were reduced potentially to avoid the production and accumulation of aberrant proteins. The proteostatic pathways were shifted toward autophagy, which might serve as a compensatory response when protein homeostasis is impaired and which we find to be required for the animals to withstand DNA damage.

We observed many similarities between the proteome alterations upon DNA damage and those during aging and starvation. We detected significant changes of metabolic pathways involved in the synthesis and use of carbohydrate, amino acid, and lipids upon persistent DNA damage. Many enzymes involved in FA biosynthesis showed significant decreases in abundance, similarly to the reports in proteomic studies of IIS-deficient worms (Depuydt et al., 2014), upon starvation (Larance et al., 2015), and during aging (Narayan et al., 2016). Our quantitative lipid profiling of worms undergoing persistent DNA damage showed a dimpling also of fat biosynthesis and a differential regulation of the complex downstream targets. Therefore, it will be interesting to investigate the role of regulators of lipid metabolism in the DNA damage response.

The network analysis combining proteome and phosphoproteome datasets reveals an intricate connection of differentially regulated signaling nodes and assigns central roles for the IIS regulator DAF-2 and the EGF- and AMPK-like signaling pathways in response to DNA damage. Mutations affecting the AMP-activated protein kinase AAK-2, playing a central role in controlling energy metabolism and regulating longevity through the CeTOR and IIS pathways (Curtis et al., 2006), lead to an increased to DNA damage sensitivity. Members both of the CeTOR and IIS pathways in response to DNA damage. Members both of the CeTOR and IIS pathways were upregulated upon UV treatment, consistent with chromatin remodeling modulating longevity through the CeTOR and IIS pathways (Curtis et al., 2006), lead to an increased to DNA damage sensitivity. Members both of the CeTOR and IIS pathways were upregulated upon UV treatment, consistent with chromatin remodeling modulating longevity through the CeTOR and IIS pathways (Curtis et al., 2006).

In conclusion, our analysis of the proteome, lipidome, and phosphoproteome after UV treatment in NER-deficient C. elegans provides a comprehensive picture of the response processes to persistent DNA damage in a metazoan animal model. The identified proteins and pathways open up previously unexplored avenues for establishing a complete model of organismal responses to persistent DNA damage. This complex in vivo analysis provides a starting point to translate the insight into the signaling networks involved in an animal’s response to UV treatment to higher organisms and ultimately to humans, thus helping decipher outcomes of NER deficiency syndromes and gaining a better understanding of the consequences of DNA damage in the aging process.

**EXPERIMENTAL PROCEDURES**

**C. elegans Strains**

Strains were maintained at 20°C on nematode growth medium (NGM) with E. coli strain OP50. N2 (Bristol; WT), TG38 aak-2[gt33], RB754 aak-2[ok524], BJS724 atg-3[p5412], BJS725 atg-9[pbp564], BJS21 xpc-1[tm3886];csh-1[ok2335], BJS173 xpc-1[tm3886];csh-1[ok2335];daf-16[mu86];zIs356[daf-16::GFP; rol-6[ua1006]], and DA2123 [adls2122 [lgl-1p::GFP::lgl-1p; rol-6[ua1006]].

**Proteome**

xpc-1;csb-1 double mutants were synchronized by bleaching and fed for 3 hr or left under starvation. Six hours after UV (310 nm, Phillips UV6, Waldmann UV236B) or mock treatment, worms were collected in extraction buffer (50 mM HEPES KOH [pH 7.5], 300 mM NaCl, 1 mM EDTA, 1% [v/v] Triton X, 0.1% [w/v] sodium deoxycholate, 10% [v/v] glycerol, and complete protease inhibitor cocktail; Roche Diagnostics) and frozen in liquid nitrogen before resuspension in extraction buffer and homogenization with zirconia beads (four cycles, 6,000 x 2; 20 s; Precellys24 Homogenizer with Cryolys Cooling Unit). Supernatant was collected after 15 min of centrifugation at 4°C. The total protein concentration was measured using the Pierce 660 nm Protein assay (Thermo Fisher Scientific).

**Phosphoproteome**

Protein pellets were re-suspended in 6 M urea and 2 M thiourea in 10 mM HEPES buffer with a Bioreactor, digested in solution with Lys-C and trypsin enzymes, and then enriched with phosphopeptide with the use of TiO2 beads before LC-MS/MS.

**Statistical Analysis**

MS raw files were analyzed with MaxQuant version 1.5.2.8 software and the UniProt reference proteome database for C. elegans. Protein and peptide identifications were controlled by a decoy-database approach FDR estimation to 0.01. GO annotations were imported on the basis of UniProt entries with Perseus. Statistical analyses (correlation calculations and visualization) were performed in the software package Rstudio version 0.99.489 (2009–2015) and network analysis in Cytoscape with WormBase as the reference network. Significantly altered protein quantities were determined by two-sided t tests and correction for multiple testing via estimation of the FDR to 5% with a permutation-based algorithm (number of permutations = 500, fudge factor s0 = 0.1). Significant differences between developmental stages were assessed by two-tailed t tests. For lipid classes, independent two-group t tests were applied.

**Lipid Analysis**

Lipids were extracted from 500,000 L1 larvae. Triacylglycerols and free FAs were quantified by analytical TLC. Relative amounts of SLs were determined by liquid chromatography coupled to electrospray ionization tandem MS (LC-ESI-MS/MS). Glycerophospholipids were analyzed by ESI-MS/MS with direct infusion of the lipid extract (Shotgun Lipidomics).

**Protein Expression**

Worm pellet was sonicated 2 x 5 s at 40% power and boiled 5 min at 94°C in Laemmli buffer, separated on SDS-PAGE gels (4%–12% resolving gel; Invitrogen), transferred to a nitrocellulose membrane (Protran, 0.2 μm), dried, and probed with primary antibodies against GFP (JL-8 Living Colors; Clontech) and tubulin (mouse monoclonal; Sigma-Aldrich) in a 10% Roti-Block (Roht) and imaged with the Odyssey Infrared Imaging System (Li-Cor Bioscience).

**ACCESSION NUMBERS**

The extended protein–protein interaction network is available at http://bffacility.uni-koeln.de/schumacher/suppdata. The accession number for the MS proteomics data reported in this paper is ProteomeXchange Consortium: PXD005649.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, six figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.08.028.

**AUTHOR CONTRIBUTIONS**

D.E. and B.S. conceived the study. D.E, H.N., V.B., L.C.-R., M.M.M., and S.B. performed the experiments. D.E., H.N., S.B., and M.K. analyzed the data. D.E., H.N., and B.S. wrote the manuscript.
ACKNOWLEDGMENTS

We thank P. Frommolt for informatics support, R. Nakad and A. Lopes for comments on the manuscript, and the Caenorhabditis Genetics Center (National Center for Research Resources) and the National Bioresource Project (Ministry of Education, Culture, Sports, Science, and Technology of Japan) for strains. D.E. received a fellowship from the FP7 ITN CodeAge 316354 and, with B.S., the Dieter Platt Foundation award. B.S acknowledges funding from Deutsche Forschungsgemeinschaft (Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases; SFB 829, SFB 670, and KFO 286), the European Research Council (Starting Grant 260383), Marie Curie (FP7 ITN CodeAge 316354, aDDress 316390, and MARRIAGE 316964), FLAG-ERA JTC 2015 (G-Immunomucins, SCHU 2494/3-1), the German-Israeli Foundation (GIF 1104-68.11/2010), Deutsche Krebshilfe (109453), Bundesministerium für Bildung und Forschung (Sybacol FKK0315893), and the COST Actions (GENIE, BM1408).

Received: March 20, 2017
Revised: June 30, 2017
Accepted: August 4, 2017
Published: August 29, 2017

REFERENCES

Alfano, L., Costa, C., Caporaso, A., Altiere, A., Indovina, P., Macaluso, M., Gior- dano, A., and Pentimalli, F. (2018). NONO regulates the intra-S-phase checkpoint in response to UV radiation. Oncogene 35, 567–576.
Altarejos, J.Y., and Montminy, M. (2011). CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat. Rev. Mol. Cell Biol. 12, 141–151.
Bar, D.Z., Davidovich, M., Lamm, A.T., Zer, H., Wilson, K.L., and Gruenbaum, Y. (2014). BAF-1 mobility is regulated by environmental stresses. Mol. Biol. Cell 25, 1127–1136.
Baudrimont, A., Penkner, A., Woglar, A., Mamnun, Y.M., Hulek, M., Struck, C., Schnabel, R., Lodl, J., and Jantsch, V. (2011). A new thermosensitive smc-3 allele reveals involvement of cohesin in homologous recombination in C. elegans. PLoS ONE 6, e24799.
Ben-Zvi, A., Miller, E.A., and Morimoto, R.I. (2009). Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc. Natl. Acad. Sci. U S A 106, 14914–14919.
Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C.C., Li, G.-W., Zhou, S., King, D., Shen, P.S., Welbeazhn, J., et al. (2012). A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. Cell 151, 1042–1054.
Buxbaum, J.N., Ye, Z., Reizach, N., Friske, L., Levy, C., Das, P., Golde, T., Maslah, E., Roberts, A.R., and Bartafi, T. (2008). Transthyretin protects Alzheimer’s mice from the behavioral and biochemical effects of Abeta toxicity. Proc. Natl. Acad. Sci. U S A 105, 2681–2686.
Curtis, R., O’Connor, G., and DiStefano, P.S. (2006). Aging networks in Caeno- rabditis elegans: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways. Aging Cell 5, 119–126.
Cutler, R.G., Thompson, K.W., Camandola, S., Mack, K.T., and Mattson, M.P. (2014). Sphingolipid metabolism regulates development and lifespan in Caeno- rabditis elegans. Mech. Ageing Dev. 143–144, 9–18.
Defenouille` re, Q., Yao, Y., Mouaikel, J., Namane, A., Galopier, A., Decourty, L., Larance, M., Pourkarimi, E., Wang, B., Brenes Murillo, A., Kent, R., Lamond, A.I., and Gartner, A. (2015). Global proteomics analysis of the response to starvation in C. elegans. Mol. Cell. Proteomics 14, 1899–2001.
Dittrich, C.M., Kratz, K., Sendoe, A., Gruenbaum, Y., Jiricny, J., and Henggart- ner, M.O. (2012). LEM-3 - A LEM domain containing nuclease involved in the DNA damage response in C. elegans. PLoS ONE 7, e24555.
Edelﬁz, D., and Schumacher, B. (2015). Genome instability in development and aging: insights from nucleotide excision repair in humans, mice, and worms. Biomolecules 5, 1855–1869.
Elle, I.C., Rodker, S.V., Fredens, J., and Færgeman, N.J. (2012). A method for measuring fatty acid oxidation in C. elegans. Worm 7, 26–30.
Fernandez, A.G., and Plano, F. (2006). MEL-28 is downstream of the Ran cycle and is required for nuclear-envelope function and chromatin maintenance. Curr. Biol. 16, 1757–1763.
Galy, V., Askjaer, P., Franz, C., Lopez-Iglesias, C., and Mattaj, I.W. (2006). MEL-28, a novel nuclear-envelope and kinetochore protein essential for zygotic nuclear-envelope assembly in C. elegans. Curr. Biol. 16, 1748–1756.
Garinis, G.A., Uittenboogaard, L.M., Stachelscheid, H., Fousteri, M., van ilijon, W., Breit, T.M., van Steeg, H., Mullenders, L.H.F., von der Horst, G.T.J., Bruning, J.C., et al. (2009). Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity. Nat. Cell Biol. 11, 604–615.
Halaschek-Wiener, J., Khattra, J.S., McKay, S., Pouzyrev, A., Stott, J.M., Yang, G.S., Holt, R.A., Jones, S.J.M., Marra, M.A., Brooks–Wilson, A.R., and Riddle, D.L. (2005). Analysis of long-lived C. elegans daf-2 mutants using serial analysis of gene expression. Genome Res. 15, 603–615.
Harley, M.E., Murina, O., Leitch, A., Higgs, M.R., Bicknell, L.S., Yigt, G., Blackford, A.N., Zlatanou, A., Mackenzie, K.J., Reddy, K., et al. (2016). TRAIP promotes DNA damage response during genome replication and is mutated in primordial dwarfism. Nat. Genet. 49, 36–43.
Hay, N. (2011). Interplay between FOXO, TOR, and Akt. Biochim. Biophys. Acta 1813, 1965–1970.
Horikawa, M., Nomura, T., Hashimoto, T., and Sakamoto, K. (2008). Elongation and desaturation of fatty acids are critical in growth, lipid metabolism and ontogeny of Caenorhabditis elegans. J. Biochem. 144, 149–158.
Iwasa, H., Yu, S., Xue, J., and Driscoll, M. (2010). Novel EGF pathway regulators modulate C. elegans healthspan and lifespan via EGF receptor, PLC-gamma, and IP3R activation. Aging Cell 9, 490–505.
Jorissen, R.N., Walker, F., Pouliot, N., Garrett, T.P.J., Ward, C.W., and Burgess, A.W. (2003). Epidermal growth factor receptor: mechanisms of activation and signalling. Exp. Cell Res. 284, 31–53.
Kim, E.J., Jin, X.-J., Kim, Y.K., Oh, I.K., Kim, J.E., Park, C.-H., and Chung, J.H. (2010). UV decreases the synthesis of free fatty acids and triglycerides in the epidermis of human skin in vivo, contributing to development of skin photaging. J. Dermatol. Sci. 57, 19–26.
Kniazeva, M., Euler, T., and Han, M. (2008). A branched-chain fatty acid is involved in post-embryonic growth control in parallel to the insulin receptor pathway and its biosynthesis is feedback-regulated in C. elegans. Genes Dev. 22, 2102–2110.
Lans, H., Marteijn, J.A., Schumacher, B., Hoeijmakers, J.H.J., Jansen, G., and Vermeulen, W. (2010). Involvement of global genome repair, transcription coupled repair, and chromatin remodeling in UV DNA damage response changes during development. PLoS Genet. 6, e1000941.
Larance, M., Pourkarimi, E., Wang, B., Brenes Murillo, A., Kent, R., Lamond, A.I., and Gartner, A. (2015). Global proteomics analysis of the response to star- vation in C. elegans. Mol. Cell. Proteomics 14, 1989–2001.
Lee, S., Horn, V., Julien, E., Liu, Y., Wysocka, J., Bowerman, B., Hengartner, M.O., and Herr, W. (2007). Epigenetic regulation of histone H3 serine 10 phos- phorylation status by HCF-1 proteins in C. elegans and mammalian cells. PLoS ONE 2, e1213.
Levine, B., and Klionsky, D.J. (2004). Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev. Cell 6, 463–477.
Li, J., Ebata, A., Dong, Y., Rizki, G., Iwata, T., and Lee, S.S. (2008). Caenorhabditis elegans HCF-1 functions in longevity maintenance as a DAF-16 regulator. PLoS Biol. 6, e233.
C. elegans. Cell Syst. 3

siou, M., Go¨ tz, J., and Nicholas, H.R. (2014). Altered proteostasis in aging and biological and chemical approaches to diseases of proteostasis deficiency. Annu. Rev. Biochem. 78, 959–991.

Rhee, S.G. (2001). Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. 70, 281–312.

Ribezzo, F., Shilo, Y., and Schumacher, B. (2016). Systemic DNA damage responses in aging and diseases. Semin. Cancer Biol. 37-38, 26–35.

Riedel, C.G., Down, R.H., Lorenz, G.F., Kirkenko, N.V., Heinbucher, T., West, J.A., Bowman, S.K., Kingston, R.E., Dillin, A., Asara, J.M., and Ruvkin, G. (2013). DAF-16 employs the chromatin remodeler SWI/SNF to promote stress resistance and longevity. Nat. Cell Biol. 15, 491–501.

Schade, M.A., Reynolds, N.K., Dollins, C.M., and Miller, K.G. (2005). Mutations that rescue the paralysis of Caenorhabditis elegans rics-8 (symnembrin) mutants activate the G alpha(s) pathway and define a third major branch of the synaptic signaling network. Genetics 169, 631–649.

Shaw, W.M., Luo, S., Lands, J., Ashraf, J., and Murphy, C.T. (2007). The C. elegans TGF-beta Dauer pathway regulates longevity via insulin signaling. Curr. Biol. 17, 1635–1645.

Sieburth, D., Madison, J.M., and Kaplan, J.M. (2007). PKC-1 regulates secretion of neuropeptides. Nat. Neurosci. 10, 49–57.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., and Czaja, M.J. (2009). Autophagy regulates lipid metabolism. Nature 458, 1131–1135.

Tresni, M., Warmerdam, D.O., Kolovos, P., Snijder, L., Vrouwe, M.G., Demers, J.A.A., van Uck, W.F.J., Grovers, F.G., Medema, R.H., Hoeijmakers, J.H.J., et al. (2015). The core splicingosome as target and effector of non-canonical ATM signalling. Nature 523, 53–58.

Tsou, M.-F.B., Wang, W.-J., George, K.A., Uryu, K., Stearns, T., and Jallepalli, P.V. (2009). Polo kinase and separase regulate the mitotic licensing of centriole duplication in human cells. Dev. Cell 17, 344–354.

van der Pluijm, I., Garinis, G.A., Brandt, R.M.C., Gorgels, T.G.M.F., Wijhoven, S.W.D., Didierich, K.E.M., de Witt, J., Mitchell, J.R., van Oostrom, C., Beems, R., et al. (2007). Impaired genome maintenance suppresses the growth hormone–insulin-like growth factor 1 axis in mice with Cockayne syndrome. PLoS Biol. 5, e2–e16.

Vig, J., and Suh, Y. (2013). Genome instability and aging. Annu. Rev. Physiol. 75, 645–688.

Walther, D.M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., Mann, M., and Hartl, F.U. (2015). Widespread proteome remodeling and aggregation in aging C. elegans. Cell 167, 919–932.

Wang, Y., and Levy, D.E. (2006). C. elegans STAT cooperates with DAF-7/TGF-beta signaling to repress dauer formation. Curr. Biol. 16, 89–94.

Wang, Y., Zhang, N., Zhang, L., Li, R., Fu, W., Ma, K., Li, X., Wang, L., Wang, J., Zhang, H., et al. (2016). Autophagy regulates chromatin ubiquitination in DNA damage response through elimination of SQSTM1/p62. Mol. Cell 63, 34–48.

Wolf, S., Ma, H., Burch, D., Maciel, G.A., Hunter, T., and Dillin, A. (2006). SMK-1, an essential regulator of DAF-16-mediated longevity. Cell 124, 1039–1053.

Wullschleger, S., Loewth, R., and Hall, M.N. (2006). TOR signaling in growth and metabolism. Cell 124, 471–484.

You, Y.-J., Kim, J., Coebb, M., and Avery, L. (2006). Starvation activates MAP kinase through the muscarinic acetylcholine pathway in Caenorhabditis elegans pharynx. Cell Metab. 3, 237–245.

Zeidan, Y.H., Wu, B.X., Jenkins, R.W., Obeid, L.M., and Hannun, Y.A. (2008). A novel role for protein kinase Cdelta-mediated phosphorylation of acid sphingomyelinase in UV light-induced mitochondrial injury. FASEB J. 22, 183–193.

Zhang, H., Abraham, N., Khan, L.A., and Gobet, V. (2015). RNAi-based biosynthetic pathway screens to identify in vivo functions of non-nucleic acid-based metabolites such as lipids. Nat. Protoc. 10, 681–700.

Zhu, H., Shen, H., Sewell, A.K., Knizzeva, M., and Han, M. (2013). A novel sphingolipid–TORC1 pathway critically promotes postembryonic development in Caenorhabditis elegans. eLife 2, e00429.
Supplemental Information

Multilayered Reprogramming in Response to Persistent DNA Damage in *C. elegans*

Diletta Edifizi, Hendrik Nolte, Vipin Babu, Laia Castells-Roca, Michael M. Mueller, Susanne Brodesser, Marcus Krüger, and Björn Schumacher
Figure S1, related to Figure 1 (A) Comparison between the three biological replicates of each treatment (untreated, UV treated and starvation). The figure shows a correlation matrix of the proteome dataset from xpc-1;csb-1 double mutants using Pearson correlation coefficients. Clustering dendrogram was calculated by Euclidean distance in complete mode. Almost linear correlation was observed for biological replicates. (B) Scatter plot showing the variability of untreated, starved and UV treated data.
Figure S2, related to Figure 1 Pie charts representing the distribution of Gene Ontology categories of significant up and downregulated proteins in xpc-1;csb-1 double mutants upon UV treatment (FDR<5%). Exact numbers of significant regulated protein in relation to the whole detected ones for each category are reported. The upper panel shows Gene Ontology annotations for C. elegans whereas the lower one implements GO annotation for human orthologues (e value cut-off < 10⁻⁴).
Figure S3, related to Figure 2 Uncropped immunoblots of the autophagy marker LGG-1(I) and LGG-1(II)::GFP upon mock-, UV and starvation treatment at different time points post treatment, (A) upon 4 and 6 hours post UV treatment, (B) within 48 hours time frame post UV treatment. The lower panel shows the immunoblot of the housekeeping gene Tubulin.
Figure S4, related to Figure 2, 4, 6 Tables showing *p*-values of pairwise comparisons for the developmental and lipid content measurements assays. Significant *p*-values are shown in bold. (A) Table showing *p*-values of pairwise comparisons between autophagy mutants and WT worms, both mock- and UV treated. (B) Table showing *p*-values of pairwise comparisons between *aak*-2 mutants and WT worms, both mock- and UV treated. (C) Table showing *p*-values of pairwise comparisons between treated and untreated worms in the TLC (upper table) and MS results (middle and lower table).
**Figure S5, related to Figure 5** (A) Correlation matrix of the phosphoroteome dataset from *xpc-1;csb-1* double mutants upon each treatment (untreated, UV treated and starvation). The biological replicates cluster together, within a range of correlation from 0.7 to 1 as reported in the colour key map on the top left of the panel. (B) The distribution of individually identified sites according to the residue that was phosphorylated: serine phosphorylation was the most represented, while tyrosine phosphorylation occurred on less than 1% of phosphorylation sites. The percentage of serine phosphorylation is shown in blue, of threonine phosphorylation in grey and of tyrosine in orange.
Figure S6, related to Figure 5 Interaction map of Gene Ontologies biological processes that are statistically overrepresented in our network. This map was created using the BiNGO tool (Maere et al., 2005) in the Cytoscape software. The colour gradient indicates which biological processes might have a main role in the response towards UV.
Table S1, related to Figure 1  Proteins quantified in xpc-1;csb-1 double mutants at a FDR< 1 %. More than 5000 proteins were quantified between UV and untreated conditions at least in two out of three biological replicates.

Table S2, , related to Figure 1D  1D analysis of human and C. elegans GO categories found differentially regulated upon UV treatment. The main GO categories found regulated are highlighted in different colours in a range from 1 to 6.

Table S3, related to Figure 3A  The two tables recapitulates the most represented clusters of proteins which were found up- and/or down-regulated at the proteome (xpc-1;csb-1 double mutants) and at the transcriptome (xpa-1 mutants) level after UV treatment. Red arrows refer to upregulation while blue arrows refer to downregulation.

Table S4, related to Figure 5  Phosphoroteome dataset from xpc-1;csb-1 double mutants upon each treatment (untreated, UV treated and starvation): among the 7000 detected phosphosites, we identified more than 3000 significantly modulated in response to UV treatment.
Supplemental Experimental Procedures:

**In-solution digestion.** Pellets were re-suspended in 6M Urea, 2M Thio-Urea in 10 mM Hepes buffer using a Bioruptor instrument. Clarification of lysate was done by centrifugation (14,000 rpm, 10 min). Supernatant was collected and proteins were reduced by Dithiothreitol (10 mM, room temperature, 45 min) and alkylated by Iodacetamide (55 mM, room temperature in the dark, 45 min). Lys-C was added at a 1 to 100 (enzyme to substrate) ratio and pre-digestion was performed for at least 2h at room temperature. Urea concentration was diluted to 2M using 50 mM Ammonium bicarbonate and Trypsin was added at a 1 to 100 ratio. Digestion was performed overnight at room temperature and was stopped by acidification. Peptides were desalted by C18 Water Cartridges and 50 µg peptides were used for proteome analysis while the remaining peptides were subjected for phosphopeptide enrichment.

**Phosphopeptide enrichment.** Eluted peptides were acidified to 6 % TFA and the final Acetonitrile concentration was 60 %. In total 5 extraction steps were performed at a beads to peptide ratio of 3:1. In detail, beads were dissolved in 60 % ACN, 6 % TFA, added to peptide mixture and incubated on a rotating wheel for 20 min at room temperature. This step was repeated five times and fractions were washed in 60 % ACN, 1 % TFA. Beads were transferred on C8 stage tips, pooling the last two fractions, and washed three times with each 300 µL of 60 % ACN, 1 % TFA. Then, beads were washed using 40 % ACN, 0.5 % CH₃COOH. Phosphorylated peptides were eluted by 3 x 30 µL of 40 % ACN, 3.75 % NH₄OH, dried in a speed vac and re-suspended in 2.5 % ACN, 5 % formic acid.

**Peptide analysis by Liquid Chromatography and Mass Spectrometry.** Peptides were eluted from C18 tips with 30 µL of 0.1% formic acid in 60 % acetonitrile (ACN), concentrated in a speed vac to complete dryness and re-suspended in 10 µL buffer A(0.1 % formic acid). The Liquid Chromatography tandem mass spectrometry (LC-MS/MS) equipment consisted out of an EASY nLC 1000 coupled via a nano-
spray electroionization source to the quadrupole based QExactive Plus instrument (Thermo Scientific). Peptides were separated on an in-house packed 50 cm column (1.9 μm C18 beads, Dr. Maisch) using a binary buffer system: A) 0.1% formic acid and B) 0.1% formic acid in acetonitrile as described previously (Krishnan et al., 2015). The content of buffer B was raised from 7% to 23% within 220 min and followed by an increase to 45% within 10 min. Then, the column was washed by 85% B for 5 min and re-equilibrated to 5% B within. Total gradient time was 240 min. A similar gradient shape was applied for phosphor-proteome analysis but shorten to a total gradient time of 120 min. Eluting peptides were ionized by an applied voltage of approx. 2.2 kV. MS1 spectra were acquired using a resolution of 70,000 (at 200 m/z), an Automatic Gain Control (AGC) target of 3e6 and a maximum injection time of 20 ms in a scan range of 300-1750 Th. In a data dependent mode, the 10 most intense peaks were selected for isolation and fragmentation in the HCD cell using a normalized collision energy of 25 and an isolation window of 2.0 Th for proteome and 1.8 for phosphor-proteome analysis. Dynamic exclusion was enabled and set to 20 s. The MS/MS scan properties were: 17.500 resolution at 200 m/z, an AGC target of 5e5 (for phosphor proteome analysis: 1e6) and a maximum injection time of 50 ms.

**MaxQuant and bioinformatics.** All raw files were subjected to MaxQuant 1.5.2.8 analysis using the implemented Andromeda search engine (Jürgen Cox and Mann, 2008; Jürgen Cox et al., 2011). Acquired MS/MS spectra were compared to the Uniprot reference proteome database of *C. elegans*. Using the implemented revert-algorithm, we used a FDR (Tusher et al., 2001) cutoff at the peptide-spectrum-match, protein and modification site level of 1%. For first and main MS/MS searches the peptide mass tolerance was set to 20 and 4.5 ppm, respectively. Phosphorylation (STY), Acetylation at protein N-term, and oxidation of methionine residues were defined as variable modification, while Carbamidomethylation was set as a fixed modification. The minimal score for modified peptides was 40. Re-Quantify, label-free-quantification and match-between-runs options were enabled using default settings. BLAST searches were performed using desktop version 2.2.31 by comparing *C. elegans* and human reference proteomes of the Uniprot consortium (downloaded Jan. 2015, (Nolte et al., 2014). BLAST results were accompanied by E-values and Bitscores, as well as the alignment length. An e value cutoff of 1E-4 was used. Please
note that the e value is highly dependent on the search space and varies between different databases. Gene Ontology annotations for both species were imported based on Uniprot entries using Perseus. 1D enrichment analysis was used to identify groups of proteins having similar categorical annotations between human and C. elegans (Juergen Cox and Mann, 2012). Statistical analyses were performed in the software package Rstudio (version 0.99.489, 2009-2015). Volcano plots, scatter plots, heatmaps and bar charts were created in Rstudio using the gplots package. Network analysis was performed in Cytoscape (Cline et al., 2007; Shannon et al., 2003) using Wormbase as the reference network. The extended protein-protein interaction network is available at the following link: http://bifacility.uni-koeln.de/quickngs/nas/labs/ReviewerBS/downloads. The colour gradient of the nodes indicates the grades of up or down-regulation at the proteome level while the three different shapes indicate the phosphorylation status of these proteins. Proteomics light label free quantification data (not SILAC data) from (Walther et al., 2015) were imported based on first Uniprot identifier and correlated to our dataset. Ratios were also calculated based on these values meaning that the calculated ratio might differ from the SILAC based ratio presented in the study.

**Lipid analysis by Thin Layer Chromatography.** 500,000 C. elegans L1 larvae were homogenized in 1 ml of Milli-Q water using the Precellys 24 Homogenisator (Peqlab, Erlangen, Germany) at 6,500 rpm for 30 sec. The protein content of the homogenate was routinely determined using bicinchoninic acid. Lipids were extracted and purified as previously described (Belgardt et al., 2010). Lipids were applied to 20 × 10 cm high performance thin layer chromatography (HPTLC) Silica Gel 60 plates (Merck, Darmstadt, Germany), which were pre-washed twice with chloroform/methanol 1:1 (v/v) and air-dried for 30 min. For the detection of triacylglycerols, each lane of the TLC plate was loaded with the equivalent of 40 µg of protein. The TLC solvent system used was hexane/toluene 1:1 (v/v), followed by hexane/diethyl ether/glacial acetic acid 80:20:1 (v/v/v). Standard lipids (Sigma-Aldrich, Taufkirchen, Germany) applied to the TLC plates in addition to the lipid samples were used for lipid identification. For detection of lipid bands, the TLC plates were sprayed with a phosphoric acid/copper sulfate reagent (15.6 g of CuSO4(H2O)5 and 9.4 ml of
H₃PO₄ (85 %, w/v) in 100 ml of water) and charred at 180 °C for 10 min (Yao and Rastetter, 1985).

**Lipid analysis by Mass Spectrometry.** Relative amounts of Sphingolipids (ceramides, glucosylceramides, and sphingomyelins) were determined by Liquid Chromatography coupled to Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS). Aliquots of the *C. elegans* L1 larvae homogenates (see above) being equivalent to 80 µg of protein were diluted to 100 µl with Milli-Q water. 750 µl of methanol/chloroform 2:1 (v/v) and internal standards (100 pmol Ceramide 17:0, Matreya, Pleasant Gap, PA, USA; 123 pmol Sphingomyelin 12:0, 122 pmol Glucosylceramide 12:0, both Avanti Polar Lipids, Alabaster, AL, USA) were added. Lipid extraction and LC-ESI-MS/MS analysis were performed as previously described (Schwamb et al., 2012). Sphingolipid species were monitored in the positive ion mode with their specific Multiple Reaction Monitoring (MRM) transitions. As characteristic product ions in Q3 the choline headgroup (m/z 184) was used for sphingomyelin species, and the C17 isosphingosine base after water loss (m/z 250) was used for endogenous ceramides and glucosylceramides (Menuz et al., 2009). Endogenous sphingolipids were quantified by normalizing their peak areas to those of the internal standards.

Relative amounts of glycerophospholipids (PC, PE, PI, PS, PG) were determined by ESI-MS/MS with direct infusion of the lipid extract (Shotgun Lipidomics). Aliquots of the *C. elegans* L1 larvae homogenates (see above) being equivalent to 100 µg of protein were diluted to 500 µl with Milli-Q water. 1.875 ml of methanol/chloroform 2:1 (v/v) and internal standards (135 pmol PC 17:0-14:1, 161 pmol PE 17:0-14:1, 127 pmol PI 17:0-14:1, 136 pmol PS 17:0-14:1, 155 pmol PG 17:0-14:1, Avanti Polar Lipids, Alabaster, AL, USA) were added. Lipid extraction and ESI-MS/MS analysis were performed as previously described (Kumar et al., 2015).

**Analysis of somatic arrest post UVB-irradiation.** An aged-synchronized population of adult worms was bleached, and the eggs were allowed to hatch overnight in M9 buffer. Arrested L1 larvae were put onto empty NGM plates and irradiated with 310 nm UVB light using Phillips UV6 bulbs in a Waldmann UV236B irradiation device.
or were mock-treated. (Irradiance was measured using a UVX digital radiometer and a UVX-31 probe from UVP and was generally around 0.3 mW cm\(^{-2}\)). Worms were collected in M9 buffer, concentrated by centrifugation and put on NGM plates with a pre-grown OP50 \textit{E.coli} lawn. Plates were kept at 20°C for 48 h and analysed by large particle flow cytometry, using a Union Biometrica COPAS Biosort system. Larval stages were determined by measuring ‘time of flight’ (length) and ‘laser extinction’ (optical density) of individual worms using the Biosort 5291 software and confirmed by manual inspection under a dissecting microscope.

**Protein expression after UVB-irradiation.** Worm pellet, collected in M9 buffer after starvation and UV treatment was diluted to one half of Laemmli buffer and sonicated 2 x 5 sec at 40% power and boiled 5 min at 94 °C. Proteins were separated on SDS-PAGE gels (4–12% resolving gel, Invitrogen), transferred to a nitrocellulose membrane (Protran, 0.2 µm, Whatman) using semi-dry blotting system (Trans-Blot SD, Bio-Rad) for 1 h at 25 V, and blocked with PBS milk 5 %. Membranes were incubated with primary antibodies against GFP (JL-8 Living Colors®, Clontech) and tubulin (mouse monoclonal, Sigma-Aldrich) in a 10 % Roti-Block® (Roth) PBS solution for 1 h. Membranes were washed 3 x 5 min in PBS + 0.1% Tween-20 (PBST) and incubated with fluorescent secondary antibodies in a 10 % Roti-Block® (Roth) PBS solution for 1 h. Finally, membranes were washed 3 x 5 min in PBST and proteins were imaged by scanning simultaneously at 700 and 800 nm with an Odyssey® Infrared Imaging System Using fluorescently labelled secondary antibodies and detection by an Odyssey scanner (Licor Bioscience, Lincoln, NE, USA).

**Supplemental References:**

Belgardt, B.F., Mauer, J., Wunderlich, F.T., Ernst, M.B., Pal, M., Spohn, G., Brönneke, H.S., Brodesser, S., Hampel, B., Schauss, A.C., Brüning, J.C., 2010. Hypothalamic and pituitary c-Jun N-terminal kinase 1 signaling coordinately
regulates glucose metabolism. Proc. Natl. Acad. Sci. U.S.A. 107, 6028–6033. doi:10.1073/pnas.1001796107

Cline, M.S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas, R., Avila-Campilo, I., Creech, M., Gross, B., Hanspers, K., Isserlin, R., Kelley, R., Killeoyne, S., Lotia, S., Maere, S., Morris, J., Ono, K., Pavlovic, V., Pico, A.R., Vailaya, A., Wang, P.-L., Adler, A., Conklin, B.R., Hood, L., Kuiper, M., Sander, C., Schmulevich, I., Schwikowski, B., Warner, G.J., Ideker, T., Bader, G.D., 2007. Integration of biological networks and gene expression data using Cytoscape. Nat Protoc 2, 2366–2382. doi:10.1038/nprot.2007.324

Cox, Jürgen, Mann, M., 2012. 1D and 2D annotation enrichment: a statistical method integrating quantitative proteomics with complementary high-throughput data. BMC Bioinformatics 13 Suppl 16, S12. doi:10.1186/1471-2105-13-S16-S12

Cox, Jürgen, Mann, M., 2008. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat. Biotechnol. 26, 1367–1372. doi:10.1038/nbt.1511

Cox, Jürgen, Neuhauser, N., Michalski, A., Scheltema, R.A., Olsen, J.V., Mann, M., 2011. Andromeda: a peptide search engine integrated into the MaxQuant environment. J. Proteome Res. 10, 1794–1805. doi:10.1021/pr101065j

Krishnan, R.K., Nolte, H., Sun, T., Kaur, H., Sreenivasan, K., Looso, M., Offermanns, S., Krüger, M., Schwierc, J.M., 2015. Quantitative analysis of the TNF-α-induced phosphoproteome reveals AEG-1/MTDH/LYRIC as an IKKβ substrate. Nature Communications 6, 6658. doi:10.1038/ncomms7658

Kumar, V., Bouameur, J.-E., Bär, J., Rice, R.H., Hornig-Do, H.-T., Roop, D.R., Schwarz, N., Brodesser, S., Thiering, S., Leube, R.E., Wiesner, R.J., Vijayaraj, P., Brazel, C.B., Heller, S., Binder, H., Löfler-Wirth, H., Seibel, P., Magin, T.M., 2015. A keratin scaffold regulates epidermal barrier formation, mitochondrial lipid composition, and activity. J. Cell Biol. 211, 1057–1075. doi:10.1083/jcb.201404147

Menuz, V., Howell, K.S., Gentina, S., Epstein, S., Riezman, I., Foronllaz-Mulhauser, M., Hengartner, M.O., Gomez, M., Riezman, H., Martinou, J.-C., 2009. Protection of C. elegans from anoxia by HYL-2 ceramide synthase. Science 324, 381–384. doi:10.1126/science.1168532

Nolte, H., Konzer, A., Ruhs, A., Jungblut, B., Braun, T., Krüger, M., 2014. Global protein expression profiling of zebrafish organs based on in vivo incorporation of stable isotopes. J. Proteome Res. 13, 2162–2174. doi:10.1021/pr5000335

Schwamb, J., Feldhaus, V., Baumann, M., Patz, M., Brodesser, S., Brinker, R., Claassen, J., Pallasch, C.P., Hallek, M., Wendtner, C.-M., Frenzel, L.P., 2012. B-cell receptor triggers drug sensitivity of primary CLL cells by controlling glucosylation of ceramides. Blood 120, 3978–3985. doi:10.1182/blood-2012-05-431783

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 13, 2498–2504. doi:10.1101/gr.1239303

Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proceedings of the National Academy of Sciences 98, 5116–5121. doi:10.1073/pnas.091062498

Walther, D.M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., Morimoto, R.I., Dobson, C.M., Vendruscolo, M., Mann, M., Hartl, F.U., 2015. Widespread Proteome Remodeling and Aggregation in Aging C. elegans. CELL 161, 919–
Yao, J.K., Rastetter, G.M., 1985. Microanalysis of complex tissue lipids by high-performance thin-layer chromatography. Anal. Biochem. 150, 111–116.