Supplemental Methods
Subasic et al. (2015)

E. coli strains and culture conditions

E. coli strain: AT713 (Lysine/Arginine auxotroph), obtained from Coli Genetic Stock Center (CGSC number 4529), was grown in M9 Minimal Salts Medium supplemented with either light labelled lysine ($^{14}N_2,^{12}C_6$-L-Lysine) and arginine ($^{14}N_4,^{12}C_6$-L-Arginine) or heavy labelled lysine ($^{15}N_2,^{13}C_6$-L-Lysine) and arginine ($^{15}N_4,^{13}C_6$-L-Arginine) at a final concentration of 150 mg/L. The isotope-labelled aminoacids were obtained from Cambridge Isotope Laboratories, USA. Cultures were harvested when the OD_{600} reached between 2.0 and 2.5. One liter of bacteria was concentrated to 50 ml by pelleting the bacteria at 10'000 rpm at 4°C for 10 minutes and resuspending the pellet in sterile water. ^{15}N-labelled bacteria was prepared according to the published protocol (Gouw et al. 2011).

Stable isotope labeling by amino acids in nematodes

Worms were grown for two generations at 25°C on NGM plates without peptone (3g/L NaCl, 20 g/L Bacto-Agar, 5mg/L cholesterol, 25 mM K_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$), seeded with 3 ml of concentrated bacteria. Heavy and light labeled samples of the following genotypes: wild-type, *mir-58.1*, *mir-80; mir-58.1* and *mir-80; mir-58.1; mir-81-82* were prepared by feeding with heavy and light labelled bacteria, respectively. Both generations were synchronized by bleaching as described in (Stiernagle, 2006). 40 000 and 120 000 L1 larvae that hatched on unseeded plates overnight were transferred to the plates with light and heavy labelled bacteria, respectively (20 000 worms / plate), and collected at the late L4 stage for subsequent protein and RNA isolation (33 hours post-transfer for wild-type and *mir-58.1*). Appropriate staging was determined by examining a characteristic vulval structure for at least 100 animals under an inverted microscope (Leica MZ125). *mir-80; mir-58.1* double mutant was collected 6h post wild-type and *mir-80; mir-58.1; mir-81-82* quadruple mutant 15 hours post wild-type due to an observed developmental delay. Worms were washed with M9 buffer three times before freezing in liquid nitrogen. Three biological replicates were grown and experiment was performed in 2 variants: one without fractionation (in three biological replicates) and the other with HILIC fractionation (one replicate).

Sample preparation for selected reaction monitoring

Wild-type, *mir-58.1*, *mir-80*, *mir-81-82*, *mir-80; mir-58.1*, *mir-58.1; mir-81-82*, *mir-80; mir-81-82*, *mir-80*; *mir-58.1; mir-81-82* were grown in three biological replicates for targeted proteomics measurement. With the exception of heavy labelled wild type sample grown on ^{15}N-labelled bacteria, all the other "light" nematode samples were grown on NGM agar plates with *E. coli* strain op50. Synchronous population of L4 staged larvae was prepared as indicated above. Heavy labeled worms were grown on NGM agarose plates covered with 3 ml of ^{15}N labelled bacteria prepared as described previously (Gouw et al. 2011). Due to the developmental delay of *mir-58.1; mir-81-82* triple mutant, *mir-80; mir-58.1* double mutant, and *mir-80; mir-58.1; mir-81-82* quadruple mutant, worms were collected 3h, 6h and 14h post wild-type (and other miR-58 family mutants that showed no developmental delay at 25°C: *mir-80*, *mir-81-82*, *mir-80*; *mir-81-82* and *mir-58.1*), respectively.

Protein isolation, precipitation and digestion

Proteins from all the samples were extracted with 50 mM Tris/HCl (pH 8.3), 5 mM EDTA, 8 M urea buffer and glass beads as described previously (Schrimpf et al. 2009). The protein concentrations of the purified extracts were determined by Bradford assay (using Bradford reagent, Sigma Aldrich). For SRM experiment: 50 µg of ^{15}N heavy and 50 µg of light labelled SRM samples were mixed, following trypsin digestion of the samples and cation exchange chromatography of the peptides according to the following protocol (Shiio and Aebersold 2006). Peptides were additionally desalted with C18 reversed-phase columns (Sep-Pak Vac C18, Waters), dried in vacuum centrifuge and resolubilised in 2% acetonitrile/ 0.1% formic acid to a final concentration of 1 µg/ul. For SILAC experiment, 100 ug of protein extract labelled with heavy lysine (^{15}N$_2$,^{13}C$_6$-L-Lysine) and arginine (^{15}N$_4$,^{13}C$_6$-L-Arginine) from wild type, *mir-58.1*, *mir-58.1; mir-80* and *mir-58.1; mir-80; mir-81-82 C. elegans* strains was mixed and used as the heavy internal proteome standard. 100 µg of light labelled *C. elegans* protein extracts were mixed with 100 µg of heavy labelled internal standard proteome, followed by precipitation with 6 volumes of ice-cold acetone. Protein samples were kept at -20 ºC for 16 hours, and afterwards resuspended in 8 M Urea/0.1 M NH$_4$HCO$_3$ buffer. Subsequently, proteins were reduced with 12 mM dithiotreitol for 30 min at 37 °C and alkylated for 45 min at 25 °C with 40 mM iodoacetamide, protected from light. After diluting the samples with 0.1 M NH$_4$HCO$_3$ to a final concentration of 1.5 M urea, Lysyl

endopeptidase (Wako) digestion was performed (enzyme:substrate ratio = 1:100) for 6 hours at 37 °C. Sequencing grade porcine trypsin (Promega) was supplemented (final enzyme:substrate ratio= 1:100) and the mixture was incubated for 16 h at 37 °C. Peptides were desalted by Sep-Pak tC18 cartridges (Waters, Milford, MA, USA), following elution with 50% acetonitrile. The liquid phase was evaporated in a vacuum centrifuge and the purified peptides were resolubilized in 2% acetonitrile/0.1% formic acid to 1 μg/ul concentration.

HILIC fractionation and desalting

Peptides obtained from SILAC samples were fractionated with Agilent 1100 HPLC flow system using a 4 mm HILIC column YMC-Pack Polyamine II (YMC Europe GmbH). Gradient of 0 - 50% solvent B (solvent A = 75% acetonitrile, 8mM KH_2PO_4 (pH 4.5); solvent B = 5% acetonitrile, 100 mM KH_2PO_4) was delivered to the samples in 30 min at a flow rate of 850 μl/min. 27 fractions were collected and combined in final 10 fractions based on the intensity measured by UV-light detector at 214 nm. 10 fractions were evaporated on a vacuum centrifuge to dryness, following C18 desalting purification with Zip-tip pipette tips for sample preparation (Size:P10, Millipore). Purified samples were evaporated to dryness and resolubilized in 2% acetonitrile/0.1% formic acid to 1μg/ul concentration.

Orbitap mass spectrometry and statistical analysis

Samples were analyzed by LC-MS/MS using a Thermo Easy nLC 1000 HPLC system coupled to a Thermo Orbitrap Elite hybrid mass spectrometer equipped with a Nanoflex electrospray source (Thermo Fisher Scientific). Peptides were separated on a Thermo PepMap RSLC column (15 cm length, 75 um inner diameter, 2 um particle size) at a flow rate of 300 nL/min. The following mobile phase gradient was utilized (Mobile phase A was water/acetonitrile/formic acid with volume ratios 97.85:2:0.15, mobile phase B water/acetonitrile/formic acid with volume ratios 2: 97.85:0.15): 0-120 min, 5-35% B; 120-122 min, 35-80% B; 122-132 min, 80% B. The mass spectrometer was operated in data-dependent sequencing mode, selecting the 15 most abundant precursors in the m/z range of 350-1600 per cycle from a precursor scan at a resolution of 120000. Precursors were isolated in the linear ion trap with an isolation width of 2.0 m/z and activated with normalized collision energy of 35 using a q of 0.25 and an activation time of 10 ms. Fragment ion spectra

3

were acquired in the linear ion trap in normal resolution mode. Singly charged ions were excluded from sequencing, and dynamic exclusion was set at 30 s after 1 sequencing event with an exclusion window of 10 ppm. The RAW files were searched against the Uniprot protein database for *C. elegans*. Cysteine carbmidomethylation was set as static modification. $^{13}C_6$, $^{15}N_4$-Arginine, $^{13}C_6$, $^{15}N_2$-Lysine and methionine oxidation were set as variable modification. The identification result was filtered with peptide and protein FDR 0.01. The quantification of MS measurements has been performed with MaxQuant with default settings and peptide with $\geq$ 6 amino acids were selected.

TSQ Quantum Ultra mass spectrometry and statistical analysis

Previously developed SRM assays (Jovanovic et al. 2012; Jovanovic et al. 2010) for 24 proteins predicted to be miR-58 targets by TargetScan and a control group of 22 random proteins were used on all combinations of miR-58 family mutants. Measurements were performed on Thermo Quantum Ultra Triple Quadrupole Mass Spectrometer. 1160 SRM transitions, generated as described (Jovanovic et al. 2010; Jovanovic et al. 2012), were split and analyzed in several consecutive runs (an average of 230 transitions per run). In average 2 peptides were used per a protein and 10 transitions per a peptide (5 light, 5 heavy). Retention peptides (iRT-Kit; Biognosys) were spiked in the sample as a run calibration reference. Instrument was run in scheduled SRM mode with cycle time of 3 seconds, retention time window of 12 minutes and Q1 FWHM set at 0.7. The following gradient of solvent B was used for the duration of 120 minutes with the flow rate of 250 nl/min (Solvent A: 99.8% water, 0.2% formic acid; Solvent B: 99.8% acetonitrile, 0.2% formic acid): 0 min, 5% B; 1 min, 10% B; 71 min, 35% B; 75 min, 80% B; 85 min, 80% B; 88 – 120 min, 5% B. Statistical validation of SRM experiments was performed with mProphet (Reiter et al. 2011). Targeted proteins from three biological replicates were measured. List of proteins used as target and random group with respective quantitation information is available in the Supplementary Table 4. The list of transitions used for the SRM quantifications together with retention peptides is shown in Supplementary Table 6.

RNA isolation

RNA was isolated by TRIzol (Life Technologies) according to the manufacturer's protocol and additionally purified by acidic phenol: chloroform (5:1) solution pH 4.5 (Ambion)

followed by another chloroform extraction to obtain high-grade RNA for sequencing. RNA concentration was measured using a Nanodrop device (Thermo Fisher Scientific).

Transcriptome sequencing and analysis

Transcriptome sequencing of RNA isolated from wild-type, *mir-58.1* single, *mir-80; mir-58.1* double and *mir-80; mir-58.1; mir-81-82* quadruple mutants in three biological replicates was performed in GATC Biotech. RNA quality control was assessed with Agilent Bioanalyzer 2100. 12 tagged random primed cDNA libraries were sequenced in two lanes with Illumina HiSeq 2000. mRNA sequencing reads were uploaded to the ClipZ server (Khorshid et al. 2011) for mapping and annotation. Read counts for individual transcripts were obtained from ClipZ and were normalised to the transcript length and a total of one million in each sample. mRNA abundances were calculated as the average over three replicates. Predicted target transcripts of different miR-58 family members with different types of seed matches were taken from Targetscan (Friedman et al. 2009). The differential expression analysis was performed using DESeq (Anders and Huber 2010) comparing the number of reads in three replicates of one condition with those in the replicates of another condition.

Cloning, Mammalian Cell Culture and Luciferase Reporter Assays

Seven TargetScan predicted miR-58 family targets that were highly upregulated in SRM, fractionated SILAC, unfractionated SILAC and RNA-seq: *C30B5.7*, *C37H5.13*, *cgh-1* (*C07H6.5*), *lys-1* (*Y22F5A.4*), *nas-3* (*K06A4.1*), *clec-89* (*C09D1.2*) and *isw-1* (*C09D1.2*) were selected for independent validation. Their full-length 3'UTRs (synthetically designed dsDNA gBLOCK®, IDT) were XhoI/NotI digested and cloned into linearized Luciferase reporter psiCHECK-2 Vector (Promega). Each predicted miR-58.1/80/81/82 binding site was disrupted by deleting the first six bases of the seed region yielding mutated (mut) reporter negative controls. RNA oligonucleotide transfection for Luciferase assays were used as follows: siRNA against Renilla (Microsynth AG) as a positive control, Silencer Negative Control No. 1 siRNA (Ambion) as a negative control and the miRNA mimics, miR-58.1 and miR-80 (Ambion). HeLa cells (ATCC) were grown in Dulbecco's modified Eagle's medium (Gibco, Invitrogen) supplemented with 10% fetal bovine serum (Sigma-Aldrich). For the luciferase assays, HeLa cells were seeded in 96-well plates (7000 cells/well). RNA oligonucleotides (9 nM per well), as well as mock were transfected using Oligofectamine

(Invitrogen) 4 hours post-seeding, according to manufacturer's instructions. Reporter plasmids (20 ng per well) were transfected after 24 hours post-seeding using jetPEI (Polyplus) according to manufacturer's instructions. After 48 hours, supernatants were removed and luminescence measured using the Dual-Glo® Luciferase Assay System (Promega). Values for Renilla luciferase were normalized against the Firefly luciferase and the resulting ratio was normalized against the corresponding mock control. Sequences of all the constructs used in the luciferase reporter assay are listed in the Supplementary Figure 7.

Quantitative real-time PCR on miR-58 family targets and *egl-1* induction

For the quantitative real-time PCR (qRT-PCR) on miR-58 targets RNA was isolated from the late L4 staged animals collected as indicated above. The sample for RNA isolation was taken at the same time as for protein isolation, so that the results from proteomics and transcriptomics approaches can be compared to each other. For *egl-1* induction assay, synchronized adult worms grown on 20°C were irradiated with 60 Gy once the large part of animals started laying eggs but no progeny had hatched (65 hours post transfer of synchronized L1 larvae to seeded plate for wild type and *mir-58.1* single mutant; 72 hours for *mir-80; mir-58.1* double mutant; 80 hours for *mir-80; mir-58.1; mir-81-82* quadruple mutant). Worms were collected 7h, 9h and 11 hours post-irradiation for wild-type and *mir-58.1* single mutants, *mir-80; mir-58.1* double mutant and *mir-80; mir-58.1; mir-81-82* quadruple mutant, respectively. Gravid adults were washed from plates, washed three times with M9 and frozen in liquid nitrogen for protein and RNA isolation. RNA was isolated as described above. 200 ng of RNA was used for cDNA synthesis with the SuperScript III (Invitogen) as instructed by the manufacturer. qPCR reactions were performed using MESA Green qPCR Mastermix Plus for SYBR Assay (Eurogentec) on an ABI 7900 HT Sequence Detection System coupled to ABI Prism 7900 SDS 2.2 Software (Applied Biosystems). qRT-PCR for miR-58 targets was performed in two technical and three biological replicates and *egl-1* qRT-PCR in three technical and two biological replicates. The mean measured levels for transcripts of interest were normalized to the mean of a set of internal controls: housekeeping genes *pgk-1*, *mpk-1* and *tbp-1* and two additional reliable reference genes *cdc-42* and *Y45F10D.4* (Hoogewijs et al. 2008). Primer pairs were mostly designed via Roche Universal Probe Library and are all listed in the Supplementary Table 5. 2−ΔΔCt method was used to calculate the relative transcript abundance (Livak and Schmittgen 2001)

Small RNA sequencing

1µg of total RNA isolated as described above was processed for small RNA sequencing. Four calibrator oligonucleotides (Cal 01-04 5 fMol each) were added as a reference as described previously (Hafner et al. 2012a; Hafner et al. 2012b). Briefly, RNA was dephosphorylated using FastAP (Fermentas) and radiolabelled according to the described method (Hafner et al. 2012a) with the 10-fold reduction of γ-32P-ATP (final concentration 0.05 µCi/µl). Subsequently, RNA was separated with denaturing PAA (15%) gelelectrophoresis and fractions between 18 and 25 nt in size were excised from the gel. Thereafter, preadenylated 3'-adapter (IDT DNA Technologies, 5'-TGGAATTCTCGGGTGCCAAGG-3') was ligated with truncated T4 RNA Ligase 2 (1-249, K227Q, final concentration 2000 U/µL, NEB) overnight on ice. After a denaturing 15% PAA gel separation, RNA ranging from 35 to 70 nt was cut out of the gel and extracted with 0.4 M NaCl overnight. The RNA was recovered by acidic phenol/chloroform extraction, ethanol precipitation, and addition of Glycoblue (Ambion) followed by 5'-RNA Adapter (5'-GUUCAGAGUUCUACAGUCCGACGAUC-3') ligation using T4 RNA ligase (Fermentas) at 37°C for 1 h. After an additional gel-purification step performed RNA was reverse transcribed into cDNA with SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's description. Library amplification step was performed with minimal number of PCR as determined by a preceding pilot PCR using different PCR cycle numbers. PCR was performed using Taq DNA-Polymerase (Sigma-Aldrich as described) with Index primers described in IlluminaTruSeq Small RNA Sample Prep Kit protocol. Amplicons were extracted using QiaExII (Qiagen) according to the purchaser's protocol. The recovered DNA was sequenced using single end, 50 cycle sequencing on HiSeq2000 (Illumina).

Small RNA-sequencing analysis

Small RNA sequencing data were uploaded to the ClipZ server (Khorshid et al. 2011) for adapter removal. Reads mapping to mature miRNA sequences downloaded from miRbase (release 20, Kozomara and Griffiths-Jones 2011) and to the four calibration sequences were identified using Segemehl (Hoffmann et al. 2009). miRNA abundances were normalized to the sum of reads mapping to the four calibration sequences and then averaged over the three replicates obtained for each mutant condition (except for the wild type condition in which only one sequencing sample yielded a sufficient number of reads).

miRNA qRT-PCR

miR-58 family member quantification in late L4 staged animals was carried out using an adaptation of the stem-loop qRT-PCR protocol (Chen et al. 2005). Small RNA was isolated with TRIzol and a DNase treatement was performed with Turbo DNA-free kit (Ambion) according to manufacturer's instructions. cDNA synthesis reactions were conducted on 500 ng RNA using SuperScript III kit (Invitrogen). cDNA synthesis reactions contained six stem-loop primers (final concentration 20 nM) ending with miRNA specific sequence for assayed miRNAs: miR-58.1, miR-80, miR-81 and miR-82 and two miRNAs that are stably expressed throughout the development (Kato et al. 2009) and that were used as normalization control: miR-52 and miR-250. Following program was used for reverse transcription: 1. 30 min at 16°C; 2. 30 sec at 30°C; 3. 30 sec at 42°C; 4. 1 sec at 50°C; 5. repeat steps 2-4 59 additional cycles; 6. 5 min at 85°C. qPCR reactions were performed in technical and biological triplicates using MESA Green qPCR Mastermix Plus for SYBR Assay (Eurogentec), according to the manufacturer's recommendations, on an ABI 7900 HT Sequence Detection System coupled to ABI Prism 7900 SDS 2.2 Software (Applied Biosystems). Universal reverse and miRNA specific forward primers were used for the amplification. All the primers used for the stem-loop RT reaction and miRNA qRT-PCR are listed in the Supplementary Table 5.

Apoptosis phenotypic analysis

Apoptotic germ cell corpses of different miR-58 family mutants were scored 24 hours post L4/adult molts using Differential Interference-Contrast (DIC) Microscopy. Irradiation was performed when the worms were at the L4 larval stage/adult molt, by exposing worms on agar plates to X-rays in an Isovolt irradiation machine with a dose of 60 Gy. RNAi of *T07D1.2* gene was performed as described previously (Neukomm et al. 2011). Due to the developmental delay at 20°C, *mir-80; mir-58.1; mir-81-82* quadruple mutants and *mir-80; mir-58.1; mir-81-82; Ex[ΔmiR-80]* needed 5 hours longer to reach L4 stage compared to wild-type and were scored 29 hours post reaching L4/adult molt stage. Experiment was done in three biological replicates (n=20 / replicate). For embryonic apoptotic cell corpses mixed embryos of indicated stages were scored for persistent cell corpses using DIC microscopy. Corpse persistence was measured by recording a video of

young adults mounted on a 3% agar pad and anesthetized in a droplet of 5 mM levamisole in M9. The time from the first corpse appearance until it was no longer visible was measured.

Western blot

Synchronized young adult worms were prepared as described above for *egl-1* induction qRT-PCR assay. Proteins were extracted in modified Laemmli sample buffer (60 mM Tris-Cl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.01% bromophenol blue, 1 mM PMSP, phosphatase inhibitor) by heating the sample 10 min at 95°C in a thermomixer, followed by 10 min sonication with Diagenode Bioruptor (5 cycles: 30 sec sonication, 30 sec pause, setting: high) and final 5 min heating at 95°C. Cleared protein extracts were loaded onto an SDS-PAGE gel. Subsequently proteins were transferred to a nitrocellulose membrane and probed with monoclonal anti-MAP kinase activated antibody produced in mouse (Diphosphorylated ERK-1&2, Sigma). Two additional immunoblots were performed following stripping: one with anti-MAP kinase antibody produced in rabbit (ERK-1, ERK-2, Sigma) and the other one with tubulin (Sigma) antibody that was used as a loading control.

References

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. *Genome Biol.* **11**: R106.

Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR et al. 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* **33**: e179.

Friedman RC, Farh KK, Burge CB, Bartel DP. 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **19**: 92–105.

Gouw JW, Tops, Bastiaan B J, Krijgsveld J. 2011. Metabolic labeling of model organisms using heavy nitrogen (15N). *Methods Mol. Biol.* **753**: 29–42.

Hafner M, Lianoglou S, Tuschl T, Betel D. 2012a. Genome-wide identification of miRNA targets by PAR-CLIP. *Methods* **58**: 94–105.

Hafner M, Renwick N, Farazi TA, Mihailović A, Pena, John T G, Tuschl T. 2012b. Barcoded cDNA library preparation for small RNA profiling by next-generation sequencing. *Methods* **58**: 164–170.

Hoffmann S, Otto C, Kurtz S, Sharma CM, Khaitovich P, Vogel J, Stadler PF, Hackermüller J. 2009. Fast mapping of short sequences with mismatches, insertions and deletions using index structures. *PLoS Comput. Biol.* **5**: e1000502.

Hoogewijs D, Houthoofd K, Matthijssens F, Vandesompele J, Vanfleteren JR. 2008. Selection and validation of a set of reliable reference genes for quantitative sod gene expression analysis in C. elegans. *BMC Mol. Biol.* **9**: 9.

Jovanovic M, Reiter L, Clark A, Weiss M, Picotti P, Rehrauer H, Frei A, Neukomm LJ, Kaufman E, Wollscheid B et al. 2012. RIP-chip-SRM--a new combinatorial large-scale approach identifies a set of translationally regulated bantam/miR-58 targets in C. elegans. *Genome Res.* **22**: 1360–1371.

Jovanovic M, Reiter L, Picotti P, Lange V, Bogan E, Hurschler BA, Blenkiron C, Lehrbach NJ, Ding XC, Weiss M et al. 2010. A quantitative targeted proteomics approach to validate predicted microRNA targets in C. elegans. *Nat. Methods* **7**: 837–842.

Kato M, Lencastre A de, Pincus Z, Slack FJ. 2009. Dynamic expression of small non-coding RNAs, including novel microRNAs and piRNAs/21U-RNAs, during Caenorhabditis elegans development. *Genome Biol.* **10**: R54.

Khorshid M, Rodak C, Zavolan M. 2011. CLIPZ: a database and analysis environment for experimentally determined binding sites of RNA-binding proteins. *Nucleic Acids Res.* **39**: D245-52.

Kozomara A, Griffiths-Jones S. 2011. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* **39**: D152-7.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402–408.

Neukomm LJ, Frei AP, Cabello J, Kinchen JM, Zaidel-Bar R, Ma Z, Haney LB, Hardin J, Ravichandran KS, Moreno S et al. 2011. Loss of the RhoGAP SRGP-1 promotes the clearance of dead and injured cells in Caenorhabditis elegans. *Nat. Cell Biol.* **13**: 79–86.

Reiter L, Rinner O, Picotti P, Hüttenhain R, Beck M, Brusniak M, Hengartner MO, Aebersold R. 2011. mProphet: automated data processing and statistical validation for large-scale SRM experiments. *Nat. Methods* **8**: 430–435.

Schrimpf SP, Weiss M, Reiter L, Ahrens CH, Jovanovic M, Malmström J, Brunner E, Mohanty S, Lercher MJ, Hunziker PE et al. 2009. Comparative functional analysis of the Caenorhabditis elegans and Drosophila melanogaster proteomes. *PLoS Biol.* **7**: e48.

Shiio Y, Aebersold R. 2006. Quantitative proteome analysis using isotope-coded affinity tags and mass spectrometry. *Nat Protoc* **1**: 139–145.