Supporting Information

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SI Materials and Methods

Nematode Strains. *smg-1(tm849)*, *smg-4(tm5409)*, and *atf-6(tm1153)* were obtained as described (1). All other strains were obtained through the *Caenorhabditis* Genetics Center. Genetic crosses were performed as described (2). The details of the mutant strains, except for *smg-4(tm5409)*, are shown in WormBase (http://www.wormbase.org). *smg-4(tm5409)* was generated in this study and lacks 338 bp in exon 2 gene disruption of smg-4 gene was confirmed by PCR using primers shown in Table S4.

Cell Culture and Transfection of siRNAs and DNAs. HeLa and HEK293 cells were cultured in DMEM with 10% (vol/vol) FBS. Murine immortalized hepatocytes were cultured as described (3). Transfection of plasmid DNAs or siRNAs (with or without plasmid DNAs) was performed using FuGENE HD or X-tream siRNA (Roche) transfection reagents, respectively, as described in the manufacturer's protocols.

Chemical Reagents, Primers, and Antibodies. Antibodies against CHOP (R-20; Santa Cruz Biotechnology), GRP78 (BD Biosciences), β-tubulin and UPF3B (Sigma), UPF1 and SMG6 (Abcam), phosphoserine and GABA receptor α 1 (GABRA1) (Millipore), SMG7 (Novus Biologicals), and GFP (mFx75; Wako) were purchased from the manufacturers. Thapsigargin (TG) and tunicamycin (TM) (Calbiochem) and lactacystin (Wako) were purchased from the manufacturers. siRNAs for human SMG6 and SMG1 were purchased from Invitrogen, and siRNAs for murine SMG6 and human DIS3 and EXOSC3 were purchased from Thermo Scientific. Sequences of primers and siRNAs are shown in Table S4.

DNA Plasmids. The cDNAs of human SMG6 and SMG6(ΔPIN) (4) were amplified by PCR and inserted into BglII-digested pEGFP-C1 (Clontech). To construct GABRA1-expression plasmids, the coding region of GABRA1 cDNA (1–1,059 bp) was amplified by PCR and inserted into HindIII-digested pcDNA 3.1(+) (Invitrogen) [referred to as pcDNA-GABRA1 (ex1-8)]. Next, a DNA fragment from the Eco81I restriction site in exon 8 through the termination codon in exon 9 was amplified from human genomic DNA and inserted into Eco81I/Xba1-digested pcDNA-GABRA1 (ex1-8) plasmid [referred to as pcDNA-GABRA1 (ex1-8-int-9)]. Finally, to generate the GABRA1 (S326fs328X) mutant, site-directed mutagenesis was performed on pcDNA-GABRA1 (ex1-8-int-9). The *Renilla* luciferase (LUC)-based nonsense-mediated mRNA decay (NMD) reporter and transfection control plasmid using *Firefly* luciferase (5) were kindly provided by Andreas Kulozic (University of Heidelberg, Heidelberg, Germany). Human UPF1 cDNA was amplified by PCR and inserted into HindIII-digested pCMV5-FLAG. 5′ UTR_ATF4 reporter plasmid was purchased from Addgene (ATF4-5). All PCR reactions were performed using KOD plus neo (TOYOBO), and the In-Fusion Advantage PCR Cloning System (Clontech) was used for DNA fragment insertion and site-directed mutagenesis. The human *Chop* promoter region was amplified by PCR using human genomic DNA (Promega) with the primers 5′-AGA TCT ACA GTC TGT TGC GGG AAC CAG GAA-3′ and 5′-AAG CTT ACA TGA TAC GCT CAG TGC CTT AGA-3′ (underlined sequences indicate restriction sites for BglII and HindIII, respectively). Amplified DNA fragments were cloned into the pGEM-T Easy Vector (Promega), followed by DNA sequence confirmation performed by the Sequencing Core at the University of Michigan. The confirmed DNA fragment was transferred into the BglII and HindIII sites of the pGL4.20 reporter vector (Promega). pRL4 of the control plasmid using *Renilla* luciferase was purchased from Promega.

Feeding-Mediated RNAi Treatment in Worms. Comprehensive feeding-mediated RNAi analysis was performed (6) using the *Caenorhabditis elegans* RNAi library provided by Source BioScience. Each *Escherichia coli* strain harboring dsRNA-expression plasmids was spotted onto nematode growth medium (NGM) containing 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). At 16 h after plating, L4 larvae of each strain were placed on plates and cultured at 20 °C. In the comprehensive RNAi screen, growth was analyzed at 7 d after RNAi treatment with an SZX-12 microscope (Olympus), and intestinal morphologies were analyzed at 3 d after RNAi treatment using a BX-51 microscope (Olympus). For growth analysis of worms in Fig. S7, pictures were taken with a BX-51 microscope (Olympus) after RNAi treatment at 20 °C for 4 d, and lengths of worms were analyzed using ImageJ software (National Institutes of Health).

RNA Preparation and Quantitative RT-PCR. Briefly, a 500-μL aliquot of TRIzol reagent (Invitrogen) was directly added to cells on 3.5-cm culture dishes after removal of the culture medium by aspiration. The lysate was transferred to 1.5-mL tubes and incubated at room temperature for 10 min. A 100-μL aliquot of chloroform was added and samples were centrifuged at 22,000 × *g*. The supernatant was transferred to another tube, and RNA was collected by isopropanol and ethanol precipitation. For RNA isolation from worms, worms grown on plates were suspended in M9 buffer and harvested by centrifugation at 1,000 × *g* for 1 min. After removal of the supernatant, the worms were transferred to 1.5-mL tubes and frozen in liquid nitrogen. The frozen pellets were thawed in 1 mL of TRIzol reagent and agitated by rotating for 24 h. The RNA was then isolated as described above.

cDNA was synthesized from 1 μg of total RNA in 20-μL reactions with random hexamers using the SuperScript III First Strand Synthesis System (Invitrogen). The synthesized cDNA was diluted to 250 μL with distilled water, and 4-μL aliquots were analyzed by quantitative (q)PCR in 20-μL reactions with 1 μM each gene-specific primer using POWER SYBR Green PCR Master Mix (Applied Biosystems). The PCR amplification products were analyzed by a 7500 Real-Time PCR System (Applied Biosystems), and CTs were normalized to *act-2* mRNA in *C. elegans* or ACTβ mRNA in mammals (see figure legends).

Xbp1 mRNA Splicing. cDNA products derived from X box-binding protein 1 (XBP1) were amplified by PCR with Taq DNA polymerase and a primer pair of hXBP1.3S and mXBP1.12AS. PCR products were digested with PstI (7) and separated by electrophoresis on a 3% agarose gel. XBP1 splicing was quantified using Image Gauge version 4.12 (Fujifilm).

Cell Proliferation Assay. Briefly, cells were plated onto 96-well tissue-culture plates (100 μL of medium per well) and siRNA was transfected after 24 h. At 24 h after siRNA transfection, the cells were treated with TM. After 48 h, CellTiter reagent (10 μL) was added to each well and incubated for 1 h at 37 °C in a 5% CO_2 atmosphere and A_{595nm} was measured on a Becton Dickinson instrument. The CellTiter 96 Aqueous One Solution Assay (Promega) is a colorimetric method for measuring the number of viable cells. The One Solution Assay contains MTS and PES, an electron-coupling reagent. MTS is reduced by living cells to

a colored formazan product that is measured by the amount of 490-nm absorbance.

Immunoblotting and Immunofluorescence Microscopy. In Fig. 3*B*, cells were harvested in radioimmunoprecipitation assay buffer (150 mM NaCl, 1.0% (wt/vol) Nonidet P-40, 0.5% (wt/vol) sodium deoxycholate (DOC), 0.1% (wt/vol) SDS, 50 mM Tris·HCl, pH 8.0) containing Complete Mini EDTA-free protease inhibitor mixture (Roche). Protein concentrations were determined using the DC Protein Assay System (Bio-Rad). Equal amounts of protein were separated by SDS/PAGE. In Fig. 5*A*, total lysate and immunoprecipitated (IP) samples were prepared as described below. IP samples and total lysates (10% equivalent of IP samples) were separated by SDS/PAGE. In Fig. S5, siRNA-treated cells expressing wild-type or S326fs328X mutant GABRA1 were lysed and prepared for SDS/PAGE as described (8). Proteins separated by SDS/PAGE were transferred to Immobilon-P membranes (Millipore). After incubation, the transferred membranes were treated with 1% (wt/vol) blocking solution (Roche) and incubated with primary antibodies in 1% (wt/vol) blocking solution in Fig. 3*A* or Can Get Signal immunoreaction enhancer solution (TOYOBO) in Fig. 5*A* and Fig. S6 overnight at 4 °C (1:300 dilution for α-CHOP antibody; 1:1,000 dilution for α-UPF1, α-UPF3B, α-FLAG, α-SMG6, α-GABRA1, and α-phosphorserine antibodies; 1:5,000 for α-β-tubulin, α-BiP, and α-SMG7 antibodies), and treated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (The Jackson Laboratory) as secondary antibodies for detection. Gel images were developed using LAS-3000 Mini Imaging System (Fujifilm). The intensities of the bands were quantified by using Image Gauge version 4.23 (Fujifilm).

Immunofluorescence microscopy was performed as described (3). Briefly, HeLa cells were fixed with 4% (wt/vol) paraformaldehyde in (phosphate-buffered salines) PBS at room temperature for 5 min and permeabilized with 0.1% (wt/vol) Triton X-100 in (phosphate-buffered salines) PBS at room temperature for 2 min. After blocking with 2% (wt/vol) BSA in PBS at room temperature for 30 min, cells were treated with primary antibody (1:500 dilution for α-UPF3B and SMG6; 1:1,000 dilution for α-GRP78) in the BSA/PBS blocking solution. After washing with PBS, cells were subjected to secondary antibodies (1:1,000 dilution for Alexa Fluor 488 anti-rabbit IgG or Alexa Fluor 594 anti-mouse IgG; Molecular Probes). After washing with PBS, cells were mounted using ProLong Gold antifade solution (Invitrogen). Cell images were obtained by using an LSM 510 confocal microscope (Zeiss) with multitrack scanning.

Dual Luciferase Reporter Assay and ATF4 Reporter Analysis. In Fig. S8, HEK293 cells cultured in 24-well tissue-culture plates (Falcon; Becton Dickinson) were transfected with human SMG6 siRNA #1 (Table S4) together with 200 ng per well of *Renilla*-LUC-NMD reporter plasmid and 40 ng per well of *Firefly*-LUC control plasmid (Fig. S8*B*) or with 200 ng per well of human *Chop* reporter plasmid together with 40 ng per well of *Renilla*-LUC control plasmid (Fig. S8*C*). In Fig. S12, HEK293 cells cultured in 24-well tissue-culture plates were transfected with 450 ng per well of *Renilla*-LUC-NMD reporter plasmid and 50 ng per well of *Firefly*-LUC control plasmid. Cells were harvested in 200 μL of 1× Passive Lysis Buffer (Promega), incubated on a shaker for 30 min at room temperature, and 7-μL aliquots were transferred to flat-bottom white-colored 96-well microtiter plates. Luciferase activities were analyzed using a Clarity Luminescence Microplate Reader (Promega). *Renilla* luciferase activity of each well was normalized to control *Firefly* luciferase activity. In the case of the NMD reporter, normalized values of luciferase activities of the premature termination codon (PTC) mutant form of the *Renilla* reporter were further normalized to the activity from the wild-type form of the reporter. In Fig. S8*D*, HeLa cells cultured in 24-well plates were transfected with 200 ng of 5′ UTR_ATF4-GFP reporter plasmid and 20 ng of pRL4 together with SMG6 siRNAs. At 48 h after transfection, cells were harvested in 200 μL of 1× Passive Lysis Buffer and *Renilla* luciferase activities were measured as described above. GFP expression was analyzed by immunoblotting as described above, and band intensities were normalized with *Renilla* luciferase activities.

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Fig. S1. Knockdown of *F48E8.6*, *exos-3*, *crt-1*, *arf-6*, and *4R79.2* causes ER stress. The transgenic strain *zcIs4* was subjected to RNAi-mediated knockdown of these five genes or *xbp-1*, and GFP fluorescence was observed at the L2 larval stage. The intensity of GFP fluorescence was increased in *exos-3(RNAi)*, *F48E8.6 (RNAi)*, and *atf-6(RNAi)* but decreased in *xbp-1(RNAi)* compared with worms treated with mock RNAi. DIC, differential interference contrast.

Fig. S2. TM-mediated induction of *crt-1* requires *ire-1*. Worms of each strain at the L2 larval stage were treated with 10 μg/mL TM for 3 h and RNA was analyzed by qRT-PCR. Average values are shown (n = 3). P values were calculated using the Student's t test. *$P < 0.05$; **$P < 0.01$; bars with no asterisks, $P >$ 0.05. N2, wild type.

Fig. S3. The expression of genes that exhibit synthetic lethality with *ire-1(v33)* that are not induced by ER stress is shown. Worms of each strain at the L2 larval stage were treated with 10 μg/mL TM for 3 h and RNA was analyzed by qRT-PCR. Average values ± SD are shown (n = 3). Error bars and asterisks are as described in Fig. S2.

Fig. S4. Basal expression of CRT1, DIS3, and EXOSC3 requires ATF6α in immortalized hepatocytes. The expression of each gene in wild-type and unfolded protein response (UPR) mutant [*Ire1α*$^{-/-}$, *Atf6α*$^{-/-}$, and homozygous Ser51Ala *eIF2α(AA)*] immortalized hepatocytes was analyzed by qRT-PCR and normalized to ACTβ. WDR47 is shown as a gene that is not reduced in the mutant strains. The relative mRNA expression of each gene compared with wild type is shown. Average values are shown (n = 3). Error bars and asterisks are as described in Fig. S2.

Fig. S5. The PTC form of GABRA1 accumulates upon knockdown of DIS3 or EXOSC3. HEK293 cells were transfected with wild-type and S326fs328X mutant pcDNA_GABRA1 (ex1-8-itn-9) together with DIS3 or EXOSC3 siRNA. After 72 h, where indicated, cells were treated with 10 μM lactacystin for 6 h and lysed with 2× SDS/PAGE loading buffer. The amounts of GABRA1 protein under each condition were analyzed by Western blotting. Asterisks indicate wild-type (*) and mutant (**) GABRA1. NS, nonspecific siRNA.

Fig. S6. The UPR is activated in *smg-4(tm5409)*. Differential interference contrast microscopy and fluorescence microscopy for GFP are shown in L4 larvae from *smg-4(tm5409);zcIs4* and the parental *zcIs4* worms.

Fig. S7. Deletions of *smg-1, smg-4*, or *smg-6* cause a synthetic growth defect upon *ire-1* knockdown. The deletion mutants of *smg-1*, *smg-4*, or *smg-6* were subjected to RNAi treatment of *ire-1*, *atf-6*, or *pek-1* for 4 d at 20 °C. The lengths of worms were analyzed and the population of each category of lengths is shown graphically.

Fig. S8. siRNA-mediated knockdown of SMG6 inhibits NMD and induces *Chop* transcription and ATF4 expression. The *Renilla* luciferase-based NMD reporter plasmid and a control plasmid of *Firefly* luciferase (*A* and *B*), the human *Chop* reporter and control plasmid of *Renilla* luciferase (*C*), or 5′ UTR_ATF4-GFP reporter plasmid and a control plasmid of *Renilla* luciferase (*D*) were transfected into HEK293 cells together with siRNA. Luciferase activities and ATF4-GFP expression were analyzed at 48 h after transfection and normalized as described in *SI Materials and Methods*. Average values are shown ($n = 3$). Error bars and asterisks are as described in Fig. S2.

Fig. S9. Expression of GRP78 and CHOP mRNA is increased upon knockdown of SMG6 in HeLa cells. HeLa cells were transfected with two specific SMG6 siRNAs (#1 and #2) that target different sequences and with a nonspecific siRNA as a negative control. At 72 h posttransfection, the cells were harvested for analysis of CHOP, GRP78, and SMG6 mRNAs by qRT-PCR. Average values are shown (n = 3). Error bars and asterisks are as described in Fig. S2.

Fig. S10. Knockdown of SMG1 increases CHOP expression. (*A*) Expression of CHOP mRNA, but not GRP78 mRNA, is increased upon knockdown of SMG1 in HeLa cells. HeLa cells were transfected with two specific SMG1 siRNAs or a nonspecific siRNA as a negative control. At 72 h posttransfection, cells were harvested for analysis of CHOP and GRP78 mRNAs by qRT-PCR. (*B*) HeLa cells were transfected with SMG1 siRNA. At 24 h after transfection, cells were treated with 250 ng/mL TM for 48 h, and cell viability was measured by MTS assay. Average values are shown (n = 3). Error bars and asterisks are as described in Fig. S2.

Fig. S11. Overexpression of SMG6 attenuates CHOP induction upon ER stress. HeLa cells transfected with pEGFP_hSMG6 or empty vector (EV), treated with 5 μg/mL TM for 24 h and analyzed for expression of CHOP (*A*) and GRP78 (*B*) by qRT-PCR. Average values are shown (*n* = 3). Error bars and asterisks are as described in Fig. S2.

Fig. S12. ER stress accumulates a PTC-containing mRNA. The *Renilla* luciferase-based NMD reporter plasmid and a control plasmid of *Firefly* luciferase were transfected into HEK293 cells. At 24 h after transfection, cells were treated with 10 εg/mL TM for 8 h. Then, luciferase activities were analyzed and normalized as described in *SI Materials and Methods*. Average values are shown (*n* = 3). Error bars and asterisks are as described in Fig. S2.

Table S1. Genes that exhibited synthetic growth defects by RNAi knockdown in UPR mutant strains

Gene	Description
Synthetic severe growth defect in *ire-1(v33)*–specific 50 genes (35 conserved genes; 15 worm-specific genes)	
acs-4	Acyl-CoA synthetase
arf-1.1	ADP ribosylation factor
arf-6	ADP ribosylation factor
attf-1	Nuclear DEAF-1–related transcriptional regulator (suppressin)
C14B1.9	Worm-specific
C29F9.2	Worm-specific
C44H9.4	tRNA-splicing endonuclease positive effector (SEN1)
C55A6.6	Predicted short chain-type dehydrogenase
cpl-1	Cysteine proteinase Cathepsin L
crn-2	Cell death-related nuclease
crt-1	Calreticulin
cyp-25A3	Cytochrome P450 3A4
D1044.1	Worm-specific
D1054.10	Worm-specific
dop-2	Orthologous to the human gene DOPAMINE RECEPTOR D2 (DRD2)
dve-1	SATB2 matrix attachment region-binding protein
exos-3	Exosomal 3′-5′ exoribonuclease complex subunit Rrp40
F13B12.3	Uncharacterized protein
F21G4.5	Worm-specific
F34D10.6	Serine/threonine protein phosphatase 6 regulatory subunit A
F48E8.6	Exosomal 3′-5′ exoribonuclease subunit Rrp44/Dis3
F59F5.7	Worm-specific
H10D18.6	UDP-glucuronosyl and UDP-glucosyl transferase
his-3	Histone 2A
ivd-1	Isovaleryl-CoA dehydrogenase
K06A5.8	WD repeat-containing protein
K07C5.6	RNA splicing factor: Slu7p
lrr-1	Uncharacterized conserved protein
max-1	Cytoplasmic protein Max-1, contains PH, MyTH4 and FERM domains
msp-76	Major sperm protein
nhr-182	Hormone receptors
R05D3.8	Putative uncharacterized protein
rpl-1	Large ribosomal subunit L 10a protein
ruvb-1	DNA helicase, TBP-interacting protein
sre-20	Worm-specific
srw-137	Worm-specific
srw-6/F47C10.2	Worm-specific
str-16	Worm-specific
T02D1.4	Worm-specific
T16G1.9	Worm-specific
T20D4.19	Worm-specific
tomm-20	Translocase of outer mitochondrial membrane complex, subunit TOM20
Y116A8C.13	DNA repair and recombination protein RAD54B
Y39A3A.2	Worm-specific
Y39G10AR.8	Translation initiation factor 2, γ-subunit
Y71G12B.11	Talin-1
Y72A10A.1	Worm-specific
ZK1098.4	Translation initiation factor 2B, α-subunit (eIF-2Bα/GCN3)
ZK550.4	TIF2E, transcription initiation factor IIE
4R79.2	GTP-binding protein SEC4
Synthetic severe growth defect in *ire-1(v33)* and *atf-6(tm1153)* 63 genes (53 conserved genes; 10 worm-specific genes)	
bed-2	Uncharacterized protein
C04C3.3	Pyruvate dehydrogenase E1, β-subunit
C27A7.1	Type I phosphodiesterase/nucleotide pyrophosphatase
cap-2	F-actin–capping protein, β-subunit
eif-1.A	Translation initiation factor 1A (eIF-1A)
elo-3	Long-chain fatty acid elongase
F08D12.1	Signal recognition particle, subunit SRP72
F11C3.1	Worm-specific
F11G11.4	Worm-specific
F25B3.6	Paf1/RNA polymerase II complex, RTF1 component
F28H6.2	Worm-specific
F29B9.7	Worm-specific

Table S1. Cont.

F54C4.1	Mitochondrial ribosomal protein L28
F55A3.3	FACT complex SPT16
F59A3.3	Mitochondrial/chloroplast ribosomal protein L24
fzy-1	Cell cycle regulation (APC)
H04M03.3	Dehydrogenase/reductase SDR family member
H06I04.3	Putative SAM-dependent rRNA methyltransferase SPB1
his-46	Histone H4
his-66	Histone H2B
hmg-1.2	HMG box-containing protein
hsp-4	ER-resident protein chaperone, GRP78
inft-1	Rho GTPase effector BNI1
K04G7.1	Worm-specific
K07F5.14	Uncharacterized conserved protein
mcm-6	DNA replication licensing factor, MCM6 component
mdt-4	Vitamin D receptor-interacting protein
mei-1	Katanin p60 ATPase-containing subunit A-like 1
mrps-5	Mitochondrial ribosomal protein S5
nfyc-1	Nuclear transcription factor Y (NF-Y), subunit C
nhx-2	Na/H exchanger protein 2
nuo-2	NADH ubiquinone oxidoreductase family member
par-1	Serine/threonine protein kinase
pat-6	α-Paravin
phb-2	Mitochondrial prohibitin complex
prx-13	Peroxisome biogenesis protein, PEX13
R05H10.2	Nuclear protein fibrillarin NOP77 (RRM superfamily)
rba-1	Nucleosome remodeling factor, subunit CAF1
rbx-1	SCF ubiquitin ligase, Rbx1 component
rdy-2	Worm-specific
rpl-24.2	60S ribosomal protein L30 isologue
rpl-30	60S ribosomal protein L30
sfa-1	Splicing factor 1/branch point-binding protein (RRM superfamily)
sur-6	Serine/threonine protein phosphatase 2A, regulatory subunit
T04G9.4	α-Aminoadipic semialdehyde dehydrogenase-phosphopantetheinyl transferase
T09A5.9	Protein phosphatase 1, regulatory subunit, and related proteins
T22B11.5	2-Oxoglutarate dehydrogenase
T22F7.3	Worm-specific
tag-313	Mitochondrial ribosomal protein L55
unc-112	Mitogen inducible gene product
vps-37	Vacuolar protein sorting-associated protein 37B
W02B3.3	Worm-specific
wwp-1	Ubiquitin protein ligase RSP5/NEDD4
Y116A8C.4	Isoform C of endothelin-converting enzyme
Y24D9A.5	Worm-specific
Y43F8C.8	Putative mitochondrial ribosomal protein mRpS35
Y53G8AL.2	NADH:ubiquinone oxidoreductase, NDUFA9/39-kDa subunit
Y54E10BR.2	GTP-binding ADP ribosylation factor-like protein yARL3
Y65B4BL.1	Worm-specific
Y65B4BR.5	Nascent polypeptide-associated complex subunit alpha (NACα)
Y82E9BR.2	Splicing factor U2AF, large subunit (RRM family)
ZK418.5	Uncharacterized protein
ZK546.14	Uncharacterized protein
Synthetic severe growth defect in all of UPR mutants 11 genes (9 conserved genes; 2 worm-specific genes)	
B0035.12	RNA-binding protein SART3 (RRM superfamily)
C47E12.2	Mitochondrial ADP/ATP carrier proteins
C47E12.7	Nucleolar protein NOP52/RRP1
C55C3.5	Worm-specific
cpar-1	Histones H3 and H4
F53F4.10	NADH:ubiquinone oxidoreductase, NDUFV2/24-kDa subunit
klp-16	Kinesin (KAR3 subfamily)
pan-1	Splice isoform 1 of Slit homolog 2 protein precursor of human (61.1%)
smk-1	Protein required for actin cytoskeleton organization and cell-cycle progression
str-205	Worm-specific
utx-1	DNA-binding protein jumonji/RBP2/SMCY

List of genes that cause synthetic growth defects upon RNAi knockdown in *ire-1(v33)* only, in both *ire-1(v33)* and *atf-6(tm1153)*, or in all three UPR mutants (*ire-1, atf-6*, and *pek-1*) is shown. Annotation of gene function and evolutionary conservation was derived from WormBase (version 220). Genes conserved in mammals were analyzed for intestinal morphology as the secondary screen.

Table S2. Genes that caused synthetic severe growth defect and intestinal degeneration by RNAi knockdown in *ire-1(v33)* and *atf-6(tm1153)*

Gene name	Gene annotation
wwp-1	E3-ubiquitin protein ligase RSP5/NEDD4
F08D12.1	Signal recognition particle subunit SRP72
hsp-4	ER resident chaperone, GRP78
nfyc-1	Nuclear transcription factor Y (NF-Y), subunit C
vps-37	Vacuolar protein sorting-associated protein 37B
elo-3	Long-chain fatty acid elongase
cap-2	F-actin–capping protein, β-subunit
R05H10.2	Nuclear protein fibrillarin NOP77 (RRM superfamily)
his-46	Histone H4
Y82E9BR.2	Splicing factor U2AF, large subunit (RRM family)

Genes that cause synthetic growth defects and intestinal degeneration upon RNAi treatment in both the *ire-1(v33)* and *atf-6(tm1153)* mutant strains are shown. These genes were identified from 62 genes that cause synthetic growth defects upon RNAi knockdown in both the *ire-1(v33)* and *atf-6(tm1153)* mutants, or in all three UPR mutants (Table S1) by analysis of intestinal degeneration.

Table S3. Effect of TG treatment on the expression of mouse homologies of the *C. elegans* genes obtained by RNAi screen

Gene name in mouse	Gene name in *C. elegans*	Relative abundance in TG treatment compared with mock (DMSO) treatment (SD)
GCN3	*ZK1098.4*	1.57 (0.148)
ARF6	*arf-6*	1.72 (0.299)
eIF2S3	*Y39G10AR.8*	1.94 (0.672)
RPL10a	*rpl-1*	1.35 (0.448)
CALR	*crt-1*	7.01 (0.744)
CTSL	*cpl-1*	1.95 (0.0645)
WDR47	*K06A5.8*	1.44 (0.116)
RAB13	*4R79.2*	1.91 (0.746)
EXOSC3	*exos-3*	2.23 (0.522)
DIS3	*F48E8.6*	2.64 (0.120)

See also Fig. 1*C*. Quantitative results were derived from the data in Fig. 1*E*. Gene names of the murine homologs are referred to by the official symbol from the HUGO Gene Nomenclature Committee (http://www.genenames.org/). qRT-PCR expression values represent the average from three independent biological samples. Error bars indicate ± SD. *$P < 0.05$; **$P < 0.01$; bars with no asterisks, $P > 0.05$.

Table S4. List of primers and siRNAs used in this article

For qRT-PCR analysis in Fig. 1*B*, Figs. S2 and S3, and Table 1 (for *C. elegans* genes)	
ZK1098.4 FW	5′ctcgagccaaaatttccaag3′
ZK1098.4 RV	5′tgacccagacatgaaggtga3′
crt-1 FW	5′acgagcaaggaatcgactgt3′
crt-1 RV	5′gccgaacatgacgttgtatg3′
rpl-1 FW	5′caccgtcaagttccagatga3′
rpl-1 RV	5′tggagagggagatgttggag3′
cpl-1 FW	5′attcagccaataccgcaaac3′
cpl-1 RV	5′ggtatcacgccagtcaacct3′
arf-6 FW	5′ggattgacgaggattcgaga3′
arf-6 RV	5′ggcttgcaattctggcttag3′
F48E8.6 FW	5′tcgtcggtatccagatgtga3′
F48E8.6 RV	5′cgggtgcaaatctcctgtat3′
exos-3 FW	5′gtacgtggccaaacgagaat3′
exos-3 RV	5′tcggtctccttcctgtggta3′
K06A5.8 FW	5′agttcgaaggcaattggatg3′
K06A5.8 RV	5′tggaagagacggctttgagt3′
4R79.2 FW	5′gctaggagtgccgtttatcg3′
4R79.2 RV	5′gctgaaggttttctgccatc3′
Y39G10AR.8 FW	5′cgcgaaaattacgagcaaat3′
Y39G10AR.8 RV	5′tgcaaagatactcgcaaacg3′
act-2 FW	5′atcgtcctcgactctggagat3′
act-2 RV	5′tcacgtccagccaagtcaag3′
For qRT-PCR analysis in Fig. 1 *C–E*, Fig. S4, and Table S3 (for mouse genes)	
ACTB FW	5′gatctggcaccacaccttct3′
ACTB RV	5′ggggtgttgaaggtctcaaa3′
GCN3 FW	5′ctgggcaggatctcaaagag3′
GCN3 RV	5′cttacagcagatggcgtcaa3′
ARF6 FW	5′acgccatcatcctcatcttc3′
ARF6 RV	5′agggctgcacataccagttc3′
EIF2S3 FW	5′tcagtatgctgctccaggtg3′
EIF2S3 RV	5′caggtaatgctccaacagca3′
RPL10a FW	5′cagcactgtgacgaggctaa3′
RPL10a RV	5′ggccaaaaacgcatcatact3′
CRT1 FW	5′aattttacggggacctggag3′
CRT1 RV	5′caccagtgtctggcccttat3′
CPL1 FW	5′ctgcttgggaacagccttag3′
CPL1 RV	5′ttcctcattcgtgccataca3′
WDR47 FW	5′tcctggatcaacgaatcaca3′
WDR47 RV	5′tcctcttccccttttgacct3′
RAB13 FW	5′tgtctgatcattcgctttgc3′
RAB13 RV	5′tccacggtaataggcggtag3′
EXOSC3 FW	5′catctatggcctgtgtgtgg3′
EXOSC3 RV	5′ccagctcttgcacaatttca3′
DIS3 FW	5′gcaagagcagggagagaatg3′
DIS3 RV	5′acacgtgaaggctggtatcc3′
For qRT-PCR analysis in Fig. 2*B* (for *C. elegans* genes)	
hsp-4 FW	5′gggcgtttctacaacgagaa3′
hsp-4 RV	5′ggatccaaccttcacctcaa3′
act-2 FW and RV	Same as Fig. 1*B*
For qRT-PCR analysis in Fig. S9 (for human genes)	
GRP78 FW	5′tagcgtatggtgctgctgtc3′
GRP78 RV	5′tgacacctcccacagtttca3′
CHOP FW	5′gcgcatgaaggagaaagaac3′
CHOP RV	5′tcaccattcggtcaatcaga3′
ACTB FW	5′ggacttcgagcaagagatgg3′
ACTB RV	5′aggaaggaaggctggaagag3′
For XBP1 splicing analysis in Fig. 3*B*	
hXBP1.3S	5′aaacagagtagcagctcagactgc3′
mXBP1.12AS	5′tccttctgggtagacctctgggag3′
For qRT-PCR analysis in Fig. 5*C* (for human genes)	
SMG1 FW	5′caagcgatgtcagcagatgt3′
SMG1 RV	5′tgctgacaaaagccattcag3′
UPF1 FW	5′tcaccacgaagttgctgaag3′

Table S4. Cont.

UPF1 RV	5′tggtagagcttggtgtgcag3′
UPF2 FW	5′caaggcagcaatggattttt3′
UPF2 RV	5′acatgcagggatgcaatgta3′
UPF3A FW	5′gaaaagagcgactggcaaac3′
UPF3A RV	5′cttctgccttgcagatcctc3′
UPF3B FW	5′aagggggaagataagcagga3′
UPF3B RV	5′gttcctgaagctgctccttg3′
SMG5 FW	5′aggcagccaaaatgtaccac3′
SMG5 RV	5′ctgcttttgggctgtaggag3′
SMG6 FW	5′atgggaaaggaaatgggaag3′
SMG6 RV	5′ctgccttcagcctctgaatc3′
SMG7 FW	5′tgctggccctctttatgtct3′
SMG7 RV	5′ttagccagtccatggagacc3′
ACTB FW and RV	Same as Fig. S9
For qRT-PCR analysis in Fig. 5*D* (for mouse genes)	
SMG6 FW	5′tgacctcaccctgcttatcc3′
SMG6 RV	5′tgcagtcagctgcaataacc3′
ACTB FW and RV	Same as Fig. 1*C*
siRNA for human SMG6 knockdown	
SMG6 siRNA #1	5′ggaaagcacgcaguugguaccugaa3′
SMG6 siRNA #2	5′auacacugccagcaaagccaacugg3′
siRNA for mouse SMG6 knockdown (mixture of the following siRNAs)	
5′gaagcaagccuuuacgaaa3′	
5′caaaguaaugaccguguaa3′	
5′gcgcaaagauaauaggcgu3′	
5′gaucggaaccuucgaguaa3′	
siRNA for human SMG1 knockdown (mixture of the following siRNAs)	
5′uuaaaugcagccuagggucauccuc3′	
5′uuuagguucauccuuauugcuaucc3′	
5′uagacaugacugcaguagaucuugc3′	
siRNA for mouse EXOSC3 knockdown (mixture of the following siRNAs)	
5′gugaacacaugacgucaga3′	
5′acucucagcagaagcggua3′	
5′guauuauuagaguccgaaa3′	
5′ccugaaugcuagagcgugc3′	
siRNA for mouse DIS3 knockdown (mixture of the following siRNAs)	
5′agguagaguuguaggaaua3′	
5′ugaugaagaucgugcgcga3′	
5′gcagauuaauuuugcguaa3′	
5′uggaaaugccagacgcuua3′	
For wild-type and S326fs328X mutant of GABRA1 (ex1-8-int-9)-expression plasmid	
hGABRA1_ex1-8_FW	5′gtttaaacttaagcttatgaggaaaagtccaggtctg3′
hGABRA1_ex1-8_FW	5′gctcggtaccaagcttcttttctggaaccacacttttg3′
hGABRA1_ex8-int-ex9_FW	5′cagaaactccctccctaaggtggcttatgcaacagctatggattg3′
hGABRA1_ex8-int-ex9_RV	5′aaacgggccctctagactattgatgtggtgtgggggcttttag3′
hGABRA1_S326fs328X_FW	5′tttgtgtttcagctctgattgagtttgccacagtaaac3′
hGABRA1_S326fs328X_RV	5′gagctgaaacacaaaggcatagcacacggcaataaac3′
For FLAG-tagged hUPF1-expression plasmid	
hUPF1_FLAG-CMV_FW	5′cgataagatcaagcttatgagcgtggaggcgtacgggc3′
hUPF1_FLAG-CMV_RV	5′cccgggatcaggatccttaatactgggacagccccgtcac3′
For GFP-tagged wild-type and ΔPIN mutant hSMG1-expression plasmid	
hSMG6_EGFP-C1_FW	5′gtccggactcagatctatggcggaagggctggag3′
hSMG6_EGFP-C1_RV	5′cttgagctcgagatcttcagcccacctgggccc3′
For CHOP reporter plasmid	
hCHOP-promoter_FW	5′agatctacagtctgttgcgggaaccaggaa3′
hCHOP-promoter_RV	5′aagcttacatgatacgctcagtgccttaga3′
For confirmation of gene disruption in smg-4(tm5409)	
external forward	5′tagaagatggtgccgttcgt3′
external reverse	5′tccggtctgcacccgttaaa3′
internal forward	5′gccgtgtatcggtttcctta3′
internal reverse	5′gaccgattcaaaagacggtc3′

Sequences of primers used in this study are shown. Primers for qRT-PCR analyses were designed to generate products of 100–150 bp using Primer3 software, version 0.4.0 (http://frodo.wi.mit.edu/primer3). FW, forward; RV, reverse.