

Supplementary Figure Legends

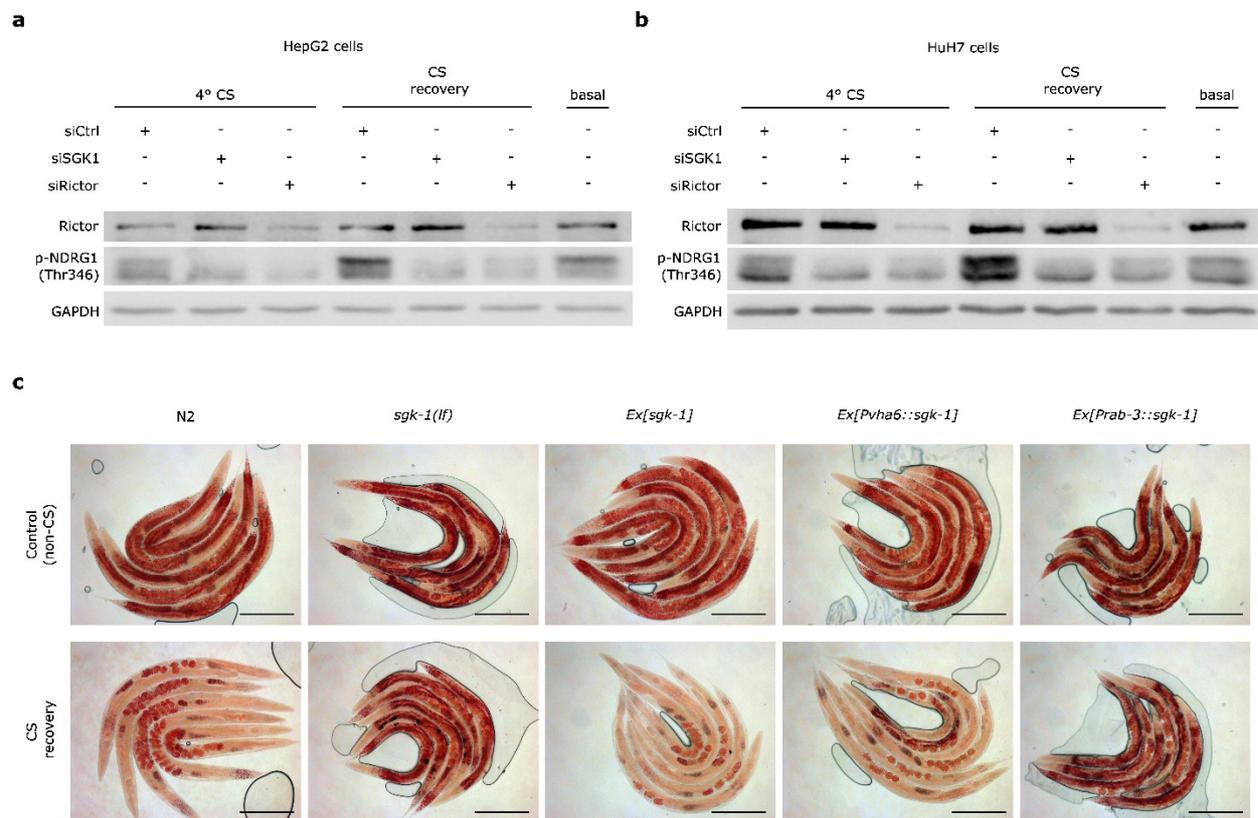


Figure S1. Mammalian SGK1 kinase activity is upregulated in CS recovery. **(a)** Immunoblot analysis of SGK1-mediated NDRG1 phosphorylation in HepG2 **(a)** and HuH-7 **(b)** human hepatocyte cell lines, exposed to CS at 4°C for 2 hr, followed by a 1 hr recovery at 37°C. Cells were transfected with scrambled short interfering RNA (siCtrl), siSGK1 and siRictor to analyze the specificity of NDRG1 phosphorylation by SGK1. We could not detect SGK1 in human hepatocyte cell lines to validate the siRNA-mediated knock down by SGK1. **(c)** ORO staining of indicated strains for tissue-specific rescue experiments in *sgk-1(lf)* mutants used for quantification in Fig. 1o. *Prab-3* drives neuronal, and *Pvha-6* intestinal expression of *sgk-1* in an *sgk-1(lf)* mutant background (n > 29). Worms were synchronized by L4 picking and exposed to a 6 hr CS at 2°C as early day 1 adults. ORO staining was performed after a 10 hr recovery period post CS. Control worms were age-matched. Scale bars: 200 μm, three independent experiments. The *sgk-1(ok358)* loss-of-function allele was used for analysis

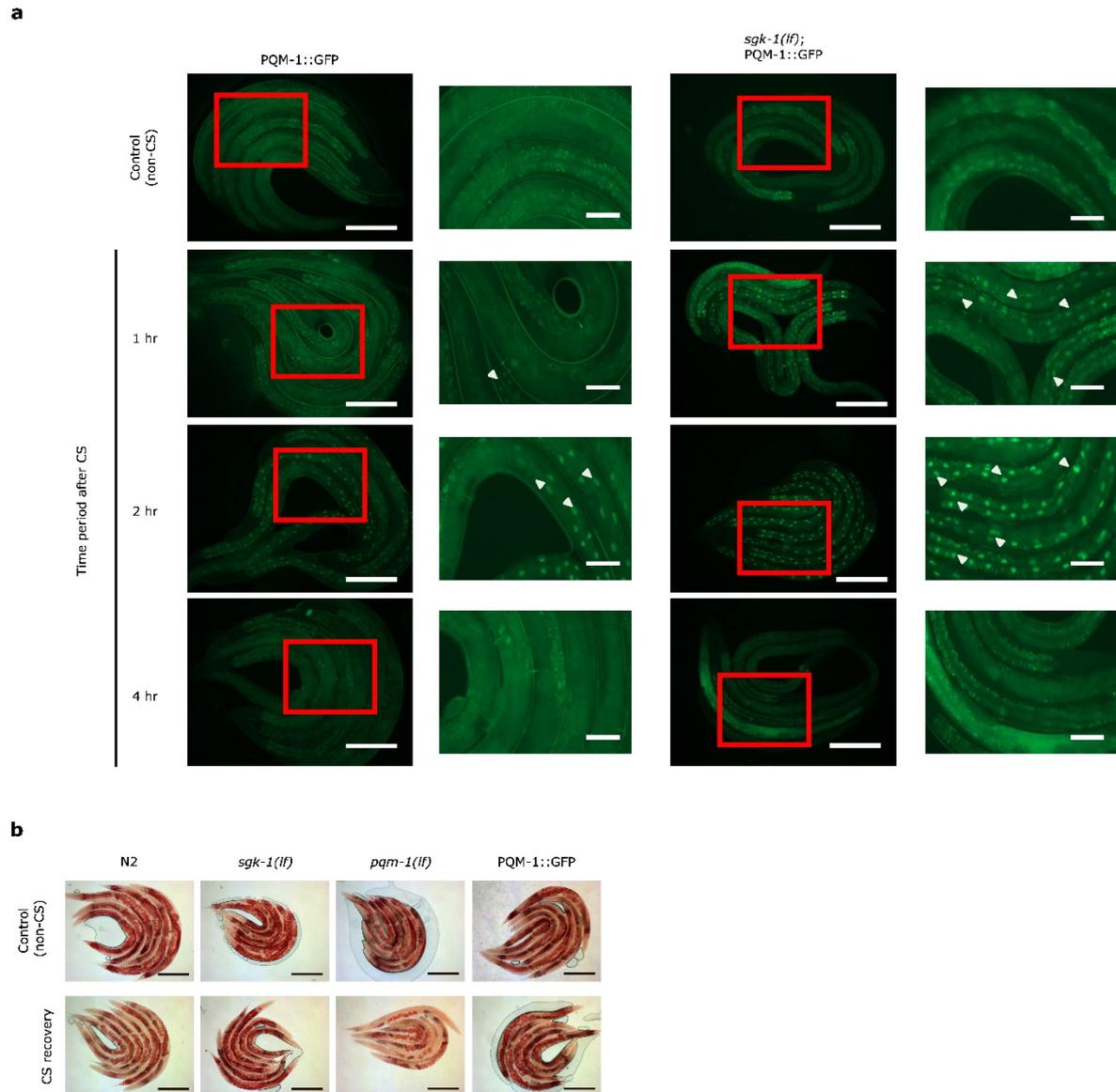


Figure S2. PQM-1 displays an SGK-1 dependent nuclear entry upon CS to regulate lipid maintenance.

a Subcellular localization analysis of a PQM-1::GFP fusion protein predominantly expressed in the intestine of worms used for quantification in Fig. 2i ($n > 24$ per condition). Subcellular localization of PQM-1::GFP was analyzed 1 hr, 2 hr and 4 hr after a 6 hr CS at 2°C during the young adulthood stage. Arrows point to intestinal nuclei in insets. **b** ORO staining of CS-recovered and control worms used for quantification in Fig. 2n ($n > 29$). Worms were synchronized by L4 picking and exposed to a 6 hr CS at 2°C as early day 1 adults (**a,b**). Control worms were age-matched. ORO staining was performed 10 hr after the CS. Scale bars: 200 μ m, three independent experiments. *sgk-1(ok358)* and *pqm-1(ok485)* loss-of-function alleles were used for analysis.

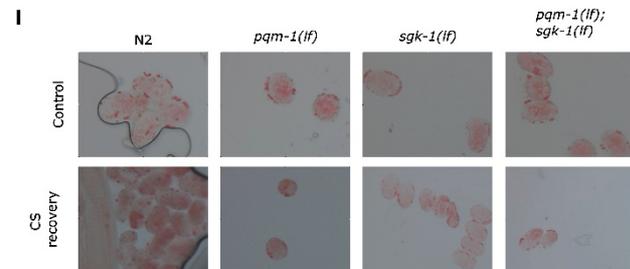
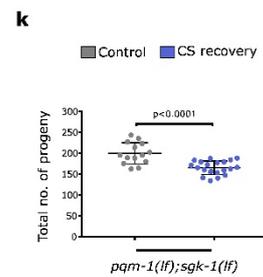
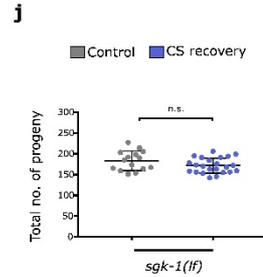
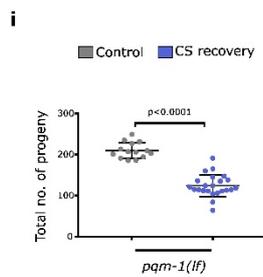
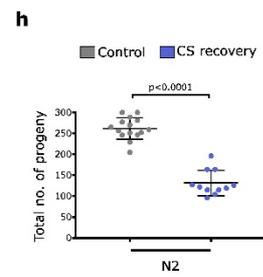
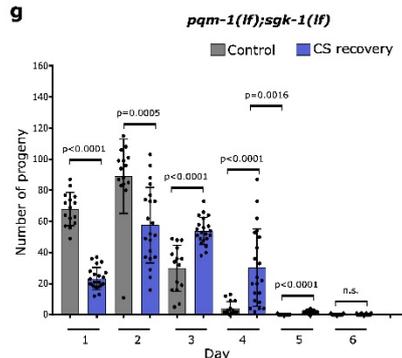
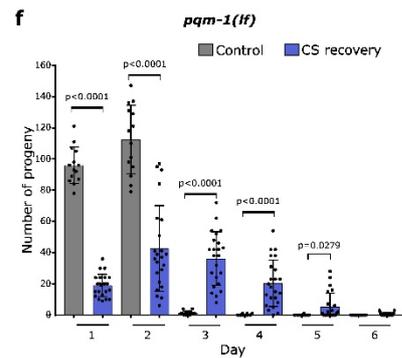
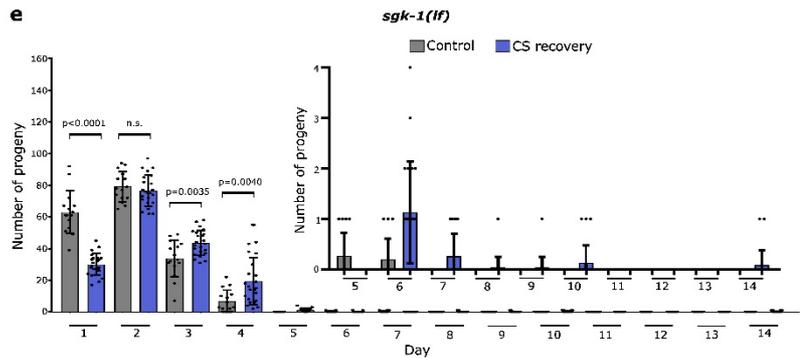
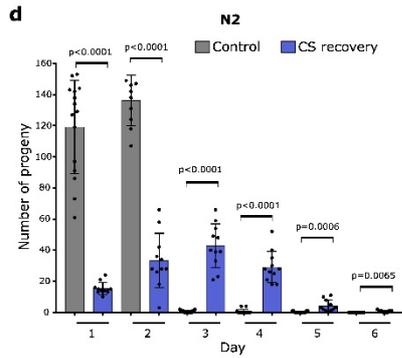
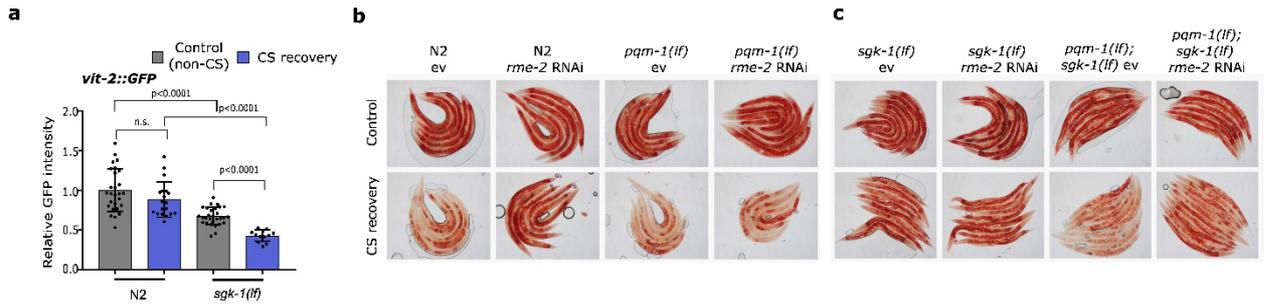


Figure S3. CS and loss of *sgk-1* affect reproduction.

a Quantification of *vit-2::gfp* reporter activity after a 6 hr CS during young adulthood and a 6 hr recovery period; three independent experiments. **b,c** ORO staining of *rme-2* RNAi treated worms used for quantification in Fig. 3q-t. ORO staining was performed 10 hr after a 6 hr CS during the young adult stage. Control worms were age-matched (n > 25). Scale bars: 200 μ m, three independent experiments. **d-g** Reproductive profiling of CS-recovered and control animals of indicated strains (n > 12 data points per condition), three independent experiments. **h-k** Brood size of CS-recovered and control animals of indicated strains (n > 10 datapoints per condition). Total numbers of progeny were calculated from reproductive profiling (**d-g**), three independent experiments. Worms were synchronized by L4 picking and exposed to a 6 hr CS at 2°C as early day 1 adults before onset of egg laying (a-k). Two-tailed t-Test was performed. *sgk-1(ok358)* and *pqm-1(ok485)* loss-of-function alleles were used for analysis. **l** ORO staining of eggs dissected out from parental hermaphrodites used for quantification in Fig. 3x.

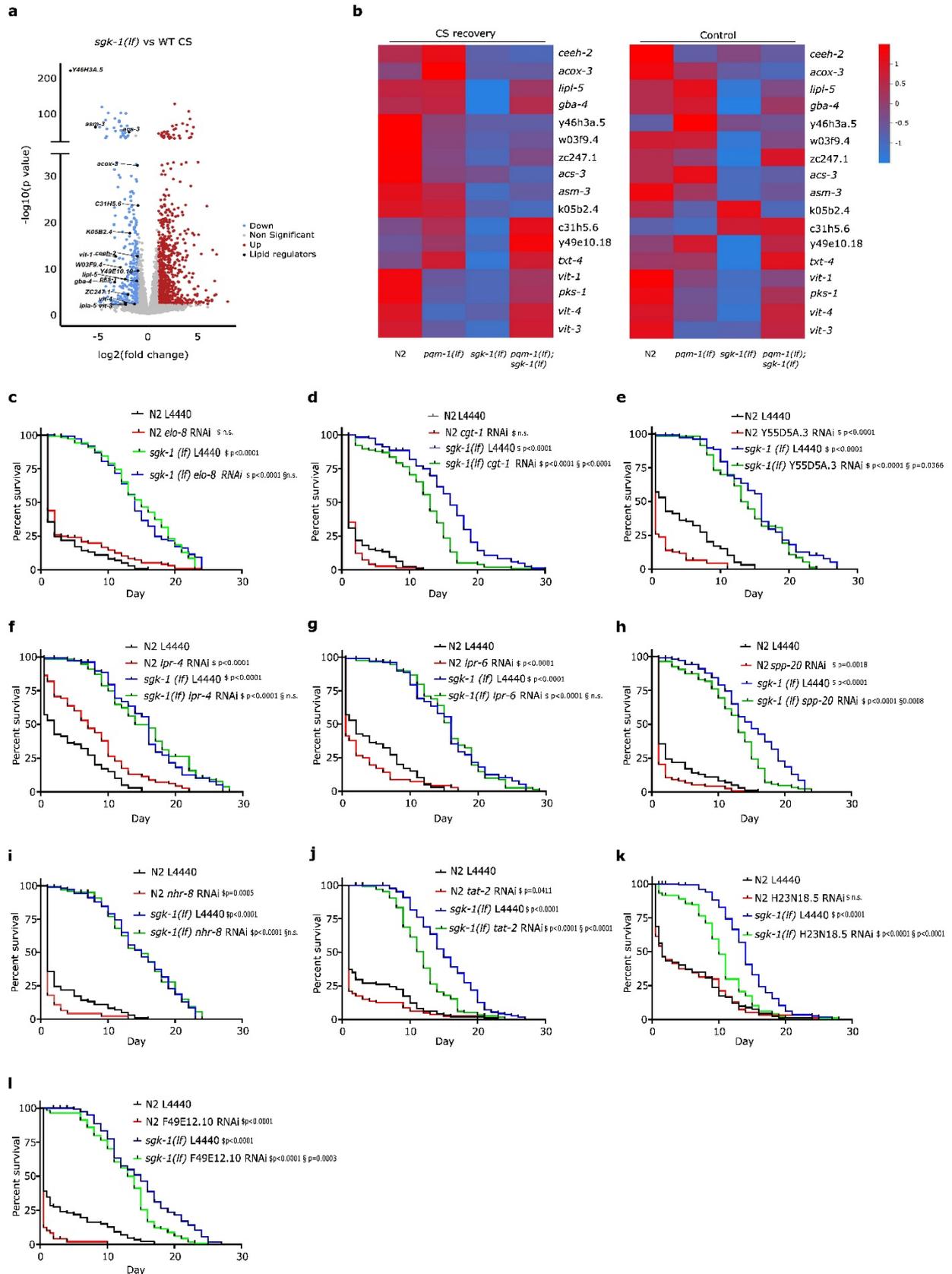


Figure S4. Identification of lipid regulators controlling post-CS survival of *sgk-1(lf)* mutants. **a** Volcano plot visualizing gene expression changes in *sgk-1(lf)* versus wild-type animals analyzed by RNA sequencing of CS-recovered young adult animals. Red and blue

dots in the Volcano plot indicate significantly upregulated and downregulated genes; black dots depict significantly downregulated lipid metabolic genes. $n = 2$ biological replicates, $FDR < 0.05$. **b** Heat maps displaying gene expression changes of lipid regulators for indicated strains in CS recovery and control conditions. Rows depict individual genes encoding lipid regulators which are downregulated in *sgk-1(lf)* versus wild type in CS recovery. A subset of these genes is differentially regulated in *sgk-1(lf)* versus *pqm-1(lf);sgk-1(lf)* mutants. Young adult animals were recovered for 2 hr after a 6 hr CS (**a-b**). **c-l** Survival analysis of animals after a 6 hr CS ($95 < n < 149$). RNAi mediated knock-down of identified lipid regulators was performed for two generations. Transcriptionally upregulated lipid regulators in *sgk-1* identified by RNA-seq analysis (**c-g**, Fig. 4) and additional genes in lipid metabolism (**h-l**) were tested. Log-rank analysis (two-sided). (\$) indicates statistical significance for a strain versus wild type (N2). (§) indicates statistical significance for a strain versus *sgk-1(lf)* mutants; three to four independent experiments. Worms were synchronized by L4 picking and exposed to CS at 2°C as early day 1 adults. *sgk-1(ok358)* and *pqm-1(ok485)* loss-of-function alleles were used for analysis.

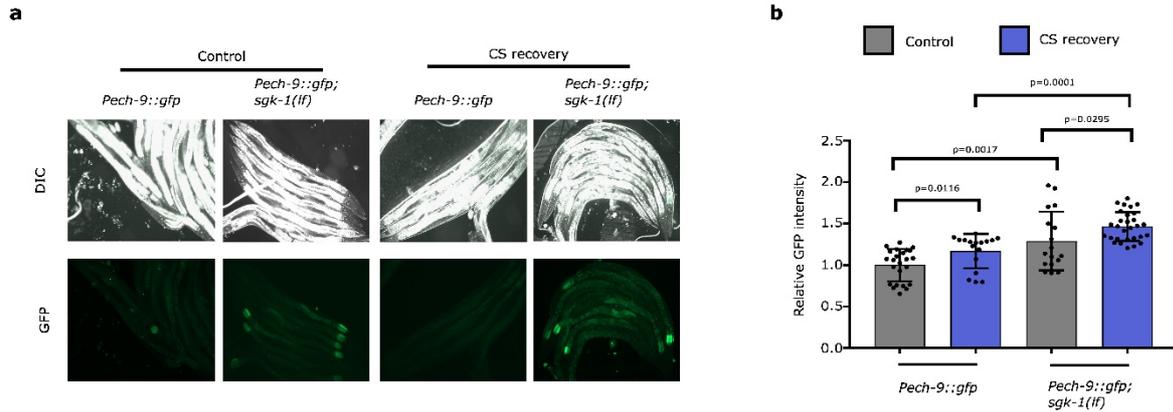


Figure S6. *ech-9* expression analysis in CS. a,b *ech-9p::gfp* reporter activity in early day-1 adult worms (a) and reporter quantification (b) in control conditions and 12 hr after a 6 hr CS at 2°C. Two-tailed *t*-test was performed, mean ± SD. Three independent experiments. *sgk-1(ok358)* loss-of-function alleles were used for analysis.

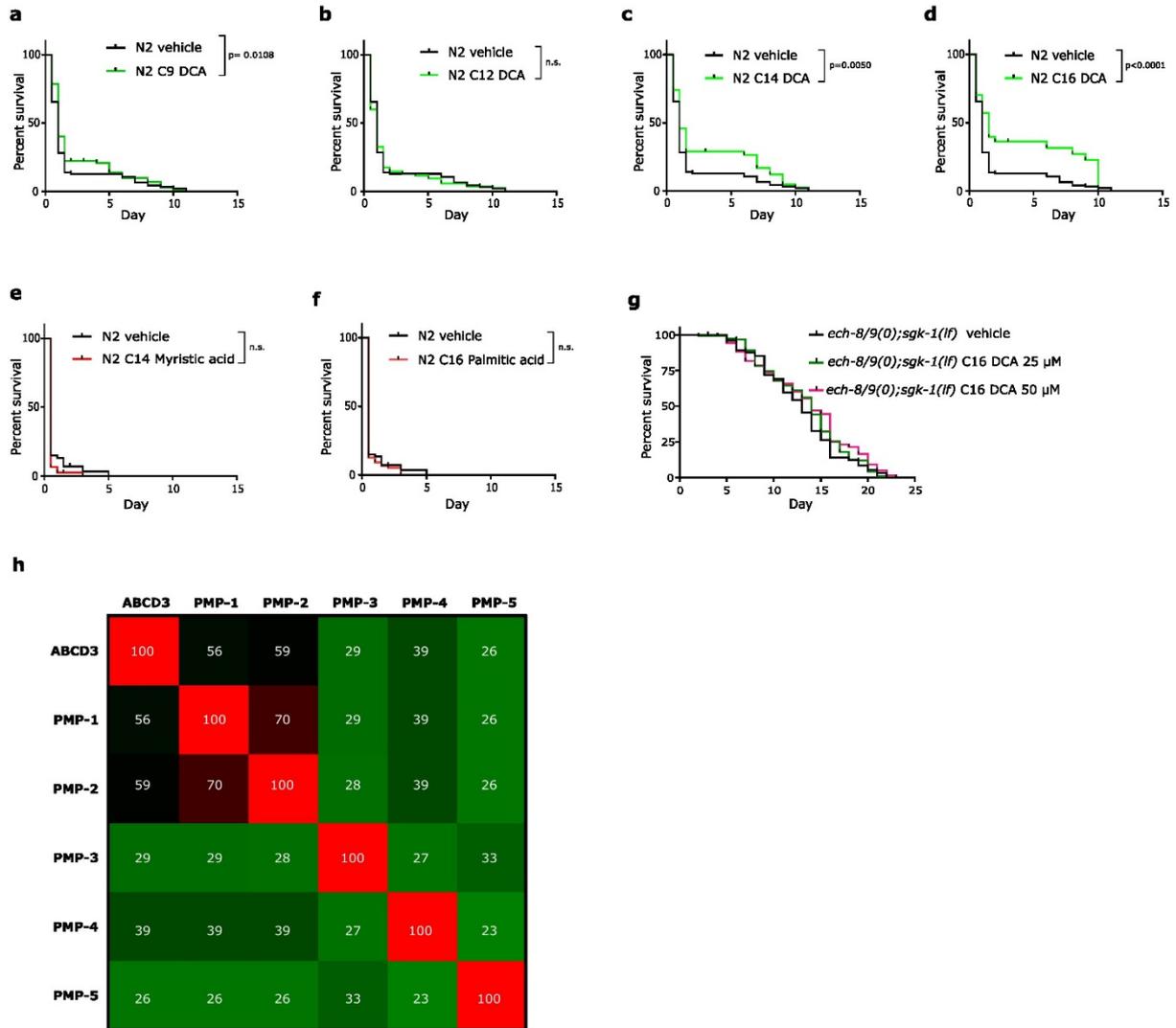


Figure S7. Effect of DCA dietary supplementation on post-CS survival. a-g Survival analysis of indicated strains. For dietary supplementation of FA, animals were exposed to 50 μ M DCAs (**a-d,g**) or 50 μ M monocarboxylic acids (**e,f**) from hatching. Log-rank analysis (two-sided). Worms were synchronized by L4 picking and exposed to a 6 hr CS at 2°C as early day 1 adults; three independent experiments. **h** Alignment score (% identity) of ABCD3 (ATP Binding Cassette Subfamily D Member 3) and *C. elegans* PMP's (Peroxisomal membrane proteins). *sgk-1(ok358)* loss-of-function and *ech-8&ech-9(by256)* null alleles were used for analysis.

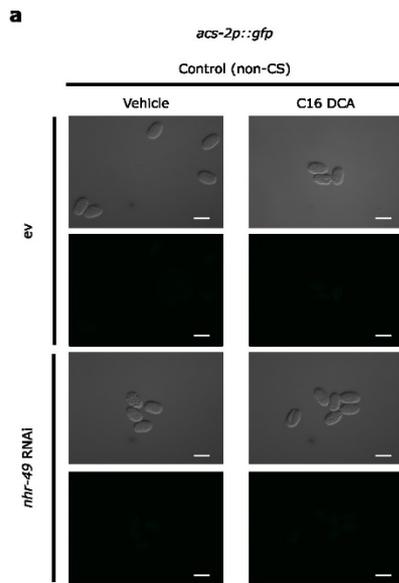


Figure S8. Hexadecanedioic acid promotes *acs-2p::GFP* reporter induction and progeny CS survival. **a** *acs-2p::GFP* reporter activity under control (non-CS) conditions used for quantification in Fig. 8g. Images of *acs-2p::GFP* reporter activity in embryos for vehicle control and C16-DCA (50 μ M) are displayed. Embryos from age-matched control parental hermaphrodites were collected in a 2 hr period as described in Fig. 8. Images of embryos originating from hermaphrodites exposed to CS conditions are displayed in Fig. 8f. Three independent experiments.

Supplementary Methods

Strains

BR7946 N2 (wild type);
BR8816 (*pqm-1(ok485)*);
BR4774 (*sgk-1(ok358)*);
KQ1564 (*sgk-1(ft15)*);
BR5901 (*rict-1(ft7)*);
BR8972 (*pqm-1(ok485);sgk-1(ok358)*);
DMS303 (*nls590[Pfat-7::fat-7::GFP + lin-15(+)]*);
BR8834 (*nls590[Pfat-7::fat-7::GFP + lin-15(+)];sgk-1(ok538)*);
BR8835 (*fat-7(wa36); sgk-1(ok358)*);
BX153 (*fat-7(wa36)*);
CF1038 (*daf-16(mu86)*);
BR7044 (*daf-16(mu86);sgk-1(ok538)*);
OP201 (*wgls201 [pqm-1::TY1::EGFP::3xFLAG(92C12) + unc-119(+)]*);
BR8844 (*wgls201 [pqm-1::TY1::EGFP::3xFLAG(92C12) + unc-119(+)];sgk-1(ok538)*);
RT130 (*pwls23[vit-2::GFP]*);
BR6982 (*sgk-1(ok538); pwls23[vit-2::GFP]*);
BR6598 (*sgk-1(ok538);Ex[Psgk-1::gfp::sgk-1(cDNA), line1]*);
BR6599 (*sgk-1(ok538);Ex[Psgk-1::gfp::sgk-1(cDNA), line2]*);
BR6600 (*sgk-1(ok538);Ex[Psgk-1::gfp::sgk-1(cDNA), line 3]*);
BR7909 (*sgk-1(ok538); byEx1588[Prab-3::mCherry::sgk-1; rol-6]*, line1);
BR7910 (*sgk-1(ok538); byEx1589[Prab-3::mCherry::sgk-1; rol-6]*, line2);
BR7911 (*sgk-1(ok538); byEx1590[Prab-3::mCherry::sgk-1; rol-6]*, line3);
BR7912 (*sgk-1(ok538); byEx1591[vha6P::mCherry::sgk-1; rol-6]*, line1);
BR7913 (*sgk-1(ok538); byEx1592[vha6P::mCherry::sgk-1; rol-6]*, line2);
BR7914 (*sgk-1(ok538); byEx1593[vha6P::mCherry::sgk-1; rol-6]*, line3);
BR7994 (*sgk-1(ok538);byEx1610[Psgk-1::EGFP::sgk-1(R83A); rol-6(su1006)]*), line1);
BR7995 (*sgk-1(ok538);byEx1611[Psgk-1::EGFP::sgk-1(R83A); rol-6(su1006)]*), line2);
BR7996 (*sgk-1(ok538);byEx1612[Psgk-1::EGFP::sgk-1(R83A); rol-6(su1006)]*), line3);
BR8336 (*sgk-1(ok538);byEx1724[Psgk-1::GFP::sgk-1(K164R); pRF4]*), line1);
BR8337 (*sgk-1(ok538);byEx1725[Psgk-1::GFP::sgk-1(K164R); pRF4]*), line2);
BR8338 (*sgk-1(ok538);byEx1726[Psgk-1::GFP::sgk-1(K164R); pRF4]*), line3);
BR8342 (*sgk-1(ok538);byEx1730[Psgk-1::GFP::sgk-1(S434A,T454A); pRF4]*), line1);

BR8343 (*sgk-1(ok538);byEx1731[Psgk-1::GFP::sgk-1(S434A,T454A); pRF4], line2*);
 BR8344 (*sgk-1(ok538);byEx1732[Psgk-1::GFP::sgk-1(S434A,T454A); pRF4], line3*);
 QC114 (*etEx2 [glo-1P::GFP::ras-2 CAAX + rol-6(su1006)]*);
 BR9320 (*pqm-1(ok485); etEx2 [glo-1p::GFP::ras-2 CAAX + rol-6(su1006)]*);
 BR8965 (*lipl-4(tm4417)*);
 GR1971 (*mgEx779 [lipl-4p::K04A8.5p::lipl-4::SL2::GFP + myo-2p::mCherry]*);
 BR9013 (*lipl-4(tm4417)V;sgk-1(ok538)X*);
 BR9014 (*mgEx779[lip1-4p::K04A8.5p::lip1-4::SL2::GFP + myo-2p::mCherry]; sgk-1(ok538)X*);
 BR9231 (*dhs-3p::dhs-3::GFP + unc-76(+);hjls37 [vha-6p::mRFP-PTS1 + Cbr-unc-119(+)]*;
sgk-1(ok538));
 CYA11 (*dhs-3p::dhs-3::GFP + unc-76(+);hjls37 [vha-6p::mRFP-PTS1 + Cbr-unc-119(+)]*).
 BR9376 (*Pech-9::3XFLAG::gfp::ech-9::3'UTR; hjls37 [vha-6p::mRFP-PTS1 + Cbr-unc-119(+)]*);
 BR9877 (*Pech-9::3XFLAG::gfp::ech-9::3'UTR; hjls37 [vha-6p::mRFP-PTS1 + Cbr-unc-119(+)]*;
sgk-1(ok538));
 BR9022 (*ech-9(by253)*);
 BR9125 (*ech-8&ech-9(by256)*)
 BR9035 (*ech-9(by253); sgk-1(538)*);
 BR9142 (*ech-8&ech-9(by256);sgk-1(ok538)*);
 BR8503 (*byls281[Pech-9::GFP::3primeUTR]*);
 BR9008 (*byls281[Pech-9::GFP::3primeUTR];sgk-1(ok538)*);

Cold stress temperature measurement

Each cold shock experiment was performed in a Binder KB E6 incubator. The temperature was recorded in 5 minutes intervals using a NIST traceable TMP117 digital temperature sensor (Texas Instruments) embedded in the agar of a reference NGM plate. In order to maximize the accuracy of the temperature reading, the sensor was operated with 64 times internal averaging performed in one second for each measurement. In the time between measurements the sensor was kept in low-power shutdown mode to prevent self-warming. The expected accuracy of the temperature readings is $\pm 0.1^{\circ}\text{C}$.

Pigmentation assay

C. elegans pigmentation was analyzed with synchronized day 1 of adulthood worms after a 6 hr CS at 2°C as described above and a recovery period of 10 hr at 20°C . Worms were

transferred to a 3% agarose pad and immobilized using 20 mM sodium azide. Worms were imaged using a Zeiss Axioimager bright field microscope (5x objective). Control worms were stage-matched to worms exposed to CS conditions. 25-30 worms were imaged for each condition with constant exposure times. The pigmentation intensity was determined in ImageJ by inverting the image, calculating the mean value within a worm region and subtracting the background around the worm.

RNAi experiments

RNAi-treated worm strains were fed (OP50(xu363))¹ containing an empty-vector construct or a construct expressing double-stranded RNA (dsRNA) against the gene of interest. Animals were grown for two generations on OP50 RNAi plates at 20°C, except for *rme-2* RNAi which was performed from hatching. RNAi clones used in this study are from Ahringer or ORFome libraries. See Table S2 for the list of used RNAi clones.

Oil Red O-based lipid staining and quantification

For lipid staining experiments, L4-synchronized worms were grown on OP50 plates to early day 1 of adulthood (onset of egg laying). Cold stress was performed at early day 1 of adulthood (13 hr post L4) at 2°C for 6 hr and recovery was performed at 20°C for 10 hr. A 10 hr recovery period was chosen, since a pronounced somatic fat-loss phenotype was observed in wild-type animals 10 hr post CS. A 0.5% Oil Red O stock solution was prepared in high-quality 100% isopropanol as described previously² For staining of worms, the stock solution was diluted to 60% with sterile water, incubated on a rocking platform at room temperature overnight and filtered through a 0.45 µm filter before staining. Control worms were stage-matched to CS exposed worms. Worms were rinsed off OP50 plates in M9 buffer, washed 3 times and resuspended in 500 µl 60% isopropanol for fixation for 2 min. Isopropanol was aspirated, and 500 µl of freshly-filtered Oil Red O working solution was added. Worm strains were incubated in a Thermomixer at 25°C using mild agitation (550 rpm) for 16-18 hr. Animals were washed three times with 500 µl of 0.001 % Triton X-100 in M9 buffer and stored until imaging on an AxioPlan 2 Imaging microscope (Carl Zeiss AG, 10x objective for worms, 20x objective for eggs). Oil Red O quantification was performed as previously described³. In brief, color images were split into RGB monochromatic images in Image J. The Oil Red intensity was determined by calculating the mean value within a worm region or within an egg area (Intensity of the green channel), adjusted by the intensity around the worm in green channel as the background. 3-7 independent biological replicates were performed for each experiment and 18-25 worms were quantified. For

quantification of the lipid content of eggs based on Oil Red O staining, eggs were dissected out of Oil Red O-stained worms followed by imaging as described above (20x objective).

FUdR treatment of *C. elegans*

To examine the effect of FUdR on *C. elegans* survival after CS, a 100 mM stock solution of FUdR was prepared in deionized water. FUdR was diluted in deionized water and distributed on NGM plates seeded with OP50 to obtain a final concentration of 100 μ M FUdR. Worms were transferred to FUdR containing plates at the L4 larval stage.

RNA sequencing

Synchronized adult day 1 worms were subjected to a 6 hr cold shock at 2 °C and recovered at 20 °C for 2 hr. A 2 hr recovery period was chosen, since PQM-1 displayed a pronounced nuclear localization 2 hr after CS. Worms were harvested and washed with M9 three times. Worms were frozen at -80 °C before RNA was isolated from biological replicates. Two biological replicates were prepared for each condition. Frozen worms were thawed and total RNA was extracted with RNeasy Kit (Qiagen) according to the manufacturer's instruction. RNA quantification was performed using a Nanodrop spectrophotometer (NanoPhotometer NP80, IMPLEN). RNA quality was evaluated by separating RNA in a 1% agarose gel. Library construction and RNA sequencing were carried out by Eurofins. RNA sequencing quality control of results and data processing were performed in Galaxy (<https://usegalaxy.eu>) according its RNA-seq analysis tutorial (<https://training.galaxyproject.org/training-material/topics/transcriptomics/tutorials/ref-based/tutorial.html>).

Briefly, FastQC, RNA STAR, feature counts, DESeq2 and go seq were utilized for reads quality control, mapping, counting, differential gene expression analysis and GO enrichment analysis. Parameters were set according to the tutorial. The *C. elegans* reference genome WBcel235.51 was used for mapping and WBcel235.96.gtf was applied for annotation. Mis-regulated genes in *sgk-1(lf)* compared to WT after CS were defined as \log_2FC (*sgk-1(lf)* vs. WT) > 1 & FDR < 0.05, or \log_2FC (*sgk-1(lf)* vs. WT) < -1 & FDR < 0.05.

Quantitative RT-PCR

Total RNA was isolated as described above for RNA-seq analysis. Quantitative RT-PCR was performed as described previously⁴. Reverse transcription was carried out using Transcriptor High Fidelity cDNA-Synthesis kit from Roche. SybrGreen real-time PCR

experiments were performed with a 1:8 dilution of cDNA using a LightCycler 96 Instrument (Roche Life Science) following the manufacturer's instructions. Data were analyzed with the $\Delta\Delta C_t$ method. Relative transcript levels were normalized to *cdc-42* and *pmp-3* as endogenous control⁵. The $\Delta\Delta C_t$ values were calculated by normalization to a wild type control sample. Primers used in this study are listed below.

Fluorescent imaging of PQM-1 subcellular localization

For analysis of PQM-1 subcellular localization worms were L4-synchronized, grown to an early day-1 of adulthood stage and exposed to a 6 hr CS as described above. Following a recovery period of 0, 1, 2 and 4 hr after CS worms were immobilized in 5 μ l of a polystyrene microspheres suspension (Polysciences)⁶ on 3% agar pads. Image for PQM-1::GFP subcellular localization were taken on a Zeiss Axioimager microscope (10x objective). Exposure time for all conditions was kept constant. Intestinal nuclear localization was scored visually for three categories: weakly nuclear (only in posterior intestinal region), moderately nuclear (anterior and posterior intestinal region) and strongly nuclear (throughout the intestine). 25-30 animals were scored per condition and three independent biological replicates were performed.

Analysis of peroxisome-lipid droplet association

Peroxisomes and lipid droplets (LDs) were visualized using a reporter strain that expresses mRFP fused to a peroxisomal localization sequence (SKL) and the LD protein DHS-3 fused to GFP (*dhs-3p::dhs-3::GFP*). Transgenic hermaphrodites were synchronized at the L4 stage and anterior intestinal cells of 6-8 day-1 of adulthood worms were analyzed following 4% paraformaldehyde fixation and mounting as described above. Peroxisomal density was calculated as described above. In some cases, where images were blurry, the plugin did not perform correctly, and quantifications were censored from further analysis. Images on Nikon eclipse Ti A1 or Zeiss LSM 880 Observer confocal microscopes were taken using the same exposure time/laser power. Peroxisomal numbers and peroxisomal-LD association were analyzed in Fiji version 1.53q. Images were merged (GFP and RFP), the peroxisomes which overlap with a LD in the same voxel were visually identified and counted in Fiji. For each image, the ratio of colocalisation between LDs and peroxisomes was calculated through dividing the number of colocalized peroxisomes with LDs by the total number of peroxisomes in each image. Peroxisome number and ratio of colocalization was plotted in Prism 7.

Fatty acid supplementation

Fatty acids (FAs) supplementation was performed as described previously^{7,8}. Briefly, FAs, including palmitic acid (CAS #57-10-3, Sigma), myristic acid (CAS #544-63-8, Sigma), azelaic acid (CAS #246379, Sigma), dodecanedioic acid (CAS #D1009, Sigma), hexadecanedioic acid (CAS #177504, Sigma), tetradecanedioic acid (CAS #821-38-5, Sigma), were freshly dissolved in absolute ethanol prior to pouring NGM plates. The working stock solution were prepared fresh before use. After adding working stock of FAs at a final concentration of 25 μ M and 50 μ M (in 0.1% ethanol) to the NGM media, the NGM media was stirred 5 minutes to ensure uniform distribution of FAs, and the plates were stored in a dark container to minimize potential light-induced oxidation. Control plates were made using the exact procedure including 0.1% ethanol (final concentration), but without the addition of FAs.

Reporter assays for progeny

Parental worms were exposed to DCAs (50 μ M) and 0.1% ethanol (solvent) from hatching. Cold-stressed parental hermaphrodites (exposed to a 6 hr CS) and non-cold stressed, age-matched parental hermaphrodites were incubated at 20°C after the CS to lay eggs. Eggs were transferred to a 3% agarose pad for imaging using a Zeiss Axioimager microscope (20x objective). For *Pacs-2::gfp* reporter quantification in eggs, eggs containing embryos predominantly at the gastrula stage were used. Exposure time for all conditions were kept constant.

Reporter analysis in *C. elegans*

For VIT-2::GFP reporter analysis worms were synchronized as described above by L4 larvae picking. Synchronized worms were exposed to a CS for 6 hr followed by a recovery period of 6 hr at 20°C (this recovery time point was chosen to allow GFP folding and maturation). Worms were immobilized in 20 mM sodium azide in M9 on a 3% agarose pad for imaging. To analyze the *lipl-4* reporter (*lipl-4p::K04A8.5p::lipl-4::SL2::GFP*) activity, the reporter signal was quantified in the indicated worm area (marked by dashed white lines) excluding the anterior area with the bleeding through signal of the coinjection marker (Fig. 5g). Fluorescent images were taken on a Zeiss Axioimager microscope (10x objective). Exposure time for all conditions were kept constant. Three independent biological replicates were performed.

Reproductive profiling

Worms were L4-synchronized as described above. Parental worms (young adults) were cold shocked for 6 hr and transferred to 20°C for recovery. After CS recovery, individual worms were singled out to small NGM plates (3 cm in diameter). Non-cold shocked, stage matched worms were singled out as controls. Parental worms were transferred every day to a fresh plate to obtain the progeny number of an individual worm per day. Plates with progeny were incubated at 20°C for 2 subsequent days, which facilitates an easy counting of grown-up offspring. At least 12 progeny profiles per worm stain and condition were determined. Progeny profiles were censored if parental worms died or bagged during reproductive profiling due to a detrimental impact of CS.

Generation of transgenic strains via microinjection

Transgenic worms were constructed by germline transformation using the gonad microinjection method as previously described⁹. The respective construct was injected into worms along with about 20 ng/μl pRF4 plasmid or 5 ng/μl pCFJ90 as a coinjection marker. The coinjection plasmid pRF4 plasmid contains *rol-6(su1006)* dominant allele, causing the phenotype of rolling worms⁹. The coinjection plasmid pCFJ90 contains *myo-2::mCherry* leading to a visible fluorescent marker in the pharynx¹⁰. Worms of the F1 generation expressing the coinjection marker were singled out on a plate and monitored until the next generation (F2). Individuals transferring the marker to the F2 generation were considered to contain a stable extrachromosomal transgene.

Cell lysis and Immunoblotting

The cells were rinsed twice with PBS and subsequently lysed using RIPA buffer (containing 150 mM NaCl, 50 mM Tris pH 8.0, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS), along with Complete Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail 2 (#P5726-5ML, Sigma), and Cocktail 3 (#P0044-1ML, Sigma), for 10 minutes prior to centrifugation. Protein concentrations were quantified using Bio-Rad Protein Assay Dye Reagent Concentrate (#500-0006) and normalized to equal levels. The lysates were combined with Laemmli Sample Buffer and denatured at 95 °C for 5 minutes. Samples were then resolved by SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% BSA in TBST for 1 hour at room temperature, the membranes were probed with primary antibodies overnight at 4 °C. Following TBST washes, the membranes were incubated with HRP-conjugated secondary antibodies for 1–2 hours. Protein detection was performed using either Pierce ECL Western Blotting Substrate (#32209) or SuperSignal

West Femto Maximum Sensitivity Substrate (#34095), and chemiluminescent signals were visualized using a LAS-4000 imaging system.

siRNA knockdown

ON-TARGET plus SMARTpool siRNAs directed against SGK1(#L-003027-00-0005), RICTOR(#L-016984-00) and non-targeting control siRNA (siControl) (#D-001810-10) were purchased from Dharmacon. HuH7 and HepG2 human hepatocyte cell lines were transfected with a final concentration of 60 nM (for RICTOR and siRNA control) and 40 nM (for SGK1) using lipofectamine RNAiMAX transfection reagent (ThermoFisher) according to manufacturer's instructions.

Statistical analysis

All data in this study are expressed as mean \pm standard deviation (SD) unless otherwise stated. Two-tailed t-test analysis, two-way ANOVA analysis, and Fisher's Exact Test were performed to compare different groups in this study (without adjustments for multiple comparisons). Confidence intervals of 95% were chosen for two-tailed t-test. Probability values below 0.05 were regarded as significant, with ns (non-significant) representing $P > 0.05$. Exact P values have been represented in each experiment. Kaplan-Meier analysis with log-rank (Mantel-Cox) method was applied using a two-sided hypothesis test (in Prism) to compare survival curves of different groups. At least three independent experiments were performed unless otherwise stated, and representative results were shown. RT-qPCR experiments were performed once using three to four biological replicates to validate RNA-seq data and reporter-based analysis.

Construction of transgenic strains

For neuronal rescue of *sgk-1*, a *sgk-1(cDNA)* construct (pBY3942) was inserted after the neuronal promoter *rab-3*. For intestinal-specific rescue, intestinal promoter *vha6* driving expression of *sgk-1* was used (pBY3915).

A point mutation in the PX domain of *sgk-1* was generated to substitute arginine 73 with alanine (pBY3966). To create a putative ATP binding deficient SGK-1 version, a point mutation was introduced into the ATP binding site, where a lysine residue at position 154 was mutated to arginine (pBY4011). To mutate putative mTOR phosphorylation sites in SGK-1, S424 and T444 were mutated to alanine (pBY4013).

To generate a transgenic strain expressing an ECH-9 fusion with GFP (*Ex[Pech-9::gfp::ech-9(genomic)]*), we constructed a plasmid, pBY4294, by amplifying 3201 bp

promoter region of *ech-9* and inserting into pEGFP-N1 vector. Additionally, we used Gibson assembly to insert 3X FLAG tag along with *ech-9* (*genomic*) sequence and its 3'UTR into the construct. Fragments were PCR amplified with overhangs.

Oligonucleotides used to generate transgenic strains

RB57 58	GGGGAATTCAATGGTGAGGAAAGATGAGGTG	<i>sgk-1</i> fwd EcoRI	pBY3 707
RB57 59	GGGCCC GGTCAGACCAAACGCGATTGGTG	<i>sgk-1</i> rev SmaI	pBY3 707
RB62 29	CCCAGCGCTATCTTCAGATGGGAGCAGTGG	Neuronal expression of SGK-1- fwd	pBY3 942
RB62 30	CCCAGCGCTGCATCTGAAAATAGGGCTACTGTAGAT	Neuronal expression of SGK-1 (rev)	pBY3 942
RB65 04	AGCTCAAGCTTCGAATTCAATGGTGAGGAAAGATGAG GT	SGK- 1(K164R)- up-fwd	pBY3 862
RB65 05	TGGACAGAATCCTCATGGCGTAGATTTTTTTGG	SGK- 1(K164R)- up-rev	pBY3 862
RB65 06	CTACGCCATGAGGATTCTGTCCAAGGAACATAT	SGK- 1(K164R)- down-fwd	pBY3 862
RB65 07	TTTCCGGGGCCAAGTACTCCGGCGTCCCACAAAATGT G	SGK- 1(K164R)- down-rev	pBY3 862
RB65 08	ATACTAGGTTTTCTGCAGTACACGCCACACAGTTTTAT TA	SGK- 1(S434A, T454A)- fwd	pBY4 013
RB65 09	GATCCGGTGGATCCCGGGTCAGACCAAACGCGATT GGTGTGCGACGAATGCAAAGTTCTCAAATCGTGATCT CGATGAGTGACAGCAAGTTGTTGCGGTGCCAACGAA GCTGAA	SGK- 1(S434A, T454A)- rev	pBY4 013
RB60 84	TCAGGAGGACCCTTGGAGGGTACGCATGTACCTTTAT AGGTGC	Intestinal expression SGK-1-fwd	pBY3 667
RB60 85	TTCAGCAGCGAACATTTTTTATGGGTTTTGGTAGGTTT TAG	Intestinal expression SGK-1 rev	pBY3 667
RB65 02	ATGGGCAAAGTTCCAGAAG	<i>ech-9</i> - fwd	pBY4 294
RB65 03	GCACTAATAAGATTGATGAAGTCATAGTTTTGATGACA TCAGTTTG	<i>ech-9</i> - rev	pBY4 294

To amplify EGFP overhang (OH) we used:

For fwd OH backbone-
 AGAATTGAAAACAATCAAAAACCTCGAGCTCAAGCTTCAAT
 For rev OH 3X FLAG-
 catgatctttataatcCTTGACAGCTCGTCCATG

To amplify *ech-9* overhang (OH) we used:
 Primers for 3X FLAG-
 For fwd w/ OH EGFP-
 GCATGGACGAGCTGTACAAGgattataaagatcatgatgg
 For rev w/ OH *ech-9*-
 CTGGAACCTTTGCCATCTTGTCATCGTCATCCT

To amplify 3'UTR overhang (OH) we used:
 Fwd. with OH to *ech-9* of 3'UTR-Region
 5'- ACTGATGTCATCAAAAATGATAAAGCGaCCGCACTAATAAGATTGAT-3
 For 3'UTR rev
 5'-GCTGATTATGATCTAGAGTCGCGGCCGCTTTTTTATTCTTAAAT-3'

Oligonucleotides used for RT-qPCR experiments

Genes	Fwd	Rev	Reference
<i>cdc-42</i>	CTGCTGGACAGGAAGATT ACG	CTCGGACATTCTCGAATGAA G	5
<i>pmp-3</i>	GTTCCCGTGTTCACTACTC AT	ACACCGTCGAGAAGCTGTA GA	
<i>vit-1</i>	GAGGTTTCGCTTTGACGGA TA	GGCTTCACATTCTCGTTCT	11
<i>vit-3,4,5</i>	CATGTGCACCATCGAAGAA CTC	CCAATGTGGTTTCAATGACA AGTTG	
<i>vit-6</i>	TTCACCCAGAAGCCAGTTC	AGGATGGGAGGCAGTAGAC	
<i>lipl-4</i>	ACAGGTATTGCGGATGTTC C	GCATTTGTTCCCAAATGAA	12
<i>fat-5</i>	CGATTTGTACGAGGATCC GGTG	CAGTGGGAGACACTGTTGA TGC	13;14
<i>fat-6</i>	TCTACCAGCTCATCTTCGA GGC	GATCACGAGCCCATTTCGAT GAC	
<i>fat-7</i>	GGAAGGAGACAGCATTCA TTGCG	GTCTTGTGGGAATGTGTGG TGG	
<i>ech-9</i>	AAAGATACGGACGGAAAA CTAACAAAG	CCAACCAAAACCGAGAATAA ACATG	this study

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