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(54) Title: METHOD FOR PROMOTING AUTOPHAGY

(57) Abstract: The technology relates to a method for promoting autophagy in a subject by increasing the level of mir-1 nucleic acid in a cell of the subject, for example by administering a therapeutically effective amount of a mir-1 nucleic acid, an interferon- β or a gene silencing agent specific for a nucleic acid encoding the TBC protein.



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METHOD FOR PROMOTING AUTOPHAGY

Technical Field

[001] The technology relates to methods for promoting autophagy in a subject by increasing the level of mir-1 in a cell of the subject.

Cross reference to related application

[002] This application claims priority to Australian provisional patent application No 2019901664 filed on 16 May 2019, which is herein incorporated by reference in its entirety.

Background

[003] Proteostasis is fundamental for cell function and survival because proteins are involved in all aspects of cellular function, ranging from cell metabolism and cell division to the cell's response to environmental challenges. Proteostasis is tightly regulated by the synthesis, folding, trafficking and clearance of proteins, all of which act in an orchestrated manner to ensure proteome stability. Protein quality control (PQC) networks are enhanced by stress response pathways, which take action whenever the proteome is challenged by environmental or physiological stress.

[004] To maintain proteostasis, cells and organisms have developed complex cellular and systemic responses that involve a plethora of molecular chaperones and proteolytic pathways. Cells utilize multiple, compartment-specific, PQC mechanisms to meticulously guide the folding, trafficking and degradation of damaged or misfolded proteins. As such, the heat shock response (HSR), unfolded protein response (UPR), endoplasmic reticulum-associated degradation (ERAD) and autophagy efficiently overcome proteotoxic stress to enable organismal function and survival. In particular, autophagy can selectively remove abnormally folded proteins via the lysosomal pathway. Common to these protective mechanisms is the activation of distinct cellular chaperone networks that alleviate toxic protein aggregation and maintain proteostasis. In particular, the Hsp70 family (and facilitating cofactors) regulates protein folding and maturation and assists in the refolding of misfolded proteins, thereby decreasing toxic protein aggregation observed in neurodegenerative disease. However, proteins that cannot be refolded are degraded through the proteasome or cleared through autophagic pathways.

[005] PQC networks enable cells to maintain proteome integrity (proteostasis) despite being persistently challenged by environmental stress and changes in internal physiology. Decline in PQC performance can lead to inefficient protein folding and accumulation of cytotoxic protein aggregates, which are associated with many human pathologies including

the neurodegenerative diseases amyotrophic lateral sclerosis, Huntington's, Parkinson's and Alzheimer's Disease.

[006] microRNA (miRNAs) are non-coding RNAs of ~21-23 nucleotides in length that post-transcriptionally regulate the expression of target genes. miRNAs predominantly interact with mRNA targets through imperfect binding to motifs in the target mRNA 3'-untranslated region (3' UTRs). This miRNA:mRNA interaction negatively impacts the stability and translational capacity of mRNA targets in a rapid and reversible manner. Imperfect binding specificity means that a single miRNA can regulate a large number of mRNA targets involved in complex cellular processes, and thereby tightly control genetic networks during development and in response to stress. Accordingly, dysregulation in miRNA-controlled processes can cause severe physiological consequences for animal behavior and survival.

[007] mir-1 is a highly conserved miRNA that is predominantly expressed in muscle, brain and blood. Multiple roles of mir-1 have been identified in the development and function of muscle tissue in various organisms.

[008] The present inventors have found that mir-1 and similar microRNAs regulate autophagy and controls the expression of specific chaperones to regulate protein aggregation and its toxicity across multiple organisms.

Summary

[009] In a first aspect, there is provided a method for promoting autophagy in a subject in need thereof, the method comprising increasing the level of mir-1 nucleic acid in the subject, reducing the level of a TBC protein in the subject, or both.

[010] The level of mir-1 nucleic acid may be increased by administering to the subject a therapeutically effective amount of a mir-1 nucleic acid, an interferon- β , or both. The autophagy may prevent, reduce or inhibit protein aggregation.

[011] In some embodiments the mir-1 nucleic acid reduces the level of a TBC protein such as TBC1D15 or tbc-7.

[012] The level of the TBC protein may be reduced by administering to the subject a therapeutically effective amount of a gene silencing agent specific for the nucleic acid encoding the TBC protein, for example an antisense oligonucleotide, ribozyme, RNAi, siRNA or miRNA.

[013] The mir-1 nucleic acid may be administered to a tissue or organ of the subject or may be administered systemically. For example, administration to a tissue or organ may be via an osmotic pump.

[014] In some embodiments the protein aggregation is toxic to the subject and may be associated with a disease. The disease may be a neurodegenerative disease such as

Huntington's disease, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, prion disease, tauopathy, frontotemporal lobar degeneration, familial dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), serpinopathy, Alexander disease, familial amyloidotic neuropathy, senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), isozyme amyloidosis, fibrinogen amyloidosis, dialysis amyloidosis, inclusion body myositis, inclusion body myopathy, cataracts, retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, hereditary lattice corneal dystrophy, cutaneous lichen amyloidosis, mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, seminal vesicle amyloid, apolipoprotein C2 amyloidosis, apolipoprotein C3 amyloidosis, Lect2 amyloidosis, insulin amyloidosis, galectin-7 amyloidosis (primary localized cutaneous amyloidosis), corneodesmosin amyloidosis, enfuvirtide amyloidosis, Cystic Fibrosis, or sickle cell disease. Accordingly, the method may be used to treat a disease associated with protein aggregation.

[015] The protein aggregation may be of any protein that forms aggregates in a cell such as amyloid β peptide, Tau protein, α -synuclein, and proteins with polyglutamine expansions.

[016] In some embodiments protein aggregation is associated with at least one of proteotoxic stress, reduced autophagy reduced expression of a heat shock protein (e.g. hsp70, hsp90, and hsp40) or reduced expression of daf-21.

[017] In one embodiment the mir-1 nucleic acid comprises the mir-1 seed sequence GGAAUGU and may be for example UGGAAUGUAAAGAAGUAUGUA.

[018] In some embodiments the mir-1 nucleic acid comprises one or more of a phosphorothioate linked nucleotide, cholesterol, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), a non-naturally occurring nucleotide, a morpholino, nucleic acid aptamer, and a peptide.

[019] In some embodiments the mir-1 nucleic acid is selected from the group consisting of UGGAAUGUAAAGAAGUAUGUA, UGGAAUGUAAAGAAGUAUGU, UGGAAUGUAAAGAAGUAUGG, and UGGAAUGUAAAGAAGUAUGUAU.

[020] In other embodiments the mir-1 nucleic acid is an expression vector comprising a promoter operatively linked to a nucleic acid comprising a sequence encoding the mir-1 seed sequence GGAAUGU. For example the nucleic acid may encode the sequence UGGAAUGUAAAGAAGUAUGUA.

[021] In some embodiments the interferon- β may be interferon- β 1a, interferon- β 1b, or both.

[022] In preferred embodiments the subject is a human subject.

[023] In a second aspect there is provided a method of treating a disease associated with protein aggregation in a subject, the method comprising increasing the level of mir-1 nucleic acid in the subject, reducing the level of a TBC protein in the subject, or both.

[024] The disease may be a neurodegenerative disease, for example one selected from the group comprising Huntington's disease, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, prion disease, tauopathy, frontotemporal lobar degeneration, familial dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), serpinopathy, Alexander disease, familial amyloidotic neuropathy, senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), isozyme amyloidosis, fibrinogen amyloidosis, dialysis amyloidosis, inclusion body myositis, inclusion body myopathy, cataracts, retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, hereditary lattice corneal dystrophy, cutaneous lichen amyloidosis, mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, seminal vesicle amyloid, apolipoprotein C2 amyloidosis, apolipoprotein C3 amyloidosis, Lect2 amyloidosis, insulin amyloidosis, galectin-7 amyloidosis (primary localized cutaneous amyloidosis), corneodesmosin amyloidosis, enfuvirtide amyloidosis, Cystic Fibrosis, and sickle cell disease.

[025] In some embodiments the treating comprises preventing, inhibiting or reducing protein aggregation in a tissue of the subject.

[026] In a second aspect there is provided use of a mir-1 nucleic acid, an interferon- β , a gene silencing agent specific for a nucleic acid encoding a TBC protein, or or a small interfering peptide for the TBC protein for the manufacture of a medicament for the promotion of autophagy or for the treatment of a disease associated with protein aggregation. The autophagy may prevent, reduce or inhibit protein aggregation.

Definitions

[027] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[028] The term "therapeutically effective amount" as used herein refers to an amount of a mir-1 nucleic acid, or combination of mir-1 nucleic acids, sufficient to prevent or inhibit the formation of protein aggregates or reduce the amount of protein aggregation in a subject or in an organ or tissue of a subject. In some embodiments the amount is sufficient to reduce the number of observable protein aggregates.

[029] As used herein, 'treatment' refers to a clinical intervention made in response to a disease, disorder or physiological condition manifested by a subject or to which a subject may be susceptible. The aim of treatment includes, but is not limited to, the alleviation or prevention of symptoms, slowing or stopping the progression or worsening of a disease, disorder, or condition and/or the remission of the disease, disorder or condition. 'Treatment' refers to one or both of therapeutic treatment and prophylactic or preventative measures. Subjects in need of treatment include those already affected by a disease or disorder or undesired physiological condition as well as those in which the disease or disorder or undesired physiological condition is to be prevented.

[030] As used herein, the terms 'subject' refers to an individual who will receive or who has received treatment (e.g., administration of a compound described herein) according to a method described herein, or who has a disease or disorder or undesired physiological condition associated with protein aggregation. A subject may also be an individual in which the disease or disorder or undesired physiological condition associated with protein aggregation is to be prevented.

[031] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this specification.

[032] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[033] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[034] As used herein, the terms "miR," "mir" and "miRNA" are used to refer to microRNA, a class of small non-coding RNA molecules that are capable of modulating RNA translation. The terms "miR", "mir" and "miRNA," unless otherwise indicated, include the mature, primary, preform of a particular microRNA as well as the seed sequence of the microRNA and sequences comprising the seed sequence, and variants thereof. For example, the terms "MiRNA- 1", "miR-1" and "mir- 1" are used interchangeably and, unless otherwise indicated, refer to microRNA-1, including miR-1, pri-miR-1, pre-miR-1, mature miR-1, miRNA-1 seed sequence, sequences comprising a miRNA-1 seed sequence, and any variants thereof.

[035] As used herein, "miRNA" or 'mir' also refers to DNA that encodes a miRNA as defined above. For example, the DNA encoding a miRNA may be cDNA or genomic DNA.

[036] As used herein, an "expression vector" refers to a nucleic acid construct, generated recombinantly or synthetically, bearing a series of specified nucleic acid elements that enable transcription of a particular gene in a host cell. Typically, gene expression is controlled using certain regulatory elements, such as promoters that may be at least one of constitutive, inducible and tissue specific.

[037] The term "operably linked" is used herein to describe the connection between regulatory elements and a gene or its coding region. Typically, gene expression is placed under the control of one or more regulatory elements, for example, without limitation, constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. A gene or coding region is said to be "operably linked to" or "operatively linked to" or "operably associated with" the regulatory elements, meaning that the gene or coding region is controlled or influenced by the regulatory element.

[038] As used herein, the term "variant" refers to a polynucleotide having a sequence substantially similar to a reference polynucleotide. A variant can comprise deletions or substitutions of one or more nucleotides, and/or additions of one or more nucleotides at the 5' end, 3' end, and/or one or more internal sites in comparison to the reference polynucleotide. Similarities and/or differences in sequences between variants and the reference polynucleotide can be detected using conventional techniques known in the art, for example polymerase chain reaction (PCR) and hybridization techniques. Variant

polynucleotides also include synthetically derived polynucleotides, such as those generated, for example, by using site-directed mutagenesis. Generally, a variant of a particular polynucleotide disclosed herein, including, but not limited to, a miRNA, will have at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more sequence identity to the reference polynucleotide as determined by sequence alignment programs known in the art.

[039] In order that the present invention may be more clearly understood, preferred embodiments will be described with reference to the following drawings and examples.

Brief Description of the Drawings

[040] **Figure 1. *mir-1* protects against proteotoxic stress.** (A) Alignment of mature *miR-1* sequences indicates deep conservation. The seed sequence of each *miR-1* family member is highlighted in blue and the conservation of *C. elegans mir-1* is highlighted in grey. *hsa* = *Homo sapiens*, *mmu* = *Mus musculus*, *gga* = *Gallus gallus*, *xtr* = *Xenopus tropicalis*, *dre* = *Danio rerio*, *dme* = *Drosophila melanogaster*, *cel* = *Caenorhabditis elegans*. (B-C) Visualization of Q40::YFP aggregates (green foci) in (B) wild-type and (C) *mir-1(gk276)* animals. Scale bar, 50 μ M. (D) Quantification of Q40::YFP aggregation in wild-type, *mir-1(gk276)*, *mir-1(n4102)* and *mir-80(nDf53)* animals. (E) Quantification of Q40::YFP aggregates in wild-type, *mir-1(gk276)* and *mir-1(gk276)* animals transgenically-expressing the *mir-1* hairpin in body wall muscle (*myo-3* promoter), pharynx (*myo-2* promoter) or intestine (*ges-1* promoter). Mutation of the *mir-1* seed sequence (Muscle *mir-1**) abrogates rescue from body wall muscle. Mature *mir-1* sequences (wild-type *mir-1* or mutated *mir-1**) used for rescue experiments are shown (box). Red nucleotides indicate the mutations in the seed sequence used in *mir-1** rescue experiments, which are predicted to hinder interactions with *mir-1* targets. (F) Body bends in wild-type, *mir-1(gk276)* and *mir-1(n4102)* mutant animals expressing α -synuclein::YFP. (G) Survival of wild-type, *mir-1(gk276)* and *mir-1(n4102)* animals after exposure to 4h of 35°C heat stress. Transgenic expression of wild-type *mir-1* hairpin, but not mutated *mir-1**, in body wall muscle rescues *mir-1(gk276)* heat stress sensitivity. All experiments were performed in triplicate and at least 10 animals were scored per experiment. Error bars show standard error of the mean (SEM). ****P<0.0001, n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[041] Figure 2. Loss of *mir-1* Enhances Organismal Sensitivity to Proteotoxic

Threats. (A) Body bends in wild type, *mir-1(gk276)* and *mir-1(n4102)* mutant animals. (B) Body bends in wild type, *mir-1(gk276)* and *mir-1(n4102)* mutant animals expressing the Q40::YFP transgene. (C) Body bends in wild type, *mir-1(gk276)* and *mir-1(n4102)* mutant

animals expressing α -synuclein::YFP. (D) Survival of wild type, *mir-1(gk276)* and *mir-1(n4102)* animals after exposure to 4h of 35°C heat stress. All experiments were performed in triplicate and at least 10 animals were scored for each experiment. Results are presented as means $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[042] **Figure 3. *mir-1* Regulates the Proteostasis Machinery.** (A) Relative mRNA levels of indicated genes in wild type and *mir-1(gk276)* mutant animals. Analyzed by qRT-PCR and two-way ANOVA analysis with two reference genes, *pmp-3* and *cdc-42*. (B-D) Relative expression of *hsp-70(F44E5.4)*, *hsp-70(C12C8.1)*, *hsp-90/daf-21* and *hsp-40* transcripts in wild type, *mir-1(gk276)* and *mir-1(n4102)* animals. Analyzed by qRT-PCR and two-way ANOVA analysis with two reference genes, *pmp-3* and *cdc-42*. (E) Quantification of Q40::YFP aggregation in *mir-1(gk276)* and three independent transgenic lines (#1-3) in which *hsp70(F44E5.5)* is expressed in body wall muscle (*myo-3* promoter). (F) Quantification of Q40::YFP aggregation in *mir-1(gk276)* and Hsp70 complex single mutants: *hsp-70(C12C8.1)(tm2318)*, *hsp-90/daf-21(p673)*, *dnj-19(gk595)* and *hip-1(gk3264)*, and in combination with *mir-1(gk276)*. All experiments were performed in triplicate. Results are presented as means $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[043] **Figure 4. *mir-1* is Required for Optimal Proteasomal Degradation.** (A) Analysis of UPS substrate (Ubv-GFP) stability at 20°C. Protein extracts of synchronized 1-day adult worms from wild type and two, independent, *mir-1* alleles (*gk276* and *n4102*) were analyzed by immunoblotting with GFP and tubulin antibodies (top). Densitometric quantification of Western blots from two independent experiments (bottom). (B) Analysis of UPS substrate (Ubv-GFP) turnover at 35°C. Protein extracts of synchronized 1-day adult worms from wild type and *mir-1(n4102)* were analyzed by immunoblotting with GFP and tubulin antibodies (top). Densitometric quantification of Western blots from two independent experiments (bottom). (C) Analysis of ERAD substrate (CPL-1*-YFP) stability at 20°C. Protein extracts of synchronized 1-day adult worms from wild type, *mir-1(n4102)* and *sel-1(e1948)* (positive control) were analyzed by immunoblotting with YFP and tubulin antibodies (top). Densitometric quantification of Western blots from two independent experiments (bottom). (D) Analysis of ERAD substrate (CPL-1*-YFP) stability at 35°C and after recovery at 20°C. Protein extracts of synchronized 1-day adult worms from wild type, *mir-1(n4102)* were analyzed by immunoblotting with YFP and tubulin antibodies (top). Densitometric quantification of Western blots from three independent experiments (bottom). (E) Quantification of endogenous poly-ubiquitinated proteins in wild type, *mir-1(gk276)* and *mir-*

1(*n4102*). (F) Quantification of endogenous poly-ubiquitinated proteins in *Q40::YFP*, *mir-1(gk276)*; *Q40::YFP* and *mir-1(n4102)*; *Q40::YFP* animals. All experiments were performed in triplicate. Results are presented as means $\pm$ SEM. *** $P < 0.001$, **** $P < 0.0001$, n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[044] **Figure 5. *mir-1* Protects Against Human Huntingtin Protein Aggregation and Toxicity.** (A) C2C12 muscle cells transfected with miR-1 mimics, miR-1 hairpin inhibitors or miRNA mimic negative controls with a mutant huntingtin-expressing vector (pHA-HTTQ74). Cells were immunostained with anti-HA antibody to detect HA-tagged HTTQ74 aggregates. Q74-expressing aggregates marked by arrows. (B) Quantification of Q74 aggregates in C2C12 muscle cells. Bars represent mean values $\pm$ SD from at least three independent experiments. P-values were calculated using t-test and were versus control (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). (C) Quantification of Q74 aggregation-induced toxicity in C2C12 muscle cells. Bars represent mean values $\pm$ SD from at least three independent experiments. P-values were calculated using t-test and were versus control (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). (D) Quantification of autophagy in C12C12 muscle cells and Hela cells.

[045] **Figure 6 Relating to Figure 1. *mir-1* Genomic Locus.** (A) Chromosomal location of *mir-1(T09B4.11)*, chromosome I, reverse strand of assembly; <http://www.wormbase.org>, WS258, showing the two deletion strains, *gk276* and *n4102* (red bars). (B) Quantitative Western blot analysis of *Q40::YFP* protein lysates from wild type, *mir-1(gk276)* and *mir-1(n4102)* animals for YFP expression using an α -GFP antibody. Quantification of three independent Western blots was performed and analyzed relative to a tubulin control. n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[046] **Figure 7. Relating to Figure 2. *mir-1* Lifespan Analysis.** (A) Lifespan analysis of wild type, *mir-1(gk276)* and *mir-1(n4102)* animals. (B) Lifespan analysis of wild type, *mir-1(gk276)* and *mir-1(n4102)* animals expressing *Q40::YFP* in body wall muscle.

[047] **Figure 8. Relating to Figure 3. *mir-1* Functions in the Insulin-like Pathway to Regulate Aggregation Formation.** (A) Quantification of *Q40::YFP* aggregation in *mir-1(gk276)*, *age-1(hx546)* and *daf-16(mu86)* single and double mutants. Experiments were performed in triplicate and at least 10 animals were scored for aggregates in each experiment. (B) Relative expression of *age-1*, *daf-2*, and *daf-16* transcripts in wild type and *mir-1(gk276)* animals. Analyzed using qRT-PCR and two-way ANOVA analysis with two reference genes, *pmp-3* and *cdc-42*. Results are presented as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. not significant to the control (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[048] **Figure 9. *mir-1* directly regulates TBC 3'UTRs in *C. elegans* in mammals.** (A) Relative *tbc-7* mRNA levels measured by quantitative real-time PCR in L4 larvae. Data normalized to values for wild-type worms. Two independent reference genes (*pmp-3* and *cdc-42*) were used. Error bars show standard error of the mean (SEM) obtained from n=3 biological replicates and 3 technical replicates each. **P<0.001, *P<0.005 (one-way ANOVA analysis, followed by Dunnett's multiple comparison test). (B) Survival of wild-type and *mir-1(gk276)* animals (incubated on control (L4440) or *tbc-7* RNAi bacteria) after exposure to 4h of heat stress (35°C) (n=30). ***P<0.001, n.s. not significant (one-way ANOVA analysis, followed by Dunnett's multiple comparison test). (C) Predicted *mir-1* binding site on the 3'UTR of *tbc-7* mRNA (green) and seed sequence in *mir-1* (blue). Mutated nucleotides in the *tbc-7* 3'UTR for experiments (E-F) are in red. (D) Indicated DNA constructs were transformed together as multi-copy extrachromosomal arrays for experiments in (E-F). (E) Expression of heterologous reporter transgenes for control *unc-54* 3'UTR (*gfp*) and wild-type and mutated *tbc-7* 3'UTR (*mCherry*) constructs in body wall muscle. (F) Quantification of *gfp* and *mCherry* fluorescence of transgenic animals calculated as CTF/total area of fluorophore (n=30). ****P<0.0001, n.s. not significant (one-way ANOVA analysis, followed by Dunnett's multiple comparison test). (G) WB of TBC1D15 and α -tubulin and (H) quantified bands from HeLa cells transfected with scrambled (Scr) or miR-1 mimics (n=5). Data are mean fluorescence intensities $\pm$ SEM. **P<0.01 (Students t-test). (I) Predicted miR-1 binding site on the 3'UTR of TBC1D15 mRNA (green) and seed sequence in miR-1 (blue). Mutated nucleotides in the TBC1D15 3'UTR for experiments (L-M) are in red. (J-K) Quantification of flow cytometry analysis of HeLa cells co-expressing Scr or miR-1 mimic together with (J) GFPd2-3'UTR TBC1D15 (n=4) or (K) mutated GFPd2-3'UTR TBC1D15_{mutant}(n=5). Data are mean fluorescence intensities $\pm$ SEM, **P<0.01 (Students t-test).

[049] **Figure 10. Relating to Figure 1. Motility Analysis.** (A-C) Quantification of body bends in wild-type and *mir-1(gk276)* mutant animals without a transgene (A), expressing the Q0::YFP transgene (B) or expressing the Q40::YFP transgene (C). All experiments were performed in triplicate (number of animals scored are shown in each bar). $\pm$ SEM. *P<0.05, **P<0.01, ****P<0.0001, n.s. not significant (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[050] **Figure 11. Relating to Figure 9. RNAi screen to identify *mir-1* targets important for the heat stress response.** Survival of wild-type and *mir-1(gk276)* animals after exposure to 4h of 35°C heat stress. Animals were incubated on RNAi bacteria to reduce expression of predicted *mir-1* targets (TargetScanWorm release 6.2). L4440 = control RNAi bacteria. n=30. ***P<0.001, wild-type compared to *mir-1(gk276)* on control RNAi bacteria.

##P<0.001 and n.s. not significant when comparing knockdown of predicted *mir-1* target to control RNAi in *mir-1(gk276)* animals (one-way ANOVA analysis, followed by Dunnett's multiple comparison test). n.a. - *hpo-18* RNAi causes lethality in wild-type and *mir-1(gk276)* animals. All experiments were performed in triplicate.

[051] **Figure 12. Relating to Figure 9. Overexpression of *tbc-7* causes Q40::YFP aggregation.** Quantification of Q40::YFP aggregates in wild-type and *mir-1(gk276)* animals transgenically-expressing *tbc-7* cDNA in body wall muscle (*myo-3* promoter). Experiments were performed in triplicate (n=30). Error bars show standard error of the mean (SEM). ****P<0.0001, ## P<0.001 compared to the control and n.s. not significant compared to *mir-1(gk276)* (one-way ANOVA analysis, followed by Dunnett's multiple comparison test).

[052] **Figure 13. Relating to Figure 9. *mir-1* and *tbc-7* are important for stress-induced autophagy.** (A) Fluorescent images of BWM expressing GFP::LGG-1/Atg8 in wild-type, *mir-1(gk276)* and *Pmyo-3::tbc-7* overexpressing animals under control conditions, immediately after heat shock for 1h at 36°C (HS) or 1h after recovery from heat shock at 15°C (HS + recovery). GFP::LGG-1 puncta = arrowheads. Scale bar, 10µM. (B-C) Quantification of GFP::LGG-1/Atg8 puncta in BWM of animals and conditions shown in (A). The values represents the number of green puncta in *mir-1(gk276)* (B) and *Pmyo-3::tbc-7* overexpressing (C) animals in comparison to one green puncta in wild-type animals for each condition. n>15. ±SEM ****P<0.0001, n.s. not significant (Welch's t-test).

[053] **Figure 14. Relating to Figure 9. *miR-1* targeting of TBC proteins is conserved.** Predicted *mir-1* binding sites are found in the 3'UTRs of mRNAs that encode TBC proteins in *C. elegans* (*tbc-7*), *D. melanogaster* (Skywalker) and humans (TBC1D15). This conservation is found in all vertebrate species examined (Targetscan). The *mir-1* seed sequences are shown in blue and the predicted *tbc-7*-related 3'UTRs are shown in green.

[054] **Figure 15. Relating to Figure 9. TBC1D15 3'UTR analysis.** Fluorescence intensity histograms from flow cytometry analysis of HeLa cells expressing Scr or miR-1 mimic together with (A) GFPd2-3'UTR-TBC1D15 or (B) GFPd2-3'UTR-TBC1D15 mutated miR-1 target sequence. Wild-type hsa TBC1D15 3'UTR = 5'-CUUUCCUUUUCGAUAACAUAUCCU-3' and mutated hsa TBC1D15 3'UTR = 5'-CUUUCCUUUUCGAUAAAAUUACU-3'.

[055] **Figure 16. Human miR-1 regulates autophagy by controlling TBC1D15 expression.** (A) WB and (B) quantification of LC3 normalised to α-tubulin from HeLa cells expressing Scr or miR-1 mimics +/- bafilomycin. Data are mean fluorescence intensities of bands ±SEM (n=3-5). **P<0.01, ***P<0.001 (one-way ANOVA with Dunnett's correction). (C) WB and (D) quantification of LC3 from HeLa cells expressing empty vector or TBC1D15 overexpression vector +/- bafilomycin. Data are mean fluorescence intensities of bands ±SEM normalised to α-tubulin (n=5). n.s. not significant to the control, ***P<0.001 (two-way

ANOVA with Bonferroni correction). (E) IF images of HeLa cells stably expressing mRFP-GFP-LC3 and transfected with empty vector or TBC1D15. Scale bar, 10 μ m. (F) Quantification of green and red vesicles and (G) red/green vesicle ratio from (E) $\pm$ SEM (n=3, 12-14 cells per replicate?). **P<0.01, ***P<0.001 (Student's t-test). (H) WB of HeLa cells co-transfected with Scr or miR-1 mimic together with empty vector or TBC1D15 +/- bafilomycin. (I) Mean band fluorescence intensities of WBs from (H) of LC3-II normalized to α -tubulin $\pm$ SEM (n=7). **P<0.01, ***P<0.001 (two-way ANOVA with Bonferroni).

[056] **Figure 17. Relating to Figure 16. miR-1 and TBC1D15 control autophagy.** (A) Quantification of the number of LC3-positive vesicles per HeLa cell $\pm$ SEM expressing Scr or independent miR-1 mimics immunostained with antibodies against LC3 (n=3). *P<0.05, **P<0.01 (one-way ANOVA with Dunnett's correction). (B) WB of HeLa cells transfected with Scr or siRNA against TBC1D15 in the presence or absence of bafilomycin. (C) Mean fluorescence intensities of LC3-II WB bands. $\pm$ SEM of LC3-II normalized to α -tubulin (n=4). *P<0.05, **P<0.01 (Student's t-test).

[057] **Figure 18. Relating to Figure 16. miR-1 overexpression induces autophagy flux.** IF images of HeLa cells stably expressing mRFP-GFP-LC3 and transfected with Scr or miR-1 mimic were recorded. (A) Quantification of green and red vesicles and (B) the red/green vesicle ratio $\pm$ SEM (n=4). **P<0.01, ***P<0.001 (Student's t-test).

[058] **Figure 19. IFN- β -induced miR-1 controls mutant Huntingtin aggregation through the autophagy pathway.** (A) Quantification of the percentage of cells containing HTT-positive aggregates expressing Scr or miR-1 mimics with EGFP-HTT_{Q74} $\pm$ SEM (n=3, 200-400 cells per replicate). ***P<0.001 (one-way ANOVA). (B) CRISPR/Cas9 ATG16L1 knockout HeLa cells co-expressing Scr or miR-1 mimics with EGFP-HTT_{Q74}. Quantification of the percentage of cells containing HTT-positive aggregates $\pm$ SEM (n=3, 200-400 cells per replicate). *P<0.05 (Student's t-test), n.s. not significant (one-way ANOVA). (C-E) Quantification of the percentage of cells containing HTT-positive aggregates in HeLa cells co-expressing EGFP-HTT_{Q74} with (C) Scr or siRNA against TBC1D15 (n=4, 200-400 cells per replicate), (D) empty vector or TBC1D15 overexpression vector (n=3, 200-400 cells per replicate), or (E) a combination of Scr or miR-1 mimic together with empty vector or TBC1D15 overexpression vector (n=6, 200-400 cells per replicate) $\pm$ SEM. (C-D) *P<0.05, **P<0.005 (Student's t-test) or (E) (two-way ANOVA with Dunnett's correction). (F) WB and (G) quantification of Tbc1d15 normalized to α -tubulin of cortical neurons from mice treated with recombinant mouse IFN- β (100U/ml) for 1-24 hours (n=4). Data are mean fluorescence intensities of bands $\pm$ SEM. n.s. not significant to the control, *P<0.05, **P<0.01, ***P<0.001 (one-way ANOVA). (H) RT-PCR of miR-1a-3p from cortical neurons treated with

recombinant mouse IFN- β (100U/ml) for 24 hours (n=3). **P<0.01 (Student's t-test). (I-J) Flow cytometry analysis of HeLa cells expressing (I) GFPd2-3'UTR TBC1D15 (n=4) or (J) mutated GFPd2-3'UTR TBC1D15_{mutant} (n=5) treated with recombinant human IFN- β (1000U/ml) for 6 or 24 hours. Data are presented as fluorescence intensity histograms (top) and mean fluorescence intensities (bottom) $\pm$ SEM. *P<0.05, ***P<0.0001 (one-way ANOVA). (K) Quantification of HeLa cells expressing EGFP-HTT_{Q74} treated with recombinant human IFN- β (1000U/ml) for 24 hours. Graph shows percentage of cells containing EGFP-HTT_{Q74}-positive aggregates (n=4, 400 cells per replicate) $\pm$ SEM. **P<0.01 (Student's t-test). (L) Quantification of GFP-Off-control and GFP-Off-miR-1 HeLa cells co-expressing EGFP-HTT_{Q74} and treated with recombinant human IFN- β (1000U/ml) for 48 hours. Graph represents percentage of cells containing EGFP-HTT_{Q74}-positive aggregates (n=5, 400 cells per replicate) $\pm$ SEM. ****P<0.0001, ***P<0.001 (two-way ANOVA with Bonferroni correction). (M) WB and (N) quantification of LC3, GFP and α -tubulin from HeLa cells stably expressing GFP-Off-Control and GFP-Off-miR-1 (miR-1 hairpin inhibitor) treated with recombinant human IFN- β (1000U/ml) for 6 hours, bafilomycin (400mM) for 4 hours or in combination (n=4) $\pm$ SEM. #P<0.05 (Student's t-test) and **P<0.01, ***P<0.001 (two-way ANOVA with Bonferroni correction).

[059] **Figure 20. Relating to Figure 19. IFN- β regulates miR-1 and TBC1D15 expression in HeLa cells.** (A) RT-PCR of miR-1-3p from HeLa cells treated with recombinant human IFN- β (1000U/ml) for 6 hours (n=3). **P<0.01 (Student's t-test). (B) WB and (C) quantification of TBC1D15 normalised to vinculin from HeLa cells treated with recombinant human IFN- β for 6 or 24 hours (n=4). Data are mean fluorescence intensities $\pm$ SEM. *P<0.05, **P<0.01 (one-way ANOVA).

[060] **Figure 21. Relating to Figure 19. TBC1D15 overexpression abrogates IFN- β - induced reduction of HTT_{Q74} aggregates.** (A) WB of TBC1D15, LC3 and α -tubulin in HeLa cells expressing empty vector or TBC1D15, treated with recombinant human IFN- β (1000U/ml) for 6 hours, bafilomycin (400mM) for 4 hours, or a combination of both. (B) Quantification of mean fluorescence intensities of LC3-II bands from (A) (n=4) $\pm$ SEM. *P<0.05, **P<0.01 (two-way ANOVA) and #P<0.05 (Student's t-test). (C) HeLa cells co-expressing EGFP-HTT_{Q74} with either empty vector or TBC1D15 overexpression vector with or without recombinant human IFN- β treatment (1000U/ml) for 24 hours. Graph represents percentage of cells containing EGFP-HTT_{Q74}-positive aggregates $\pm$ SEM. **P<0.01 (two-way ANOVA with Bonferroni correction) and #P<0.05 (Student's t-test). (D) Neuronally differentiated N2A cells with NTC1 or *Irfnb* CRISPR/Cas9 knockout co-expressing EGFP-

HTT_{Q74}. Graph represents percentage of cells containing EGFP-HTT_{Q74}-positive aggregates (n=3) $\pm$ SEM. *P<0.01, **P<0.001 (Student's t-test).

Description of Embodiments

[061] Proteostasis requires a complex interplay between numerous molecular pathways. The present inventors have identified that the regulatory capacity of mir-1 facilitates management of proteotoxic stress. In particular mir-1 directly targets and regulates autophagy and the expression of specific proteins, such as heat shock proteins, associated with proteostasis. The inventors have therefore identified a direct link between a single miRNA and maintenance of proteostasis.

[062] Accordingly, and as demonstrated herein, administration of mir-1 or interferon- β is useful for the promotion of autophagy and the prevention, inhibition or reduction of protein aggregation.

Methods

[063] Increasing the levels of mir-1, for example by administration of a mir-1 nucleic acid or an interferon- β is useful for the promotion of autophagy and the prevention, inhibition or reduction of protein aggregation. Accordingly methods are provided for the promotion of autophagy and the prevention, inhibition or reduction of protein aggregation comprising administering a mir-1 nucleic acid to a subject. In one embodiment a method for promotion of autophagy in a subject comprises contacting a cell or tissue with the a mir-1 nucleic acid or an interferon- β . This results in an increase (or promotion) of autophagy which leads to the prevention, inhibition or reduction of protein aggregation. In some embodiments this requires administration to the subject a therapeutically effective amount of a mir-1 nucleic acid or an interferon- β .

[064] Increased mir-1 levels also reduce the level of TBC proteins such as tbc-7 or TBC1D15. Therefore, reducing the level of a TBC protein is also useful for the promotion of autophagy and the prevention, inhibition or reduction of protein aggregation. Accordingly methods are provided for the promotion of autophagy and the prevention, inhibition or reduction of protein aggregation comprising administering an agent that reduces the level of a TBC protein in a subject. In one embodiment a method for promotion of autophagy in a subject comprises contacting a cell or tissue with a TBC gene silencing agent, that is a gene silencing agent specific for the nucleic acid encoding the TBC protein. Examples of suitable agents include for example antisense oligonucleotides, ribozymes, RNAi, siRNA or miRNA. Alternatively or in addition a small interfering peptide may be used to reduce the level of a TBC protein such as tbc-7 or TBC1D15.

[065] Contacting a cell or tissue with the mir-1 nucleic acid or TBC gene silencing agent may be achieved by any method known in the art. In some embodiments contacting the cell and the miRNA occurs *in vivo*. The mir-1 nucleic acid or gene silencing agent may be contacted with the cell directly, i.e. applied directly to a cell, or alternatively may be combined with the cell indirectly, e.g. by injecting the mir-1 nucleic acid into the bloodstream of a subject, which then carries the molecule to the cell.

[066] In some embodiments administering a mir-1 nucleic acid increases the level of mir-1 in a cell organ or tissue compared to the endogenous mir-1 level. The term 'endogenous' as used in this context refers to the "naturally-occurring" levels of expression and/or activity of mir-1.

mir-1 nucleic acids

[067] As used herein a 'mir-1 nucleic acid' is a nucleic acid molecule that comprises, consists of, or encodes mir-1 a precursor or variant thereof, or an miRNA with a seed region comprising the sequence GGAAUGU. As disclosed herein, a mature miRNA sequence comprises about the first 6 to about the first 24 nucleotides of a pri-mir-1 or a pre-mir-1, about the first 8 to about the first 22 nucleotides of a pre-mir-1, or about the first 10 to about the first 20 nucleotides of a pre-mir-1. In some embodiments, the mir-1 can be an isolated or purified oligonucleotide having at least 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the miRNA is a hybridizable portion of a mir-1 coding sequence or its complementary sequence. In some embodiments, the mir-1 oligonucleotide has at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides. In some embodiments, the mir-1 oligonucleotide has at least 19, 20, 21, 22, 23, 24, or 25 nucleotides. Isolated or purified polynucleotides having at least 6 nucleotides (i.e., a hybridizable portion) of a mir-1 coding sequence such as the seed sequence or its complement are used in some embodiments. In some embodiments, mir-1 nucleic acids preferably comprise at least 22 (continuous) nucleotides, or a full-length mir-1 sequence, for example as set out in Figure 1B.

[068] Nucleic acid molecules that encode mir-1 can be used in various embodiments disclosed herein. Sequences for mature mir-1 and pre-mir-1 are known in the art. Sequences for the seed sequence of mir-1 are shown in Figure 1B.

[069] In some embodiments the mir-1 is human mir-1. There are three mir-1 sequences in humans that all have the same sequence but are in different chromosomal locations. These are as follows:

hsa-mir-1-1 UGGAAUGUAAAGAAGUAUGUAU

hsa-mir-1-2 UGGAAUGUAAAGAAGUAUGUAU

hsa-mir-1-3 UGGAAUGUAAAGAAGUAUGUAU

[070] The methods disclosed herein are not limited by the source of the mir-1 nucleic acid. For example, the mir-1 nucleic acids can be naturally-occurring or synthetic. In some embodiments, the mir-1 nucleic acid can effectively reduce the expression of target polynucleotides through RNA interference. In some embodiments, a synthetic mir-1 nucleic acid has a sequence that is different from a naturally-occurring mir-1 nucleic acid and effectively mimic the naturally-occurring miRNA. For example, the synthetic mir-1 nucleic acid can have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or greater sequence similarity to naturally-occurring mir-1. In some embodiments, the at least about 95%, can be a naturally-occurring or synthetic mir-1. In some embodiments, the microRNA can be a human mir-1.

[071] In some embodiments, a synthetic mir-1 nucleic acid can have a sequence that is different from a naturally-occurring mir-1 and effectively mimic the naturally-occurring miRNA. For example, the synthetic mir-1 nucleic acid can have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or greater sequence similarity to naturally-occurring mir-1. For example, the naturally-occurring miRNA can be human mature mir-1, human pri-mir-1, or human pre-miR-1.

[072] Nucleotide sequences that encode a variant of a mir-1, with one or more substitutions, additions and/or deletions, and fragments of miR-1 as well as truncated versions of mir-1 may also be useful in the methods disclosed herein. Preferably, the mir1 variant has at least about 50% of the desired functional activity of naturally-occurring mir-1. In some embodiments, the variant of the mir-1 has at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% of the functional activity of mir-1.

[073] The substitutions, additions and/or deletions may be outside seed region, in the seed region or both.

[074] The mir-1 nucleic acid can be from a human or non-human mammal, derived from any recombinant source, synthesized in vitro or by chemical synthesis. The nucleic acid can be DNA or RNA, and can in a double-stranded, single-stranded or partially double-stranded form. The mir-1 nucleic acid can be prepared by any means known in the art to prepare

nucleic acids. For example, nucleic acids may be chemically synthesized using commercially available reagents and synthesizers by methods that are well-known in the art. Larger DNA or RNA segments can also readily be prepared by conventional methods known in the art, such as synthesis of a group of oligonucleotides, followed by ligation of oligonucleotides to build the complete segment. Unless otherwise indicated, the various embodiments are not limited to naturally occurring mir-1 sequences; mutants and variants of mir-1 sequences may also be used.

[075] In some embodiments, modified nucleotides or backbone modifications can be used to increase stability and/or optimize delivery of the mir-1 nucleic acids. Non-limiting modified nucleotides include locked nucleic acid (LNA), 2'-O-Me nucleotides, 2'-O-methoxyethyl, and 2'-fluoro. Backbone modifications include, but are not limited to, phosphorothioate and phosphonate. In some embodiments, a mir-1 nucleic acid can be modified with cholesterol to enhance delivery to target cells or tissues. The cholesterol can be linked, for example, through a hydroxyprolinol linkage on the 3' end of the microRNA.

[076] In some embodiments, the mir-1 nucleic acid can comprise ribonucleotides, deoxyribonucleotides, 2'-modified nucleotides, phosphorothioate-linked deoxyribonucleotides, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), or other forms of naturally or non-naturally occurring nucleotides. The mir-1 nucleic acid can comprise nucleobase modifications, include, but not limited to, 2-amino-A, 2-thio (e.g., 2-thio-U), G-clamp modifications, morpholinos, nucleic acid aptamers, or any other type of modified nucleotide or nucleotide derivative that is capable of base pairing. For example, in addition to naturally occurring nucleotide bases, non-naturally occurring modified nucleotide bases that can be used in the mir-1 nucleic acids disclosed herein, include, but are not limited to, 8-oxo-guanine, 6-mercaptopguanine, 4-acetylcytidine, 5-(carboxyhydroxyethyl) uridine, 2'-O-methylcytidine, 5-carboxymethylamino-methyl-2-thioridine, 5-carb 1 pseudouridine, beta-D- galactosylqueosine, 2'-Omethylguanosine, inosine, N6-isopentenyladenosine, 1- methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylaminomethylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5- methylcytidine, N.sup.6-methyladenosine, 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, beta-D-mannosylqueosine, 5- methoxycarbonylmethyluridine, 5 -methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9-beta-D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine, N-((9-beta-D- ribofuranosylpurine-6-yl) N-methylcarbamoyl) threonine, uridine- 5 -oxyacetic acid methylester uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 2-thiouridine,

5-methyluridine, N-((9-beta-D-ribofuranosylpurine-6-yl) carbamoyl) threonine, 2'-O-methyl-5-methyluridine, 2'-O-methyluridine, wybutosine, and 3-(3-amino-3-carboxypropyl) uridine.

[077] The mir-1 nucleic acids disclosed herein can also be attached to a peptide or a peptidomimetic ligand which may affect pharmacokinetic distribution of the mir-1 nucleic acid such as by enhancing cellular recognition, absorption and/or cell permeation. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long. A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen.

[078] In some embodiments, a mir-1 nucleic acid is modified with cholesterol to enhance delivery to target cells. The cholesterol can be linked, for example, through a hydroxyprolinol linkage on the 3' end of the miRNA.

[079] Also disclosed herein are nucleic acid constructs for expressing a mir-1 nucleic acid. In some embodiments, expression constructs that comprise an expression vector and a coding sequence for a mir-1 nucleic acid inserted therein can be used to deliver the mir-1 nucleic acid to a target cell (e.g., a neuron). In addition to mir-1 nucleic acid coding sequence, the expression construct may contain one or more additional components, including regulatory elements such as a promoter, an enhancer or both. In some embodiments, mir-1 nucleic acid is associated with a regulatory element that directs the expression of the coding sequence in a target cell or tissue.

[080] It will be appreciated by those skilled in the art that the choice of expression vectors and/or regulatory elements to which the mir-1 nucleic acid encoding sequence is operably linked generally depends on the functional properties desired, e.g., miRNA transcription, and the host cell to be transformed. Examples of expression regulatory elements include, but are not limited to, tissue or cell specific promoters, inducible promoters, constitutive promoters, enhancers, and other regulatory elements. In some embodiments, the mir-1 nucleic acid sequence is operably linked with a tissue specific promoter. In some embodiments, the expression vector can replicate and direct expression of mir-1 nucleic acid in the target cell or tissue, for example in the brain. Expression control elements that can be used for regulating the expression of an operably linked coding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, enhancers, and other regulatory elements. In some embodiments, the promoter is the U6 promoter or CMV promoter. In some embodiments, the promoter is a tissue-specific promoter. In some embodiments, the promoter is a promoter specific to a target cell type. In some embodiments, the promoter is a promoter specific to macrophages.

[081] In some embodiments, the expression vector integrates into the genome of the host cell (e.g., a neuron). In some embodiments, the expression construct is maintained extrachromosomally in the host cell comprising the expression vector.

[082] Those skilled in the art will appreciate that any methods, expression vectors, expression control elements and target cells suitable for adaptation to the expression of a miRNA or anti-miRNA in target cells can be used herein and can be readily adapted to the specific circumstances.

Gene silencing agents for TBC proteins

[083] As used herein 'gene silencing' refers to a group of sequence-specific regulatory mechanisms (e.g. RNA interference (RNAi), transcriptional gene silencing, post-transcriptional gene silencing, quelling, co-suppression, and translational repression) mediated by RNA molecules which result in the inhibition or 'silencing' of the expression of a corresponding protein-coding gene. A 'gene silencing agent' is any agent that can be used for gene silencing. Gene silencing agents include antisense oligonucleotides, ribozymes, RNAi, siRNA, miRNA, or combinations thereof.

[084] The nucleic acid sequences of TBC coding regions are known and it is therefore within the knowledge of the skilled person to develop suitable gene silencing agents to reduce the levels of TBC proteins such as tbc-7, TBC1D15 or their homologs.

[085] In some embodiments the TBC gene silencing agents are nucleic acids. These may comprise one or modifications as set out above for mir-1 nucleic acids. These nucleic acids may be expressed from a vector for example as set out above for mir-1 nucleic acids.

[086] In some embodiments gene silencing agents are nucleic acids and can therefore be administered to a subject using the same methods as set out below for mir-1 nucleic acids.

Administration of mir-1 nucleic acids

[087] In the methods described herein a mir-1 nucleic acid is administered to a subject. In particular the mir-1 nucleic acid is delivered to a target cell, tissue or organ. In some embodiments, a mir-1 nucleic acid is delivered to a target cell, tissue or organ or an expression vector encoding the mir-1 nucleic acid is delivered to a target cell, tissue or organ where the mir-1 nucleic acid is expressed. In some embodiments, delivery is systemic and the expression vector is taken up into target cells, tissues or organs. In some embodiments the expression vector may be taken up by non-target cells, tissues or organs, but preferably does not have a significant negative effect on such cells or tissues, or on the subject as a whole.

[088] Methods for delivery of oligonucleotides and expression constructs to target cells are known in the art and include the methods are described briefly below. Target cells can be,

for example neurons. In some embodiments, the mir-1 nucleic acid or expression vector is delivered to the target cell, tissue or organ *in vivo*. In some embodiments, the mir-1 nucleic acid or expression vector is delivered to the target cell *ex vivo*. In some embodiments, the mir-1 nucleic acid or expression vector is delivered to the target cell *in vitro*.

[089] In some embodiments, the target cell is a neuron. The neuron may be present in a subject or may be in culture outside of the subject. In some embodiments, the mir-1 nucleic acid or expression vector is delivered to a target organ or tissue. Non-limiting examples of target organs and tissues include organs and tissues where protein aggregation is known to occur, for example and without limitation, the brain, nervous system and muscle.

[090] In some embodiments the mir-1 nucleic acids are delivered systemically, such as by intravenous injection. Additional routes of administration may include, for example, oral, topical, intrathecal, intraperitoneal, intranasal, intraocular, and intramuscular. In some embodiments, mir-1 nucleic acids or expression vectors can be delivered *ex vivo* to cells harvested from a subject and then cells containing the mir-1 nucleic acid are reintroduced to the subject.

[091] Delivery of the mir-1 nucleic acid or expression vector to a target cell can be achieved in a variety of ways. In some embodiments, a transfection agent is used. As used herein, the term "delivery vehicle" refers to a compound or compounds that enhance the entry of the mir-1 nucleic acid into cells. Examples of delivery vehicles include protein and polymer complexes (polyplexes), combinations of polymers and lipids (lipopolyplexes), multilayered and recharged particles, lipids and liposomes (lipoplexes, for example, cationic liposomes and lipids), polyamines, calcium phosphate precipitates, polycations, histone proteins, polyethylenimine, polylysine, and polyampholyte complexes. In some embodiments, the delivery vehicle comprises a transfection agent. Transfection agents may be used to condense nucleic acids. Transfection agents may also be used to associate functional groups with a polynucleotide. Non-limiting examples of functional groups include cell targeting moieties, cell receptor ligands, nuclear localization signals, compounds that enhance release of contents from endosomes or other intracellular vesicles (such as membrane active compounds), and other compounds that alter the behavior or interactions of the compound or complex to which they are attached (interaction modifiers). For delivery *in vivo*, complexes made with sub-neutralizing amounts of cationic transfection agent can be used.

[092] In some embodiments, the mir-1 nucleic acid or expression vector can be delivered using an exosome or exosome-like vesicle. For example the mir-1 nucleic acid may be introduced into an exosome-producing cell and exosomes containing the mir-1 nucleic acid may be isolated from those cells. Alternatively, exosomes may be isolated or prepared

according to any method known in the art and the mir-1 nucleic acid introduced into the exosomes.

[093] In some embodiments, the mir-1 nucleic acid or expression vector can be delivered systemically. In some embodiments, the mir-1 nucleic acid or expression vector can be delivered in combination with one or more pharmaceutically acceptable carriers. Polymer reagents for delivery of the mir-1 nucleic acid or expression vector may incorporate compounds that increase their utility. These groups can be incorporated into monomers prior to polymer formation or attached to polymers after their formation. A vector transfer enhancing moiety is a molecule that modifies a nucleic acid complex and can direct it to a cell location (such as tissue cells) or location in a cell (such as the nucleus) either in culture or in a whole organism. By modifying the cellular or tissue location of the complex, the desired localization and activity of the miRNA, anti- miRNA or expression vector can be enhanced. The transfer enhancing moiety can be, for example, a protein, a peptide, a lipid, a steroid, a sugar, a carbohydrate, a nucleic acid, a cell receptor ligand, or a synthetic compound. The transfer enhancing moieties can, in some embodiments, enhance cellular binding to receptors, cytoplasmic transport to the nucleus and nuclear entry or release from endosomes or other intracellular vesicles.

[094] Nuclear localizing signals (NLSs) can also be used to enhance the targeting of the mir-1 nucleic acid or expression vector into proximity of the nucleus and/or its entry into the nucleus. Such nuclear transport signals can be a protein or a peptide such as the SV40 large Tag NLS or the nucleoplasmin NLS. These nuclear localizing signals interact with a variety of nuclear transport factors such as the NLS receptor (karyopherin alpha) which then interacts with karyopherin beta. The nuclear transport proteins themselves can also, in some embodiments, function as NLS since they are targeted to the nuclear pore and nucleus.

[095] Those skilled in the art will be able to select and use an appropriate system for delivering the mir-1 nucleic acid or expression vector to target organs or tissues or to target cells *in vitro*, *ex vivo*, or *in vivo* without undue experimentation.

[096] In some embodiments local delivery of mir-1 nucleic acid or expression vector to target organs or tissues is desirable. In particular, delivery of the mir-1 nucleic acid or expression vector to the brain is desirable for the reduction or inhibition of protein aggregation in neurons.

[097] There are a number of strategies to enable localised delivery of therapeutic mir-1 nucleic acid or expression vector to the brain. These include drug delivery devices

[098] Osmotic mini-pumps, such as the Alzet® osmotic pump, can be used for effective local delivery of the mir-1 nucleic acid or expression vector at a sustainable therapeutic

concentration. The pumps with their reservoirs are commonly implanted into subcutaneous tissue, and deliver the mir-1 nucleic acid or expression vector to the target tissue via silicone tubes and cannulas. Osmotic mini-pumps depend on osmotic pressure for steady state drug delivery and have already been applied clinically for the delivery of various molecules such as dopamine. Osmotic pumps have been successfully delivered to the brain parenchyma, lateral ventricles and the epidural/intrathecal spaces of the spine.

[099] Localised delivery of mir-1 nucleic acids or expression vectors may be achieved by grafting of cells. This method has been used as cell-replacement therapy for neurodegenerative disorders such as Parkinson's and Huntington's disease, epilepsy, ischemia and in the treatment of brain tumours. In addition to cell replacement therapy, cells containing a mir-1 nucleic acid or expression vector may also be grafted to the CNS to facilitate delivery of either therapeutic genes or recombinant.

Mir-1 nucleic acid dosage

[0100] The effective dose level of the administered mir-1 nucleic acid, expression vector or interferon- β will depend upon a variety of factors including: the type of condition being treated and the stage of the condition; the activity and nature of the mir-1 nucleic acid or expression vector employed; the composition employed; the age, body weight, general health, sex and diet of the subject; the, time of administration; the route of administration; the duration of the treatment; drugs used in combination or coincidental with the treatment, together with other related factors well known in medicine.

[0101] A skilled person would be able, by routine experimentation, to determine an effective, non-toxic dosage which would be required to treat applicable conditions. These will most often be determined on a case-by-case basis.

[0102] Generally, an effective dosage is expected to be in the range of about 0.0001 mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001 mg to about 750mg-per kg body weight per 24 hours; about 0.01 mg to about 500mg per kg body weight per 24 hours; about 0.1 mg to about 500mg per kg body weight per 24 hours; about 0.1 mg to about 250mg per kg body weight per 24 hours; or about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range of about 10mg to about 200mg 20 per kg body weight per 24 hours.

[0103] Alternatively, an effective dosage may be up to about 5000mg/m². Generally, an effective dosage is expected to be in the range of about 10 to about 5000mg/m², typically about 10 to about 2500mg/m², about 25 to about 2000mg/m², about 50 to about 1500mg/m², about 50 to about 1000mg/m², or about 75 to about 600mg/m². Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the condition being-

treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques.

[0104] It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests

[0105] The efficacy of a treatment regime may also be evaluated by determining the level of expression of mir-1 ligand in the sample from a subject treated with a mir-1 nucleic acid or an expression vector. After a period of time the level of expression of a mir-1 nucleic acid in a further sample from the subject is determined and a change in the level of mir-1 nucleic acid expression may be indicative of the efficacy of the treatment regime.

[0106] The sample may comprise blood plasma or blood serum. The level of expression of mir-1 in the sample may be predictive of the amount of inhibition of protein aggregation and the treatment regime may be adjusted accordingly. Typically an elevated level of expression mir-1 is indicative of inhibition of protein aggregation.

Interferon- β

[0107] As used herein a 'interferon- β ' is used to refer to full length mammalian interferon- β . Any interferon- β can be used in the methods of the inventions. In some embodiments the interferon- β refers to functional fragments and derivatives that retain the ability to increase endogenous mir-1 levels in a cell of a subject. In a preferred embodiment the interferon- β is human interferon- β . In some embodiments the interferon- β is a peptide mimetic of interferon- β . Suitable interferon- β peptide mimetics are known in the art.

[0108] Human interferon- β (IFN- β) is a 166 amino acid glycoprotein produced by fibroblasts, as well as other cells, after induction by viral infection or by double-stranded RNA. Three forms of IFN- β are in clinical use for treating a variety of human disorders, these forms are:

- a. natural human IFN- β produced from human fibroblasts (n-IFN- β);
- b. recombinant human IFN- β such as that produced in *E.coli* (IFN- β -1b). In some embodiments this form contains a serine substitution for cystine at position 17); and
- c. recombinant human IFN- β produced in Chinese hamster ovary cells (IFN- β -1a). IFN- β -1a typically contains the natural human amino acid sequence.

[0109] Both n-IFN- β and IFN- β -1a are glycosylated with a single N-linked complex carbohydrate moiety whereas IFN- β -1b is not glycosylated.

Administration of Interferon- β

[0110] In the methods described herein an interferon- β is administered to a subject.

Methods for administration of interferons are known in the art and include the methods are described briefly below.

[0111] Interferon- β may be administered as a formulation comprising a pharmaceutically effective amount of the compound, in association with one or more pharmaceutically acceptable excipients including carriers, vehicles and diluents. The term "excipient" herein means any substance, not itself a therapeutic agent, used as a diluent, adjuvant, or vehicle for delivery of a therapeutic agent to a subject or added to a pharmaceutical composition to improve its handling or storage properties or to permit or facilitate formation of a solid dosage form such as a tablet, capsule, or a solution or suspension suitable for oral, parenteral, intradermal, subcutaneous, or topical application. Excipients can include, by way of illustration and not limitation, diluents, binding agents, wetting agents, polymers, lubricants, glidants, stabilizers, and substances added to improve appearance of the composition. Acceptable excipients include (but are not limited to) mannitol, sorbitol, lactose, sucrose, starches, polyvinyl alcohol, and polyethylene glycols, and other pharmaceutically acceptable materials. Examples of excipients and their use is described in Remington's Pharmaceutical Sciences, 20th Edition (Lippincott Williams & Wilkins, 2000). The choice of excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

[0112] The interferon- β may be formulated for parenteral administration, including intravenous, intramuscular, subcutaneous, intravitreal, or intraperitoneal administration, fluid unit dosage forms may be prepared by combining the interferon- β and a sterile vehicle, typically a sterile aqueous solution which is preferably isotonic with the blood of the recipient. Depending on the vehicle and concentration used, the interferon- β may be either suspended or dissolved in the vehicle or other suitable solvent. In preparing solutions, the compound may be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, agents such as a local anaesthetic, anti-inflammatory agent, preservative and buffering agents can be dissolved in the vehicle. A surfactant or wetting agent may be included in the composition to facilitate uniform distribution of the interferon- β .

[0113] In one or more preferred embodiments the interferon- β is formulated as an injectable solution, suspension or emulsion.

[0114] Lyophilized formulations are preferably reconstituted with a solution consisting primarily of water (e.g., USP WFI, or water for injection) or bacteriostatic water (e.g., USP

WFI with 0.9% benzyl alcohol). Alternatively, solutions comprising buffers and/or excipients and/or one or more pharmaceutically acceptable carriers may be used.

[0115] In some embodiments, the interferon- β is administered using an implant, for example, a biodegradable implant such as those made from, for example, polylactic acid (PLA), polyglycolic acid, poly(lactide-co-glycolide) (PLGA), cross-linked gelatin derivatives, hypromellose, polyesters and/or polycaprolactones; or a non-biodegradable implant such as those made from, for example, polyvinyl alcohol, ethylene vinyl acetate, silicon and/or polysulfone capillary fiber.

[0116] In some embodiments, the interferon- β is formulated in a sustained release formulation or depot. Exemplary sustained release formulations or depots include a microsphere; matrix; emulsion; lipid-based; polymer-based; nanomicelle; micelle; nanovesicle such as a liposome, noisome, transfersome, discome, pharmacosome, emulsome or spanlastic, especially a liposome; microparticle; nanoparticle such as a nanocapsule or nanosphere composed of e.g. lipids, proteins, natural or synthetic polymers such as albumin, sodium alginate, chitosan, PLGA, PLA and/or polycaprolactone; or in situ gel such as an in situ hydrogel drug delivery system.

[0117] The amount of therapeutically effective interferon- β that is administered and the dosage regimen to promote autophagy depends on a variety of factors, including the age, weight, sex, and medical condition of the subject, the severity of the disease, the route and frequency of administration, the particular interferon- β employed, as well as the pharmacokinetic properties (e.g., adsorption, distribution, metabolism, excretion) of the individual treated, and thus may vary widely. Such treatments may be administered as often as necessary and for the period of time judged necessary by the treating physician. One of skill in the art will appreciate that the dosage regime or therapeutically effective amount of the compound to be administered may need to be optimized for each individual.

[0118] The pharmaceutical compositions may contain active ingredient in the range of about 1 mg to 200 mg, typically in the range of about 1 mg to 50 mg and more typically between about 1 mg and 30 mg. A daily dose of about 0.01 mg/kg to 100 mg/kg body weight, typically between about 0.1 mg/kg and about 50 mg/kg body weight, may be appropriate, depending on the route and frequency of administration. The daily dose will typically be administered in one or multiple doses per day or per week.

[0119] The interferon- β may be administered in combination with other agents, for example, known treatments of protein aggregation diseases such as those set out below.

Diseases and Conditions

[0120] Interferon- β regulates mir-1 expression and mir-1 is expressed in cells including muscle and brain that are involved in many conditions associated with protein aggregation.

In many cases this protein aggregation is toxic to the cells and to the subjects. Such conditions can be referred to as 'proteopathies'. Methods using interferon- β and/or a mir-1 nucleic acid or expression vector are provided herein for preventing, inhibiting or reducing protein aggregation. Those methods are also applicable to the treatment or prevention of conditions associated with protein aggregation.

[0121] Conditions to which methods are applicable include, but are not limited to Alzheimer's disease, cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, prion disease, synucleinopathy such as Parkinson's disease, tauopathy, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, trinucleotide repeat disorder such as Huntington's disease, familial dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), serpinopathy, Alexander disease, familial amyloidotic neuropathy, senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, Aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), Isozyme amyloidosis, fibrinogen amyloidosis, dialysis amyloidosis, inclusion body myositis, inclusion body myopathy, cataracts, retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, hereditary lattice corneal dystrophy, cutaneous lichen amyloidosis, mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, seminal vesicle amyloid, apolipoprotein C2 amyloidosis, apolipoprotein C3 amyloidosis, Lect2 amyloidosis, insulin amyloidosis, galectin-7 amyloidosis (primary localized cutaneous amyloidosis), corneodesmosin amyloidosis, enfuvirtide amyloidosis, Cystic Fibrosis, sickle cell disease.

[0122] The methods are applicable to prevent, inhibit reduce protein aggregation of a protein selected from Alzheimer's amyloid β peptide ($A\beta$), Tau protein, prion protein, α -synuclein, TDP-43, fused in sarcoma (FUS) protein, superoxide dismutase, ubiquilin-2 (UBQLN2), proteins with polyglutamine expansions, ABri, Adan, Cystatin C, Notch3. glial fibrillary acidic protein (GFAP), seipin, transthyretin, serpin, immunoglobulin light chain, immunoglobulin heavy chain, amyloid A protein, islet amyloid polypeptide, medin (lactadherin), apolipoprotein AI, apolipoprotein AII, apolipoprotein AIV, gelsolin, lysozyme, fibrinogen, beta-2 microglobulin, crystalline, rhodopsin, calcitonin, atrial natriuretic factor, prolactin, keratoepithelin, keratin, keratin intermediate filament protein, lactoferrin, surfactant protein C (SP-C), odontogenic ameloblast-associated protein, semenogelin I, spolipoprotein C2 (ApoC2), apolipoprotein C3 (ApoC3), leukocyte chemotactic factor-2

(Lect2), insulin, galectin-7, corneodesmosin, enfuvirtide, cystic fibrosis transmembrane conductance regulator (CFTR) protein, and hemoglobin.

Combination Therapy

[0123] The terms 'combination therapy' or 'adjunct therapy' in defining use of interferon- β and/or a mir-1 nucleic acid together with one or more other pharmaceutical agents, are intended to embrace administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single formulation having a fixed ratio of these active agents, or in multiple, separate formulations of each agent.

[0124] In accordance with various embodiments of the present invention one or more of interferon- β , a mir-1 nucleic acid and a TBC gene silencing agent may be formulated or administered in combination with one or more additional therapeutic agents. Thus, in accordance with various embodiments of the present invention, at least one of interferon- β , a mir-1 nucleic acid and a TBC gene silencing agent may be included in combination treatment regimens with surgery and/or other known treatments or therapeutic agents, and/or adjuvant or prophylactic agents.

[0125] A number of agents are available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for treatment of the diseases and conditions listed above as part of combination drug therapy. Suitable agents which may be used in combination therapy will be recognized by those of skill in the art. Suitable agents are listed, for example, in the Merck Index, An Encyclopaedia of Chemicals, Drugs and Biologicals, 12th Ed., 1996, and subsequent editions, the entire contents of which are incorporated herein by reference.

[0126] For example, when used to promote autophagy in a subject with Alzheimer's at least one of the interferon- β , a mir-1 nucleic acid and a TBC gene silencing agent, may be administered with an anti-Alzheimer's agent, such as one or more of galantamine, rivastigmine, and donepezil. In other embodiments, when used for the treatment of Huntington's disease either or both of interferon- β , and a mir-1 nucleic acid may be administered with, for example, Tetrabenazine (Xenazine) antipsychotic drugs, such as haloperidol (Haldol) and chlorpromazine, or risperidone or quetiapine. Alternatively, interferon- β , and a mir-1 nucleic acid may be administered with an additional anti-Parkinson's agent such as levodopa, dopamine agonists, MAO B inhibitors, catechol O-methyltransferase (COMT) inhibitors, anticholinergics, amantadine, and deep brain stimulation.

[0127] Combination regimens may involve the active agents being administered together, sequentially, or spaced apart as appropriate in each case. Combinations of active agents including at least two of the interferon- β , mir-1 nucleic, and the TBC gene silencing agent may be synergistic.

[0128] The co-administration of at least one of the interferon- β , the mir-1 nucleic acid and the TBC gene silencing agent with an additional agent may be effected by the agents being in the same unit dose as another active agent, or one or more other active agent(s) may be present in individual and discrete unit doses administered at the same, or at a similar time, or at different times according to a dosing regimen or schedule. Sequential administration may be in any order as required, and may require an ongoing physiological effect of the first or initial compound to be current when the second or later compound is administered, especially where a cumulative or synergistic effect is desired.

[0129] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

EXAMPLES

Experimental Procedures

***Caenorhabditis elegans* strains**

[0130] All *C. elegans* strains were cultured at 20°C as previously described (Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics 77, 71-94) unless otherwise stated. The following strains were used:

- N2 (Bristol strain, wild-type)
- RJP3690 mir-1(gk276)I
- RJP3691 mir-1(n4102)I
- AM141 rmls133[Punc-54::Q40::YFP]X
- RJP3636 rmls133[Punc-54::Q40::YFP]X; mir-1(gk276)I
- RJP3672 rmls133[Punc-54::Q40::YFP]X; mir-1(n4102)I
- RJP3584 rmls133[Punc-54::Q40::YFP]X; mir-80(nDf53)III
- RJP3596 rpls194[Pmyo-3::mir-1; Pelt-2::gfp]; rmls133[Punc-54::Q40::YFP]X; mir-1(gk276)I
- RJP3636 rpEx195[Pmyo-2::mir-1; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X; mir-1(gk276)I

- RJP3660 rpEx329[Pges-1::mir-1; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X; mir-1(gk276)I
- RJP3657 rpEx196[Pmyo-3::mir-1mut; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X; mir-1(gk276)I
- NL5901 pkIs2386[Punc-54::alphasynuclein::YFP+unc119(+)
- RJP3679 pkIs2386[Punc-54::alphasynuclein::YFP+unc119(+); mir-1(gk276)I
- RJP3595 pkIs2386[Punc-54::alphasynuclein::YFP+unc119(+); mir-1(n4102)I
- RJP3683 rpEx330[Pmyo-3::hsp-70(F44E5.4); Pmyo-2::mCherry]; rmls133[Punc-54::Q40::yfp]; mir-1(gk276)I line 1
- RJP3684 rpEx330[Pmyo-3::hsp-70(F44E5.4); Pmyo-2::mCherry];
- rmls133[Punc-54::Q40::yfp]; mir-1(gk276)I line 2
- RJP3685 rpEx330[Pmyo-3::hsp-70(F44E5.4); Pmyo-2::mCherry];
- rmls133[Punc-54::Q40::yfp]; mir-1(gk276)I line 3
- RJP3740 rmls133[Punc-54::Q40::yfp]; hsp-70(tm2318)
- RJP3739 rmls133[Punc-54::Q40::yfp]; hsp-70(tm2318); mir-1(gk276)I,
- RJP3751 rmls133[Punc-54::Q40::yfp]; hip-1(gk3264)
- RJP3752 rmls133[Punc-54::Q40::yfp]; hip-1(gk3264); mir-1(gk276)I, RJP3754
- rmls133[Punc-54::Q40::yfp]; dnj-19(gk595)
- RJP3753 rmls133[Punc-54::Q40::yfp]; dnj-19(gk595); mir-1(gk276)I
- hhIs64[unc-119(+); Psur-5::UbiV-GFP]III; hhIs113[Pnhx-2::cpl-1W32AW35A::yfp; Pmyo-2::mCherry]I
- hhIs64[unc-119(+); Psur-5::UbiV-GFP]III; mir-1(n4102)I
- hhIs64[unc-119(+); Psur-5::UbiV-GFP]III; mir-1(gk276)I
- hhIs113[Pnhx-2::cpl-1W32AW35A::yfp; Pmyo-2p::mCherry]I; mir-1(n4102)I
- hhIs113[Pnhx-2::cpl-1W32AW35A::yfp; Pmyo-2::mCherry]I; sel-1(e1948)V
- RJP3678 rmls133[Punc-54::Q40::yfp]; age-1(hx546)
- RJP3675 rmls133[Punc-54::Q40::yfp]; age-1(hx546); mir-1(gk276)
- RJP3673 rmls133[Punc-54::Q40::yfp]; daf-16(mu86)
- RJP3680 rmls133[Punc-54::Q40::yfp]; daf-16(mu86); mir-1(gk276)
- RJP3636 mir-1(gk276)I; rmls133[Punc-54::Q40::YFP]X
- RJP3672 mir-1(n4102)I; rmls133[Punc-54::Q40::YFP]X
- RJP3584 mir-80(nDf53)III ; rmls133[Punc-54::Q40::YFP]X
- RJP3596 mir-1(gk276)I rpls194[Pmyo-3::mir-1; Pelt-2::gfp]; rmls133[Punc-54::Q40::YFP]X

- RJP3636 mir-1(gk276)I; rpEx195[Pmyo-2::mir-1; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X
- RJP3660 mir-1(gk276)I; rpEx329[Pges-1::mir-1; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X
- RJP3657 mir-1(gk276)I; rpEx196[Pmyo-3::mir-1*; Pmyo-2::mCherry]; rmls133[Punc-54::Q40::YFP]X
- NL5901 pkIs2386[Punc-54:: α -synuclein::YFP + unc119(+)
- RJP3679 mir-1(gk276)I; pkIs2386[Punc-54:: α -synuclein::YFP + unc119(+)
- RJP3595 mir-1(n4102)I; pkIs2386[Punc-54:: α -synuclein::YFP + unc119(+)
- RJP3920 rpEx1674[Ptbc-7::GFP + Pttx-3::mCherry].

Generation of transgenic strains

[0131] All constructs were injected into young adult hermaphrodites as complex arrays with PvuII digested bacterial DNA (80 ng/ μ l) and Pmyo-2::mCherry (5 ng/ μ l) or Pelt- 2::gfp as co-transformation marker.

RNA-mediated interference

[0132] RNAi clones were obtained from the Ahringer C. elegans RNAi feeding library. All clones were sequenced and verified before use. Experiments were performed as follows; YA staged animals were moved to RNAi bacteria-seeded NGM plates and left to produce progeny for three days. Then 10 L4 staged animals were picked to plates seeded with 50 μ l RNAi bacteria and left at 20°C for 24 hrs. 4 plates with 10 worms were assayed for each of three replicates. Then animals were heat shocked for 5 hrs at 35°C in a single layer in a ventilated incubator to ensure an equal distribution of heat. After heat shock the animals were left to recover for 17 hrs at 20°C and then scored for survival by touching with a platinum wire and the animals that did not respond were scored as dead.

Quantification of aggregates

[0133] The total number of aggregates was counted in body wall muscles using a Zeiss, AXIO Imager M2 fluorescence microscope at magnification 40x. All experiments were performed on L4 animals and in triplicates with at least 10 worms counted per replicate.

Thrashing assay

[0134] To assay motility, animals at day 3 or day 7 post L4 were placed in 10 μ l of M9 liquid, allowed to recover for 10 sec. and then number of body bends was counted for one minute. A total of 10 worms were counted per each of three replicates. Animals not moving at all were censored from the experiments.

Heat shock assay

[0135] Five young adult worms were cultured on nematode growth medium (NGM) plates seeded with 300 µl of OP50 *Escherichia coli* bacteria to produce progeny at 20°C for three days. Ten L4 larval staged animals were incubated on NGM plates seeded with 50 µl of 24 hours old OP50 bacteria at 20°C for 24 hours. Four plates with ten worms were assayed for each of three replicates. Animals were heat shocked for 4 hours at 35°C in a single layer in a ventilated incubator to ensure an equal distribution of heat. After heat shock, animals were recovered for 17 hours at 20°C and scored for survival by touching with a platinum wire. Animals that did not respond were scored as dead.

RNA preparation and qRT-PCR analysis

[0136] RNA sequencing and RT-qPCR experiments were performed in triplicate. RNA was isolated from synchronised L4 animals: 2400 animals/sample for RNA-seq and 400 animals/sample for qPCR validation. Samples were washed 3 times in M9 buffer, resuspended in TRIzol (Invitrogen) and frozen in liquid nitrogen. Samples were repeatedly thawed at 37°C, vortexed for 30 seconds, then re-frozen in liquid nitrogen a total of 7 times. Homogenates were mixed with chloroform (Sigma), centrifuged and RNA within the upper phase was purified using the RNeasy mini kit (Qiagen) as per kit instructions, and included DNase digestion. For qPCR analysis, 300ng of purified RNA was converted to cDNA using the ImProm II Reverse Transcription System (Promega), as per kit instructions, using an OligodT:Random primer ratio of 1:3. Samples were diluted to 5ng/µl, qPCR analysis was performed using LightCycler 480 SYBR Green (Roche). RNA expression levels were normalized to two reference genes, *cdc-42* and *pmp-3*. The oligonucleotides used are available on request. For detection of miR-1-3p and miR-191, RNA was extracted with QIAzol (Qiagen) and purified with a miRNeasy mini kit (Qiagen cat. no. 217004). TaqMan™ MicroRNA Reverse Transcription kit was used for cDNA synthesis (20ng RNA from cortical neurons and 100ng RNA from HeLa cells) using miR-specific TaqMan probes according to manufacturer description. For quantitative real-time PCR miR-1-3p and miR-191 specific TaqMan probes were used according to manufacturer description using an Applied Biosciences Step-One Real Time PCR machine for detection.

RNA sequencing library construction and transcriptome analysis

[0137] RNA sequencing was performed at Micromon Genomics (Monash University). mRNA samples were converted to indexed Illumina sequencing libraries using Illumina's TruSeq Stranded mRNA Sample Prep Kit, employing oligo (dT)-conjugated beads to enrich for polyadenylated transcripts. Libraries were quantitated using a Qubit DNA HS kit (Invitrogen, Carlsbad CA., USA), sized using an AATI Fragment Analyzer (Advanced Analytical Technologies Inc., USA), and sequenced on an Illumina NextSeq500 configured

to produce 75 nt paired-end reads. Fastq files were generated by bcl2fastq, trimming 3' adapter sequences.

[0138] The sequencing reads in fastq format were processed using the RNAsik pipelining tool, version 1.5.0 as follows. Reads were assessed for quality and duplication using FastQC v0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and mapped to the *C. elegans* genome (version WBcel235, downloaded from Ensembl) using STAR v2.5.2b (Dobin et al., Bioinformatics 29, 15-21 (2013)). Uniquely mapping read-pairs were assigned to annotated transcript exons (including splice-junctions) contained in the Ensembl GTF file for genome build WBcel235 using FeatureCounts v1.5.2 (Liao, et al, Bioinformatics 30, 923-930 (2014)), aggregating at the gene level to produce genewise counts for each sample. This gene-count matrix was loaded into the Degust tool (<http://degust.erc.monash.edu>) for differential gene expression analysis. Genes that failed to accrue at least 10 counts in at least one sample were filtered out and the samples were normalized for library size by the TMM method (Robinson, & Oshlack, Genome Biol 11, R25 (2010)). Testing for differential gene expression between the miR-1 and N2 conditions was then performed using Limma-voom (Law, et al, Genome Biol 15, R29 (2014)).

Molecular cloning

[0139] *mir-1* rescue constructs were generated by PCR amplification of the *mir-1* hairpin and cloned downstream of the *myo-3*, *myo-2* or *ges-1* promoters. A standard site-directed mutagenesis protocol was used to generate the *mir-1* construct with mutated seed sequence mir-1mut. The Pmyo-3::hsp70 construct was generated by amplification of the hsp-70(F44E5.5) cDNA and then cloned downstream of the myo-3 promoter.

[0140] A standard site-directed mutagenesis protocol was used to generate the mir-1 construct with mutated seed sequence mir-1*. The human TBC1D15 3'UTR was amplified from genomic DNA and subcloned into the pCAG-GFPd2 vector, a gift from Connie Cepko lab (Addgene plasmid #14760; <http://n2t.net/addgene:14760>; RRID:Addgene 79148) using NotI and Bsu36I. Forward primer: TATATGCGGCCGCTCACTGTTCTTGCTTTTTTGGG and reverse primer: CCATTAATTAATAATGTCTTCAGAATGCTCCTGAGGGTGC.

Protein quality control assays

[0141] Worms were lysed in SDS sample buffer and equal volumes applied to SDS-PAGE gels. Western blotting was performed using antibodies against GFP/YFP (Clontech) and Tubulin (Sigma). Digital sample detection was performed using the Infrared Odyssey CLx Imager (Li-cor). Densitometric quantification was performed using the software Image Studio Version 4.0 (Li-cor). For heat stress assays of strains carrying the UFD substrate: Synchronized 1 day adult worms were incubated for 2 hours at 35°C and collected for

protein analysis at the indicated time points. Heat stress with strains carrying the ERAD substrate: Synchronized L4 larval stage worms were incubated for 2 hours at 35°C and collected for protein analysis after 24 hours of recovery at 20°C.

Microscopy

[0142] Animals were anaesthetized with 20mM NaN₃ on 5% agarose pads and images were taken by an AXIO Imager M2 fluorescence microscope and Zen software (Zeiss).

Mammalian cell culture

[0143] Hela and C2C12 cells were kept in DMEM containing 10% Foetal bovine serum (FBS), 1% glutamine and 1% Penicillin/Streptomycin (P/S). The media was changed every 2-3 days and the cells were split every 3-4 days.

[0144] For primary cortical neuron (CN) cultures cortexes were dissected from 1-day-old mice pups and processed as previously described (P. Ejlerskov et al., Cell 163, 324-339 (2015)). Neurons were cultured in Neurobasal medium (Gibco) containing B27 (2%) and gentamicin for 8-10 days on culture plates pre-coated with poly-D-lysine (137,500 cells/cm²). Half of the medium was changed every 3-4 days. HeLa and N2A neuroblastoma cells were maintained in DMEM containing GlutaMax, 10% Foetal bovine serum (FBS) and 1% Penicillin/Streptomycin (P/S). Media was changed every 2-3 days and cells were split every 3-4 days. For neuronal differentiation, N2A cells were cultured for 4 days in DMEM medium containing GlutaMax, 1% FBS and 20 µM retinoic acid.

Transfection

[0145] Cells were seeded at a density of 20000 cells/cm² and the following day miR mimics (Dharmacon; cat. no. C-300585-05-0005, C-300586-05-0005, C-310377-07-0005, C-310376-07-0005) or miR inhibitors (Dharmacon; cat. no. IH-300585-06-0005, IH-300586-06-0005, IH-310376-08-0005 and IH-310377-08-0005) mRFP-GFP-LC3, pEF6-myc-TBC1D15 (Addgene plasmid# 79148; <http://n2t.net/addgene:79148>; RRID:Addgene 79148), EGFP-HTT_{Q74} (vector backbone pEGFP-C1; HTT exon1), GFPd2-3'UTR-TBC1D15, GFPd2-3'UTR-TBC1D15_{mutant}, were transfected with Lipofectamine 2000 or Mirus TransIT according to manufacturer's protocol. The following day the cells were treated with bafilomycin A1 (400 nM) for 4 hours or were untreated. When transfecting with miR-1 mimics or siRNA knockdown oligos the medium was changed the following day and bafilomycin A1 was added 48 hours after initial transfection. For co-transfections with miR-1 mimics cells were transfected with miR-1 24 hours prior to EGFP-HTT_{Q74}, TBC1D15, empty vector, GFPd2-3'UTR-TBC1D15 or GFPd2-3'UTR-TBC1D15_{mutant}.

Immunofluorescence staining and imaging

[0146] After 48-72 hours of eGFP-HTT_{Q74} expression the cells were fixed in 4% PFA for 10 minutes, blocked in blocking buffer (5% normal goat serum, 1% bovine serum albumin, and 0.25% triton-X-100) and incubated with LC3B antibodies (Cosmo, cat.no. CAC-CTB-LC3-2-IC) dissolved 1:150 in blocking buffer over night at 4°C. The following day the cells were washed three times in PBS⁻, incubated with Alexa fluor secondary antibodies (Invitrogen) 1:1000 and phalloidin 633 (Molecular Probes, cat. no. A22287) 1:400 in blocking buffer for 60 minutes at room temperature. Subsequently, the nuclei were stained with DAPI (Sigma, D9564) 1:1000 in PBS⁻, washed three times in PBS⁻, and mounted on glass slides with mounting media. Images were acquired with a Zeiss 880 confocal microscope, equipped with a live cell imaging incubator, using the 405nm, 488nm, and 568nm, and 633nm lasers and a pinhole of 0.8 μ m. For live cell imaging, cells were maintained at 37°C and 5% CO₂ in a humidified incubator and images were acquired at a speed of 1 per second for 2 minutes. Movies were generated as avi files in ImageJ and displayed at a speed of 10 frames per second. LC3B- (on fixed cells detected with antibody) and mRFP-GFP-LC3-positive (live cell imaging) vesicles were quantified using ImageJ and Volocity. Each image contained 2-4 cells and a total of 12-24 cells were scored in each technical replicate. EGFP-HTT_{Q74} positive aggregates were quantified by manual counting using the 63x objective on a Zeiss Axio Imager M2 microscope. Each condition was set up in duplicates and 200-400 cells were counted per technical replicate.

Flow cytometry analysis

[0147] HeLa cells were transfected with scrambled or miR-1 mimic one day prior to transfection with GFPd2-3'UTR-TBC1D15 or GFPd2-3'UTR-TBC1D15 Δ . The following day, cells were placed on ice and stained with live/dead cell marker (Invitrogen, cat. no. L23102) according to manufactures protocol before analysis in with Accuri C6 flow cytometer using the FL1-A channel for detection of GFP signal and FL4-A for detection of dead cells. The cells were analysed in technical duplicates measuring the mean intensity fluorescence for 15,000-20,000 cells per well in the live cell population.

Western blot analysis: *C. elegans*

[0148] Young adult animals were picked directly into SDS sample buffer, boiled for 15 mins at 95°C and cooled on ice. The solution was centrifuged for 10 mins at 3000rpm and equal amounts of sample were loaded onto a protein gel, separated by SDS page and protein analysis was assayed with GFP antibody (Roche), PolyUbiquitin antibody (Sigma) and α -tubulin (12G10 - Developmental Studies Hybridoma Bank, University of Iowa).

Western blot analysis: Mammalian cells

[0149] Cells were lysed in triton-X-100 buffer (1% triton-X-100, 100 mM NaCl, 50 mM Tris-HCl, 1 mM EGTA, and 10 mM MgCl₂) containing phosphatase inhibitor cocktail 2 and 3 (Sigma, cat.no. P5726 and P0044), and complete protease inhibitor cocktail (Roche, cat. no. 11873580001) for 5 minutes at room temperature and then kept on ice. The lysis suspensions were harvested and centrifuged at 16,100 g for 5 minutes at 4°C and the supernatants collected. The protein concentrations were measured with Biorad DCTM protein assay (cat. no. 5000112) reagent and 25 µg of sample was run on SDS-PAGE gels and transferred to PVDF Immobilon-FV membranes (Millipore, cat. no. IPFL20200). Membranes were blocked in either 5% milk or 5% BSA and subsequently incubated with primary antibodies overnight. The following day membranes were washed three times in PBS with 0.1% tween-20 (PBS-T), and incubated with species specific secondary antibodies coupled to 680 nm or 800 nm fluorophores (Li-Cor) in 5% milk or 2% BSA. Finally, the membranes were washed three times in PBS-T and signal detected in Li-Cor Odyssey scanner using the 700 nm and 800 nm emission filters. Mean fluorescence band intensities were quantified using Image Studio Lite version 5.2.

Autophagy experiments***C. elegans***

[0150] Animals were incubated at 15°C for two generations prior to the experiment. Young adult animals were then heat shocked for 1h at 36°C (heat shock) or recovered for 1h at 15°C (heat shock + recovery) before being imaged by confocal microscopy.

Mammalian cells

[0151] For autophagy flux assays, cells were treated with bafilomycin A1 (400nM) for 4 hours and subsequently lysed in triton-X-100 buffer and processed for WB as described above. When co-treating HeLa cells with recombinant human IFN-β, cells were pre-treated with IFN-β (1000U/ml) for 2 hours before the addition of bafilomycin (400nM) for 4 hours.

Statistical analysis

[0152] All statistical analysis was performed using the GraphPad Prism 5.0 software. Student's *t*-test, one- way or two-way ANOVA analysis followed by Dunnetts or Bonferroni's multiple comparison tests were used. Data is presented as means ± SEM.

RESULTS**Example 1: *mir-1* Prevents Polyglutamine Aggregation**

[0153] miR-1 is a highly conserved miRNA that is detected in muscle, neurons and circulatory body fluid of multiple metazoan phyla (Figure 1A). Intriguingly, miR-1 expression

is depleted in a *Drosophila melanogaster* model of Alzheimer's disease and human miR-1 is reduced in the cerebrospinal fluid of patients with Parkinson's disease.

[0154] To gain insight into the function of miR-1 in the regulation of protein aggregation mir-1 function was assayed in a *C. elegans* transgenic polyglutamine model for protein conformation disorders. This model expresses a polypeptide of 40 glutamine residues fused to yellow fluorescent protein (Q40:YFP), hereafter referred to as Q40. Q40 is expressed in body wall muscle. Using two independently-derived *mir-1* deletion alleles, *mir-1(gk276)* and *mir-1(n4102)*, it was found that loss of *mir-1* increased the number Q40 protein aggregates present in the body wall muscle, without affecting expression levels of the Q40 transgene (Figure 1 and 6). This phenotype was not due to a non-specific change in the miRNA profile of muscle cells as loss of the muscle-specific *mir-80*, did not affect Q40 protein aggregation (Figure 1).

[0155] In *C. elegans*, *mir-1* is expressed in muscle of the body wall muscle and pharynx. To characterize the functional locale of *mir-1* in regulating Q40 aggregation, tissue-specific rescue experiments were performed. It was found that expression of *mir-1* in body wall muscle rescues the excessive Q40 aggregation phenotype in *mir-1(gk276)* animals (Figure 1E and 6). In contrast, expression of *mir-1* in the pharynx did not rescue the Q40 protein aggregation phenotype (Figure 1). In mammals miR-1 has been detected in serum and is therefore characterized as a circulating miRNA. To investigate whether expressing *mir-1* in a distal tissue could have an effect on protein aggregation in the body wall muscles *mir-1* was expressed under control of an intestinal promoter. No suppression of Q40 protein aggregation was observed (Figure 1). Therefore, *mir-1* acts cell autonomously in body wall muscle cells to control protein aggregation.

[0156] The majority of miRNAs function through imperfect interactions with the 3'UTR of target mRNAs causing instability and/or translational repression. Therefore the inventors investigated whether disrupting the target binding properties of *mir-1* would affect its rescuing ability. To this end, two essential nucleotides in the binding sequence of the *mir-1* hairpin were mutated, which would prevent the canonical miRNA:mRNA target binding, this failed to rescue the Q40 protein aggregation of *mir-1* mutant animals when expressed in body wall muscle (Figure 1). *mir-1* acts to prevent Q40 protein aggregation in body wall muscle and does so through established means of canonical miRNA:mRNA target interactions.

[0157] Two conserved nucleotides in the *mir-1* seed sequence were mutated and the rescue experiment was repeated (Figure 1E). Expressing mutated *mir-1* (*mir-1**) in body wall muscle failed to rescue the Q40 protein aggregation phenotype of *mir-1(gk276)* animals (Figure 1E). The expression of expanded polyglutamine repeats in muscle is toxic and

progressively affects muscle function and *C. elegans* motility. It was found that Q40 toxicity was exacerbated in *mir-1* mutant animals in an age-dependent manner (Figure 10). To determine if *mir-1* has an effect on motility in a non-proteotoxic environment, the inventors tested the motility of animals expressing the control Q40::YFP transgene as well as wild-type and *mir-1(gk276)* animals devoid of transgenes (Figure 10). A slight decrease in motility in *mir-1* mutant animals expressing Q40::YFP and with no transgene was observed (Figure 10), indicating that loss of *mir-1* causes defects in muscle proteostasis, which are exacerbated when animals are overloaded with protein aggregates. Taken together, *mir-1* prevents Q40 protein accumulation in body wall muscle and protects against proteotoxicity. While not being bound by theory it is presumed that this occurs through 3'UTR-directed regulation of its target gene(s).

Example 2: *mir-1* Protects Against Proteostatic Threats.

[0158] Expression of expanded repeats of polyglutamine in muscle is toxic and progressively affects muscle function and *C. elegans* motility. To investigate if loss of *mir-1* affects polyglutamine toxicity the motility of animals expressing Q40 in wild-type and *mir-1* mutant animals (Figure 2) was measured. For comparison, the motility of animals expressing the control Q0::YFP transgene as well as wild-type, *mir-1(gk276)* and *mir-1(n4102)* animals devoid of transgenes was also measured (Figures 2 and 7). An age-dependent decrease in motility in the Q40 model in both *mir-1* deletion alleles (Figure 2), indicating increased polyglutamine toxicity was observed. Also observed was a slight decrease in motility in *mir-1* mutant animals expressing Q0::YFP and with no transgene (Figures 2 and 7).

[0159] To explore the possibility that *mir-1* generally protects against proteotoxic stress two stress paradigms were examined. First, a Parkinson's disease (PD) model where the aggregation-prone peptide α -synuclein is expressed in body wall muscle. This model elicits age-dependent decline in motility that is associated with accumulation of α -synuclein aggregates (J. F. Cooper et al., Parkinsons Disease 1, 15022 (2015)). It was found that loss of *mir-1* caused a ~50% reduction in motility, presumably through toxicity caused by α -synuclein inclusions (Figure 1F and Figure 2). Second, *mir-1* function in heat stress sensitivity was examined. This is a more general proteotoxic stress (Figure 1G). Elevated temperature places added pressure on the protein folding machinery causing endogenous proteins to misfold and form toxic aggregates (Wallace et al., Cell 162, 1286-1298 (2015)). The heat stress sensitivity of both *mir-1* mutant alleles at 35°C was examined and it was found that *mir-1* is required for survival in these conditions (Figure 2). In addition to acute environmental stress, the aging process can gradually lead to the accumulation of misfolded

proteins. Therefore, the inventors investigated whether *mir-1* is coupled to lifespan regulation. Surprisingly, it was found that both *mir-1* mutant alleles exhibit wild type lifespan (Figure 7). This suggests that *mir-1* primarily functions when an animal is challenged with proteostatic stress and/or that parallel pathways overcome proteostasis defects during aging. Taken together the data presented herein show that *mir-1* is required for *C. elegans* to manage the proteotoxic effects of aggregation-prone proteins and acute heat stress conditions. However, accumulation of misfolded proteins in *mir-1* mutant animals is not detrimental to *C. elegans* lifespan suggesting that PQC pathways are at least partially functional.

[0160] Worms with and without Q40 did not show any change in lifespan compared to wild type.

[0161] It was found that loss of *mir-1* caused severe heat stress sensitivity and that resupplying with *mir-1*, but not mutated *mir-1* (*mir-1**), in body wall muscle rescues this phenotype (Figure 1G). In addition to acute environmental stressors, the aging process causes accumulation of misfolded proteins. Surprisingly, *mir-1* mutant animals exhibit wild-type lifespan (Figure 7A), demonstrating that *mir-1* primarily acts to combat proteotoxic challenges and/or that parallel pathways overcome proteostasis defects during aging. Alternatively, the activities of *mir-1* in controlling protein aggregation are uncoupled from lifespan regulation. Together, this data shows that *mir-1* plays a broad role in protecting against the accumulation of aggregation-prone proteins and the toxic effect of acute heat stress.

Example 3: *mir-1* functions in the insulin/IGF-1-like pathway to regulate protein aggregation.

[0162] The insulin-like signalling (ILS) pathway functions to drive the expression of stress related genes, such as molecular chaperones. Prominent components of the ILS pathway, DAF-2, the insulin-like growth factor receptor, AGE-1, a phosphatidylinositol 3-kinase, and DAF-16, the FOXO transcription factor, have been shown to regulate protein aggregation. To investigate if *mir-1* functions in the ILS pathway to control protein aggregation, the inventors initially analysed the level of *daf-2*, *age-1* and *daf-16* mRNA by quantitative real-time polymerase chain reaction (qRT-PCR) and found that these were not affected in *mir-1* mutants (Figure 8). Next, genetic analysis was performed to examine the effect on protein aggregation by eliminating the expression of these genes in the *Q40; mir-1(gk276)* mutant background. From Figure 8 it can be seen that the epistasis analysis shows that *mir-1* functions downstream of AGE-1 and upstream of DAF-16 in the ILS pathway to regulate Q40 protein aggregation.

Example 4: *mir-1* Controls the Expression of Specific Heat Shock Protein Genes Involved in Proteostasis.

[0163] Maintenance of cellular protein pools requires the activity of multiple genetic pathways, ensuring correct protein folding and clearance of damaged proteins. These pathways include the heat shock response (HSR), ubiquitin proteasome system (UPS), unfolded protein response (UPR), ER-associated degradation pathway (ERAD) and autophagy.

[0164] To explore the premise that *mir-1* controls the expression of specific branches of these PQC pathways to prevent toxic protein aggregation, the inventors conducted expression analysis of relevant genes by RT-qPCR. It was found that the expression level of genes related to PQC were largely unaffected in *mir-1* mutants compared to wild type (Figure 3). However, the inventors found that two members of the Hsp70 family, *C12C8.1* and *F44E5.4*, are downregulated in *mir-1(gk276)* mutant animals. This regulatory effect was confirmed using the independent *mir-1(n4102)* allele (Figure 3).

[0165] The Hsp70 family functions to prevent protein aggregation through reactivation of denatured proteins, targeting proteins for degradation and disassembly of protein complexes. The inventors therefore asked whether transgenic expression of Hsp70 in *mir-1* mutant animals could rescue Q40 aggregate formation. Indeed, resupplying *hsp-70/F44E5.4* in body wall muscle reduced number of Q40 aggregates in *mir-1* mutant animals. This indicates that reduced expression of Hsp70 proteins in *mir-1* mutant animals disturbs the proteostasis machinery. Hsp70 is proposed to function in a multi-step chaperone reaction cycle which requires co-chaperones to licence the fate of misfolded protein substrates. Two common Hsp70-interacting chaperones are Hsp90 and Hsp40. Hsp40 is a co-factor for Hsp70 that supports ATP-driven binding of polypeptides to Hsp70. Hsp90 functions in the same network as Hsp70 to regulate protein refolding. Interestingly, the expression of both co-chaperones is downregulated at the mRNA level in both *mir-1* deletion mutants. This suggests that *mir-1* is required to control the expression of a discrete subset of molecular factors that are part of the chaperone reaction cycle.

[0166] Finally, epistasis analysis was performed to ask whether *hsp-70*, *hsp-40* and another Hsp70-interacting protein, *hip-1*, function in the same genetic pathway as *mir-1* to control Q40 protein aggregation. Loss of any of these genes causes accumulation of a similar number of Q40 aggregates as *mir-1* mutant animals and that double mutant combinations with the *mir-1* mutant were not additive (Figure 3). These data support the conclusion that *mir-1* controls the expression of the Hsp70 multi-chaperone complex to buffer the formation or enhance the degradation of toxic protein aggregates.

Example 5: *mir-1* Regulates Misfolded Protein Clearance Pathways.

[0167] Apart from their crucial role in re-folding of misfolded proteins, Hsp70 and its co-chaperones also support proteasome function. Two independent *in vivo* degradation assays that enable quantitative analysis of proteasomal function were performed to investigate the role of *mir-1* on proteasomal degradation (Figure 4). First, the inventors monitored the UPS, which is responsible for the degradation of superfluous and damaged proteins. A ubiquitin fusion degradation substrate (*sur-5::UbV-GFP* reporter strain) that expresses ubiquitin fused to GFP in somatic cells in *C. elegans* was used as a substrate. This substrate is efficiently degraded by the UPS in wild type animals, however, induction of protein-folding stress reduces the turnover of UbV-GFP. It was found that in *mir-1* mutant animals UbV-GFP protein levels are increased suggesting an important role for *mir-1* in the control of UPS function (Figure 4). Next, the inventors assayed the requirement for *mir-1* in the ER-associated degradation (ERAD) pathway, through which misfolded proteins are translocated from the ER to the cytoplasm for degradation by the proteasome. The inventors employed an ERAD substrate, CPL-1*::YFP, which accumulates in the ER when ERAD is inhibited. In consensus with the UPS substrate experiment, it was found that CPL-1*::YFP also accumulates in *mir-1* mutant animals (Figure 4).

[0168] Previously, it was shown that acute heat stress leads to enhanced turnover of UbV-GFP. The inventors also found this to be the case in wild type animals, however, *mir-1* mutant animals continued to accumulate high levels of UbV-GFP substrate (Figure 4). Likewise, upon heat stress the ERAD substrate CPL-1*::YFP accumulated by 20-fold (Figure 4) and the level of unprocessed CPL-1*::YFP continued to increase (40-fold) even when *mir-1* mutant animals were recovered at 20°C for 24 hours (Figure 4). Together, these data show that *mir-1* is required for efficient protein turnover in the cytoplasm and ER and that loss of *mir-1* disrupts the degradation of protein substrates after proteotoxic stress.

[0169] It is clear that loss of *mir-1* impairs degradation of specific protein substrates. However no difference in the pool of ubiquitinated proteins was observed in animals lacking *mir-1* compared to wild type animals. Accordingly *mir-1* does not modulate general protein degradation.

[0170] Autophagy is another cytoprotective mechanism that plays a role in protein homeostasis by facilitation degradation and recycling of cytosolic components. Autophagy in wild type and *mir-1* mutants was monitored by analysing levels of lipid-bound *atg-8/lgg-1*, which is a central molecule for the formation of autophagosomes and can be used as readout for autophagy activity. It was found that loss of *mir-1* resulted in a significant decrease in autophagy in mammalian cells.

Example 6: miR-1 Controls Huntingtin Protein Aggregation in Mammalian Cells.

[0171] The mature *mir-1* sequence is completely conserved from worms to humans (Figure 1B), suggesting an evolutionary conserved function for *mir-1* in regulating protein aggregation. The inventors investigated the role of human miR-1 in aggregation of a mutant huntingtin exon 1 fragment with 74 polyQ repeats (pHA-HTTQ74) in the C2C12 mouse myoblast cell line. First, the expression of miR-1 in C2C12 cells was knocked down using miR-1 hairpin inhibitors and found that, as in *C. elegans*, polyQ aggregation increased (Figure 5). To further explore if overexpression of miR-1 could impact protein aggregation in other cell types miR-1 mimics were overexpressed in HeLa cells expressing HTT_{Q74}. As in C2C12 cells, Q74 aggregation was reduced in this epithelial-derived cell line (Figure 5).

[0172] The reduction of HTT_{Q74} aggregates upon overexpression of miR-1 suggests that it may have a therapeutic effect, even in non-muscle cells. To explore this possibility, the inventors overexpressed miR-1 mimics in HeLa cells expressing HTT_{Q74}. As in C2C12 cells, HTT_{Q74} aggregation was reduced in this epithelial-derived cell line.

Example 7: *mir-1* targets *tbc-7*.

[0173] The data presented herein provides evidence that *mir-1* targets an mRNA or mRNAs, that encode vital regulators of proteotoxic stress. To identify these targets, the inventors used two complementary approaches. First, RNA sequencing was used to identify differentially expressed genes in *mir-1(gk276)* animals compared to wild-type (data not shown). Next, the expression of predicted *mir-1* target genes (TargetScanWorm release 6.2) was knocked down using RNA-mediated interference (RNAi) to identify regulators of *mir-1(gk276)* heat stress sensitivity (Figure 11). It was found that *tbc-7* mRNA, a highly conserved predicted *mir-1* target, is elevated in *mir-1(gk276)* animals (Figure 9A).

[0174] Further, reducing *tbc-7* expression fully suppressed heat stress sensitivity of *mir-1(gk276)* animals (Figure 9 and Figure 11). TBC-7 is uncharacterized and predicted to encode a Rab GTPase-activating protein (Rab GAP) member of the Ire-2/Bub2/CDC16 (TBC) family. To determine the *tbc-7* expression pattern, a transgene driving green fluorescent protein (*gfp*) under the control of a *tbc-7* promoter (*Ptbc-7::gfp*) was generated. Fluorescence was detected in the intestine, head cells and importantly in body wall muscle, the site-of-action for *mir-1* in regulating proteotoxic stress. This confirms single-cell transcriptional profiling that detected *tbc-7* expression in multiple tissues, including in body wall muscle. (

[0175] To assess whether *mir-1* directly regulates *tbc-7* expression *in vivo*, the inventors used a well-established 3'UTR sensor assay (Pedersen et al., Science 341, 1404-1408 (2013)). The inventors generated a transgene expressing two reporters in body wall muscle: one red fluorescent protein (*mCherry*) 'sensor' reporter controlled by the *tbc-7* 3'UTR and

another with a *gfp* 'control' reporter controlled by the *unc-54* 3'UTR, which does not contain any *mir-1* binding sites (Figure 9C-F). In wild-type animals expressing this transgene, robust *gfp* expression and weak *mCherry* expression was detected (Figure 9E-F). When the same transgene was transferred into the *mir-1(gk276)* mutant, high *mCherry* expression was observed, suggesting that *mir-1* directly represses the *tbc-7* 3'UTR (Figure 9E-F). Further, when the predicted *mir-1* binding site was disrupted in the *tbc-7* 3'UTR *mCherry* sensor, high red fluorescence in wild-type animals was observed, confirming that *mir-1* regulation is required to repress *tbc-7* expression (Figure 9E-F). As *mir-1* directly downregulates *tbc-7* expression, the inventors hypothesized that overexpressing *tbc-7* in body wall muscle (the site of *mir-1* action) would phenocopy a *mir-1* mutant phenotype in wild-type animals. It was found that overexpressing *tbc-7* in wild-type body wall muscle causes increased accumulation of Q40 aggregates, but did not enhance the Q40 aggregation phenotype of *mir-1(gk276)* animals (Figure 12).

[0176] This data reveals that a *mir-1/tbc-7* regulatory axis is important for managing toxic protein aggregates in *C. elegans*. TBC proteins control vesicular transport in cells by enhancing Rab GTPase hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). The Rab GTPase guanine-binding status is important for interaction-specificity with effector molecules. Therefore, TBCs can precisely control the specificity and rate of vesicular transport routes and thus have been functionally associated with autophagy. To examine the function of *mir-1* and *tbc-7* in autophagy, the inventors used a *C. elegans* strain that expresses GFP-tagged LGG-1/Atg8 reporter, to enable visualization of autophagosomes as fluorescent puncta (Figure 13). When autophagy is activated, cytosolic LGG-1/Atg8 is conjugated to phosphatidylethanolamine at the phagophore membrane by the ATG5-ATG12-ATG16L1 complex, forming lipidated LGG-1-II/Atg8 that is present in vesicular autophagosome structures. Thus, the number of GFP::LGG-1-positive puncta can be used as readout of autophagic activity. The inventors found that the number of GFP::LGG-1 puncta in *mir-1(gk276)* mutant BWM was not different from wild-type in standard laboratory conditions (Figure 13). However, loss of *mir-1* abrogates the autophagic heat stress response, as does *tbc-7* overexpression in body weight muscle (Figure 13). Together, these data indicate that *mir-1* regulation of *tbc-7* expression is required to control autophagy-dependent stress responses.

Example 8: miR-1 controls autophagy.

[0177] The mature *mir-1* sequence is completely conserved from worms to humans (Figure 1A). The inventors found that the *Drosophila* and human orthologs of TBC-7 - Skywalker and TBC1D15, respectively - are also predicted targets of miR-1 (Figure 14). This posits an evolutionary conserved function for miR-1 in controlling autophagy through TBC protein

regulation. The inventors have shown herein that *mir-1* directly regulates *tbc-7* expression in *C. elegans*. To determine if miR-1 function is conserved, the inventors measured TBC1D15 protein in mammalian cells (Figure 9G-H). It was found that miR-1 overexpression reduced TBC1D15 protein levels in HeLa cells (Figure 9G-H). Additionally, a *gfp* 'sensor' reporter containing the wild-type TBC1D15 3'UTR is downregulated by miR-1 overexpression, and this downregulation requires the miR-1 binding site (Figure 9I-K and Figure 15). These data show that, as in *C. elegans*, miR-1 directly interacts with the 3'UTR of a TBC protein-encoding mRNA to downregulate its expression.

[0178] To examine the role of miR-1/TBC1D15 on autophagy in mammalian cells, the inventors measured LC3-positive vesicular structures and LC3-II protein abundance (Fig 3). Overexpression of miR-1 in HeLa cells increased both the number of LC3-positive puncta per cell and total LC3-II protein levels (Figure 16A-B and Figure 17A). In the presence of the vesicular ATPase inhibitor bafilomycin A1 (BafA1), which inhibits lysosomal acidification and consequently blocks the autophagy flux, the LC3-II levels were even further increased when expressing miR-1 mimics, supporting the autophagy-promoting effect of miR-1 (Figure 16A-B). TBC1D15 knockdown cells exhibited the same phenotype as miR-1 overexpression (Figure 17B-C), suggesting that miR-1-mediated downregulation of TBC1D15 promotes autophagy flux. To support this, miR-1 overexpression also increases the autolysosome/autophagosome ratio, scored with a mRFP-GFP-LC3 reporter (Figure 11A-C), confirming that this is indeed due to an increase in autophagy flux rather than a blockage of the pathway.

[0179] TBC1D15 was overexpressed in HeLa cells to further characterize its role in autophagy. It was found that TBC1D15 overexpression increased LC3-II levels, which did not further increase in the presence of Baf A1, indicating a block in the autophagy pathway (Figure 16C-D). This was further validated by TBC1D15 overexpression in HeLa cells expressing the mRFP-GFP-LC3 reporter, which revealed large stationary autophagosomes and decreased autolysosome/autophagosome ratio (Figure 16E-G and movies). Co-expressing TBC1D15 and miR-1 did not change basal LC3-II levels, nor in the presence of Baf A1, when compared to cells expressing TBC1D15 with scrambled control (Figure 16H-I). Thus, ectopic expression of TBC1D15, which does not contain the endogenous 3'UTR, masks miR-1-induced autophagy flux presumably via its blocking effect on autophagy. Together, these data show that miR-1 regulation of TBC1D15 controls autophagy and that unrestricted expression of TBC1D15 causes a late-stage block in autophagy flux.

Example 9: The miR-1/TBC1D15 axis can be used to reduce the accumulation of polyglutamine aggregates in human cells.

[0180] The collective data presented herein suggests that manipulation of the miR-1/TBC1D15 axis could be used to reduce the accumulation of polyglutamine aggregates in human cells. To examine this possibility, the inventors expressed EGFP-tagged mutant human huntingtin exon 1 fragment with 74 polyQ repeats (HTT_{Q74}) in HeLa cells and manipulated miR-1 and TBC1D15 levels (Figure 19). Overexpression of two independently derived miR-1 mimics reduced the number of cells containing HTT_{Q74} aggregates (Figure 19)

[0181] Conversely, CRISPR/Cas9 knockdown of ATG16L1, a protein essential for autophagosome formation, inhibits miR-1-induced reduction of HTT_{Q74} aggregates, confirming that miR-1 acts through the autophagy pathway (Fig. 4B). It was also found that TBC1D15 knockdown lowered, and overexpression increased, the percentage of HTT_{Q74} positive cells (Figure 19C-D). TBC1D15 overexpression also blunted the ability of miR-1 overexpression to reduce the percentage of cells containing HTT_{Q74} aggregates (Figure 19E), showing that correct regulation of TBC1D15 is important to prevent the accumulation of toxic protein aggregates.

[0182] The inventors next examined the therapeutic potential of boosting miR-1 expression to reduce of HTT_{Q74} accumulation through the autophagy pathway. The cytokine interferon- β (IFN- β) positively regulates miR-1 expression in hepatic cells. IFN- β can promote autophagy flux and alleviate models of neurodegenerative disease. In mouse primary cortical neurons, the inventors found that IFN- β induced miR-1 expression by 2-fold and concomitantly decreased Tbc1d15 protein levels (Figure 19F-H). To examine whether IFN- β can induce autophagy and reduce HTT_{Q74} accumulation through miR-1/TBC1D15, the inventors used HeLa cells as a model. It was found that IFN- β also induces miR-1 and reduces TBC1D15 levels in HeLa cells (Figure 20). IFN- β regulation of TBC1D15 requires an intact miR-1 3'UTR binding site as a wild-type TBC1D15 3'UTR *gfp* sensor, but not a miR-1 binding site-mutated TBC1D15 3'UTR *gfp* sensor, is downregulated by IFN- β (Figure 19 I-J).

[0183] To establish if IFN- β depends on miR-1 to control autophagy and HTT_{Q74} accumulation, the inventors performed genetic knockdown and overexpression experiments. First, it was found that IFN- β treatment reduces HTT_{Q74} aggregate accumulation but this was abolished in cells stably expressing a hairpin inhibitor against miR-1 (Off-miR-1) (Figure 19 K-L). The dependency of miR-1 for IFN- β -mediated reduction of HTT_{Q74} aggregates is likely through the autophagy pathway. Indeed, the inventors found

that in the presence of Baf A1, IFN- β causes a further increase in LC3-II levels, however, this is diminished in Off-miR-1 HeLa cells (Figure 19M-N). These data indicate that IFN- β enhancement of autophagy flux, and reduction of HTT_{Q74} accumulation, is dependent on miR-1 induction.

[0184] In this example, the inventors have shown that the beneficial effects of miR-1 overexpression on autophagy and HTT_{Q74} accumulation is abrogated by an autophagy block caused by TBC1D15 overexpression. The inventors tested whether disruption of autophagy flux by TBC1D15 would prevent IFN- β promotion of autophagy. Treating control cells with either IFN- β or Baf A1 caused an increase in LC3-II levels (Figure 21A-B). Additionally, co-treatment with IFN- β and Baf A1 generates a further increase in LC3-II levels, supporting the role of IFN- β in promoting autophagy flux (Figure 21A-B). TBC1D15 overexpression blocks autophagy (Figure 16D). Neither IFN- β nor Baf A1, either independently or in combination, further increased LC3-II levels caused by TBC1D15 overexpression, further confirming that excess TBC1D15 causes a late-stage autophagy block (Figure 21A-B). Correct regulation of TBC1D15 is important, as TBC1D15 overexpression abrogated IFN- β -mediated reduction of HTT_{Q74} accumulation (Figure 21C). Neurons from mice lacking the *Ifnb* gene display a late-stage block in autophagy, causing accumulation of α -synuclein aggregates and Lewy bodies. Similarly, neuronally-differentiated N2A cells with CRISPR/Cas9 knockout of the *Ifnb* gene increased the number of cells with HTT_{Q74} aggregates (Figure 21D). The data herein suggests that these disease-causing phenotypes may in part be explained by dysregulation of TBC1D15.

DISCUSSION

[0185] Taken together, the foregoing examples show that *mir-1* performs an evolutionarily conserved, protective function against protein degradation in multiple cell types and cellular compartments. The examples identify a highly conserved regulatory axis through which the miR-1 gene controls the accumulation of aggregation-prone proteins in *C. elegans* and mammalian cells. In *C. elegans*, *mir-1* functions autonomously to prevent polyglutamine aggregate accumulation in body wall muscle and abrogates the detrimental effects of α -synuclein and heat stress on behaviour and physiology. In mammalian cells, miR-1 protects against the accumulation of mutant huntingtin in HeLa cells and neurons. The inventors determined that miR-1 performs these protective roles by controlling the expression of TBC proteins - *tbc-7* in worms and TBC1D15 in mammals. This conserved mechanistic relationship maintains appropriate levels of autophagic flux to enable toxic protein aggregates to be efficiently removed. The examples establish that deficits in miR-1 and

TBC protein function contribute to the etiology of protein aggregation disorders and their manipulation by IFN- β provides a therapeutic opportunity to treating these diseases.

[0186] The cellular proteome is continuously challenged by intrinsic and extrinsic factors. Protein quality control programs safeguard the proteome and protect against toxic aggregate formation through chaperone-assisted formation, secretion and degradation of proteins. This study describes a previously unknown link between *mir-1* and proteostasis. Using multiple protein aggregation models and environmental stress it is demonstrated herein that *mir-1* acts in *C. elegans* muscle to regulate protein aggregation and to prevent systemic effects of proteotoxicity. This study further shows that the role of *mir-1* in protein aggregation is conserved in mammals and that miR-1 can reduce aggregation of Huntingtin protein in multiple cell types.

[0187]

***mir-1* and Hsp70 Proteins**

[0188] Loss of *mir-1* causes an increase in protein aggregation and toxicity through an imbalance in pathway(s) that control proteostasis. Through systematic analysis of known components of common degradation pathways, it was found that two different Hsp70 genes and their facilitating cofactors Hsp40 and Hsp90 are downregulated at the mRNA level when *mir-1* is not expressed. Genetic analyses support the hypothesis that *mir-1* functions in the same inherent pathway(s) as these regulators of proteostasis.

[0189] The Hsp70 family represents one of the most abundant and highly conserved chaperone families and, supported by co-chaperones, regulates virtually all aspects of cellular proteostasis. Lack of *mir-1* reduced the expression of Hsp70, Hsp40 and Hsp90. Downregulation of these genes and the fact that none of the chaperones contain canonical *mir-1* target sites in their 3'UTR, point to an indirect or non-canonical *mir-1*-driven regulation of chaperone expression.

[0190] Hsp70 activity is associated with efficient degradation by the ubiquitin/proteasome system and, amongst other functions, assists efficient substrate delivery and loading of the proteolytic complex to the proteasome. Furthermore, the two isoforms of Hsp70 analyzed herein in *C. elegans* share 100% identity with isoform 1 of Heat Shock cognate 71 kDa protein (HSP8A), which is known to participate in the ERAD quality control pathway. As disclosed herein the degradation of substrates specific for both the UPS and ERAD pathways was impaired. Accordingly, through the regulation of Hsp70 and co-chaperone expression, *mir-1* affects proteasomal degradation.

***mir-1* as a Proteome Defence Molecule**

[0191] From the foregoing examples it is evident that protein folding and degradation is tightly controlled within cells and requires the activation of numerous molecules and pathways and *mir-1* is a crucial component of this system that affects multiple facets of cellular proteostasis. However, analysis of the organismal effect of *mir-1* loss in *C. elegans* in standard laboratory conditions has not identified gross morphological or behavioural phenotypes in locomotion, pharyngeal pumping, defecation, egg retention, brood size and lifespan. Only by overburdening the organism with aggregation-prone peptides or exposure to insults that induce proteostatic stress (e.g. heat stress) was the function of *mir-1* in proteostasis identified. Accordingly, *mir-1* is an important molecule required for safeguarding the proteome under stress and the protective function for *mir-1* is evolutionarily conserved in mammals. The foregoing demonstration that toxic protein aggregation can be reduced in multiple mammalian cell types by expressing *mir-1* indicates that administration of *mir-1* or a *mir-1* mimic is a viable therapeutic option for a plethora of protein aggregation disorders.

[0192] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the technology as shown in the specific embodiments without departing from the spirit or scope of technology as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. A method for promoting autophagy in a subject in need thereof, the method comprising increasing the level of mir-1 nucleic acid in the subject, reducing the level of a TBC protein in the subject, or both.
2. The method of claim 1 wherein the level of mir-1 nucleic acid is increased by administering to the subject a therapeutically effective amount of a mir-1 nucleic acid, an interferon- β , or both.
3. The method of claim 1 or 2, wherein the autophagy prevents, reduces or inhibits protein aggregation.
4. The method of any one of claims 1 to 3, wherein the mir-1 nucleic acid decreases expression of the TBC protein.
5. The method of claim 4, wherein the TBC protein is TBC1D15 or tbc-7.
6. The method of any one of claims 1 to 3, wherein the level of the TBC protein is reduced by administering to the subject a therapeutically effective amount of a gene silencing agent specific for the nucleic acid encoding the TBC protein, or administering to the subject a therapeutically effective amount of a small interfering peptide for the TBC protein.
7. The method of claim 6, wherein the gene silencing agent is an antisense oligonucleotide, ribozyme, RNAi, siRNA or miRNA.
8. The method of any one of claims 2 to 6, wherein the mir-1 nucleic acid is administered to a tissue or organ of the subject.
9. The method of claim 6 or 7, wherein the gene silencing agent is administered to a tissue or organ of the subject.
10. The method of any one of claims 3 to 9, wherein the protein aggregation is toxic to the subject.
11. The method of any one of claims 3 to 10, wherein the protein aggregation is selected from the group consisting of aggregation of amyloid β peptide, Tau protein, α -synuclein, and proteins with polyglutamine expansions.
12. The method of any one of claims 3 to 11, wherein the protein aggregation is associated with a disease.
13. The method of claim 12, wherein the disease is a neurodegenerative disease.
14. The method of claim 13, wherein the neurodegenerative disease is selected from the group consisting of Huntington's disease, Parkinson's disease, Alzheimer's disease, amyotrophic

lateral sclerosis, cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, prion disease, tauopathy, frontotemporal lobar degeneration, familial dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), serpinopathy, Alexander disease, familial amyloidotic neuropathy, senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), isozyme amyloidosis, fibrinogen amyloidosis, dialysis amyloidosis, inclusion body myositis, inclusion body myopathy, cataracts, retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, hereditary lattice corneal dystrophy, cutaneous lichen amyloidosis, mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, seminal vesicle amyloid, apolipoprotein C2 amyloidosis, apolipoprotein C3 amyloidosis, Lect2 amyloidosis, insulin amyloidosis, galectin-7 amyloidosis (primary localized cutaneous amyloidosis), corneodesmosin amyloidosis, enfuvirtide amyloidosis, Cystic Fibrosis, and sickle cell disease

15. The method of any one of claims 3 to 11, wherein the protein aggregation is associated with proteotoxic stress.

16. The method of any one of claims 3 to 15, wherein the protein aggregation is associated with reduced autophagy.

17. The method of any one of claims 3 to 16, wherein the protein aggregation is associated with reduced expression of a heat shock protein.

18. The method of claim 17, wherein the heat shock protein is selected from the group consisting of hsp70, hsp90, and hsp40.

19. The method of any one of claims 3 to 17, wherein the protein aggregation is associated reduced expression of daf-21.

20. The method of any one of claims 1 to 19, wherein the mir-1 nucleic acid comprises the mir-1 seed sequence GGAAUGU.

21. The method of any one of claims 1 to 20, wherein the mir-1 nucleic acid comprises the sequence UGGAAUGUAAAGAAGUAUGUA.

22. The method of claim 21, wherein the mir-1 nucleic acid is UGGAAUGUAAAGAAGUAUGUA.

23. The method according to any one of claims 1 to 22, wherein the mir-1 nucleic acid comprises one or more of a phosphorothioate linked nucleotide, cholesterol, a peptide nucleic

acid (PNA), a locked nucleic acid (LNA), a non-naturally occurring nucleotide, a morpholino, nucleic acid aptamer, and a peptide.

24. The method of claim 23, wherein the mir-1 nucleic acid is selected from the group consisting of UGGAAUGUAAAGAAGUAUGUA, UGGAAUGUAAAGAAGUAUGU, UGGAAUGUAAAGAAGUAUGG, and UGGAAUGUAAAGAAGUAUGUAU.

25. The method of any one of claims 1 to 24, wherein mir-1 nucleic acid is an expression vector comprising a promoter operatively linked to a nucleic acid comprising a sequence encoding the mir-1 seed sequence GGAAUGU.

26. The method of any one of claims 1 to 25, wherein the interferon- β is interferon- β 1a, interferon- β 1b or an interferon- β mimetic.

27. The method of any one of claims 1 to 26 wherein the subject is a human subject.

28. A method of treating a disease associated with protein aggregation in a subject, the method comprising increasing the level of mir-1 nucleic acid in the subject, reducing the level of a TBC protein in the subject, or both.

29. The method of claim 28, wherein the disease is a neurodegenerative disease.

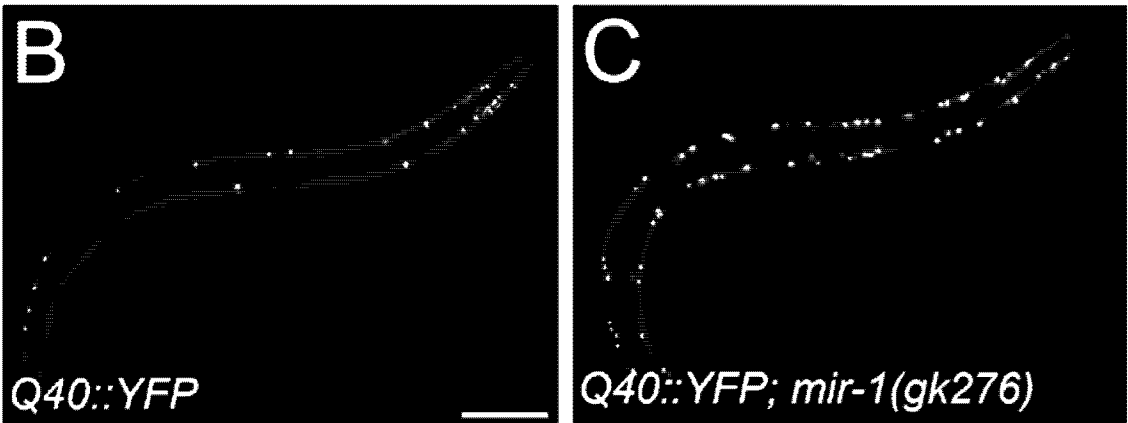
30. The method of claim 29, wherein the neurodegenerative disease is selected from the group consisting of Huntington's disease, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, cerebral β -amyloid angiopathy, Retinal ganglion cell degeneration in glaucoma, prion disease, tauopathy, frontotemporal lobar degeneration, familial dementia, hereditary cerebral hemorrhage with amyloidosis, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), serpinopathy, Alexander disease, familial amyloidotic neuropathy, senile systemic amyloidosis, AL (light chain) amyloidosis, AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), isozyme amyloidosis, fibrinogen amyloidosis, dialysis amyloidosis, inclusion body myositis, inclusion body myopathy, cataracts, retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, hereditary lattice corneal dystrophy, cutaneous lichen amyloidosis, mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, seminal vesicle amyloid, apolipoprotein C2 amyloidosis, apolipoprotein C3 amyloidosis, Lect2 amyloidosis, insulin amyloidosis, galectin-7 amyloidosis (primary localized cutaneous amyloidosis), corneodesmosin amyloidosis, enfuvirtide amyloidosis, Cystic Fibrosis, and sickle cell disease.

31. The method of any one of claims 28 to 30 wherein the treating comprises preventing, inhibiting or reducing protein aggregation in a tissue of the subject.
32. Use of a mir-1 nucleic acid or an interferon- β for the manufacture of a medicament for the promotion of autophagy, or for the treatment of a disease associated with protein aggregation.
33. The use of claim 32, wherein the autophagy prevents, reduces or inhibits protein aggregation.

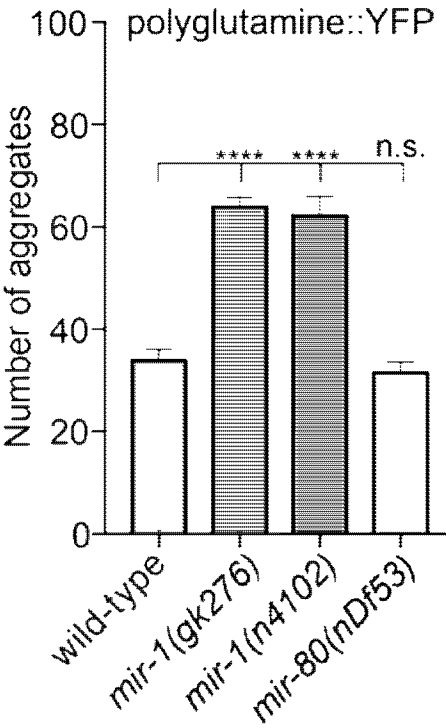
Figure 1

A

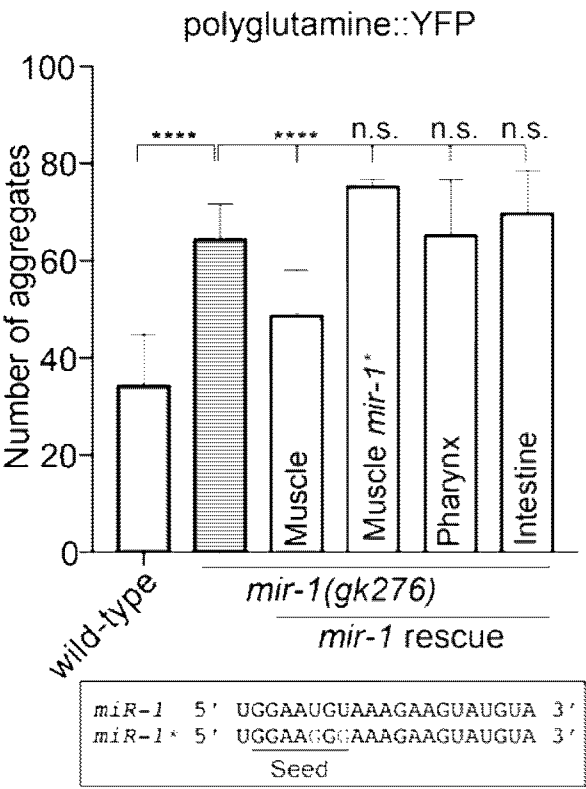
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gga-miR-1	UGGAAUGUAAAAGAAGUAUGUA
xtr-miR-1	UGGAAUGUAAAAGAAGUAUGUA
dre-miR-1	UGGAAUGUAAAAGAAGUAUGUAU
dme-miR-1	UGGAAUGUAAAAGAAGUAUGG
cel-miR-1	UGGAAUGUAAAAGAAGUAUGUA



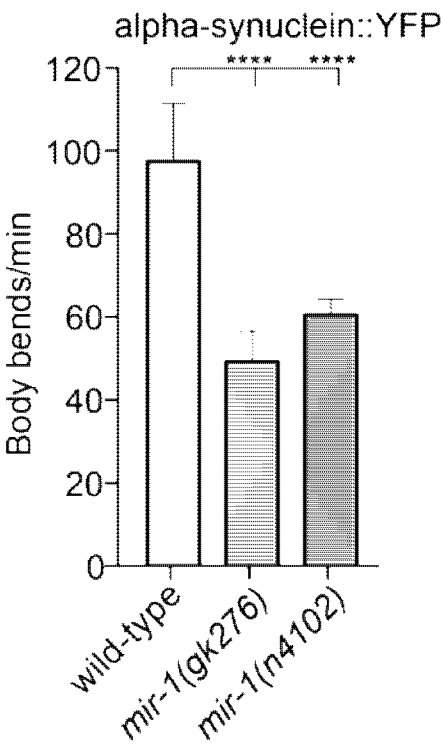
D



E



F



G

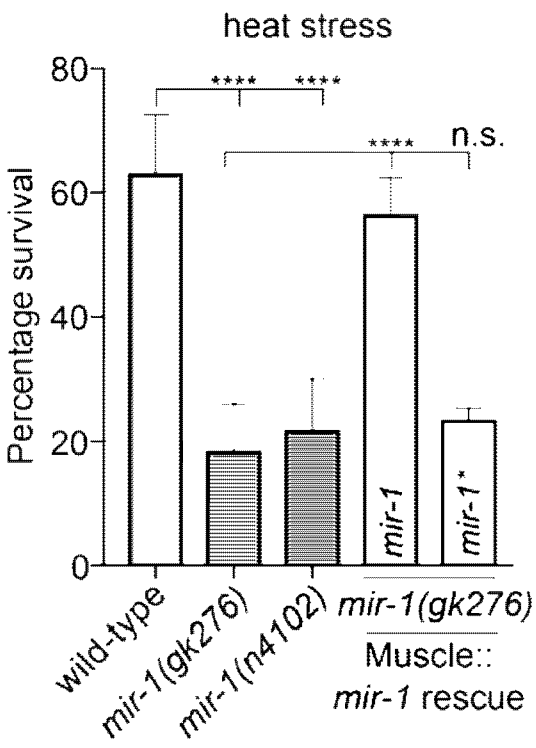


Figure 2

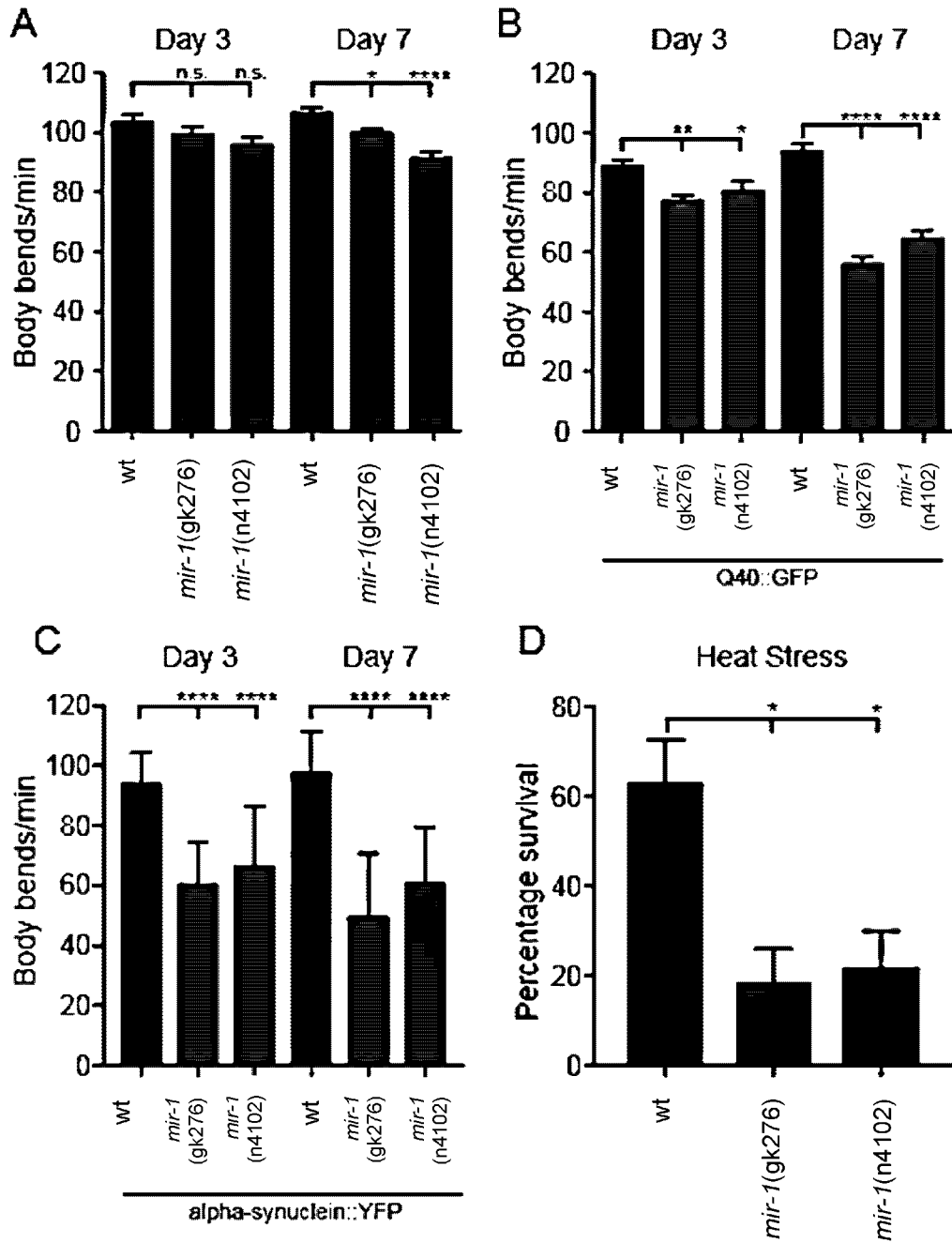


Figure 3

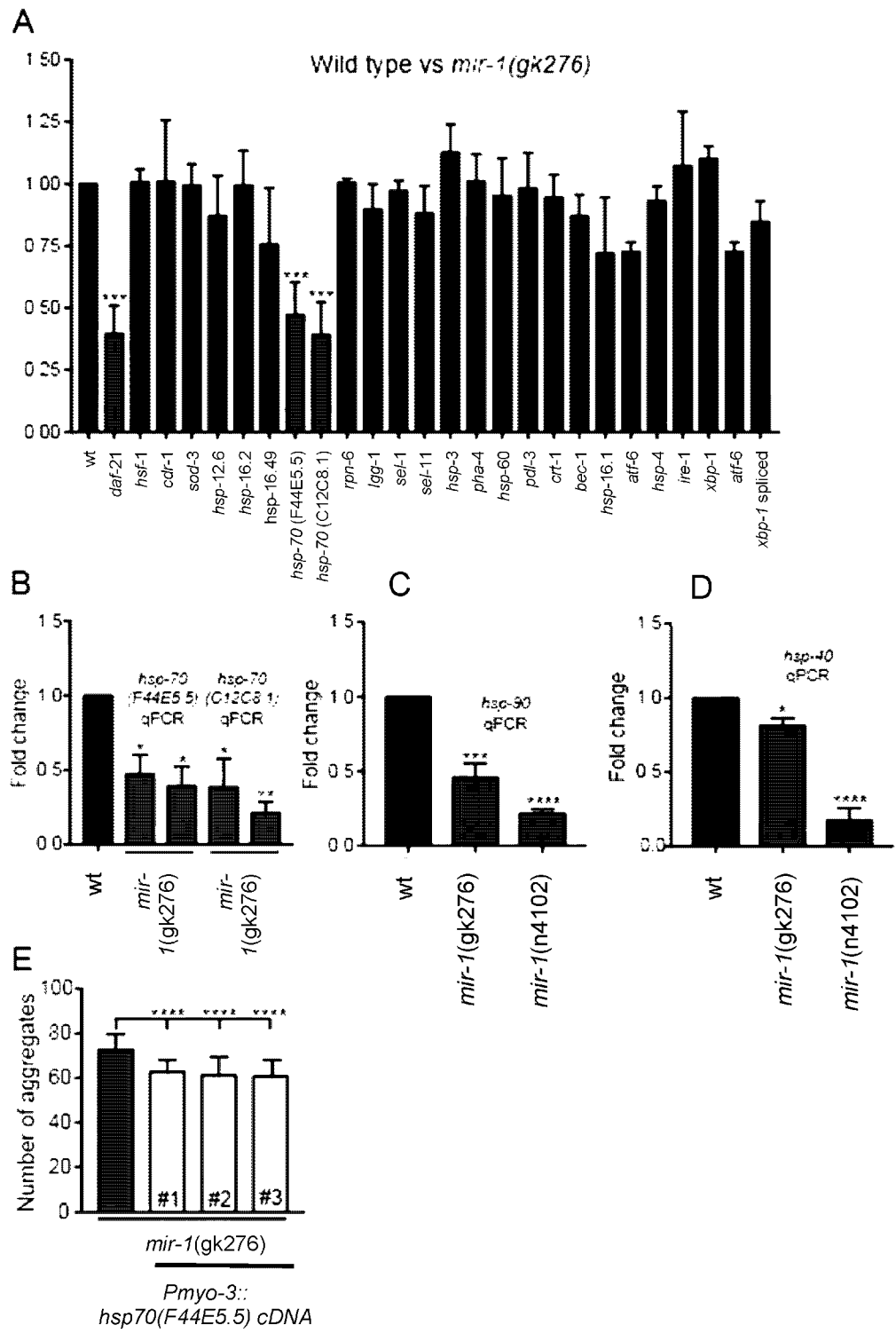


Figure 4

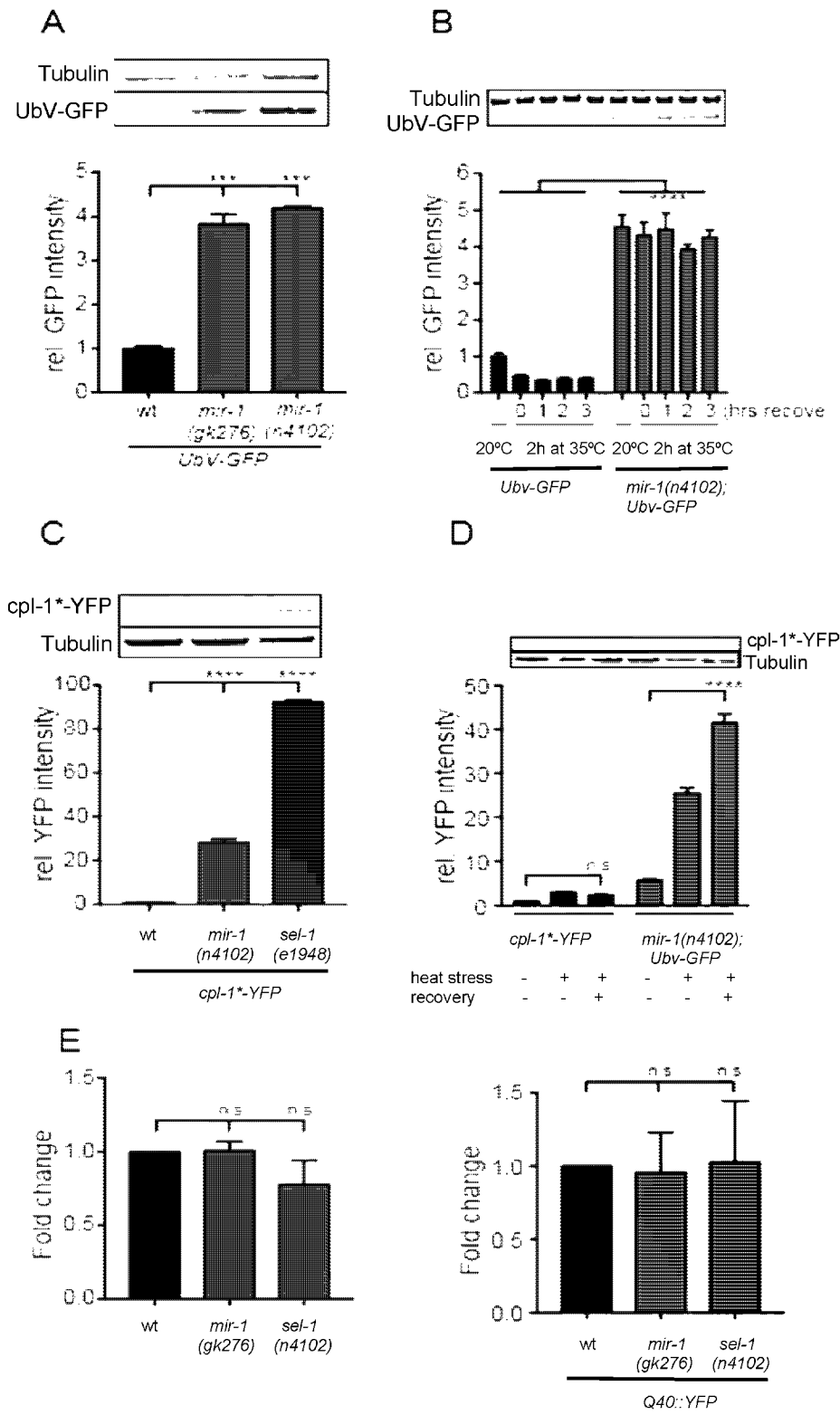
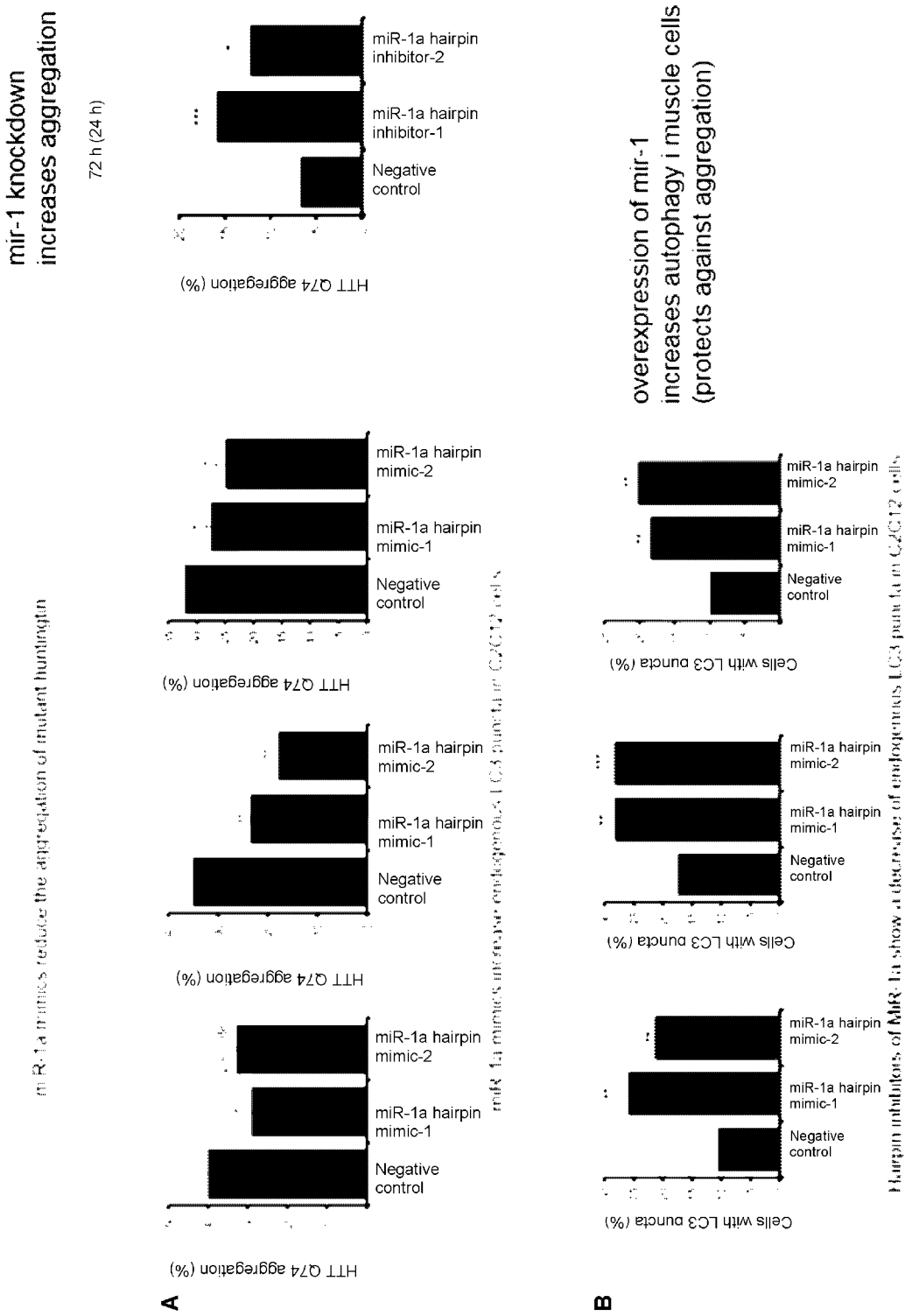


Figure 5



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Figure 5 (cont.)

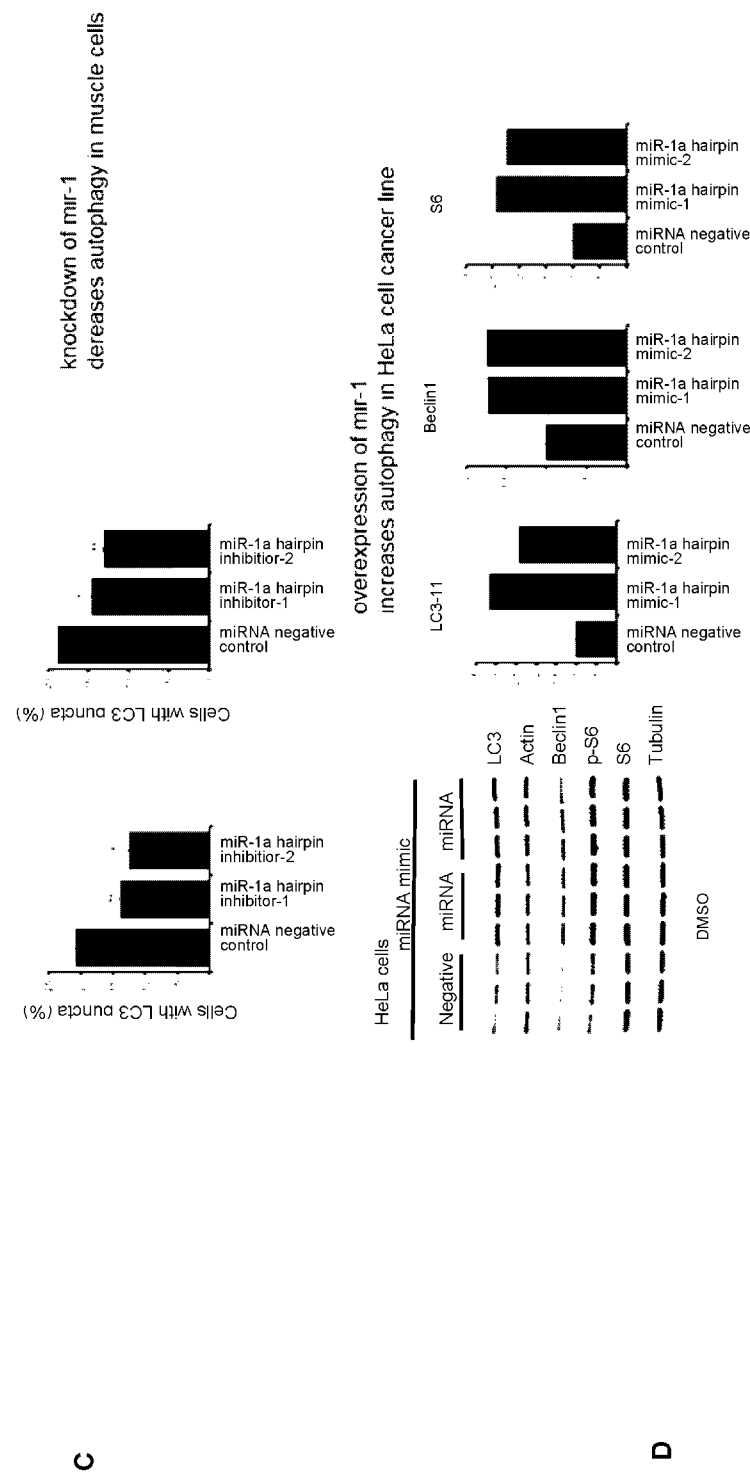
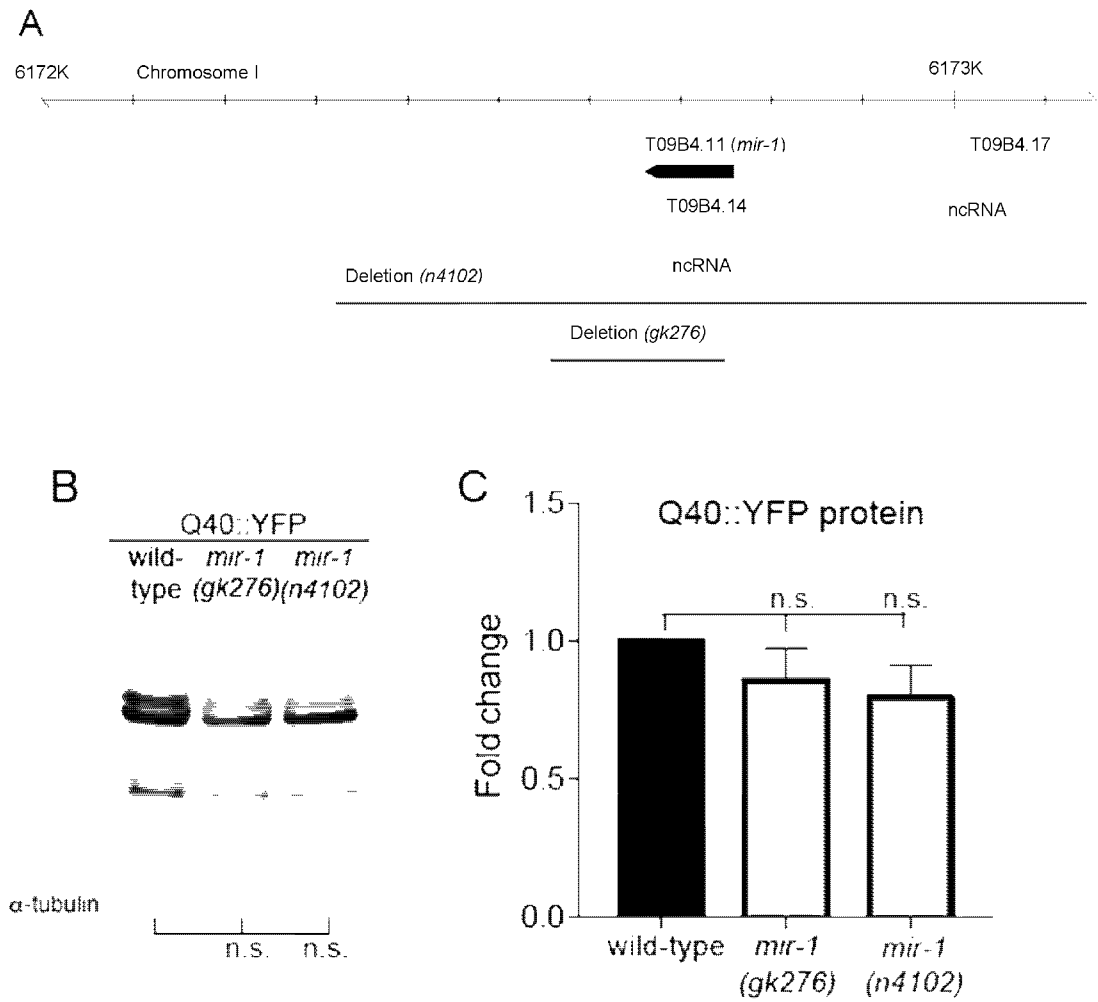


Figure 6



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Figure 7

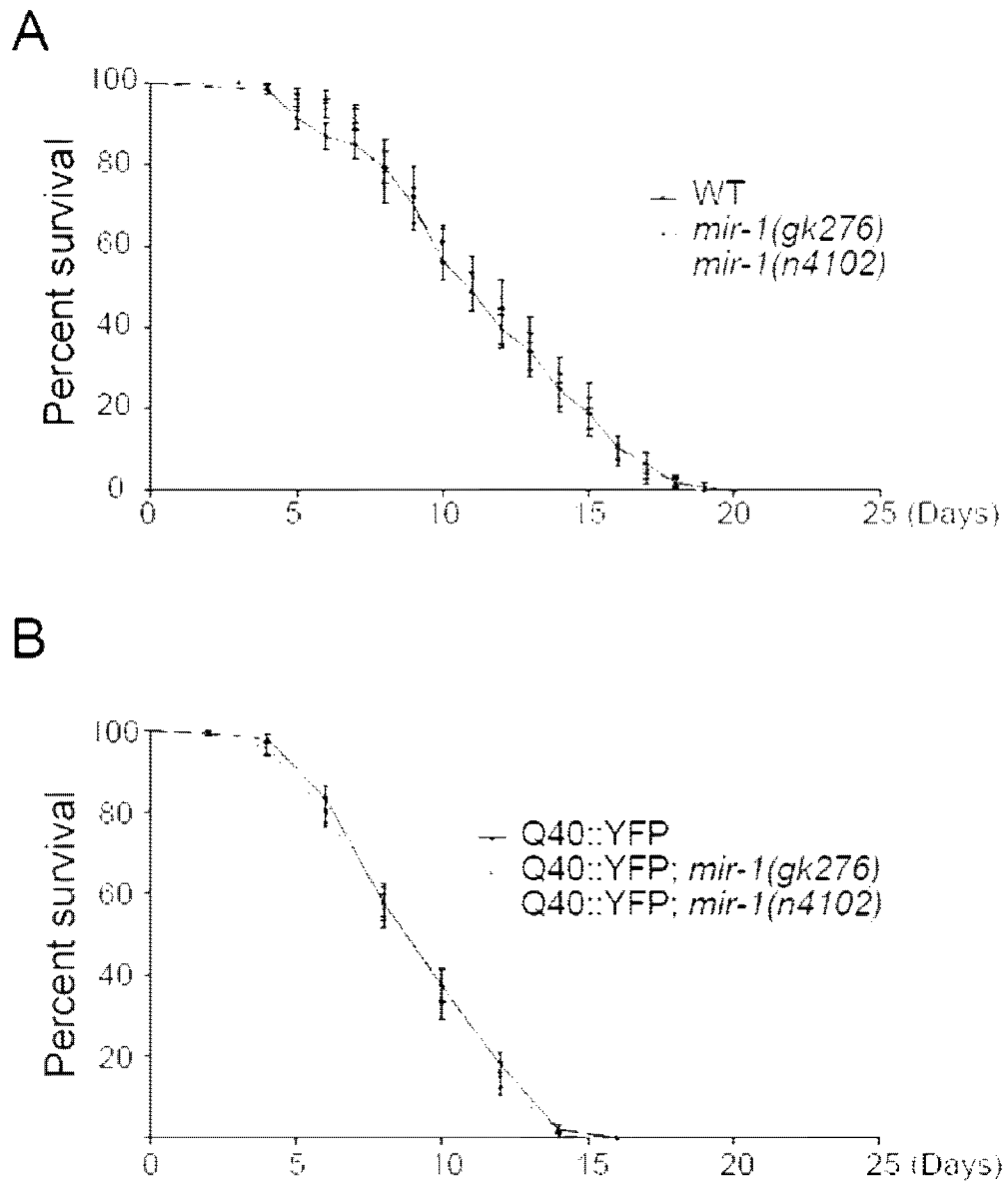


Figure 8

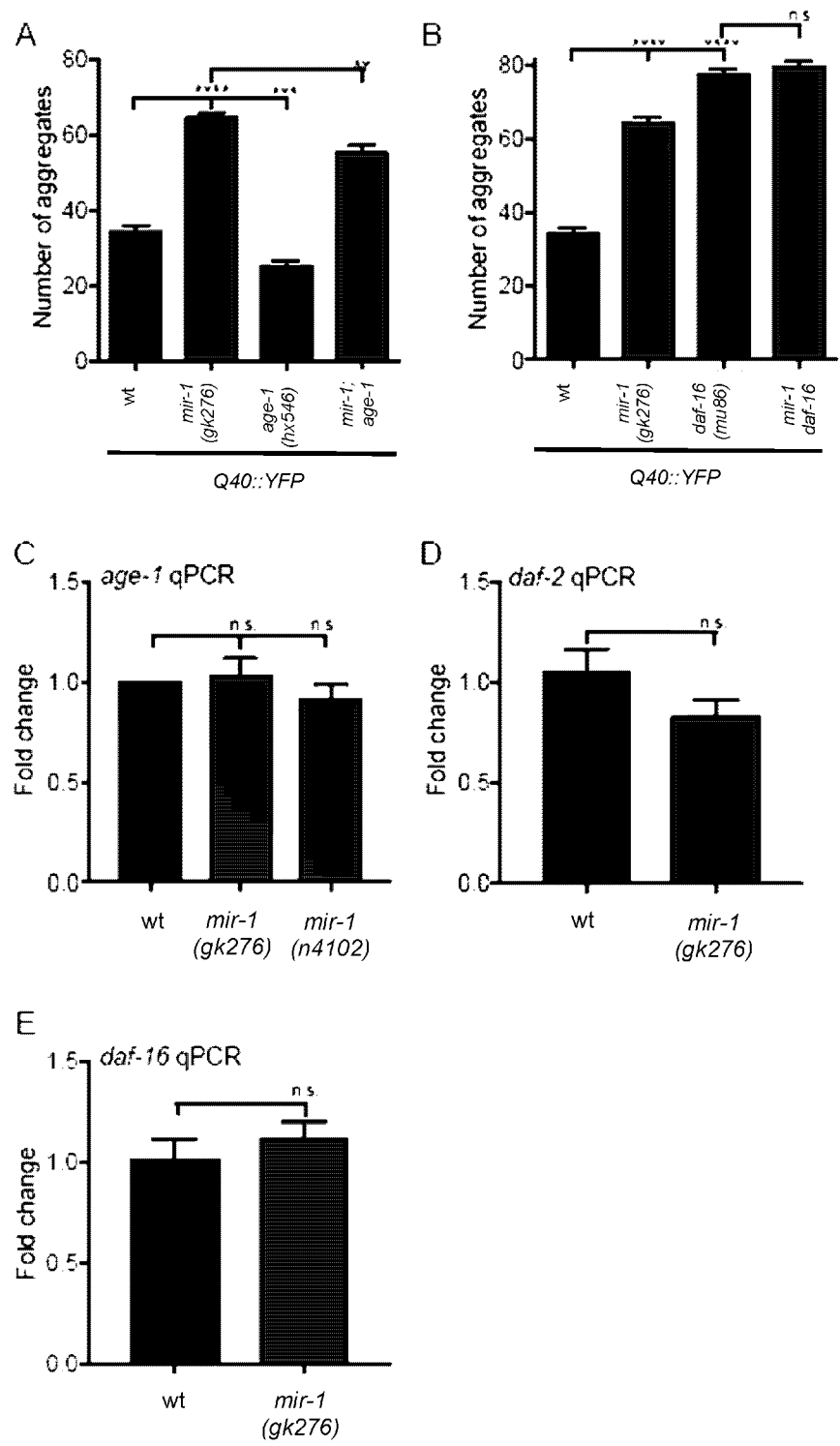
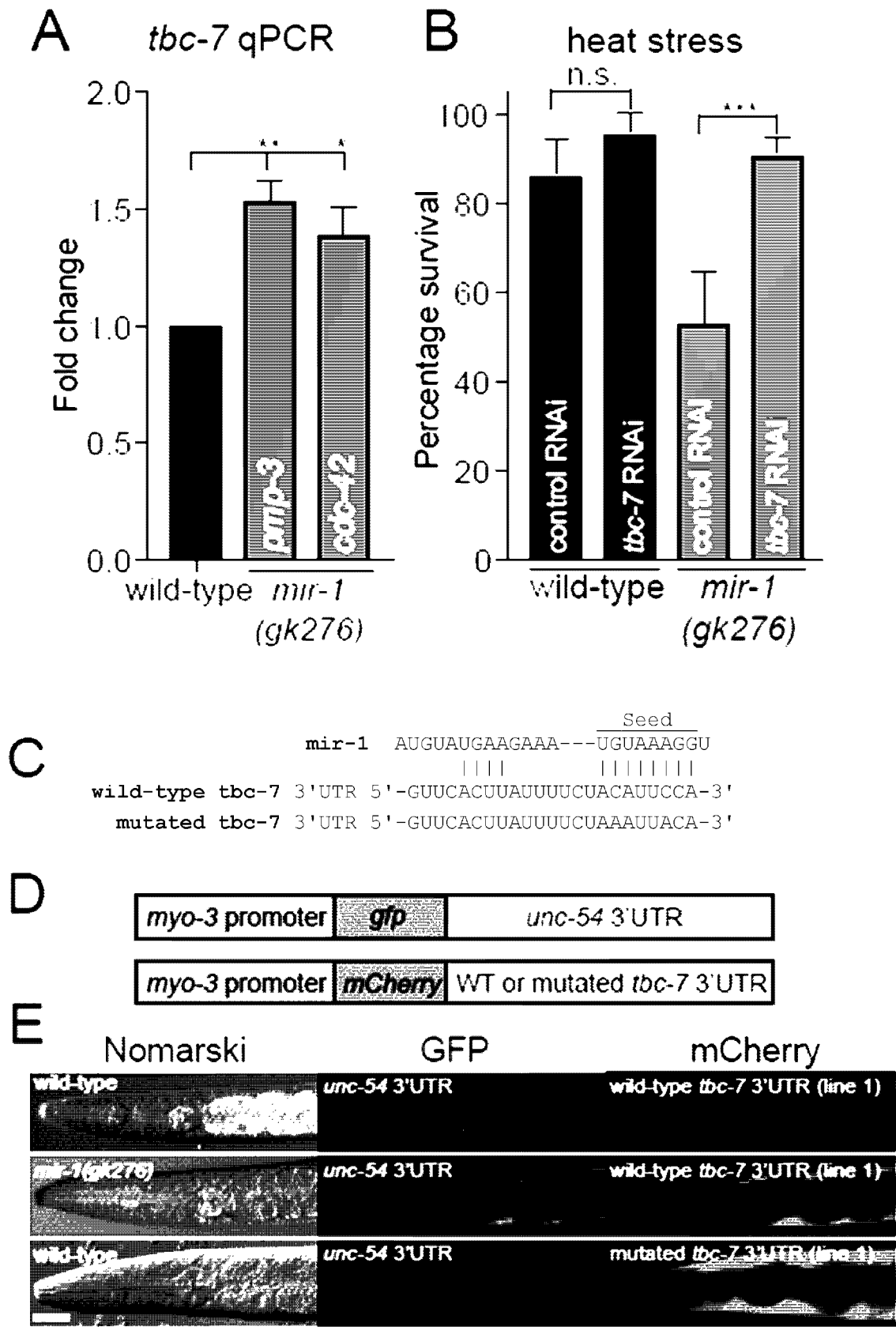


Figure 9



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Figure 9 (cont)

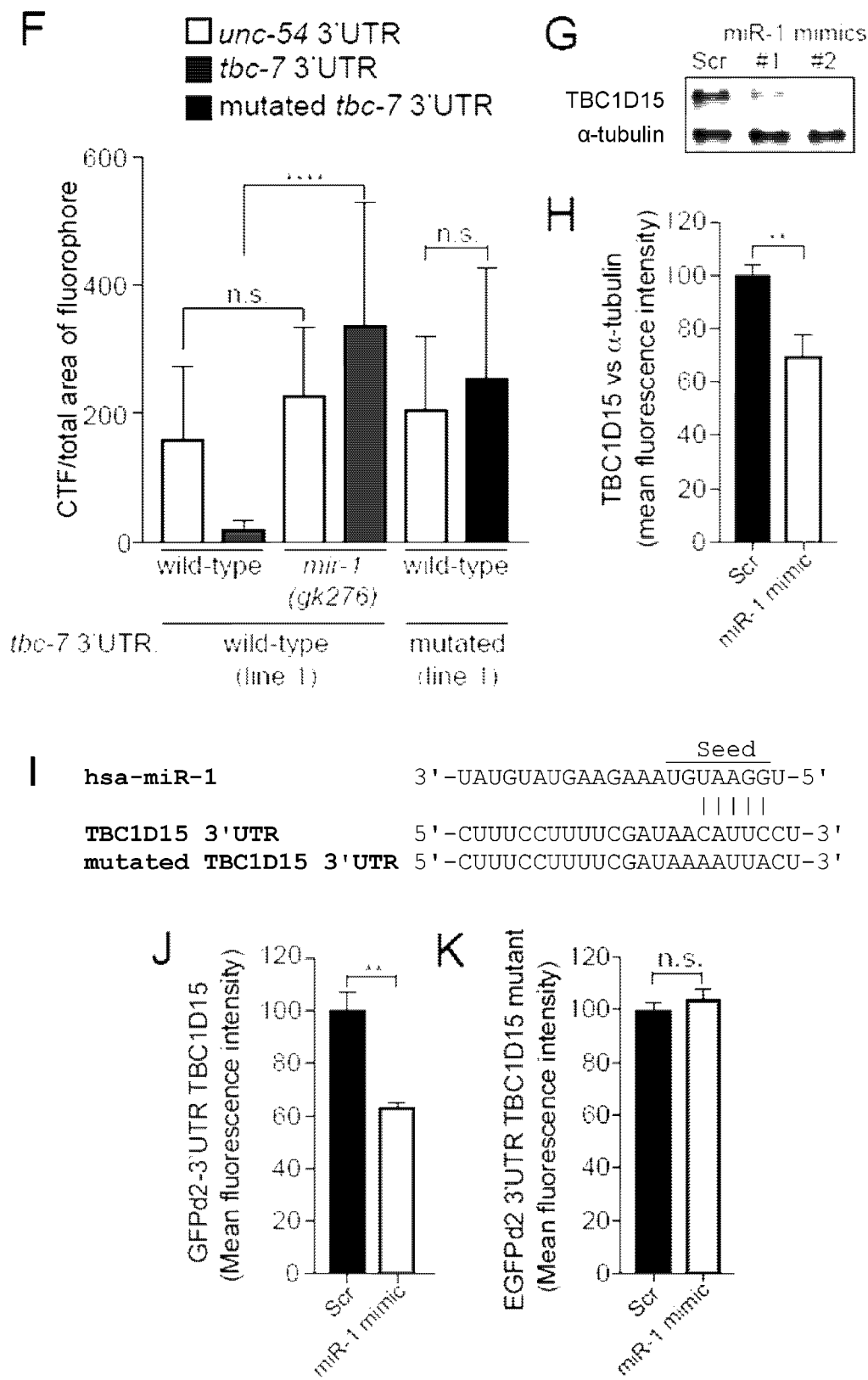


Figure 10

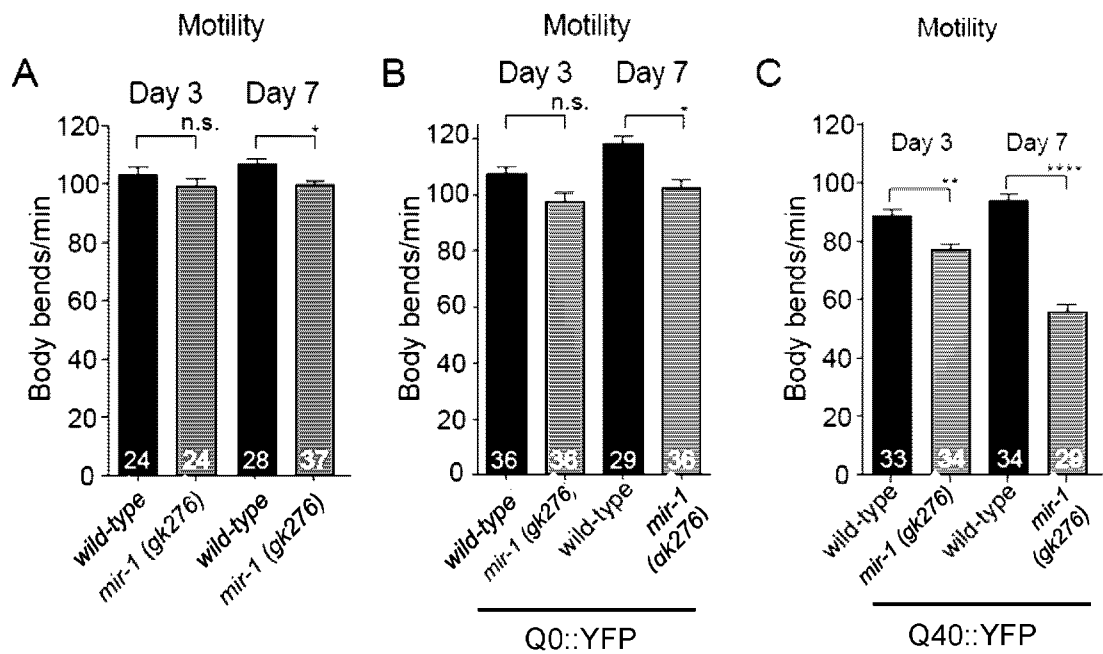


Figure 11

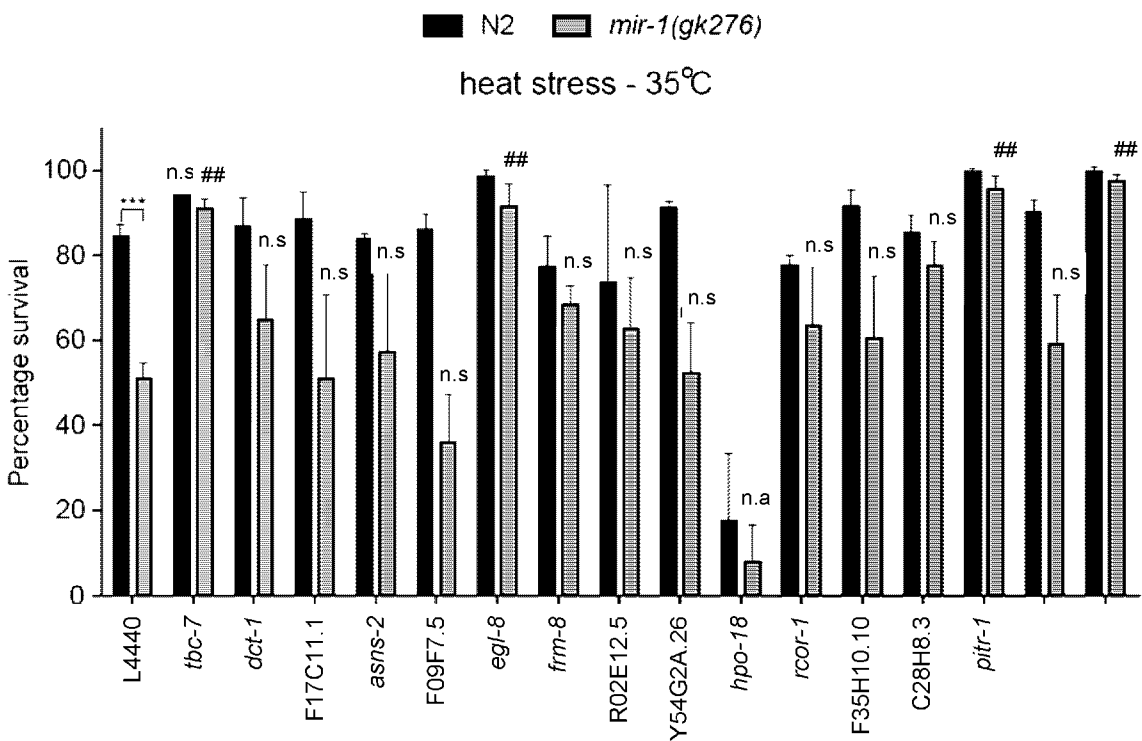


Figure 12

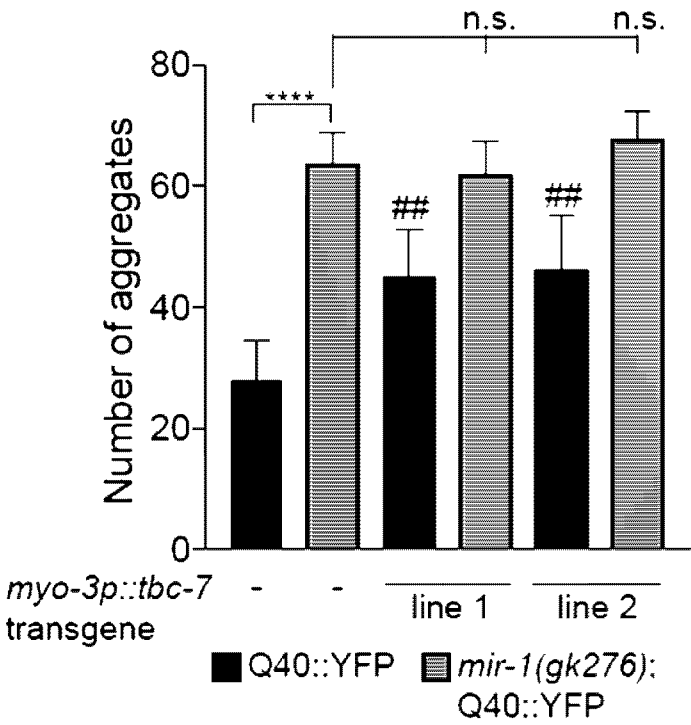
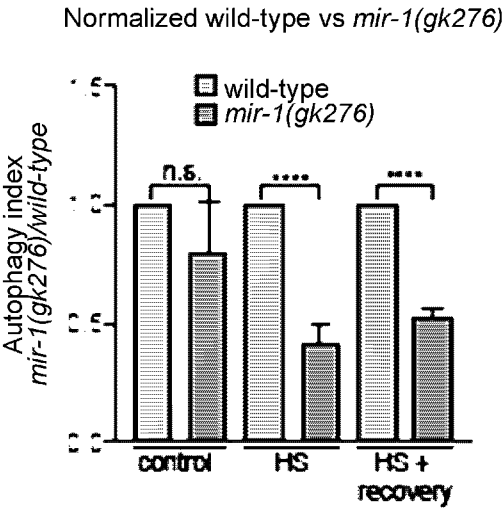


Figure 13

A



B

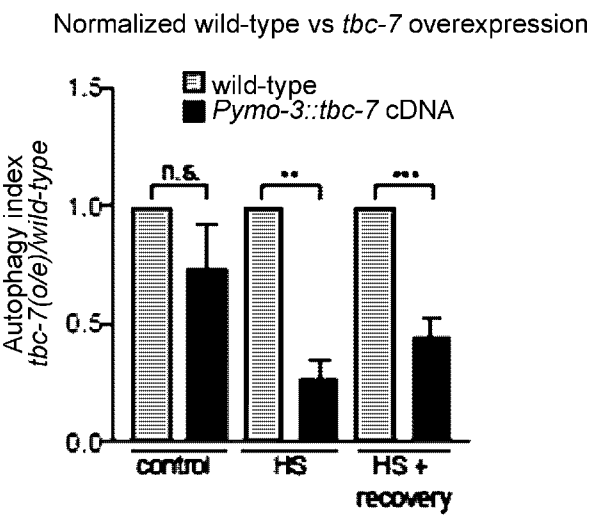


Figure 14

C. elegans

3' AUGUAUGAAGAAA---UGUAAGGU 5' *cel-mir-1*
 |||| |||||
 5' GUUCACUUAUUUUCUACAUUCCA 3' *tbc-7* 3'UTR

D. melanogaster

3' GAGGUAUGAAGAAUGUAAGGU 5' *dme-miR-1*
 |||||
 5' AUUAUUUUAAGAUUCCAUAUCCA 3' Skywalker 3'UTR

H. sapiens

3' UAUGUAUGAAGAAUGUAAGGU 5' *hsa-miR-1*
 |||||
 5' CUUCCUUUUCGAUAACAUAUCCU 3' TBC1d15 3'UTR

Figure 15

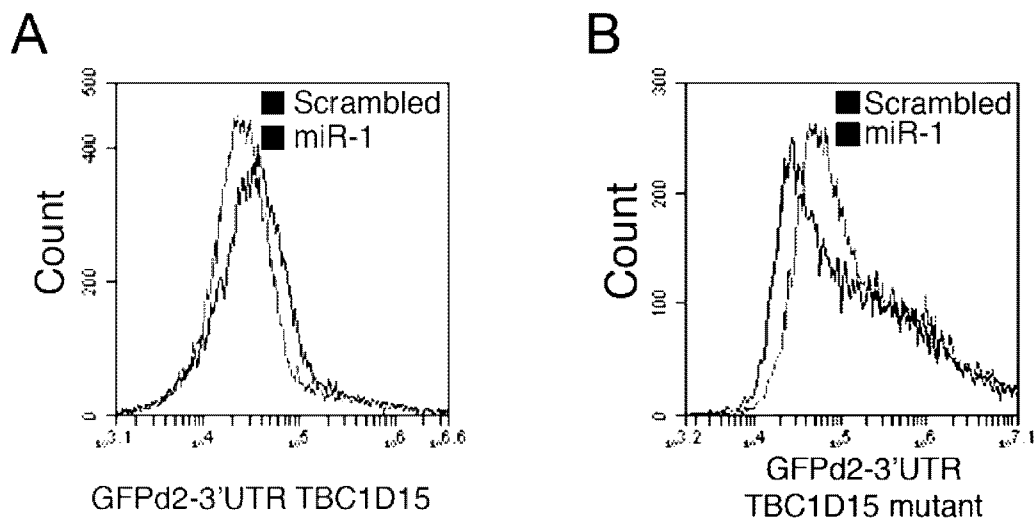


Figure 16

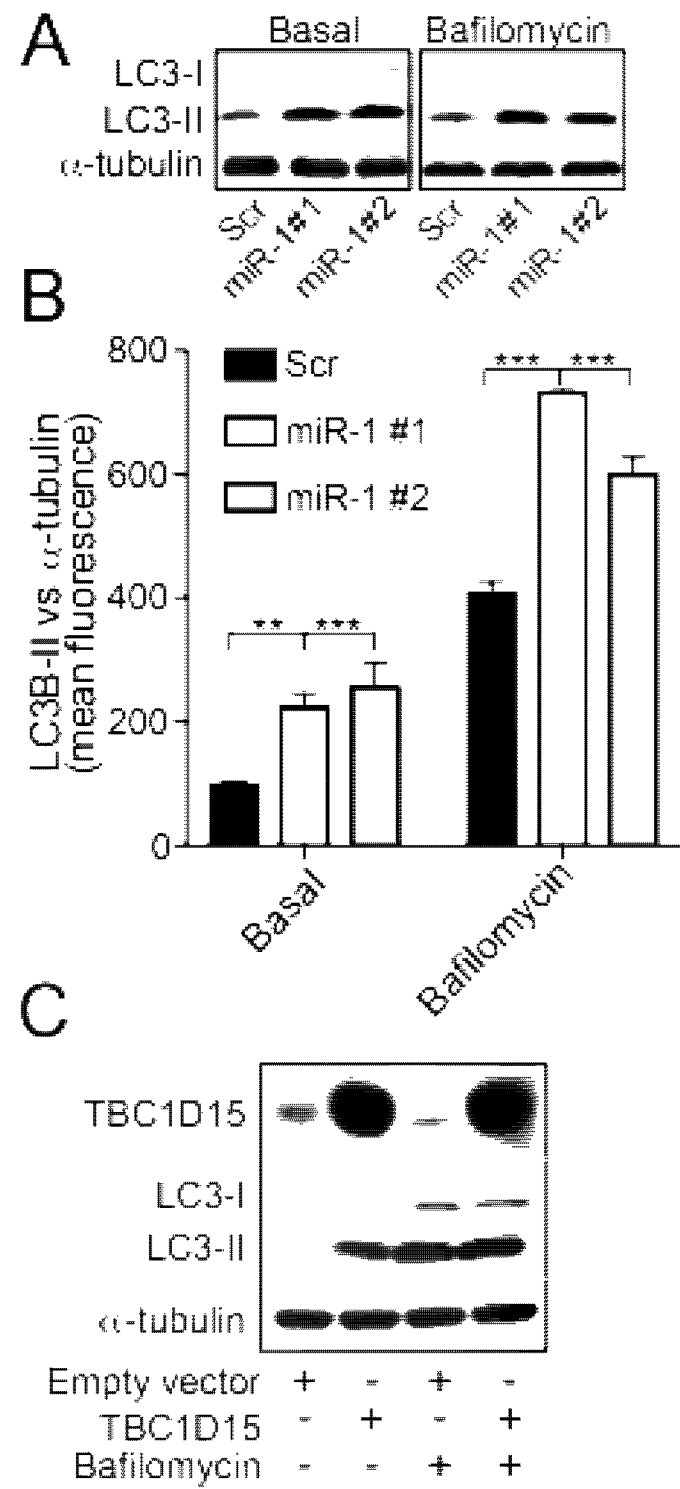


Figure 16 (cont.)

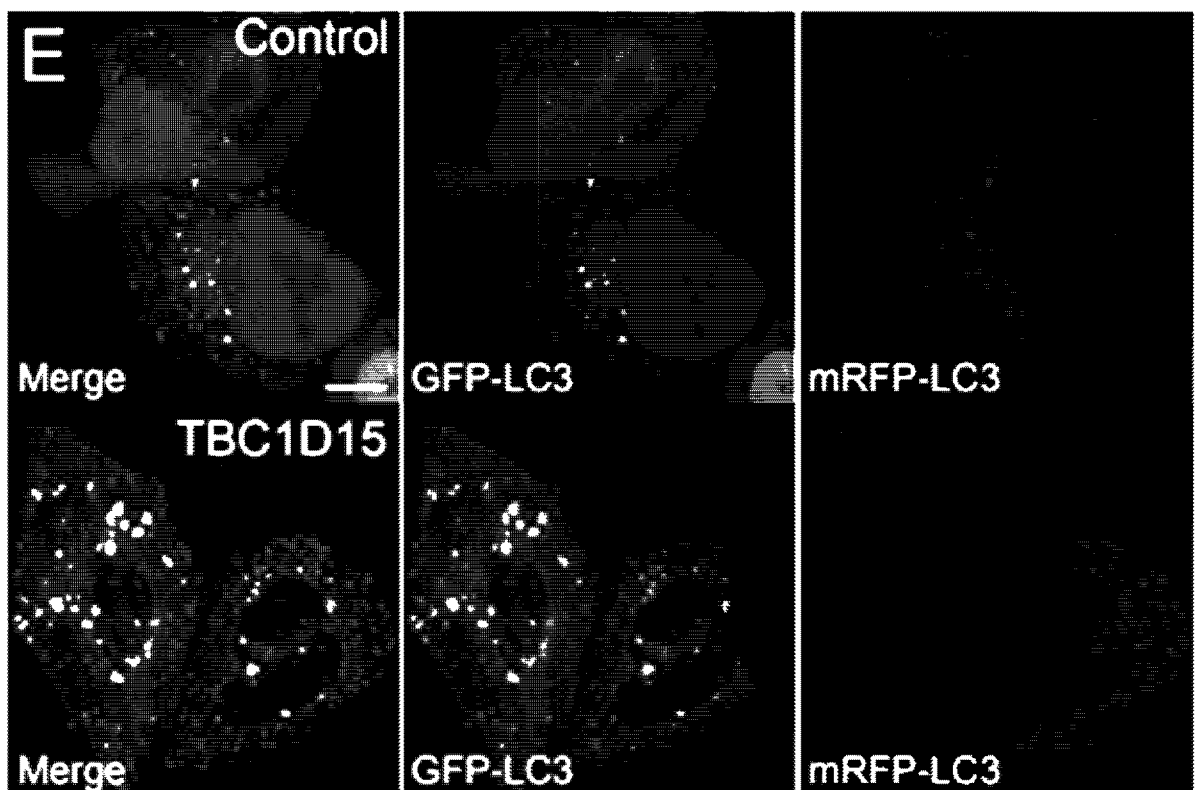
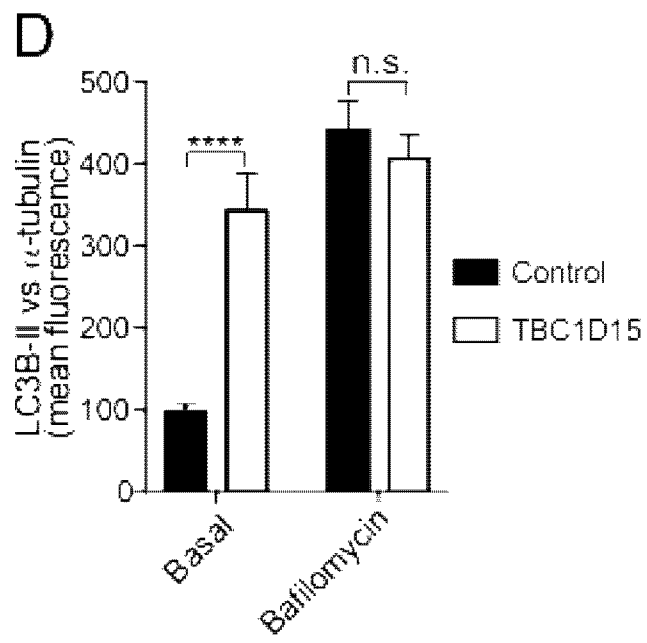


Figure 16 (cont)

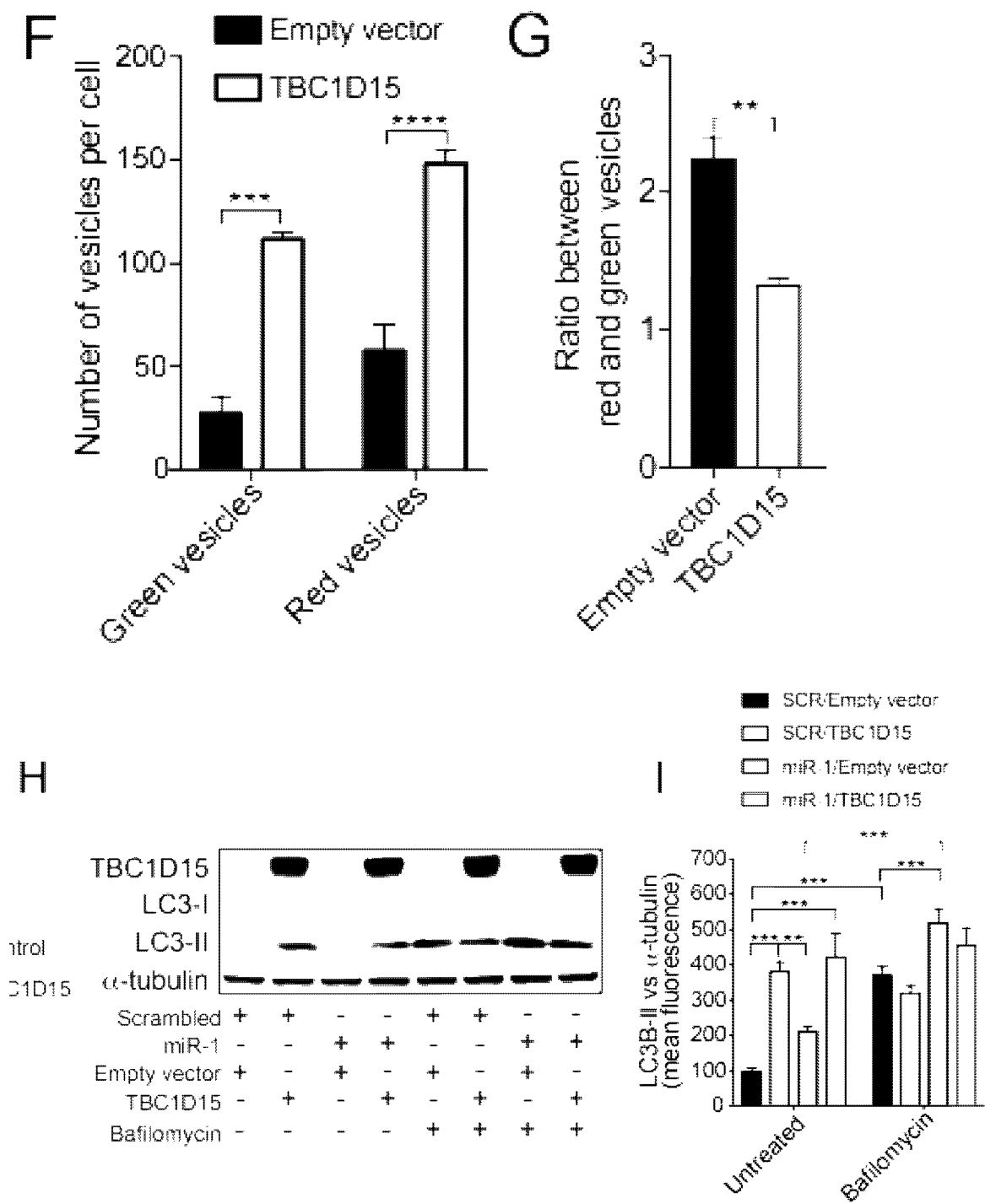
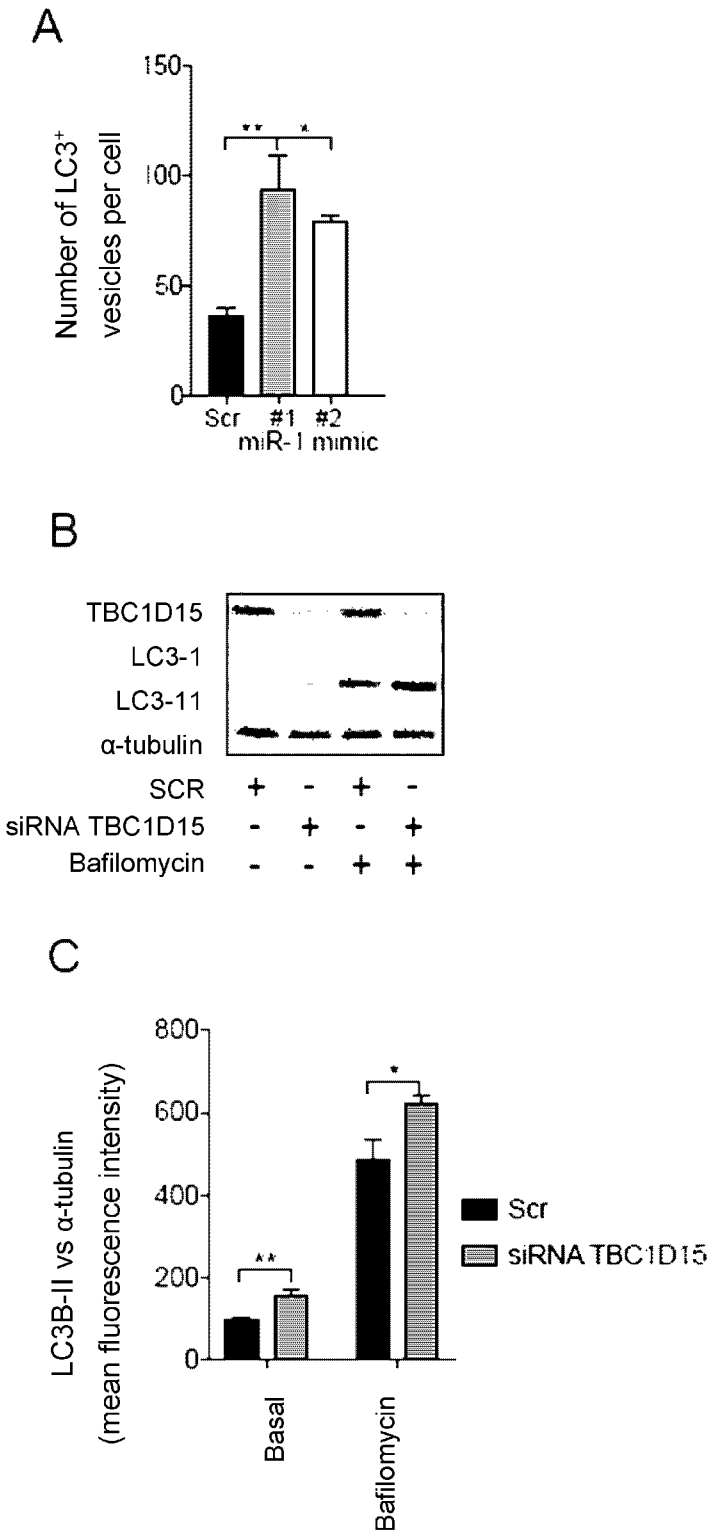


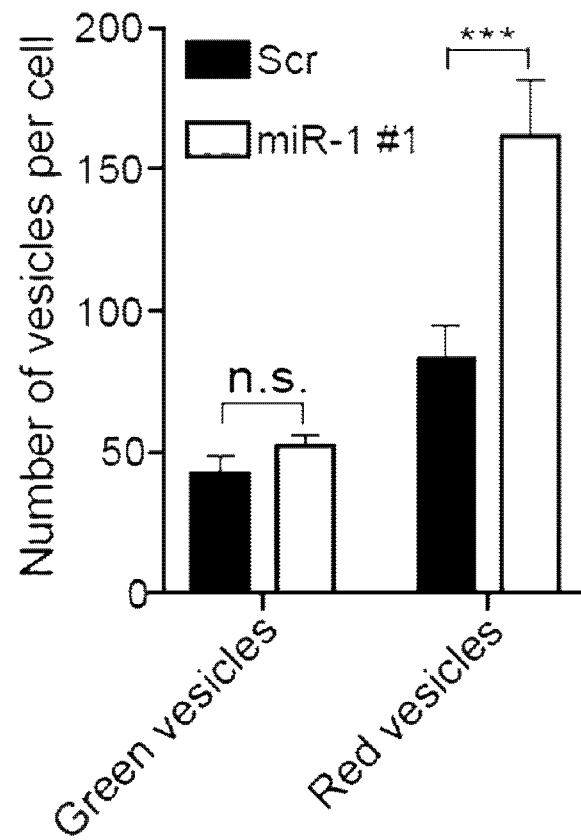
Figure 17



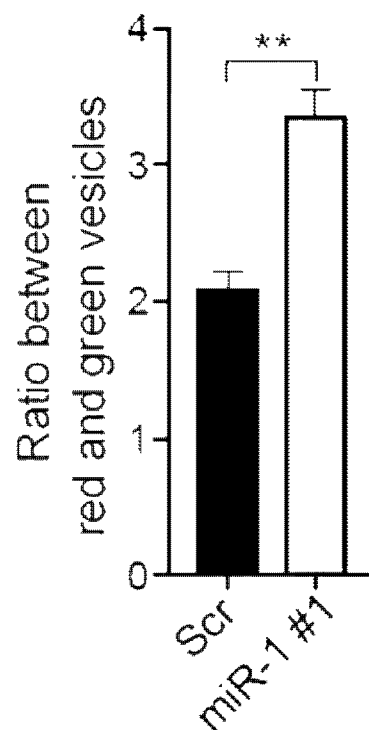
19/27

Figure 18

A



B



20/27

Figure 19

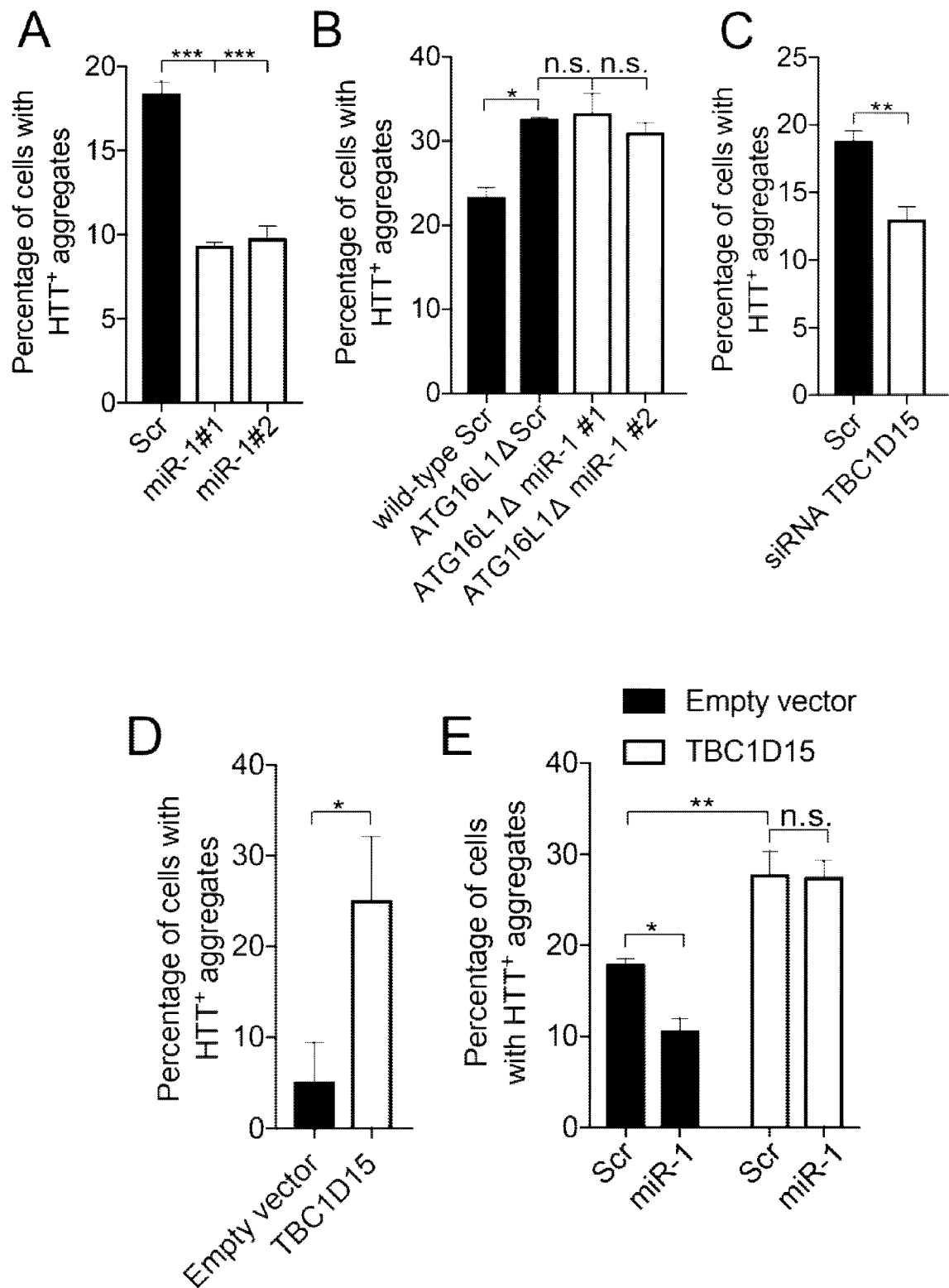
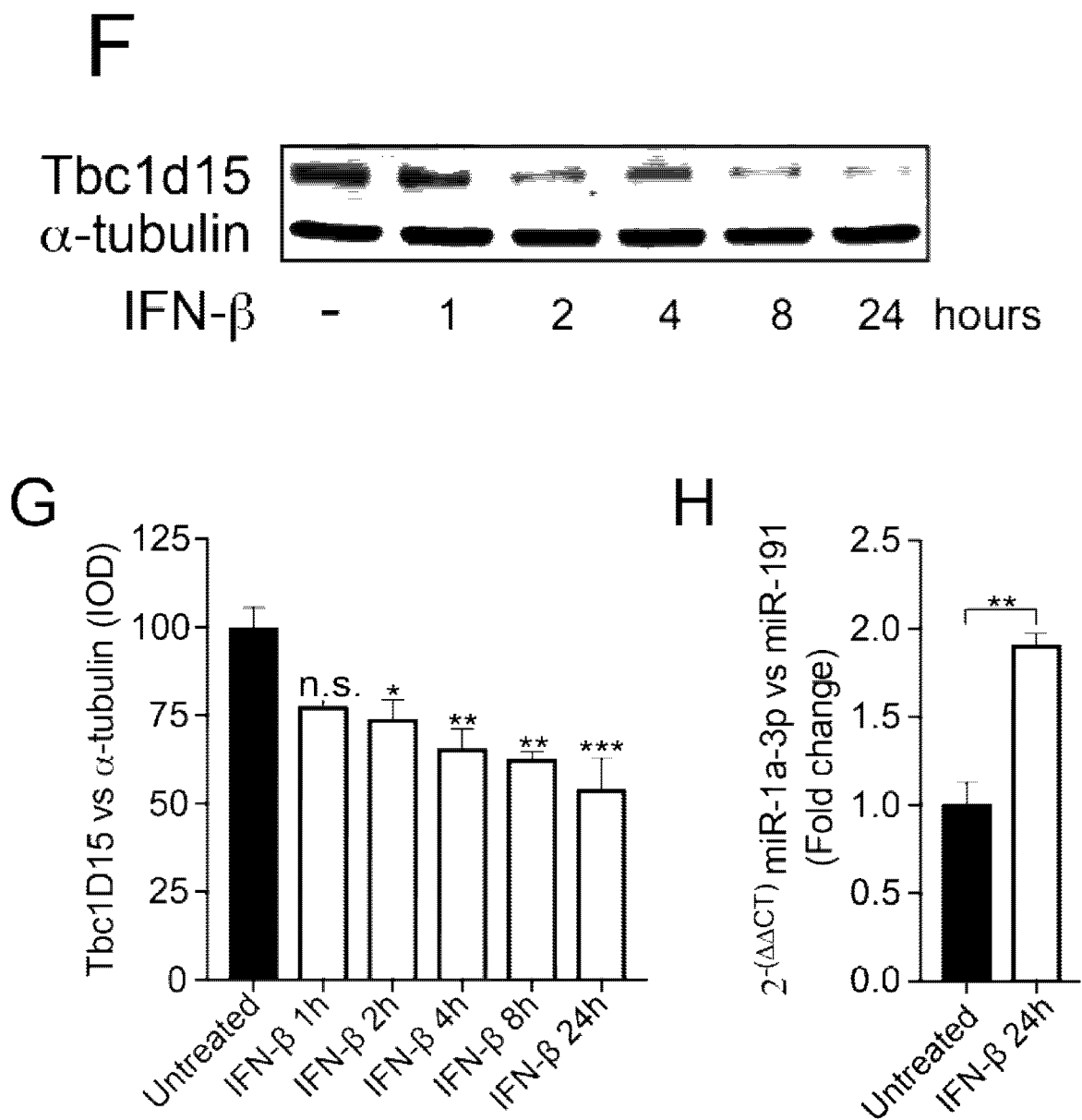


Figure 19 (cont)



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Figure 19 (cont)

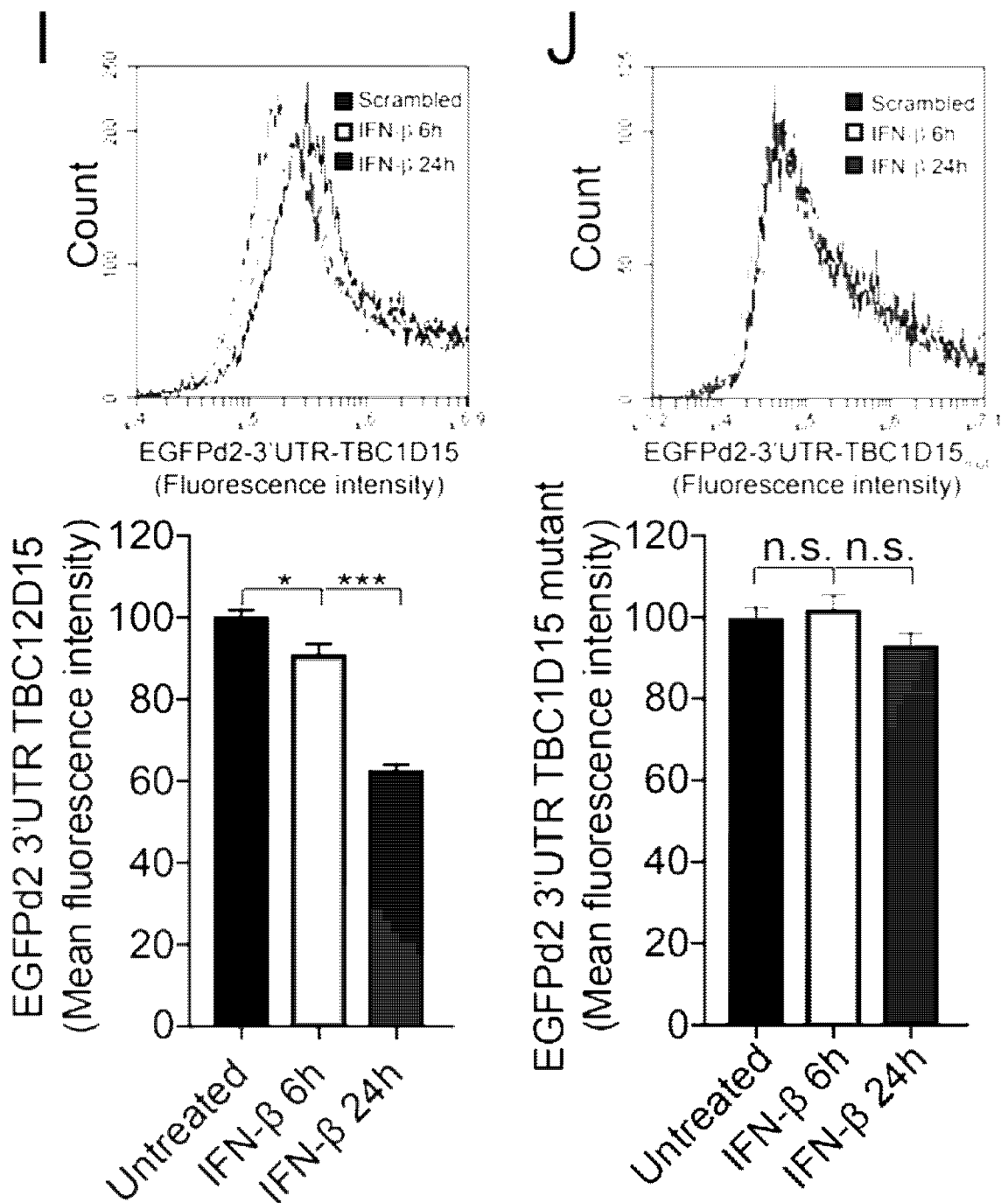


Figure 19 (cont)

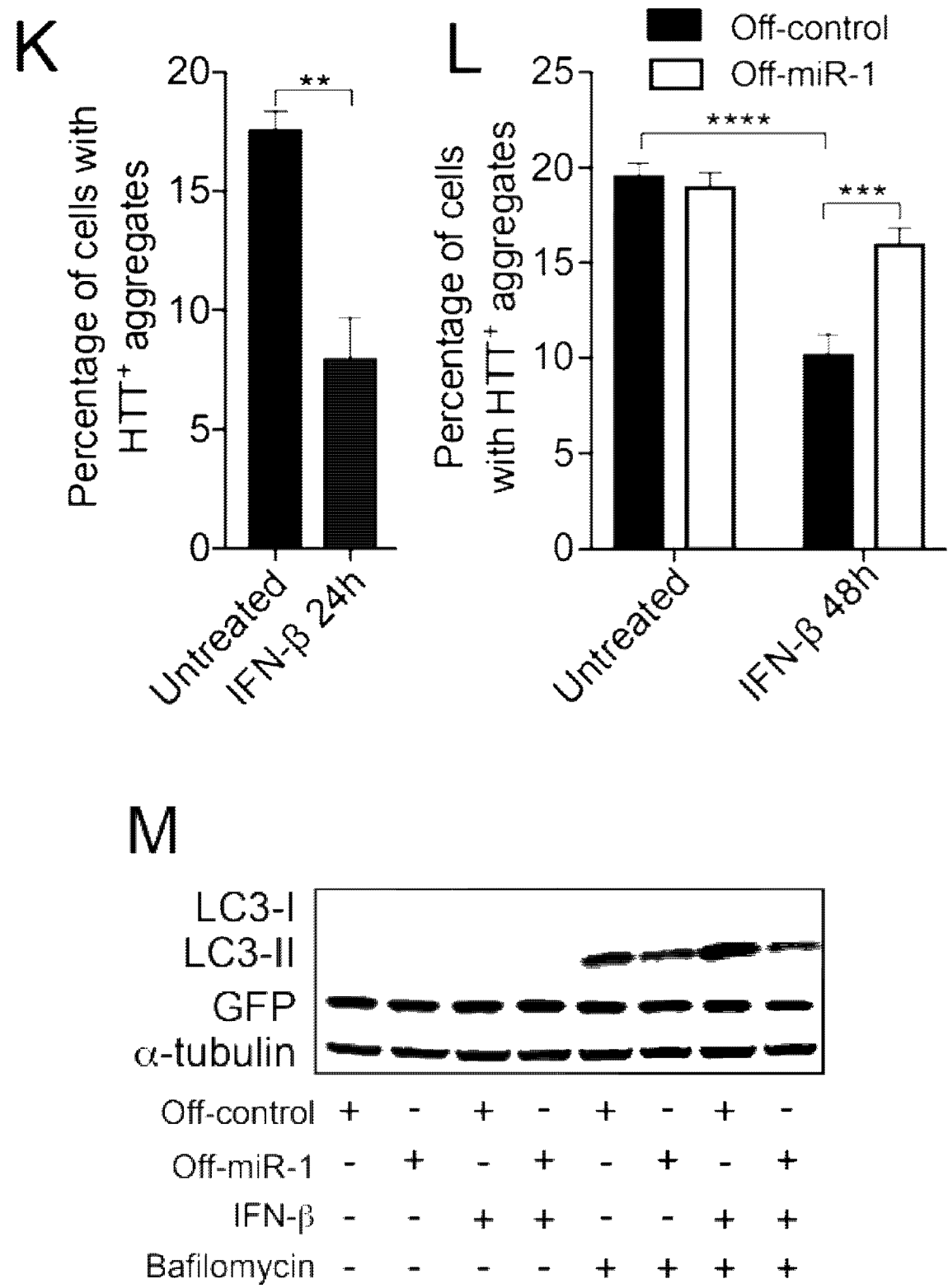


Figure 19 (cont)

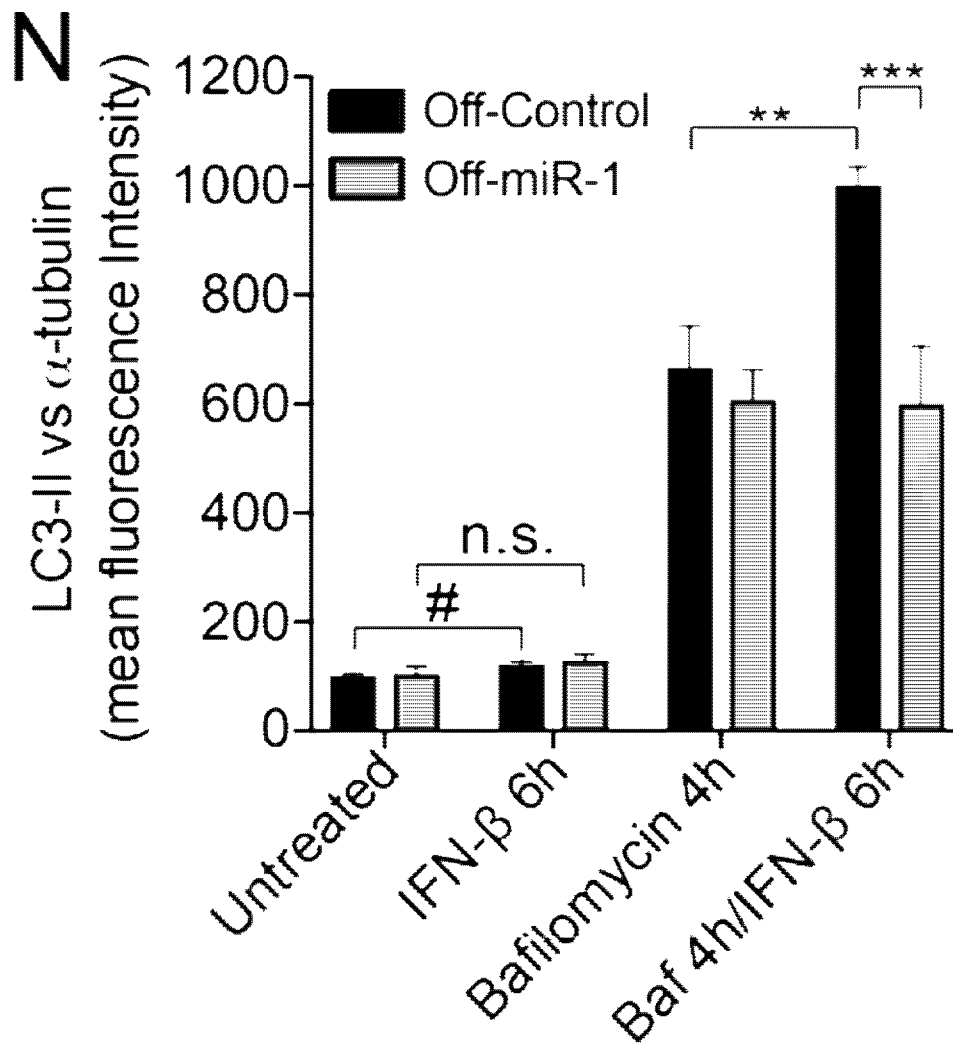


Figure 20

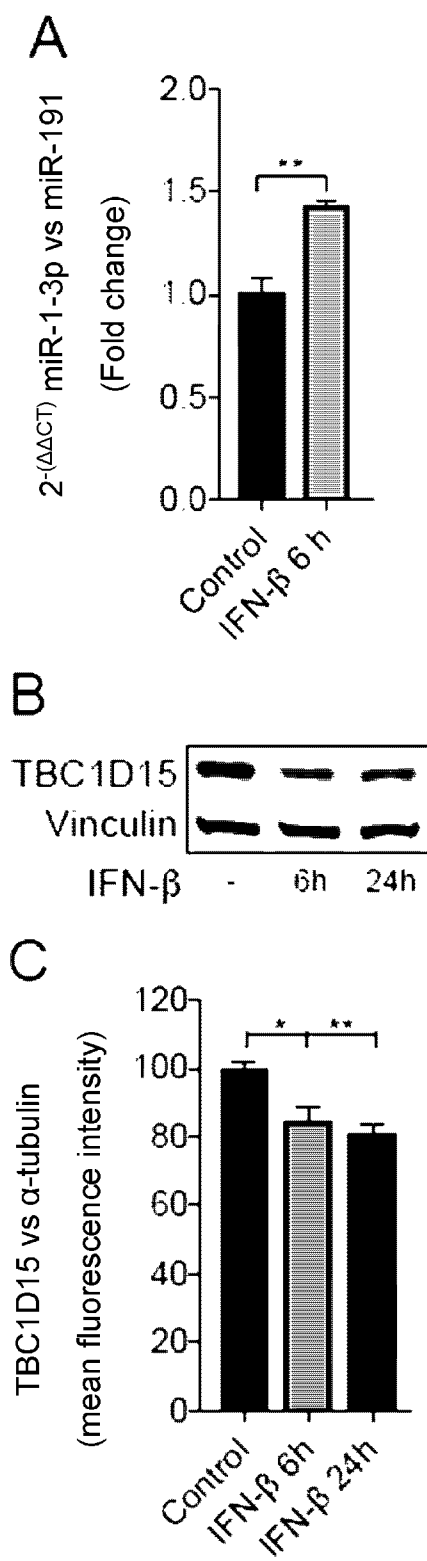
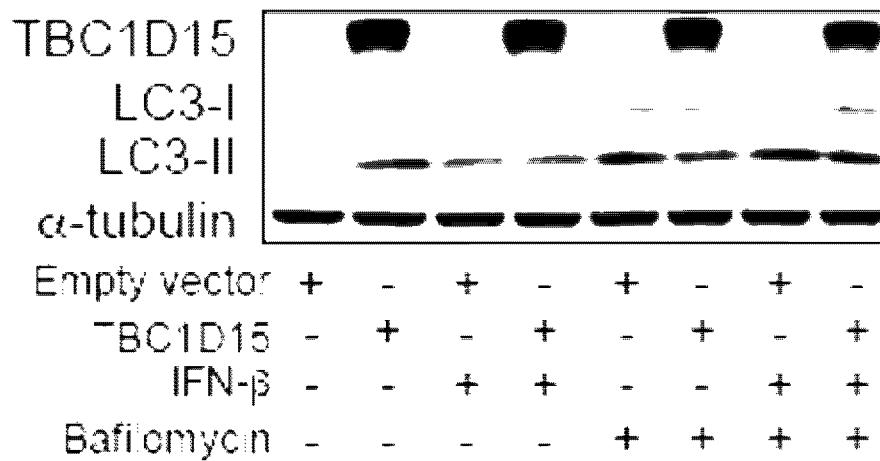


Figure 21

A



B

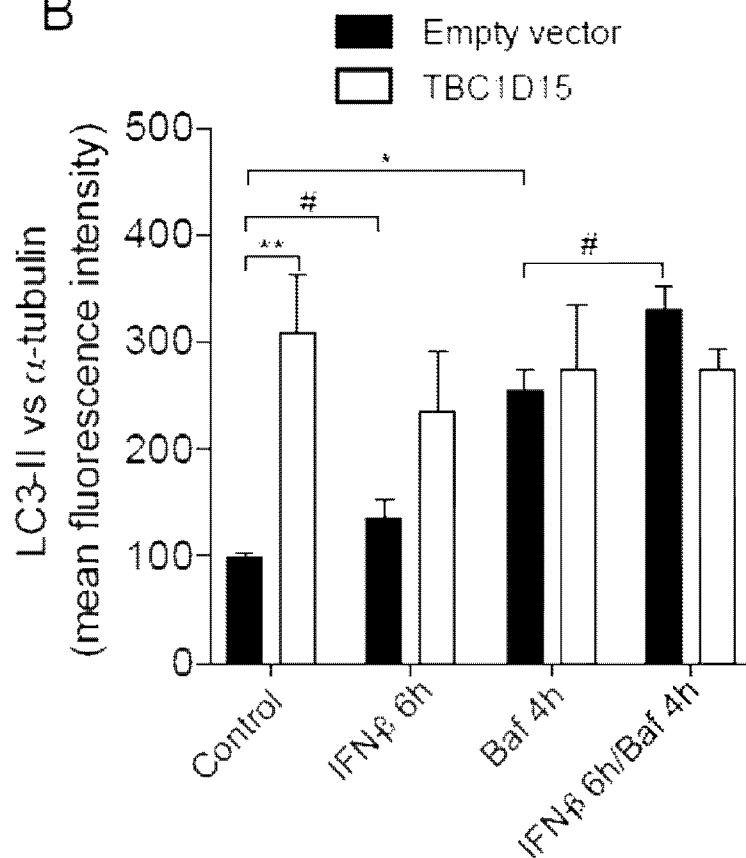
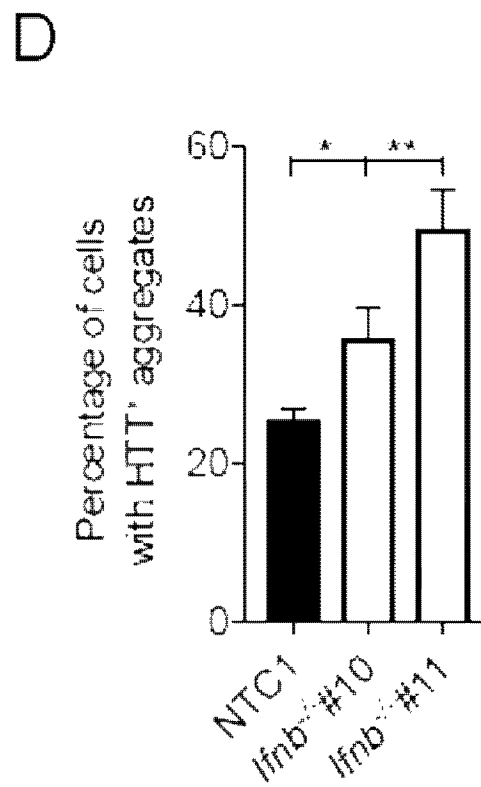
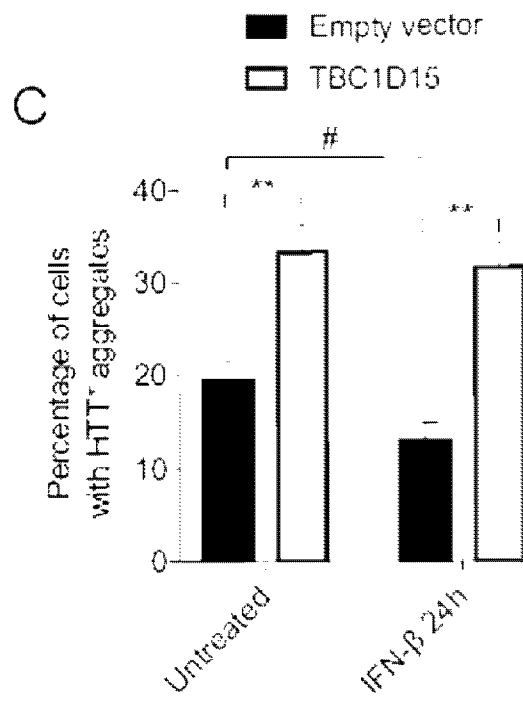


Figure 21 (cont)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2020/050478

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/7105 (2006.01) A61K 38/21 (2006.01) A61P 25/28 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATENW (includes WPIAP, EPODOC, and all full text databases in English), MEDLINE, BIOSIS, EMBASE, CAPLUS, clinicaltrials.gov, and classification marks and keywords (A61K 38/215, IFN-beta, mir-1, TBC protein, autophagy, proteotoxic, neurodegeneration, tauopathy, amyloid, alpha-synuclein, Alzheimer's disease, Parkinson's disease, Huntington's disease, heat shock proteins, Daf21, and related terms)

Internal databases provided by IP Australia (INTESS and PAMS NOSE), ESPACENET, PUBMED, and keywords (Monash, Cambridge, Pocock, Nchammer, Rubinsztein, Ejlerskov)

GENOMEQUEST, REGISTRY, and sequences (sequences disclosed in claims 21, 22, and 24)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			