Supporting Information

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SI Materials and Methods

***Caenorhabditis elegans* Strains.** All strains were maintained at 16 °C, 20 °C, or 25 °C using standard culture methods and fed with *Escherichia coli* strain OP50 (1). The following mutants and alleles were used in this study: LGII: *osm-8(n1518)*, *age-1(hx546)*; *LGIII: osm-7(n1515)*, *daf-2(e1370)*, *drIs20 [vha-6p::Q44-YFP, rol-6 (su1006)]*; LGX: *osm-11(n1604)*. *drIs20* was constructed by UV mutagenesis-mediated integration of strain GF80 ($^{+/+}$*; dgEx80 [vha-6p::Q44-YFP; rol-6(su1006)]*) (2). Integration was carried out by exposing ~50 *dgEx80* L4 animals to 30,000 μJ/cm^2 generated from a UV cross-linker (Stratagene). After singling the progeny of ~500 F1 animals, an integrated line segregating 100% *rol-6* animals was isolated and outcrossed three times to wild-type animals to generate *drIs20 [Pvha-6::Q44-YFP;rol-6(su1006)]*, which was used in all subsequent studies. All double-mutant combinations were constructed using standard crossing strategies. Strain genotypes were verified by visible recessive phenotypes and DNA sequencing.

Fluorescence Microscopy and Fluorescence Recovery After Photobleaching Analysis. For all aggregation assays, worms were imaged on a fluorescence-equipped stereo dissecting microscope (Leica MZ16FA). Worms with ≥10 distinguishable aggregates, defined as fluorescent puncta that could be defined from adjacent/background fluorescence on all edges, were considered aggregated animals (2). For aggregate morphology and time-lapse imaging experiments, levamisole (1 mM)-anesthetized animals were imaged on thin agarose pads containing the indicated levels of NaCl using a fluorescence inverted microscope (Leica DMI4000B) and digital camera (Leica DFC340Fx). Deconvolution of Z-stacked (0.2-μm step size) images was carried out in Leica image analysis software (advanced fluorescence 6000 software). For fluorescence recovery after photobleaching analysis, animals were imaged using a confocal microscope (Zeiss LSM510) with a 40×/1.2 N.A. water-immersion lens and bleached with five scans from a 488-nm laser at 100% power. Images (128 × 128 pixels) were collected every 250 ms after bleaching. The kinetics and time constants of recovery were calculated by fitting the data with the Hill function in OriginLab software. *C. elegans* immunohistochemistry was carried out on manually dissected intestines. Briefly, intestines were released from *drIs20* animals by manual dissection in 100 mM K_2HPO_4 and then fixed for 1 h at 4 °C in 100 mM K_2HPO_4 + 3% (vol/vol) paraformaldehyde (EM Biosciences). Fixed worms were washed five times in BT buffer (20 mM H_3BO_3, 10 mM NaOH, 0.5% Triton X-100), permeabilized for 1 h at 4 °C in BTB buffer [BT + 2% (vol/vol) β-mercaptoethanol], and blocked for 1 h at 4 °C in Aba buffer (1× PBS, 0.5% BSA, 0.5% Triton X-100). Samples were incubated overnight at 4 °C in anti-ubiquitin antibody diluted in Aba buffer (1:100 dilution; Dako), washed five times in Aba buffer, and then incubated for 2 h at 4 °C in a goat anti-rabbit Alexa 594 secondary antibody (1:1,000 dilution; Molecular Probes). After being washed five times in Aba buffer, samples were mounted in ProLong antifade (Invitrogen) reagent for imaging.

PolyQ Protein Biochemistry. To isolate osmotic aggregates, synchronized *drIs20* young adults (24 h post-L4 stage) were exposed to 500 mM NaCl NGM plates for 4 h to induce the formation of osmotic aggregates. For aging aggregates, young adult animals were aged on NGM plates containing fluorodeoxyuridine for 4 d after the L4 stage. Aged and osmotically stressed animals were processed in parallel using identical solutions and conditions. Specifically, the animals were rinsed from the plates with M9 solution and washed twice. One hundred microliters of washed pelleted worms were added to 500 μL of RIPA buffer [150 mM NaCl, 50 mM Tris·HCl (pH 7.5), 1 mM EGTA, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, Roche Complete Protease Inhibitor (0.1%), 0.5 mM PMSF] and were sonicated on ice (15 cycles, 30% power, 2-s pulse, 5-s rest; Branson Ultrasonics sonicator). Following sonication, the homogenate was subjected to a low-speed spin (400 × *g*, 5 min). The low-speed supernatant (500 μL) was then subjected to ultracentrifugation (100,000 × *g*, 30 min). The supernatant was removed and the pellet was resuspended in 500 μL of RIPA buffer + 0.1% SDS and sonicated on ice (1 cycle, 20% power, 2-s pulse). This extraction process was repeated for RIPA buffer + 0.5%, 1.0%, 1.5%, and 2.0% (wt/vol) SDS. The pellet from the 2% SDS extraction was then solubilized in 100 μL of urea buffer [30 mM Tris·HCl, 7 M urea, 2 M thiourea, 4% (wt/vol) CHAPS, 0.5 mM PMSF, Roche Complete Protease Inhibitor (0.1%)]. Ten microliters of each fraction (2% of the total fraction, except for the urea fraction, which was 10% of the total fraction) was added to 10 μL of 2× SDS sample buffer and then loaded onto a 10% (wt/vol) SDS/PAGE gel. Proteins were transferred onto nitrocellulose and then probed with a monoclonal anti-GFP antibody (1:1,000; Roche), which was detected using enhanced chemiluminescence. Band intensity was quantified using gel analysis routines in ImageJ (National Institutes of Health). The total amount of protein present in each sample was calculated and the amount of protein in each sample was calculated as a percentage of the total. The extraction and quantification procedure was repeated on four separate occasions with independently isolated samples.

HEK Cell Experiments. HEK293 cells were grown in Dulbecco's minimal essential media (Gibco) with 10% (vol/vol) heat-inactivated FBS (Gibco) and penicillin/streptomycin on laminin/polylysine-coated glass coverslips. Plasmids were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations. One day after transfection, the cells were placed into either isotonic or hypertonic PBS and kept at 37 °C for up to 3 h. For heat-stress experiments, cells were incubated at 42 °C for 3 h. For oxidative stress, cells were incubated in PBS containing the indicated amounts of paraquat, EtOH, sorbitol, sucrose, or NaCl for 3 h. Antibody staining was carried out as previously described (3) using a polyclonal anti-ubiquitin antibody (1:100; Dako).

1. Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77(1):71–94.
2. Mohri-Shiomi A, Garsin DA (2008) Insulin signaling and the heat shock response modulate protein homeostasis in the *Caenorhabditis elegans* intestine during infection. *J Biol Chem* 283(1):194–201.
3. Mojsilovic-Petrovic J, et al. (2009) FOXO3a is broadly neuroprotective in vitro and in vivo against insults implicated in motor neuron diseases. *J Neurosci* 29:8236–8247.

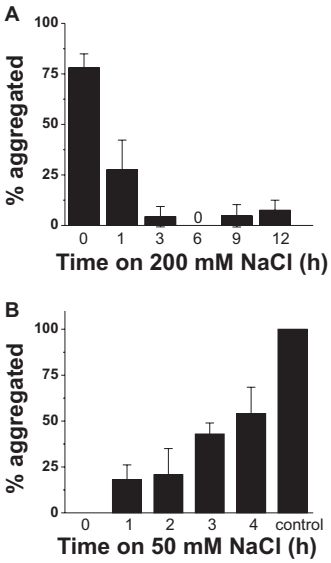


Fig. S1. Acquisition and loss of adaptation-mediated protection against osmotically induced polyQ aggregation. (*A*) Young adult (24 h post-L4 stage) *drIs20* hermaphrodites were grown their entire life on 50 mM NaCl NGM plates and then transferred to NGM plates containing 200 mM NaCl for the indicated periods. Animals were then placed on NGM plates containing 500 mM NaCl, and the percentage of animals containing aggregates was quantified after 4 h. Data shown are mean ± SD. $n = 4$ replicates, ≥40 animals per time point. (*B*) L4 stage *drIs20* hermaphrodites were transferred to NGM containing 200 mM NaCl for 24 h and then transferred to NGM plates containing 50 mM NaCl for the indicated periods. Animals were then placed on NGM plates containing 500 mM NaCl, and the percentage of animals containing aggregates was quantified after 4 h. Control, animals that were never exposed to 200 mM NaCl. Data shown are mean ± SD. $n = 4$ replicates, ≥40 animals per time point.

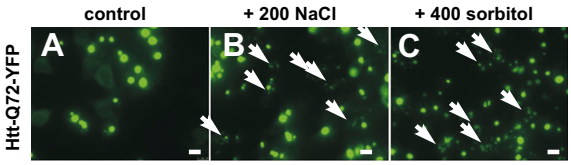


Fig. S2. Osmotically induced polyQ aggregation in mammalian cells is due to an osmotic effect. HEK293 cells transfected with HTT-Q72-YFP were exposed to control (*A*), 200 mM NaCl (*B*), or 400 mM sorbitol (*C*) for 3 h and then imaged. Arrows indicate cells containing cytoplasmic polyQ aggregates that are not observed in control cells. (Scale bars, 10 μm.)

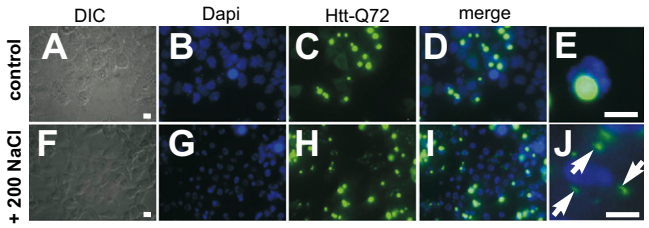


Fig. S3. Osmotically induced polyQ aggregates do not colocalize with nuclear DNA in HEK293 cells. HTT-Q72-YFP–expressing HEK cells exposed to control (*Upper*) or hypertonic 200 NaCl for 3 h (*Lower*). Arrows point to osmotically induced aggregates that do not colocalize with DAPI-stained nuclei. DIC, differential interference contrast. [Scale bars, 10 μm (*A–D* and *F–I*); 5 μm (*E* and *J*).]

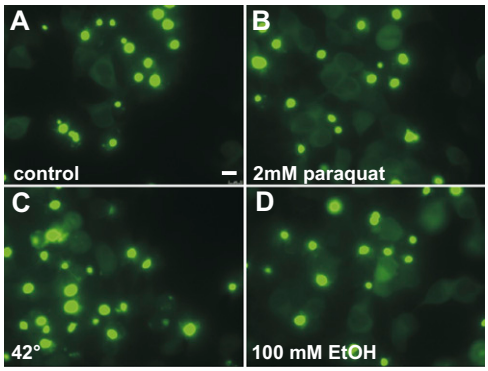


Fig. S4. Stress-induced polyQ aggregation does not occur in response to oxidative stress, heat stress, or ethanol stress in HEK293 cells. HTT-Q72-YFP–expressing HEK cells were exposed to the indicated stressors for 3 h and then imaged. (Scale bar, 10 μm.)

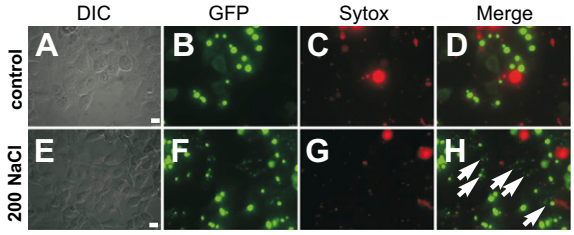


Fig. S5. Cells that contain osmotically induced polyQ aggregates are viable. HTT-Q72-YFP–expressing HEK293 cells exposed to isotonic PBS (*A–D*) or PBS + 200 mM NaCl (*E–H*) labeled with the vital dye Sytox Orange. Arrows point to cells with osmotically induced aggregates, which do not costain with Sytox dye. (Scale bars, 10 μm.)

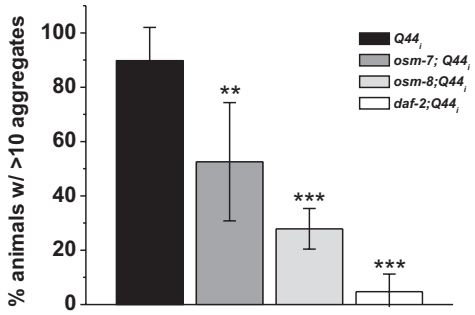


Fig. S6. Osmotic stress resistance mutants protect against aging-induced polyQ aggregation. Animals were aged for 7 d after the L4 stage, and the number of animals with fewer than 10 visible aggregates was counted. $n = 5$ trials, ≥10 worms per trial. Results are representative of two independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$.

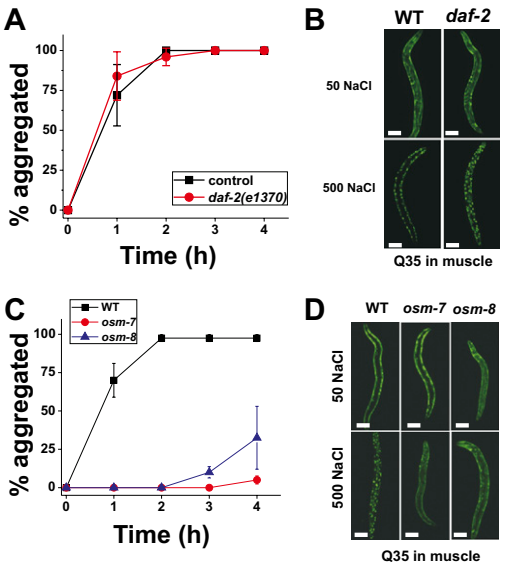
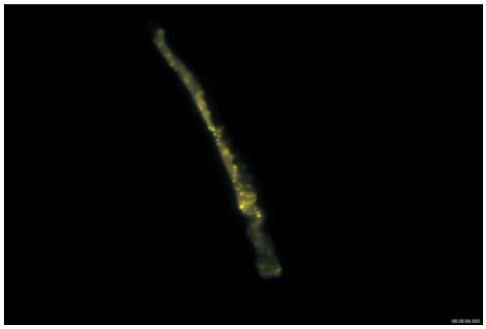


Fig. S7. Genetic activation of the osmotic stress-response pathway but not the insulin-like growth factor pathway blocks osmotically induced Q35-YFP aggregation in muscle. (*A*) Percentage of aggregated young adult animals that express muscle Q35-YFP following exposure to 500 mM NaCl for wild type and *daf-2(e1370)*. $n \geq 40$ animals for each genotype. (*B*) Representative images of wild-type or *daf-2(e1370)* animals expressing intestinal Q44-YFP following 4 h of exposure to either 50 or 500 mM NaCl. (Scale bars, 100 μm.) (*C*) Percentage of aggregated young adult animals that express muscle Q35-YFP following exposure to 500 mM NaCl for wild type, *osm-7(n1515)*, and *osm-8(n1518)*. $n \geq 40$ animals for each genotype. (*D*) Representative images of wild type, *osm-7(n1515)*, and *osm-8(n1518)* animals expressing Q35-YFP after 4 h of exposure to either 50 or 500 mM NaCl. (Scale bars, 100 μm.)



Movie S1. Osmotically induced aggregates are not derived from larger aging-type aggregates. Young adult hermaphrodite expressing Q44-YFP in the intestine was placed on a pad with 500 mM NaCl, and a Z stack of images was collected every 4 min. The movie represents the time series of collapsed Z sections.

Movie S1