Supporting Information

Fig. S1. Phylogenetic tree of *Caenorhabditis elegans* ULPs and other ULPs/SENP s. *C. elegans* ULPs are labeled in bold font. Colors represent different ULP/SENP families. Human SENPs 1–3; 5–8 are shown as well as ULPs of *Drosophila melanogaster* (Dme) and *Saccharomyces cerevisiae* (Sce). (Scale bar: branch length, which is expressed as the expected number of substitutions per site.)

Fig. S2. *ulp-4::gfp* signal is reduced by *ulp-4* RNAi. We tested the activity of *ulp-4* RNAi on worms expressing *ulp-4p::ulp-4::GFP*. (A) The stereotypic expression pattern of *ulp-4::GFP* in day-1 adult hermaphrodites fed with empty vector RNAi. Hermaphroditic-specific neuron (HSN) expression (white arrowhead) and expression in head muscles (white arrows) are shown. (B) *ulp-4* RNAi activity resulted in the elimination of GFP signal from body wall muscle cells and HSN neurons. When the worms were treated with *ulp-4* RNAi for more than two generations, the signal was also reduced in pharyngeal cells. (Scale bar: 50 µm.)
Fig. S3. *hmgs-1* knockdown is rescued by mevalonate supplementation. (A) A schematic representation of mevalonate pathway metabolism. Cholesterol production is absent in *C. elegans* but other branches of the pathway are conserved. (B) Worms fed with *hmgs-1* RNAi show a significant reduction in the level of pharyngeal pumping. This effect can be fully rescued by supplementation of 20 mM mevalonate indicating that *hmgs-1* knockdown phenotypes stem from impaired mevalonate pathway flux. 20mM mevalonate does not affect the pumping rate of WT worms; precluding a nonspecific rescue by mevalonate supplementation. Bars represent SEs. Number of worms, *n* = 10 WT worms in each condition.
A comparison between HMGS-1 and human HMG-CoA synthase 1 (HMGCS1) modifications. (A) Prediction of sumoylation sites in HMGS-1 and HMGCS1 proteins. Lysine (K) highlighted in red are the residues predicted to be sumoylated. (B) HMGCS1 undergoes ubiquitination and acetylation at multiple sites. Human HMGCS1 modifications are based on data available from PhosphoSitePlus (www.phosphosite.org/homeAction.do). Sequences were aligned using ClustalW (www.ebi.ac.uk/Tools/msa/clustalw2) and visualized using Jalview software (1).

Fig. S5. A model of HMGS-1 structure and its alignment with human HMGCS1. (A) Predicted HMGS-1 3D structure based on a Protein Data Bank structural search (www.rcsb.org/pdb/home/home.do). HMGS-1 shares the same structure with the human proteins HMGCS2 (42%) and HMGCS1 (40%) with confidence of 100%. The sumoylated Lys408 is labeled in red. (B) Alignment between HMGS-1 and its functional human ortholog HMGCS1. On the HMGCS1 structure, K426 which is the closest to the sumoylated residue of HMGS-1 is labeled in blue. This residue was found to undergo ubiquitination in human HMGCS1. The Phyre server (1) was used to search for proteins with a HMGS-1–related structure.

Molecular characterization of the *ulp-4* locus and its requirement for normal fat homeostasis. (A) RT-PCR analyses of tm3886 within the *ulp-4* locus. The *ulp-4* N-terminal region is in green (exons 1–3). This is followed by the C-terminal region (exons 4–5) that is labeled in gray. This region harbors the *ulp-4* catalytic domain (the conserved cysteine is labeled “©”). RT-PCR analyses revealed that in the strain homozygous for the tm3886 deletion, a truncated transcript still exists. Because the tm3886 deletion introduces a frame shift followed by a stop codon before the catalytic site, this transcript presumably does not code for a functional ULP-4 protein. (B–E) Worms’ fat level was determined by Oil-O-Red staining. (C) *ulp-4*(tm3688) mutant worms have less fat than the WT control (B). (D) The level of fat is marginally increased in worms overexpressing *ulp-4::gfp*. (E) *ulp-4::gfp* construct partially rescues the fat loss of *ulp-4(-)* mutants, indicating that the *ulp-4::gfp* construct is functional. (Scale bar: 50 μm.)
Fig. S7. Opposite effects of *ulp-4* loss and *ulp-4* overexpression (*ulp-4*::GFP) on *C. elegans* lifespan. (A) Lifespan analyses of WT, *ulp-4* mutants, and worms overexpressing *ulp-4* under its own promoter. (B) *ulp-4* mutants have a longer median and maximal lifespan than WT controls, whereas *ulp-4* overexpression exhibits an opposite effect. The effect of *ulp-4* on *C. elegans* lifespan is significant based on multiple statistical analyses. (C) All lifespan experiments were held at 20 °C on plates seeded with *Escherichia coli*, strain OP50, at the same time.
**Fig. S8.** Proteomic analysis of HMGS-1 interactors. (A) A diagram of the metabolic network discovered by the survey of HMGS-1 interactors. This analysis suggests that acetyl-CoA and acetoacetyl-CoA synthesis is physically coupled to condensation by HMGS-1, presumably in a hand-off type of mechanism. (B) Statistics of the Kyoto Encyclopedia of Genes and Genomes (KEGG, www.genome.jp/kegg) components enriched in the immunoprecipitated fraction based on a KEGG analysis performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/home.jsp) (1). (C) The list of ubiquitin-proteasome-related proteins interacting with HMGS-1. HMGS-1 itself, labeled in red, was found as the most enriched protein in the immunoprecipitated fraction, demonstrating the potency of our assay. The list is comprised of fifteen proteins identified by Eukaryotic Orthologous Groups of proteins analysis (http://genome.jgi-psf.org/help/kogbrowser.jsf) and five proteins that were identified manually.

Fig. S9. ClustalW sequence alignment between C. elegans ULP-4 small ubiquitin-like modifier protease and human SENP3 and SENP6. Specific acidic residues are labeled in red, and acidic regions (rich in acidic residues) are underlined in blue. Acidic regions are not present in other human SENPs. The alignment was annotated using Jalview software (1).


Movie S1. WT worms on hmg-1 RNAi. A movie of 30 frames per second showing day-1 adult WT worms grown from egg to adulthood on hmg-1 RNAi. Treatment with hmg-1 RNAi results in slow development, small body size, partial sterility, and severe worm paralysis.
Movie S2. WT worms on hmgS-1 RNAi with 20 mM mevalonate. A movie of 30 frames per second showing day-1 adult WT worms grown from egg to adulthood on hmgS-1 RNAi in the presence of 20 mM mevalonate. The severe hmgS-1 RNAi phenotypes shown in Movie S1 are fully rescued by the supplementation of mevalonate. This rescue demonstrates that hmgS-1 knockdown phenotypes stem from an impaired mevalonate pathway flux. This result links HMGS-1 activity directly to the mevalonate pathway.

Other Supporting Information Files

Dataset S1 (XLSX)