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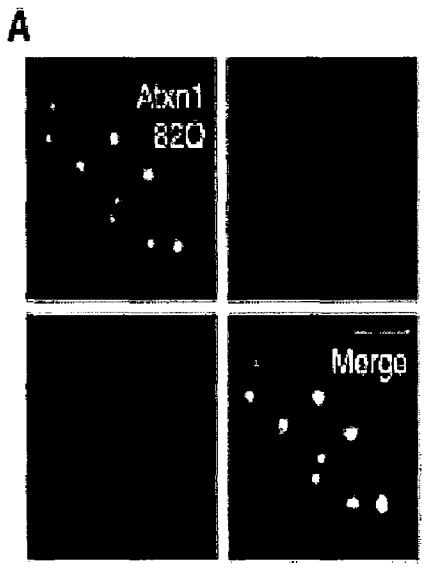

Figure 1A

(57) Abstract: The present invention relates to compositions and methods for promoting the degradation of misfolded proteins and protein aggregates. The compositions and methods may be used to treat a disorder associated with misfolded proteins or protein aggregates. In certain instances, the compositions and methods relate to modulators of one or more TRIM proteins or one or more STUbLs.

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TITLE OF THE INVENTION

Compositions and Methods for Degradation of Misfolded Proteins

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 62/168,309 filed on May 29, 2015, the contents of which are incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under CA088868, GM060911, CA182675, CA184867, and P30 AI045008, awarded by the National Institute of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Proteins are the most abundant macromolecules of the cell and are critical to virtually all physiological processes. To perform their biological functions, the majority of proteins need to fold into and maintain their native conformations. Although the native conformation of a protein is determined by its amino acid sequence, the folding process is extraordinarily complex and highly prone to error, and its utility can be further limited in situations of genetic mutations, biogenetic inaccuracies, and posttranslational damages (Dobson, 2003, Nature, 426: 884-890; Goldberg, 2003, Nature, 426: 895-899). Proteins that have adopted aberrant conformations, and the aggregates formed by them, pose a constant threat to cell viability and function. Failure to eliminate these proteins is closely linked to the pathogenesis of various debilitating human diseases (Selkoe, 2003, Nature, 426: 900-904; Taylor et al., 2002, Science, 296: 1991-1995)

To contend with protein misfolding, cells employ two broad sets of protein quality control (PQC) systems: systems that assist proteins in achieving their native conformations, and systems that eliminate misfolded proteins once they are formed. The former consist mainly of a large number of molecular chaperones and their cochaperones, which in an ATP-dependent manner protect proteins in their nonnative state and reduce

misfolding and aggregation. Notable examples include (1) heat shock protein 70 (Hsp70), which aids the folding of a wide range of proteins; (2) Hsp60/chaperonin, which forms a macromolecular cage to encapsulate relatively small proteins for uninterrupted folding; and (3) HSP90, which most commonly acts on proteins involved in cell signaling and transcription (Hartl et al., 2011, Nature, 475: 324-332).

Systems that remove misfolded proteins include protein disaggregases. For example, Hsp100 proteins in prokaryotes or lower eukaryotes (e.g., ClpB in bacteria and Hsp104 in yeast) can resolubilize protein aggregates, functioning in concert with Hsp70 and its cochaperone Hsp40 (Glover and Linquist, 1998, Cell, 94: 73-82). Nevertheless, given that protein misfolding is inevitable and often cannot be reversed due to mutations, biogenetic errors, or irreparable damages, cells ultimately rely on degradative systems to maintain protein quality. Yet, these systems are still poorly understood. Although the ubiquitin-proteasome pathway, along with autophagy, must be an important part of these systems, the critical issue of how they selectively recognize misfolded proteins and target them for degradation remains elusive (Goldberg, 2003, Nature, 426: 895-899; Tyedmers et al, 2010, Nat Rev Mol Cell Biol, 11: 777-788).

Furthermore, compared to the other cellular compartments such as the endoplasmic reticulum (Buchberger et al., 2010, Mol Cell, 40: 238-252), the PQC systems in the nucleus are conspicuously unclear. Misfolded proteins in the nucleus can be particularly damaging to postmitotic mammalian cells (e.g., neurons and cardiac myocytes), which are unable to remove these proteins through the breakdown of the nuclear envelope during mitosis. The importance of understanding PQC in this cellular compartment is emphasized by the formation of neuronal intranuclear inclusions that are associated with various dominantly inherited neurodegenerative diseases, including Huntington's disease (HD) and several types of spinocerebellar ataxias (SCAs). These diseases are caused by an expansion within the relevant genes of a CAG repeat, which encodes a polyQ stretch. They are manifested when the polyQ stretch exceeds a threshold length that is disease specific, and become progressively more severe as its length increases (Orr and Zoghbi, 2007, Annu Rev Neurosci, 30: 575-621).

Thus, there is a need in the art for compositions and methods for eliminating misfolded proteins. The present invention satisfies this unmet need.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a composition for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates, wherein the composition comprises a modulator of one or more TRIM proteins. In one embodiment, the modulator increases the expression or activity of the one or more TRIM proteins. In one embodiment, the modulator is at least one of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

In one embodiment, the modulator increases the expression or activity of at least one of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

In one embodiment, the composition comprises an isolated peptide comprising one or more TRIM proteins. In one embodiment, the isolated peptide further comprises a cell penetrating peptide (CPP) to allow for entry of the isolated peptide into a cell. In one embodiment, the CPP comprises the protein transduction domain of HIV tat.

In one embodiment, the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

In one embodiment, the disease or disorder is a polyQ disorder. In one embodiment, the disease or disorder is a neurodegenerative disease or disorder selected from the group consisting of Spinocerebellar ataxia (SCA) Type 1 (SCA1), SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), a transmissible spongiform encephalopathy (prion disease), a tauopathy, and Frontotemporal lobar degeneration (FTLD). In one embodiment, the disease or disorder is selected from the group consisting of AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV

amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis. In one embodiment, the disease or disorder is cancer associated with p53 mutant aggregates.

In one aspect, the present invention provides a method for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates in a subject in need thereof, where the method comprises administering to the subject a composition comprising a modulator of one or more TRIM proteins. In one embodiment, the modulator increases the expression or activity of the one or more TRIM proteins. In one embodiment, the modulator is at least one of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

In one embodiment, the modulator increases the expression or activity of at least one of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

In one embodiment, the composition comprises an isolated peptide comprising one or more TRIM proteins. In one embodiment, the isolated peptide further comprises a cell penetrating peptide (CPP) to allow for entry of the isolated peptide into a cell. In one embodiment, the CPP comprises the protein transduction domain of HIV tat.

In one embodiment, the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

In one embodiment, the disease or disorder is a polyQ disorder. In one embodiment, the disease or disorder is a neurodegenerative disease or disorder selected

from the group consisting of Spinocerebellar ataxia (SCA) Type 1 (SCA1), SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), a transmissible spongiform encephalopathy (prion disease), a tauopathy, and Frontotemporal lobar degeneration (FTLD). In one embodiment, the disease or disorder is selected from the group consisting of AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis. In one embodiment, the disease or disorder is cancer associated with p53 mutant aggregates.

In one embodiment, the method comprises administering the composition to at least one neural cell of the subject.

In one aspect, the present invention provides a composition for treating or preventing a disease or disorder associated with degradation of functional mutant protein, where the composition comprises a modulator of one or more TRIM proteins. In one embodiment, the modulator increases the expression or activity of the one or more TRIM proteins. In one embodiment, the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid. In one embodiment, the disease or disorder is cystic fibrosis.

In one aspect, the present invention provides a method for treating or preventing a disease or disorder associated with degradation of functional mutant protein in a subject in need thereof, where the method comprises administering to the subject a composition comprising a modulator of one or more TRIM proteins. In one embodiment, the modulator increases the expression or activity of the one or more TRIM proteins. In

one embodiment, the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid. In one embodiment, the disease or disorder is cystic fibrosis.

In one aspect, the present invention provides a composition for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates, where the composition comprises a modulator of one or more SUMO-targeted ubiquitin ligase (STUbl). In one embodiment, the modulator increases the expression or activity of one or more STUbLs. In one embodiment, the modulator is at least one of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid. In one embodiment, the modulator increases the expression or activity of RNF4.

In one aspect, the present invention provides a method for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates in a subject in need thereof, where the method comprises administering to the subject a composition comprising a modulator of one or more STUbLs. In one embodiment, the modulator increases the expression or activity of one or more STUbLs. In one embodiment, the modulator is at least one of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid. In one embodiment, the modulator increases the expression or activity of RNF4.

In one aspect, the present invention provides a method for producing a recombinant protein, where the method comprises administering a modulator of one or more TRIM proteins to cell modified to express a recombinant protein. In one embodiment, the modulator comprises an isolated peptide comprising one or more TRIM proteins. In one embodiment, the modulator comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended

drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1, comprising Figure 1A through Figure 1L, depicts the results of example experiments demonstrating that PML promotes the degradation of Atxn1 82Q and other nuclear misfolded proteins. (Figure 1A) HeLa cells transfected with Atxn1 82Q-GFP were stained with anti-PML antibody (red) and DAPI (blue). Individual and merged images are shown. Scale bar, 10 μm. (Figure 1B) Atxn1 82Q-GFP was expressed alone or together with PML in HeLa cells. Left, representative fluorescence images of cells. Scale bar, 20 μm. Right, quantification of cells based on sizes of Atxn1 82Q-GFP inclusions. (Figure 1C) Atxn1 82Q-GFP was expressed alone or together with PML in HeLa cells (left), or alone in HeLa cells that were previously treated with control (−) or PML siRNA. Cell lysate fractions (when indicated) and whole-cell lysates (WCL) were analyzed by filter retardation assay (for SR fraction) or western blot (WB; for the rest). Molecular weight standards (in kDa) and relative ratios of SS or SR Atxn1 versus actin are indicated. (Figure 1D) Steady-state levels of FLAG-Atxn1 82Q or 30Q when expressed alone or together with PML in HeLa cells (left), or when expressed alone in HeLa cells that were treated with control or PML siRNA (right), analyzed by WB. (Figure 1E) Effect of PML on the stability of total FLAG-Atxn1 82Q protein, analyzed by a pulse-chase assay and autoradiography. The relative amounts of ^{35}S-labeled Atxn1 82Q are indicated. (Figure 1F and Figure 1G) Effect of PML overexpression (Figure 1F) and knockdown (Figure 1G) on the stability of Atxn1 82Q-GFP, analyzed by CHX treatment and WB. (Figure 1H) Effect of PML on Atxn1 82Q-GFP levels in the absence or presence of MG132. (Figure 1I) Top, relative percentages of Httex1p 97QP-expressing cells with cytoplasmic (left) and nuclear (right) inclusions, in the absence or presence of PML (means + SD, n = 3). Bottom, representative fluorescence images of transfected cells immunostained with an anti-Htt antibody. Arrowheads indicate Httex1p 97QP aggregates. (Figure 1J and Figure 1K) Levels of HA-Httex1p 97QP and HA-Httex1p 97QP(KR) (Figure 1J) and GFP-TDP-43 (Figure 1K) in cells with and without PML overexpression. Virtually all Htt aggregates were in the SR fraction. (Figure 1L) Stability

of nFucDM-GFP in control and PML-depleted cells, analyzed by CHX treatment and WB.

Figure 2, comprising Figure 2A through Figure 2E, depicts the results of example experiments demonstrating the recognition of misfolded proteins by PML. (Figure 2A) Binding of GST-Htt 25Q and GST-Htt 103Q to immobilized FLAG-PML and FLAG-GFP (negative control), analyzed by an in vitro pull-down assay followed by WB (top and bottom) and Ponceau S staining (middle). *IgG heavy chain. (Figure 2B) Binding of GST-PML (shown on the right) and the control GST protein to native (N) and urea-denatured (D) luciferase (luc) immobilized on Ni-NTA beads, analyzed as in (Figure 2A). *Nonspecific proteins from BL21 bacterial lysates that bound to the control beads. (Figure 2C) Binding of indicated GST-Htt fusions to FLAG-PML CC conjugated on anti-Flag M2 beads or to control beads, analyzed by WB (top and middle) and Coomassie staining (bottom). (Figure 2D) Binding of PML F12/SRS2 (shown on the right) to a peptide library derived from luciferase. The N-terminal amino acid of the first peptide and the number of the last peptide spotted in each row are indicated. (Figure 2E) The occurrence of each amino acid in PML SRS2-binding peptides relative to its occurrence in the luciferase peptide array (set at 100%).

Figure 3, comprising Figure 3A through Figure 3F, depicts the results of example experiments demonstrating that SUMO2/3 is involved in the ubiquitination and PML-mediated degradation of Atxn1 82Q. (Figure 3A) SUMO1 and SUMO2/3 modification of Atxn1 82Q in HeLa cells treated without or with MG132. For better comparison of modified Atxn1 82Q, Denaturing immunoprecipitation (d-IP) products with a similar level of unmodified Atxn1 82Q were analyzed by WB. *Nonspecific bands. (Figure 3B) Localization of Atxn1 82Q (detected by anti-FLAG antibody, red) in GFP-SUMO2- or GFP-SUMO3-expressing U2OS cells treated with or without MG132. Scale bar, 10 μm. (Figure 3C) SUMO2/3 modification of Atxn1 82Q and 30Q in HeLa cells, analyzed by d-IP followed by WB (top) and Ponceau S staining (bottom). (Figure 3D) Atxn1 82Q was expressed alone or together with SUMO1 or HA-SUMO2 KR in HeLa cells that were previously transfected with the indicated siRNA or un-transfected (−). Cells were treated with or without MG132. SUMOylation and ubiquitination of FLAG-Atxn1 82Q was analyzed by d-IP and WB. (Figure 3E) Levels of Atxn1 82Q-GFP

expressed alone or together with increasing amounts of PML in HeLa cells pretreated with the indicated siRNA. (Figure 3F) Effect of PML on Atxn1 82Q-GFP levels in the presence or absence of SUMO1 or SUMO2 KR.

Figure 4, comprising Figure 4A through Figure 4F, depicts the results of example experiments demonstrating that PML promotes SUMOylation of Atxn1 82Q. (Figure 4A) SUMOylation of FLAG-Atxn1 82Q in HeLa cells in the absence or presence of HA-PML cells, and without or with MG132 treatment. The amount of Atxn1 82Q DNA used for transfected was adjusted to yield comparable levels of the unmodified protein. (Figure 4B and Figure 4C) SUMOylation of FLAG-Atxn1 82Q (Figure 4B) and FLAG-nFlucDM-GFP (Figure 4C) in control and PML siRNA-transfected HeLa cells treated without or with MG132. (Figure 4D and Figure 4E) SUMOylation of purified HA-Atxn1 82Q-FLAG was performed in the presence of recombinant FLAG-PML, FLAG-PML M6, and SUMO2 as indicated. In (Figure 4D), the amounts of different d-IP products were adjusted to yield a similar level of unmodified Atxn1 82Q (middle). (Figure 4F) Levels of Atxn1 82Q-GFP in HeLa cells in the absence or presence of increasing amounts of PML or PML M6.

Figure 5, comprising Figure 5A through Figure 5I, depicts the results of example experiments demonstrating a role of RNF4 in the degradation of Atxn1 82Q. (Figure 5A) Levels of Atxn1 82Q-GFP in HeLa cells without and with RNF4 overexpression. (Figure 5B and Figure 5C) Effect of RNF4 overexpression on Atxn1 82Q-GFP stability in control and PML-depleted HeLa cells, analyzed by CHX treatment and WB. Relative SS Atxn1 82Q/actin ratios are shown in (Figure 5C). (Figure 5D) Levels of FLAG-Atxn1 82Q in HeLa cells pretreated with a control siRNA (−) or a combination of three RNF4 siRNAs. (Figure 5E) Representative fluorescent images of Atxn1 82Q-GFP in control and RNF4-knockdown HeLa cells. Scale bar, 20 μm (Figure 5F) Localization of Atxn1 82Q-GFP and endogenous RNF4 in HeLa cells treated with vehicle (DMSO) or MG132. Scale bar, 10 μm. (Figure 5G and Figure 5H) Levels of FLAG-Atxn1 82Q and FLAG-Atxn1 30Q (Figure 5G) or HA-Httex1p 97QP and HA-Httex1p 97QP(KR) (Figure 5H) in HeLa cells without and with RNF4 overexpression. (Figure 5I) Stability of nFlucDM-GFP in HeLa cells stably expressing shCtrl and shRNF4 (left) and the levels of RNF4 in these cells (right).

Figure 6, comprising Figure 6A through Figure 6I, depicts the results of example experiments demonstrating that RNF4 promotes the ubiquitination and degradation of SUMO2/3-modified Atxn1 82Q. (Figure 6A and Figure 6B) Levels of SUMOylated FLAG-Atxn1 82Q (Figure 6A) and FLAG-nFlucDM-GFP (Figure 6B), in the absence or presence of RNF4, in HeLa cells treated with or without MG132. d-IP products with similar levels of unmodified proteins, as well as WCL, were analyzed. (Figure 6C) Levels of SUMOylated FLAG-Atxn1 82Q in HeLa cells that were pretreated with a control siRNA or a combination of RNF4 siRNAs, analyzed as in (Figure 6A). (Figure 6D and Figure 6E) Unmodified and SUMO2-modified FLAG-Atxn1 82Q proteins conjugated on M2 beads (+), or control M2 beads (−), were incubated with ubiquitination reaction mixture, in the absence or presence of GST-RNF4. (Figure 6D) A schematic diagram of the experimental design. (Figure 6E) WB analysis of FLAG-Atxn1 82Q (left) and GST-RNF4 (right). (Figure 6F and Figure 6G) Localization of Atxn1 82Q-GFP and RNF4 proteins (detected by anti-FLAG antibody) in HeLa. Scale bar, 10 μm. (Figure 6H) Effect of the indicated RNF4 proteins on Atxn1 82Q-GFP levels in HeLa cells. (Figure 6I) Effect of PML overexpression on Atxn1 82Q-GFP levels in HeLa cells that were pretreated with control or RNF4 siRNA.

Figure 7, comprising Figure 7A through Figure 7I, depicts the results of example experiments demonstrating that PML deficiency exacerbates behavioral and pathological phenotypes of the SCA1 mouse model. (Figure 7A and Figure 7B) Retention times (average + SEM) on accelerating Rotarod at 7 (Figure 7A) and 11 (Figure 7B) weeks of age, with the number of animals indicated in parenthesis. (Figure 7C and Figure 7D) Cerebellar sections of 12-week-old animals were stained with hematoxylin. (Figure 7C) Quantification of molecular layer thickness (means ± SEM, n = 3 mice/genotype). (Figure 7D) Representative images of the staining. Scale bar, 200 μm. (Figure 7E and Figure 7F) Cerebellar sections of 1-year-old animals were stained with an anti-calbindin antibody. (E) Quantitation of Purkinje cells, graphed as the average number of soma per 1 mm length (means ± SEM, n = 4 mice/genotype). (F) Representative images of the staining. Scale bar, 200 μm. (Figure 7G and Figure 7H) Sections of the cerebellar cortex from 12-week-old mice were stained with an anti-ubiquitin antibody and counterstained with hematoxylin. (Figure 7G) Percentage of Purkinje cells with aggregates (means ±

SEM, n = 3 mice/genotype). (Figure 7H) Representative images of the immunohistochemistry staining. Arrowheads indicate the ubiquitin positive aggregates in Purkinje cell bodies. Scale bar, 50 μm. No ubiquitin-positive aggregates were observed in Purkinje cells in mice without $Atxn1^{tg/-}$ (see Figure 14D). (Figure 7I) A model for PQC by the PML-RNF4 system. PML recognizes misfolded proteins through SRSs and conjugates them with poly-SUMO2/3 chain through its SUMO E3 ligase activity (a). RNF4 ubiquitinates SUMOylated-misfolded proteins (b) and targets them for proteasomal degradation (c).

Figure 8, comprising Figure 8A through Figure 8G, depicts the results of example experiments demonstrating that PML co-localizes with Atxn1 82Q aggregates and decreases insoluble Atxn1 82Q. (Figure 8A) Atxn1 82Q-GFP was expressed in PML-deficient ($PML^{-/-}$) mouse embryonic fibroblasts (MEFs) together with each of the indicated PML isoforms. Cells were stained with anti-PML antibody (red) and DAPI (blue). (Figure 8B) Levels of FLAG-Atxn1 82Q in HeLa cells treated with a control siRNA (-), PML siRNA #4, or PML siRNA #9. Cell lysates were analyzed by Western blot and filter retardation assays. The ratios of Atxn1 82Q in the SS fraction versus actin, normalized to the control, are shown. (Figure 8C) HeLa cells were transfected with a control siRNA, PML siRNA #4 (which targeted the 5'UTR of the PML mRNA), or PML siRNA #4 plus a plasmid expressing the open reading frame of PML (hence resistant to the siRNA). Levels of Atxn1 82Q-GFP in various fractions were analyzed. (Figure 8D) HeLa cells were treated with control siRNA, PML siRNA #4, and PML siRNA #9 and then transfected with FLAG-Atxn1 82Q. The levels of the FLAG-Atxn1 82Q transcript were determined by quantitative RT-PCR normalized to levels of 18S rRNA. (Figure 8E) Atxn1 30Q-GFP was expressed in HeLa cells in the absence or presence of PML. Cells were then treated with CHX for the indicated times. (Figure 8F) HeLa cells were transfected with nFlucDM-GFP and the corresponding wild-type luciferase (WT) protein. Endogenous PML was detected by an anti-PML antibody (red), and DNA by DAPI (blue). Scale bar: 10 μm. (Figure 8G) PML knockdown leads to accumulation of insoluble mutant luciferase. HeLa cells were transfected with control or PML siRNA and then with nFluc-GFP or nFlucDM-GFP. The SR fraction contained very small amounts of nFlucDM-GFP.

Figure 9, comprising Figure 9A through Figure 9E, depicts the results of example experiments demonstrating the interaction of PML with pathogenic Htt proteins. (Figure 9A) PML preferentially interact with pathogenic Htt. Lysates of 293T cells expressing FLAG-GFP or FLAG-PML were incubated with GST-Htt 25Q or GST-Htt 103Q immobilized on glutathione beads (lanes 1-3 and 5-7), or with control glutathione beads (lanes 4 and 8). The input and pull-down fractions were analyzed by Western blot. (Figure 9B) Schematic representation of wild-type PML isoforms IV (aa 1-633) (called PML in the present work) and various deletion fragments (F1-F10). The RING domain (R), B1 box, B2 box, and coiled-coil region (CC) are labeled. Substrate recognition sites SRS1 and SRS2 are indicated by lines. The interaction of PML with pathogenic Htt proteins (Htt 103Q or 52Q) and denatured luciferase are shown. ND: not done. (Figure 9C) Interactions of full-length PML and PML deletion mutants with Htt. PML proteins were generated by coupled *in vitro* transcription/translation in the presence of [^{35}S]Met, and were incubated with purified GST-Htt 103Q, GST-Htt 25Q, or GST immobilized on glutathione beads. [^{35}S]Met-labeled proteins in the input and pull-down samples were analyzed by autoradiography, and GST proteins in the pull-down samples were analyzed by Coomassie staining. The three pull-down sample sets were analyzed at the same time and in the same way, including the amount of samples and the exposure duration of autoradiography. The input samples were exposed for a shorter period of time. (Figure 9D) Sequences of Htt 52Q and the CC-destabilizing (cc-) mutant. The amino acids in Htt 52Q that are changed to Pro in Htt 52Q cc- are shown in red. (Figure 9E) Interaction of purified PML CC region (shown on the right) with cellulose-bound luciferase peptide scans was assayed as in Figure 2D. * TEV protease.

Figure 10, comprising Figure 10A through Figure 10C, depicts the results of example experiments demonstrating the interaction of PML with denatured luciferase. (Figure 10A) Interaction of PML fragments with denatured luciferase. In vitro-translated, [^{35}S]-labeled full-length FLAG-PML and FLAG-PML deletion mutants were incubated with native or denatured luciferase immobilized on beads, or control beads. The input and beads-bound PML proteins were analyzed by autoradiography, and luciferase by Coomassie blue staining. *, Non-specific bands. The three pull down sample sets were analyzed at the same time and in the same way, including the amount of samples and the

exposure duration of autoradiography. The input samples were exposed for a shorter period of time. (Figure 10B and Figure 10C) Identification of the second substrate recognition site (SRS2) on PML. (Figure 10B) PML deletion mutations encompassing amino acids at the C-terminus and summary of their interaction with denatured luciferase. (Figure 10C) In vitro-translated, [^{35}S]-labeled GST-fusions of PML fragments or GST were tested for interaction with luciferase as described in (Figure 10A).

Figure 11, comprising Figure 11A through Figure 11G, depicts the results of example experiments demonstrating the modification of misfolded proteins by SUMO2/3. (Figure 11A) HeLa cells were transfected with FLAG-Atxn1 82Q or FLAG-Atxn1 82Q (5KR) and were treated with or without MG132. FLAG-Atxn1 82Q and FLAG-Atxn1 82Q (5KR) were isolated by d-IP, and their SUMO2/3 modification was analyzed by Western blot. (Figure 11B) HeLa cells were transfected without or with GFP-TDP-43 and treated with vehicle (DMSO) (-) or MG132 (+). d-IP was performed using an anti-GFP antibody or a control antibody. d-IP products were analyzed by Western blot. (Figure 11C) HeLa cells were transfected with nFluc-GFP, nFlucSM-GFP, and nFlucSM-GFP (each was also N-terminally tagged with the FLAG epitope) and treated with or without MG132. nFluc proteins were isolated by d-IP. The whole cell lysates (WCL) and IP products were analyzed by Western blot. Note that the difference between the SUMO2/3 modifications of WT luciferase versus SM/DM luciferase (top, lanes 1-3) was not due to a change in the overall SUMO2/3 conjugation in the WCL (bottom). (Figure 11D) Atxn1 82Q-GFP was expressed in HeLa cells pre-treated with control (Ctrl), SUMO2/3, or SUMO1 siRNA. Cell lysates were analyzed by Western blot. (Figure 11E and Figure 11F) HeLa cells were treated with control (Ctrl) siRNA, SUMO2/3 siRNA, or SUMO1 siRNA (Figure 11E), or treated with these siRNAs and then transfected with GFP (Figure 11F). Cell lysates were analyzed by Western blot. (Figure 11G) PML and PML M6 proteins used for *in vitro* SUMOylation assay. FLAG-PML and FLAG-PML M6 were expressed in 293T cells and purified by anti-FLAG (M2) beads. The proteins were analyzed by Coomassie staining along with BSA standards (left) and by Western blot (right). The two additional bands (arrowheads) presented in both PML and PML M6 lanes (left) were determined to be PML fragments based on both

Western blot (right) and by mass spectrometry analysis. A schematic representation of M6 mutant is shown at the bottom. *: Point mutations are described elsewhere herein.

Figure 12, comprising Figure 12A through Figure 12I, depicts the results of example experiments demonstrating that RNF4 promotes degradation of misfolded proteins. (Figure 12A) Representative fluorescence images of HeLa cells expressing Atxn1 82Q-GFP alone or together with RNF4. Scale bar: 20 μm. Images are from the same experiment as that shown in Figure 1B. (Figure 12B) The half-life of Atxn1 82Q-GFP in HeLa cells with and without RNF4 overexpression. Samples are from the same experiments as those shown in Figure 1F. (Figure 12C) HeLa cells were transfected with control siRNA (-), RNF4 siRNA alone, or RNF4 siRNA plus an siRNA-resistant RNF4. Cell lysates were analyzed by Western blot. (Figure 12D) FLAG-Atxn1 82Q was expressed in HeLa cells that were pre-treated with control siRNA and the indicated RNF4 siRNA. FLAG-Atxn1 82Q was isolated by d-IP with anti-FLAG M2 beads. WCL and IP products were analyzed by Western blot with indicated antibodies. (Figure 12E) HeLa cells expressing both Atxn1 82Q-GFP and FLAG-RNF4 were treated with vehicle (DMSO) or MG132. Exogenous (Exo.) RNF4 was detected anti-FLAG antibody. In control cells treated with DMSO, exogenous RNF4 showed partial co-localization with Atxn1 82Q-GFP aggregates. Upon MG132 treatment, complete co-localization of exogenous RNF4 with Atxn1 82Q aggregates was observed in 100% of cells. Scale bar: 10 μm. (Figure 12F) Western blot analysis of HeLa cells transfected with GFP-TDP-43 alone or together with increasing amounts of RNF4. (Figure 12G) GFP-TDP-43 was expressed in cells pre-treated with a control siRNA (-) or the indicated RNF4 siRNA. 500 cells were counted in each experiment. Percentages of cells with GFP-TDP-43 foci are shown. (Figure 12H) HeLa cells treated with RNF4 siRNA were transfected with GFP-TDP-43. Cells were stained with an anti-RNF4 antibody (red) and DAPI (blue). Note that TDP-43 formed aggregates in a cell in which RNF4 was knocked down (filled arrowhead), but was diffused in a control cell (open arrowhead). (Figure 12I) HeLa cells were transfected with GFP-TDP-43 and treated with MG132. Endogenous RNF4 was immunostained with anti-RNF4 antibody (red). Note that RNF4 also formed nuclear foci in cells with no TDP-43 (Figure 12E – Figure 12H).

Figure 13, comprising Figure 13A through Figure 13F, depicts the results of example experiments demonstrating that SUMO2/3 are involved in RNF4-mediated degradation of Atxn1 82Q. (Figure 13A) Atxn1 82Q-GFP and/or RNF4 were expressed in HeLa cells pre-treated with control siRNA, SUMO2/3 siRNA, and SUMO1 siRNA. Cell lysates were analyzed by Western blot. (Figure 13B) U2OS cells stably expressing GFP-SUMO2 were transfected first with the indicated RNF4 siRNAs or a control siRNA (-) and then with FLAG-Atxn1 82Q. The percentages of transfected cells with GFP-SUMO2-positive Atxn1 82Q aggregates are shown (mean + SD, n=3). In each experiment, 200 cells were counted. Representative images of the transfected cells are shown on the right. GFP-SUMO2 does not form aggregated structure in cells without Atxn1 82Q expression. (Figure 13C) Schematic representation of wild-type and mutant RNF4 proteins. The SUMO-interaction motifs (SIMs) 1 to 4 and the RING domain are shown. *: Point mutations are described elsewhere herein. (Figure 13D and Figure 13E) Purified recombinant GST fusions of rat (r) RNF4 and RNF4 CS1 (Figure 13D) or human RNF4 and RNF4 SIMm (Figure 13E) were incubated with ubiquitin E1, E2 (UbcH5a), and ubiquitin (Ub) as indicated, plus Mg^{2+}-ATP. The reaction mixtures were analyzed by Western blot using an anti-GST antibody. (Figure 13F) Expression of Atxn1 82Q-GFP in cells treated with the indicated combinations of control, PML, and RNF4 siRNAs as indicated. Cell lysates were analyzed by Western blot.

Figure 14, comprising Figure 14A through Figure 14D, depicts the results of experiments demonstrating that PML deficiency reduces arborization of Purkinje cell dendrites but does not result in aggregates in Purkinje cells. (Figure 14A) Midsagittal cerebellar sections of 12-week-old mice were stained with an antibody against the Purkinje cell-specific protein calbindin. Fluorescence intensity was plotted from a rectangular area from the preculminate fissures (n = 2 mice for $PML^{+/+}$, and n = 3 mice for all the other genotypes). $PML^{-/-}$ mice showed significant loss of dendritic arborization compared to $PML^{+/+}$ mice (ANOVA, p = 0.031). (Figure 14B) Representative confocal images of calbindin immunofluorescence. Scale bar: 100 μm. (Figure 14C) Quantitation of Purkinje cell density in 12-week $PML^{+/+}$, $PML^{+/-}$ and $PML^{-/-}$ mice with and without $Atxn1^{tg/-}$, graphed as the average number of soma per 1 mm length (means + SEM, n = 3 mice/genotype). (Figure 14D) Immunohistochemical staining of the cerebellar cortex

sections from 12-week-old $PML^{+/+}$ and $PML^{-/-}$ mice without $Atxn1^{tg/-}$. The sections were stained with an anti-ubiquitin antibody and counterstained with hematoxylin. Scale bar: 50 μm. Note that no ubiquitin positive aggregates were detected in those sections. The stained sections of $PML^{+/+}$:$Atxn1^{tg/-}$ and $PML^{-/-}$:$Atxn1^{tg/-}$ mice are shown in Figure 7G.

Figure 15, comprising Figure 15A through Figure 15D, depicts the results of example experiments demonstrating the co-localization of TRIM27, TRIM32, and TRIM5δ with EGFP-Atxn1 82Q and Httex1p 97QP. (Figure 15A) Atxn1 82Q-GFP was expressed in HeLa cells together with the indicated FLAG-tagged TRIM proteins. Cells were stained with anti-FLAG antibody (red) and DAPI (blue). Scale bar: 10 μm. (Figure 15B and Figure 15C) Httex1p 97QP was expressed in HeLa cells together with FLAG-tagged TRIM5δ, TRIM27 (Figure 15B), and TRIM32 (Figure 15C). Cells were immunostained with anti-huntingtin (green) and anti-FLAG (red) antibodies. Note that TRIM32 co-localized with Httex1p 97QP regardless of the cellular localization of Httex1p 97QP. (Figure 15D) HeLa cells were transfected with Atxn1 82Q-GFP. Endogenous TRIM27 was detected by anti-TRIM27 antibody (red). Arrowheads indicate endogenous TRIM27 bodies that were co-localized with the Atxn1 82Q aggregates. Scale bar: 10 μm.

Figure 16, comprising Figure 16A through Figure 16D, depicts the results of example experiments demonstrating the reduction of aggregated Atxn1 82Q by TRIM27, TRIM32, and TRIM5δ. (Figure 16A and Figure 16B) Levels of Atxn1 82Q-GFP when expressed alone (-) or together with the indicated FLAG-tagged TRIM proteins in HeLa cells. The cell lysates were analyzed by Western blot. In the left panel of (Figure 16A), the ratios of Atxn1 82Q in the SS fraction versus actin are given, and the expression of TRIM11 could be detected after a prolonged exposure. (Figure 16C) Expression levels of Atxn1 82Q in HeLa cells treated with control (-) or TRIM27 siRNA. *, a non-specific band. (Figure 16D) PIASy does not inhibit the levels of Atxn1 82Q protein. Western blot analysis of HeLa cells transfected with Atxn1 82Q-GFP, PIASy, and PML as indicated.

Figure 17, comprising Figure 17A through Figure 17C, depicts the results of example experiments demonstrating that TRIM27 and TRIM32 reduce aggregated Atxn1 82Q independent of PML. (Figure 17A) Partial co-localization of TRIM27 with

PML. HeLa cells transfected with FLAG-TRIM27 were immunostained with anti-FLAG (green) and anti-PML (red) antibodies. (Figure 17B) Co-localization of TRIM27 with Atxn1 82Q-GFP aggregates in $PML^{+/+}$ and $PML^{-/-}$ MEFs. FLAG-TRIM 27 was stained by anti-FLAG (red) antibody. (Figure 17C) Atxn1 82Q-GFP was expressed alone or together with the indicated TRIM proteins in $PML^{+/+}$ and $PML^{-/-}$ MEFs. Extracts were analyzed by filter retardation assay. To better compare the effects of TRIM proteins on the aggregates, a lighter exposure is shown on the right panel.

Figure 18, comprising Figure 18A through Figure 18G, depicts the results of example experiments demonstrating that TRIM proteins depend on SUMO2/3 and the proteasome to remove insoluble Atxn1 82Q. (Figure 18A-Figure 18F) Atxn1 82Q-GFP was expressed alone or together with TRIM5δ, TRIM27, and TRIM32 in cells treated without or with MG132 (Figure 18A-Figure 18C), or in control or SUMO2/3 knockdown cells (Figure 18D-Figure 18F). Cell lysates were analyzed by Western blot. (Figure 18G) SUMOylation of purified HA-Atxn1 82Q-FLAG was performed in the presence of recombinant FLAG-TRIM11 and SUMO2 as indicated. HA-Atxn1 82Q-FLAG was isolated using denaturing immunoprecipitation. The reaction mixtures and IP samples were analyzed by Western blot.

Figures 19, comprising Figure 19A through Figure 19F, depicts the results of example experiments demonstrating the localization of TRIM proteins in relation to Atxn1 82Q. Atxn1 82Q-GFP (green) was expressed in HeLa cells together with the indicated HA-tagged TRIM proteins. Cells were immunostained with anti-HA antibody (red). The representative localization patterns for each TRIM protein are shown. (Figure 19A) The representative localization patterns of TRIM1 - TRIM 16. (Figure 19B) The representative localization patterns of TRIM17 - TRIM32. (Figure 19C) The representative localization patterns of TRIM33 - TRIM41 and TRIM44 - TRIM 41. (Figure 19D) The representative localization patterns of TRIM52, TRIM54-TRIM58, TRIM62-TRIM66, TRIM73-TRIM74, and TRIM76. TRIM proteins that showed co-localization with Atxn1 82Q-GFP in a substantial number of cells are indicated in yellow. Scale bar: 10 μm.

Figure 20, comprising Figure 20A and Figure 20B, depicts the results of a systematic analysis of TRIM proteins on Atxn1 82Q and Httex1p 97QP. Atxn1 82Q-GFP

(Figure 20A) or Httex1p 97QP (Figure 20B) was co-expressed with the indicated TRIM proteins in HeLa cells. Cell lysates were analyzed by Western blot. TRIM proteins labeled red and green are those that reduced and increased the levels of the polyQ proteins, respectively, while TRIM proteins labeled in black had no observable effect. Note that the effects of TRIM proteins can be influenced by their levels of expression, as described elsewhere herein.

Figure 21, comprising Figure 21A through Figure 21C, depicts the results of example experiments demonstrating that recombinant TAT-TRIM11 reduces the levels of misfolded proteins. HeLa cells were transfected with Atxn1 82Q-GFP, Atxn1 30Q, or Htt 97Q-GFP, and were subsequently incubated with recombinant TRIM11 or SUMO2 proteins. (Figure 21A) Treatment of HeLa cells with TRIM11 led to strong reduction in the levels of Atxn1 82Q. (Figure 21B) Treatment of HeLa cells with TRIM11 also led to strong reduction in the levels of Htt 97Q. (Figure 21C) SUMO2 had minimal effect on the levels of Atxn1 82Q.

Figure 22 depicts the amino acid sequence of Tat-TRIM11 used in example experiments.

Figure 23, comprising Figure 23A through Figure 23F, depicts results of experiments demonstrating RNF4 staining of human brain samples. Anti-RNF4 (green) immunostaining of HD brain tissues. RNF4 showed diffused nuclear localization (Figure 23A – Figure 23C) or formed foci (Figure 23D – Figure 23F) (indicated by arrowheads). Scale bar: 10 μm.

Figure 24, comprising Figure 24A through Figure 24H, depicts results of experiments demonstrating co-localization of RNF4 with neuronal inclusions of SCA1 patients. Immunostaining of SCA1 brain tissues with anti-polyQ (1C2) and anti-RNF4 antibodies. Scale bar: 10 μm.

Figure 25, comprising Figure 25A through Figure 25L, depicts results of experiments demonstrating co-localization of RNF4 with neuronal inclusions of SCA1 patients. Figure 25 depicts immunostaining of HD brain tissues with anti-Htt, anti-ubiquitin and two separate RNF4 antibodies. Note that Htt and ubiquitin form ring-like structures (Figure 25A – Figure 25H) or evenly distributed inclusions (Figure 25I – Figure 25L) with the RNF4 signal in the center.

Figure 26, comprising Figure 26A through Figure 26H, depicts results of experiments demonstrating co-localization of RNF4 with inclusions in neurons of HD patients. Shown are Htt and RNF4 immunostaining of HD brain tissues. Two different RNF4 antibodies (#1 and #2) were used. Scale bar: 10 μm.

Figure 27, comprising Figure 27A through Figure 27D, depicts results from experiments demonstrating SUMOylation of Atxn1 82Q, p53 and alpha-Synuclein. Figure 27A depicts experiments where SUMOylation of Atxn1 82Q was analyzed when TRIM11 WT, TRIM11 MUT or PML was co-expressed. Before lysis, 10 μM MG132 was added for 4 hours. Figure 27B depicts in vitro SUMOylation of purified Atxn1 82Q incubated with TRIM11 WT or TRIM11 MUT in the presence of E1, E2 and SUMO2. Figure 27C depicts in vitro SUMOylation of purified Flag-p53 incubated with TRIM11 or PML in the presence of E1, E2 and SUMO2. Figure 27D depicts in vitro SUMOylation of alpha-Synuclein incubated with TRIM11 WT in the presence or absence of E1, E2 and SUMO2.

Figure 28, comprising Figure 28A through Figure 28G, depicts results from experiments demonstrating TRIM11 is recruited to Atxn1 82Q aggregates. Figure 28A depicts immunofluorescence analysis of transfected GFP-Hsp70 in 293T cells. Figure 28B depicts immunofluorescence analysis of transfected GFP-TRIM11 in 293T cells. Figure 28C depicts immunofluorescence analysis showing that Hsp70 can be recruited into the aggregates of Atxn1 82Q. Figure 28D depicts immunofluorescence analysis showing that TRIM11 can be recruited into the aggregates of Atxn1 82Q. Figure 28E depicts immunoblotting analysis detergent-soluble and insoluble fractions of cells transfected with Atxn1 82Q, TRIM11 or Hsp70. Where indicated, 10 μM MG132 is added for 3 hours. Figure 28F depicts immunoblotting analysis of detergent-soluble and insoluble fractions of cells transfected with Atxn1 82Q, TRIM11 WT (wild type) or TRIM11 MUT (mutation). Where indicated, 10 μM MG132 is added for 3 hours. Figure 28G depicts immunoblotting where HCT116 cells are transfected with the indicated plasmids. After 48 hours, cells were lysed and then stained with 20 μM Thioflavin-T (ThT).

Figure 29, comprising Figure 29A through Figure 29C, depicts results from experiments demonstrating TRIM11 binding to Atxn1 82Q. Figure 29A depicts

purified experiments where Flag-Atxn1 82Q immobilized on beads was incubated with GST or GST-TRIM11. Figure 29B experiments where depicts purified Flag-Atxn1 82Q or Flag-Atxn1 30Q immobilized on beads was incubated with GST or GST-TRIM11. Figure 29C depicts binding of GST-TRIM11 and GST protein to native (N) and urea-denatured (D) luciferase (luc) immobilized on Ni-NTA beads.

Figure 30, comprising Figure 30A through Figure 30D, depicts results from experiments demonstrating TRIM11 reduces cellular aggregates. Figure 30A depicts experiments where HCT116 cells stably expressing GFP-Atxn1 82Q were lysed and then stained with 20 μM Thioflavin-T (ThT). Figure 30B depicts sedimentation analysis of GFP-Atxn1 82Q- HCT116 cells transfected with TRIM11. Figure 30C depicts experiments where HCT116 cells stably expressing GFP-Atxn1 82Q were transfected with TRIM11 WT or TRIM11 MUT. After 48 hours, cells were lysed and stained with 20μM ThT. Figure 30D depicts sedimentation analysis of GFP-Atxn1 82Q- HCT116 cells transfected with TRIM11 WT or TRIM11 MUT.

Figure 31, comprising Figure 31A through Figure 31H, depicts results from experiments demonstrating TRIM11 acts as a molecular chaperone to prevent aggregate formation. Figure 31A depicts experiments where luciferase (10 nM) was incubated with 200 nM GST, 200 nM GST-TRIM11 or 200 nM Hsp70 at 45°C with the indicated time. Native luciferase activity was set as 100%. N=3. Figure 31B depicts experiments where GFP (0.45 uM) was incubated with 200 nM GST, 200 nM GST-TRIM11 or 200 nM Hsp70 at 45°C with the indicated time. Native GFP fluorescence was set as 100%. N=3. Figure 31C depicts the activity of transfected luciferase in HCT116 measured without heat shock as control. After 30 min heat shock at 45°C or after 3 hour recovery at incubator, the luciferase activities were relative to the control. Figure 31D depicts the activity of transfected luciferase in HCT116 measured without heat shock as control. After 60 min heat shock at 45°C or after 1.5 hour or 3 hour recovery at incubator, the luciferase activities were relative to the control. Figure 31E depicts immunoblotting analysis of HCT116 cells stably expressing control vector or Flag-TRIM11. Figure 31F depicts ThT analysis showing the prevention of beta-amyloid fibrils formation by GST, TRIM11 or Hsp70. Figure 31G depicts a sedimentation assay showing the prevention of Atxn1 82Q aggregates formation by Lysozyme, GST or TRIM11. The results were

shown by immunoblotting and dot-blot assay. Figure 31H depicts a sedimentation assay showing the prevention of p53 aggregates formation by L TRIM11. Where indicated, E1, E2, SUMO2 or ATP was applied. The results were shown by immunoblotting and dot-blot assay.

Figure 32, comprising Figure 32A through Figure 32G, depicts results from experiments demonstrating HSF1 is not required for regulating the transcription of TRIM11. Figure 32A depicts experiments where HCT116 cells were treated with or without heat shock (42°C) for 1 hour and then recovered for different time. Total cell lysates were subjected to immunoblotting with the indicated antibodies. Figure 32B depicts experiments whereHCT116 cells stably expressing Flag-TRIM11 were treated with or without heat shock (42°C) for 1 hour and then recovered for different time. Total cell lysates were subjected to immunoblotting with the indicated antibodies. Figure 32C depicts experiments where HeLa cells were treated with or without heat shock (42°C) for 1 hour and then recovered for different time. Total cell lysates were subjected to immunoblotting with the indicated antibodies. Figure 32D depicts experiments where A549 cells were treated with or without As_2O_3 for 30 min and then recovered for different time. Total cell lysates were subjected to immunoblotting with the indicated antibodies. Figure 32E depicts experiments where A549 cells were treated with or without H_2O_2 for 100 min and then recovered for different time. Total cell lysates were subjected to immunoblotting with the indicated antibodies. Figure 32F depicts semi-quantitative PCR analysis of *TRIM11*, *HSP70*, *HSP90* and *GAPDH* in response to heat shock. Figure 32G depicts experiments where A549 cells stably expressing vector or HSF1 were treated with or without heat shock and recovered for 3 hours. Immunoblotting and semi-quantitative PCR were analyzed.

Figure 33, comprising Figure 33A through Figure 33F, depicts results from experiments demonstrating that p53 is a factor in upregulating TRIM11 in heat shock response. Figure 33A depicts immunoblotting of HCT116 p53 wild type or p53 null cells treated with heat shock and recovered. Figure 33B depicts qPCR analysis of TRIM11 mRNA level in HCT116 p53 wild type or p53 null cells treated with heat shock and recovered. Figure 33C depicts immunoblotting and semi-quantitative PCR analysis of A549 cells stably expressing control (Ctrl) or p53 shRNA treated with or without heat

shock and recovered for 3 hours. Figure 33D depicts immunoblotting and semi-quantitative PCR analysis of HCT116 cells transfected with Ctrl or p53 siRNA treated with or without heat shock and recovered for 3 hours. Figure 33E depicts crystal violet analysis of survival of HCT116 cells which were heated and recovered for 24 hours. Where indicated, KRIBB11 was added. Figure 33F depicts relative cell numbers of results presented in Figure 33E analyzed by OD490.

Figure 34, comprising Figure 34A through Figure 34G, depicts results from experiments demonstrating TRIM11 acts as a disaggregase to resolve preformed aggregates. Figure 34A depicts disaggregation and reactivation of preformed luciferase aggregates using increasing concentrations of Lysozyme, GST or GST-TRIM11 (n=3). Figure 34B depicts a sedimentation assay showing that heat-aggregated luciferase resolved by GST or GST-TRIM11. The results were shown by immunoblotting. Figure 34C depicts disaggregation and reactivation of preformed GFP aggregates using increasing concentrations of Lysozyme, GST or GST-TRIM11 (n=3). Figure 34D depicts a sedimentation assay showing that heat-aggregated GFP resolved by GST or GST-TRIM11. The results were shown by immunoblotting. Figure 34E depicts a sedimentation assay showing preformed Atxn1 82Q aggregates resolved by Lysozyme, GST or TRIM11. The results were shown by immunoblotting. Figure 34F depicts a sedimentation assay showing that preformed Atxn1 82Q aggregates (left) and p53 aggregates (right) disaggregated by 1 μM Hsp70 and 0.5 μM Hsp40. Figure 34G depicts a sedimentation assay showing that preformed Atxn1 82Q aggregates disaggregated by 0.5 μM GST, 0.5 μM TRIM11, 1 μM Hsp70, 0.5 μM Hsp40 or 1 μM Hsp104.

Figure 35, comprising Figure 35A through Figure 35D, depicts results from experiments demonstrating full length TRIM11 is required for refolding activity. Figure 35A depicts a schematic diagram of TRIM11 structure. Figure 35B depicts disaggregation and reactivation of preformed luciferase aggregates using increasing concentrations of the indicated proteins (n=3). Figure 35C depicts a sedimentation assay showing heated luciferase aggregates disaggregated by GST, TRIM11, RBC or B30.2. Figure 35D depicts disaggregation and reactivation of preformed luciferase aggregates using the indicated proteins (n=3).

Figure 36, comprising Figure 36A and Figure 36B depicts results from experiments demonstrating TRIM11 binding to substrates is required for TRIM11 disaggregation function. Figure 36A depicts purified Flag-Atxn1 82Q immobilized on beads was incubated with GST, GST-TRIM11, GST-RBC or GST-B30.2. Figure 36B depicts purified Flag-Atxn1 82Q immobilized on beads was incubated with GST, GST-TRIM11 or other TRIM11 fragments.

Figure 37, comprising Figure 37A through Figure 37E depicts results from experiments demonstrating TRIM11 performs disaggregation independently of its SUMO E3 ligase activity. Figure 37A depicts experiments where luciferase (10 nM) was incubated with 200 nM GST, 200 nM GST-TRIM11 WT or MUT at 45°C with the indicated time. Native luciferase activity was set as 100%. N=3. Figure 37B depicts disaggregation and reactivation of preformed luciferase aggregates by 200 nM GST, 200 nM GST-TRIM11 WT or MUT. Figure 37C depicts the binding of GST, GST-TRIM11 WT or MUT to native (N) and urea-denatured (D) luciferase (luc) immobilized on Ni-NTA beads. Figure 37D depicts immunofluorescence analysis of transfected GFP-TRIM11 MUT in 293T cells. Figure 37E depicts immunofluorescence analysis show that TRIM11 MUT can be recruited into the aggregates of Atxn1 82Q.

Figure 38, comprising Figure 38A through Figure 38E, depicts results from experiments demonstrating TRIM11 can also preform alpha-Synuclein amyloid fibril formation and disaggregate preformed alpha-Synuclein fibers. Figure 38A depicts ThT analysis showing prevention of alpha-Syn fibrils formation by GST, TRIM11, Hsp70, Hsp40 or Hsp104. Figure 38B depicts ThT analysis showing prevention of alpha-Syn fibrils formation by TRIM11 in a dose-dependent manner. Figure 38C depicts EM images of the fiber formation of alpha-Syn monomer incubated with GST or GST-TRIM11. Figure 38D depicts a sedimentation assay showing that preformed alpha-Syn fiber disaggregated by TRIM11 and Hsp104 A503S. Figure 38E depicts ThT analysis showing disaggregation of preformed alpha-Syn fibrils by GST, TRIM11 or Hsp104.

Figure 39, comprising Figure 39A through Figure 39D, depicts results from experiments demonstrating TRIM21 has similar disaggregation functions to TRIM11. Figure 39A depicts binding of GST, GST-TRIM11 WT or MUT to native (N) and urea-denatured (D) luciferase (luc) immobilized on Ni-NTA beads. Figure 39B

depicts a sedimentation assay showing that heat-aggregated luciferase resolved by GST or GST-TRIM21. Figure 39C depicts Disaggregation and reactivation of preformed luciferase aggregates using increasing concentrations of GST or GST-TRIM21 (n=3). Figure 39D depicts a luciferase assay where luciferase (10 nM) was incubated with 200 nM GST or 200 nM GST-TRIM21 at 45ºC for 1 min. Native luciferase activity was set as 100%. N=3.

Figure 40, comprising Figure 40A through Figure 40E, depicts results from experiments demonstrating PML and Atxn1 82Q disaggregation. Figure 40A depicts commassie blue staining of purified Flag-PML6 from 293T cells. Figure 40B depicts disaggregation and reactivation of preformed GFP aggregates using increasing concentrations of Lysozyme or Flag-PML6 (n=3). Figure 40C depicts disaggregation and reactivation of preformed GFP aggregates using Lysozyme, Flag-PML6 or GST-TRIM11 (n=3). Figure 40D depicts commassie blue staining of purified Flag-PML4 fragments from 293T cells. Figure 40E depicts disaggregation and reactivation of preformed GFP aggregates using different PML4 fragments (n=3).

Figure 41, comprising Figure 41A and Figure 41B, depicts results from experiments demonstrating TRIM11 in mouse primary hippocampal neurons. Figure 41A depicts MTT analysis showing that GST or TRIM11 incubated alpha-Syn-induced cell death. Figure 41B depicts immunofluorescence analysis of p-alpha-Syn or p62 in alpha-Syn fiber treated Hippocampal neuron cells.

Figure 42, comprising Figure 42A through Figure 42C, depicts results from experiments demonstrating TRIM11 is upregulated in response to heat shock in cortical and hippocampal neurons. Figure 42A depicts immunoblotting of mouse primary cortical neurons treated with heat shock for 30 minutes at 42ºC and recovered for 3 hours. Figure 42B depicts immunoblotting of mouse primary hippocampal neurons treated with heat shock for 30 min at 42C° and recovered for 3 hours. Figure 42C depicts semi-quantitative PCR analysis of *TRIM11*, *HSP70*, *HSP90* and *GAPDH* in response to heat shock in hippocampal neurons.

DETAILED DESCRIPTION

The present invention is related to the discovery of the role of members of the tripartite motif (TRIM) family of proteins and the SUMO-dependent ubiquitin ligase RNF4 in the recognition and degradation of misfolded proteins, which play a role in the pathology of a variety of neurodegenerative disorders.

In one aspect, the present invention provides compositions and methods to treat or prevent a disease or disorder associated with misfolded protein or protein aggregates. It is demonstrated herein that TRIM proteins have roles in targeting misfolded proteins for proteosomal degradation, as chaperone protein, and in disaggregating protein aggregates or inclusions. Thus, in certain aspects, the present invention can be used to eliminate intracellular or extracellular misfolded proteins, protein aggregates, or protein inclusions.

For example, in certain embodiments, the invention provides compositions and methods to treat or prevent a neurodegenerative disorder in a subject in need thereof. For example, in certain embodiments, the invention provides compositions and methods for the treatment or prevention of neurodegenerative disorders that are poly-glutamine (polyQ) disorders, where repeats of the CAG codon encode proteins with polyglutamine tracts that can result in misfolded protein aggregates. Exemplary polyQ disorders include, but are not limited to Spinocerebellar ataxia (SCA) Type 1 (SCA1), SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, and Dentatorubral-pallidoluysian atrophy (DRPLA). In certain embodiments, the invention provides compositions and methods for the treatment of neurodegenerative disorders associated with misfolded proteins or protein aggregates, including, but not limited to, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), transmissible spongiform encephalopathies (prion disease), tauopathies, and Frontotemporal lobar degeneration (FTLD). However, the present invention is not limited to the treatment or prevention of neurodegenerative disorders. Rather, the invention encompasses the treatment or prevention of any disease or disorder associated with a misfolded protein or protein aggregate. Other such diseases and disorders include, but is not limited to AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen

amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis. In certain embodiments, the invention encompasses the treatment or prevention of cancer associated with p53 mutant aggregates, including but not limited to bladder carcinoma, astrocytoma, pharynx carcinoma, lymphoma, and adenocarcinoma.

In one aspect, the invention encompasses the use of one or more TRIM proteins to stabilize a misfolded protein. In certain aspects, stabilization of a functional misfolded protein via one or more TRIM proteins described herein can treat or prevent a disease or disorder associated with the misfolded protein. For example, in one embodiment, stabilization of mutant cystic fibrosis transmembrane conductance regulator (CFTR), via one or more TRIM proteins described herein, would allow mutant CFTR to function instead of being degraded. It is envisioned that using TRIM proteins to stabilize misfolded proteins can be used to treat cystic fibrosis and other diseases associated with degradation of partially functional proteins. Stabilization of proteins, via one or more TRIM proteins described herein, can be used to treat any disease or disorder associated with degradation of functional mutant protein, including but not limited to cystic fibrosis and lysosomal storage diseases such as Gaucher's disease and Fabry's disease.

In one aspect, the present invention provides compositions and methods to increase the expression, activity, or both of a TRIM protein. In certain embodiments, the composition comprises a nucleic acid molecule, expression vector, protein, peptide, small molecule, or the like, which increases the expression, activity, or both of one or more TRIM proteins.

In one aspect, the present invention provides compositions and methods to increase the expression, activity, or both of one or more SUMO-targeted ubiquitin ligases (STUbL). In certain embodiments, the composition comprises a nucleic acid molecule, expression vector, protein, peptide, small molecule, or the like, which increases the expression, activity, or both of one or more STUbLs.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, or ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term "abnormal" when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (e.g., age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the "normal" (expected) respective characteristic. Characteristics which are normal or expected for one cell or tissue type, might be abnormal for a different cell or tissue type.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

A disease or disorder is "alleviated" if the severity of a sign or symptom of the disease or disorder, the frequency with which such a sign or symptom is experienced by a patient, or both, is reduced.

An "effective amount" or "therapeutically effective amount" of a compound is that amount of a compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered. An "effective amount" of a delivery vehicle is that amount sufficient to effectively bind or deliver a compound.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of a compound, composition, vector, or delivery system of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound, composition, vector, or delivery system of the invention or be shipped together with a container which contains the identified compound, composition, vector, or delivery system. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

The terms "patient," "subject," "individual," and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in vivo, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of a disease or disorder, for the purpose of diminishing or eliminating those signs or symptoms.

As used herein, "treating a disease or disorder" means reducing the severity and/or frequency with which a sign or symptom of the disease or disorder is experienced by a patient.

The phrase "biological sample" as used herein, is intended to include any sample comprising a cell, a tissue, or a bodily fluid in which expression of a nucleic acid or polypeptide is present or can be detected. Samples that are liquid in nature are referred to herein as "bodily fluids." Biological samples may be obtained from a patient by a variety of techniques including, for example, by scraping or swabbing an area of the

subject or by using a needle to obtain bodily fluids. Methods for collecting various body samples are well known in the art.

As used herein, an "immunoassay" refers to any binding assay that uses an antibody capable of binding specifically to a target molecule to detect and quantify the target molecule.

By the term "specifically binds," as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific.

In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

A "coding region" of a mRNA molecule consists of the nucleotide residues of the mRNA molecule which are matched with an anti-codon region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues comprising codons for amino acid residues which are not present in the mature protein encoded by the mRNA molecule (e.g., amino acid residues in a protein export signal sequence).

"Complementary" as used herein to refer to a nucleic acid, refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

"Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in its normal context in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural context is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, i.e., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, i.e., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, i.e., RNA or DNA or proteins, which naturally accompany it in the cell. The term

therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (i.e., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

The term "polynucleotide" as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric "nucleotides." The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR, and the like, and by synthetic means.

As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides

include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

As used herein, "conjugated" refers to covalent attachment of one molecule to a second molecule.

"Variant" as the term is used herein, is a nucleic acid sequence or a peptide sequence that differs in sequence from a reference nucleic acid sequence or peptide sequence respectively, but retains essential biological properties of the reference molecule. Changes in the sequence of a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid, or may result in amino acid substitutions, additions, deletions, fusions and truncations. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a nucleic acid or peptide can be a naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and peptides may be made by mutagenesis techniques or by direct synthesis.

As used herein, a "modulator of one or more TRIM proteins" is a compound that modifies the expression, activity or biological function of the TRIM protein as compared to the expression, activity or biological function of the TRIM protein in the absence of the modulator.

As used herein, a "modulator of RNF4" is a compound that modifies the expression, activity or biological function of RNF4 as compared to the expression, activity or biological function of RNF4 in the absence of the modulator.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from

1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

In one aspect, the present invention provides compositions and methods to treat or prevent a disease or disorder associated with misfolded proteins or protein aggregates. For example, the present invention provides compositions and methods to increase the recognition and elimination of misfolded proteins. In certain embodiments, the invention provides for the SUMO-mediated ubiquitination and eventual degradation of misfolded protein. In certain embodiments, the invention provides for the disaggregation of protein aggregates or inclusions. The present invention can thus be used to eliminate misfolded proteins, protein aggregates, or protein inclusions, both intracellularly or extracellularly.

The present invention is related to the discovery of the role of members of the tripartite motif (TRIM) family of proteins and the SUMO-targeted ubiquitin ligase (STUbL), RNF4, in the recognition and degradation of misfolded proteins, which play a role in the pathology of a variety of diseases and disorders, including many neurodegenerative diseases and disorders.

The data presented herein demonstrates that members of the TRIM protein family co-localize with misfolded proteins and mediate the degradation of misfolded proteins. Further, it is described herein that RNF4 is an ubiquitin ligase which mediates the ubiquitination and degradation of misfolded proteins. Thus, the present invention provides compositions that increase the expression, activity, or both of one or more TRIM proteins, one or more STUbLs, or a combination thereof. For example, it is demonstrated that nucleic acid molecules encoding a TRIM protein and recombinant TRIM proteins promotes the degradation of misfolded protein.

In one embodiment, the composition comprises a modulator of the expression or activity of one or more TRIM proteins. For example, in one embodiment, the modulator increases the expression or activity of one or more TRIM proteins. The one or more TRIM proteins, include, any member of the TRIM protein family, including

mammalian and non-mammalian members. In certain embodiments, the modulator increases the expression or activity of one or more of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

In one embodiment, the composition comprises a modulator of one or more STUbLs. For example, in one embodiment, the modulator increases the expression or activity of one or more STUbLs. Exemplary STUbLs include, but are not limited to RNF4 and RNF111 (also known as Arkadia).

In one embodiment, the composition comprises a modulator of one or more TRIM proteins and a modulator of one or more STUbLs.

The present invention provides a method for treating or preventing a disease or disorder associated with misfolded proteins or protein aggregates, in a subject in need. It is found herein that increasing the level of expression or activity of a TRIM protein or STUbL, can promote the degradation of misfolded protein, thereby treating the disease or disorder in the subject.

Examples of neurodegenerative diseases or disorders which may be treated or prevented by the compositions and methods of this invention include, but are not limited to polyQ disorders such as SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), transmissible spongiform encephalopathies (prion disease), tauopathies, and Frontotemporal lobar degeneration (FTLD). However, the present invention is not limited to the treatment or prevention of neurodegenerative disorders. Rather, the invention encompasses the treatment or prevention of any disease or disorder associated with a misfolded protein or protein aggregate. Other such diseases and disorders include, but is not limited to AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis,

lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis. In certain embodiments, the invention encompasses the treatment or prevention of cancer associated with p53 mutant aggregates, including but not limited to bladder carcinoma, astrocytoma, pharynx carcinoma, lymphoma, and adenocarcinoma.

In one aspect, the invention encompasses the use of one or more TRIM proteins to stabilize a misfolded protein. In certain aspects, stabilization of a functional misfolded protein via one or more TRIM proteins described herein can treat or prevent a disease or disorder associated with the misfolded protein, including but not limited to cystic fibrosis and lysosomal storage diseases such as Gaucher's disease and Fabry's disease.

In one aspect, the present invention provides a method to diagnose a disease or disorder associated with a misfolded protein or protein aggregate. For example, in one embodiment, one or more TRIM proteins or one or more STUbLs are used as diagnostic markers.

In one aspect, the present invention is directed to compositions and methods for manufacture of a recombinant protein of interest. For example, in certain embodiments, the one or more TRIM proteins and one or more STUbLs can be used to disaggregate protein aggregates of recombinant protein of interest.

Compositions

In various embodiments, the present invention includes modulator compositions and methods of preventing and treating a disease or disorder associated with misfolded protein or protein aggregates. In various embodiments, the modulator compositions and methods of preventing or treating of the invention modulate the level or activity of a gene, or gene product. In some embodiments, the modulator composition of the invention is an activator that increases the level or activity of a gene, or gene product.

It will be understood by one skilled in the art, based upon the disclosure provided herein, that modulating a gene, or gene product, encompasses modulating the level or activity of a gene, or gene product, including, but not limited to, modulating the transcription, translation, splicing, degradation, enzymatic activity, binding activity, or combinations thereof. Thus, modulating the level or activity of a gene, or gene product, includes, but is not limited to, modulating transcription, translation, degradation, splicing, or combinations thereof, of a nucleic acid; and it also includes modulating any activity of polypeptide gene product as well.

In one embodiment, the modulator increases the expression or activity of a gene or gene product by increasing production of the gene or gene product, for example by modulating transcription of the gene or translation of the gene product. In one embodiment, the modulator increases the expression or activity of a gene or gene product by providing exogenous gene or gene product. For example, in certain embodiments, the modulator comprises an isolated nucleic acid encoding one or more TRIM proteins or one or more STUbLs. In one embodiment, the modulator comprises an isolated nucleic acid encoding one or more TRIM proteins and one or more STUbLs. In certain embodiments, the modulator comprises an isolated peptide comprising one or more TRIM proteins or one or more STUbLs. In one embodiment, the modulator comprises an isolated peptide comprising one or more TRIM proteins and one or more STUbLs. In one embodiment, the modulator increases the expression or activity of a gene or gene product by inhibiting the degradation of the gene or gene product. For example, in one embodiment, the modulator decreases the ubiquitination, proteosomal degradation, or proteolysis of one or more TRIM proteins or one or more STUbLs. In one embodiment, the modulator increases the stability or half-life of a gene product.

In various embodiments, the modulated gene, or gene product, is one or more TRIM proteins. For example, it is described herein that TRIM proteins recognize misfolded proteins and mediate the degradation of misfolded proteins and protein aggregates. In one embodiment, the gene or gene product is one or more STUbLs. For example, it is described herein that RNF4, a member of the STUbl family of proteins, mediates the degradation of misfolded proteins and protein aggregates.

Modulation of a gene, or gene product, can be assessed using a wide variety of methods, including those disclosed herein, as well as methods known in the art or to be developed in the future. That is, the routineer would appreciate, based upon the disclosure provided herein, that modulating the level or activity of a gene, or gene product, can be readily assessed using methods that assess the level of a nucleic acid encoding a gene product (e.g., mRNA), the level of polypeptide gene product present in a biological sample, the activity of polypeptide gene product present in a biological sample, or combinations thereof.

The modulator compositions and methods of the invention that modulate the level or activity of a gene, or gene product, include, but should not be construed as being limited to, a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), or combinations thereof. One of skill in the art would readily appreciate, based on the disclosure provided herein, that a modulator composition encompasses a chemical compound that modulates the level or activity of a gene, or gene product. Additionally, a modulator composition encompasses a chemically modified compound, and derivatives, as is well known to one of skill in the chemical arts.

In one embodiment, the modulator composition of the present invention is an agonist, which increases the expression, activity, or biological function of a gene or gene product. For example, in certain embodiments, the modulator of the present invention is an agonist of one or more TRIM proteins or one or more STUbLs.

Further, one of skill in the art would, when equipped with this disclosure and the methods exemplified herein, appreciate that modulators include such modulators as discovered in the future, as can be identified by well-known criteria in the art of pharmacology, such as the physiological results of modulation of the genes, and gene products, as described in detail herein and/or as known in the art. Therefore, the present invention is not limited in any way to any particular modulator composition as exemplified or disclosed herein; rather, the invention encompasses those modulator compositions that would be understood by the routineer to be useful as are known in the art and as are discovered in the future.

Further methods of identifying and producing modulator compositions are well known to those of ordinary skill in the art. Alternatively, a modulator can be synthesized chemically. Further, the routineer would appreciate, based upon the teachings provided herein, that a modulator composition can be obtained from a recombinant organism. Compositions and methods for chemically synthesizing modulators and for obtaining them from natural sources are well known in the art and are described in the art.

One of skill in the art will appreciate that a modulator can be administered as a small molecule chemical, a polypeptide, a peptide, an antibody, a nucleic acid construct encoding a protein, an antisense nucleic acid, a nucleic acid construct encoding an antisense nucleic acid, or combinations thereof. Numerous vectors and other compositions and methods are well known for administering a protein or a nucleic acid construct encoding a protein to cells or tissues. Therefore, the invention includes a peptide or a nucleic acid encoding a peptide that is modulator of a gene, or gene product. For example, the invention includes a peptide or a nucleic acid encoding a peptide that comprises one or more TRIM proteins, one or more STUbLs, or a combination thereof. (Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

Peptides

In one embodiment, the composition of the present invention comprises one or more peptides. For example, in one embodiment, a peptide of the composition comprises an amino acid sequence of one or more TRIM proteins. For example, in one embodiment, the peptide comprises one or more of TRIM5δ, TRIM 11, TRIM19, TRIM 21, TRIM27, and TRIM32. In certain embodiments, the peptide comprises an amino acid sequence of one or more STUbLs. For example, in one embodiment, the peptide comprises one or more of RNF4 and RNF111 (Arkadia).

Exemplary amino acid sequences of TRIM proteins, and cDNA nucleotide sequences encoding TRIM proteins is provided in Table 1 below.

Table 1: TRIM protein nucleotide and amino acid sequences

Name	Nucleotide Accession Number	Nucleotide Sequence	Protein Accession Number	Amino Acid Sequence
TRIM1	NM_012216.3	SEQ ID NO: 1	NP_036348.2	SEQ ID NO: 2
TRIM2	NM_015271.4	SEQ ID NO: 3	NP_056086.2	SEQ ID NO: 4
TRIM3	NM_006458.3	SEQ ID NO: 5	NP_006449.2	SEQ ID NO: 6
TRIM4	NM_033017.3	SEQ ID NO: 7	NP_148977.2	SEQ ID NO: 8
TRIM5	NM_033034.2	SEQ ID NO: 9	NP_149023.2	SEQ ID NO: 10
TRIM6	NM_001003818.2	SEQ ID NO: 11	NP_001003818.1	SEQ ID NO: 12
TRIM7	NM_033342.3	SEQ ID NO: 13	NP_203128.1	SEQ ID NO: 14
TRIM8	NM_030912.2	SEQ ID NO: 15	NP_112174.2	SEQ ID NO: 16
TRIM9	NM_015163.5	SEQ ID NO: 17	NP_055978.4	SEQ ID NO: 18
TRIM10	NM_006778.3	SEQ ID NO: 19	NP_006769.2	SEQ ID NO: 20
TRIM11	NM_145214.2	SEQ ID NO: 21	NP_660215.1	SEQ ID NO: 22
TRIM12	NM_023835.2	SEQ ID NO: 23	NP_076324.2	SEQ ID NO: 24
TRIM13	NM_005798.4	SEQ ID NO: 25	NP_005789.2	SEQ ID NO: 26
TRIM14	NM_014788.3	SEQ ID NO: 27	NP_055603.2	SEQ ID NO: 28
TRIM15	NM_033229.2	SEQ ID NO: 29	NP_150232.2	SEQ ID NO: 30
TRIM16	NM_006470.3	SEQ ID NO: 31	NP_006461.3	SEQ ID NO: 32
TRIM17	NM_016102.3	SEQ ID NO: 33	NP_057186.1	SEQ ID NO: 34
TRIM18	NM_000381.3	SEQ ID NO: 35	NP_000372.1	SEQ ID NO: 36
TRIM19	NM_033238.2	SEQ ID NO: 37	NP_150241.2	SEQ ID NO: 38
TRIM20	NM_000243.2	SEQ ID NO: 39	NP_000234.1	SEQ ID NO: 40
TRIM21	NM_003141.3	SEQ ID NO: 41	NP_003132.2	SEQ ID NO: 42
TRIM22	NM_006074.4	SEQ ID NO: 43	NP_006065.2	SEQ ID NO: 44
TRIM23	NM_001656.3	SEQ ID NO: 45	NP_001647.1	SEQ ID NO: 46
TRIM24	NM_015905.2	SEQ ID NO: 47	NP_056989.2	SEQ ID NO: 48
TRIM25	NM_005082.4	SEQ ID NO: 49	NP_005073.2	SEQ ID NO: 50
TRIM26	NM_003449.4	SEQ ID NO: 51	NP_003440.1	SEQ ID NO: 52
TRIM27	NM_006510.4	SEQ ID NO: 53	NP_006501.1	SEQ ID NO: 54
TRIM28	NM_005762.2	SEQ ID NO: 55	NP_005753.1	SEQ ID NO: 56
TRIM29	NM_012101.3	SEQ ID NO: 57	NP_036233.2	SEQ ID NO: 58
TRIM30	NM_009099.2	SEQ ID NO: 59	NP_033125.2	SEQ ID NO: 60
TRIM31	NM_007028.4	SEQ ID NO: 61	NP_008959.3	SEQ ID NO: 62
TRIM32	NM_012210.3	SEQ ID NO: 63	NP_036342.2	SEQ ID NO: 64
TRIM33	NM_015906.3	SEQ ID NO: 65	NP_056990.3	SEQ ID NO: 66
TRIM34	NM_021616.5	SEQ ID NO: 67	NP_067629.2	SEQ ID NO: 68
TRIM35	NM_171982.4	SEQ ID NO: 69	NP_741983.2	SEQ ID NO: 70
TRIM36	NM_018700.3	SEQ ID NO: 71	NP_061170.2	SEQ ID NO: 72
TRIM37	NM_015294.4	SEQ ID NO: 73	NP_056109.1	SEQ ID NO: 74

TRIM38	NM_006355.4	SEQ ID NO: 75	NP_006346.1	SEQ ID NO: 76
TRIM39	NM_021253.3	SEQ ID NO: 77	NP_067076.2	SEQ ID NO: 78
TRIM40	NM_001286633.1	SEQ ID NO: 79	NP_001273562.1	SEQ ID NO: 80
TRIM41	NM_033549.4	SEQ ID NO: 81	NP_291027.3	SEQ ID NO: 82
TRIM42	NM_152616.4	SEQ ID NO: 83	NP_689829.3	SEQ ID NO: 84
TRIM43	NM_138800.1	SEQ ID NO: 85	NP_620155.1	SEQ ID NO: 86
TRIM44	NM_017583.5	SEQ ID NO: 87	NP_060053.2	SEQ ID NO: 88
TRIM45	NM_025188.3	SEQ ID NO: 89	NP_079464.2	SEQ ID NO: 90
TRIM46	NM_025058.4	SEQ ID NO: 91	NP_079334.3	SEQ ID NO: 92
TRIM47	NM_033452.2	SEQ ID NO: 93	NP_258411.2	SEQ ID NO: 94
TRIM48	NM_024114.3	SEQ ID NO: 95	NP_077019.2	SEQ ID NO: 96
TRIM49	NM_020358.2	SEQ ID NO: 97	NP_065091.1	SEQ ID NO: 98
TRIM50	NM_178125.3	SEQ ID NO: 99	NP_835226.2	SEQ ID NO: 100
TRIM51	NM_032681.3	SEQ ID NO: 101	NP_116070.2	SEQ ID NO: 102
TRIM52	NM_032765.2	SEQ ID NO: 103	NP_116154.1	SEQ ID NO: 104
TRIM54	NM_032546.3	SEQ ID NO: 105	NP_115935.3	SEQ ID NO: 106
TRIM55	NM_184085.1	SEQ ID NO: 107	NP_908973.1	SEQ ID NO: 108
TRIM56	NM_030961.2	SEQ ID NO: 109	NP_112223.1	SEQ ID NO: 110
TRIM58	NM_015431.3	SEQ ID NO: 111	NP_056246.3	SEQ ID NO: 112
TRIM59	NM_173084.2	SEQ ID NO: 113	NP_775107.1	SEQ ID NO: 114
TRIM60	NM_152620.2	SEQ ID NO: 115	NP_689833.1	SEQ ID NO: 116
TRIM61	NM_001012414.2	SEQ ID NO: 117	NP_001012414.1	SEQ ID NO: 118
TRIM62	NM_018207.2	SEQ ID NO: 119	NP_060677.2	SEQ ID NO: 120
TRIM63	NM_032588.3	SEQ ID NO: 121	NP_115977.2	SEQ ID NO: 122
TRIM64	NM_001136486.1	SEQ ID NO: 123	NP_001129958.1	SEQ ID NO: 124
TRIM65	NM_173547.3	SEQ ID NO: 125	NP_775818.2	SEQ ID NO: 126
TRIM66	NM_014818.1	SEQ ID NO: 127	NP_055633.1	SEQ ID NO: 128
TRIM67	NM_001004342.3	SEQ ID NO: 129	NP_001004342.3	SEQ ID NO: 130
TRIM68	NM_018073.7	SEQ ID NO: 131	NP_060543.5	SEQ ID NO: 132
TRIM69	NM_080745.4	SEQ ID NO: 133	NP_542783.2	SEQ ID NO: 134
TRIM70	NM_001037330.1	SEQ ID NO: 135	NP_001032407.1	SEQ ID NO: 136
TRIM71	NM_001039111.2	SEQ ID NO: 137	NP_001034200.1	SEQ ID NO: 138
TRIM72	NM_001008274.3	SEQ ID NO: 139	NP_001008275.2	SEQ ID NO: 140
TRIM73	NM_198924.3	SEQ ID NO: 141	NP_944606.2	SEQ ID NO: 142
TRIM74	NM_198853.2	SEQ ID NO: 143	NP_942150.1	SEQ ID NO: 144
TRIM75	NM_001033429.2	SEQ ID NO: 145	NP_001028601.1	SEQ ID NO: 146
TRIM76	NM_153610.4	SEQ ID NO: 164	NP_705838.3	SEQ ID NO: 165
TRIM77	NM_001146162.1	SEQ ID NO: 166	NP_001139634.1	SEQ ID NO: 167

The invention should also be construed to include any form of a peptide having substantial homology to the peptides disclosed herein. Preferably, a peptide which

is "substantially homologous" is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to amino acid sequence of the peptides disclosed herein.

In one embodiment, the composition of the invention comprises a peptide, a fragment of a peptide, a homolog, a variant, a derivative or a salt of a peptide described herein. For example, in certain embodiments, the composition comprises a peptide comprising one or more TRIM proteins, a fragment of one or more TRIM proteins, a homolog of one or more TRIM proteins, a variant of one or more TRIM proteins, a derivative of one or more TRIM proteins, or a salt of one or more TRIM proteins. In certain embodiments, the composition comprises a peptide comprising one or more STUbLs, a fragment of one or more STUbLs, a homolog of one or more STUbLs, a variant of one or more STUbLs, a derivative of one or more STUbLs, or a salt of one or more STUbLs.

In one embodiment, the composition comprises a combination of the peptides described herein. For example, in certain embodiments, the composition comprises a peptide comprising one or more TRIM proteins and a peptide comprising one or more STUbLs. In one embodiment, the composition comprises a peptide comprising one or more TRIM proteins and one or more STUbLs.

In certain embodiments, the peptide comprises a targeting domain, which targets the peptide to a desired location. For example, in certain embodiments, the targeting domain binds to a targeted cell, protein, or protein aggregate, thereby delivering the therapeutic peptide to a desired location. For example, in one embodiment, the targeting domain is directed to bind to a protein or protein aggregate associated with a disease or disorder, including but not limited to the proteins and protein aggregates of amyloid-beta, alpha-synuclein, tau, prions, SOD1, TDP-43, FUS, p53 mutants, and proteins associated with polyglutamine repeats, such as huntingtin, ataxins.

In certain embodiments, the targeting domain comprises a peptide, nucleic acid, small molecule, or the like, which has the ability to bind to the targeted cell, protein, or protein aggregate. For example, in one embodiment, the targeting domain comprises

an antibody or antibody fragment which binds to a targeted cell, protein, or protein aggregate.

The peptide of the present invention may be made using chemical methods. For example, peptides can be synthesized by solid phase techniques (Roberge J Y et al (1995) Science 269: 202-204), cleaved from the resin, and purified by preparative high performance liquid chromatography. Automated synthesis may be achieved, for example, using the ABI 431 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The peptide may alternatively be made by recombinant means or by cleavage from a longer polypeptide. The composition of a peptide may be confirmed by amino acid analysis or sequencing.

The variants of the peptides according to the present invention may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, (ii) one in which there are one or more modified amino acid residues, e.g., residues that are modified by the attachment of substituent groups, (iii) one in which the peptide is an alternative splice variant of the peptide of the present invention, (iv) fragments of the peptides and/or (v) one in which the peptide is fused with another peptide, such as a leader or secretory sequence or a sequence which is employed for purification (for example, His-tag) or for detection (for example, Sv5 epitope tag). The fragments include peptides generated via proteolytic cleavage (including multi-site proteolysis) of an original sequence. Variants may be post-translationally, or chemically modified. Such variants are deemed to be within the scope of those skilled in the art from the teaching herein.

The peptides of the invention can be post-translationally modified. For example, post-translational modifications that fall within the scope of the present invention include signal peptide cleavage, glycosylation, acetylation, isoprenylation, proteolysis, myristoylation, protein folding and proteolytic processing, etc. Some modifications or processing events require introduction of additional biological machinery. For example, processing events, such as signal peptide cleavage and core

glycosylation, are examined by adding canine microsomal membranes or Xenopus egg extracts (U.S. Pat. No. 6,103,489) to a standard translation reaction.

The peptides of the invention may include unnatural amino acids formed by post-translational modification or by introducing unnatural amino acids during translation. A variety of approaches are available for introducing unnatural amino acids during protein translation. By way of example, special tRNAs, such as tRNAs which have suppressor properties, suppressor tRNAs, have been used in the process of site-directed non-native amino acid replacement (SNAAR). In SNAAR, a unique codon is required on the mRNA and the suppressor tRNA, acting to target a non-native amino acid to a unique site during the protein synthesis (described in WO90/05785). However, the suppressor tRNA must not be recognizable by the aminoacyl tRNA synthetases present in the protein translation system. In certain cases, a non-native amino acid can be formed after the tRNA molecule is aminoacylated using chemical reactions which specifically modify the native amino acid and do not significantly alter the functional activity of the aminoacylated tRNA. These reactions are referred to as post-aminoacylation modifications. For example, the epsilon-amino group of the lysine linked to its cognate tRNA ($tRNA_{LYS}$), could be modified with an amine specific photoaffinity label.

The peptides of the invention may be conjugated with other molecules, such as proteins, to prepare fusion proteins. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins provided that the resulting fusion protein retains the functionality of the peptide of the invention.

Cyclic derivatives of the peptides the invention are also part of the present invention. Cyclization may allow the peptide to assume a more favorable conformation for association with other molecules. Cyclization may be achieved using techniques known in the art. For example, disulfide bonds may be formed between two appropriately spaced components having free sulfhydryl groups, or an amide bond may be formed between an amino group of one component and a carboxyl group of another component. Cyclization may also be achieved using an azobenzene-containing amino acid as described by Ulysse, L., et al., J. Am. Chem. Soc. 1995, 117, 8466-8467. The components that form the bonds may be side chains of amino acids, non-amino acid components or a combination of the two. In an embodiment of the invention, cyclic

peptides may comprise a beta-turn in the right position. Beta-turns may be introduced into the peptides of the invention by adding the amino acids Pro-Gly at the right position.

It may be desirable to produce a cyclic peptide which is more flexible than the cyclic peptides containing peptide bond linkages as described above. A more flexible peptide may be prepared by introducing cysteines at the right and left position of the peptide and forming a disulphide bridge between the two cysteines. The two cysteines are arranged so as not to deform the beta-sheet and turn. The peptide is more flexible as a result of the length of the disulfide linkage and the smaller number of hydrogen bonds in the beta-sheet portion. The relative flexibility of a cyclic peptide can be determined by molecular dynamics simulations.

The peptides of the invention may be converted into pharmaceutical salts by reacting with inorganic acids such as hydrochloric acid, sulfuric acid, hydrobromic acid, phosphoric acid, etc., or organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid, benezenesulfonic acid, and toluenesulfonic acids.

Peptides of the invention may also have modifications. Modifications (which do not normally alter primary sequence) include in vivo, or in vitro chemical derivatization of polypeptides, e.g., acetylation, or carboxylation. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are peptides which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Such variants include those containing residues other than naturally-occurring L-amino acids, e.g., D-amino acids or non-naturally-occurring synthetic amino acids. The peptides of the invention may further be conjugated to non-amino acid

moieties that are useful in their therapeutic application. In particular, moieties that improve the stability, biological half-life, water solubility, and/or immunologic characteristics of the peptide are useful. A non-limiting example of such a moiety is polyethylene glycol (PEG).

Covalent attachment of biologically active compounds to water-soluble polymers is one method for alteration and control of biodistribution, pharmacokinetics, and often, toxicity for these compounds (Duncan et al., 1984, Adv. Polym. Sci. 57:53-101). Many water-soluble polymers have been used to achieve these effects, such as poly(sialic acid), dextran, poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA), poly(N-vinylpyrrolidone) (PVP), poly(vinyl alcohol) (PVA), poly(ethylene glycol-co-propylene glycol), poly(N-acryloyl morpholine (PAcM), and poly(ethylene glycol) (PEG) (Powell, 1980, Polyethylene glycol. In R. L. Davidson (Ed.) Handbook of Water Soluble Gums and Resins. McGraw-Hill, New York, chapter 18). PEG possess an ideal set of properties: very low toxicity (Pang, 1993, J. Am. Coll. Toxicol. 12: 429-456) excellent solubility in aqueous solution (Powell, supra), low immunogenicity and antigenicity (Dreborg et al., 1990, Crit. Rev. Ther. Drug Carrier Syst. 6: 315-365). PEG-conjugated or "PEGylated" protein therapeutics, containing single or multiple chains of polyethylene glycol on the protein, have been described in the scientific literature (Clark et al., 1996, J. Biol. Chem. 271: 21969-21977; Hershfield, 1997, Biochemistry and immunology of poly(ethylene glycol)-modified adenosine deaminase (PEG-ADA). In J. M. Harris and S. Zalipsky (Eds) Poly(ethylene glycol): Chemistry and Biological Applications. American Chemical Society, Washington, D.C., p 145-154; Olson et al., 1997, Preparation and characterization of poly(ethylene glycol)ylated human growth hormone antagonist. In J. M. Harris and S. Zalipsky (Eds) Poly(ethylene glycol): Chemistry and Biological Applications. American Chemical Society, Washington, D.C., p 170-181).

A peptide of the invention may be synthesized by conventional techniques. For example, the peptides of the invention may be synthesized by chemical synthesis using solid phase peptide synthesis. These methods employ either solid or solution phase synthesis methods (see for example, J. M. Stewart, and J. D. Young, Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford Ill. (1984) and G. Barany and R. B. Merrifield, The Peptides: Analysis Synthesis, Biology editors E. Gross

and J. Meienhofer Vol. 2 Academic Press, New York, 1980, pp. 3-254 for solid phase synthesis techniques; and M Bodansky, Principles of Peptide Synthesis, Springer-Verlag, Berlin 1984, and E. Gross and J. Meienhofer, Eds., The Peptides: Analysis, Synthesis, Biology, suprs, Vol 1, for classical solution synthesis.)

The peptides may be chemically synthesized by Merrifield-type solid phase peptide synthesis. This method may be routinely performed to yield peptides up to about 60-70 residues in length, and may, in some cases, be utilized to make peptides up to about 100 amino acids long. Larger peptides may also be generated synthetically via fragment condensation or native chemical ligation (Dawson et al., 2000, Ann. Rev. Biochem. 69:923-960). An advantage to the utilization of a synthetic peptide route is the ability to produce large amounts of peptides, even those that rarely occur naturally, with relatively high purities, i.e., purities sufficient for research, diagnostic or therapeutic purposes.

Solid phase peptide synthesis is described by Stewart et al. in Solid Phase Peptide Synthesis, 2nd Edition, 1984, Pierce Chemical Company, Rockford, Ill.; and Bodanszky and Bodanszky in The Practice of Peptide Synthesis, 1984, Springer-Verlag, New York. At the outset, a suitably protected amino acid residue is attached through its carboxyl group to a derivatized, insoluble polymeric support, such as cross-linked polystyrene or polyamide resin. “Suitably protected” refers to the presence of protecting groups on both the alpha-amino group of the amino acid, and on any side chain functional groups. Side chain protecting groups are generally stable to the solvents, reagents and reaction conditions used throughout the synthesis, and are removable under conditions which will not affect the final peptide product. Stepwise synthesis of the oligopeptide is carried out by the removal of the N-protecting group from the initial amino acid, and coupling thereto of the carboxyl end of the next amino acid in the sequence of the desired peptide. This amino acid is also suitably protected. The carboxyl of the incoming amino acid can be activated to react with the N-terminus of the support-bound amino acid by formation into a reactive group, such as formation into a carbodiimide, a symmetric acid anhydride, or an “active ester” group, such as hydroxybenzotriazole or pentafluorophenyl esters.

Examples of solid phase peptide synthesis methods include the BOC method which utilized tert-butyloxcarbonyl as the alpha-amino protecting group, and the FMOC method which utilizes 9-fluorenylmethyloxcarbonyl to protect the alpha-amino of the amino acid residues, both which methods are well-known by those of skill in the art.

Incorporation of N- and/or C-blocking groups may also be achieved using protocols conventional to solid phase peptide synthesis methods. For incorporation of C-terminal blocking groups, for example, synthesis of the desired peptide is typically performed using, as solid phase, a supporting resin that has been chemically modified so that cleavage from the resin results in a peptide having the desired C-terminal blocking group. To provide peptides in which the C-terminus bears a primary amino blocking group, for instance, synthesis is performed using a p-methylbenzhydrylamine (MBHA) resin, so that, when peptide synthesis is completed, treatment with hydrofluoric acid releases the desired C-terminally amidated peptide. Similarly, incorporation of an N-methylamine blocking group at the C-terminus is achieved using N-methylaminoethyl-derivatized DVB, resin, which upon HF treatment releases a peptide bearing an N-methylamidated C-terminus. Blockage of the C-terminus by esterification can also be achieved using conventional procedures. This entails use of resin/blocking group combination that permits release of side-chain peptide from the resin, to allow for subsequent reaction with the desired alcohol, to form the ester function. FMOC protecting group, in combination with DVB resin derivatized with methoxyalkoxybenzyl alcohol or equivalent linker, can be used for this purpose, with cleavage from the support being effected by TFA in dicholoromethane. Esterification of the suitably activated carboxyl function, e.g. with DCC, can then proceed by addition of the desired alcohol, followed by de-protection and isolation of the esterified peptide product.

The peptides of the invention may be prepared by standard chemical or biological means of peptide synthesis. Biological methods include, without limitation, expression of a nucleic acid encoding a peptide in a host cell or in an in vitro translation system.

Included in the invention are nucleic acid sequences that encode the peptide of the invention. In one embodiment, the invention includes nucleic acid sequences encoding the amino acid sequence of one or more TRIM proteins or one or

more STUbLs. Accordingly, subclones of a nucleic acid sequence encoding a peptide of the invention can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Laboratory, Cold Springs Harbor, New York (2012), and Ausubel et al. (ed.), *Current Protocols in Molecular Biology*, John Wiley & Sons (New York, NY) (1999 and preceding editions), each of which is hereby incorporated by reference in its entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for a particular activity.

Combined with certain formulations, such peptides can be effective intracellular agents. However, in order to increase the efficacy of such peptides, the one or more peptides of the invention can be provided a fusion peptide along with a second peptide which promotes "transcytosis", e.g., uptake of the peptide by cells. For example, in one embodiment, the peptide may comprise a cell-penetrating domain, for example a cell-penetrating peptide (CPP) to allow for the peptide to enter a cell. In one embodiment, the CPP is derived from HIV Tat.

To illustrate, the one or more peptides of the present invention can be provided as part of a fusion polypeptide with all or a fragment of the N-terminal domain of the HIV protein Tat, e.g., residues 1-72 of Tat or a smaller fragment thereof which can promote transcytosis. In one embodiment, the peptide comprises the protein transduction domain of HIV Tat (YGRKKRRQRRR; (SEQ ID NO: 163)). In other embodiments, the one or more peptides can be provided a fusion polypeptide with all or a portion of the antenopedia III protein. Other cell-penetrating domains that mediate uptake of the peptide are known in the art, and are equally applicable for use in a fusion peptide of the present invention.

Nucleic Acids

In one embodiment, the composition of the invention comprises one or isolated nucleic acids. For example, in one embodiment, the one or more isolated nucleic acids encodes one or more TRIM proteins. For example, in one embodiment, the one or more isolated nucleic acids encodes one or more of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17,

TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30. In certain embodiments, the one or more isolated nucleic acids encodes one or more STUbLs. For example, in one embodiment, the one or more isolated nucleic acids encodes one or more of RNF4 and RNF111 (Arkadia).

Exemplary nucleotide sequences encoding TRIM proteins is found in Table 1.

In certain embodiments, a peptide corresponding to one or more TRIM proteins or one or more STUbLs is expressed from the one or more nucleic acids in a cell in vivo or in vitro using known techniques.

The nucleotide sequence of the isolated nucleic acids include both the DNA sequence that is transcribed into RNA and the RNA sequence that is translated into a polypeptide. According to other embodiments, the nucleotide sequences are inferred from the amino acid sequence of the peptides of the invention. As is known in the art several alternative nucleotide sequences are possible due to redundant codons, while retaining the biological activity of the translated peptides.

Further, the invention encompasses an isolated nucleic acid comprising a nucleotide sequence having substantial homology to a nucleotide sequence encoding a disclosed herein. Preferably, the nucleotide sequence of an isolated nucleic acid is "substantially homologous," that is, is about 60% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to a nucleotide sequence of an isolated nucleic acid encoding a peptide of the invention.

In one embodiment, the composition comprises a combination of the nucleic acid molecules described herein. For example, in certain embodiments, the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins and an isolated nucleic acid molecule encoding one or more STUbLs. In one

embodiment, the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins and one or more STUbLs.

Thus, the invention encompasses expression vectors and methods for the introduction of exogenous DNA into cells with concomitant expression of the exogenous DNA in the cells such as those described, for example, in Sambrook et al. (2012, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

The desired nucleic acid encoding one or more one or more TRIM proteins or one or more STUbLs can be cloned into a number of types of vectors. However, the present invention should not be construed to be limited to any particular vector. Instead, the present invention should be construed to encompass a wide plethora of vectors which are readily available and/or well-known in the art. For example, a desired polynucleotide of the invention can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

In specific embodiments, the expression vector is selected from the group consisting of a viral vector, a bacterial vector and a mammalian cell vector. Numerous expression vector systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-vector based systems can be employed for use with the present invention to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2012), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and

one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

For example, vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. In a preferred embodiment, the composition includes a vector derived from an adeno-associated virus (AAV). Adeno-associated viral (AAV) vectors have become powerful gene delivery tools for the treatment of various disorders. AAV vectors possess a number of features that render them ideally suited for gene therapy, including a lack of pathogenicity, minimal immunogenicity, and the ability to transduce postmitotic cells in a stable and efficient manner. Expression of a particular gene contained within an AAV vector can be specifically targeted to one or more types of cells by choosing the appropriate combination of AAV serotype, promoter, and delivery method

In one embodiment, the encoding sequence is contained within an AAV vector. More than 30 naturally occurring serotypes of AAV are available. Many natural variants in the AAV capsid exist, allowing identification and use of an AAV with properties specifically suited for skeletal muscle. AAV viruses may be engineered using conventional molecular biology techniques, making it possible to optimize these particles for cell specific delivery of nucleic acid sequences, for minimizing immunogenicity, for

tuning stability and particle lifetime, for efficient degradation, for accurate delivery to the nucleus, etc.

Thus, expression of one or more TRIM proteins or one or more STUbLs can be achieved by delivering a recombinantly engineered AAV or artificial AAV that contains one or more encoding sequences. The use of AAVs is a common mode of exogenous delivery of DNA as it is relatively non-toxic, provides efficient gene transfer, and can be easily optimized for specific purposes. Exemplary AAV serotypes include, but is not limited to AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9.

Desirable AAV fragments for assembly into vectors include the cap proteins, including the vp1, vp2, vp3 and hypervariable regions, the rep proteins, including rep 78, rep 68, rep 52, and rep 40, and the sequences encoding these proteins. These fragments may be readily utilized in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV serotype sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. As used herein, artificial AAV serotypes include, without limitation, AAV with a non-naturally occurring capsid protein. Such an artificial capsid may be generated by any suitable technique, using a selected AAV sequence (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from a different selected AAV serotype, non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or from a non-viral source. An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a "humanized" AAV capsid. Thus exemplary AAVs, or artificial AAVs, suitable for expression of one or more TRIM proteins or one or more STUbLs, include AAV2/8 (see U.S. Pat. No. 7,282,199), AAV2/5 (available from the National Institutes of Health), AAV2/9 (International Patent Publication No. WO2005/033321), AAV2/6 (U.S. Pat. No. 6,156,303), and AAVrh8 (International Patent Publication No. WO2003/042397), among others.

For expression of the desired polynucleotide, at least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a TATA box, such as the

promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 genes, a discrete element overlying the start site itself helps to fix the place of initiation.

Additional promoter elements, i.e., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either co-operatively or independently to activate transcription.

A promoter may be one naturally associated with a gene or polynucleotide sequence, as may be obtained by isolating the 5′ non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a polynucleotide sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding polynucleotide segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a polynucleotide sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a polynucleotide sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," *i.e.,* containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein (U.S. Patent 4,683,202, U.S. Patent 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription

and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. Those of skill in the art of molecular biology generally know how to use promoters, enhancers, and cell type combinations for protein expression, for example, see Sambrook et al. (2012). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

In one embodiment, the promoter or enhancer specifically directs expression of the one or more TRIM proteins or one or more STUbLs in the intestinal epithelium in neural tissue. For example, in certain embodiments, the promoter or enhancer specifically directs expression of the one or more TRIM proteins or one or more STUbLs in a neuron, astrocyte, oligodendrocyte, Perkinje cell, pyramidal cell, or the like.

In order to assess the expression of the desired polynucleotide, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other embodiments, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers are known in the art and include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. Reporter genes that encode for easily assayable proteins are well known in the art. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a protein whose expression is manifested by some easily detectable property, e.g.,

enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (see, e.g., Ui-Tei et al., 2000 FEBS Lett. 479:79-82). Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of siRNA polynucleotide and/or polypeptide expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2012, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

Regardless of the method used to introduce exogenous nucleic acids into a host cell, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

Any DNA vector or delivery vehicle can be utilized to transfer the desired polynucleotide to a cell *in vitro* or *in vivo*. In the case where a non-viral delivery system is utilized, a preferred delivery vehicle is a liposome. The above-mentioned delivery systems and protocols therefore can be found in Gene Targeting Protocols, 2ed., pp 1-35 (2002) and Gene Transfer and Expression Protocols, Vol. 7, Murray ed., pp 81-89 (1991).

"Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes may be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers. However, the present invention also encompasses compositions that have different structures in solution than the normal vesicular structure. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

In one embodiment, the composition of the invention comprises in vitro transcribed (IVT) RNA encoding one or more components of the one or more TRIM proteins or one or more STUbLs. In one embodiment, an IVT RNA can be introduced to a cell as a form of transient transfection. The RNA is produced by in vitro transcription using a plasmid DNA template generated synthetically. DNA of interest from any source can be directly converted by PCR into a template for in vitro mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any other appropriate source of DNA. The desired template for in vitro transcription is one or more TRIM proteins or one or more STUbLs.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the DNA is a full length gene of interest of a portion of a gene. The gene can include some or all of the 5' and/or 3' untranslated regions (UTRs). The gene can include exons and introns. In one embodiment, the DNA to be used for PCR is a human gene. In another embodiment, the DNA to be used for PCR is a human gene including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

In one embodiment, the composition of the present invention comprises a modified nucleic acid encoding one or more one or more TRIM proteins or RNF4 described herein. For example, in one embodiment, the composition comprises a nucleoside-modified RNA. In one embodiment, the composition comprises a nucleoside-modified mRNA. Nucleoside-modified mRNA have particular advantages over non-modified mRNA, including for example, increased stability, low immunogenicity, and enhanced translation. Nucleoside-modified mRNA useful in the present invention is further described in U.S. Patent No. 8,278,036, which is incorporated by reference herein in its entirety.

Modified Cell

The present invention includes a composition comprising a cell which comprises one or more TRIM proteins, one or more STUbLs, a nucleic acid encoding a one or more TRIM proteins, a nucleic acid encoding a one or more STUbLs or a combination thereof. In one embodiment, the cell is genetically modified to express a protein and/or nucleic acid of the invention. In certain embodiments, genetically modified cell is autologous to a subject being treated with the composition of the invention. Alternatively, the cells can be allogeneic, syngeneic, or xenogeneic with respect to the subject. In certain embodiment, the cell is able to secrete or release the expressed protein into extracellular space in order to deliver the peptide to one or more other cells.

The genetically modified cell may be modified in vivo or ex vivo, using techniques standard in the art. Genetic modification of the cell may be carried out using an expression vector or using a naked isolated nucleic acid construct.

In one embodiment, the cell is obtained and modified ex vivo, using an isolated nucleic acid encoding one or more proteins described herein. In one embodiment, the cell is obtained from a subject, genetically modified to express the protein and/or nucleic acid, and is re-administered to the subject. In certain embodiments, the cell is expanded ex vivo or in vitro to produce a population of cells, wherein at least a portion of the population is administered to a subject in need.

In one embodiment, the cell is genetically modified to stably express the protein. In another embodiment, the cell is genetically modified to transiently express the protein.

Substrates

The present invention provides a scaffold or substrate composition comprising a protein of the invention, an isolated nucleic acid of the invention, a cell expressing the protein of the invention, or a combination thereof. For example, in one embodiment, a protein of the invention, an isolated nucleic acid of the invention, a cell a cell expressing the protein of the invention, or a combination thereof is incorporated within a scaffold. In another embodiment, a protein of the invention, an isolated nucleic acid of the invention, a cell expressing the protein of the invention, or a combination

thereof is applied to the surface of a scaffold. The scaffold of the invention may be of any type known in the art. Non-limiting examples of such a scaffold includes a, hydrogel, electrospun scaffold, foam, mesh, sheet, patch, and sponge.

Therapeutic Methods

The present invention also provides therapeutic methods for a disease or disorder associated with protein misfolding, protein aggregates, or a combination thereof.

In various embodiments, diseases and disorders treatable by the methods of the invention include, but are not limited to: polyQ disorders such as SCA1, SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), transmissible spongiform encephalopathies (prion disease), tauopathies, Frontotemporal lobar degeneration (FTLD), AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis. In certain embodiments, the method comprises the treatment or prevention of cancer associated with p53 mutant aggregates, including but not limited to bladder carcinoma, astrocytoma, pharynx carcinoma, lymphoma, and adenocarcinoma.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease associated with protein misfolding or protein aggregates that is already established. Particularly, the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant signs or symptoms of the

disease or disorder do not have to occur before the present invention may provide benefit. Therefore, the present invention includes a method for preventing a disease or disorder associated with protein misfolding or protein aggregates, in that a modulator composition, as discussed previously elsewhere herein, can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder.

One of skill in the art, when armed with the disclosure herein, would appreciate that the prevention of a disease associated with protein misfolding or protein aggregates, encompasses administering to a subject a modulator composition as a preventative measure against the development of, or progression of a disease associated with protein misfolding or protein aggregates. As more fully discussed elsewhere herein, methods of modulating the level or activity of a gene, or gene product, encompass a wide plethora of techniques for modulating not only the level and activity of polypeptide gene products, but also for modulating expression of a nucleic acid, including either transcription, translation, or both.

Additionally, as disclosed elsewhere herein, one skilled in the art would understand, once armed with the teaching provided herein, that the present invention encompasses methods of treating, or preventing, a wide variety of diseases associated with protein misfolding or protein aggregates, where modulating the level or activity of a gene, or gene product treats or prevents the disease. Various methods for assessing whether a disease is associated protein misfolding or protein aggregates are known in the art. Further, the invention encompasses treatment or prevention of such diseases discovered in the future.

In one aspect, the method comprises use of one or more TRIM proteins to stabilize a misfolded protein. In certain aspects, stabilization of a functional misfolded protein via one or more TRIM proteins described herein can treat or prevent a disease or disorder associated with the misfolded protein. For example, in one embodiment, stabilization of mutant cystic fibrosis transmembrane conductance regulator (CFTR), via one or more TRIM proteins described herein, would allow mutant CFTR to function instead of being degraded. It is envisioned that using TRIM proteins to stabilize misfolded proteins can be used to treat cystic fibrosis and other diseases associated with degradation of partially functional proteins. Stabilization of proteins, via one or more

TRIM proteins described herein, can be used to treat any disease or disorder associated with degradation of functional mutant protein, including but not limited to cystic fibrosis and lysosomal storage diseases such as Gaucher's disease and Fabry's disease.

The invention encompasses administration of a modulator of a gene, or gene product. To practice the methods of the invention; the skilled artisan would understand, based on the disclosure provided herein, how to formulate and administer the appropriate modulator composition to a subject. The present invention is not limited to any particular method of administration or treatment regimen.

In one embodiment, the method comprises administering to the subject in need an effective amount of a composition that increases the expression or activity of one or more TRIM protein or one or more STUbLs.

For example, in one embodiment, the method comprises administering to the subject in need an effective amount of a composition that increases the expression or activity of one or more of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

In one embodiment, the method comprises administering to the subject in need an effective amount of a composition that increases the expression or activity of one or more of RNF4 and RNF111 (Arkadia).

In one embodiment, the method comprises administering to the subject an effective amount of a composition that increases the expression or activity of one or more TRIM proteins and one or more STUbLs.

In one embodiment, the method comprises increasing the expression or activity of the one or more TRIM proteins or one or more STUbLs in at least one neural cell of the subject. For example, in certain embodiments, the method comprises increasing the expression or activity of the one or more TRIM proteins or one or more STUbLs in a at least one neuron, astrocyte, oligodendrocyte, Perkinje cell, pyramidal cell, or the like.

In one embodiment, the method comprises contacting the neural tissue of a subject with an effective amount of a composition that increases the expression or activity of one or more components of the one or more TRIM proteins or one or more STUbLs. For example, in certain embodiments, the method comprises contacting a neuron, astrocyte, oligodendrocyte, Perkinje cell, pyramidal cell, or the like, of a subject with an effective amount of a composition that increases the expression or activity of one or more TRIM proteins or one or more STUbLs.

One of skill in the art will appreciate that the modulators of the invention can be administered singly or in any combination. Further, the modulators of the invention can be administered singly or in any combination in a temporal sense, in that they may be administered concurrently, or before, and/or after each other. One of ordinary skill in the art will appreciate, based on the disclosure provided herein, that the modulator compositions of the invention can be used to prevent or to treat a disease or disorder associated with a misfolded protein or protein aggregate, and that a modulator composition can be used alone or in any combination with another modulator to effect a prophylactic or therapeutic result.

In various embodiments, any of the modulators of the invention described herein can be administered alone or in combination with other modulators of other molecules associated with a disease associated with protein misfolding or protein aggregates. In various embodiments, any of the modulators of the invention described herein can be administered alone or in combination with other therapeutic or preventative agents which may be used to treat or prevent a disease associated with protein misfolding or protein aggregates. Exemplary therapeutic agents which may be used in combination with the modulators of the present invention include, but is not limited to, anti-amyloid-β antibodies and anti-tau antibodies.

Gene Therapy

Contacting cells in a subject with a nucleic acid composition that encodes a protein that increases the expression or activity of one or more TRIM proteins or one or more STUbLs can inhibit or delay the onset of one or more symptoms of a disease or disorder associated with protein misfolding or protein aggregates.

In one embodiment, the nucleic acid composition of the present invention encodes one or more peptides. For example, in one embodiment, a nucleic acid composition can encode a peptide that comprises an amino acid sequence of one or more TRIM proteins. For example, in one embodiment, the nucleic acid composition encodes a peptide comprising one or more of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30. In certain embodiments, the nucleic acid composition encodes a peptide comprising the amino acid sequence of one or more STUbLs. For example, in one embodiment, the nucleic acid composition encodes a peptide comprising one or more of RNF4 and RNF111 (Arkadia).

The invention should also be construed to include any form of a nucleic acid encoding a peptide having substantial homology to the peptides disclosed herein. Preferably, a peptide which is "substantially homologous" is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to amino acid sequence of the peptides disclosed herein.

In one embodiment, the composition of the invention comprises a nucleic acid encoding a peptide, a fragment of a peptide, a homolog, a variant, a derivative or a salt of a peptide described herein. For example, in certain embodiments, the composition comprises a nucleic acid encoding a peptide comprising one or more TRIM proteins, a fragment of one or more TRIM proteins, a homolog of one or more TRIM proteins, a variant of one or more TRIM proteins, or a derivative of one or more TRIM proteins. In certain embodiments, the composition comprises a nucleic acid encoding a peptide comprising one or more STUbLs, a fragment of one or more STUbLs, a homolog of one or more STUbLs, a variant of one or more STUbLs, or a derivative of one or more STUbLs.

According to the present invention, a method is also provided of supplying protein to a cell which carries a normal, or a mutant gene, associated with diminished or insufficient activity of one or more TRIM proteins or one or more STUbLs. Supplying protein to a cell with a mutant gene should allow normal functioning of the recipient cells. The nucleic acid encoding a peptide may be introduced into the cell in a vector such that the nucleic acid remains extrachromosomal. In such a situation, the nucleic acid will be expressed by the cell from the extrachromosomal location. More preferred is the situation where the nucleic acid or a part thereof is introduced into the cell in such a way that it integrates into the cell's genome or recombines with the endogenous mutant gene present in the cell. Vectors for introduction of genes both for recombination, for integration, and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the practitioner.

As generally discussed above, a nucleic acid, where applicable, may be employed in gene therapy methods in order to increase the level or activity of the peptides of the invention even in those persons in which the wild type gene is expressed at a "normal" level, but the gene product is insufficiently functional.

"Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups by uncharged groups. One or more TRIM proteins or one or more STUbLs of the present invention can be delivered using gene therapy methods, for example locally in neural cell or tissue or systemically (e.g., via vectors that selectively target specific tissue types, for example, tissue-specific adeno-associated viral vectors). In some embodiments, primary cells harvested from the individual can be transfected ex vivo with a nucleic acid encoding any of the peptides of the present invention, and then returned the transfected cells to the individual's body.

Gene therapy methods are well known in the art. See, e.g., WO96/07321 which discloses the use of gene therapy methods to generate intracellular antibodies.

Gene therapy methods have also been successfully demonstrated in human patients. See, e.g., Baumgartner et al., Circulation 97: 12, 1114-1123 (1998), Fatham, C.G. 'A gene therapy approach to treatment of autoimmune diseases', Immun. Res. 18:15-26 (2007); and U.S. Patent No. 7,378089, both incorporated herein by reference. See also Bainbridge JWB et al. "Effect of gene therapy on visual function in Leber's congenital Amaurosis". N Engl J Med 358:2231-2239, 2008; and Maguire AM et al. "Safety and efficacy of gene transfer for Leber's Congenital Amaurosis". N Engl J Med 358:2240-8, 2008.

There are two major approaches for introducing a nucleic acid encoding a peptide or protein (optionally contained in a vector) into a patients cells; in vivo and ex vivo. For in vivo delivery, in certain instances, the nucleic acid is injected directly into the patient, sometimes at the site where the protein is most required. For ex vivo treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, e.g., U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. Commonly used vectors for ex vivo delivery of the gene are retroviral and lentiviral vectors.

Gene therapy would be carried out according to generally accepted methods, for example, as described by Friedman et al., 1991, Cell 66:799-806 or Culver, 1996, Bone Marrow Transplant 3:S6-9; Culver, 1996, Mol. Med. Today 2:234-236. In one embodiment, cells from a patient would be first analyzed by the diagnostic methods known in the art, to ascertain the expression or activity of one or more TRIM proteins or one or more STUbLs. A virus or plasmid vector, containing a copy of the gene or a functional equivalent thereof linked to expression control elements and capable of replicating inside the cells, is prepared. The vector may be capable of replicating inside the cells. Alternatively, the vector may be replication deficient and is replicated in helper cells for use in gene therapy. Suitable vectors are known, such as disclosed in U.S. Pat.

No. 5,252,479 and PCT published application WO 93/07282 and U.S. Pat. Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500. The vector is then injected into the patient. If the transfected gene is not permanently incorporated into the genome of each of the targeted cells, the treatment may have to be repeated periodically.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors or as the basis for repairing gene transfer vectors, including papovaviruses (e.g., SV40, Madzak et al., 1992, J. Gen. Virol. 73:1533-1536), adenovirus (Berkner, 1992;Curr. Topics Microbiol. Immunol. 158:39-66), vaccinia virus (Moss, 1992, Current Opin. Biotechnol. 3:518-522; Moss, 1996, PNAS 93:11341-11348), adeno-associated virus (Russell and Hirata, 1998, Mol. Genetics 18:325-330), herpesviruses including HSV and EBV (Fink et al., 1996, Ann. Rev. Neurosci. 19:265-287), lentiviruses (Naldini et al., 1996, PNAS 93:11382-11388), Sindbis and Semliki Forest virus (Berglund et al., 1993, Biotechnol. 11:916-920), and retroviruses of avian (Petropoulos et al., 1992, J. Virol. 66:3391-3397), murine (Miller, 1992, Hum. Gene Ther. 3:619-624), and human origin (Shimada et al., 1991; Helseth et al., 1990; Page et al., 1990; Buchschacher and Panganiban, 1992, J. Virol. 66:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses, although adenovirus and adeno-associated virus are also being used.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation; mechanical techniques, for example microinjection; membrane fusion-mediated transfer via liposomes; and direct DNA uptake and receptor-mediated DNA transfer (Curiel et al., 1992, Am. J. Respir. Cell. Mol. Biol 6:247-252). Viral-mediated gene transfer can be combined with direct in vitro gene transfer using liposome delivery, allowing one to direct the viral vectors to the tumor cells and not into the surrounding non-dividing cells. Injection of producer cells would then provide a continuous source of vector particles. This technique has been approved for use in humans with inoperable brain tumors.

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an

adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged. For other techniques for the delivery of adenovirus based vectors see U.S. Pat. Nos. 5,691,198; 5,747,469; 5,436,146 and 5,753,500.

Liposome/DNA complexes have been shown to be capable of mediating direct in vivo gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized in vivo uptake and expression have been reported in tumor deposits, for example, following direct in situ administration.

Expression vectors in the context of gene therapy are meant to include those constructs containing sequences sufficient to express a polynucleotide that has been cloned therein. In viral expression vectors, the construct contains viral sequences sufficient to support packaging of the construct. If the polynucleotide encodes a protein, expression will produce the protein. If the polynucleotide encodes an antisense polynucleotide or a ribozyme, expression will produce the antisense polynucleotide or ribozyme. Thus in this context, expression does not require that a protein product be synthesized. In addition to the polynucleotide cloned into the expression vector, the vector also contains a promoter functional in eukaryotic cells. The cloned polynucleotide sequence is under control of this promoter. Suitable eukaryotic promoters include those described above. The expression vector may also include sequences, such as selectable markers and other sequences described herein.

In certain embodiments, the method comprises the use of gene transfer techniques which target an isolated nucleic acid directly to neural tissue. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of a nucleic acid molecule (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell/tissue type. These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, co-infection with adenovirus can be included to disrupt endosome function.

Pharmaceutical Compositions and Formulations

The invention also encompasses the use of pharmaceutical compositions of the invention or salts thereof to practice the methods of the invention. Such a pharmaceutical composition may consist of at least one modulator composition of the invention or a salt thereof in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one modulator composition of the invention or a salt thereof, and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The compound or conjugate of the invention may be present in the pharmaceutical composition in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

In one embodiment, the pharmaceutical compositions useful for practicing the methods of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. A composition useful within the methods of the invention may be directly administered to the skin, vagina or any other tissue of a mammal. Other contemplated formulations include liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the

type and severity of the disease being treated, the type and age of the veterinary or human subject being treated, and the like.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of multiple daily doses (e.g., about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions that are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist may design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

In one embodiment, the compositions of the invention are formulated using one or more pharmaceutically acceptable excipients or carriers. In one embodiment, the pharmaceutical compositions of the invention comprise a therapeutically effective amount of a compound or conjugate of the invention and a

pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers that are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate or gelatin. In one embodiment, the pharmaceutically acceptable carrier is not DMSO alone.

Formulations may be employed in admixtures with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, vaginal, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, e.g., other analgesic agents.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and

solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" that may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an anti-oxidant and a chelating agent that inhibits the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition that may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for

example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive,

sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the subject either prior to or after a diagnosis of disease. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously

infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a subject, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to prevent or treat disease. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 1 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

The compound may be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject.

A medical doctor, e.g., physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a disease in a subject.

In one embodiment, the compositions of the invention are administered to the subject in dosages that range from one to five times per day or more. In another embodiment, the compositions of the invention are administered to the subject in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It will be readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention will vary from subject to subject depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any subject

will be determined by the attending physical taking all other factors about the subject into account.

Compounds of the invention for administration may be in the range of from about 1 mg to about 10,000 mg, about 20 mg to about 9,500 mg, about 40 mg to about 9,000 mg, about 75 mg to about 8,500 mg, about 150 mg to about 7,500 mg, about 200 mg to about 7,000 mg, about 3050 mg to about 6,000 mg, about 500 mg to about 5,000 mg, about 750 mg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 50 mg to about 1,000 mg, about 75 mg to about 900 mg, about 100 mg to about 800 mg, about 250 mg to about 750 mg, about 300 mg to about 600 mg, about 400 mg to about 500 mg, and any and all whole or partial increments therebetween.

In some embodiments, the dose of a compound of the invention is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound (i.e., a drug used for treating the same or another disease as that treated by the compositions of the invention) as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

In one embodiment, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound or conjugate of the invention, alone or in combination with a second pharmaceutical agent; and instructions for using the compound or conjugate to treat, prevent, or reduce one or more symptoms of a disease in a subject.

The term "container" includes any receptacle for holding the pharmaceutical composition. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, i.e., the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, e.g., treating or preventing a disease in a subject, or delivering an imaging or diagnostic agent to a subject.

Routes of administration of any of the compositions of the invention include oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intracerebral, epidural, intracerebroventricular, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

Diagnostic Methods

The present invention provides a method to diagnose a subject having or at risk for developing a disease or disorder associated with protein misfolding or protein aggregates. For example, in one embodiment, the method comprises using the level of expression or activity of one or more TRIM proteins or one or more STUbLs as diagnostic markers. In one embodiment, the method comprises detecting the presence of a genetic mutation in a nucleic acid encoding one or more TRIM proteins or one or more STUbLs.

In one embodiment, the method is used to diagnose a subject as having a disease or disorder associated with protein misfolding or protein aggregates. In one embodiment, the method is used to diagnose a subject as being at risk for developing a disease or disorder associated with protein misfolding or protein aggregates. In one embodiment, the method is used to evaluate the effectiveness of a therapy for a neurodegenerative disease or disorder associated with protein misfolding or protein aggregates.

In one embodiment, the method comprises collecting a biological sample from a subject. Exemplary samples include, but are not limited to blood, urine, feces, sweat, bile, serum, plasma, tissue biopsy, and the like. For example, in one embodiment, the sample comprises at least one cell of neural tissue. In one embodiment, the sample comprises a neuron, astrocyte, oligodendrocyte, Perkinje cell, pyramidal cell, or the like.

Methods for detecting a reduced expression or activity of one or more TRIM proteins or one or more STUbLs comprise any method that interrogates a gene or its products at either the nucleic acid or protein level. Such methods are well known in the art and include, but are not limited to, nucleic acid hybridization techniques, nucleic acid reverse transcription methods, and nucleic acid amplification methods, western blots, northern blots, southern blots, ELISA, immunoprecipitation, immunofluorescence, flow cytometry, immunocytochemistry. In particular embodiments, disrupted gene transcription is detected on a protein level using, for example, antibodies that are directed against specific proteins. These antibodies can be used in various methods such as Western blot, ELISA, immunoprecipitation, flow cytometry, or immunocytochemistry techniques.

Methods of manufacturing recombinant protein

In certain embodiments, the present invention provides a method of using one or more TRIM proteins, one or more STUbLs, or a combination thereof, in the production of a recombinant protein of interest. For example, the one or more TRIM proteins, one or more STUbLs, or a combination thereof, can be used to disaggregate protein aggregates of the recombinant protein of interest, thereby allowing for the production and collection of the recombinant protein of interest.

In certain embodiments, the method comprises administering to a cell one or more TRIM proteins, one or more STUbLs, a nucleic acid molecule encoding one or more TRIM proteins, a nucleic acid molecule encoding one or more STUbLs, or a combination thereof. In certain embodiments, the cell is modified to express the recombinant protein of interest. The cell may be of any expression system, including, but not limited to a yeast expression system, bacterial expression system, insect expression system, or mammalian expression system.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: A Cellular System that Degrades Misfolded Proteins and Protects against Neurodegeneration

Misfolded proteins compromise cellular function and cause disease. How these proteins are detected and degraded is not well understood. The experiments presented herein show that PML (also known as TRIM19) and the SUMO-dependent ubiquitin ligase RNF4 act together to promote the degradation of misfolded proteins in the mammalian cell nucleus. PML selectively interacts with misfolded proteins through distinct substrate recognition sites and conjugates these proteins with the small ubiquitin-like modifiers (SUMOs) through its SUMO ligase activity. SUMOylated misfolded proteins are then recognized and ubiquitinated by RNF4 and are subsequently targeted for proteasomal degradation. Further, it is demonstrated herein that PML deficiency exacerbates polyglutamine (polyQ) disease in a mouse model of spinocerebellar ataxia 1 (SCA1). These findings reveal a mammalian system that removes misfolded proteins through sequential SUMOylation and ubiquitination and define its role in protection against protein-misfolding diseases.

The promyelocytic leukemia protein (PML; also known as TRIM19) is a member of the tripartite motif (TRIM) family of proteins, which contain an N-terminal TRIM/BRCC region, consisting of a RING domain, one or two B boxes, and a coiled-coil (CC) motif, followed by a variable C-terminal region. PML is predominantly a nuclear protein and is the eponymous component of PML nuclear bodies. It is implicated in a wide variety of cell processes, including apoptosis, transcription, DNA damage signaling, and antiviral responses (Bernardi and Pandolfi, 2007, Nat Rev Mol Cell Biol, 8: 1006-1016). Notably, PML also colocalizes with aggregates formed by polyQ proteins associated with SCAs (Skinner et al., 1997, Nature, 389, 971-974; Takahashi et al., 2003, Neurobiol Dis, 13: 230-237) and, upon overexpression, promotes degradation of at least one of them (mutant ataxin-7) (Janer et al., 2006, J Cell Biol, 174: 65-76). Despite the potential importance of these observations, the role of PML in the removal of misfolded proteins is not well understood. In particular, it is unclear whether PML plays a broad role in the removal of nuclear misfolded proteins. The critical issue of the molecular mechanisms by which PML removes misfolded proteins is unaddressed. Moreover, the physiological relevance of the effect of PML on misfolded proteins is not known.

The materials and methods employed in these experiments are now described.

Plasmids

All proteins are of human origin unless otherwise indicated. Plasmids for expressing the following proteins in mammalian cells were made in pRK5 by PCR, and each was fused with HA, FLAG, or 6xHis tag, or GST or GFP protein at the NH2- or COOH-terminus as indicated: FLAG-PML mutants (isoform IV unless otherwise indicated); GST-PML; Atxn1 82Q-GFP, HA-Atxn1 82Q-FLAG, FLAG-Atxn1 82Q; HA-Httex1p 97QP and HA-Httex1p 97QP(KR); FLAG-nFluc-GFP, FLAG-nFlucSM-GFP, and FLAG-nFlucDM-GFP; HA-RNF4, HA-RNF4 SIMm, HA-RNF4-FLAG, and HA-RNF4 SIMm-FLAG; and HA-SUMO2 KR. Atxn1 82Q plasmids were made based on the FLAG-Atxn1 82Q/pcDNA plasmid provided by H. Orr (Riley et al., 2005, J Biol Chem, 280: 21942-21948); Httex1p 97QP and Httex1p 97QP(KR) (in which K6, K9, and K15 were changed to Arg) are based on Steffan et al., 2004, Science, 304: 100-104; and nFluc plasmids based on Gupta et al., 2011, Nat Methods, 8: 879-884. Each nFluc protein was fused to the SV40 nuclear localization signal (PKKKRKV) (SEQ ID NO: 147) at the NH2-terminus and to GFP at the COOH-terminus. In FlucDM, R188 and R261 were changed to Glu; In FlucSM, R188 was changed to Glu (Gupta et al., 2011, Nat Methods, 8: 879-884). The template for PCR amplification of RNF4 was purchased from Open Biosystems (gene accession number: NM002938). In RNF4 SIMm, the following resides within SIMs were changed to Ala: I36, L38, and V39 (SIM1); I46, V47, and L49 (SIM2); V57, V58, and V59 (SIM3); and V67, V68, I69 and V70 (SIM4). In SUMO2 KR, the internal SUMOylation consensus site Lys11 was mutated to Arg.

For bacterial expression, GST fusions of Htt 25Q, Htt 103Q, Htt 52Q, Htt 52Q cc-, PML CC-FLAG, RNF4, and RNF4 SIMm were constructed in pGEX-1ZT, a derivative of pGEX-1λT with additional cloning sites. Htt 25Q, Htt 52Q and Htt 103Q contained the Htt amino acids 1-17 followed by a polyQ stretch of the indicated length (Krobitsch and Lindquist, 2000, Proc Natl Acad Sci USA, 97: 1589-1594). Htt 52Q and Htt 52Q cc- cDNAs were assembled by joining synthetic oligos. FLAG-PML F12 (571-

633)-6xHis was constructed in pET28a. All plasmids generated for this study were confirmed by DNA sequencing.

The following plasmids were previously described: FLAG-PML, FLAG-PML M6 (which had the C57S, C60S, C129A, C132A, C189A, and H194A mutations), 6xHis-SUMO1, and 6xHis-SUMO2 (Chu and Yang, 2011, Oncogene, 30: 1108-1116); FLAG-Atxn1 82Q and FLAGAtxn1 30Q (Riley et al., 2005, J Biol Chem, 280: 21942-21948); luciferase-6xHis (a *Photinus pyralis* luciferase variant) (Sharma et al., 2010, Nat Chem Biol, 6: 914-920); GST-rRNF4 (where "r" denotes rat origin, same below), GSTrRNF4 CS1 (in which C136 and C139 were changed to Ser), FLAG-rRNF4, and FLAGrRNF4 CS (in which C136, C139, C177, and C180 were changed to Ser) (Hakli et al., 2004, FEBS Lett, 560: 56-62); and PML isoforms I, II, III, IV, and VI (used in Figure 8A) (Xu et al., 2005, Mol Cel, 17: 721-732).

siRNAs

PML and RNF4 siRNAs were purchased from Qiagen, and the sense strand sequences were: PML#4, CTCCAAGATCTAAACCGAGAA (SEQ ID NO: 148); PML#9, CACCCGCAAGACCAACAACAT (SEQ ID NO: 149); RNF4#5, CCCTGTTTCCTAAGAACGAAA (SEQ ID NO: 150); RNF4#6, TAGGCCGAGCTTTGCGGGAAA (SEQ ID NO: 151); RNF4#8, AAGACTGTTTCGAAACCAACA (SEQ ID NO: 152). RNF4 was knocked down with either siRNAs individually or in combination at an equal molar ratio. SUMO1 siRNA (Thermo Scientific, siGENOME SMARTpool M-016005-03-0005) was a pool of 4 target-specific siRNA duplexes. The sense strand sequences were: TCAAGAAACUCAAAGAATC (SEQ ID NO: 153), GACAGGGTGTTCCAATGAA (SEQ ID NO: 154), GGTTTCTCTTTGAGGGTCA (SEQ ID NO: 155), and GAATAAATGGGCATGCCAA (SEQ ID NO: 156). SUMO2/3 siRNAs (Santa Cruz sc-37167) was a pool of three different siRNA duplexes, and sense strand sequences were CCCAUUCCUUUAUUGUACA (SEQ ID NO: 157), CAGAGAAUGACCACAUCAA (SEQ ID NO: 158), and CAGUUAUGUUGUCGUGUAU (SEQ ID NO: 159).

Cell culture and transfection

HeLa cells (from ATCC) and U2OS cells expressing GFP-SUMO2 or GFP-SUMO3 (Mukhopadhyay et al., 2006, J Cell Biol, 174: 939-949) were maintained in standard culture conditions. DNA plasmids were transfected into cells using Lipofectamine 2000. When PML and Atxn1 were co-transfected, either FLAG-PML plus Atxn1 82Q/30Q-GFP or HA-PML plus FLAG-Atxn1 82Q/30Q was used. HA-RNF4 and HA-RNF4-FLAG plasmids were used for testing protein expression and cellular localization, respectively.

siRNAs using Lipofectamine 2000 or RNAiMAX (Invitrogen), according to the manufacturer's instructions. For knockdown experiments, two rounds of siRNA transfection were performed on consecutive days. When both DNA and siRNA were transfected, DNA was transfected 4-6 hours after treatment with combined RNF4 siRNAs, and a day after treatment with other siRNAs. MG132 (Sigma) was added 24 hours after the last transfection at 7.5-10 μM (final concentration) for 4-5 hours.

Generation of RNF4 shRNA stable cell lines

shRNA against human RNF4, cloned into pLKO.1, was obtained from Thermo Scientific. The antisense sequence of shRNF4 is TGGCGTTTCTGGGAGTATGGG (SEQ ID NO: 160) (TRCN0000017054). For lentiviral production, 293T cells were transfected with lentiviral vectors, Gag helper plasmid, Rev helper plasmid, and VSVG helper plasmid. Virus-containing media was collected at 48 hours and 72 hours and spun for 5 minutes at 100 g. HeLa cells were transduced using virus-containing supernatant with polybrene and selected with puromycin. The pLKO.1 vector was used to create control stable cells.

Cell lysate fractionation, filter retardation assay, and Western blot

Cell lysates were made in NP-40-containing buffer and fractionated into supernatant (NS) and pellet by centrifugation. Both fractions were boiled in buffer containing 2% SDS and analyzed by western blot. A portion of the pellet was analyzed by a filter retardation assay for SR species.

Samples were prepared as described with modifications (Janer et al., 2006, J Cell Biol, 174: 65-76). Cells were harvested and lysed for 30 min on ice in buffer

containing 50 mM Tris, pH 8.8, 100 mM NaCl, 5 mM $MgCl_2$, 0.5% NP-40, 2 mM DTT, 250 IU/ml benzonase (Sigma), 1 mM PMSF, 1x complete protease cocktail (Roche), and 20 mM N-Ethylmaleimide (NEM; Sigma). Protein concentrations were determined by Bradford assay (Bio-Rad Labs). The whole cell lysates were centrifuged at 13,000 rpm for 15 minutes at 4 °C. The supernatant, containing NP-40-soluble (NS) proteins, was analyzed by SDS-PAGE. The pellet was resuspended in the pellet buffer (20 mM Tris, pH 8.0, 15 mM MgCl2, 2 mM DTT, 250 IU/ml benzonase, 1 mM PMSF, 1X complete protease cocktail, and 20 mM NEM) and incubated for 30 minutes on ice. The pellet fraction was boiled in 2% SDS, 50 mM DTT. One portion of the boiled pellet fraction was resolved by SDS-PAGE, and proteins entering the gel (SDS-soluble, SS) were detected by Western blot. The other portion was applied to a membrane filter with 0.2 μm pore size as previously described (Wanker et al., 1999, Methods Enzymol, 309: 375-386), and the SDS-resistant (SR) aggregates retained on the filter was analyzed by immunoblotting.

Primary antibodies against the following proteins were used for Western blot with product information and dilutions indicated: PML (rabbit, H-238, 1:1,000 and goat, N-19, 1:500), ubiquitin (mouse, P4D1, 1:10,000), and HA (rabbit, Y-11, 1:500) (Santa Cruz Biotechnology); FLAG (mouse, M2, 1:7,500), actin (rabbit, 1:10,000), and β-tubulin (mouse, 1:5,000) (Sigma); GFP (mouse, 1:4,000) (Clonetech); GST (goat, 1:1000, GE Healthcare Life Sciences); SUMO1 (mouse, 1:500, Invitrogen); SUMO2/3 (rabbit, 1:250, Abgent); HA (for transfected HA-RNF4) (rat, 3F10, horseradish peroxidase or HRP-conjugated, 1:10,000) (Roche); RNF4 (mouse, 1:500, Abnova, and a mouse monoclonal antibody developed by Abmart using antigen peptide DLTHNDSVVI (SEQ ID NO: 161), 1:1,000). Transfected FLAG-PML was detected by anti-FLAG antibody, and transfected HA-PML and HA-RNF4 were detected by HA antibody.

The secondary antibodies were either conjugated to HRP (Santa Cruz Biotechnology), or labeled with IRD Fluor 800 or IRD Fluor 680 (LI-COR, Inc.). Western blots were developed using ECL reagents and analyzed using ImageJ, or scanned with the Odyssey infrared imaging system, and analyzed using Image Studio Lite (LI-COR, Inc.).

Immunofluorescence of cultured cells

Cells cultured on coverslips were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.2% Triton X-100 for 15 minutes, blocked with 1% BSA, and incubated with antibodies as indicated. Cells were mounted with medium containing DAPI (for DNA detection) (Vector Labs), and the images were acquired with a Nikon Eclipse E800 or Olympus IX81 microscope. The following primary antibodies were used with product information and concentrations indicated: PML (rabbit, H-238 and mouse, PG-M3, 1:100), RNF4 (goat, C-15, 1:25) (Santa Cruz Biotechnology), and FLAG (for transfected FLAG-PML and HA-RNF4-FLAG) (mouse, M2, 1:2,000) (Sigma). Secondary antibodies were FITC-conjugated anti-mouse, anti-rabbit (Zymed), and anti-goat (Invitrogen) IgGs; Texas Red-conjugated anti-mouse and anti-rabbit IgGs (Vector labs); and Rhodamine Red-X conjugated anti-goat (Jackson ImmunoResearch Labs).

For quantification of Atxn1 82Q, Httex1p 97QP, and SUMO2-positive Atxn1 82Q aggregates, approximately 400, 500, and 200 cells, respectively, from ten or more randomly selected fields were examined. The sizes of Atxn1 82Q inclusions were measured using ImageJ, and cells were categorized based on the largest inclusion in cells. *P*-value for the proportions of cells with aggregates of various sizes in the presence and the absence of PML was calculated using a chi-squared test. For cells transfected with Httex1p 97QP, approximately 30% of them had either cytoplasmic or nuclear aggregates.

Assays of protein half-life

Cells were pulse labeled in Met- and Cys-free DMEM medium supplemented with [^{35}S]Met and [^{35}S]Cys, and then cultured in regular DMEM. Alternatively, cells were treated with CHX. Immunoprecipitated [^{35}S]Atxn1 82Q or unlabeled Atxn1 82Q in cell lysate was analyzed by autoradiography or western blot.

For pulse-chase analysis, HeLa cells were transfected with FLAG-Atxn1 82Q alone or together with a moderate amount of PML. 17 h after transfection, cells were cultured in Met and Cys-free DMEM medium for 30 min, and then pulse labeled for 30 min with [^{35}S]Met and [^{35}S]Cys (100 μCi/ml each). Afterwards, cells were rinsed twice with PBS and chased in DMEM with 10% FBS for 0-18 h. Cells were lysed in IP-lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 0.5% NP-40, and 2 mM DTT) containing

2% SDS and 50 mM DTT, and boiled at 95 °C for 10 minutes. The whole cell lysates were centrifuged at 13,000 rpm for 15 minutes. The supernatants were diluted 20-fold in IP-lysis buffer and incubated with anti-FLAG M2 beads at 4 °C overnight. The beads were sequentially washed with IP lysis buffer with additional 0, 0.5 M, and 1 M KCl, and boiled in a 2% SDS sample buffer. Samples were resolved by SDS-PAGE and analyzed by autoradiography. To better compare the half-life of Atxn1 82Q under different conditions, exposures with similar signal intensity at 0 hours were presented.

For cycloheximide (CHX) treatment of Atxn1 82Q-transfected cells, 150 μg/ml CHX was added to cell culture medium 4-5 hours after transfection. Cells were harvested and snap-frozen on dry ice at indicated time points, lysed, and fractioned for Western blot analysis. For CHX treatment of nFlucDM-transfected cells, 50 μg/ml CHX was added to cell culture medium 17 hours after transfection. Cells were harvested at indicated time points, and whole cell lysates were used for Western blot analysis.

Quantitative RT-PCR analysis

Total RNA was extracted using TRIzol (Invitrogen). cDNA synthesis was carried out by reverse transcription of total RNA using the First Strand cDNA Synthesis Kit (Marligen Biosciences). A Taqman Gene Expression Assay (Applied Biosystems) with human Atxn1 (Hs00165656_m1) and 18s rRNA (4333760F) primers/probe sets were used for qPCR analysis.

Protein purification

FLAG-PML, FLAG-PML M6, and FLAG-Atxn1 82Q-HA were expressed in 293T cells and purified by anti-FLAG M2 beads (Sigma) as previously described (Tang et al., 2006, Nat Cell Biol, 8: 855-862; Tang et al., 2004, J Biol Chem, 279: 20369-20377) with modifications. Cells were lysed in IP-lysis buffer (50mM Tris, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 0.5% NP-40 and 2 mM DTT) supplemented with 1 mM PMSF, and 1x complete protease cocktail. For PML purification, IP-lysis buffer was also supplemented with 20 μM ZnCl2. The lysates were centrifuged at 13,000 rpm for 15 minutes. Supernatants were incubated with anti-FLAG M2 beads at 4°C for 4 hours to overnight. M2 beads were sequentially washed with IP lysis buffers containing 0, 0.5,

and 1 M KCl, and with an elution buffer (50 mM Tris, pH 7.5, 150 mM NaCl, and 2 mM DTT). The bound proteins were eluted in the elution buffer containing 0.1-0.3 mg/ml 3xFLAG peptide (Sigma). The major additional bands observed in the FLAG-PML and FLAG-PML M6 preps were derived from PML based on both Western blot and mass spectrometry analyses (Figure 11G).

GST fusion of PML was expressed in 293T cells and purified using glutathione-Sepharose™ 4B beads (GE Healthcare Life Sciences) with similar lysis and wash conditions as described above. GST fusions of rRNF4, rRNF4 CS1, RNF4, and RNF4 SIMm were expressed in *Escherichia coli* BL21 DE3 or Rosetta 2 (EMD Chemicals) and purified as previously described (Hakli et al., 2001, J Biol Chem, 276: 23653-23660). The bacteria were grown at 37°C to A 600nm = 0.6 - 0.8, and were induced for protein expression with 0.3 mM IPTG for 3 hours at 30°C. GST-tagged proteins were purified with glutathione beads. GST and GST fusions of Htt 25Q, Htt 103Q were purified similarly except that 0.1 mM IPTG was used for inducing protein expression. The bound proteins were eluted in the elution buffer containing 30 mM glutathione (Sigma).

Luciferase-6xHis was expressed in BL21 DE3 as previously described (Sharma et al., 2010, Nat Chem Biol, 6: 914-920). To generate immobilized native luciferase, Luc (N), Ni-NTA beads (Qiagen) were incubated with bacterial lysates and washed according to the manufacturer's instructions. Denatured luciferase, Luc (D), was generated by treating immobilized Luc (N) with 8 M urea for 5 minutes. A luciferase activity assay showed that only 0.2% of enzymatic activity remained after urea treatment. For control beads, lysates from bacteria expressing no luciferase were incubated with Ni-NTA beads in parallel.

To generate PML mutants used for luciferase peptide scans, FLAG-PML F12 (571-633)-6xHis and GST-PML CC-FLAG were expressed in BL21 DE cells at room temperature with 0.1 mM IPTG induction for 3 hours and 1 hour, respectively. Flag-PML F12 (571-633)-6xHis was purified first using M2 beads and the FLAG peptide elution, and was subjected to a second purification using Ni-NTA beads, according to the manufacturer's instructions. To generate the PML CC domain, a TEV protease cleavage site was introduced between GST and PML CC. The GST-PML CC-FLAG conjugated

glutathione beads were incubated with TEV protease (Sigma) according to the manufacturer's instructions to release PML CC-FLAG from the GST moiety (and from the beads). PML CC-FLAG used for pull-down assay was generated by incubating the purified PML CC-FLAG proteins with M2 beads and washed as described above.

Pull-Down Assays

For FLAG pull-down assays, FLAG-PML, FLAG-GFP and PML CC-FLAG bound to anti-FLAG M2 beads were prepared as described above. Purified GST-Htt 25Q, GST-Htt 103Q, GST-Htt 52Q or GST-Htt 52Q cc- were centrifuged at 13,000 rpm for 15 minutes to remove any aggregated proteins. FLAG-PML (2.5 μg) or FLAG-GFP (1.1 μg) at comparable molarity was incubated with GST-Htt 25Q or GST-Htt 103Q (2.5 μg each) in the absence or presence of Hsp70 (2.5 μg) and Hsp40 (1.4 μg) (Enzo Life Sciences) in a final volume of 200 μl assay buffer (50 mM Tris, pH 7.5, 150 mM NaCl, and 2 mM DTT, 0.5% NP-40) for 2 hours at 4 °C. The beads were washed three times with IP-lysis buffer in Compact Reaction Columns (Affymetrix/USB) and boiled in 2% SDS sample buffer. Samples were analyzed by Western blot. The PML CC pull-down assay was performed similarly, except M2 beads containing 1.6 μg PML CC-FLAG or control M2 beads were incubated with 5 μg GST or GST-Htt proteins.

For GST pull-down assay, 2 μg each of GST, GST-Htt 25Q, and GST-Htt 103Q proteins that bound to glutathione-Sepharose™ 4B beads were incubated with 400 μg lysates from 293T cells expressing FLAG-PML protein at 4 °C for 4 hours. The beads were washed three times with IP-lysis buffer in Compact Reaction Columns and boiled in 2% SDS sample buffer. Samples were analyzed by Western blot. For detecting the interaction between PML mutants and Htt, [^{35}S]Met-labeled full-length and mutant PML proteins were generated using the SP6 Coupled Transcription/Translation System (Promega) and incubated with GST, GST-25Q and GST-Htt 103Q that bound to beads in 150 μl IP-lysis buffer at 4 °C overnight. Beads were washed and boiled as described above. Input and pull-down samples were analyzed by autoradiography and Coomassie blue staining. For autoradiography, pull-down samples from the same experiments that were resolved on different gels were subjected to the same exposure time.

For the luciferase pull-down assay, 3 μg of Luc (N) or Luc (D) bound Ni-NTA beads or control beads prepared from an equal amount of bacterial lysate were incubated with purified GST (1 μg), GST-PML (3 μg) in the absence or presence of Hsp70 (3 μg) and Hsp40 (1.7 μg), or [^{35}S]Met-labeled full-length and mutant PML proteins in 200 μl PBS containing 15 mM Imidazole, 1 mM DTT, 0.5% Triton X-100 and 0.5% NP-40 at 4 °C for 4 hours. The beads were washed three times with PBS containing 20 mM Imidazole, 1 mM DTT, 0.5% Triton X-100 and 0.5% NP-40 and boiled. The input and pull-down samples were analyzed as described above.

Screening of cellulose-bound peptides for binding to PML domains

A peptide library (13-mers overlapping by ten amino acids) for *Photinus pyralis* luciferase was prepared by automated spot synthesis (JPT peptide Technologies). The peptide array membrane was probed with purified PML SRS1 and SRS2 fragments. The peptide array membrane was blocked with Odyssey Blocking buffer (LI-COR, Inc) and incubated with FLAG-PML F12(571-633)-HisX6 or PML F4/CC-FLAG (150 nM each) in TBS-T (50 mM TRIS, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 0.05% Tween and 1 mM DTT) at 4 °C for 2 hours. The membrane was washed and blotted with mouse anti-Flag and anti-mouse-HRP antibodies following the manufacturer's instructions. The blots were developed using ECL reagents. Background signal on blots with anti-Flag and anti-mouse-HRP antibodies alone was minimal even at long exposures. The peptide array membrane was regenerated according to the manufacturer's protocol.

SUMOylation and Ubiquitination Analysis

Cells or in vitro reaction mixtures were boiled in buffer containing 2% SDS and then diluted in buffer without SDS or passed through a Bio-Spin chromatography column to reduce the SDS concentration. Proteins were immunoprecipitated (denaturing IP or d-IP) and analyzed by western blot.

In vivo SUMOylation and ubiquitination assays

Cells were transfected with FLAG-Atxn1, FLAG-nFluc-GFP, and other expression plasmids as indicated. For the experiments shown in Figure 4A, a SUMO2-

expressing plasmid was also used. Twenty four hours after transfection, cells were treated with 7.5 μM MG132 or DMSO for 5 hours or left untreated, and harvested in IP-lysis buffer supplemented with 2% SDS and 50 mM DTT. For denaturing immunoprecipitation (d-IP), cell lysates were boiled at 95°C for 10 minutes. One aliquot was saved for Western blot analysis. The rest of the lysates were either diluted 20-fold in IP-lysis buffer or passed through a Bio-Spin chromatography column (Bio-Rad) equilibrated with IP-lysis buffer to reduce the SDS concentration. Lysates were then incubated with anti-FLAG (M2) beads at 4°C for 4 hours or overnight. The beads were washed as described for FLAG-tagged protein purification, and boiled in 2% SDS sample buffer. Proteins from beads were analyzed by Western blot with anti-FLAG, -SUMO2/3, -SUMO1, -ubiquitin, and other antibodies as indicated. To better compare the levels of ubiquitinated or SUMOylated species, d-IP products containing similar levels of unmodified proteins were often used for Western blot analysis.

In vitro SUMOylation assays

Components for *in vitro* ubiquitination and SUMOylation reactions were purchased from Boston Biochem. *In vitro* SUMOylation assays were performed at 37 °C for 1.5 hours in 30 μl reaction buffer (50 mM Tris pH 7.5, 5.0 mM Mg2+-ATP, and 2.5 mM DTT) containing purified HA-Atxn1 82Q-FLAG (600 ng /200 nM), FLAG-PML (for Figure 4D, 50 and 200 ng or 22 and 90 nM; for Figure 4E 100 ng or 45 nM) or FLAG-PML M6 (100 ng or 45 nM), SAE1/SAE2 (125 nM), Ubc9 (1 μM), His-SUMO2 (25 μM), Hsp70 (420 ng/200 nM), Hsp40 (240 ng/200 nM) and BSA (0.1 μg/ml). The reaction mixtures were denatured by the addition of 30 μl IP-lysis buffer containing 2% SDS and 50 mM DTT and heating at 95°C for 10 minutes. One aliquot of the heated reaction mixes were saved for Western blot analysis, and the rest were diluted 20-fold in IP-lysis buffer without SDS. HA-Atxn1-FLAG was immunoprecipitated by anti-HA beads (Roche) and analyzed for SUMO2/3 modification using anti-SUMO2/3 antibodies.

In vitro ubiquitination assays

In vitro assays for RNF4 self-ubiquitination were performed at 37°C for 1 hour in 10 μl reaction buffer (50 mM Tris pH 7.5 and 2.5 mM DTT) containing purified

GST-RNF4 protein (250 ng/530 nM), UBE1 (125 nM), UbcH5a (625 nM), ubiquitin (2.5 μg/30 μM), and Mg^{2+}-ATP (2.5 mM). The reaction mixtures were heated at 95°C for 10 minutes and analyzed by Western blot.

For *in vitro* ubiquitination of SUMOylated Atxn1 82Q, a mix of SUMOylated and unmodified Atxn1 82Q proteins was prepared as ubiquitination reaction substrate. M2 beads conjugated with FLAG-Atxn1 82Q-HA (1.5 μg/300 nM) and control M2 beads were mixed with 0.75 μM SAE1/SAE2, 12.5 μM Ubc9, 125 μM His-SUMO2, and 2.5 mM DTT in a total volume of 50 μl of Mg2+-ATP-Energy Regeneration Solution containing 5 mM ATP (Boston Biochem). To achieve sufficient Atxn1 82Q SUMOylation, the reaction was performed at 37 °C for 24 hours, and the reaction buffer was replaced after 12 hours. Beads were then washed sequentially with IP-lysis buffer with additional 0, 0.5, and 1 M KCl and with ubiquitination reaction buffer (50 mM Tris pH 7.5 and 150 mM NaCl) (Tang et al., 2006, Nat Cell Biol, 8: 855-862).

Atxn1 82Q beads and control beads were then incubated at 37 °C for 1 hour with ubiquitination reaction mixes in 20 μl volume containing GST-rRNF4 (0, 40, 160 and 500 ng, or 0, 43, 170, and 530 nM), UBE1 (100 nM), UbcH5a (500 nM), ubiquitin (5 μg/30 μM), and Mg^{2+}-ATP (2.5 mM) in reaction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, and 2.5 mM DTT). Afterwards, beads were separated from the supernatant and washed with IP-lysis buffer. Atxn1 82Q was denatured and released from the beads by the addition of IP-lysis buffer containing 2% SDS and 50 mM DTT and heat at 95°C for 10 minutes. After dilution, Atxn1 82Q was immunoprecipitated with M2 beads. The IP products and the supernatant from the reaction were analyzed by Western blot.

Mouse breeding and genotyping

The heterozygous B05 transgenic mice (*Atxn1*$^{tg/-}$), which harbor the human *SCA1*-coding region with 82 CAG repeats driven by a Purkinje cell-specific promoter element, were provided (Burright et al., 1995, Cell, 82: 937-948). *PML*$^{-/-}$ mice were provided (Wang et al., 1998, Science, 279: 1547-1551). *Atxn1*$^{tg/-}$ mice (on FVB background) were mated with *PML*$^{-/-}$ (on 129Sv background). *PML*$^{+/-}$:*Atxn1*$^{tg/-}$ mice from

the F1 generation were mated with *PML*$^{-/-}$ or *PML*$^{+/+}$ to generate mice used for Rotarod tests and pathology. The mating scheme did not affect the Rotarod performance, formation of aggregates, molecular layer thickness, or dendritic arborization of the F2 generation of *PML*+ or *PML*$^{+/-}$:*Atxn1*$^{tg/-}$ mice. The mouse genotype was determined by PCR either as described (Burright et al., 1995, Cell, 82: 937-948) (for *Atxn1*) or according to suggestions by the NCI Mouse Repository (for *PML*).

Accelerating Rotarod test

An accelerating Rotarod apparatus (47600, Ugo Basile, Italy) was used to measure motor coordination and balance. Only naïve animals were used. Each animal was given three trials per day for four consecutive days, with a 1 hour rest between trials. For each trial, mice was placed on the Rotarod with increasing speed, from 4-80 rpm, over 10 minutes. Their latency to fall off the Rotarod (in seconds) was recorded.

Immunostaining and pathological analysis of mouse cerebellum

Paraffin-embedded cerebellar midsagital sections were stained with indicated antibodies and visualized using a Leica SP5 II laser scanning confocal microscope, or stained with hematoxylin and visualized using an Olympus BX51 microscope.

Immunohistochemistry and immunofluorescence were performed as previously described with modifications (Duda et al., 2000, J Neuropathol Exp Neurol, 59: 830-841; Emmer et al., 2011, J Biol Chem 286: 35104-35118). Paraffin-embedded cerebella were cut into 10-μm sections. For molecular layer measurements, three haematoxylin stained midsagittal sections with 100 μm intervals were analyzed per mouse. Twenty measurements at the primary fissure for each section were averaged.

To quantify Purkinje cell dendritic arborization, midsagittal sections of cerebella were stained with an antibody against the Purkinje cell-specific protein calbindin (mouse, CB-955, 1:250; Sigma). Twenty 0.5 μm optical sections were accumulated with Leica SP5 II laser scanning confocal microscope. The brightest continuous 12 sections (6 μm) were projected for maximum intensity. The fluorescence intensity profile from the same region of preculminate fissure was plotted using ImageJ.

To quantify Purkinje cells, midsagittal sections were stained with anti-calbindin antibody and comparable regions were used for cell counting. The length of the Purkinje cell layer was measured by drawing segmented line along Purkinje cell soma center using ImageJ. For each mouse, 350 – 900 neurons along approximately 30 mm were measured. The Purkinje cell density was determined by dividing the number of cells by the length of Purkinje cell layer.

To determine the number of Purkinje cells with aggregates, midsagittal sections were stained with anti-ubiquitin (mouse, Ubi-1, MAB1510, 1:2,000; Millipore) antibody. Three hundred cells or more were counted from the same brain regions per mouse. Images were taken using an Olympus BX51 microscope mounted with a DP71 Olympus digital camera.

Four mice per genotype were used for counting Purkinje cells of 1-year-old mice, two $PML^{+/+}$ mice per genotype for quantification of dendritic arborization, and three mice per genotype for the rest of the studies.

Statistical analysis

The numbers of cells with aggregates were analyzed by chi-squared test and Student's t-test when appropriate. The behavioral scores and cerebellar pathology were analyzed by two-way ANOVA with repeated measurements and Student's t-test. All the data were analyzed using Prism5 software or Microsoft Excel 2008.

The results of the experiments are now described

PML Promotes Proteasomal Degradation of Pathogenic Ataxin-1 Protein

SCA1 is a fatal neurological disorder characterized by progressive ataxia and loss of neurons, especially cerebellar Purkinje cells. It is caused by the expansion of a polyQ stretch in the *SCA1* gene product, Ataxin-1 (Atxn1) (Orr and Zoghbi, 2007, Annu Rev Neurosci, 30: 575-621). To investigate the role of PML in eliminating nuclear misfolded proteins, a cell culture model was generated in which a pathogenic Atxn1 protein with 82 contiguous glutamines that was C-terminally fused to the enhanced green fluorescent protein, Atxn1 82Q-GFP, was expressed in HeLa cells. Similar to pathogenic

Atxn1 proteins in human SCA1 patients and mouse SCA1 transgenic models (Skinner et al., 1997, Nature, 389: 971-974), Atxn1 82Q-GFP was localized to the nucleus, exhibiting a diffuse localization pattern with markedly higher concentration in microscopically visible inclusions (Figure 1A and Figure 1B). Atxn1 82Q-GFP also yielded both NP-40-soluble (soluble or NS) and NP-40-insoluble (aggregated) species in cell lysates. The latter could be further divided into SDS-soluble (SS) and SDS-resistant (SR) species (Figure 1C).

Concordant with previous reports (Skinner et al., 1997, Nature, 389: 971-974), endogenous PML colocalized with Atxn1 82Q-GFP inclusions, accumulating in bodies adjacent to them and also being distributed within (Figure 1A). PML is expressed as several isoforms (Nisole et al., 2013, Front Oncol, 3: 125). Five major PML isoforms (I, II, III, IV, and VI) were examined and it was found that all five colocalized with Atxn1-GFP inclusions (Figure 8A). For subsequent analyses, the commonly used isoform IV (hereafter called PML unless otherwise noted) was chosen.

When co-expressed with Atxn1 82Q, PML significantly decreased the size of Atxn1 82Q-GFP nuclear inclusions (Figure 1B). It also reduced the steady-state levels of the Atxn1 82Q-GFP protein, especially the aggregated SS and SR species (Figure 1C, left). To evaluate the effect of endogenous PML (all isoforms), it was knocked down using two independent small interfering RNAs (siRNAs). This noticeably raised the levels of Atxn1 82Q-GFP, especially aggregated species (Figure 1C, right). Silencing PML also increased the steady-state levels of a FLAG-tagged Atxn1 82Q protein (Figure 8B). The effect of PML siRNA on Atxn1 82Q could be reversed by an siRNA-resistant form of PML (Figure 8C), ruling out off-target effects of the siRNA.

To evaluate whether PML specifically reduces pathogenic Atxn1 proteins, a nonpathogenic ataxin-1 protein, Atxn1 30Q, was used. Forced expression of PML did not reduce the abundance of Atxn1 30Q-GFP, while knockdown of PML did not significantly augment it either (Figure 1D), underscoring the selective effect of PML on pathogenic Atxn1 proteins.

PML did not inhibit the transcription of the *Atxn1 82Q* gene (Figure 8D). To determine whether PML promotes the degradation of the Atxn1 82Q protein, a pulse-chase assay was performed. In the absence of co-transfected PML, total [^{35}S]-labeled

Atxn1 82Q protein was rather stable, and its levels declined only ~20% in 18 hours. By contrast, in the presence of PML, total [^{35}S]Atxn1 82Q protein was destabilized, and its levels declined ~80% over the same period of time (Figure 1E).

Experiments were conducted using cycloheximide (CHX) to block protein synthesis and to investigate the degradation of the pre-existing Atxn1 82Q protein. Forced expression of PML accelerated the degradation of aggregated Atxn1 82Q, reducing its half-life from ~8 hours to ~2 hours, while having a minimal effect on soluble Atxn1 82Q (Figure 1F). Conversely, silencing PML prolonged the half-life of aggregated Atxn1 82Q and, to a lesser extent, the half-life of soluble Atxn1 82Q (Figure 1G). The ability of PML to remove aggregated Atxn1 82Q was markedly diminished by the proteasome inhibitor MG132 (Figure 1H). In contrast, PML did not alter the half-life of Atxn1 30Q (Figure 8E). Collectively, these results indicate that PML targets pathogenic, but not normal, Atxn1 protein for proteasomal degradation.

A General Role for PML in Degrading Nuclear Misfolded Proteins

To assess whether PML plays a broad role in degrading misfolded proteins in the nucleus, two additional proteins linked to neurodegeneration were tested: (1) a pathogenic fragment of huntingtin (Htt) encoded by the first exon of the *HD* gene, Httex1p 97QP (Steffan et al., 2004, Science, 304: 100-104); and (2) TAR DNA-binding protein 43 (TDP-43), which is associated with both amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) (Chen-Plotkin et al., 2010, Nat Rev Neurosci, 6: 211-220). Httex1p 97QP formed microscopically visible inclusions in both the nucleus and the cytoplasm (Figure 1I), while TDP-43 formed inclusions mainly in the nucleus. PML reduced the nuclear, but not the cytoplasmic, Httex1p 97QP inclusions (Figure 1I), and decreased the amount of aggregated Httex1p 97QP (Figure 1J, lanes 1 and 2). PML also lowered the amount of aggregated, but not soluble, TDP-43 (Figure 1K).

To extend these analyses, a structurally destabilized mutant of the model chaperone substrate firefly luciferase (FlucDM) was used, which was developed as a probe for the capacity of cellular PQC systems (Gupta et al., 2011, Nat Methods, 8: 879-884). Endogenous PML partially co-localized with a nuclear form of FlucDM (nFlucDM-

GFP), which formed inclusions, but not with the wild-type counterpart (nFlucWT-GFP), which displayed diffuse localization (Figure 8F). Silencing PML noticeably elevated the levels of aggregated nFlucDM-GFP (Figure 8G) and extended the half-life of total nFlucDM-GFP protein (Figure 1L). Taken together, these results indicate that PML facilitates the removal of multiple misfolded proteins in the mammalian cell nucleus.

Recognition of Misfolded Proteins by Distinct Sites on PML

To investigate the mechanism by which PML degrades misfolded proteins, it was first examined whether PML is able to directly recognize these proteins. For these experiments a pathogenic (103Q) and a nonpathogenic (25Q) Htt fragment, each being fused to glutathione S-transferase (GST), were used. In an in vitro assay with purified recombinant proteins, immobilized FLAG-PML, but not the control protein FLAG-GFP, pulled down GST-Htt 103Q (Figure 2A), indicating a specific and direct interaction between PML and Htt 103Q. FLAG-PML also pulled down GST-Htt 25Q. However, this interaction was substantially weaker than the PML-Htt 103Q interaction (Figure 2A). In a reciprocal experiment, immobilized GST-Htt 103Q proteins also interacted more strongly with FLAG-PML than immobilized GST-Htt 25Q did (Figure 9A). Hsp70 and Hsp40, which recognize a broad range of misfolded proteins, did not enhance the PML-Htt 103Q interaction (Figure 2A). These results suggest that PML can directly associate with polyQ proteins and preferentially with the pathogenic form.

It was also examined whether PML selectively binds to denatured luciferase. 6xHis-tagged luciferase that was immobilized on Ni-NTA beads was either denatured with urea or kept in the native form. Denatured, but not native, luciferase specifically interacted with GST-PML, and the Hsp70/Hsp40 system did not enhance this interaction (Figure 2B). Thus, PML can directly recognize misfolded, but not native, luciferase.

To understand the molecular basis for the interaction of PML with misfolded proteins, it was sought to identify the substrate recognition sites (SRSs) of PML, as well as the structural features on substrates that these SRSs discern. It was previously shown that, in a manner dependent on its length, polyQ and the flanking regions form CC structures, which facilitate the assembly of polyQ proteins into an

oligomeric or aggregated state and also mediate the interaction of polyQ proteins with CC-containing proteins (Fiumara et al., 2010, Cell, 143: 1121-1135). Thus, it was hypothesized that PML, via its CC region within the TRIM/RBCC motif, interacts with pathogenic polyQ proteins. A panel of PML fragments (F1–F5) was constructed, where each fragment either contained or lacked the CC region (Figure 9B). A fragment containing the CC region (F1) interacted with Htt 103Q, while two fragments lacking this region (F2 and F3) did not (Figure 9B and Figure 9C). Moreover, the CC region alone (F4) bound to Htt 103Q, while deleting this region from the entire PML protein (F5 or ΔCC) greatly diminished this binding. Thus, PML recognizes Htt 103Q almost exclusively through the CC region. Similar to the full-length PML, PML CC displayed a clear binding preference for the pathogenic Htt 103Q to the nonpathogenic Htt 25Q (Figure 2C). PML CC also strongly interacted with another pathogenic Htt construct, Htt 52Q (Figure 2C). Thus, PML CC likely constitutes an SRS (called SRS1).

To test whether PML CC recognizes the homologous CC structure in Htt proteins, the residues in Htt 52Q that were predicted to be involved in the CC formation were mutated, yielding Htt 52Q cc- (Figure 9D). A similar mutation was previously shown to reduce the formation of the CC structure in Htt 72Q (Fiumara et al., 2010, Cell, 143: 1121-1135). Indeed, compared to Htt 52Q, Htt 52Q cc- displayed a noticeably reduced propensity to form aggregates and a substantially weaker interaction with PML CC (Figure 2C). Therefore, PML CC/SRS1 likely interacts with the CC structure on pathogenic Htt proteins.

Given that PML also promotes the degradation of non-polyQ proteins such as luciferase and TDP-43 (Figure 1 and Figure 8), it was reasoned that PML might contain at least another SRS that could discern non-CC structural features on misfolded proteins. To test this possibility, the panel of PML fragments was examined for interaction with denatured luciferase. Although the CC region alone could interact with denatured luciferase, significant levels of interaction were also observed in two fragments (F2 and F5) that lacked this region but contained the C terminus (aa 361–633) (Figure 9B and Figure 10A). Using additional deletion constructs within the C terminus (F6–F18, Figure 9B and Figure 10B), it was found that a stretch of 63 amino acids (aa 571–633) was sufficient for binding to denatured luciferase. Either NH_2- or COOH-terminal

deletions of this stretch abolished the binding (Figure 10). Thus, the last 63 amino acids of PML likely constitute another SRS (called SRS2).

To investigate the linear sequences in luciferase that can be recognized by PML SRS2, purified PML SRS2 was used to screen a cellulose-bound peptide library that represented the complete sequence of luciferase. The library consisted of 180 peptides, each containing 13 amino acid residues that overlapped adjacent peptides by ten. Similar to chaperones such as Hsp70 and ClpB (Rudiger et al., 1997, EMBO J, 16: 1501-1507; Schlieker et al., 2004, Nat Struct Mol Biol, 11: 607-615), PML SRS2 only bound to a subset of these peptides (Figure 2D), indicating its ability to distinguish peptides with different amino acid compositions. An analysis of the relative occurrence of all 20 amino acids in PML SRS2-interacting peptides versus all peptides in the library showed that PML SRS2 strongly favored aromatic (Phe, Trp, and Tyr) and positively charged (Arg and Lys) residues, and disfavored negatively charged residues (Asp and Glu) (Figure 2E). This amino acid preference was similar to that of ClpB, except that SRS2 had an additional preference for Leu and His, which are disfavored by ClpB (Schlieker et al., 2004, Nat Struct Mol Biol, 11: 607-615).

For comparison, the binding of PML CC/SRS1 to the peptide library was tested. Consistent with the notion that this region recognizes higher-order structures instead of linear sequences, PML CC/SRS1 weakly bound to only a few peptides (Figure 9E). Based on these results, it was concluded that PML contains at least two regions that can recognize misfolded proteins: the CC region within the TRIM/RBCC motif (SRS1) and the 63 amino acid stretch at its C terminus (SRS2), which can discern CC structures and exposed peptides enriched in both aromatic and basic amino acids, respectively.

Involvement of SUMOylation in the Degradation of Atxn1 82Q

Experiments were conducted to investigate how PML promotes the degradation of misfolded proteins upon recognition. Misfolded proteins associated with neurodegeneration are frequently modified by SUMO, although the role of this modification remains unclear (Martin et al., 2007, Nat Rev Neurosci, 8: 948-959). Mammalian cells express three major SUMO proteins, SUMO1–SUMO3. SUMO2 and SUMO3 are nearly identical to each other in their sequence (collectively called

SUMO2/3) and are approximately 50% identical to SUMO1 (Wilkinson and Henley, 2010, Biochem J, 428: 133-145). The modification of Atxn1 82Q by these SUMO proteins and their involvement in Atxn1 82Q degradation was investigated.

Atxn1 82Q was modified by both exogenous (Riley et al., 2005, J Biol Chem, 280: 21942-21948) and endogenous (Figure 3A, left) SUMO1, and this modification was weaker than that of Atxn1 30Q (Riley et al., 2005, J Biol Chem, 280: 21942-21948). Atxn1 82Q was also modified by endogenous SUMO2/3 (Figure 3A, right), and co-localized with GFP-SUMO2/3 in the nucleus (Figure 3B). The sites in Atxn1 82Q that were conjugated with SUMO1 and SUMO2/3 might be different, because a mutant Atxn1 82Q with impaired SUMO1 conjugation, Atxn1 82Q (5KR) (Riley et al., 2005, J Biol Chem, 280: 21942-21948), showed no defect in SUMO2/3 conjugation (Figure 11A).

Of note, Atxn1 82Q was more strongly modified by endogenous SUMO2/3 compared to Atxn1 30Q (Figure 3C), correlating with the different responses of these Atxn1 proteins to PML-mediated degradation (Figure 1 and Figure 8). Similarly, TDP-43 was modified by endogenous SUMO2/3 (Figure 11B). SUMO 2/3 also modified FlucDM, as well as another structurally destabilized luciferase mutant, FlucSM (Gupta et al., 2011, Nat Methods, 8: 879-884). The SUMO2/3 modification of these luciferase mutants was also stronger than that of wild-type luciferase (Figure 11C).

Moreover, proteasome inhibition enhanced the co-localization of Atxn1 82Q with GFP-SUMO2/3 (Figure 3B). It also increased SUMO2/3-modified Atxn1 82Q concurrently with ubiquitinated Atxn1 82Q, but not SUMO1-modified Atxn1 82Q (Figure 3A and Figure 3D, lanes 2 and 3). Likewise, proteasome inhibition increased SUMO2/3-modified TDP-43 and luciferase mutants (Figure 11B and Figure 11C).

To assess the role of SUMO proteins in the ubiquitination and proteasomal degradation of Atxn1 82Q, SUMO2/3 and SUMO1 were silenced separately using siRNA. Silencing SUMO2/3, but not SUMO1, effectively reduced ubiquitination of Atxn1 82Q (Figure 3D, lanes 4–7). Silencing SUMO2/3 also raised the levels of Atxn1 82Q, especially the aggregated form, but not the levels of the control protein GFP (Figure 11D - Figure 11F), and it diminished the ability of PML to remove aggregated Atxn1 82Q (Figure 3E).

SUMO2 and SUMO3 contain an internal SUMOylation consensus site that enables the formation of polychains. SUMO1 does not contain this site, and when conjugated to the SUMO2/3 chain, it can terminate the chain elongation (Wilkinson and Henley, 2010, Biochem J, 428: 133-145). Therefore, two additional strategies were used to inhibit the modification of Atxn1 82Q by SUMO2/3. First, SUMO1 was overexpressed. This strongly reduced Atxn1 82Q conjugation to SUMO2/3 and, at the same time, impaired conjugation of Atxn1 82Q to ubiquitin (Figure 3D, lanes 8 and 9) and its degradation by PML (Figure 3F, left). Second, a SUMO2 mutant that was deficient in chain formation, SUMO2 KR, was used. Overexpression of SUMO2 KR effectively reduced the amount of SUMO2/3-modified Atxn1 82Q species, especially those of high molecular weights. It also reduced ubiquitin-modified Atxn1 82Q species (Figure 3D, lanes 12 and 13) and blunted the ability of PML to degrade Atxn1 82Q (Figure 3F, right). Moreover, a SUMOylation-defective Htt mutant, Httex1p 97QP(KR) (Steffan et al., 2004, Science, 304: 100-104), was used, and it was found that it was resistant to the PML-mediated degradation (Figure 1J). Taken together, these results show that ubiquitination and degradation of Atxn1 82Q and likely other misfolded proteins are dependent on their modification by SUMO2/3.

PML as a SUMO E3 Ligase of Atxn1 82Q

It was previously demonstrated that PML possesses SUMO E3 ligase activity that enhances the efficiency and specificity of SUMOylation (Chu and Yang, 2011, Oncogene, 30: 1108-1116). Hence, it was examined whether PML promotes SUMOylation of Atxn1 82Q. When co-expressed with Atxn1 82Q in cells, PML strongly increased SUMO2/3 modification of Atxn1 82Q, both in the absence and in the presence of the proteasome inhibitor MG132 (Figure 4A). Conversely, silencing PML markedly reduced SUMO2/3-modified Atxn1 82Q under these conditions (Figure 4B). Similarly, silencing PML reduced SUMO2/3 modification of nFlucDM (Figure 4C).

To confirm the SUMO E3 activity of PML toward Atxn1 82Q, in vitro SUMOylation assays were performed with purified recombinant proteins. In the absence of PML, Atxn1 82Q was weakly modified by SUMO2 (Figure 4D and Figure 4E), consistent with previous observations that SUMOylation can proceed in vitro without a

SUMO E3 ligase (Wilkinson and Henley, 2010, Biochem J, 428: 133-145). Of note, PML augmented Atxn1 82Q SUMOylation in a dose-dependent manner (Figure 4D and Figure 4E). In contrast, a SUMO E3-defective mutant, PML M6 (Chu and Yang, 2011, Oncogene, 30: 1108-1116), failed to do so (Figure 4E and Figure 11G); PML M6 was also ineffective at reducing aggregated Atxn1 82Q (Figure 4F). These results suggest that PML is a SUMO E3 ligase of Atxn1 82Q, and that this activity is involved in Atxn1 82Q degradation.

A Role for RNF4 in Degrading Misfolded Proteins

Proteins conjugated with a poly-SUMO2/3 chain can be recognized and ubiquitinated by RNF4, a RING domain ubiquitin ligase with four tandem SUMO-interacting motifs (SIMs) (Sun et al., 2007, EMBO J, 26: 4102-4112). However, the role of RNF4 in degrading misfolded proteins remains undefined. It was found that forced RNF4 expression strongly reduced the steady-state levels of aggregated Atxn1 82Q in cell lysates (Figure 5A), as well as the number of Atxn1 82Q inclusions in the nucleus (Figure 12A). RNF4 also shortened the half-life of aggregated, but not soluble, Atxn1 82Q (Figure 5B, lanes 1–12; Figure 5C and Figure 12B). Conversely, knocking down endogenous RNF4 with three siRNAs, individually or in combination, increased total and aggregated Atxn1 82Q proteins in cell lysates (Figure 5D, Figure 12C and Figure 12D), as well as Atxn1 82Q inclusions in the nucleus (Figure 5E). An siRNA-resistant form of RNF4 could reverse the effect of RNF4 knockdown (Figure 12C), indicative of the specificity of the siRNA.

Moreover, both endogenous and exogenous RNF4 proteins normally displayed a diffuse nuclear distribution pattern with minimal or moderate co-localization with Atxn1 82Q inclusions. However, upon proteasome blockage, RNF4 became highly enriched in Atxn1 82Q inclusions (Figure 5F and Figure 12E), likely reflecting a stalled attempt of RNF4 in clearing Atxn1 82Q. In contrast to its effect on Atxn1 82Q, RNF4 did not reduce the levels of Atxn1 30Q (Figure 5G). Collectively, these results demonstrate a role for RNF4 in eliminating pathogenic Atxn1 proteins.

To assess a general effect of RNF4 on misfolded proteins, it was tested on Httex1p 97QP, TDP-43, and nFlucDM. Forced expression of RNF4 markedly reduced

Httex1p 97QP, especially the aggregated form, while having a much weaker effect on Httex1p 97QP(KR) (Figure 5H). Likewise, forced expression of RNF4 decreased the levels of TDP-43 (Figure 12F), whereas silencing RNF4 augmented the percentage of TDP-43-expressing cells with nuclear inclusions (Figure 12G and Figure 12H). Upon proteasome inhibition, endogenous RNF4 became highly enriched in TDP-43 inclusions (Figure 12I), similar to its accumulation in Atxn1 82Q inclusions under the same conditions (Figure 5F). Moreover, silencing RNF4 prolonged the half-life of nFlucDM (Figure 5I). Collectively, these observations suggest that RNF4 plays a critical role in the degradation of diverse misfolded proteins.

RNF4 Mediates Ubiquitination and Degradation of SUMO2/3-Modified Atxn1 82Q

Similar to PML, the ability of RNF4 to eliminate misfolded proteins was dependent on SUMO2/3, as this ability was compromised in cells devoid of SUMO2/3, but not in cells devoid of SUMO1 (Figure 13A). Of note, forced expression of RNF4 preferentially reduced SUMO2/3-modified Atxn1 82Q and nFlucDM over the unmodified proteins (Figure 6A and Figure 6B). Conversely, silencing RNF4 increased SUMO2/3-modified Atxn1 82Q (Figure 6C) and enhanced the localization of GFP-SUMO2 to the Atxn1 82Q inclusions (Figure 13B). These results show that RNF4 targets SUMO2/3-modified misfolded proteins for degradation.

To confirm that RNF4 ubiquitinates SUMO2/3-conjugated misfolded proteins, an in vitro ubiquitination assay was performed using a mixture of unmodified and SUMO2-modified Atxn1 82Q proteins (Figure 6D). In the presence of increasing doses of RNF4, the SUMO2-modified Atxn1 82Q proteins, which were of relatively low molecular weight (Figure 6E, lanes 1, 6, and 9), were progressively converted to higher-molecular-weight species that were also modified by ubiquitin (lanes 2–4, 7–9, and 12–14). In contrast, the unmodified Atxn1 82Q protein was not ubiquitinated (lanes 1–4). Therefore, RNF4 is a ubiquitin ligase for SUMO2/3-modified, but not unmodified, Atxn1 82Q protein.

RNF4 possesses both ubiquitin ligase and SUMO-binding activities (Sun et al., 2007, EMBO J, 26: 4102-4112). To ascertain the involvement of these activities in degrading Atxn1 82Q, RNF4 mutants defective in either ubiquitin ligase (CS and CS1) or

SUMO-binding (SIM^m) activity were generated (Figure 13C). CS and CS1, albeit losing their ubiquitin E3 activity (Figure 13D), were still able to co-localize with Atxn1 82Q inclusions (Figure 6F). SIM^m, on the other hand, retained a substantial level of ubiquitin ligase activity (Figure 13E), but failed to co-localize with Atxn1 82Q inclusions (Figure 6G). Neither of the RNF4 mutant classes was capable of removing aggregated Atxn1 82Q (Figure 6H). Collectively, these results suggest that RNF4 binds to SUMO2/3-modified misfolded proteins via its SIM region and ubiquitinates these proteins via its ligase activity.

Although forced expression of PML led to effective clearance of aggregated Atxn1 82Q in control cells, this ability was greatly diminished in RNF4-depleted cells (Figure 6I). Reciprocally, forced expression of RNF4, albeit highly effective in accelerating Atxn1 82Q degradation in control cells, failed to do so in PML-depleted cells (Figure 5B, lanes 19–24 versus lanes 7–12; and Figure 5C). Moreover, in PML-depleted cells, which displayed high Atxn1 82Q levels, silencing RNF4 did not further elevate the levels of Atxn1 82Q (Figure 13F). These results indicate mutual dependence of PML and RNF4 in the degradation of Atxn1 82Q.

PML Deficiency Exacerbates Behavioral and Pathological Phenotypes in a Mouse Model of SCA1

The results described above revealed a PQC system that degrades Atxn1 82Q and likely other nuclear misfolded proteins through sequential PML-mediated SUMOylation and RNF4-mediated ubiquitination. To investigate the physiological role of this system, a mouse model of SCA1 (B05), which expresses the *Atxn1 82Q* transgene ($Atxn1^{tg/-}$) in the cerebellar Purkinje cells, was used. Resembling human SCA1 patients, B05 mice develop ataxia and neurological abnormalities with increasing age (Burright et al., 1995, Cell, 82: 937-948). The loss of RNF4 in mice results in embryonic lethality (Hu et al., 2010, Proc Natl Acad Sci, 107: 15087-15092), precluding the analysis of its effect on B05 mice. However, *PML*-knockout ($PML^{-/-}$) mice are viable and appear to develop normally (Wang et al., 1998, Science, 279: 1547-1551). B05 mice were crossbred with $PML^{-/-}$ and *PML*-wild-type ($PML^{+/+}$) mice and littermates of all genotypes—$PML^{+/+}$,

PML$^{+/-}$, and *PML*$^{-/-}$, *PML*$^{+/+}$:*Atxn1*$^{tg/-}$, *PML*$^{+/-}$:*Atxn1*$^{tg/-}$, and *PML*$^{-/-}$:*Atxn1*$^{tg/-}$— were compared for both motor performance and neuropathology.

Motor performance—including balance, coordination, and endurance—was evaluated using a Rotarod apparatus with accelerating speed. To determine whether any potential behavioral defects were due to a progressively diminished capacity, as opposed to a developmental impairment, mice at different ages were examined. To rule out the influence of the long-term motor memory, only naive animals were used, each being tested for 4 consecutive days.

At 7 weeks of age, all mice performed similarly on the Rotarod (Figure 7A). Although some differences were observed among mice of distinct genotypes, they were not statistically significant (ANOVA p = 0.53), suggesting that *PML*$^{-/-}$ mice did not have pre-existing impairments in their motor functions. At 11 weeks of age, all mice lacking the *Atxn1 82Q* transgene (*PML*$^{+/+}$, *PML*$^{+/-}$, and *PML*$^{-/-}$) still showed no statistical difference in their performance (ANOVA p = 0.33) (Figure 7B), and *PML*$^{+/+}$ and *PML*$^{+/+}$:*Atxn1*$^{tg/-}$ also performed similarly. These observations suggest that either *PML* deficiency or *Atxn1 82Q* transgene expression alone was insufficient to cause motor defects at this age. Interestingly, *PML*$^{-/-}$:*Atxn1*$^{tg/-}$ showed severe impairments in Rotarod performance compared to either *PML*$^{+/+}$:*Atxn1*$^{tg/-}$ or *PML*$^{-/-}$ mice. Although these three groups of animals were comparable at the beginning of the 4 consecutive testing days, unlike the other two groups, *PML*$^{-/-}$:*Atxn1*$^{tg/-}$ mice showed minimal improvement over time. The lack of improvement of *PML*$^{-/-}$:*Atxn1*$^{tg/-}$ mice on the Rotarod was reminiscent of *Atxn1*$^{tg/-}$ mice at advanced stages (Clark et al., 1997, J Neurosci, 17: 7385-7395). The *PML* heterozygous counterparts (*PML*$^{+/-}$:*Atxn1*$^{tg/-}$ mice) displayed an intermediate impairment on the Rotarod (ANOVA p = 0.0004 for the three *Atxn1*$^{tg/-}$ groups) (Figure 7B). Thus, *PML* deficiency aggravates motor defects of the *Atxn1*$^{tg/-}$ mice.

The major neuropathological phenotype of the *Atxn1*$^{tg/-}$ mice is the degeneration of Purkinje cells, a constituent of the top layer (the molecular layer) of the cerebellar cortex. This degeneration is manifested initially in the shrinkage of the molecular layer and the atrophy of Purkinje cell dendrites, and later in the loss of Purkinje cell bodies (Burright et al., 1995, Cell, 82: 937-948; Clark et al., 1997, J Neurosci, 17: 7385-7395). At 12 weeks of age, *PML*$^{+/-}$ and *PML*$^{-/-}$ mice showed only a

slight and statistically insignificant shrinkage in the molecular layers, while $PML^{+/+}$:$Atxn1^{tg/-}$ mice exhibited a discernible shrinkage, compared to $PML^{+/+}$ mice (Figure 7C and Figure 7D). Because $PML^{+/+}$:$Atxn1^{tg/-}$ mice performed similarly on the Rotarod to $PML^{+/+}$ mice (Figure 7B), neurodegeneration in $PML^{+/+}$:$Atxn1^{tg/-}$ mice might not have reached a critical threshold. This nonlinear correlation between behavioral and pathological phenotypes of the SCA1 transgenic model has been previously observed (Gehrking et al., 2011, Hum Mol Genet, 20: 2204-2212). Importantly, compared to $PML^{+/+}$:$Atxn1^{tg/-}$ mice, $PML^{+/-}$:$Atxn1^{tg/-}$ and $PML^{-/-}$:$Atxn1^{tg/-}$ mice displayed a moderate and a strong further reduction, respectively, in the thickness of molecular layer (Figure 7C and Figure 7D). This correlated with worsening performance of these animals on the Rotarod (Figure 7B). Thus, PML deficiency aggravates the shrinkage of the molecular layer in $Atxn1^{tg/-}$ mice.

Dendritic arborization of Purkinje cells was also examined by immunofluorescence staining with an antibody against the Purkinje cell-specific protein calbindin. At 12 weeks of age, the fluorescence intensity of Purkinje cell dendrites in all groups containing the *Atxn1 82Q* transgene was reduced to very low levels that precluded precise comparison (Figure 14A and Figure 14B). Of note, compared to $PML^{+/+}$ littermates, $PML^{-/-}$ mice already showed a strong reduction in dendritic arborization of Purkinje cells, while $PML^{+/-}$ mice showed an intermediate reduction (Figure 14A and Figure 14B). These results indicate that PML itself has a role in protecting against neurodegeneration.

Despite the thinning of the molecular layer and the loss of Purkinje cell dendrites that were associated with *PML* deficiency, no significant difference in Purkinje cell population was observed among 12-week-old animals of different genotypes (Figure 14C). At 1 year of age, $PML^{-/-}$ mice displayed only a mild (11.0%) and statistically insignificant ($p = 0.107$) reduction in the number of Purkinje cells compared to $PML^{+/+}$ mice, while $PML^{+/+}$:$Atxn1^{tg/-}$ mice displayed a noticeable reduction (Figure 7E and Figure 7F). Of note, $PML^{-/-}$:$Atxn1^{tg/-}$ mice showed a significant further reduction in Purkinje cell density compared to $PML^{+/+}$:$Atxn1^{tg/-}$ mice (~24%, $p = 0.0023$), and $PML^{+/-}$:$Atxn1^{tg/-}$ mice showed an intermediate cell loss (Figure 7E and Figure 7F).

Again, these results demonstrate that PML deficiency worsens the neuropathological defects caused by the *Atxn1 82Q* transgene.

Neurodegeneration of B05 mice is accompanied by the formation of ubiquitin-positive Atxn1 82Q inclusions in Purkinje cells (Clark et al., 1997, J Neurosci, 17: 7385-7395). To determine the effect of PML on Atxn1 82Q nuclear inclusions, Purkinje cells with these inclusions were quantified in mice at 12 weeks of age. *PML* deficiency alone did not result in the formation of aggregates (Figure 14D), but it significantly increase the number of aggregate-containing Purkinje cells in Atxn1 82Q transgenic mice (Figure 7G and Figure 7H). Collectively, these results suggest that endogenous PML plays a role in preventing accumulation of misfolded proteins in SCA1 animals and suppressing the progression of this neurodegenerative disease.

PQC system that degrades misfolded proteins

Presented herein is evidence for a PQC system that degrades misfolded proteins in mammalian cell nuclei. This system comprises a recognition branch, PML, which selectively binds to misfolded proteins and marks these proteins with poly-SUMO2/3 chains, and an effector branch, RNF4, which ubiquitinates SUMOylated misfolded proteins and targets them for proteasomal degradation. This relay system likely provides a critical link between misfolded proteins and the proteasome in mammalian cells, and it may play an important role in the protection against neurodegeneration and other proteopathies (Figure 7I).

Selective Recognition of Misfolded Proteins by PML

The exquisite selectivity of this system resides in PML, which contains at least two SRSs. SRS1, consisting of the CC region within the TRIM/RBCC motif, favors CC structures on pathogenic polyQ proteins and perhaps other misfolded proteins. SRS2, consisting of the C-terminal 63 amino acids, recognizes short peptides enriched in both aromatic (Phe, Trp, and Tyr) and positively charged (Arg and Lys) amino acids. SRS2 is similar to the bacterial Hsp100 ClpB (Schlieker et al., 2004, Nat Struct Mol Biol, 11: 607-615), except that SRS2 also favors peptides containing Leu, a residue that is often exposed in misfolded proteins and is engaged by Hsp70 (Rudiger et al., 1997, EMBO J,

16: 1501-1507). Thus, SRS2 displays a hybrid substrate specificity of ClpB and Hsp70. These observations indicate that PML can recognize structures or regions that are commonly found in misfolded proteins.

A Role for SUMOylation in Degrading Misfolded Proteins

Conjugation to SUMO is a major posttranslational modification, occurring on numerous proteins and vital to most eukaryotic life. Yet, beyond the generalization that it alters protein-protein interactions, the physiological function of SUMOylation remains elusive (Wilkinson and Henley, 2010, Biochem J, 428: 133-145). A prominent feature of the PML-RNF4 system is the involvement of SUMO2/3 modification prior to ubiquitination (Figure 3, Figure 4, and Figure 11). It was observed previously that conjugation of SUMO2/3 to cellular proteins is markedly enhanced by protein-denaturing stresses (Saitoh and Hinchey, 2000, J Biol Chem, 275: 6252-6258). The evidence presented in the current study provides an explanation for this observation, and suggests that a principal physiological function of SUMO2/3 modification is likely to facilitate the degradation of misfolded proteins, acting in concert with ubiquitination.

SUMO conjugation enhances protein solubility (Panavas et al., 2009, Methods Mol Biol, 497: 303-317). Because aggregated proteins cannot be effectively degraded by the proteasome (Verhoef et al., 2002, Hum Mol Genet, 11: 2689-2700), enhancing protein solubility may be a beneficial effect conferred by PML prior to ubiquitination. Moreover, the extent of SUMOylation may enable the "triage decision" as to whether a given misfolded protein is selected for refolding or degradation. Consistent with this notion, conjugation to a single SUMO appears to be sufficient to enhance protein solubility (Panavas et al., 2009, Methods Mol Biol, 497: 303-317), and thus may facilitate refolding. In contrast, conjugation to SUMO2/3 chains is needed for effective recognition by the four tandem SIMs on RNF4 for ubiquitination and degradation (Tatham et al., 2008, Nat Cell Biol, 10: 538-546). Such chains may form after unsuccessful refolding attempts.

SUMOylation of misfolded proteins has been reported to either promote or inhibit neurodegenerative diseases (Martin et al., 2007, Nat Rev Neurosci, 8: 948-959). These seemingly contradictory observations may be reconciled by the distinct functions

of SUMO1 and SUMO2/3 in the removal of misfolded proteins (Figure 3), and by the dichotomy between the functions of SUMOylation: enhancing solubility of an abnormal protein (which may enhance its toxicity) and promoting its degradation. Thus, the outcome of elevated SUMOylation likely depends on whether it can be matched by the cellular degradative capacity.

A Potential Major PQC System

Proteins in the nucleus may harbor mutations or sustain acute and chronic damages, as proteins elsewhere do. The highly crowded environment of the nucleus likely makes it especially challenging to maintain protein quality. The ubiquitin-proteasome pathway is expected to be the main degradative system in the nucleus, where autophagy is not known to operate. Previous studies have implicated a few ubiquitin ligases, such as yeast San1 and Doa10 and mammalian UHRF-2 and E6-AP, in the degradation of nuclear misfolded proteins (Cummings et al., 1999, Neuron, 24: 879-892; Deng and Hochstrasser, 2006, Nature, 443: 827-831; Gardner et al., 2005, Cell, 120: 803-815; Iwata et al., 2009, J Biol Chem, 284: 9796-9803). Nevertheless, the predominantly nuclear localization of PML, along with the potent effect of PML and RNF4 on diverse misfolded nuclear proteins, suggests that the PML-RNF4 system is likely a major PQC system in mammalian cell nuclei.

The TRIM family of proteins is shared among metazoans, from approximately 20 members in *C. elegans* to over 70 in mice and humans (Ozato et al., 2008, Nat Rev Immunol, 8: 849-860). It was previously demonstrated that at least several other TRIM proteins also possess SUMO E3 activity (Chu and Yang, 2011, Oncogene, 30: 1108-116). Given their localization to the cytoplasm in addition to the nucleus (Ozato et al., 2008, Nat Rev Immunol, 8: 849-860), it is speculated that TRIM proteins also participate in PQC in the cytoplasm. The rapid expansion of the TRIM proteins during evolution might in part be a response to the increasing complexity of managing protein quality in cells of longer-living animals.

RNF4 is conserved among vertebrates (Sun et al., 2007, EMBO J, 26: 4102-4112). SUMO-dependent ubiquitin ligases are also present in low eukaryotic species (Sun et al., 2007, EMBO J, 26: 4102-4112), and are involved in the degradation

of at least one mutant yeast transcription factor (Wang and Prelich, 2009, Mol Cell Biol, 29: 1694-1706). Thus, it is also possible that systems analogous to the PML-RNF4 system may play a role in maintaining protein quality in these organisms.

The PML-RNF4 System and Neurodegeneration

PML$^{-/-}$ mice have been extensively characterized for a variety of phenotypes including tumorigenesis (Wang et al., 1998, Science, 279: 1547-1551). The present study indicates a role for PML in protection from neurodegeneration (Figure 7 and Figure 14). Neurodegenerative disorders including SCAs and HD are usually late-onset diseases. Accumulating evidence suggests a progressive decline in PQC during aging (Balch et al., 2008, Science, 319: 916-919). The strong effect of the PML-RNF4 on pathogenic proteins associated with SCA1, HD, and ALS and of PML deficiency on the progression of the SCA1 mouse model, along with the accumulation of PML in neuronal inclusions in patients with various neurodegenerative diseases (Skinner et al., 1997, Nature, 389, 971-974; Takahashi et al., 2003, Neurobiol Dis, 13: 230-237), suggests that insufficiency or dysfunction of the PML-RNF4 system may have a role in these diseases. Thus, the PML-RNF4 system and analogous systems would be valuable targets in their treatment.

Example 2: TRIM proteins can recognize misfolded proteins and promote their degradation

The tripartite motif-containing (TRIM) family consists of a large number of proteins in metazoan cells, ranging from approximately twenty in *C. elegans* to over seventy in mice and humans. These proteins share at their N-termini the characteristic TRIM or RBCC motif, which is comprised of a RING domain, one or two B-boxes (which, like the RING domain, a coordinated by zinc ions), and a coiled coil region. This is followed by C-terminal region that are much more variable among TRIM proteins and contains distinct motifs (Hatakeyama, 2011, Nat Rev Cancer, 11: 792-804; Ozato et al., 2008, Nat Rev Immunol, 8: 849-860). TRIM proteins modulate a range of cellular processes including those that protect against cancer and viral infection. Biochemically, a number of TRIM proteins exhibit ubiquitin E3 ligase activity, which is attributed to the

RING domain within the TRIM/RBCC region. In addition, at least several TRIM proteins possess E3 ligases for protein conjugation to SUMO (small ubiquitin-like modifier). However, an outstanding issue has remained as to the substrates of the TRIM family of SUMO E3.

As described above, it was demonstrated that PML (promyelocytic leukemia protein; also known as TRIM19) plays a critical role in the elimination of misfolded proteins. PML was initially identified as the product of a gene involved in the t(15;17) chromosomal translation that is associated with the majority of acute promyelocytic leukemia. It is the major structural and the namesake component of the PML nuclear bodies. It was shown that PML is able to specifically bind to and promote the degradation of a range of misfolded protein. Via distinct regions, PML can discern common features found in misfolded proteins, including peptides enriched in aromatic amino acid residues and coiled coil structures. PML then tags misfolded proteins with poly-SUMO2/3 chains through its SUMO E3 activity. This permits modified misfolded proteins to be recognized by the SUMO-targeted ubiquitin ligase (STUbL) RNF4, which ubiquitinates misfolded proteins and target them for with the consequential degradation in the proteasome. The role of PML in protein quality control is important for the protection against neurodegenerative diseases, as PML deficiency exacerbates behavioral as well as neuropathological phenotypes of a mouse model of spinocerebellar ataxia type 1 (SCA1), a progressive and lethal disease caused by the expansion of a polyglutamine (polyQ) stretch in ataxin-1. Given the existence of a large number if TRIM proteins, the results obtained using PML raises an important question as to whether other TRIM proteins, like PML, are able to recognize and degrade misfolded proteins. In the experiments presented herein, a panel of TRIM proteins is analyzed and it is observed that the ability to recognize and degrade misfolded proteins is prevalent among TRIM proteins, indicating a critical role for this family in protein quality control in metazoan cells.

The materials and methods employed in these experiments are now described.

Plasmids

FLAG-TRIM27, FLAG-TRIM32, and FLAG-TRIM5δ were made in pRK5 by PCR. Templates for PCR amplification were purchased from Open Biosystems, and the corresponding gene accession numbers are BC013580, BC003154 and CV029096, respectively. All three genes are of human origin. The following plasmids were previously described: FLAG-PML (isoform IV) (Chu and Yang, 2011, Oncogene, 30: 1108-116); Atxn1 82Q-GFP, FLAG-Atxn1 82Q, and HA-Httex1p 97QP (Guo et al., 2014, Mol Cell, 55(1): 15-30); FLAG-TRIM11 (Ishikawa et al., 2006, FEBS Lett, 580: 4784-4792); FLAG-TRIM22 (the long form) (Barr et al., 2008); HA-TRIM39 (Lee et al., 2009, Exp Cell Res, 315: 1313-1325); HA-tagged TRIM1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 32 (Reymond et al., 2001, EMBO J, 20: 2140-2151; Uchil et al., 2008, 2008, PLoS Pathog, 4: e16); and the remaining TRIM expression plasmids, expressing proteins with either an N-terminal HA-tag or C-terminal V5 tag (Versteeg et al., 2013, Immunity, 38: 384-398). For analyzing the effect of TRIM proteins on the levels of Atxn1 82Q and Httex1p 97QP, Trim11 (NM_145214.2), Trim15 (NM_033229.2), Trim28 (NM_005762.2), Trim34 (NM_021616.5), Trim39 (NM_021253.3), Trim42 (NM_152616.4), Trim43 (NM_138800.1), Trim65 (NM_173547.3), Trim67 (NM_001004342.3), Trim70 (NM_001037330.1), Trim71 (NM_001039111.2) and Trim75 (NM_001033429.2) were constructed into pcDNA 3.1(-) vector containing a 5'-HA tag. All the other Trim plasmids were obtained (Versteeg et al., 2013, Immunity, 38: 384-398).

siRNAs

SUMO2/3 siRNAs (Santa Cruz sc-37167) was a pool of three different siRNA duplexes with the sense strand sequences of 5'-CCCAUUCCUUUAUUGUACA-3' (SEQ ID NO: 157), 5'-CAGAGAAUGACCACAUCAA-3' (SEQ ID NO: 158), and 5'-CAGUUAUGUUGUCGUGUAU-3' (SEQ ID NO: 159).

TRIM27 siRNA was purchased from Qiagen, with the sense strand sequences being 5'-AACTCTTAGGCCTAACCCAGA-3' (SEQ ID NO: 162).

Cell culture and transfection

HeLa cells were obtained from ATCC. *PML*$^{+/+}$ and *PML*$^{-/-}$ MEF cells were derived from the embryos of mice with the corresponding genotypes. Cells were maintained in standard culture conditions. DNA plasmids were transfected into cells using Lipofectamine 2000, and siRNAs were transfected into cells in two rounds on consecutive days using either Lipofectamine 2000 or RNAiMAX (Invitrogen), according to the manufacturer's instructions. When both DNA and siRNA were transfected, DNA was transfected a day after the second round of siRNA transfection. MG132 (Sigma) was added 24 hours after the last transfection at 7.5-10 μM (final concentration) for 4-5 hours.

Immunofluorescence

Cells cultured on coverslips were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.2% Triton X-100 for 15 minutes, and incubated sequentially with primary and secondary antibodies. Primary antibodies were anti-HA, anti-FLAG (mouse mAb M2, 1:2,000) (Sigma), and anti-TRIM27. Secondary antibodies were FITC-conjugated anti-mouse, anti-rabbit (Zymed), and anti-goat (Invitrogen) IgGs; Texas Red-conjugated anti-mouse and anti-rabbit IgGs (Vector labs); and Rhodamine Red-X conjugated anti-goat (Jackson ImmunoResearch Labs). Afterwards, cells were mounted with medium containing DAPI (Vector Labs), and the images were acquired with a Nikon Eclipse E800 or Olympus IX81 microscope.

Cell lysate fractionation, Western blot, and filter retardation assay

Samples were prepared as described (Guo et al., 2014, Mol Cell, 55(1): 15-30). Briefly, cells were lysed on ice in NP-40 lysis buffer (50 mM Tris, pH 8.8, 100 mM NaCl, 5 mM MgCl2, 0.5% NP-40, 2 mM DTT) supplemented with 250 IU/ml benzonase (Sigma), 1 mM PMSF, 1x complete protease cocktail (Roche), and 20 mM N-Ethylmaleimide (NEM; Sigma). Cell lysates were centrifuged at 13,000 rpm for 15 minutes at 4 °C. The supernatant, containing NP-40-soluble (NS) proteins, was analyzed by SDS-PAGE and Western blot. The pellet was resuspended in the pellet buffer (20 mM Tris, pH 8.0, 15 mM MgCl2, 2 mM DTT) supplemented with 250 IU/ml benzonase, 1 mM PMSF, 1X complete protease cocktail, and 20 mM NEM. The pellet fraction was boiled in 2% SDS plus 50 mM DTT and was resolved by SDS-PAGE. Proteins entering

the gel (SDS-soluble, SS) were detected by Western blot. For filter retardation (dot blot) assay, a portion of the boiled pellet was applied to a membrane filter with 0.2 μm pore size, and the SDS-resistant (SR) aggregates retained on the filter was analyzed by immunoblotting. Primary antibodies were: anti-HA (rabbit, Y-11, 1:500) (Santa Cruz Biotechnology); anti-FLAG (mouse, M2, 1:7,500) and anti-actin (rabbit, 1:10,000) (Sigma); anti-GFP (mouse, 1:4,000) (Clonetech); and SUMO2/3 (rabbit, 1:250, Abgent). The secondary antibodies were either conjugated to HRP (Santa Cruz Biotechnology), or labeled with IRD Fluor 800 or IRD Fluor 680 (LI-COR, Inc.). Western blots were developed using ECL reagents and analyzed using ImageJ, or scanned with the Odyssey infrared imaging system, and analyzed using Image Studio Lite (LI-COR, Inc.).

Protein purification and in vitro SUMOylation assays

FLAG-TRIM27 and HA-Atxn1 82Q-FLAG were expressed in 293T cells and purified by anti-FLAG M2 beads (Sigma) as previously described (Tang et al., 2006, Nat Cell Biol, 8: 855-862; Tang et al., 2004, J Biol Chem, 279: 20369-20377) with modifications. Cells were lysed in IP-lysis buffer (50mM Tris, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 0.5% NP-40 and 2 mM DTT) supplemented with 1 mM PMSF and 1x complete protease cocktail. For TRIM27 purification, IP-lysis buffer was also supplemented with 20 μM $ZnCl_2$. The lysates were incubated with anti-FLAG M2 beads at 4°C for 4 hours to overnight. M2 beads were washed with IP-lysis buffers containing 0, 0.5, and 1 M KCl, and with the elution buffer (50 mM Tris, pH 7.5, 150 mM NaCl, and 2 mM DTT). The bound proteins were eluted in the elution buffer containing 0.1-0.3 mg/ml 3xFLAG peptide (Sigma).

Other components for *in vitro* SUMOylation reactions were purchased from Boston Biochem. *In vitro* SUMOylation assays were performed at 37 °C for 1.5 hours in 30 μl reaction buffer (50 mM Tris pH 7.5, 5.0 mM Mg2+-ATP, and 2.5 mM DTT) containing purified HA-Atxn1 82Q-FLAG (600 ng /200 nM), FLAG-TRIM27, SAE1/SAE2 (125 nM), Ubc9 (1 μM), His-SUMO2 (25 μM), and BSA (0.1 μg/ml). The reaction mixtures were denatured by the addition of 30 μl IP-lysis buffer containing 2% SDS and 50 mM DTT and heating at 95°C for 10 minutes. One aliquot of the heated reaction mixes were saved for Western blot analysis, and the rest were diluted 20-fold in

IP-lysis buffer without SDS. HA-Atxn1 82Q-FLAG was immunoprecipitated by anti-HA beads (Roche) and analyzed for SUMO2/3 modification using an anti-SUMO2/3 antibody.

The results of the experiments are now described.

Co-localization of TRIM27, TRIM32, and TRIM5δ with pathogenic Ataxin-1 and huntingtin proteins

It was previously showed that PML/TRIM19 can recognize and promote the degradation of various nuclear misfolded proteins including Atxn1 82Q, a pathogenic ataxin 1 protein with a stretch of eight-two glutamine that is associated with spinocerebellar ataxia type 1 (SCA1) (Guo et al., 2014, Mol Cell, 55(1): 15-30). The TRIM family of proteins in humans and mice consists of over seventy members that can be distinguished based on structural features of the region C-terminal to the conserved TRIM/RBCC motif (Ozato et al., 2008, Nat Rev Immunol, 8: 849-860). The majority of TRIM proteins contain in this region one or more conserved domains, the most common of which include the PRY-SPRY domain (present in ~40 TRIM proteins) and multiple NHL domains (present in 4 TRIM proteins). In addition, several TRIM proteins do not contain a recognizable motif in the C-terminal region. To examine whether other human TRIM proteins, like PML, are able to recognize misfolded proteins, TRIM27 (with a PRY-SPRY domain), TRIM32 (with multiple NHL domains), and TRIM5δ (a short splicing variant of TRIM5 with no known domain) were first chosen. When expressed in HeLa cells, these three TRIM proteins displayed overlapping yet distinct cellular localization patterns: TRIM27 and TRIM32 were localized in both the cytoplasm and the nucleus, while TRIM5δ was localized only in the cytoplasm. Nevertheless, all three proteins are concentrated in the speckled bodies in their respective compartment(s) (Figure 15A).

Experiments were conducted to examine the co-localization of TRIM27, TRIM32, and TRIM5δ with Atxn1 82Q, as well as with a fragment of the huntingtin protein (Htt) containing a stretch of ninety-seven glutamines (Httex1p 97Q), which is associated with HD. Atxn1 82Q, which was expressed as a fusion of enhanced green

fluorescence protein (GFP), formed inclusions only in the nucleus, while HA-tagged Httex1p 97QP formed inclusions in both the nucleus and cytoplasm (Guo et al., 2014, Mol Cell, 55(1): 15-30) (Figure 15A and Figure 15B). Exogenous TRIM27 and TRIM32 co-localized with Atxn1 82Q-GFP inclusion in the nucleus (Figure 15A) and with HA-Httex1p 97QP inclusions in both the nucleus and cytoplasm (Figure 15B and Figure 15C). Moreover, endogenous TRIM27 also co-localized with Atxn1 82QGFP inclusion (Figure 15D). Exogenous TRIM5δ co-localized only with the cytoplasmic HA-Httex1p 97QP inclusions (Figure 15B and Figure 15C). Thus, despite the differences in their C-terminal sequence, TRIM27, TRIM32, and TRIM5δ are able to recognize misfolded protein formed in their respective cellular compartment(s).

TRIM27, TRIM32, and TRIM5δ reduce the levels of both insoluble and soluble Atxn1 82Q proteins

To examine whether these TRIM proteins are able to reduce the levels of misfolded proteins, each of them was expressed with FLAG-tagged Atxn1 82Q in HeLa cells. The levels of FLAG-Atxn1 82Q in cell lysates that were NP40-soluble (soluble or NS) and NP-40-insoluble, but SDS-soluble (aggregated or SS), were analyzed. Of note, each TRIM protein was able to reduce the levels of soluble and aggregated Atxn1 82Q protein (Figure 16A and Figure 16B). TRIM27 and TRIM32 also decreased the levels of Atxn1 82Q protein that were resistant to both NB-40 and SDS (SR), which were detected by a filter retardation assay and likely represented β-amyloid structure (see below).

When expressed at similar levels, all three TRIM proteins, especially TRIM32, showed stronger activity than PML in reducing the levels of soluble Atxn1 82Q (Figure 16A). Moreover, while the activity of TRIM27 and TRIM5δ was somewhat weaker than, the activity of TRIM32 in reducing aggregated Atxn1 82Q was comparable to that of PML (Figure 16A and Figure 16B). TRIM27 was chosen for further analysis. The expression of endogenous TRIM27 was knocked down using siRNA, which led to a significant increase in the levels of both soluble and aggregated Atnx1 82Q proteins (Figure 16C). Together, these results suggest that similar to PML, TRIM27, TRIM32, and TRIM5δ are capable of removing misfolded proteins. In contrast to these TRIM

proteins, PIASy, a member of the PIAS family of SUMO E3s, was unable to reduce the levels of either soluble or aggregated Atxn1 82Q (Figure 16D).

The effect of TRIM27 and TRIM32 on Atxn1 82Q is independent of PML

TRIM27 is partially co-localized in PML nuclear bodies (Cao et al., 1998, J Cell Sci, 111(Pt 10): 1319-1329) (Figure 17A). To examine whether TRIM27 and the other nucleus-localized TRIM protein, TRIM32, rely on PML to degrade misfolded protein, PML-wild type ($PML^{+/+}$) and PML-deficient ($PML^{-/-}$) mouse embryonic fibroblasts (MEFs) were used. Despite the co-localization of TRIM27 with the PML nuclear bodies, TRIM27 was present in the Atxn1 82Q aggregates even in the absence of PML (Figure 17B). The levels of SDS-insoluble Atxn1 82Q aggregates were markedly higher in $PML^{-/-}$ compared to $PML^{+/+}$ MEFs (Figure 17C), consistent with a role for PML in removing Atxn1 82Q (Guo et al., 2014, Mol Cell, 55(1): 15-30). Re-introducing PML decreased the levels of the aggregate Atxn1 82Q. Of note, TRIM27 and TRIM32 were as effective as PML in reducing aggregated Atxn1 82Q in both $PML^{+/+}$ and $PML^{-/-}$ cells (Figure 17C). Together, these results suggest that TRIM27 and TRIM32 can eliminate Atxn1 82Q independently of PML.

TRIM27, TRIM32, and TRIM5δ target Atxn1 82Q for proteasomal degradation in a SUMO2/3-dependent manner

It was previously found that PML promotes proteasomal degradation of misfolded proteins via its SUMO E3 activity (Guo et al., 2014, Mol Cell, 55(1): 15-30). When cells were treated with the proteasome inhibitor MG132, the ability of TRIM27, TRIM32, and TRIM5δ to reduce soluble and especially insoluble Atxn1 82Q protein was significantly impaired, suggesting that these three TRIM proteins also target Atxn1 82Q for proteasomal degradation (Figure 18A - Figure 18C). In addition, when endogenous SUMO2/3 was knocked down with siRNAs, TRIM27, TRIM32, and TRIM5δ could no longer effectively reduce the levels of Atxn1 82Q (Figure 18D - Figure 18F). Hence, these TRIM proteins also rely on SUMO2/3 in degrading Atxn1 82Q.

Using TRIM27 as an example, it was next examined whether TRIM proteins other than PML can act as SUMO E3 ligases for Atxn1 82Q. When incubated

with SUMOylation reaction components, including SUMO E1 (SAE1/SAE2), E2 (Ubc9), SUMO2, and ATP, Atxn1 82Q was minimally conjugated to SUMO2. However, conjugation of Atxn1 82Q to SUMO2 was enhanced in a TRIM27 dose-dependent manner (Figure 18G), suggesting that TRIM27 is a SUMO E3 for Atxn1 82Q.

Co-localization with misfolded proteins is a prevalent property of TRIM proteins

Prompted by these observations, the remaining TRIM proteins were tested for their ability to recognize Atxn1 82Q. With the exception of two (TRIM12 and TRIM30), which are present in mice but not in humans, all the other TRIM proteins are of human origin. Each TRIM was tagged with either an HA, Flag, or V5 epitope, and was co-expressed with Atxn1 82Q-EGFP in HeLa cells.

A previous survey of a subset of TRIM proteins showed that these proteins identify with compartments in both the cytoplasm and nucleus (Reymond et al., 2001, EMBO J, 20: 2140-2151). Among the TRIM proteins tested here, eleven (TRIM42, 43, 53, 59-61, 67, 70-72, and 75) could not be detected by immunofluorescence. Among the remaining sixty-three TRIM proteins, five of them (TRIM22, 28, 33, 65, and 66) were localized exclusively in the nucleus, a localization pattern similar to PML. However, the possibility that certain isoforms of these TRIM proteins that were not tested here may be present in the cytoplasm cannot be excluded. For example, such cytoplasmic isoforms have been shown for PML. Twenty-seven TRIM proteins (TRIM1-5, 7, 9, 10, 13, 18, 20, 24, 25, 29, 34, 36, 37, 39, 45-47, 50, 54, 63, 69, and 76) were localized mainly or exclusively in the cytoplasm. Twenty-seven TRIM proteins (TRIM6, 8, 11, 12, 16, 21, 23, 27, 30-32, 35, 38, 40, 44, 48, 49, 51, 52, 55, 56, 58, 62, 64, 68, 73, and 74) were present in a substantially amount in both the nucleus and cytoplasm. A number of the TRIM proteins either formed speckled or filamentous structures in the nucleus or the cytoplasm, others were localized diffusedly, and a few of them displayed perinuclear localization. Often, a TRIM protein exhibited the combination of these localization patterns either in the same or different cells (Figure 19 and Table 2).

Fourteen TRIM proteins (TRIM6, 8, 11, 19, 21, 22, 27, 28, 30, 32, 33, 35, 38, and 51), including PML/TRIM19, co-localized with the nuclear Atxn1 82Q inclusions in a substantial number of cells (Figure 19 and Table 2), representing nearly 43% of the

thirty-two TRIM proteins that were present either partially or exclusively in the nucleus. These TRIM proteins are from distinct subgroups with different C-terminal sequences. Six proteins (TRIM6, 11, 21, 22, 35, and 38), like TRIM27, are members of the subgroup IV, containing a SPRY motif that is often preceded by a PRY motif. TRIM8, like PML, is a member of the group V, containing no known domains. TRIM28 and TRIM33 are members of group VI, containing a PHD-BR motif. TRIM32 belongs to the subgroup VII, with a five NHL repeats. TRIM51 is considered a TRIM-like protein, lacking the two B-boxes but containing the RING domain and the coiled-coil region; it also contains a SPRY domain, like members of the subgroup IV. Together, these results show that a substantial number of TRIM proteins, despite the divergence in their C-terminus, possess the ability to recognize misfolded proteins.

Degradation of misfolded proteins mediated by other TRIM proteins

To examine the role of TRIM proteins in protein quality control, all human TRIM proteins except for TRIM53 (pseudogene) and TRIM57 (same as TRIM59), as well as TRIM12 and TRIM30 (of mouse origin), were tested for the ability to degrade Atxn1 82Q. Thirty-five of the human TRIM proteins (TRIM3, 4, 5, 6, 7, 9, 11, 13-17, 19, 20, 21, 24, 25, 28, 29, 34, 39, 43-46, 49, 50, 52, 58, 59, 65, 67, 70, 74, and 75) and mouse TRIM30 were able to reduce the levels of NS and/or SS fractions of Atxn1 82Q to different extents (Figure 20A and Table 2). Several TRIM proteins showed strong activity, including TRIM4, 5, 9, 11, 16, 17, 20, 30, 39, 43, 65, 70, 75 (Figure 20A and Table 2), and the one with strongest activity appeared to be TRIM11 (Figure 16A, Figure 20A and Table 2). Of note, TRIM27 and TRIM32, which were shown above to be able to reduce Atxn1 82Q, did not display an effect in this assay. This difference was likely due to the expression levels. In the experiments described above, TRIM27 and TRIM32 were cloned into the plasmid pRK5. But in the experiments described in this section, TRIM27 and TRIM32 were cloned into pcDNA. Stronger expression from the pRK5 plasmids were consistently observed as compared to expression from the pcDNA plasmid. Therefore, even for the ones that did not display an activity to degrade Atxn1 82Q, they might do so when their expression levels were elevated. It is therefore concluded that the ability to promote degradation of Atxn1 82Q is prevalent among TRIM proteins.

Of note, a few TRIM proteins enhanced, rather than inhibited, the expression of Atxn1 82Q, including TRIM26, 33, 42, 47, 48, 66, 69, and 76. This suggests a diverse effect of different TRIM proteins on Atxn1 82Q.

In parallel, the effect of TRIM proteins on Httex1p 97QP was also examined (Figure 20B). Only a few TRIM proteins were able to reduce the levels of Httex1p 97QP in the NS and/or SS fractions, including TRIM3, 11, 30, 68, 74, and 75. The majority of the TRIM proteins were able to increase the levels of Httex1p 97QP, including TRIM1, 4, 6-10, 12-15, 21, 23-28, 32-39, 41-47, 49-51, 54-56, 58, 60-67, 69-73, 76, and 77. Unlike Atxn1 82Q, only a small fraction of cells expressing Httex1p 97QP contained inclusion. Consistently, the portion of Httex1p 97QP protein in the SS fraction was very low compared to the portion of Atxn1 82Q in the same fraction. Also, in the previous study, PML (cloned in the pRK5 plasmid) was able to reduce the levels of Httex1p 97QP, while here PML (cloned in pcDNA) was not. Thus, the effect of TRIM proteins on Httex1p 97QP, like their effect of Atxn1 82Q, might also be influenced by their expression levels. These results suggest different effects of TRIM proteins on different misfolded proteins, which, among others, can be influenced by their expression levels.

TRIM proteins

Previously it has been shown that PML is able to eliminate a range of misfolded proteins presented in the nucleus of mammalian cells. The experiments presented herein systematically analyzed nearly all TRIM proteins and find that unprecedented large number of them can recognize misfolded proteins. Still, this number is likely to be an underestimate, because a single misfolded protein, Atxn1 82Q, was mainly used. At least two substrate recognition sites (SRSs) have been identified in PML, the coiled-coil region within the RBCC motif and the region comprising the last sixty amino acids. These SRSs can discern coiled-coil structures and peptides enriched in aromatic acid residues (Phe, Trp, and Tyr), respectively, which are found in some denatured proteins. TRIM proteins are most divergent in their C-terminal region (Hatakeyama, 2011, Nat Rev Cancer, 11: 792-804; Ozato et al., 2008, Nat Rev Immunol, 8: 849-860). It is likely that other TRIM proteins may process specific ability to

recognized misfolded proteins of distinct structural features. Also, it is likely that many TRIM proteins that can recognize misfolded proteins in the cytoplasm. Hence, the present observations underscore the critical importance of this large family in protein quality control.

Of note, TRIM5δ was able to promote Atxn1 82Q degradation despite of its cytoplasmic localization. It is possible that TRIM5δ can recognize soluble, misfolded Atxn1 82Q in the cytoplasm before it is imported into the nucleus. Also, TRIM27 and TRIM32 show strong ability to degrade soluble Atxn1 82Q, while PML shows less activity. This might be related to the fact that, unlike PML, TRIM27 and TRIM32 are partially localized to the cytoplasm, where Atxn1 82Q protein (and virtually all the other proteins) is generated. Hence, by recognizing misfolded proteins that are destined to the nucleus, the cytoplasmic TRIM proteins may play an important role in protein quality control in the nucleus.

PML degrades misfolded proteins in a manner that is dependent on its SUMO E3 activity (Guo et al., 2014, Mol Cell, 55(1): 15-30). Likewise, TRIM5δ, TRIM27, and TRIM32 rely on SUMO to remove Atxn1 82Q. An in vitro assay also confirms the SUMO E3 activity of TRIM27 towards Atxn1 82Q. Hence, TRIM proteins might employ similar mechanism to rid cells of misfolded proteins. The most relevant SUMO proteins for degrading misfolded proteins are SUMO2/3. The poly-chains formed by SUMO2/3 facilitate the recognition by the multiple SIMs on RNF4 (Tatham et al., 2008, Nat Cell Biol, 10: 538-546). Consistent with a role in protein quality control, SUMO2/3 in unstressed cells are mainly un-conjugated forms but become conjugated to target proteins after protein damaging stresses (Golebiowski et al., 2009, Sci Signal, 2: ra24; Saitoh and Hinchey, 2000, J Biol Chem, 275: 6252-6258). Nevertheless, given the diversity among the TRIM proteins and the ubiquitin E3 ligase activity associated with some (Meroni and Diez-Roux, 2005, Bioessays, 27: 1147-1157), it is still possible that some TRIM proteins may primarily function as ubiquitin E3s for misfolded proteins.

Currently, it is unclear why some TRIM proteins can degrade misfolded proteins such as Atxn1 82Q, while others cannot. Also, among the TRIM proteins tested, TRIM11 possesses a remarkably potent activity to reduce Atxn1 82Q. It remains to be determined what may account for its activity. Some TRIM proteins can enhance the

expression levels of Atxn1 82Q and especially Httex1p 97QP. This further underscores the functional diversity of these proteins. TRIM proteins can function as chaperones to prevent protein misfolding and as disaggregase to dissolve previously formed protein aggregates. These activities may in part account for the effect of TRIM proteins to stabilize Atxn1 82Q and especially Httex1p 97QP. This suggests another important usage of TRIM proteins in the therapy of diseases associated with misfolded proteins. A number of human diseases are linked closely to the degradation of mutant proteins that are partially functional. A notable example is cystic fibrosis (CF), which is caused by mutations in the gene cystic fibrosis transmembrane conductance regulator (CFTR). These mutations are degraded in the cytoplasm. Therapies are being developed to stabilize the mutant CFTR, allowing mutant CFTR to reach the member to fulfill its function. It is envisioned that using TRIM proteins may achieve such a goal in CF and other diseases associated with degradation of partially functional proteins.

Table 2: Summary of the effect of cellular localization of TRIM proteins and their co-localization with Atxn1 82Q.

Name	Other common names	Domains	Species	Isoforms used in this study	Cellular localization	Pattern	Atxin1 82Q aggregates colocalizaiton
TRIM1	MID2, FXY2, RNF60	**R B1 B2 CC COS FN3 SPRY**	h		C	diffuse, foci, filament	-
TRIM2	CMT2R, RNF86	**R B2 CC FIL NHL**	h		C	diffuse, foci	-
TRIM3	BERP, HAC1, RNF22, RNF97	**R B2 CC FIL NHL**	h		C	diffuse	-
TRIM4	RNF87	**R B2 CC SPRY**	h		C	foci	-
TRIM5	RNF88	**R B2 CC SPRY (gamma does not have SPRY)**	h	γ	C	foci	-
TRIM6	RNF89	**R B2 CC PRY SPRY**	h		C, N	diffuse	**
TRIM7	GNIP, RNF90	**R B2 CC PRY SPRY**	h		C	foci	-
TRIM8	GERP, RNF27	**R B1 B2 CC**	h		C, N	foci	***
TRIM9	RNF91, SPRING	**R B1 B2 CC COS FN3 PRY SPRY**	h		C	foci, filament	-
TRIM10	RNF9, HERF1, RFB30	**R B2 CC PRY SPRY**	h		C	foci, filament	-
TRIM11	BIA1, RNF92	**R B2 CC PRY SPRY**	h		C, N	diffuse	***
TRIM12	2310043C0 1Rik	**R B2 CC**	mouse only		C, N	diffuse, foci	-
TRIM13	CAR, LEU5, RFP2, DLEU5, RNF77	**R B2 CC TM**	h		Perinuclear	diffuse, foci	-
TRIM14		B2 CC PRY/SPRY	h		C, N	foci	-
TRIM15	RNF93, ZNFB7, ZNF178	**R B2 CC PRY SPRY**	h		C	foci	-
TRIM16	EBBP	B2 CC PRY/SPRY	h		C, N, perinuclear	diffuse	-
TRIM17	TERF, RBCC, RNF16	**R B2 CC PRY SPRY**	h		C	diffuse	-
TRIM18	MID1, FXY. RNF59	**R B1 B2 CC COS FN3 PRY SPRY**	h		C	filament	-
TRIM19	PML	**R B1 B2 CC**	h		N	foci	***
TRIM20	PRYRIN, MEFV	**B2 CC PRY/SPRY**	h		C	diffuse, foci	-
TRIM21	Ro52, SSA1, SSA/Ro,	**R B2 CC PRY SPRY**	h		C, N	diffuse, foci, filament	***

	RNF81						
TRIM22	STAF50, GPSTAF50, RNF94	**R B2 CC SPRY**	h	short and long	N	foci	***
TRIM23	ARD1, ARFD1, RNF46	**R B1 B2 CC ARF**	h		C, N, perinuclear	diffuse, foci	-
TRIM24	TIF1α, TF1A, TIF1A, TIF1, PTC6, RNF82	**R B1 B2 CC PHD BR**	h		C, perinuclear	foci	-
TRIM25	EFP, Z147, RNF147, ZNF147	**R CC PRY SPRY**	h		C	diffuse, foci	-
TRIM26	AFP, RNF95, ZNF173	**R B2 CC PRY SPRY**	h		C	diffuse, foci	-
TRIM27	RFP, RNF76	**R B2 CC PRY SPRY**	h		C, N	foci	***
TRIM28	KAP, TIF1β, TF1B, TIF1B, RNF96, PPP1R157	**R B1 B2 CC PHD BR**	h		N	diffuse	***
TRIM29	ATDC	**B1 B2 CC**	h		C	filament	-
TRIM30	RPT1, RGD1563970	R B2 CC PRY/SPRY	mouse only		C, N	diffuse, foci	***
TRIM31	RNF, HCG1, HCGI, C6orf13	**R B2 CC**	h		C, N	diffuse, foci	#
TRIM32	HT2A, BBS11, TATIP, LGMD2H	**R B2 CC NHL**	h		C, N	foci	**
TRIM33	TIF1γ, TF1G, TIF1G, ECTO, PTC7, RFG7	**R B1 B2 CC PHD BR**	h		N	diffuse, foci	***
TRIM34	IFP1, RNF21	**R B2 CC SPRY**	h		C	foci	-
TRIM35	HLS5, MAIR	**R B2 CC PRY SPRY**	h		C, N	foci	**
TRIM36	RNF98, HAPRIN, RBCC728	**R B1 B2 CC COS FN3 SPRY**	h		C	filament	-
TRIM37	MUL, POB1, TEF3	**R B2 CC MATH**	h		C	foci	-
TRIM38	RoRet, RNF15	**R B2 PRY SPRY**	h		C, N	foci	***
TRIM39	TFP, RNF23	**R B2 CC PRY SPRY**	h		C	foci	-
TRIM40	RNF35	**R B2 CC**	h		C, N	diffuse	#
TRIM41	RINCK	**R CC B2 CC**	h		C	foci	-

		PRY SPRY (According to Nat imm review)					
TRIM42	T4A1, PPP1R40	**R B1 B2 CC COS FN3**	h		N/A	N/A	N/A
TRIM43		**R B2 CC SPRY**	h		N/A	N/A	N/A
TRIM44	DIPB, MC7, HSA249128	B2 CC	h		C, N	diffuse	-
TRIM45	RNF99	**R B1 B2 CC FIL NHL**	h		C	foci	-
TRIM46	TRIFIC, GENEY	**R B2 CC COS FN3 SPRY**	h		C	filament	-
TRIM47	GOA, RNF100	**R B2 CC PRY**	h		C	foci	-
TRIM48	RNF101	**R B2 SPRY**	h		C, N	diffuse	#
TRIM49	RNF18, TRIM49A, TRIM49L2	**R B2 SPRY**	h		C, N	diffuse	-
TRIM50		**R B2 CC PRY SPRY**	h		C	foci	-
TRIM51	SPRYD5A	R B2 SPRY	h		C, N	foci	*
TRIM52	RNF102	**R B2**	h		C, N	diffuse	#
TRIM53		**R SPRY (According to Nat imm review)**	h		N/A	N/A	N/A
TRIM54	MURF, RNF30, muRF3, MURF-3	**R B2 CC COS**	h		C	filament	-
TRIM55	MURF-2, RNF29, muRF2	**R B2 CC COS**	h		C, N	foci, filament	***
TRIM56	RNF109	**R B2 CC**	h		C, N	diffuse, foci	-
TRIM58	BIA2	**R B2 CC PRY SPRY**	h		C, N	diffuse, foci	-
TRIM59	TRIM57, MRF1, TSBF1, IFT80L, RNF104	**R B2 CC TM**	h		N/A	N/A	N/A
TRIM60	RNF33, RNF129	**R B2 CC PRY SPRY**	h		N/A	N/A	N/A
TRIM61	RNF35	**R B2 CC**	h		N/A	N/A	N/A
TRIM62	DEAR1	**R B2 CC PRY SPRY**	h		C, N	foci	-
TRIM63	MURF1, RF1, RNF28	**R B2 CC COS**	h		C	filament	-
TRIM64	C11orf28	**R B2 CC SPRY**	h		C, N	diffuse	***
TRIM65	4732463G12Rik	**R B2 CC SPRY**	h		N	diffuse	***
TRIM66	TIF1δ,TIF	B1 B2 CC PHD BR	h		N	foci, diffuse	-

	1D, C11orf29,						
TRIM67	TNL	**R B1 B2 CC COS FN3 SPRY**	h		N/A	N/A	N/A
TRIM68	GC109, RNF137, SS-56, SS56	**R B2 PRY SPRY**	h		C, N	diffuse	-
TRIM69	HSD-34, HSD34, RNF36, Trif	**R CC PRY SPRY**	h		C	filament	-
TRIM70		CC PRY/SPRY	h		N/A	N/A	N/A
TRIM71	LIN-41, LIN41	**R B1 B2 CC FIL NHL**	h		N/A	N/A	N/A
TRIM72	MG53	**R B2 CC PRY SPRY**	h		N/A	N/A	N/A
TRIM73	TRIM50B	**R B2 CC**	h		C, N	foci	***
TRIM74	TRIM50C	**R B2 CC**	h		C, N	foci, diffuse	**
TRIM75		**R B2 CC PRY SPRY**	h		N/A	N/A	N/A
TRIM76	CMYA5, SPRYD2, C5orf10	B CC FN3, PRY/SPRY	h		C	diffuse	-

-	No co-localization
#	Faintly distributed around aggregates
*	<5% cells
**	5-20% cells
***	>20% cells

Table 3. Summary of the effect of TRIM proteins on Atxn1 82Q.

	Subfamily	Soluble WB	IS WB	Total flow	Detection WB	WB fraction Trims
Trim1	I	-	-	-	Normal	S/IS
Trim2	VII	-	-	-	Normal	S
Trim3	VII	-	↓	-	Normal	S/IS
Trim4	IV	-	↓↓		Normal	S/IS
Trim5	IV	-	↓↓		Normal	S/IS
Trim6	IV	-	↓		None	
Trim7	IV	-	↓		None	
Trim8	V	-	-	-	Normal	S/IS
Trim9	I	↓	↓↓	↓↓	Low	S
Trim10	IV	-	-	↓		IS
Trim11	IV	↓↓↓	↓↓↓	↓↓↓	None	
Trim12		-	-	-	Low	IS
Trim13	XI	-	↓/-	-	Normal	IS
Trim14	F IV	-	↓/-	↓	Normal	IS

Trim15	IV	-	↓/-	↓	Normal	IS
Trim16	F IV	↓	↓	↓	None	
Trim17	IV	↓	↓↓	↓	Normal	S/IS
Trim18	I	-	-	-	Normal	S
Trim19	V	↓	-	↓	Normal	S/IS
Trim20	F IV	↓↓	↓↓↓	↓↓↓	Low	**S**
Trim21	IV	-	↓	↓	Normal	S/IS
Trim22	IV	-	-	↓	Normal	S/IS
Trim23	IX	-	-	↓	Low	S/IS
Trim24	VI	-	↓	↓↓	None	
Trim25	IV	-	-	↓	None	
Trim26	IV	-	↑	↑	Normal	IS
Trim27	IV	-	-	-	Normal	IS
Trim28	VI	-	↓	-	Normal	S
Trim29		-	↓	-	Normal	IS
Trim30		-	↓↓	↓↓	Low	IS
Trim31	V	-	-	↓	Normal	S
Trim32	VII	-	-	-	Normal	IS
Trim33	VI	↑	↑	-	None	
Trim34	IV	-	↓	↓	Normal	IS
Trim35	IV	-	-	-	Normal	IS
Trim36	I	-		↑	None	
Trim37	VIII	-		↑	None	
Trim38	IV	-		↑	Low	S/IS
Trim39	IV	-		↓	Normal	S/IS
Trim40	V	-		-	Normal	S
Trim41	IV	-		-	Normal	S/IS
Trim42	III	↑	↑	↑	Normal	S/IS
Trim43	IV	-	↓↓	↓	Normal	IS
Trim44		-	↓	-	Normal	S/IS
Trim45	XI	-	↓	-	Normal	IS
Trim46	I	-	↓	↓	Normal	S/IS
Trim47	IV	-	↑	↓	Normal	S/IS
Trim48	IV	-		↓	Low	IS
Trim49	IV	↓	↓↓	↓	Low	S/IS
Trim50	IV	-	↓↓	↓	Normal	S/IS
Trim51	F IV	-	-	-	None	
Trim52	V		↓	↓	Normal	S
Trim54	II	-	-	-	none	
Trim55	II	-	-	↑	Normal	S
Trim56	V	-	-	-	None	
Trim58	XI	-	-	↑	Normal	S/IS
Trim59	XI	↓	↓	-	Normal	S/IS

Trim60	IV	-	-	↑	None	
Trim61	V	-	-	↑	None	
Trim62	IV	-	-	-	None	
Trim63	II	-	-	↑	Normal	
Trim64	IV	-	↑	-	Normal	S/IS
Trim65	IV	↓	↓↓	↓	Normal	S/IS
Trim66	F IV	-	↑	↑	None	
Trim67	I	-	↓	↓/-	Normal	S/IS
Trim68	IV	-	-	↓	Normal	S
Trim69	IV	↑	↑	↑	None	
Trim70	F IV	-	↓↓	↓↓	low	S/IS
Trim71	VII	-	-	-	None	
Trim72	IV	-	-	↑	Normal	S/IS
Trim73	V	-	-	-	Normal	
Trim74	V	-	↓	↓	Normal	
Trim75	IV	-	↓↓	↓	Normal	
Trim76		-	↑	↑	Normal	S/IS
Trim77		-	-	↑		

-	No Change
↓	10-25%
↓↓	25-50%
↓↓↓	>50%
↓/-	Change < 10%

Table 4. Summary of the effect of TRIM proteins on Httex1p 97QP

	Subfamily	Soluble WB	IS WB	Total flow
Trim1	I	-	↑	-
Trim2	VII	-	-	-
Trim3	VII	-	↓	-
Trim4	IV	-	↑	-
Trim5	IV	-	-	-
Trim6	IV	-	↑	-
Trim7	IV	-	↑	-
Trim8	V	-	↑	-
Trim9	I	-	↑	-
Trim10	IV	-	↑	-
Trim11	IV	↓	↓	↓↓↓
Trim12		-	↑	↑

Trim13	XI	-	↑	↑
Trim14	F IV	-	↑↑	↑
Trim15	IV	-	↑↑	-
Trim16	F IV	-	↑	↓
Trim17	IV	-	↑	-
Trim18	I	-	↑	-
Trim19	V	-	↑/-	↓
Trim20	F IV	-	↑/-	↓
Trim21	IV	-	↑	↑
Trim22	IV	-	-	-
Trim23	IX	-	↑	↑
Trim24	VI	-	↑	↓
Trim25	IV	-	↑	↑
Trim26	IV	-	↑	↑
Trim27	IV	-	↑	↑
Trim28	VI	-	↑	↑
Trim29		-	-	↓
Trim30		-	↓	↓↓
Trim31	V	-	-	-
Trim32	VII	-	↑	↑
Trim33	VI	-	↑	↑
Trim34	IV	-	↑	↑
Trim35	IV	-	↑	↑
Trim36	I	-	↑	↑
Trim37	VIII	-	↑	↑
Trim38	IV	-	↑	↑
Trim39	IV	-	↑	-
Trim40	V	-	-	↑
Trim41	IV	-	↑	↑

Example 3: Delivery of recombinant TRIM11 into mammalian cells promotes the degradation of misfolded proteins

As described above, it has been demonstrated that members of the tripartite motif-containing (TRIM) family play an important role in the recognition and degradation of misfolded proteins. Initially using PML/TRIM19 as an example, it was shown that PML can specifically bind to misfolded proteins via distinct regions that recognize structure features commonly found in misfolded proteins. PML then uses its SUMO E3 activity to tag the misfolded proteins with poly-SUMO2/3 chains. This allows misfolded proteins to be recognized by a SUMOylation-targeted ubiquitin ligase

(STUbL) RNF4, with the consequential ubiquitination and proteasomal degradation of misfolded proteins. It was further shown that this PML-RNF4-mediated sequential SUMOylation and ubiquitination system plays an important role in the protection against neurodegenerative diseases.

Subsequently, the vast majority of all known human TRIM proteins were survey, and it was found that a substantially number of them are able to localize to the inclusion formed by misfolded proteins such as pathogenic ataxin 1 (Atxn1 82Q) and huntingtin (Htt 97Q) proteins. By testing representative TRIM proteins, it was shown that many TRIM proteins are also capable of degrading misfolded proteins in a SUMO-dependent manner. Of note, among the TRIM proteins that were tested, one TRIM proteins, TRIM11, exhibits a particularly strong activity to reduce misfolded proteins.

In the experiments presented herein, it was sought to develop recombinant TRIM11 as an agent for degrading intracellular misfolded proteins associated with neurodegeneration. For this, the HIV Tat-derived peptide was used, which is able to deliver proteins into mammalian cells (Figure 22). TRIM11 was fused with the Tat-derived peptide, the fusion protein was expressed in bacteria, and the protein was purified to homogeneity using affinity resin, followed by gel filtration column. For comparison, Tat-peptide-fused SUMO2 protein was also purified in parallel.

HeLa cells were transfected with Atxn1 82Q-GFP, Atxn1 30Q, or Htt 97Q-GFP, and were subsequently incubated with recombinant TRIM11 or SUMO2 proteins. As shown in Figure 21A, treatment of HeLa cells with TRIM11 led to strong reduction in the levels of Atxn1 82Q. It also led to strong reduction in the levels of Htt 97Q (Figure 21B). In contrast, SUMO2 had minimal effect on the levels of Atxn1 82Q (Figure 21C). These data suggest that delivery of TRIM11 into mammalian cells reduces the levels of misfolded proteins, providing a proof or concept evidence for targeting the TRIM-RNF4 system for the therapy of neurodegeneration and other protein-misfolding diseases.

Example 4: Localization of RNF4 to neuronal inclusions in SCA1 and HD patients

RNF4 deficiency in mice results in embryonic lethality (Hu et al, 2010, PNAS 107:15087-92), precluding the analysis of its deficiency on mouse models of

neurodegeneration. Previous studies showed that PML, along with SUMO and ubiquitin, co-localizes with neuronal inclusions in polyglutamine disease patients (Dorval and Fraser; 2006, J BIol Chem 281:9919-24; Martin et al., 2007, Nat Rev Neurosci 8:948-59; Skinner et al., 1997, Nature 389:971-4; Takahasi et al., 2003, Neurobiol Dis 13:230-70), suggesting the involvement of PML in these diseases. The data presented herein analyzes the localization of RNF4 in post-mortem brain tissues of human SCA1 and HD patients.

The materials and methods employed in these experiments are now described.

HD patient tissues (globus pallidus) were provided by the Harvard Brain Tissue Resource Center: AN06564, AN12127 and AN12029 (aggregates observed), and AN09048, AN19685, AN14942, AN13612 and AN17467 (no aggregates observed); *SCA1* patient tissues (basis points) were obtained, National Ataxia Foundation. Immunohistochemistry and immunofluorecence were performed as previously described with modifications (Duda et al., 2000, J Neuropathol Exp Neurol 59:830-41; Emmer et al., 2011, J Biol Chem 286:35104-18). HD and SCA1 patient brains were embedded in paraffin and cut into 6 □m sections. Sections were stained for anti-RNF4 (#1: Rabbit, 11-25, 1:300, Sigma; #2: Goat, C15, 1:25, Santa Cruz), anti-Huntingtin (mouse, MAB5374, 1:500; Millipore), anti-ubiquitin (mouse, Ubi-1, MAB1510, 1:2,000; Millipore), and anti-polyQ (mouse MAB1574, 1C2, 1:1,000; Millipore) as indicated. Control rabbit antibodies were used to confirm the specificity of anti-RNF4 staining.

The results of the experiments are now described.

When detected in patient neurons without inclusions, RNF4 tended to distribute diffusely throughout the nucleus or form nuclear foci (Figure 23). In the two cases of SCA1 that were examined, nuclear inclusions reactive to an anti-polyQ antibody (1C2) were present. RNF4 was found in five out of sixteen and four out of seventeen 1C2-reactive nuclear inclusions, respectively (Figure 24).

Among the HD patients, cytoplasmic inclusions reactive to either anti-huntingtin or anti-ubiquitin antibody were present in the globus pallidus region in three out of eight patient samples examined. This was in agreement with previous observations that nuclear inclusions were rare in adult-onset HD cases (DiFiglia et al., 1997, Science 277:1990-3). Nevertheless, RNF4 co-localized with approximately 20% of the inclusions in the globus pallidus region based on the results with two separate anti-RNF4 antibodies (Figure 25 and Figure 26). The inclusions containing RNF4 tended to show RNF4 immunoreactivity surrounded by a ring of huntingtin or ubiquitin signals (Figure 25 and Figure 26). The partial co-localization of RNF4 with the polyQ aggregates in SCA1 and HD patients is reminiscent of the partial co-localization of PML with polyQ aggregates (Skinner et al., 1997, Nature 389:971-4; Takahashi et al., 2003, Neurobiol Dis 13:230-7), and it may reflect the blockage of the PML/TRIM-RNF4 system in the disease-affected neurons.

Example 5: Molecular chaperone and protein diaggregase activities of TRIM proteins

The data described herein demonstrates that TRIM11 can function as a molecular chaperone and disaggregase. In one aspect, TRIM11 can prevent aggregate formation. On the other hand, TRIM11 is not only able to refold stress-induced non-amyloid aggregates (luciferase and GFP aggregates), but also disaggregate amyloid aggregates (Atxn1 82Q aggregates and alpha-Synuclein fibrils). Further, it is also shown herein that TRIM11 is also a SUMO E3 ligase that can SUMOylate Atxin1 82Q and subsequent degradation. Therefore, TRIM11 may serve as a link between protein disaggregation and degradation.

The materials and methods employed in these experiments are now described.

siRNA transfection

Mouse TRIM11 siRNA (sc-76735) was purchased from Santa Cruz. In the first day, hippocampal neuron cells (15000cells/well) were plated in 96 well plates with plating medium. In the second day (about 18 hours after plating), completely change the

plating medium to neuronal medium. In the third day, siRNA (1pmol) was transfected into cells by Lipofectamine RNAiMAX. The transfection methods were carried out according to the manufacturer's instructions.

Cell culture

HCT16 cells were cultured with Mccoy 5A medium. A549 cells were cultured with RPMI1640 medium. HeLa and HEK293T cells were cultured with DMEM medium.

These culture mediums all contain 10% Fetal Bovine Serum (FBS). Primary neuron cells were obtained. Neuron cells were cultured according to previously described methods. Simply, the cells were first plated with plating medium (Combine Neurobasal medium, B27, GlutaMAX , penicillin/streptomycin and 10% FBS by the indicated dilution ratios). After 18-24 h, completely change the plating medium to neuronal medium (Combine Neurobasal medium, B27, GlutaMAX and penicillin/streptomycin by the indicated dilution ratios). Except notification, all cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

Immunofluorescence

Cells plated on coverslips were fixed with 4% paraformaldehyde for 10 minutes at room temperature. Cells were further permeabilized with methanol for 10 minutes at 20°C. Cells were washed with PBS and then blocked by 2% bovine serum albumin (BSA) for 30 min at room temperature. Cells were incubated with the primary antibody overnight at 4°C, followed by incubation with a fluorescent secondary antibody for 1 hour at room temperature. Finally, the coverslips were mounted to glass slides with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories; H-1200). For antibodies dilution, anti-HA (1:100), anti-p-a-Syn (1:100) and anti-p62 (1:200) were applied.

Antibodies

Anti-TRIM11 (ABC926) antibody was purchased from Millipore. Monoclonal anti-Flag antibody, rabbit Flag antibody and anti-Flag agarose beads were

purchased from Sigma. Anti-HA affinity matrix (clone 3F10, 11815016001) was purchased from Roche. Anti-HSF1 (sc-9144) was purchased from Santa Cruz. Anti-a-Synuclein Phopho-Ser129 (825701) was purchased from Biolegend. Anti-Hsp70 (ADI-SPA-810-D) was purchased from Enzo. Anti-a-Synuclein (2642) and anti-Hsp90 (4874) was purchased from Cell signaling technology.

Protein fractionation, filter retardation assay and Immunoblotting

Cells were lysed with the lysis buffer (50 mM Tris, pH 8.8, 100 mM NaCl, 5 mM $MgCl_2$, 0.5% IGEPAL CA-630, 1 mM DTT, 250 IU/ml benzonase (Sigma), 1 mM PMSF and 1x complete protease cocktail (Roche)) for 30 minutes on ice. The supernatant was obtained by centrifuging 13000rpm for 15-20 minutes at 4°C. The pellet was further resuspended in the pellet buffer (20 mM Tris, pH 8.0, 15 mM MgCl2, 1 mM DTT, 250 IU/ml benzonase, 1 mM PMSF and 1X complete protease cocktail) for 30 minutes on ice, followed by directly boiling with 2% SDS buffer. Protein concentration was measured by Bradford assay (Bio-Rad Labs). All protein samples were subjected to immunoblotting by SDS-PAGE. The supernatant was considered as a soluble fraction. The pellet was considered as an insoluble fraction (SDS-soluble). The other portion was subjected to filter retardation assay. Simply, the pellet samples were filtered through a membrane with 0.2μM pore size, so that the SDS-resistant aggregates retained on the membrane were analyzed by immunoblotting.

Protein purification

Flag-Atxn1-82Q-HA was transfected in 293T cells and purified by anti-Flag M2 beads. For getting highly purified proteins, the beads were extensively washed with increased concentrations of NaCl.

His-Luciferase was expressed in BL21 DE3 cells purified. To generate immobilized native or denatured luciferase, native luciferase was first purified from bacterial cell lysates according to the manufacturer's instructions. Second, denatured luciferase was generated by incubating native luciferase with 8 M urea for 5 minutes

In vivo and in vitro sumoylation assays

For in vivo sumoylation assays, cells were transfected with the indicated plasmids. After 48 hours, cells were lysed in lysis buffer (50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 0.5% Trition, 1 mM DTT, 1 mM PMSF and 1x complete protease cocktail) supplemented with 2% SDS and 50 mM DTT. Cell lysates were further boiled at 95°C for 10 minutes. One aliquot was saved for input. The rest of cell lysates were diluted 10-fold in lysis buffer and then incubated with anti-HA beads at 4°C overnight. The beads were extensively washed and analyzed by immunoblotting with the indicated antibodies. For in vitro sumoylation assays, His-SUMO-2 (UL-753), UbcH9 (E2-465) and SUMO E1 (E-315) were purchased from Boston Biochem.

Plasmids

Flag-TRIM11 was constructed by inserting KpnI/XbaI TRIM11 cDNA into KpnI/XbaI digested pcDNA3.1-Flag vector. Flag-TRIM11mutation (12/13EE to 12/13AA) was generated by site-directed mutation. GST-TRIM11 was constructed by inserting BglII/SalI TRIM11 cDNA into BamHI/SalI digested pGEX-1λT vector. GFP-Hsp70 and GFP-TRIM11 were constructed by inserting Hsp70 and TRIM11 cDNA into pEGFP-C1 vector. All the constructs were verified by DNA sequencing.

Luciferase reactivation assay

To generate aggregates, firefly luciferase (100nM) in luciferase refolding buffer (LRB; 25 mM HEPES-KOH [pH 7.4], 150 mM KAOc, 10 mM MgAOc, 10 mM DTT) was heated at 45°C for 8-10 minutes. Aggregated luciferase (10nM) was incubated with the indicated concentrations of TRIM11 or other proteins at 25°C for 90 minutes. For Hsp104/Hsp70-Hsp40 di-chaperone system, 5mM ATP and an ATP regeration system (1mM creatine phosphate, 0.5uM creatine kinase) were required. For in vivo luciferase refolding assay, cells in 96 well plate were transiently transfected with wild type luciferase . After 24 hours, cells were heated at 42°C or 45°C for 1 hour or 30 minutes, respectively. Prior to heat shock, 20 μg/ml cycloheximide was added into culture medium. After heat shock, cells were transferred to 37°C incubator for another 1.5 or 3 hours. Luciferase activity was measured with Promega Luciferase System.

GFP Disaggregation Assay

To generate aggregates, GFP (4.5μM) in buffer A (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 20 mM $MgCl_2$, 5 mM DTT, 0.1 mM EDTA, 10% (v/v) glycerol) was incubated for 15 minutes at 85°C. GFP aggregates (0.45μM) were incubated with the indicated proteins with different amounts at 25°C for 60 minutes. Disaggregation of GFP aggregates was detected by measuring fluorescence at 510nm upon excitation at 395nm (Infinite M200 pro).

Reagents

Luciferase (L9506), Mg-ATP (A9187), phosphocreatine (P1937) and creatine kinase (C3755) were purchased from Sigma. KRIBB11 (385570) was purchased from Millipore. Beta-Amyloid (1-42) (RP10017) was purchased from GenScript.

The results of the experiments are now described.

PML (TRIM19) SUMOylated misfolded protein that can be recognized by RNF4 to be degraded through proteasome. However, it is unknown whether TRIM11 is also a SUMO E3 ligase. According to the structural analysis of TRIMs, two highly conserved glutamates (Glu9 and Glu10) of TRIM25 are required for its ubiquitin E3 ligase activity. Therefore mutated TRIM11 (Glu12/Glu13 to Ala12/Ala13) were generated that lacked E3 ligase activity (Figure 27A and Figure 27B). In vivo SUMOylation assay showed that Atxn1 82Q was efficiently SUMOylated by wild type TRIM11, as well as PML (Figure 27A). To further confirm this, in vitro SUMOylation assay showed that purified Atxn1 82Q could be significantly SUMOylated by TRIM11. Therefore, TRIM11 was also a SUMO E3 ligase for Atxn1 82Q. In cells, TRIM11 like Hsp70 was mainly localized in the cytoplasm (Figure 28A and Figure 28B). Intriguingly, TRIM11 or Hsp70 could be recruited into the aggregates of Atxn1 82Q (Figure 28C and Figure 28D), suggesting that there might be an interaction between TRIM11 and Atxn1 82Q. To test this, pull down analysis presented that TRIM11 selectively bound to Atxn1 82Q (Figure 29A). Importantly, TRIM11 preferentially interacted with pathologic form Atxn1 82Q, not Atxn1 30Q (Figure 29B). Therefore it was hypothesized that TRIM11

had a potential to selectively bind to misfolded proteins. To investigate the prediction, native or denatured luciferase beads were further generated and then a pull down assay was performed. As shown in Figure 29C, in contrast to control GST proteins, TIRM11 specifically bound to denatured luciferase, which indicated that TRIM11 was capable of binding misfolded proteins.

It has been demonstrated that Hsp70, a molecular chaperone, can suppress the expression of pathology associated Atxn1 82Q in *SCA1* mice. Additionally, Hsp70 reduced the protein level of the detergent-insoluble fraction of Atxn1 82Q, which was consistent with the results presented herein (Figure 28E). It was next investigated whether there was a similarity between TRIM11 and Hsp70 for aggregate formation. To study this, the solubilized feature of Atxn1 82Q was analyzed by detergent fractionation. As show in Figure 28E, TRIM11, like Hsp70, reduced the detergent-insoluble fraction of Atxn1 82Q, suggesting that TRIM11 could control protein aggregation. Moreover, MG132 treatment moderately increased the insoluble Atxn1 82Q fraction (Figure 28E), which was consistent with the previous report that the proteasome is necessary for controlling Atxn1 82Q aggregate formation. Interestingly, when Atxn1 82Q was co-expressed with wild type TRIM11 or mutant TRIM11, the mutant TRIM11 had a lower ability to reduce the detergent-insoluble fraction of Atxn1 82Q by comparing with wild type TRIM11 (Figure 28F), suggesting that E3 ligase activity of TRIM11 was required for the reduction of Atxn1 82Q aggregates. To further test whether TRIM11 had an effect on amyloid like aggregates in cells, wild type or mutant TRIM11 was transfected into the cells and ThT staining showed that wild type and mutant TRIM11 could both down-regulate the level of amyloid like aggregates (Figure 28G). Stable overexpression of Atxn1 82Q moderately enhanced the ThT staining (Figure 30A). Similarly, TRIM11 could also reduce the detergent-insoluble fractions of Atxn1 82Q in stable cells (Figure 30B). Importantly, wild type TRIM11 had a stronger ability than mutant TRIM11 could to reduce the cellular aggregates (Figures 30C and 30D).

Due to the similarity of Hsp70 and TRIM11, it was hypothesized that TRIM11 might function as molecular chaperone for controlling protein aggregation. Generally, chaperones have a capacity of preventing aggregation-prone misfolded proteins. This is the most primary and effective way to control protein aggregation.

Therefore, the prevention function of TRIM11 in the formation of aggregate was investigated. Luciferase activity was rapidly decreased without chaperone in response to heat shock (Figure 31A). However, incubation of TRIM11 as well as Hsp70 could efficiently protect luciferase from heat inactivation (Figure 31A). Similarly, TRIM11 also protect GFP proteins against heat (Figure 31C). These results suggested that TRIM11 might function as molecular chaperone like Hsp70. In cells, stable overexpression of TRIM11 was able to moderately protect luciferase against heat shock (Figure 31C and Figure 31D). What's more, TRIM11 could also obviously recover the heat inactivation of luciferase (Figure 31C and Figure 31D), which further confirmed the chaperone like function of TRIM11. Importantly, TRIM11 overexpression did not change the protein level of Hsp70 (Figure 31E). Next, Alzheimer disease associated beta-amyloid (1-42) was used as a substrate to study the prevention function of TRIM11. As shown in Figure 31F, TRIM11 could inhibit amyloid fiber formation by ThT analysis. Additionally, Atxn1 82Q was purified to test the process of aggregate formation. Control protein GST could not prevent Atxn1 aggregate formation, while TRIM11 efficiently blocked the formation of aggregate (Figure 31G). Moreover, TRIM11 could also prevent amyloid like aggregate of Atxn1 82Q (Figure 31G). It is known that p53 is prone to form aggregate in vitro. As shown in Figure 31H, TRIM11 significantly prevented p53 from denaturation. Intriguingly, p53 could be SUMOylated by TRIM11 in vitro (Figure 27C) and SUMOylation of p53 could block amyloid like aggregate but promoted oligomer formation. To sum up, TRIM11 may function as a molecular chaperone to prevent aggregate formation.

To determine if TRIM11 could be upregulated in response to heat shock HCT116 cells stably expressing vector or TRIM11 were used. In control stable cells, TRIM11 was increased during the recovery after heat shock (Figure 32A). However, in TRIM11 expressing cells, exogenous TRIM11 protein could not be upregulated (Figure 32B), meaning that the up regulation of TRIM11 induced by heat was not due to the change of protein stability. Heat-induced TRIM11 up regulation was further confirmed in HeLa cells (Figure 32C). Because many stress can induce protein misfolding, whether TRIM11 could be induced by other stress was examined. As shown in Figures 30D and 30E, the protein level of TRIM11 was upregulated in response to As_2O_3 and H_2O_2

treatment. The mRNA level of TRIM11 could be induced in response to heat shock (Figure 32F), which is similar with Hsp70. To investigate the mechanisms by which TRIM11 was induced by heat shock, A549 cells were generated which stably express HSF1, a key transcriptional factor to control heat responsive proteins. Surprisingly, overexpression of HSF1 down- regulated TRIM11 protein level with or without heat shock treatment (Figure 32G), suggesting that HSF1 was possible not required for regulating the transcription of TRIM11.

Another key transcriptional factor, p53, can be activated during the heat shock response. p53 is also able to directly increase the transcription of TRIMs, for example TRIM21 and TRIM24. Therefore, it was investigated whether TRIM11 could be controlled by p53 in response to heat shock. As shown in Figure 33A, TRIM11 was increased in response to heat shock in p53 wild type but not p53 null HCT116 cells. Accordingly, the mRNA level of TRIM11 only was enhanced in p53 wild type HCT116 cells (Figure 33B), suggesting that p53 contributed to TRIM11 transcription. To further confirm the importance of p53, the protein and mRNA level of TRIM11 could not be upregualted in A549 cells stably expressing p53 shRNA (Figure 33C). Similar phenomenon was confirmed again in p53 knockdown HCT116 cells (Figure 33D). Together, these results strongly implied that p53 might be a key factor to upregulate TRIM11 in heat shock response. HSF1 is considered as a safeguard for cell survival following heating stress. KRIBB11, a chemical inhibitor, can be used to inhibit HSF1 activity. As shown in Figure 33E and Figure 33F, with KRIBB11 treatment, p53 nulls were more sensitive to heat shock, which implied that p53 also contributed to cell survival in response to heating stress.

Hsp70 may promote the dissolution of some kinds of protein aggregates. Next, it was investigated whether TRIM11 could disaggregate protein aggregates. As expected, TRIM11 resolubilized heat-inactivated luciferase from insoluble aggregates (Figure 34A) and recovered luciferase activity in a dose-dependent manner (Figure 34B). Also, the solubilized function of TRIM11 was further confirmed using preformed GFP aggregates as substrates (Figure 34C and Figure 34D). These results suggested that TRIM11 could disaggregate disordered aggregates. Next, assays were performed using Atxn1 82Q aggregates as substrates. As shown in Figure 34E, TRIM11 could efficiently

resolubilized Atxn1 82Q aggregates. Notably, Hsp70/Hsp40 di-chaperone only disaggregated amyloid like structure of Atxn1 82Q into the pellet (Figure 34F), suggesting that TRIM11 might act in a different way. As expected, TRIM11, as well as Hsp104, significantly recovered amyloid like structure into the supernatant (Figure 34G).

To determine which functional domain of TRIM11 was required for disaggregation activity of TRIM11 heated luciferase aggregates were used as substrates. As shown in Figure 35B, TRIM11 could efficiently recover heat inactivation of luciferase activity, but RBC or B30.2 fragments of TRIM11 had weaker reactivation ability. Consistently, in sedimentation assay, TRIM11 had a stronger activity than its two fragments to resolubilize preformed luciferase aggregates (Figure 35C). Further, each single domain almost lost the refolding activity by comparing with full length TRIM11 (Figure 35D). Furthermoe, full length TRIM11 very strongly interacted with Atxn1 82Q, but RBC or B30.2 did not bound to Atxn1 82Q (Figure 36A). Similarly, single domain of TRIM11 did not bind to Atxn1 82Q (Figure 36B). These results strongly suggested that binding to substrates might be required for TRIM11 disaggregation function.

It was next investigated whether SUMO E3 ligase activity is required for protein disaggregation. Here mutant TRIM11 was used to test its disaggregation activity. As shown in Figure 37A, mutant TRIM11 maintained the ability to prevent luciferase from heat inactivation like wild type TRIM11. Moreover, mutant TRIM11 also had a similar potential with wild type TRIM11 to recover denatured luciferase activity (Figure 37B). Mutant TRIM11 was still able to selectively bind to denature luciferase (Figure 37C). All these results indicated that in vitro, TRIM11 performed its disaggregation function independently of its E3 activity. Notably, mutant TRIM11 was largely localized in the nucleus (Figure 37D), which was different from wild type TRIM11. Further, mutant TRIM11 could also be co-localized with Atxn1 82Q.

Previous studies showed that Hsp110, Hsp70, and Hsp40, the metazoan protein disaggregase system, could not efficiently disaggregate amyloid. Therefore, it was investigated whether TRIM11 could disaggregate amyloid fiber. Here alpha-synuclein was applied as a client. Firstly, it was determined whether TRIM11 could also prevent alpha-Synuclein fiber formation. As shown in Figure 38A, TRIM11, as well as Hsp70 and Hsp104, efficiently inhibit the formation of alpha-Synuclein amyloid fiber

(Figure 38A). Also, the inhibition was a dose-dependent manner (Figure 38B). Indeed, electron microscopy (EM) revealed that alpha-Synuclein fibers were exhibited by control GST protein, whereas no fiber observed by TRIM11 (Figure 38C). Next, alpha-Synuclein fibers were used to study the disaggregation of TRIM11. As shown in Figure 38D, with TRIM11 or mutant Hsp104 treatment, solubilization of alpha-Synuclein fibers increased in a dose-dependent manner. Accordingly, fiber disassembly was obvious with TRIM11 or Hsp104 treatment by ThT fluorescence (Figure 38E).

There are over 70 TRIM family members with common N-terminus and different C-terminus. Therefore, next whether other TRIM proteins also had similar function like TRIM11 was investigated. Therefore, TRIM21 was selected because it shares the same domains with TRIM11. In vitro pull down assay revealed that TRIM21 also preferentially bound to denatured luciferase (Figure 39A). Similarly, TRIM21 could moderately solubilized heated luciferase aggregates (Figure 39B) and recovered heat inactivation of luciferase (Figure 39C). TRIM21 also was able to protect luciferase against heat inactivation (Figure 39D). PML could degrade Atxn1 82Q aggregates through proteasome. So to investigate whether disaggregation and degradation of Atxn1 aggregates were coupled by PML, PML from was purified 293T cells (Figure 40A). As shown in Figure 40B, PML could recover heat inactivation of GFP fluorescence in a dose-dependent manner. However, the recover ability was weaker than TRIM11 (Figure 40C). Several fragments of PML were also purified from 293T cells (Figure 40D). Interestingly, one fragment (361-633) of PML might be necessary for the reactivation of heated GFP aggregates (Figure 40E).

To better understand the role of TRIM11 in controlling formation of alpha-Synuclein aggregates, mouse primary hippocampal neurons were used as a model. Alpha-Synuclein fibrils (PEF) were generated with GST or TRIM11 in vitro (GST-PEF and TRIM11-PEF). Two weeks after PEF addition, about 30% neuron death induced by PEF aggregates (Figure 41A). However, TRIM11-PEF has a lower toxic to neurons (Figure 41A). Indeed, immunofluorescence revealed that there were significant alpha-Synuclein aggregates that recapitulated features of the Lewy bodies in Parkinson's disease brains, whereas there were no big aggregates following addition of TRIM11-PEF (Figure 41B), which further showed that TRIM11 had a prevention function in formation

of alpha-Synuclein fibers. Notably, in two kinds of neurons (cortical and hippocampal neurons), TRIM11 could be up-regulated in response to heat shock (Figure 42A and Figure 42B). Moreover, the mRNA level of TRIM11 was also increased in hippocampal neurons (Figure 42C). It was of interest that TRIM11 was likely involved in controlling neurodegenerative disease.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A composition for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates, the composition comprising a modulator of one or more TRIM proteins.

2. The composition of claim 1, wherein the modulator increases the expression or activity of the one or more TRIM proteins.

3. The composition of claim 1, wherein the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

4. The composition of claim 1, wherein the modulator increases the expression or activity of at least one selected from the group consisting of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as “PML”), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

5. The composition of claim 1, wherein the composition comprises an isolated peptide comprising one or more TRIM proteins.

6. The compositing of claim 5, wherein the isolated peptide further comprises a cell penetrating peptide (CPP) to allow for entry of the isolated peptide into a cell.

7. The composition of claim 6, wherein the CPP comprises the protein transduction domain of HIV tat.

8. The composition of claim 1, wherein the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

9. The composition of claim 1, wherein the disease or disorder is a polyQ disorder.

10. The composition of claim 1, wherein the disease or disorder is a neurodegenerative disease or disorder selected from the group consisting of Spinocerebellar ataxia (SCA) Type 1 (SCA1), SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), a transmissible spongiform encephalopathy (prion disease), a tauopathy, and Frontotemporal lobar degeneration (FTLD).

11. The composition of claim 1, wherein the disease or disorder is selected from the group consisting of AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis.

12. The composition of claim 1, wherein the disease or disorder is cancer associated with p53 mutant aggregates.

13. A method for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates in a subject in need thereof, the method comprising administering to the subject a composition comprising a modulator of one or more TRIM proteins.

14. The method of claim 13, wherein the modulator increases the expression or activity of the one or more TRIM proteins

15. The method of claim 13, wherein the modulator is at least one selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

16. The method of claim 13, wherein the modulator increases the expression or activity of at least one selected from the group consisting of human TRIM3, TRIM4, TRIM5, TRIM6, TRIM7, TRIM9, TRIM11, TRIM13, TRIM14, TRIM15, TRIM16, TRIM17, TRIM19 (also referred to herein as "PML"), TRIM20, TRIM21, TRIM24, TRIM25, TRIM27, TRIM28, TRIM29, TRIM32, TRIM34, TRIM39, TRIM43, TRIM44, TRIM45, TRIM46, TRIM49, TRIM50, TRIM52, TRIM58, TRIM59, TRIM65, TRIM67, TRIM69, TRIM70, TRIM74 and TRIM75; and mouse TRIM30.

17. The method of claim 13, wherein the composition comprises an isolated peptide comprising one or more TRIM proteins.

18. The method of claim 17, wherein the isolated peptide further comprises a cell penetrating peptide (CPP) to allow for entry of the isolated peptide into a cell.

19. The method of claim 18, wherein the CPP comprises the protein transduction domain of HIV tat.

20. The method of claim 13, wherein the composition comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

21. The method of claim 13, wherein the disorder is a polyQ disorder.

22. The method of claim 13, wherein the disease or disorder is a neurodegenerative disorder elected from the group consisting of Spinocerebellar ataxia (SCA) Type 1 (SCA1), SCA2, SCA3, SCA6, SCA7, SCA17, Huntington's disease, Dentatorubral-pallidoluysian atrophy (DRPLA), Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), a transmissible spongiform encephalopathy (prion disease), a tauopathy, and Frontotemporal lobar degeneration (FTLD).

23. The method of claim 13, wherein the disease or disorder is selected from the group consisting of AL amyloidosis, AA amyloidosis, Familial Mediterranean fever, senile systemic amyloidosis, familial amyloidotic polyneuropathy, hemodialysis-related amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, Finnish hereditary amyloidosis, lysozyme amyloidosis, fibrinogen amyloidosis, Icelandic hereditary cerebral amyloid angiopathy, type II diabetes, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary cerebral hemorrhage with amyloidosis, pituitary prolactinoma, injection-localized amyloidosis, aortic medial amyloidosis, hereditary lattice corneal dystrophy, corneal amyloidosis associated with trichiasis, cataract, calcifying epithelial odontogenic tumor, pulmonary alveolar proteinosis, inclusion-body myostis, and cuteaneous lichen amyloidosis.

24. The method of claim 13, wherein the disease or disorder is cancer associated with p53 mutant aggregates.

25. The method of claim 13, wherein the method comprises administering the composition to at least one neural cell of the subject.

26. A composition for treating or preventing a disease or disorder associated with degradation of functional mutant protein, the composition comprising a modulator of one or more TRIM proteins.

27. The composition of claim 26, wherein the modulator increases the expression or activity of the one or more TRIM proteins.

28. The composition of claim 26, wherein the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

29. The composition of claim 26, wherein the disease or disorder is cystic fibrosis.

30. A method for treating or preventing a disease or disorder associated with degradation of functional mutant protein in a subject in need thereof, the method comprising administering to the subject a composition comprising a modulator of one or more TRIM proteins.

31. The method of claim 30, wherein the modulator increases the expression or activity of the one or more TRIM proteins

32. The method of claim 30, wherein the modulator is at least one selected from the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

33. The method of claim 30, wherein the disease or disorder is cystic fibrosis.

34. A composition for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates, the composition comprising a modulator of one or more SUMO-targeted ubiquitin ligase (STUbl).

35. The composition of claim 34, wherein the modulator increases the expression or activity of one or more STUbLs.

36. The composition of claim 34, wherein the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

37. The composition of claim 34, wherein the modulator increases the expression or activity of RNF4.

38. A method for treating or preventing a disease or disorder associated with misfolded protein or protein aggregates in a subject in need thereof, the method comprising administering to the subject a composition comprising a modulator of one or more STUbLs.

39. The method of claim 38, wherein the modulator increases the expression or activity of one or more STUbLs.

40. The method of claim 38, wherein the modulator is at least one of the group consisting of a chemical compound, a protein, a peptide, a peptidomemetic, an antibody, a ribozyme, a small molecule chemical compound, a nucleic acid, a vector, and an antisense nucleic acid.

41. The method of claim 38, wherein the modulator increases the expression or activity of RNF4.

42. A method for producing a recombinant protein comprising administering a modulator of one or more TRIM proteins to cell modified to express a recombinant protein.

43. The method of claim 42, wherein the modulator comprises an isolated peptide comprising one or more TRIM proteins.

44. The method of claim 42, wherein the modulator comprises an isolated nucleic acid molecule encoding one or more TRIM proteins.

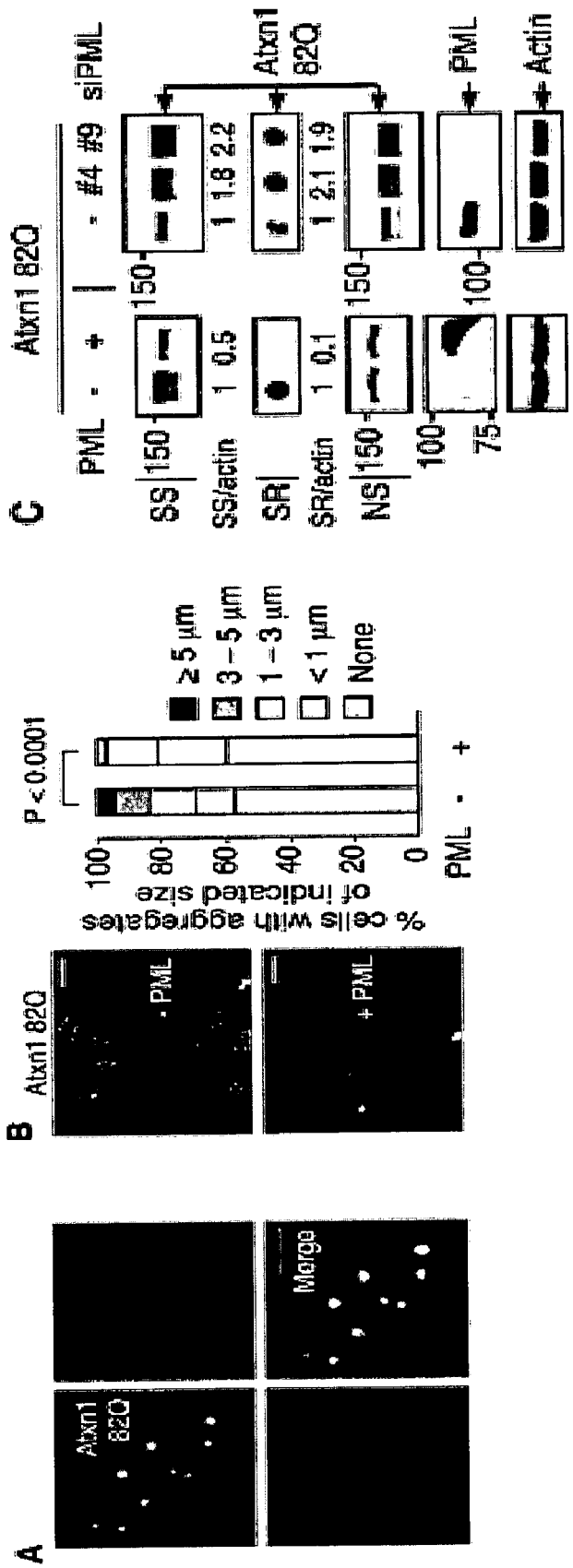


Figure 1A – Figure 1C

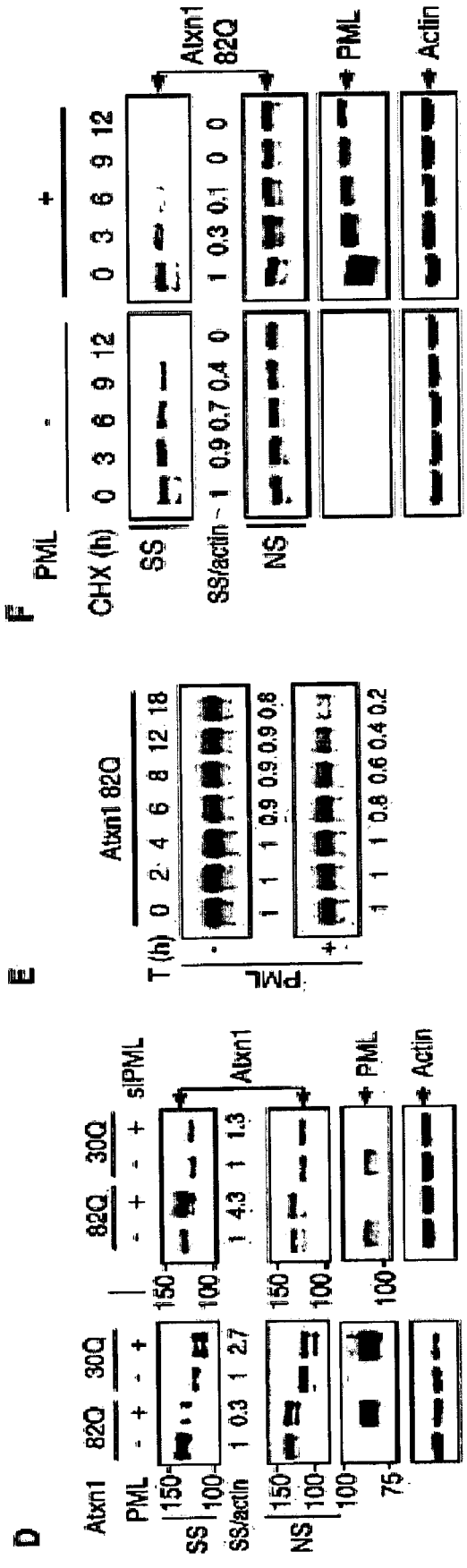


Figure 1D – Figure 1F

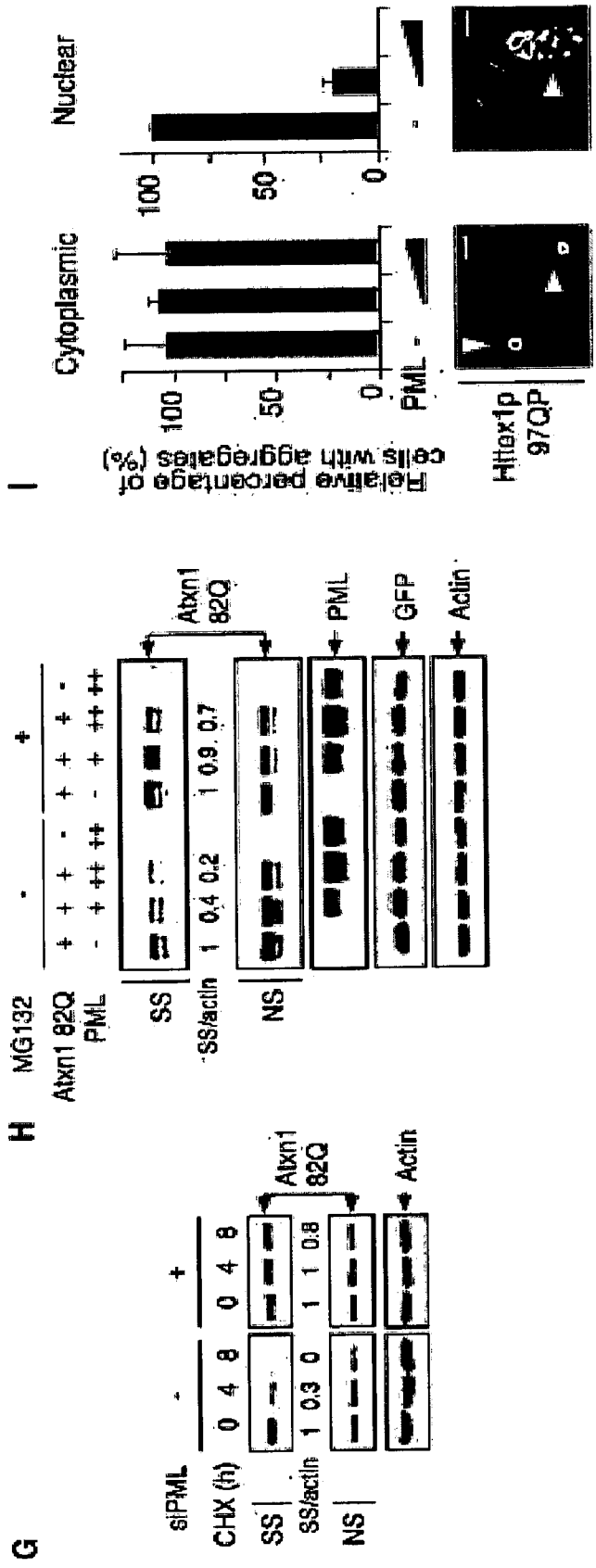


Figure 1G – Figure 1I

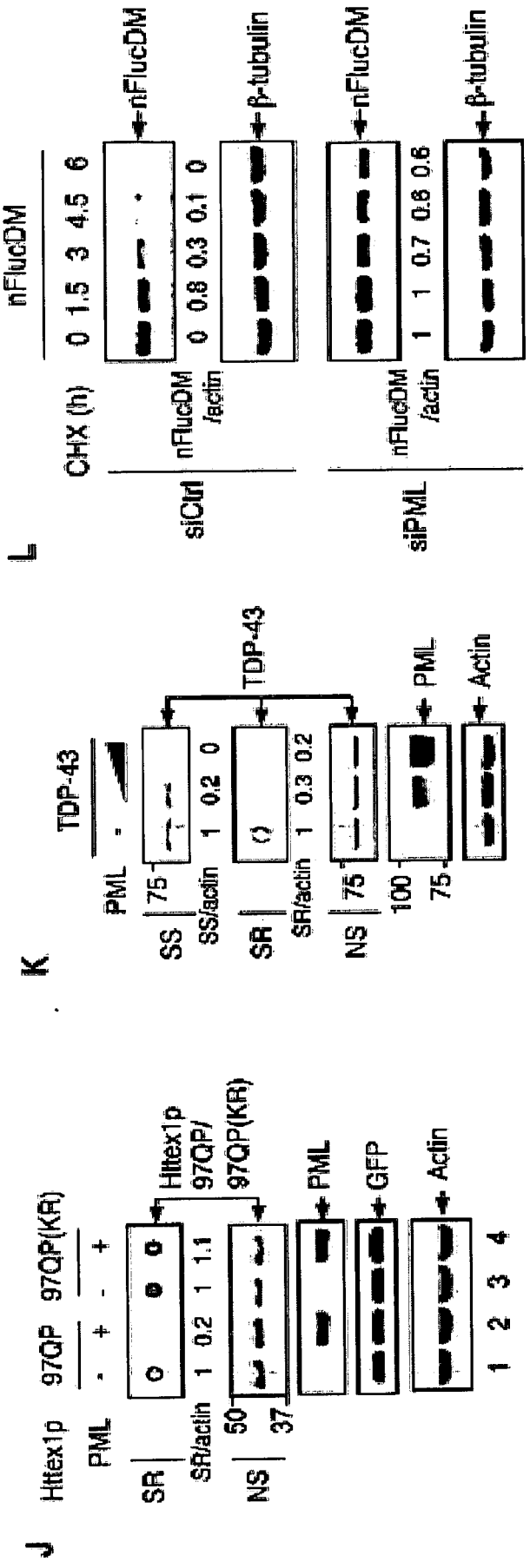


Figure 1J – Figure 1L

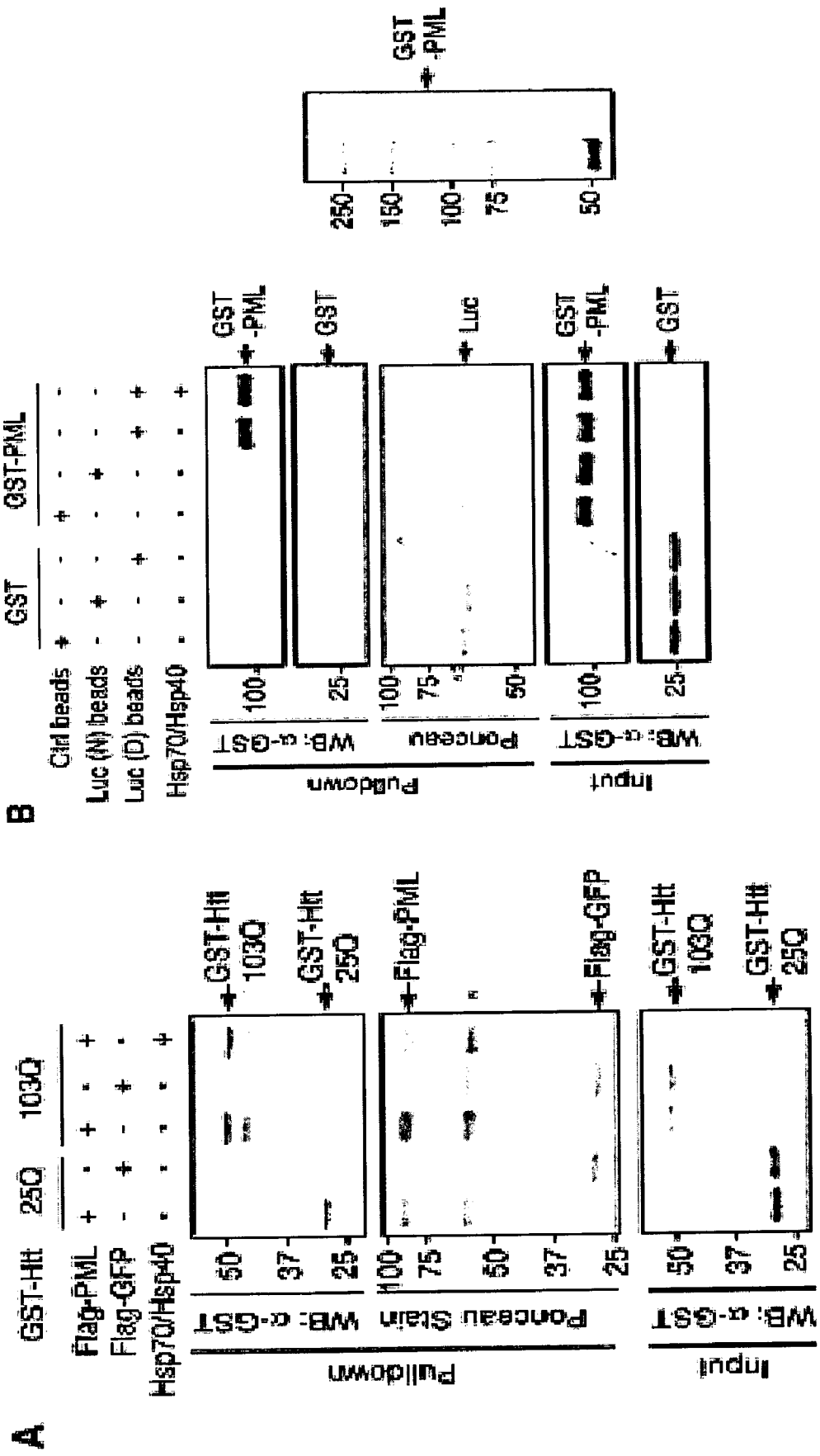


Figure 2A – Figure 2B

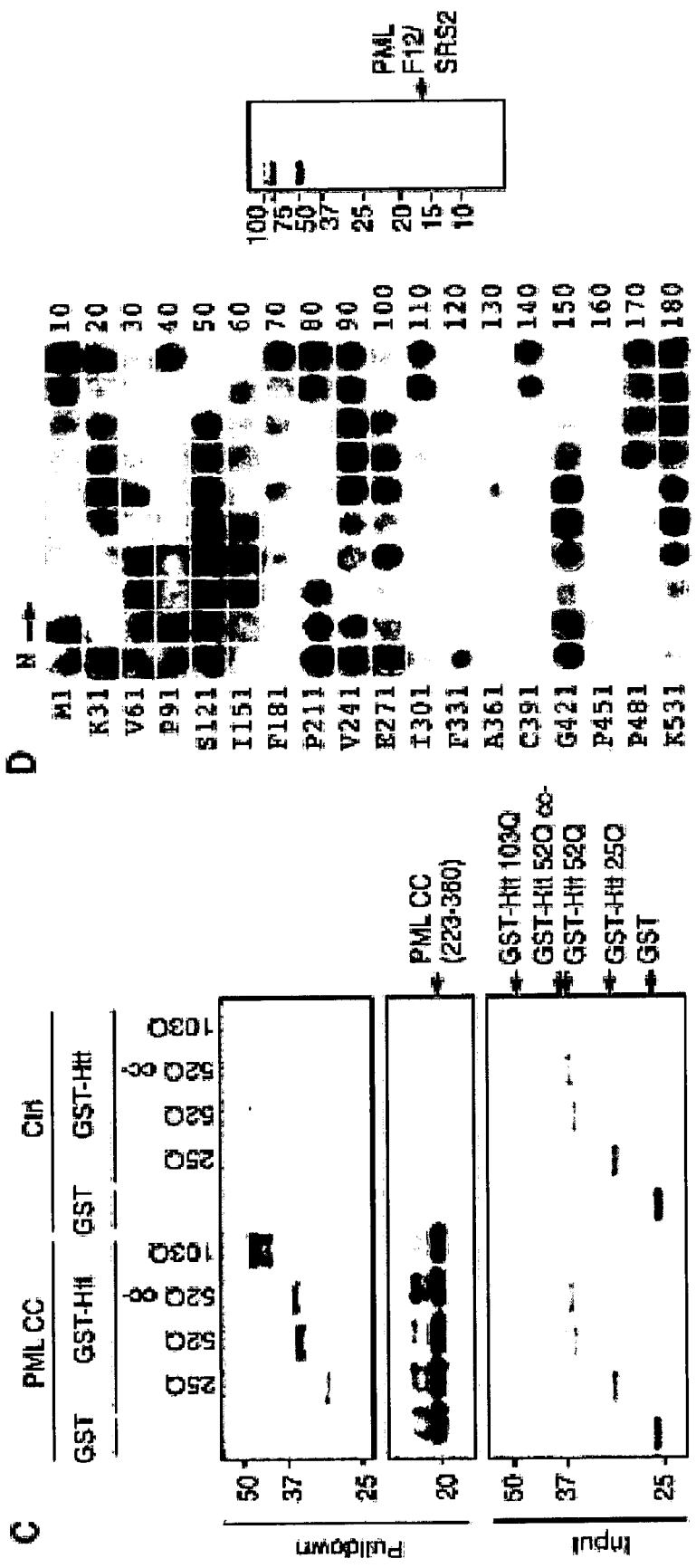


Figure 2C – Figure 2D

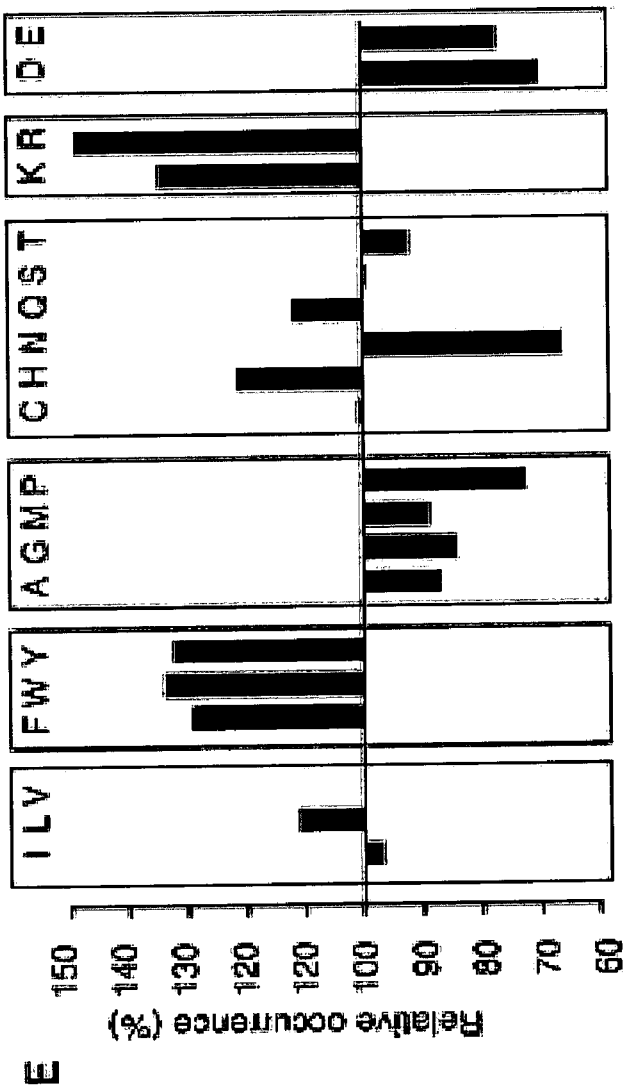

E
Relative occurrence (%)
150
140
130
120
120
100
90
80
70
60
ILV
FWY
AGMP
CHNQST
KR
DE

Figure 2E

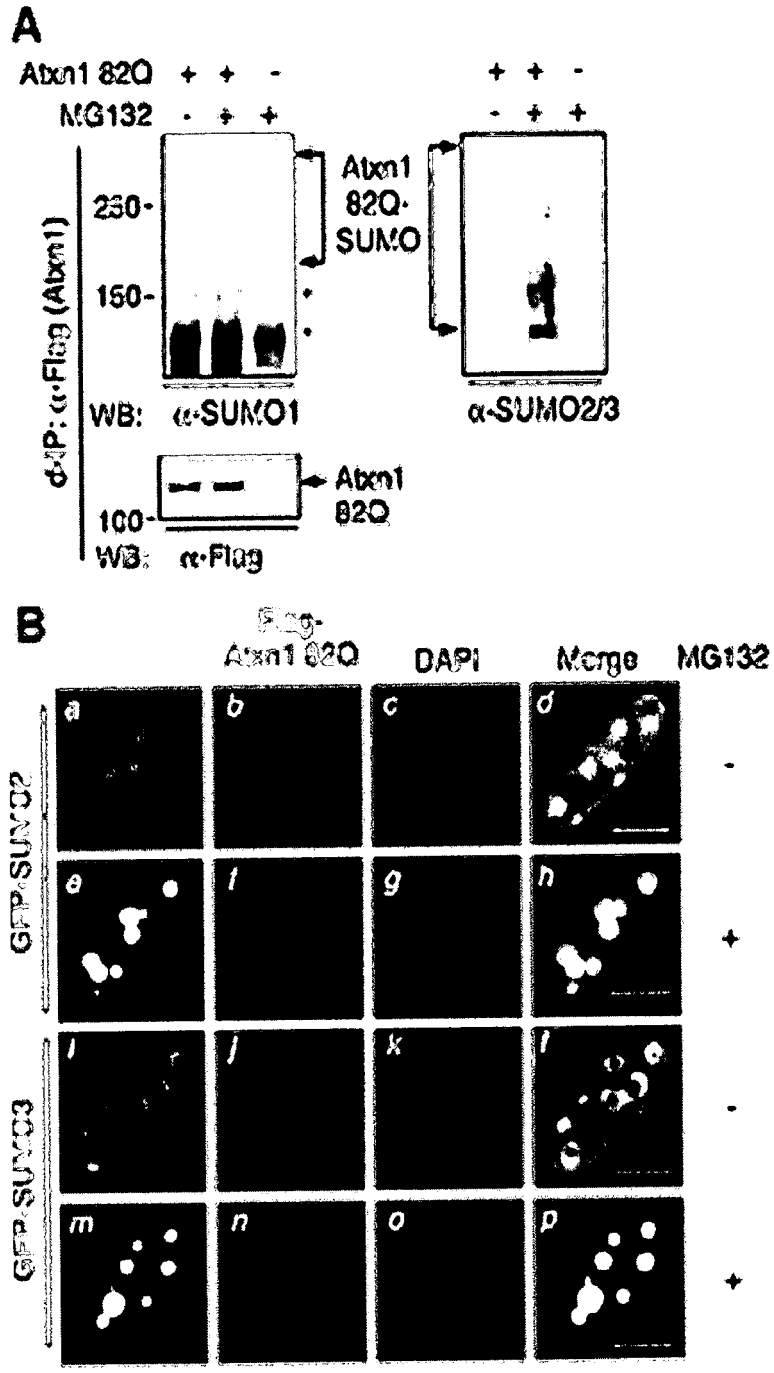


Figure 3A – Figure 3B

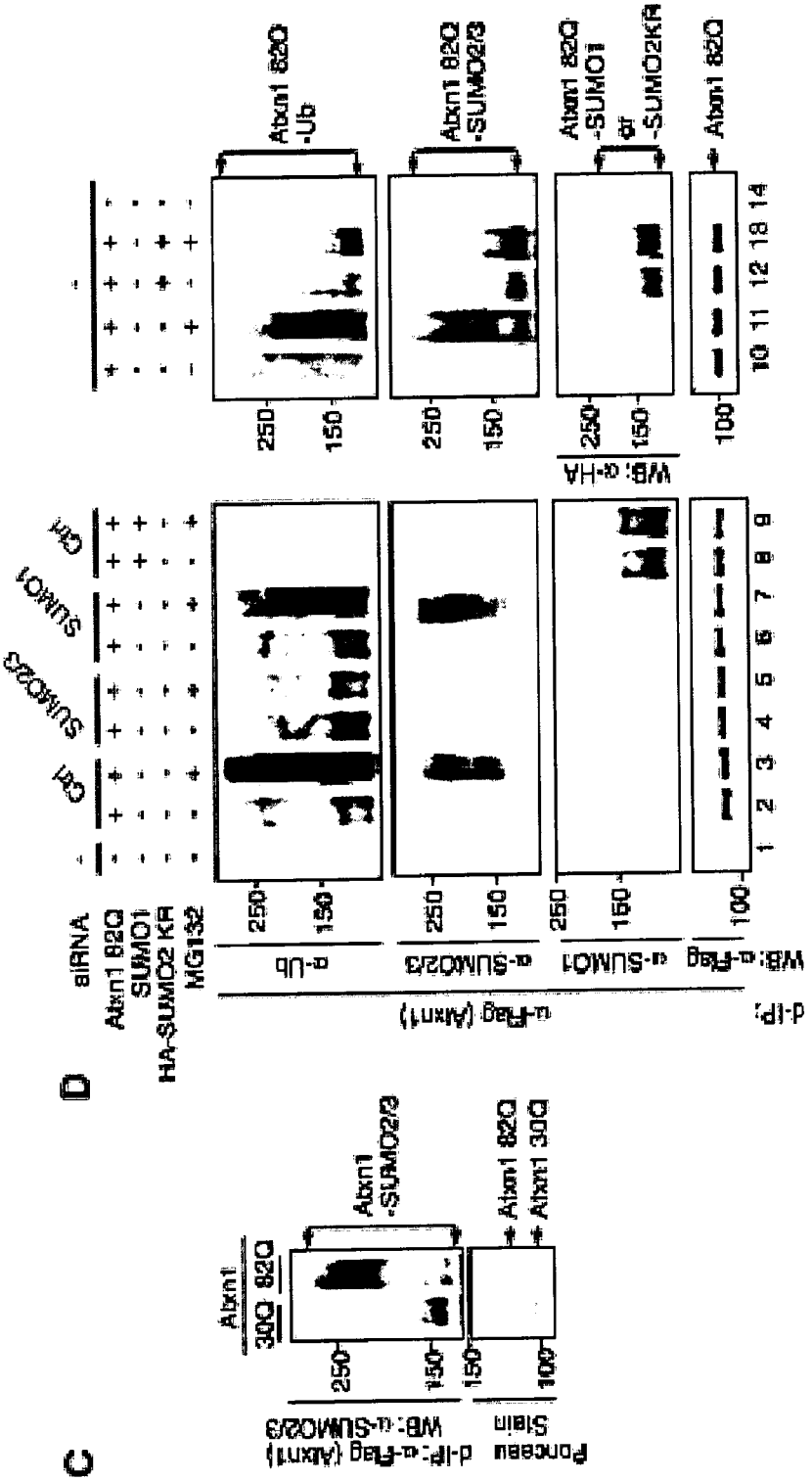


Figure 3C – Figure 3D

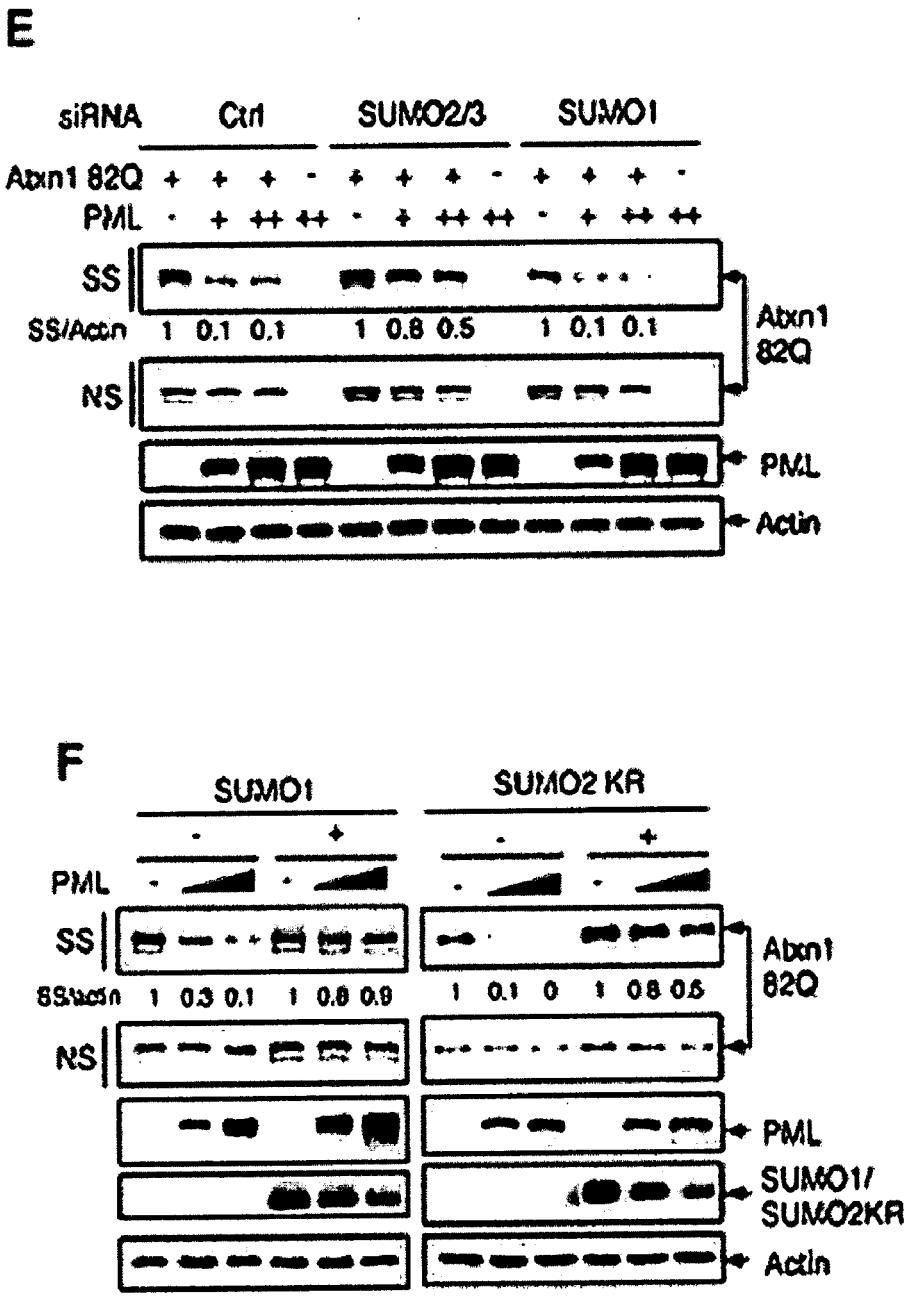


Figure 3E – Figure 3F

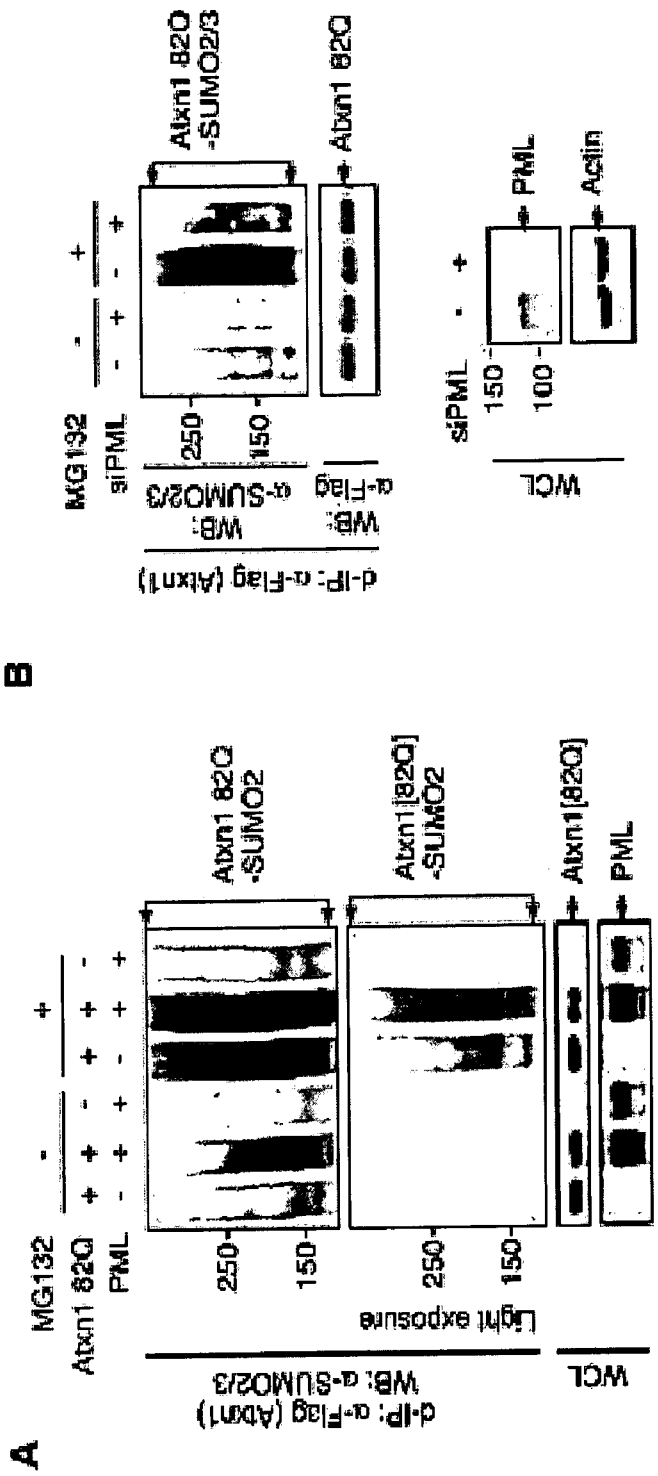


Figure 4A – Figure 4B

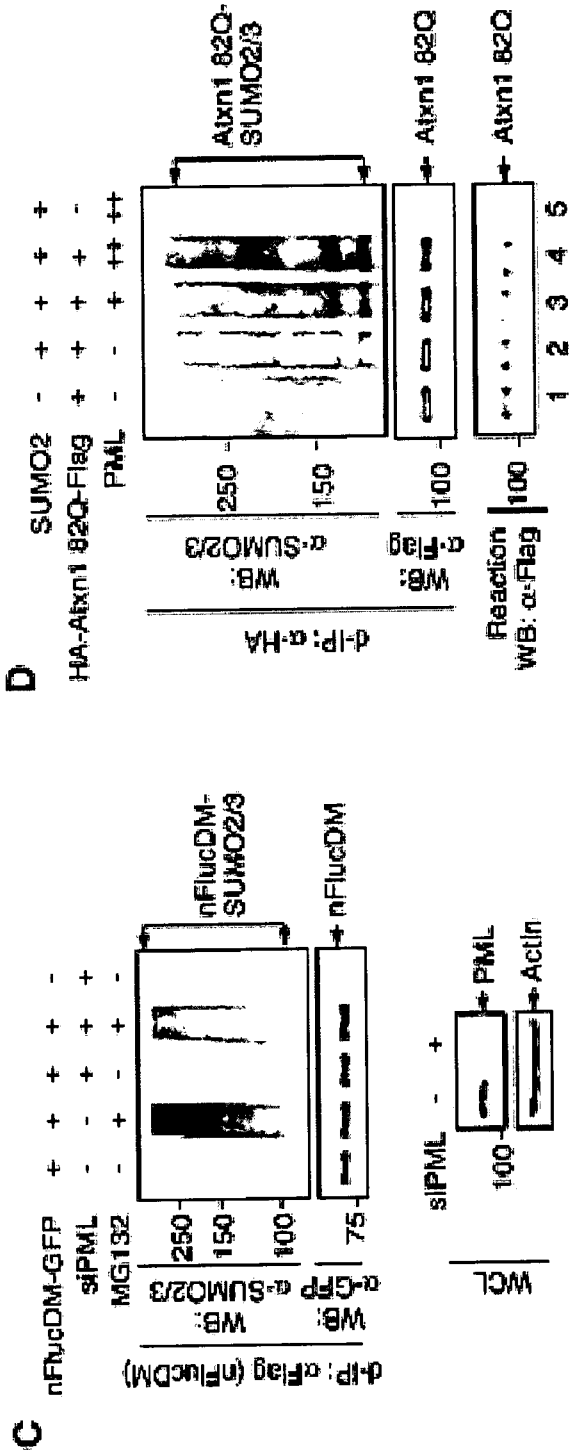
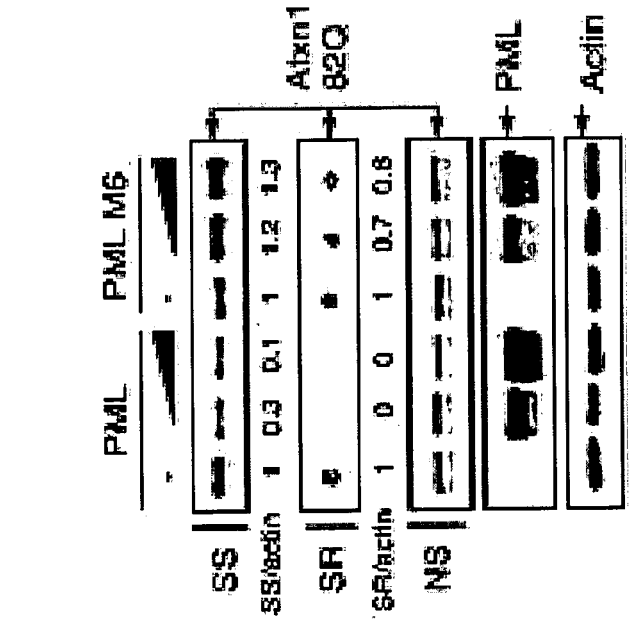


Figure 4C – Figure 4D



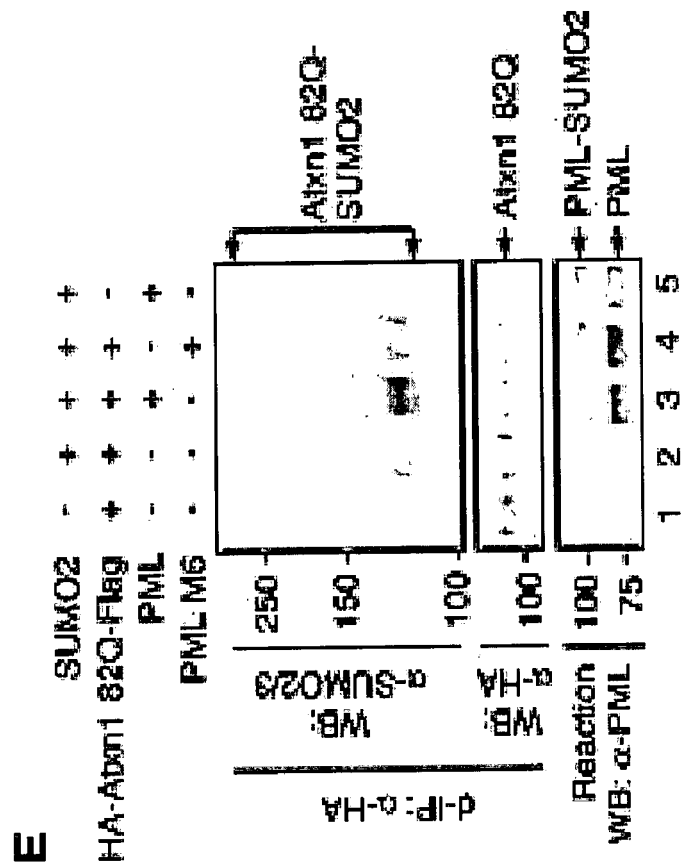


Figure 4E – Figure 4F

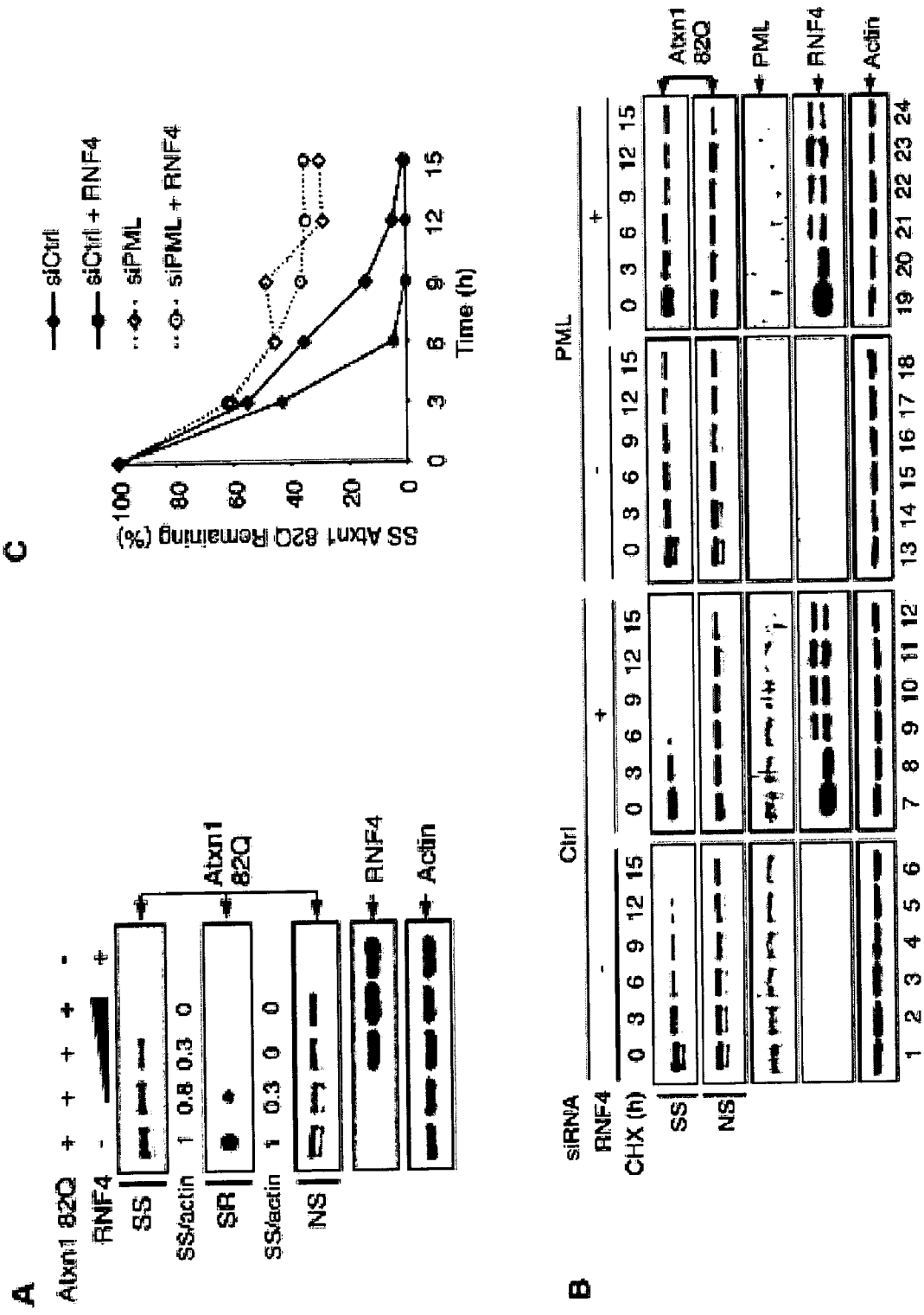


Figure 5A – Figure 5C

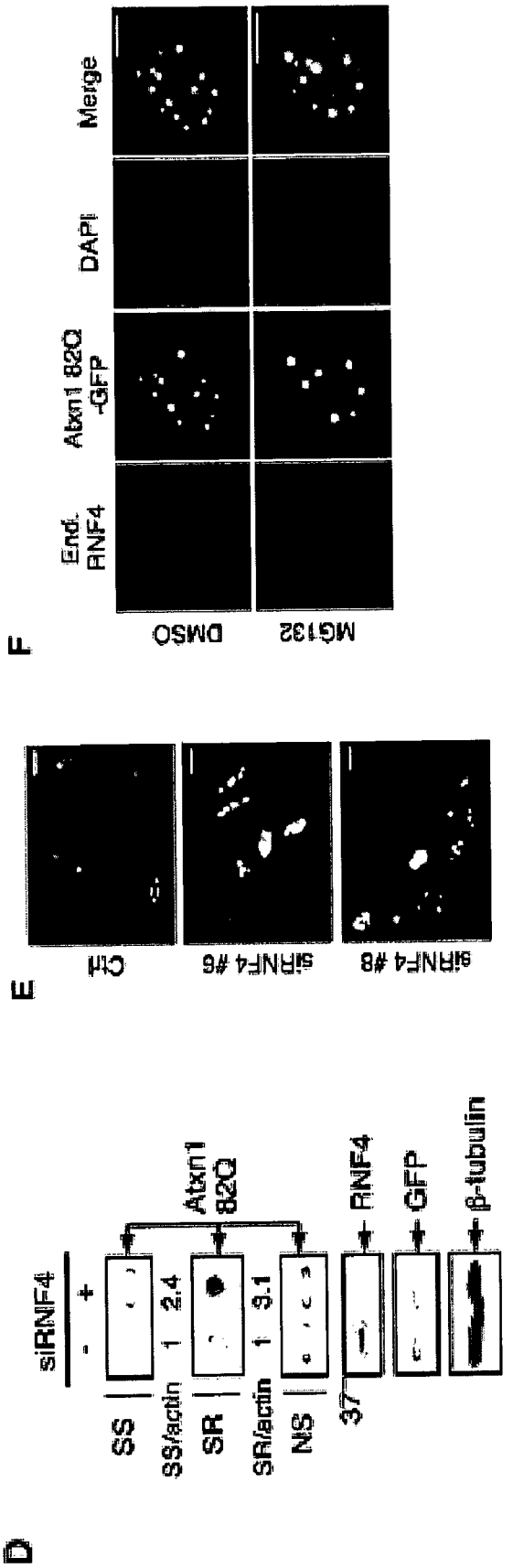


Figure 5D – Figure 5F

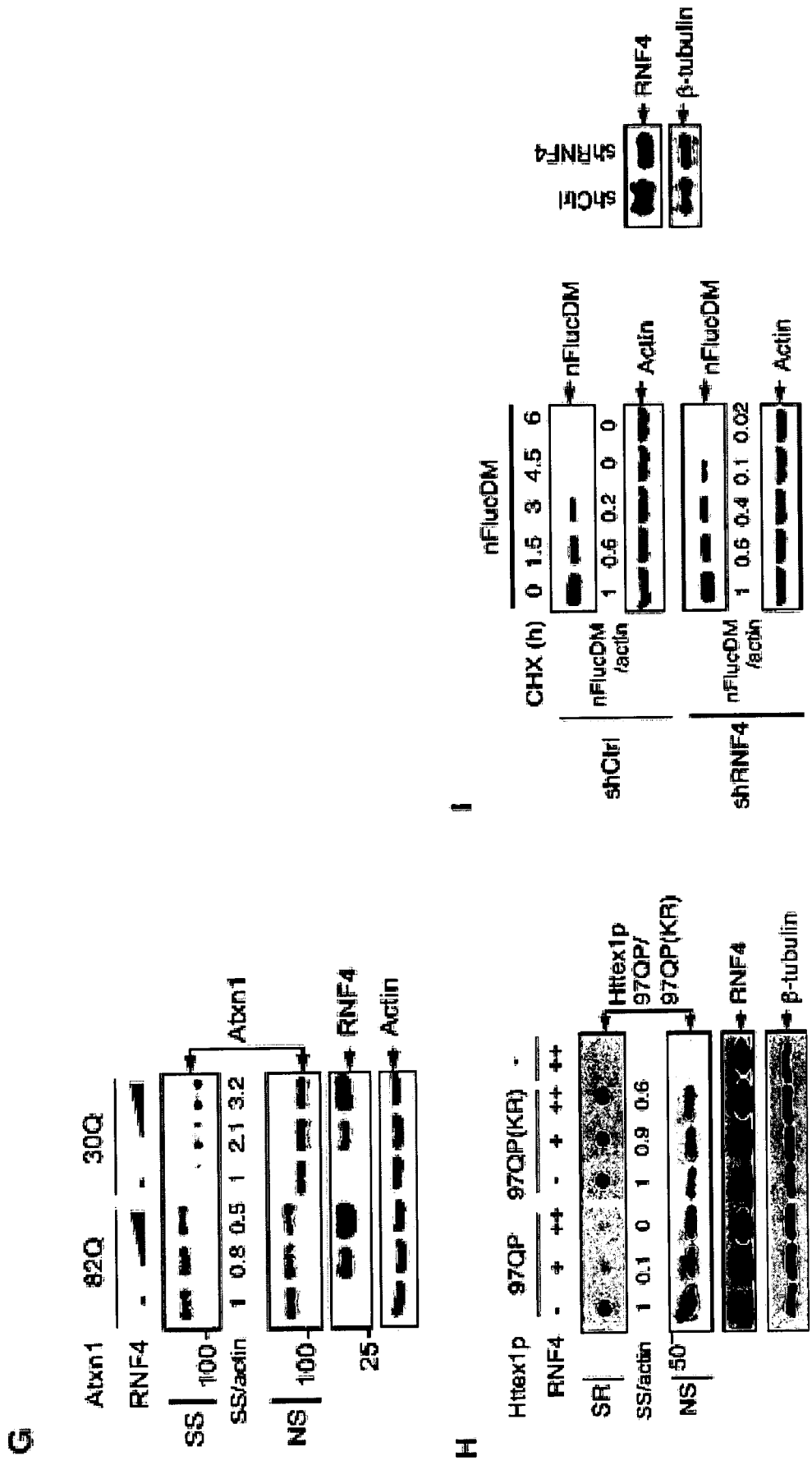


Figure 5G – Figure 5I

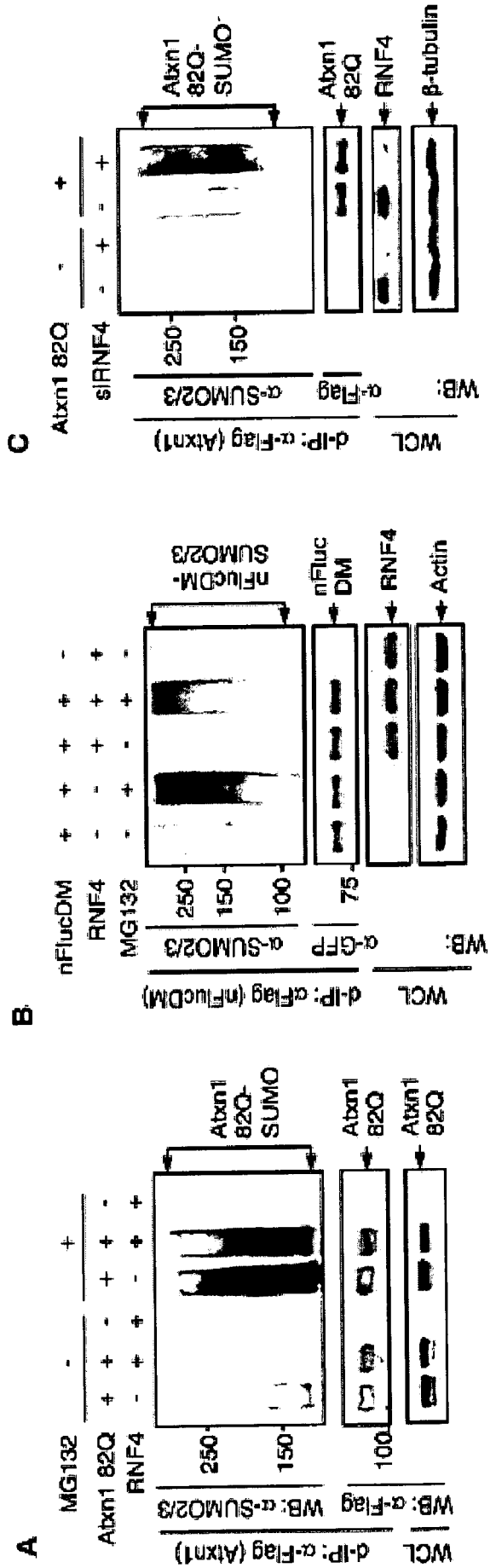


Figure 6A – Figure 6C

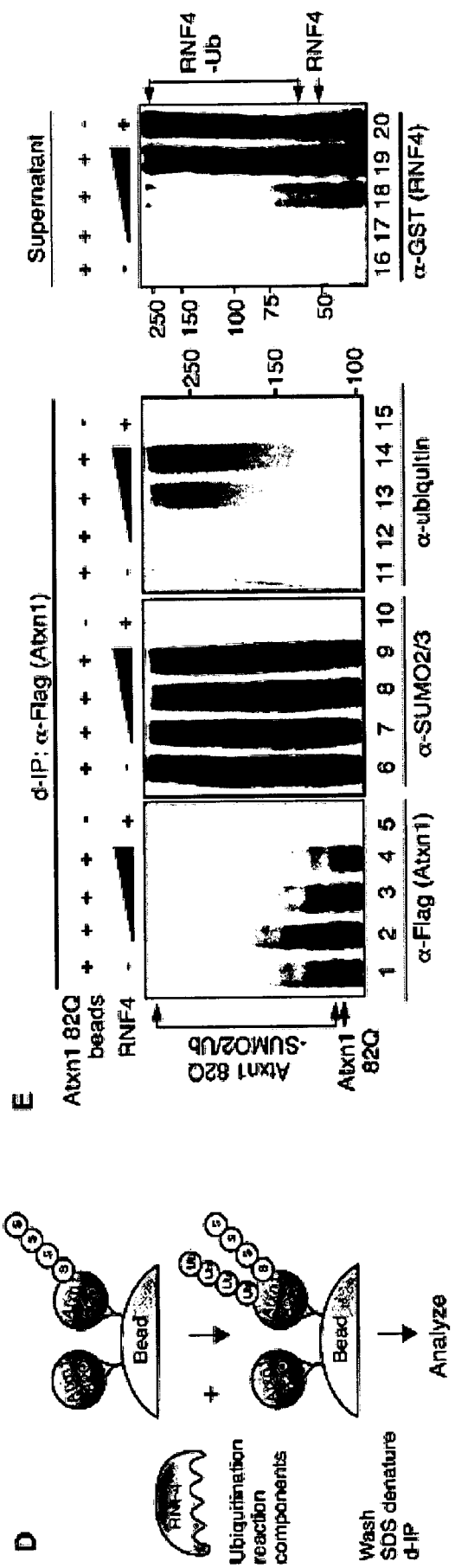


Figure 6D – Figure 6E

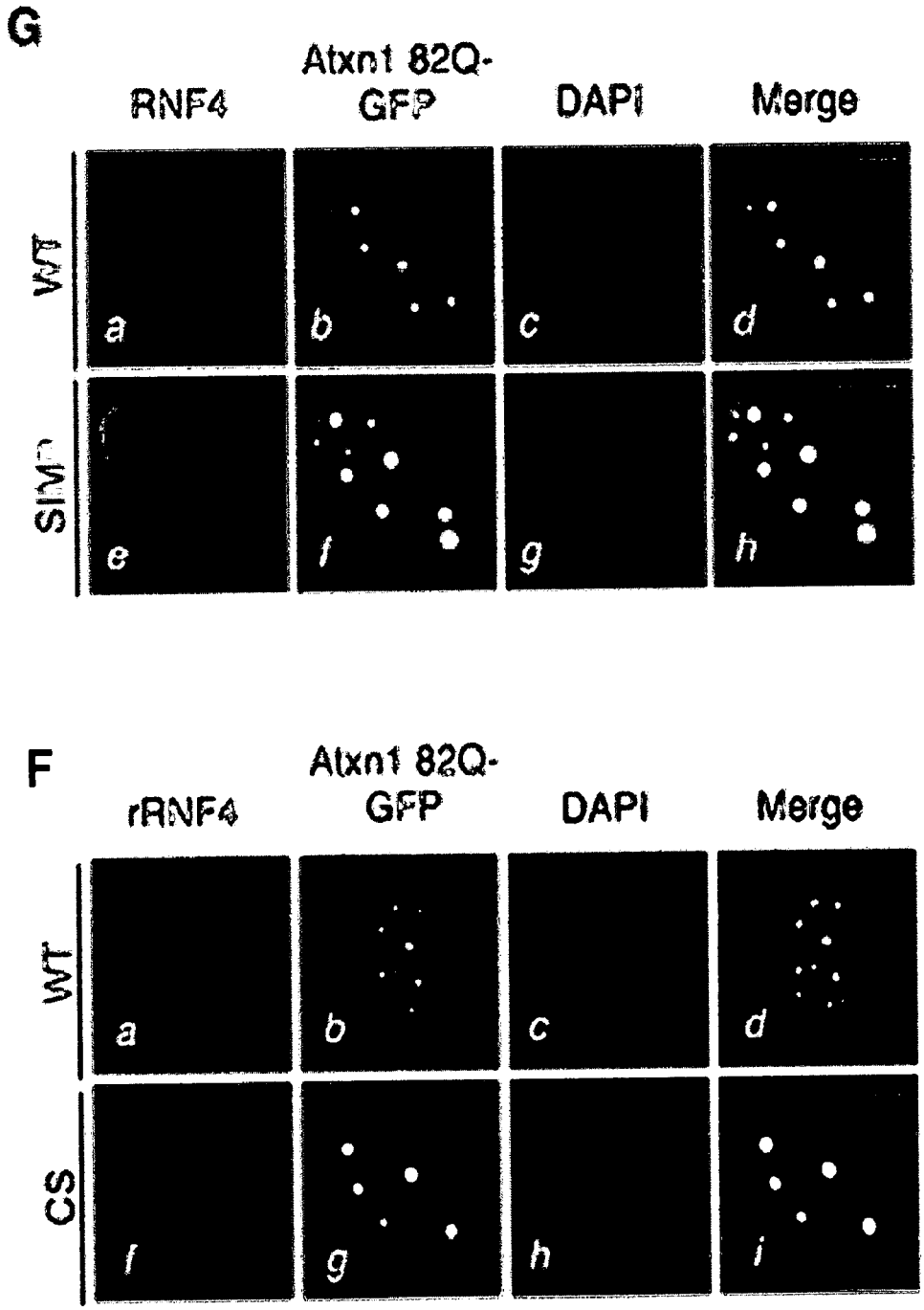
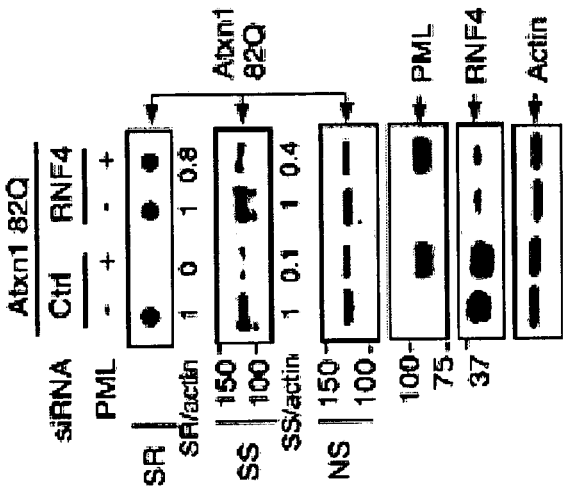


Figure 6F – Figure 6G



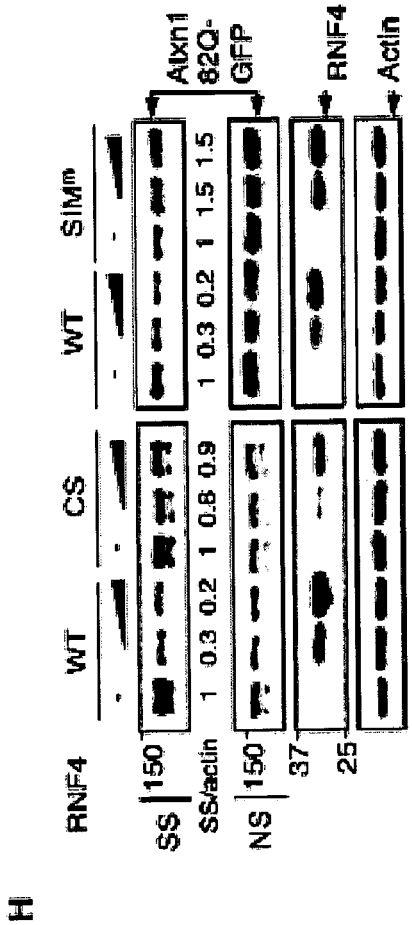


Figure 6H – Figure 6I

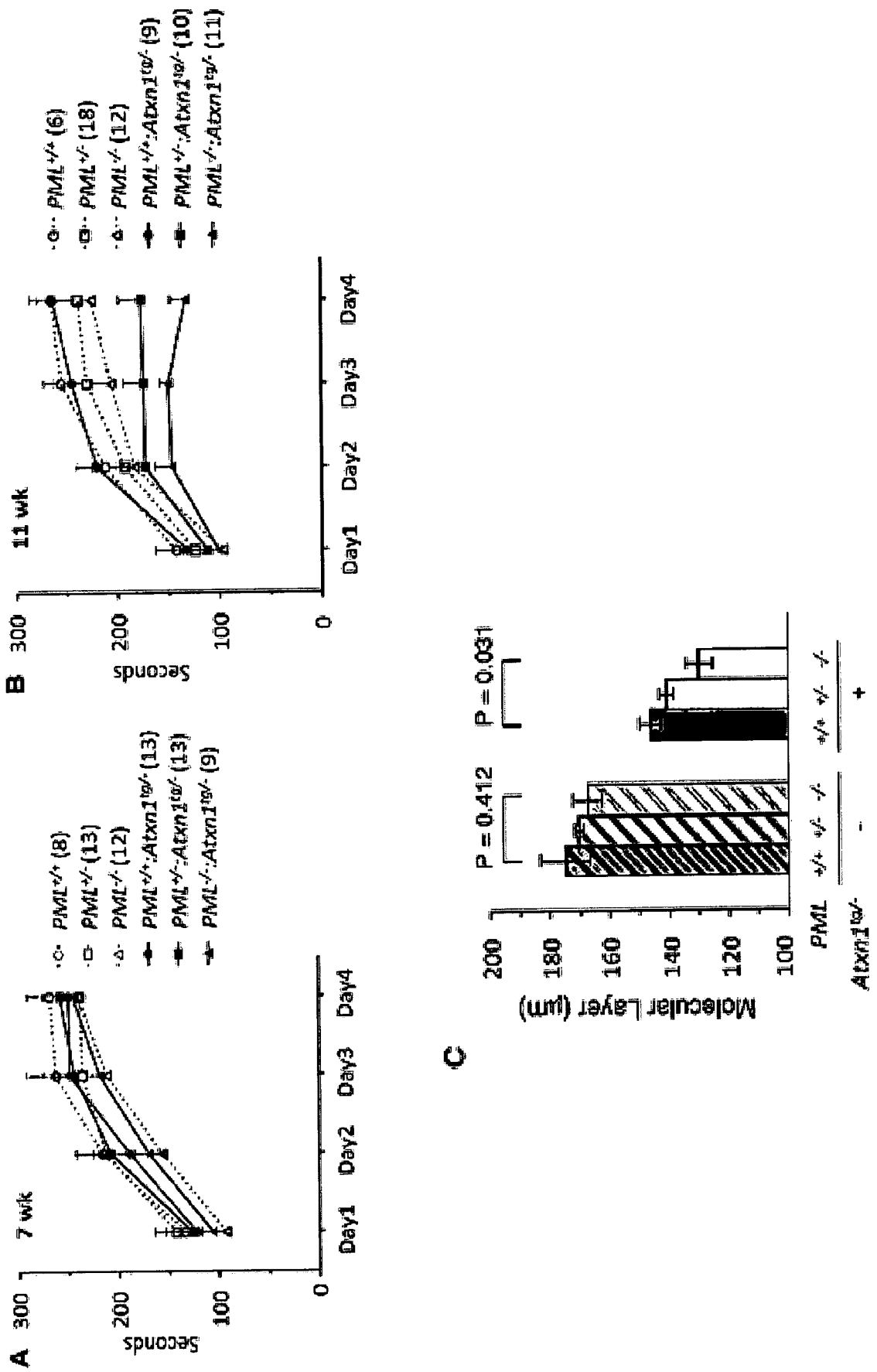


Figure 7A – Figure 7C

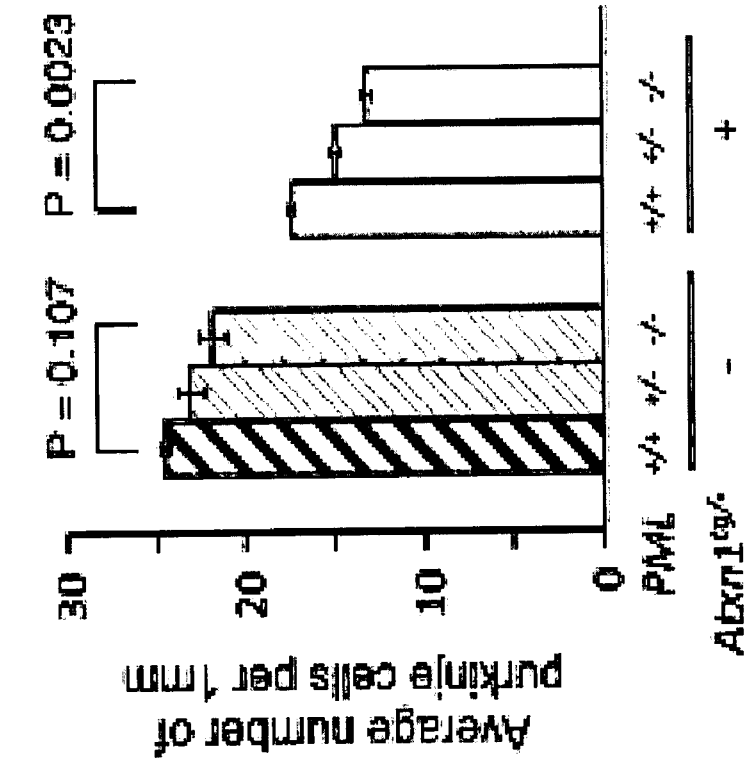




Figure 7D – Figure 7E

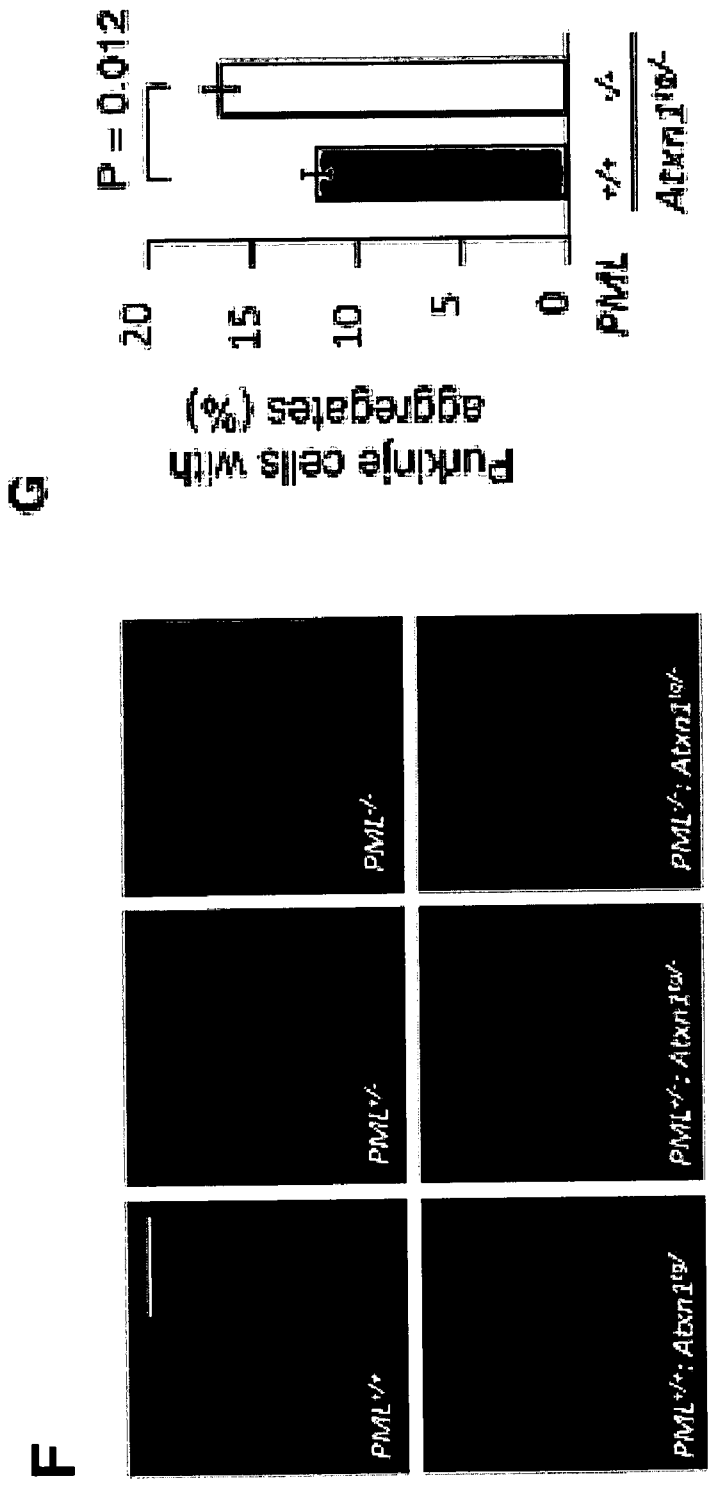


Figure 7F – Figure 7G

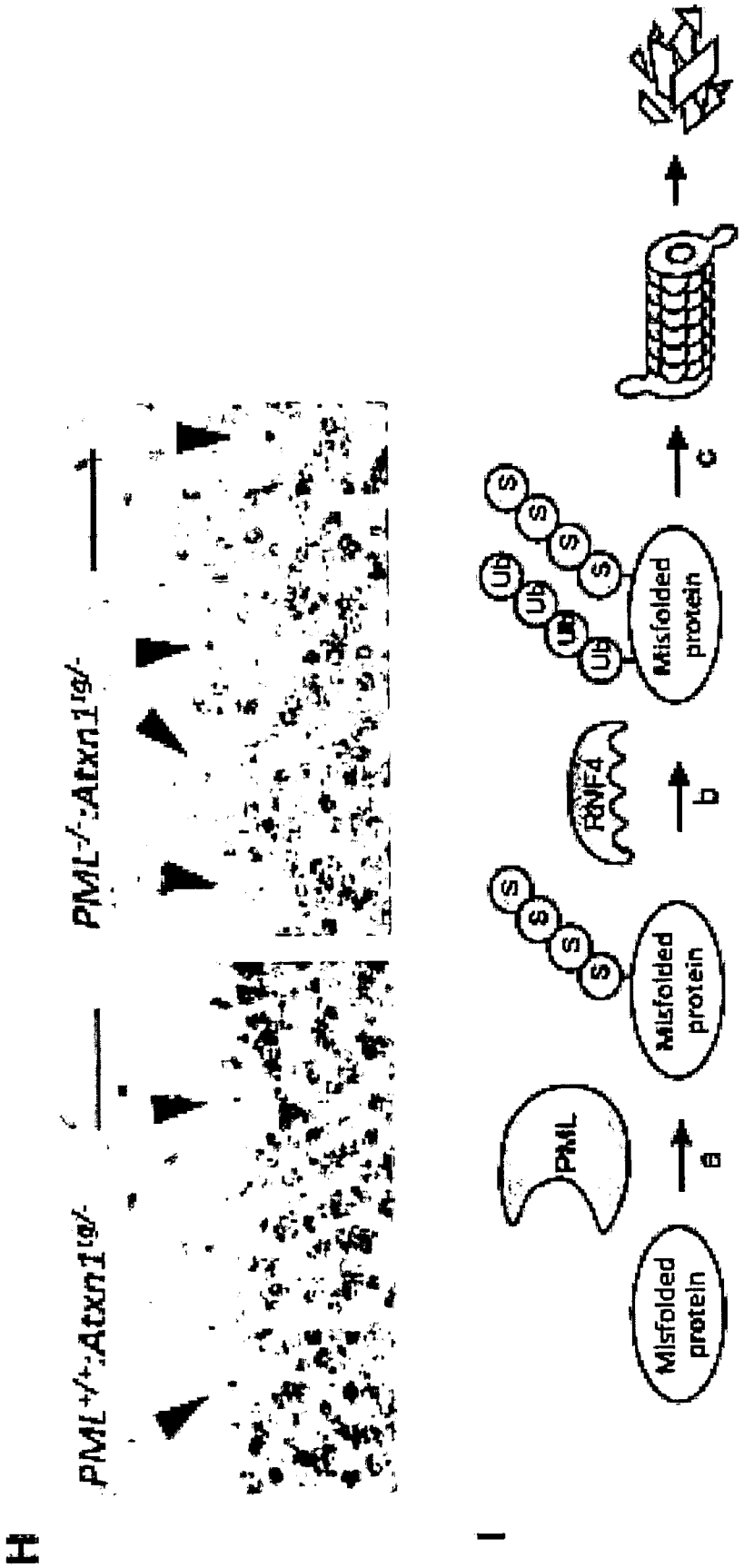


Figure 7H – Figure 7I

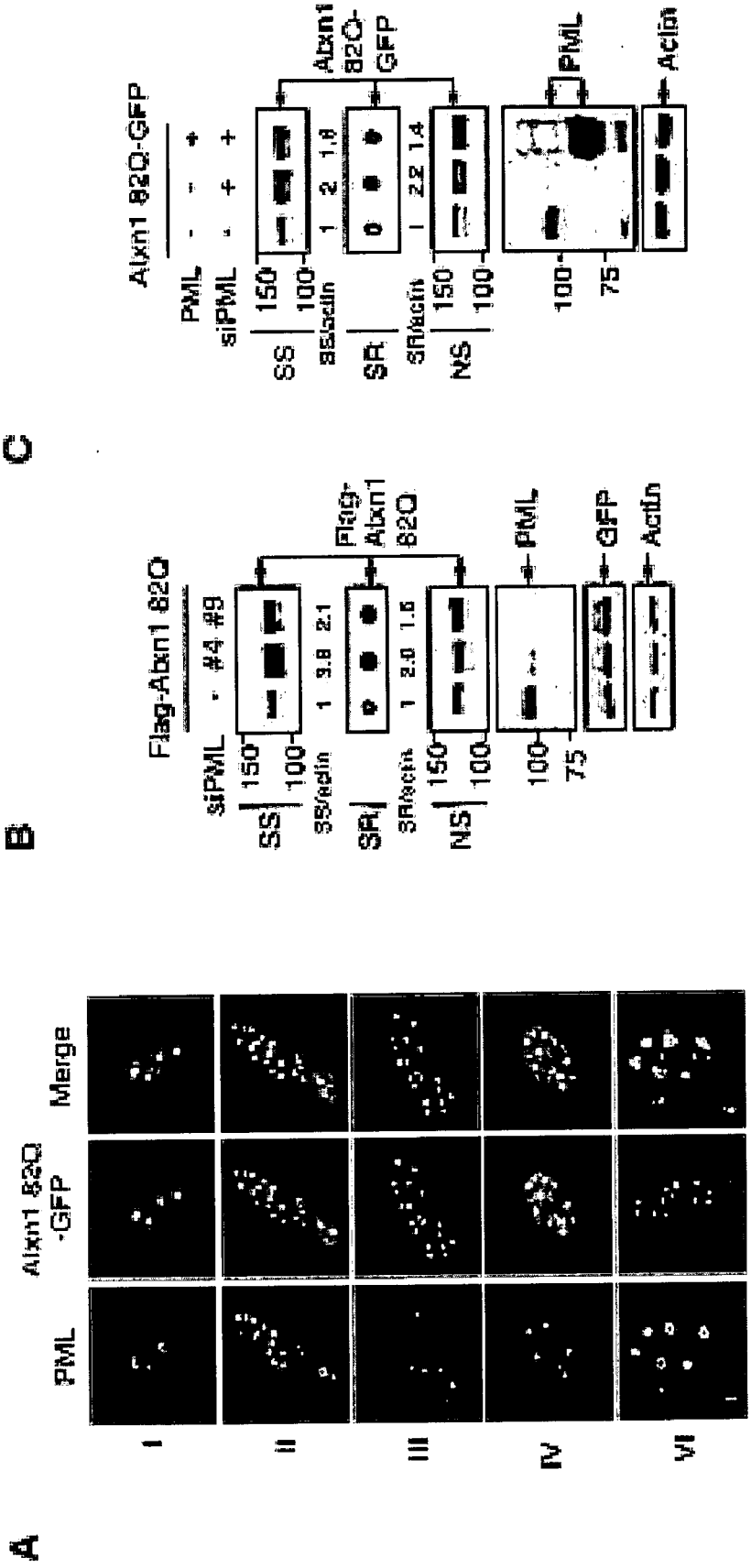


Figure 8A – Figure 8C

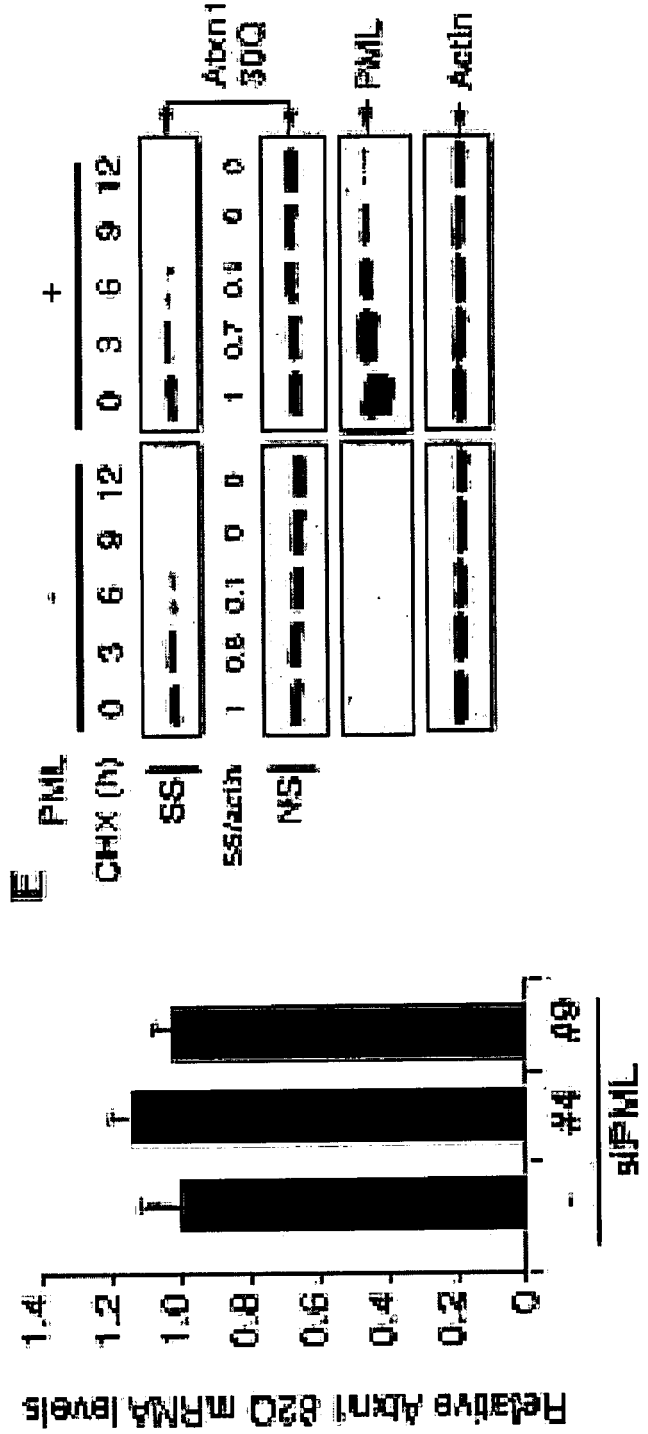


Figure 8D – Figure 8E

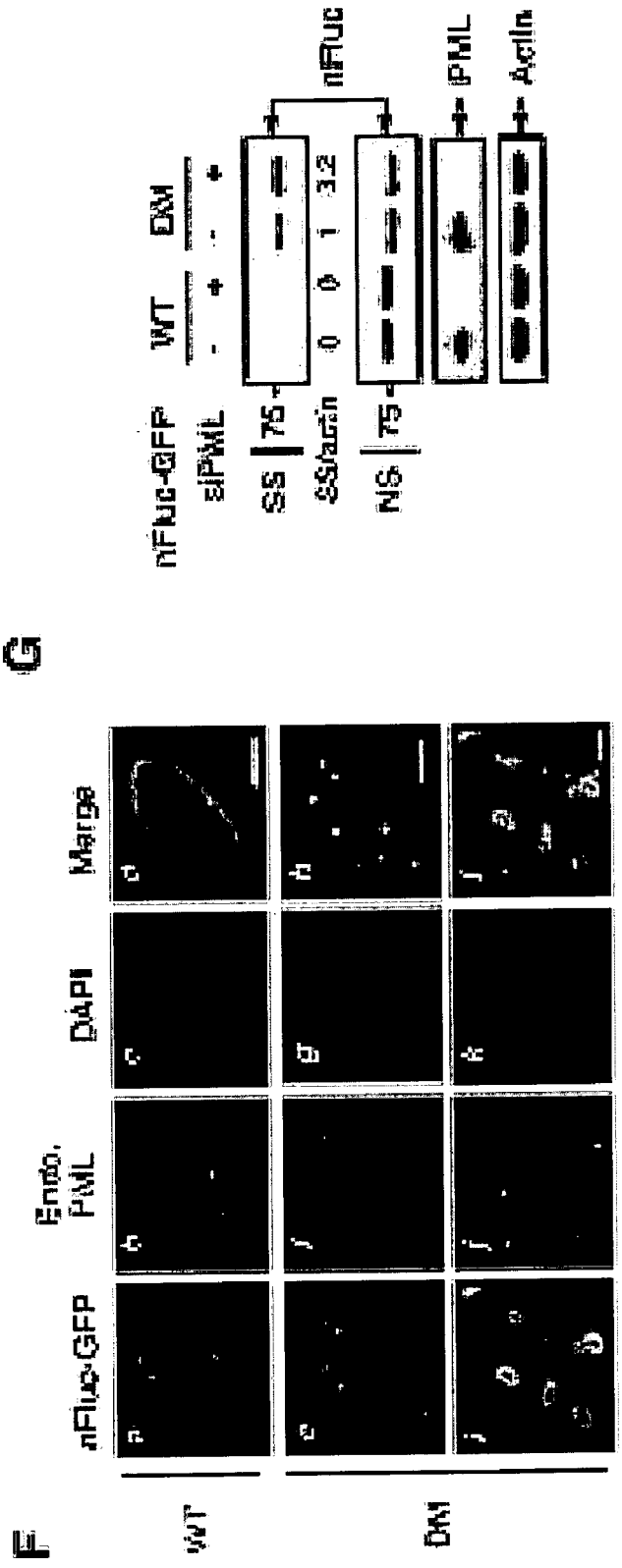


Figure 8F – Figure 8G

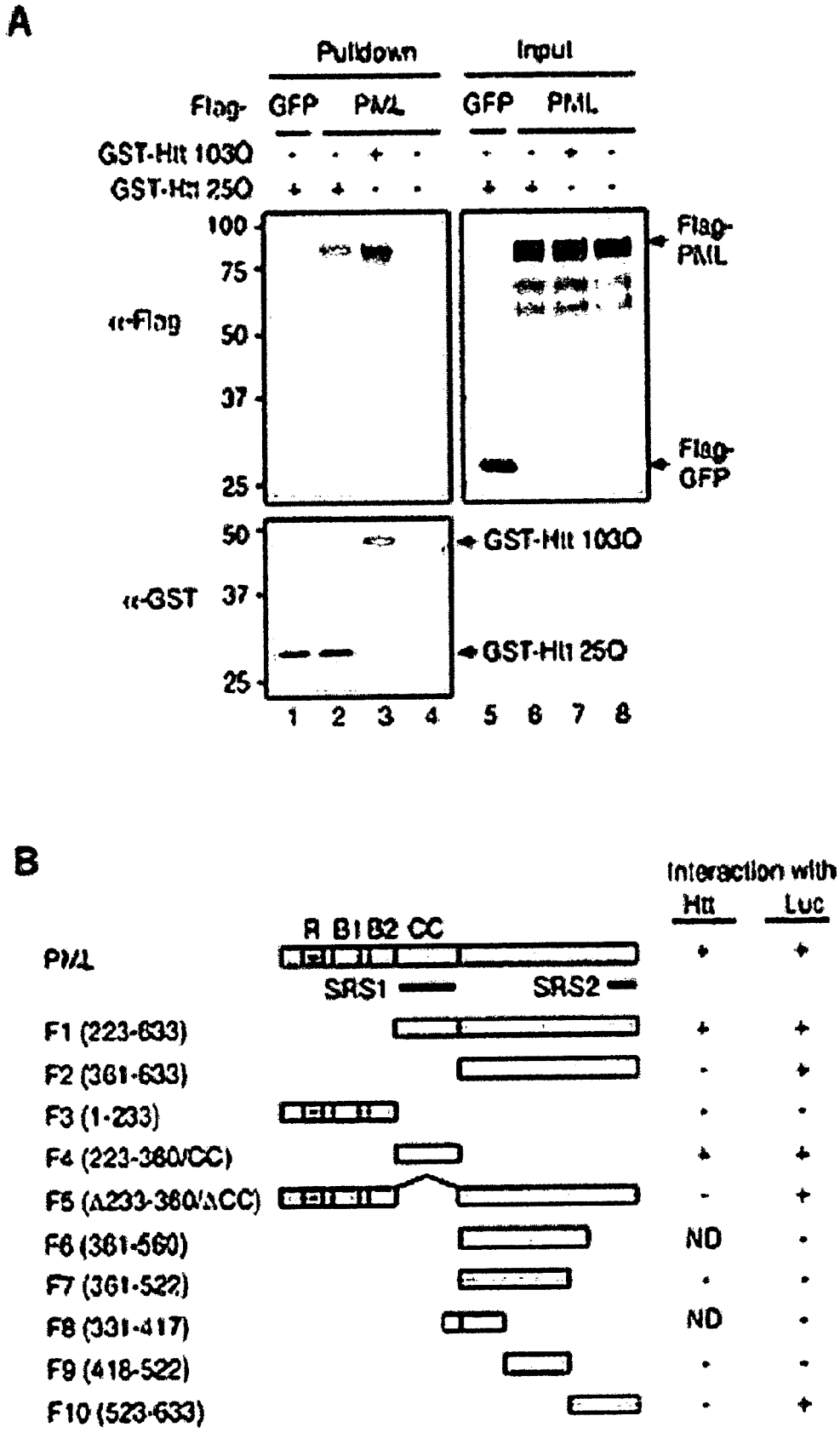


Figure 9A – Figure 9B

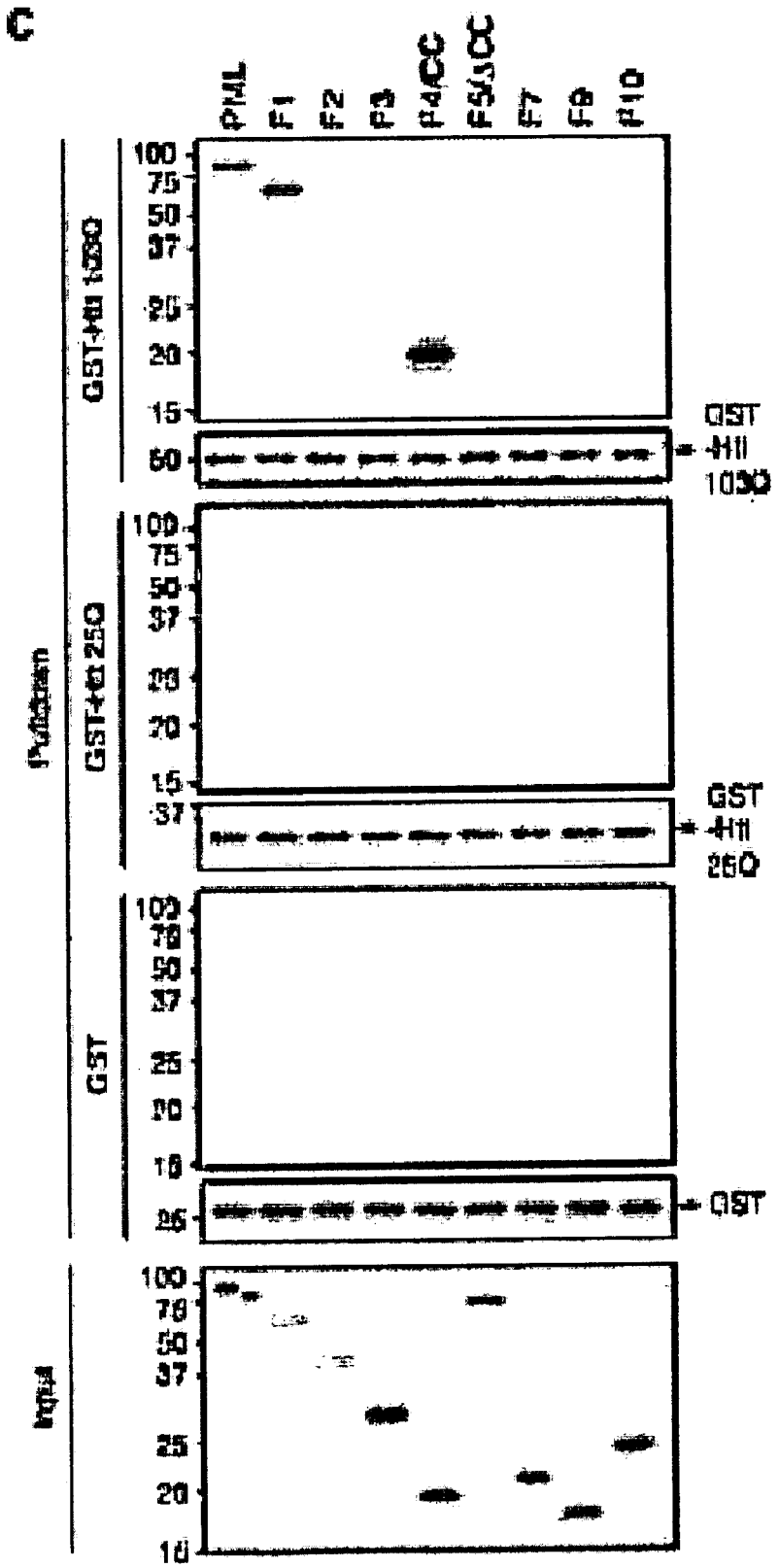

C
PML
F1
F2
F3
P4CC
F5ΔCC
F7
F8
F10
Pulldown
GST-Hil 1030
GST-Hil 250
GST
Input
100
75
50
37
25
20
15
GST-Hil 1030
GST-Hil 250
GST

Figure 9C

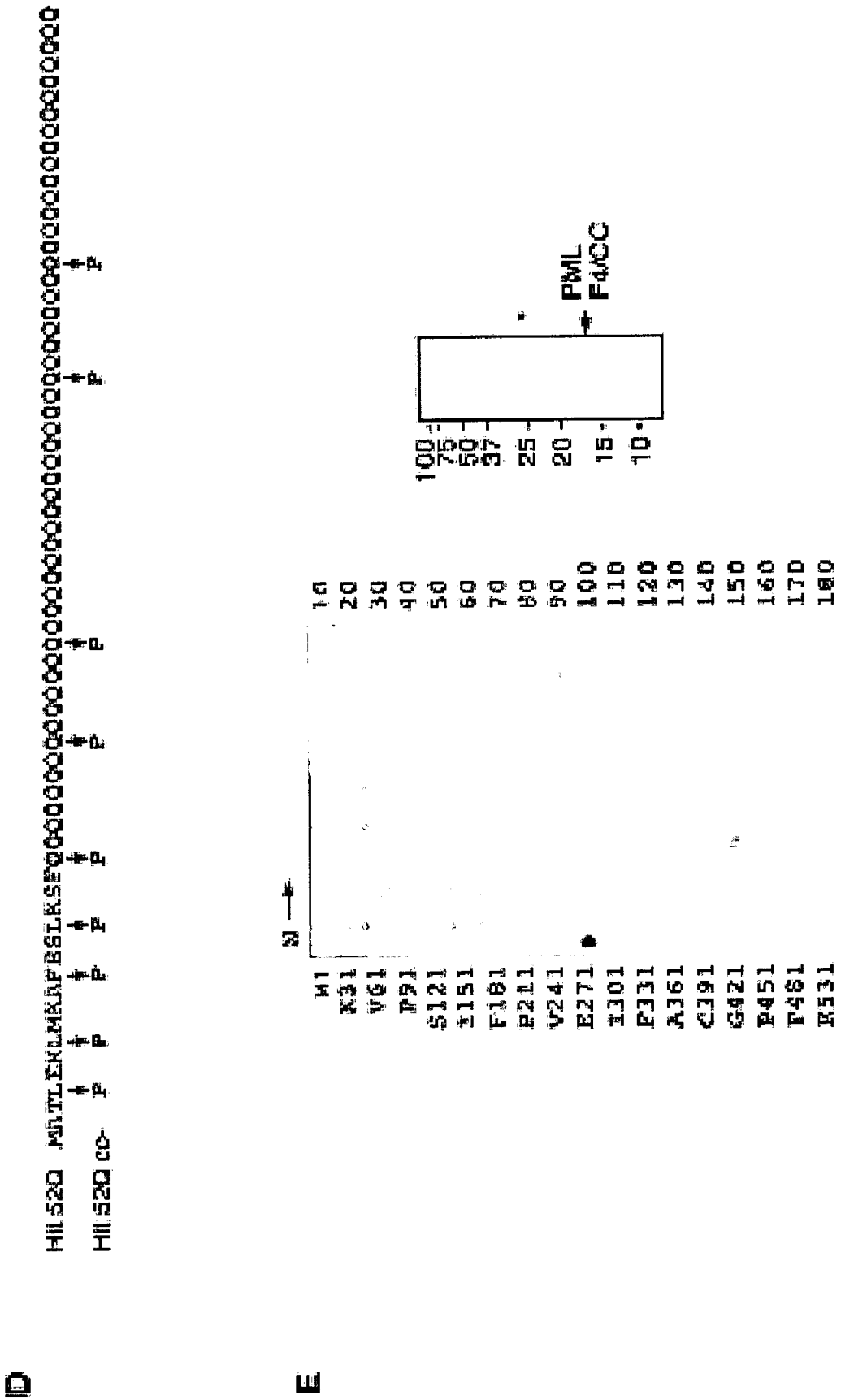


Figure 9D – Figure 9E

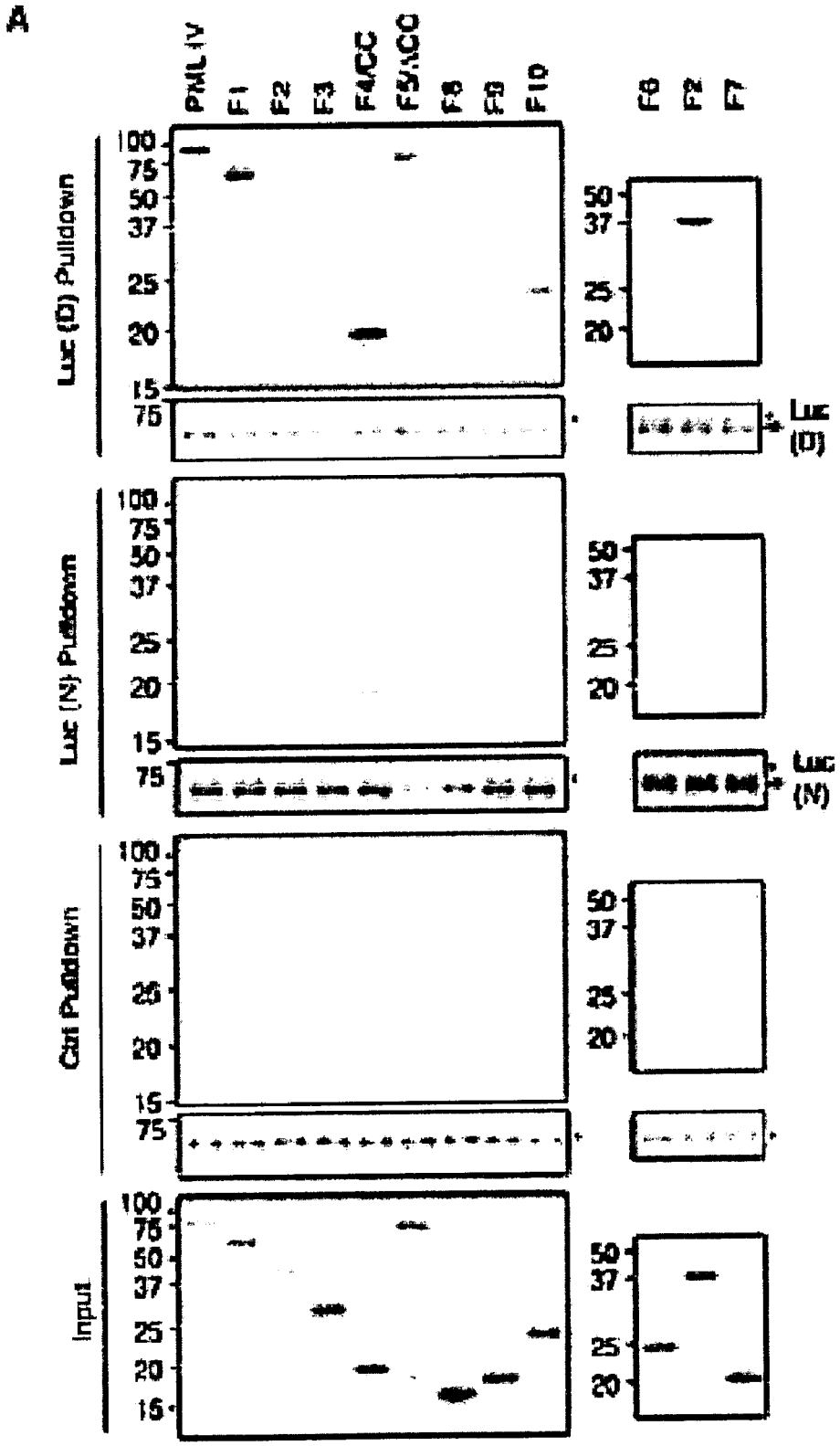

A
PML IV
F1
F2
F3
F4CC
F5NCC
F6
F9
F10
F6
F2
F7
Luc (D) Pulldown
Luc (N) Pulldown
Ctrl Pulldown
Input
100
75
50
37
25
20
15
Luc (D)
Luc (N)

Figure 10A

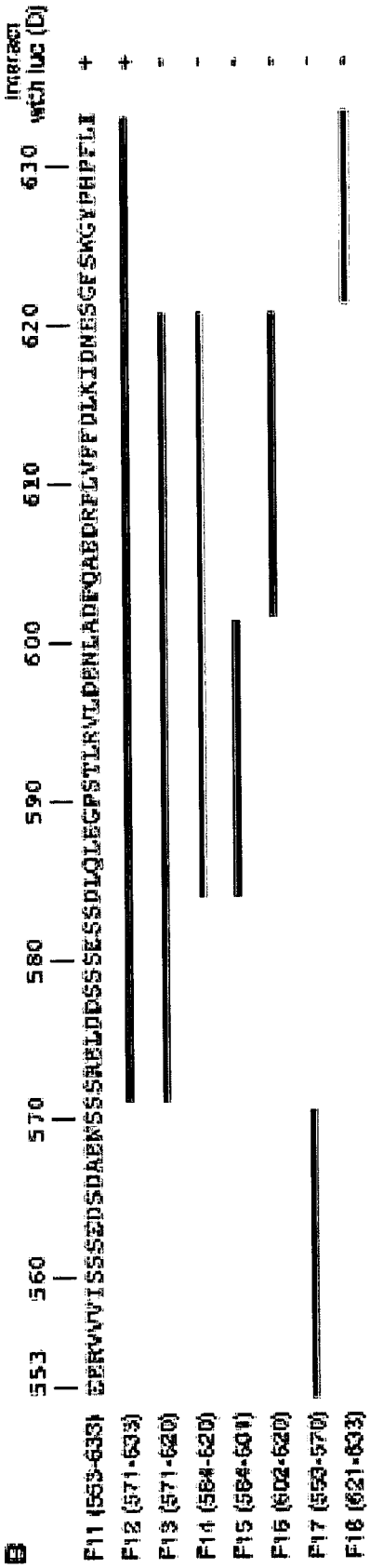

B
553
560
570
580
590
600
610
620
630
Interact with luc (D)
F11 (553-633)
F12 (571-633)
F13 (571-620)
F14 (584-620)
F15 (584-601)
F16 (602-620)
F17 (553-570)
F18 (621-633)

Figure 10B

C

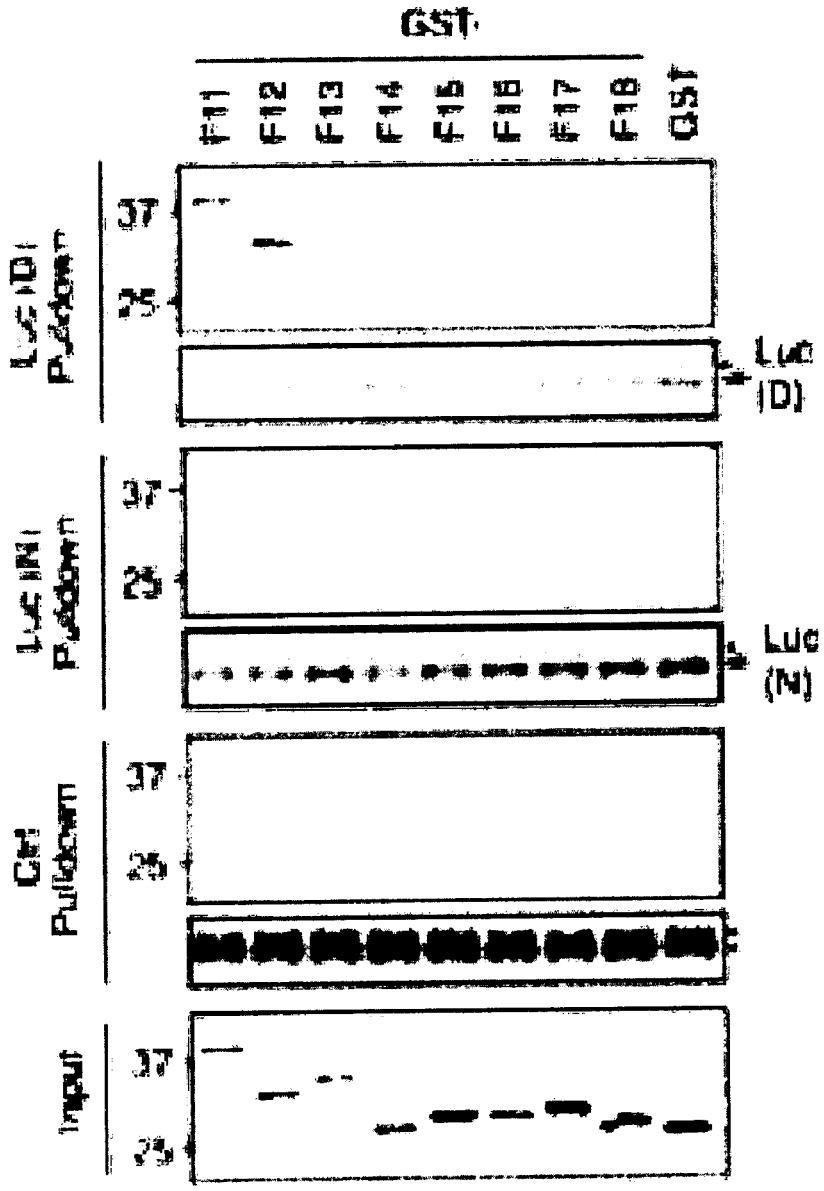

GST
F11
F12
F13
F14
F15
F16
F17
F18
GST
37
25
Luc (D)
Luc (N)
Ctrl
Pulldown
Input

Figure 10C

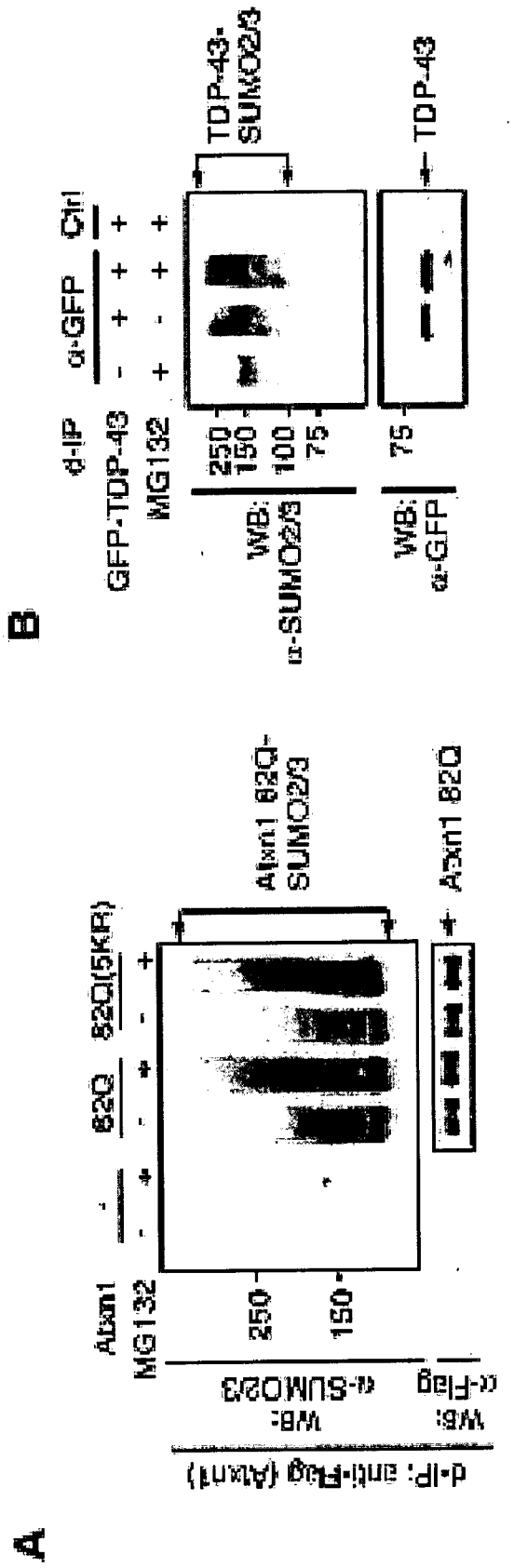


Figure 11A – Figure 11B

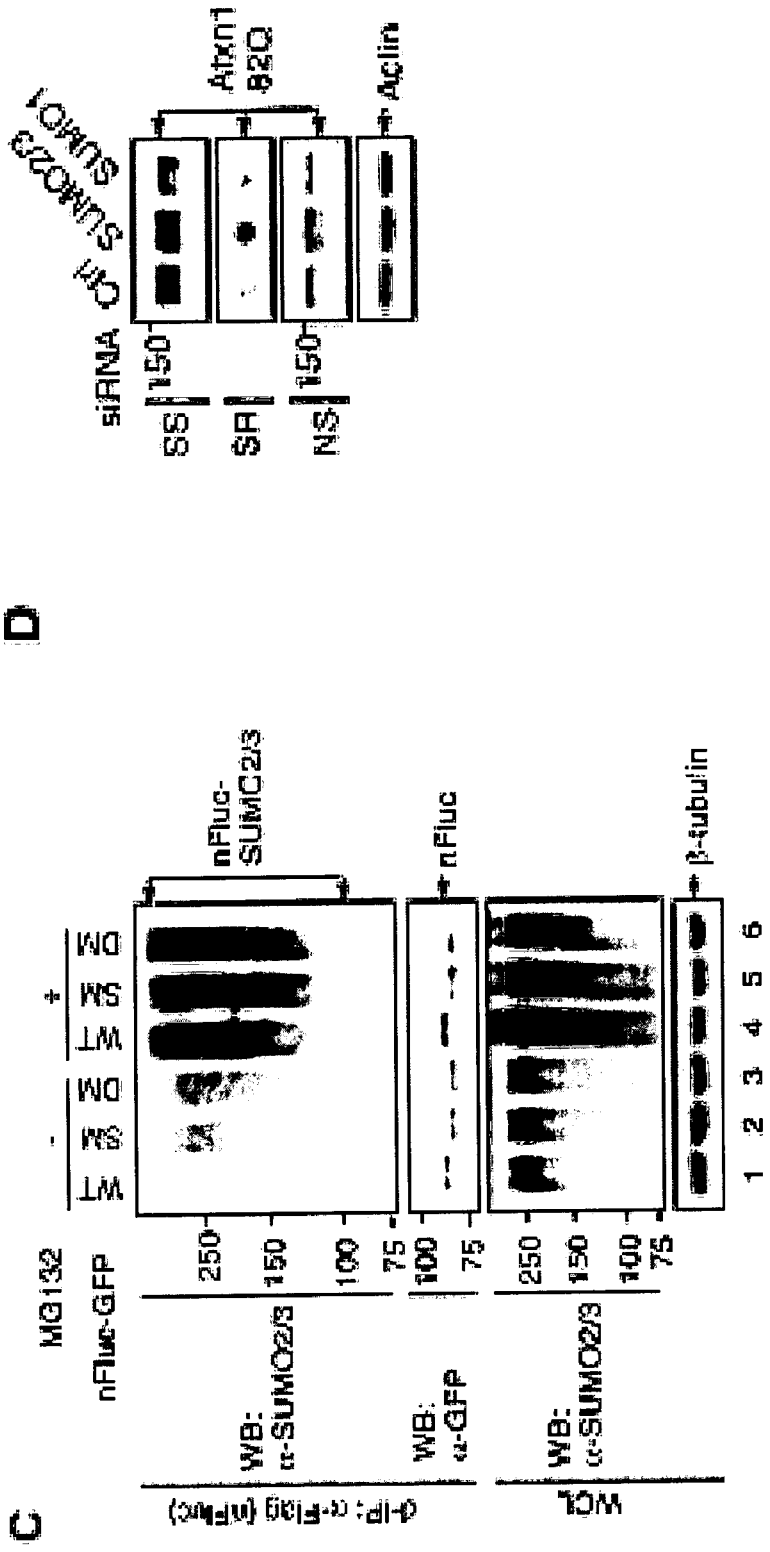


Figure 11C – Figure 11D

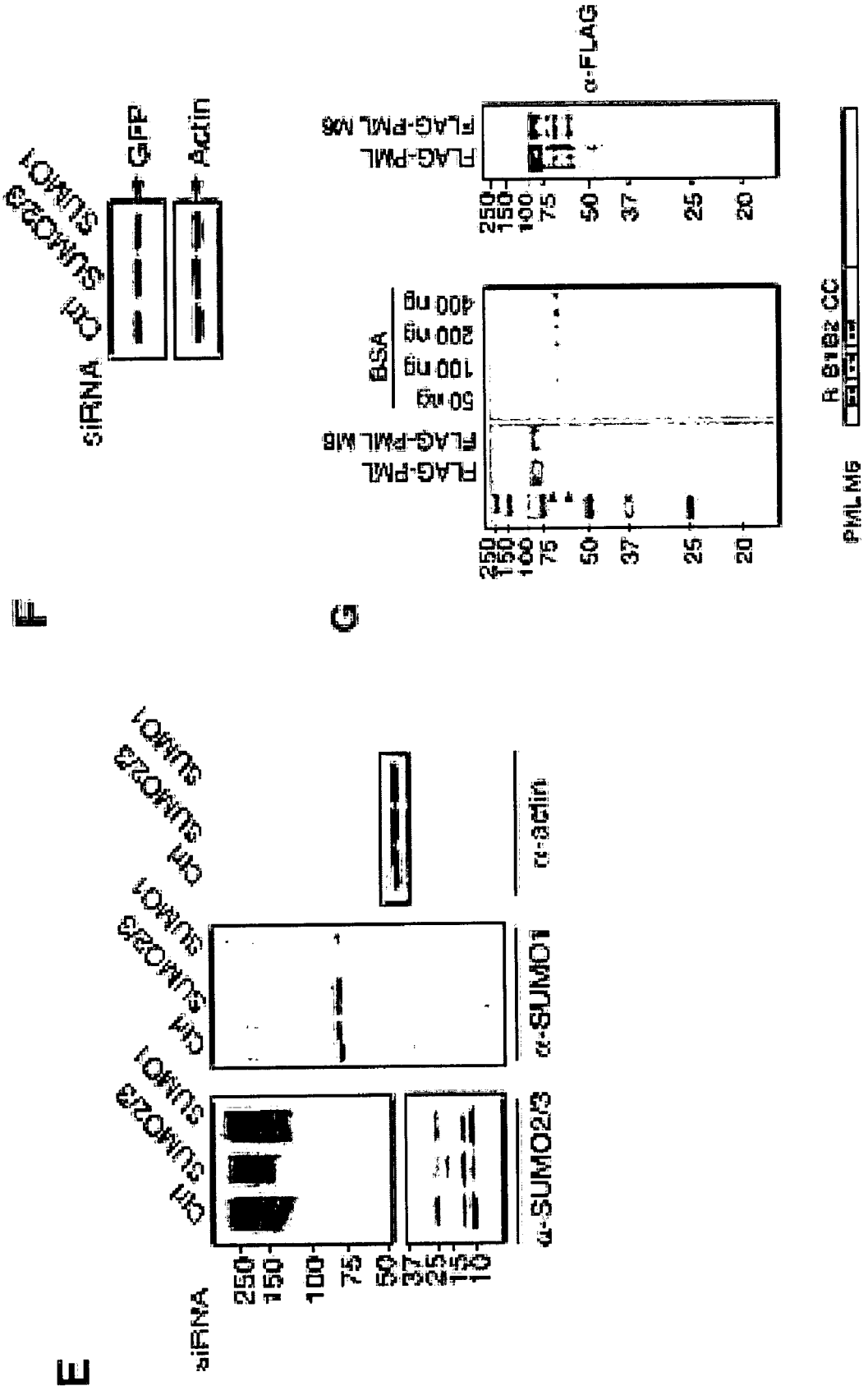


Figure 11E – Figure 11G

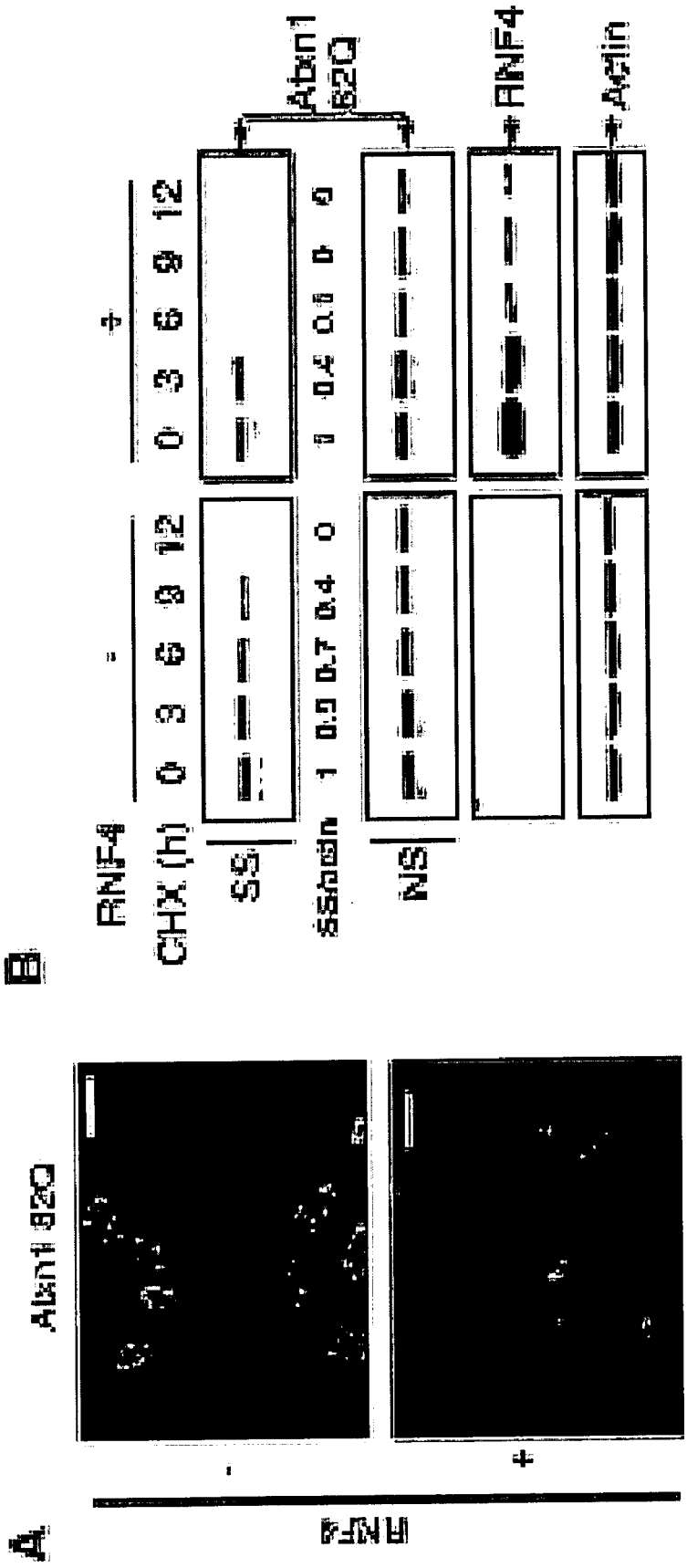
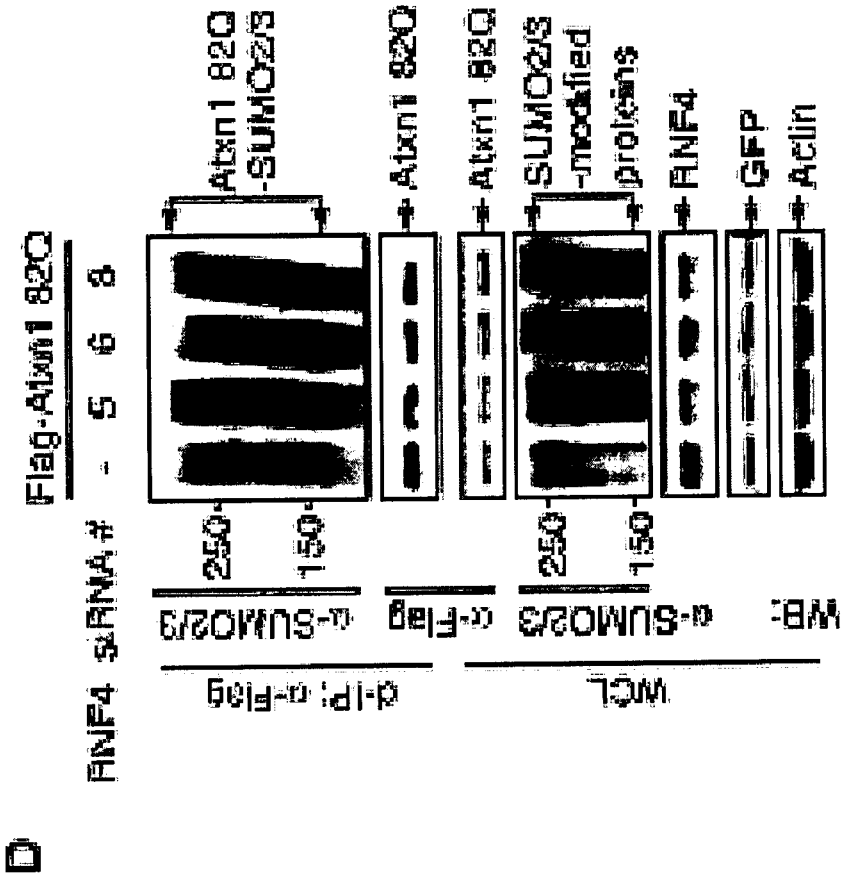


Figure 12A – Figure 12B



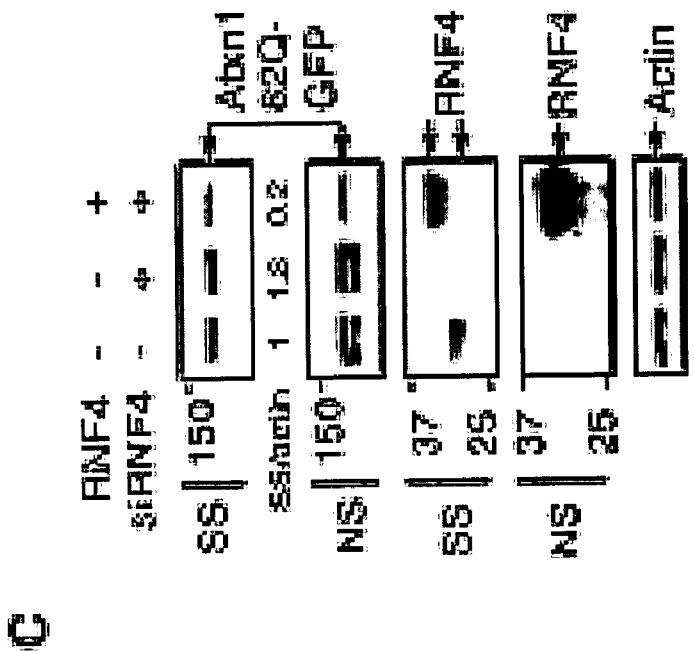


Figure 12C – Figure 12D

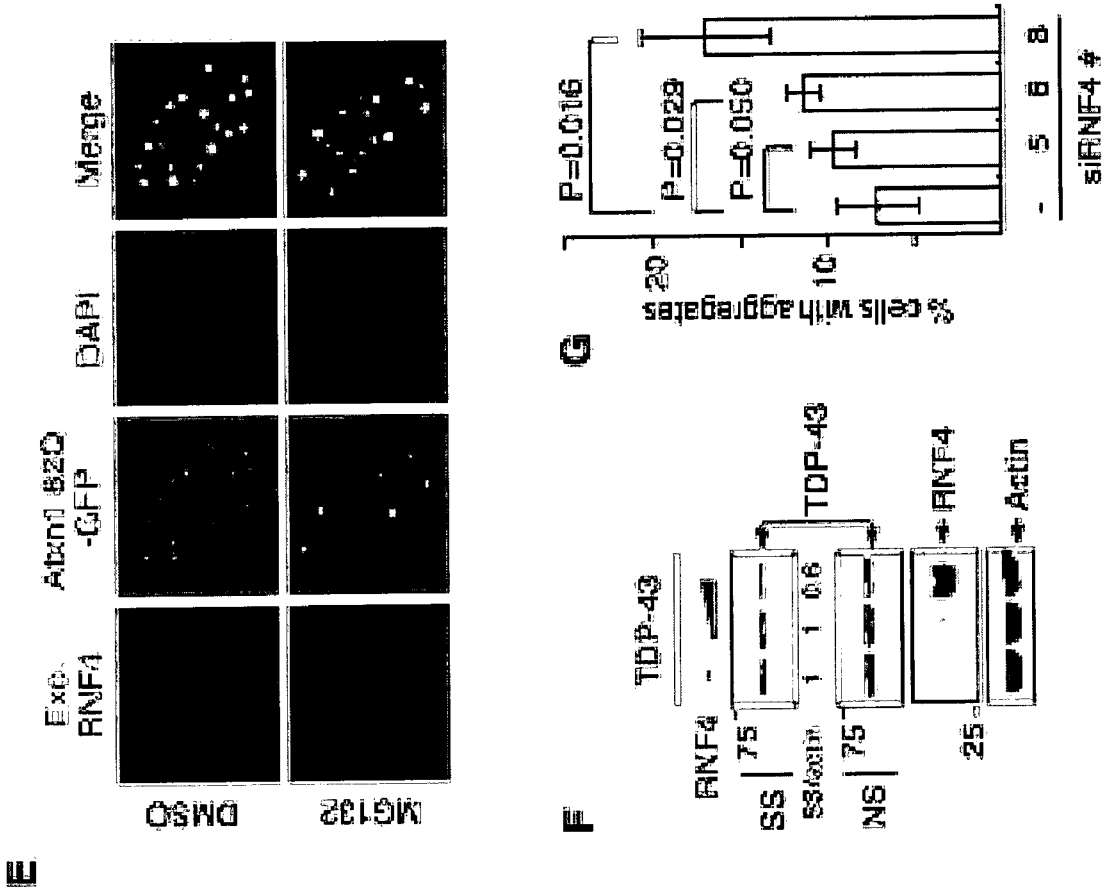


Figure 12E – Figure 12G

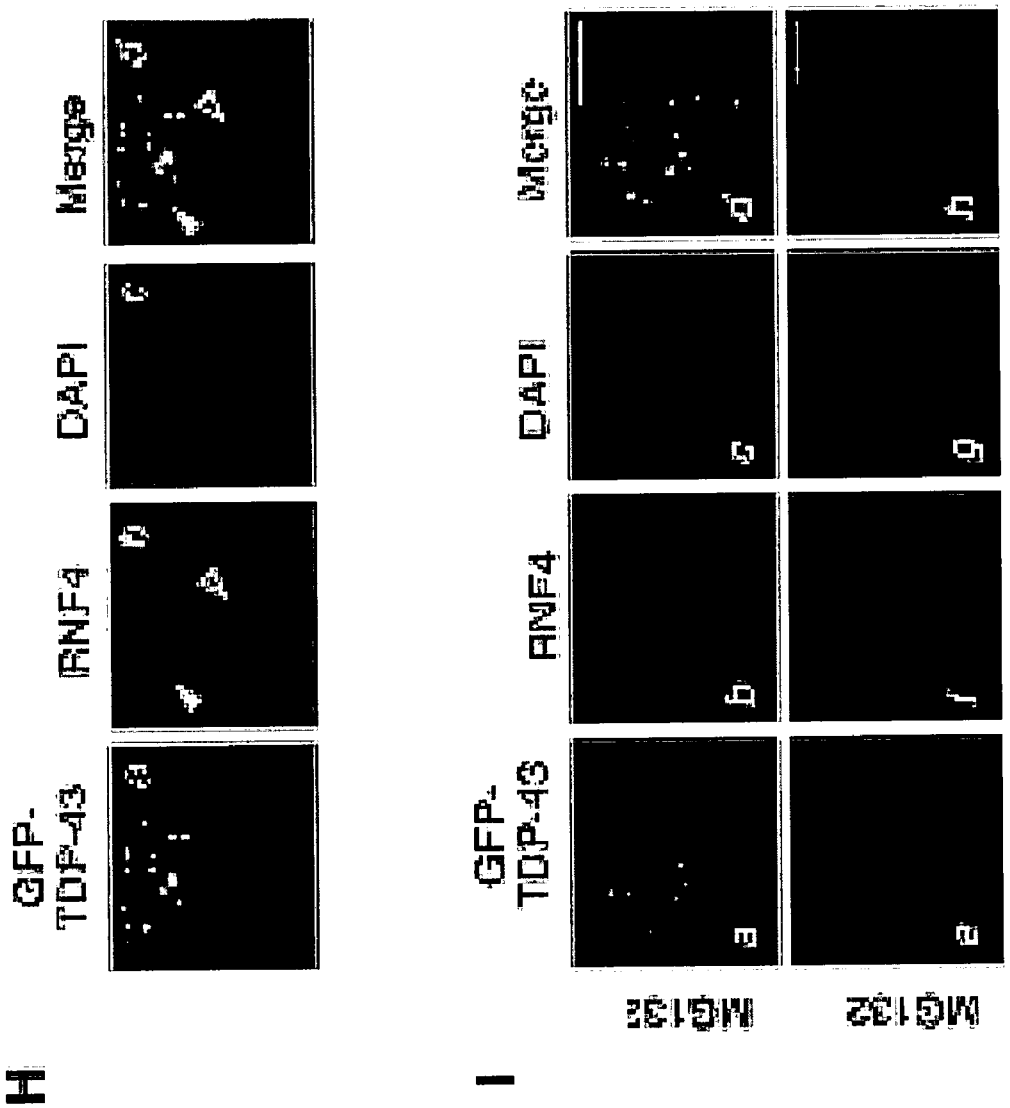


Figure 12H – Figure 12I

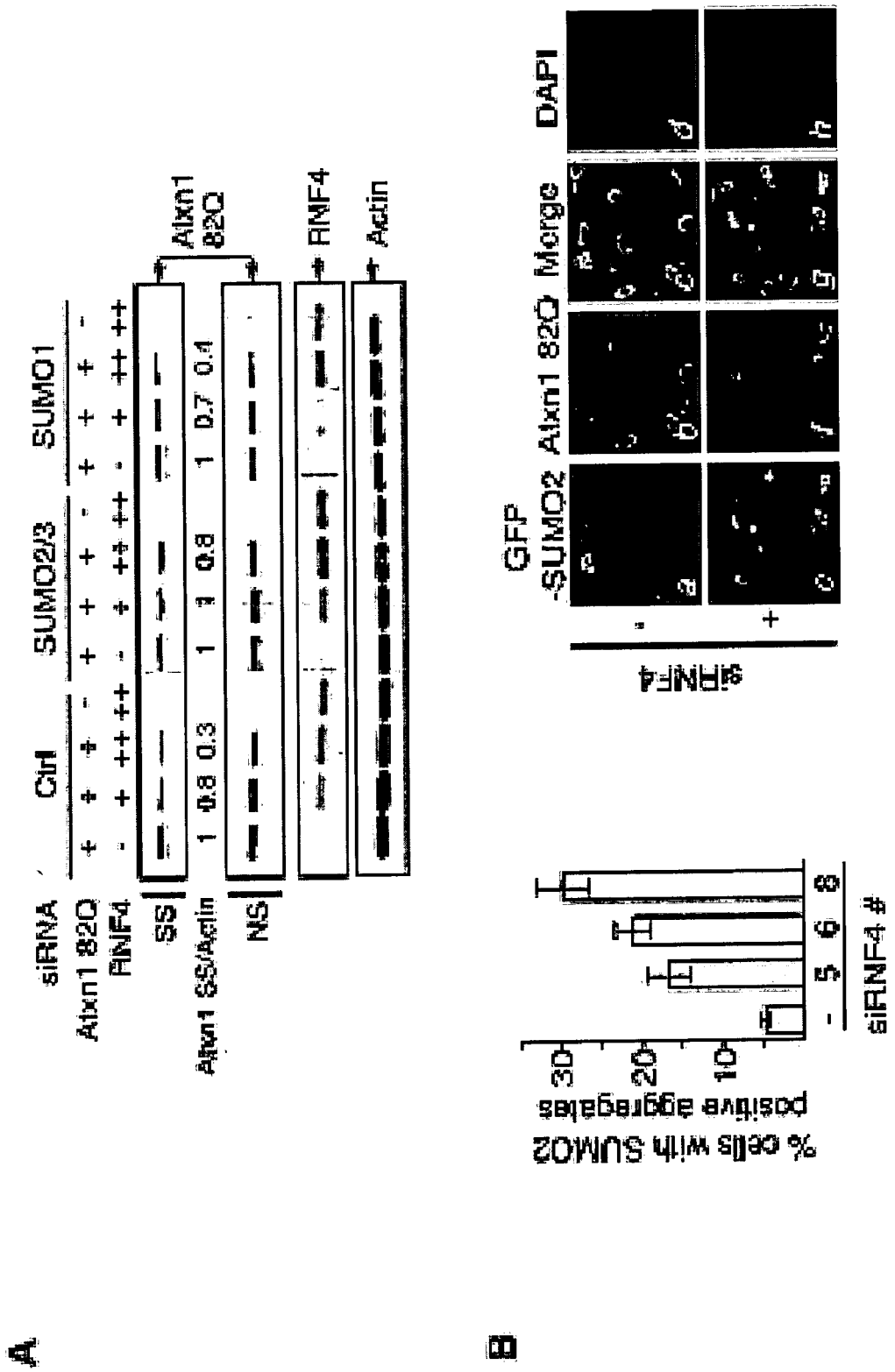


Figure 13A – Figure 13B

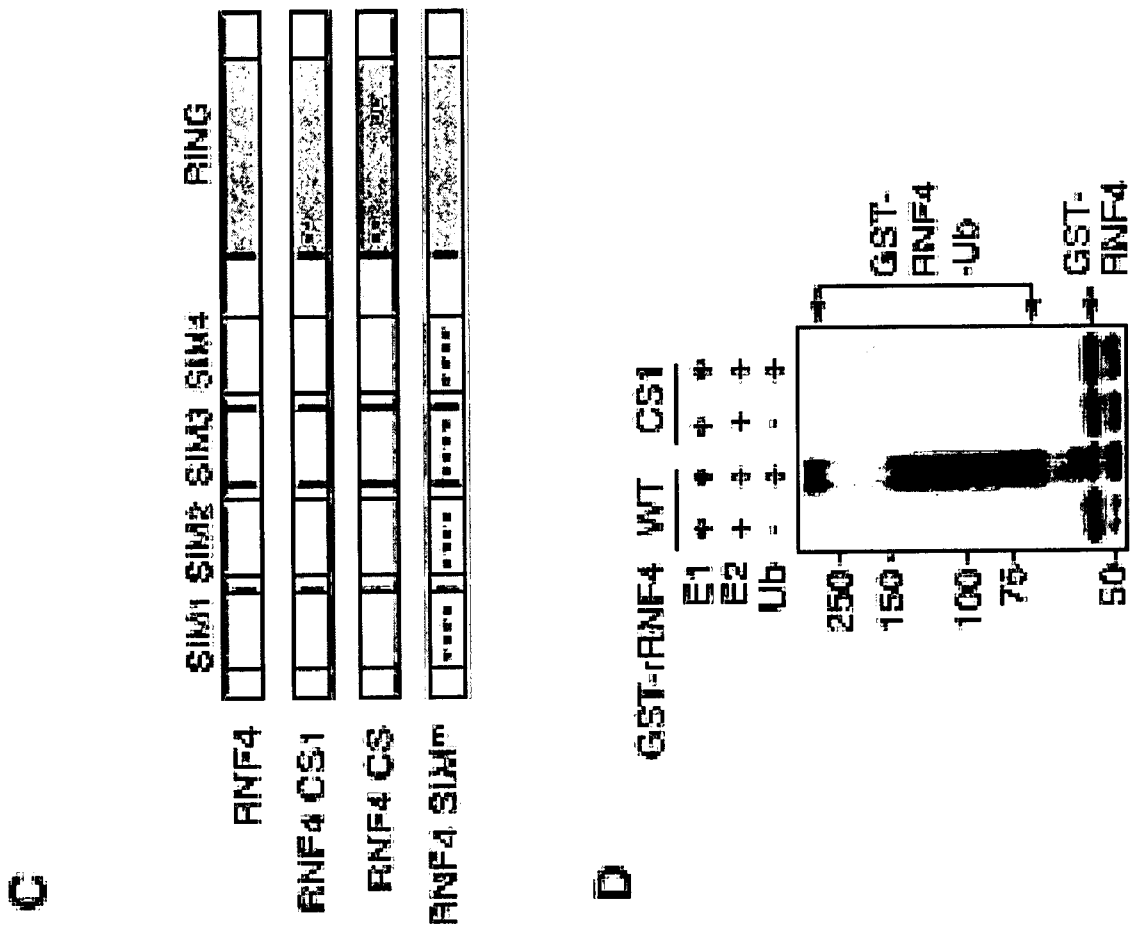
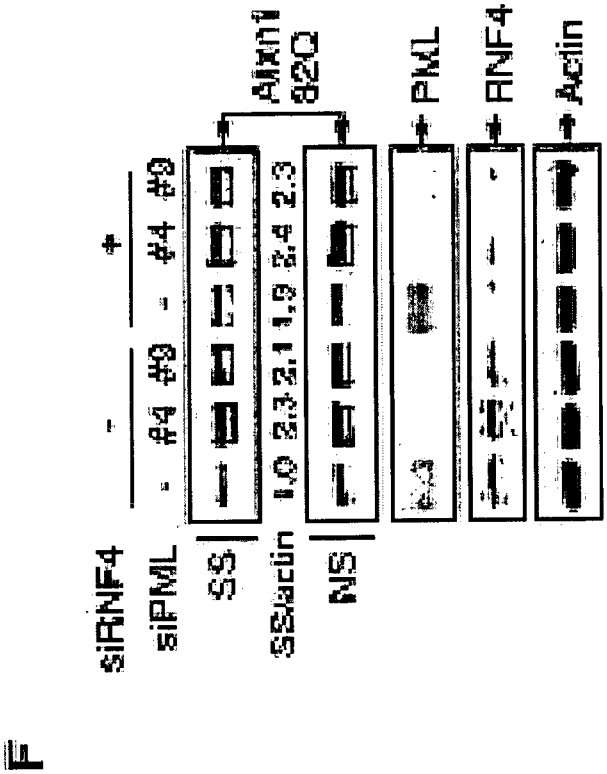


Figure 13C – Figure 13D



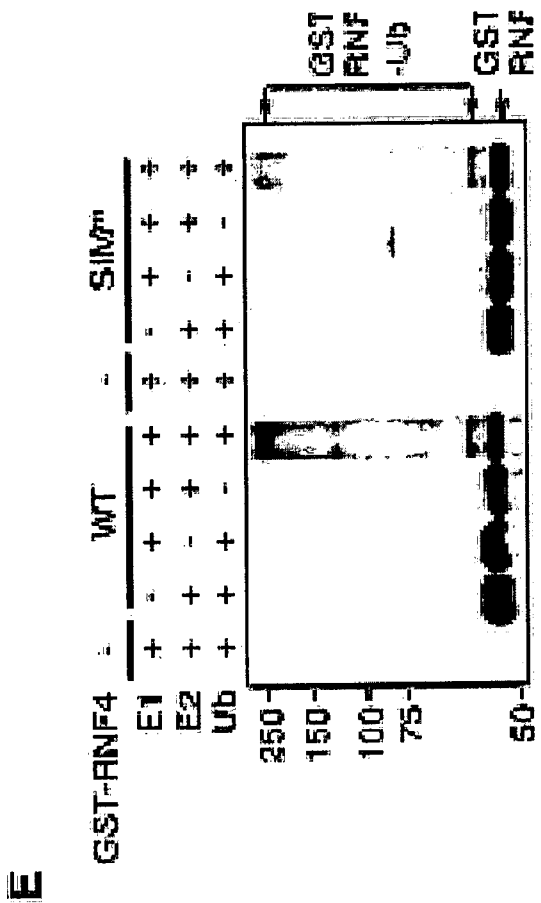


Figure 13E – Figure 13F

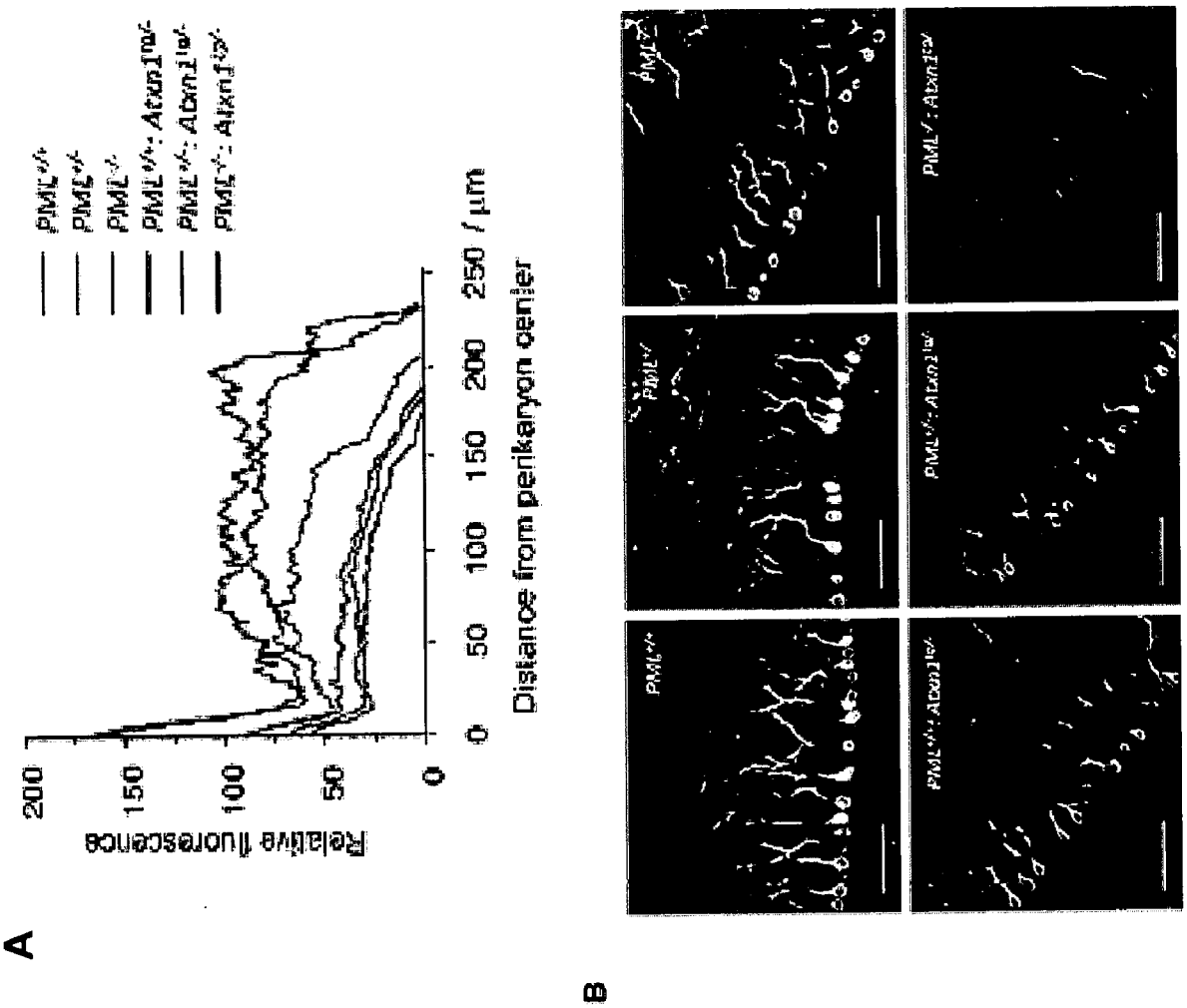


Figure 14A – Figure 14B

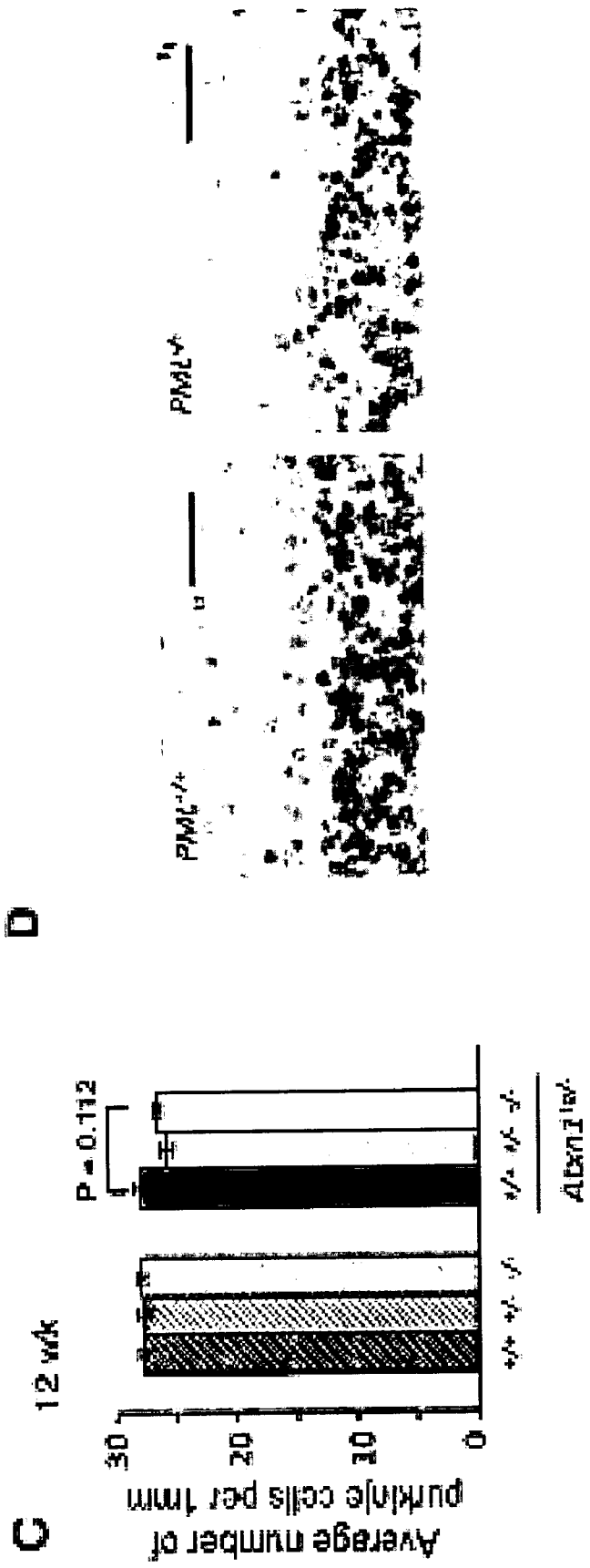


Figure 14C – Figure 14D

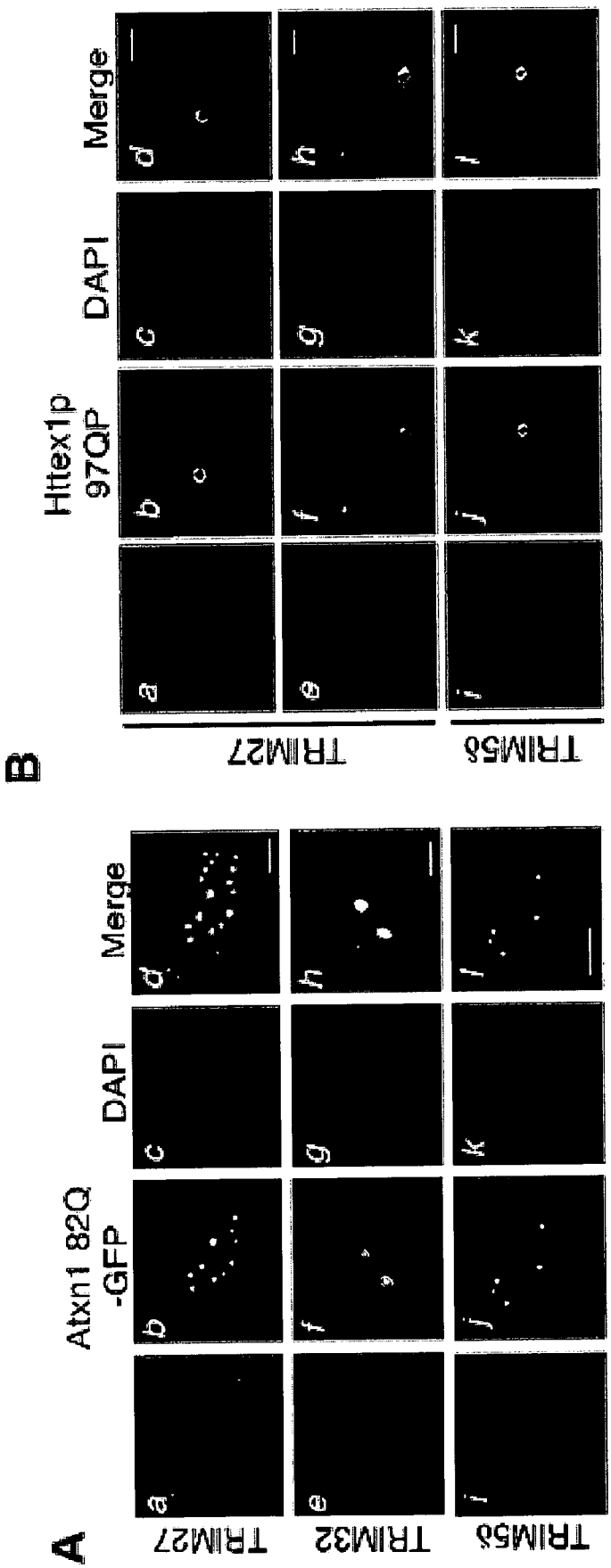


Figure 15A – Figure 15B

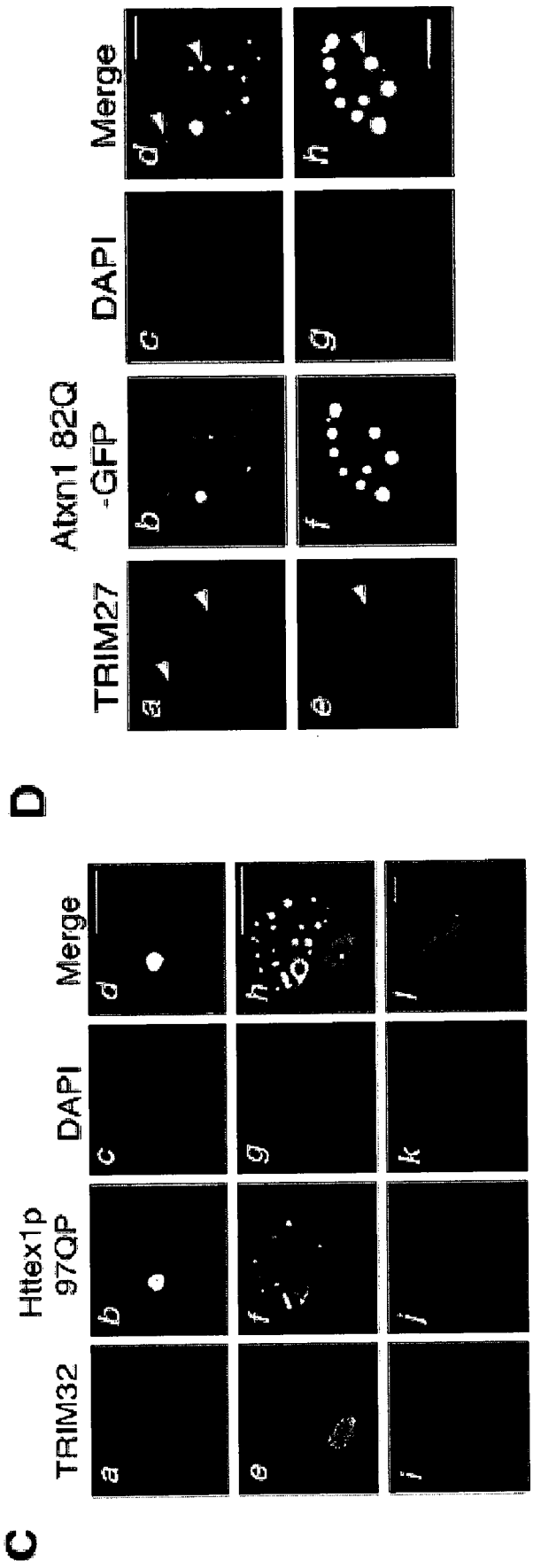
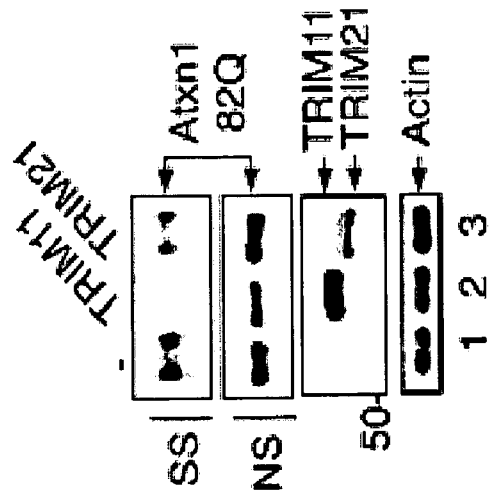


Figure 15C – Figure 15D


TRIM11
TRIM21
-
Atxn1
82Q
TRIM11
TRIM21
Actin
SS
NS
50
1 2 3

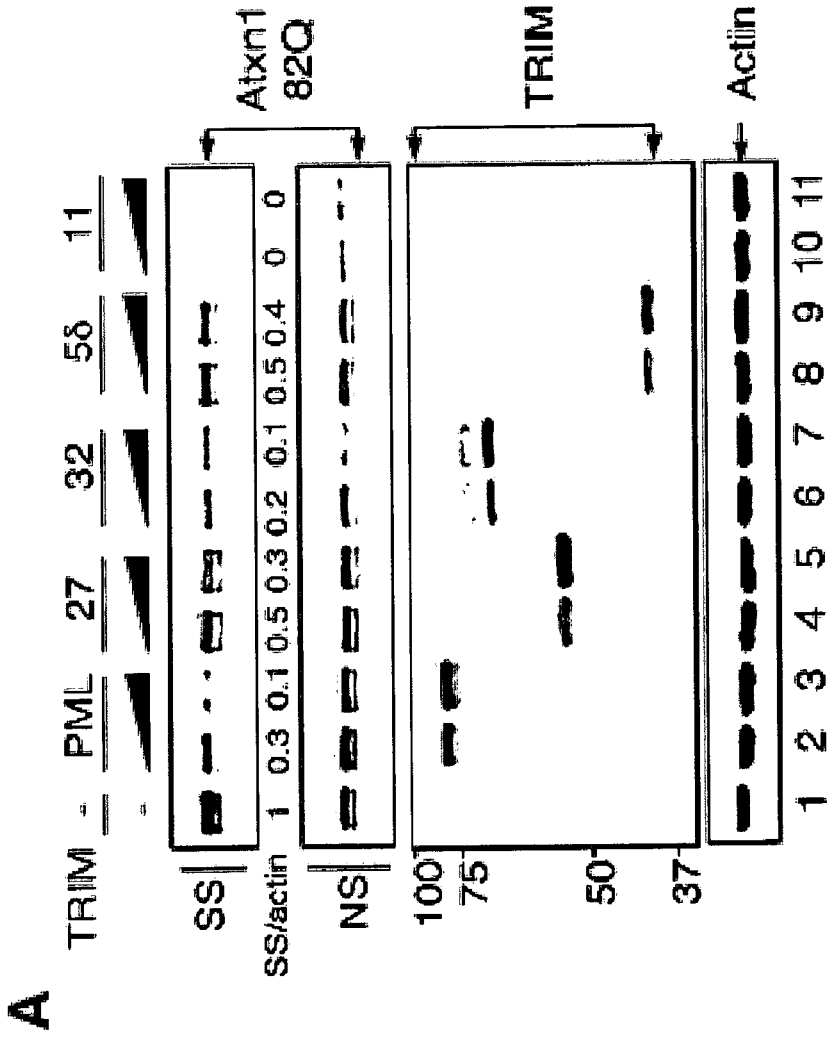

A
TRIM - PML 27 32 5δ 11
SS
SS/actin 1 0.3 0.1 0.5 0.3 0.2 0.1 0.5 0.4 0 0
NS
Atxn1
82Q
TRIM
Actin
100
75
50
37
1 2 3 4 5 6 7 8 9 10 11

Figure 16A

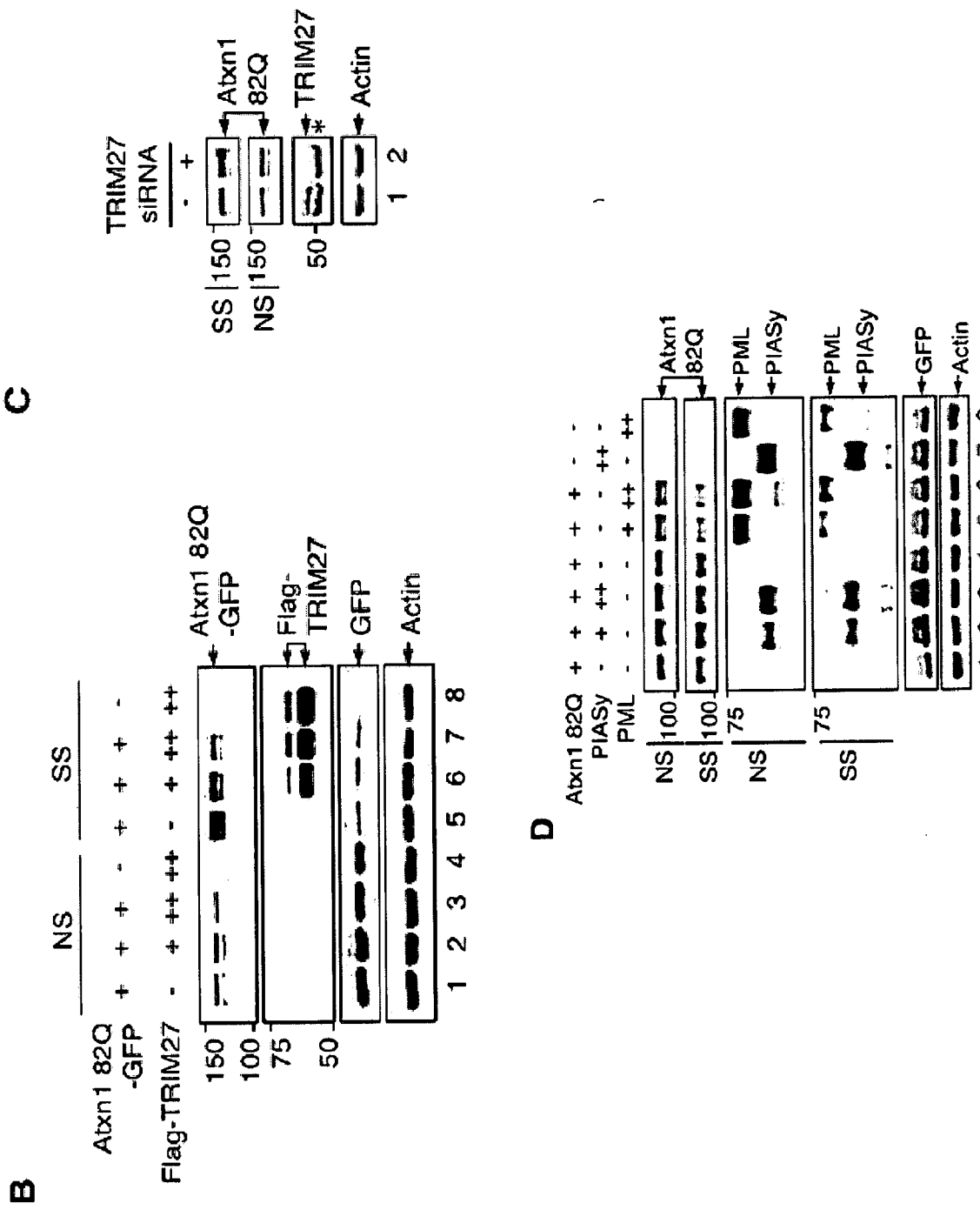


Figure 16B – Figure 16D

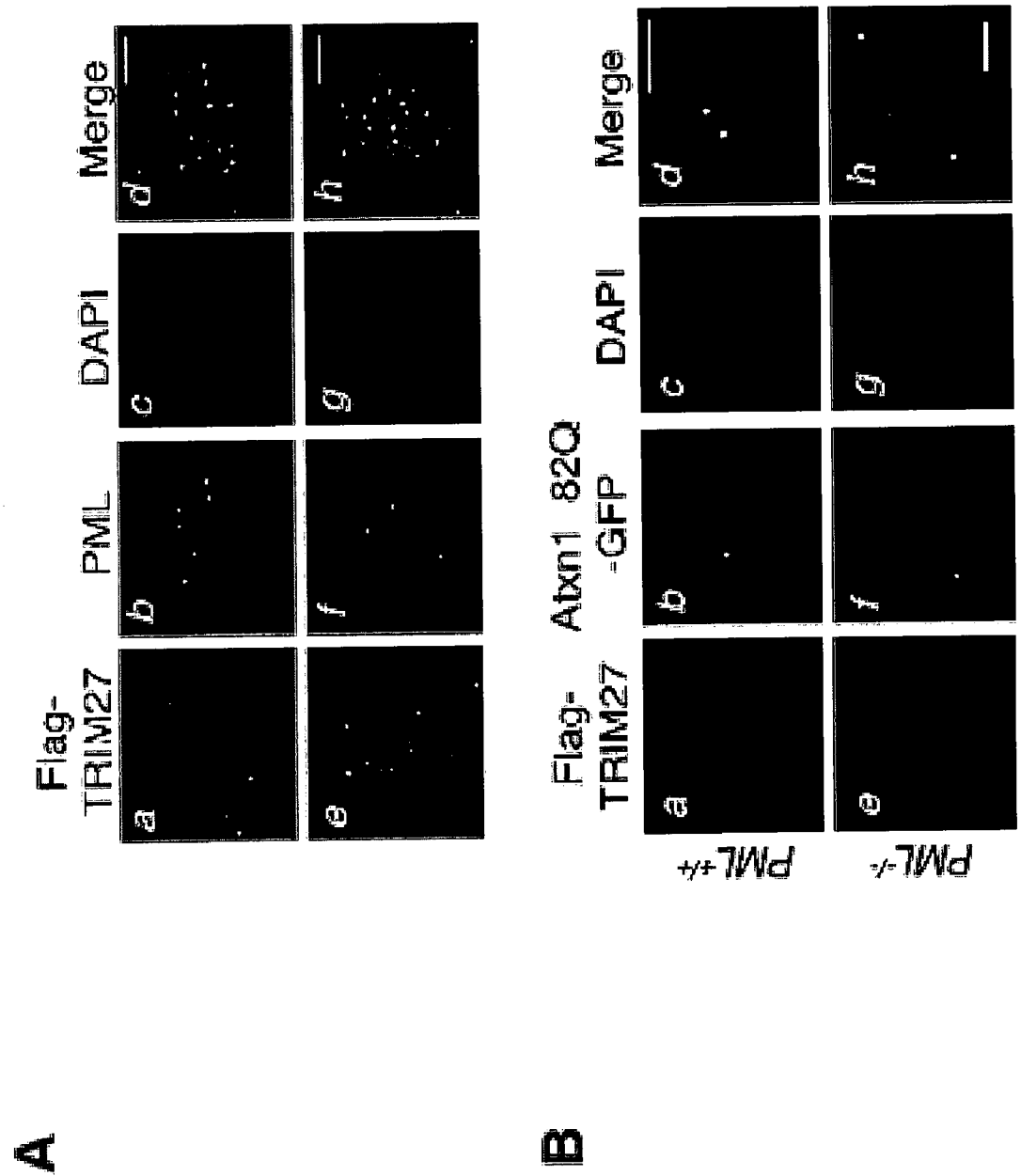


Figure 17A – Figure 17B

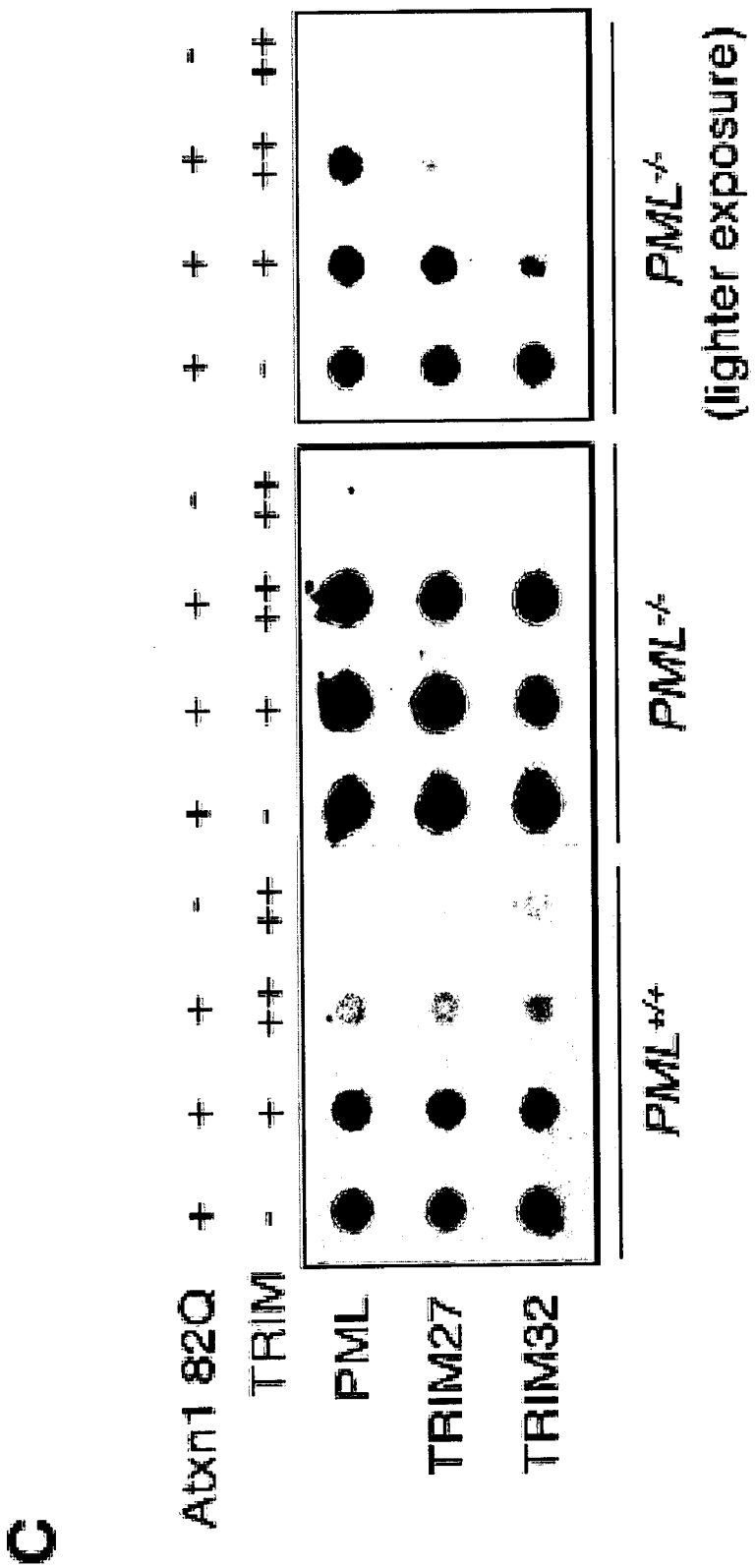

C
Atxn1 82Q
TRIM
PML
TRIM27
TRIM32
PML+/+
PML-/-
PML-/-
(lighter exposure)

Figure 17C

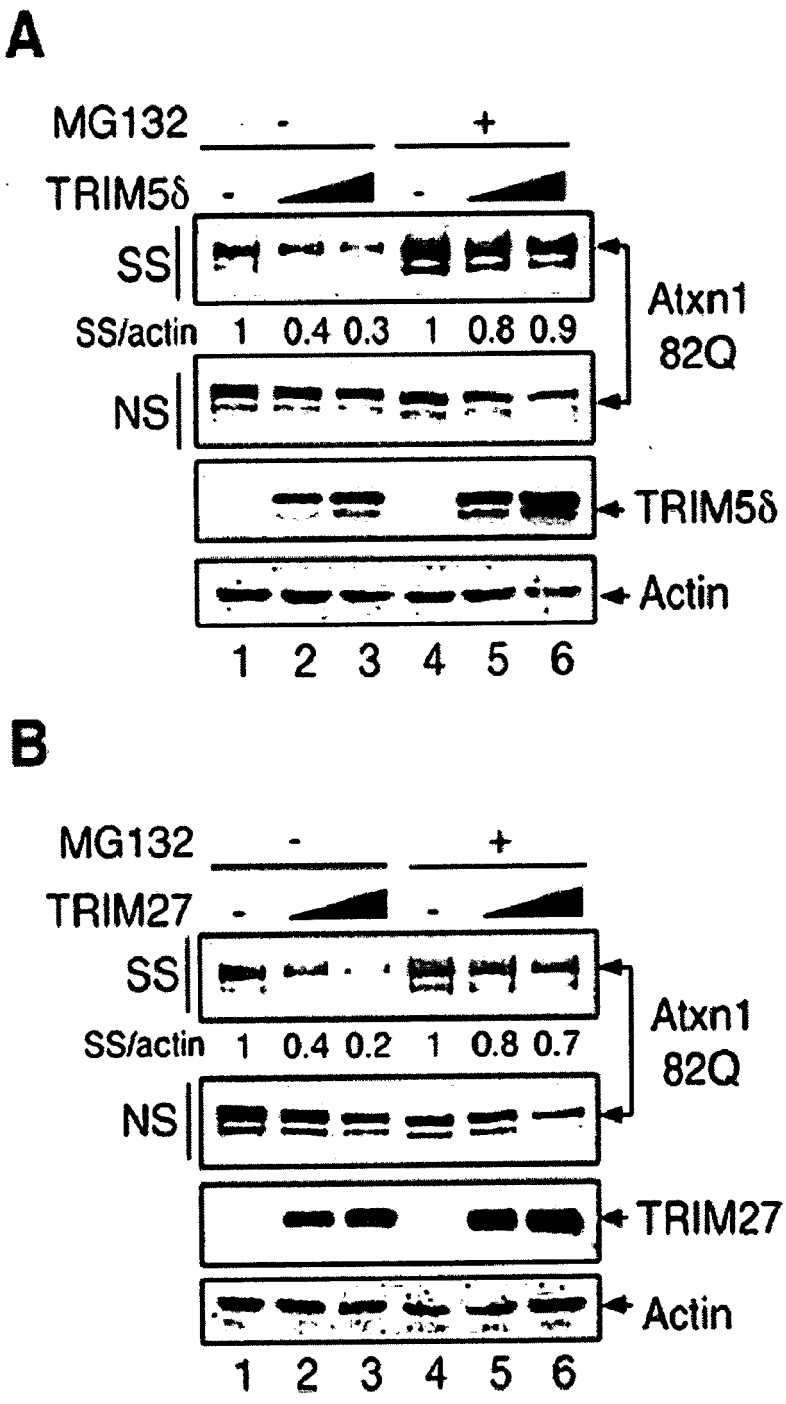
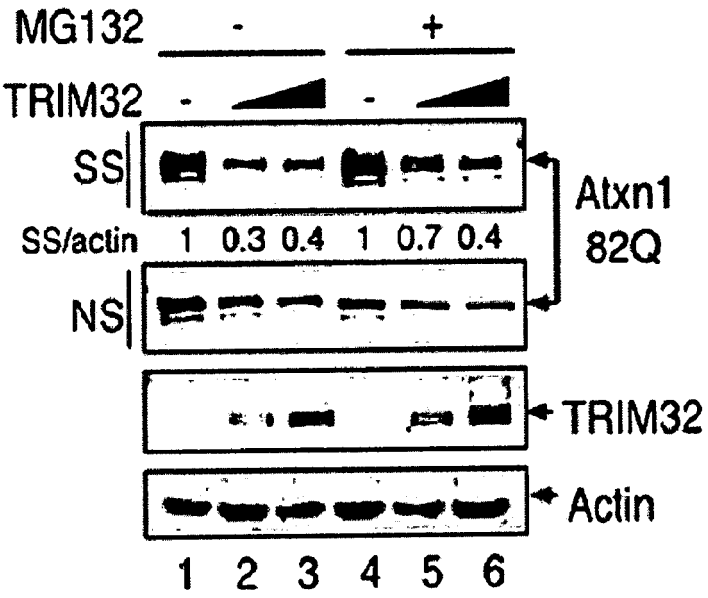


Figure 18A – Figure 18B

C



D

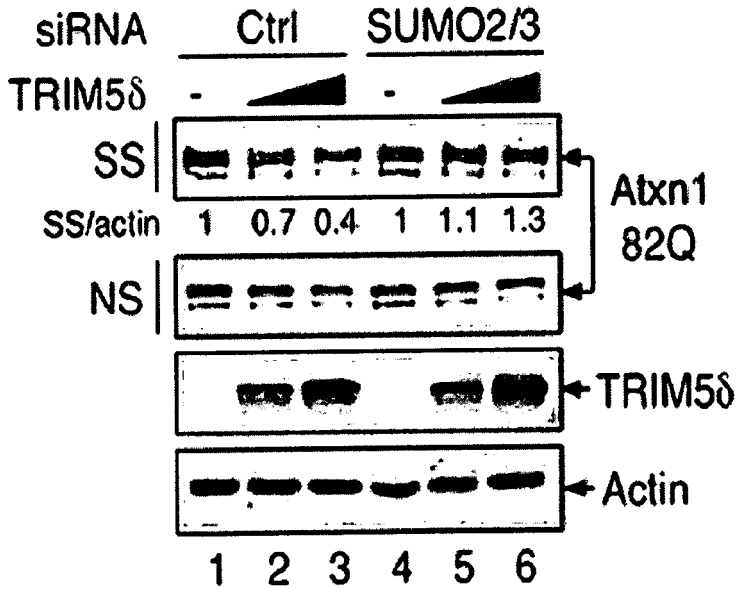


Figure 18C – Figure 18D

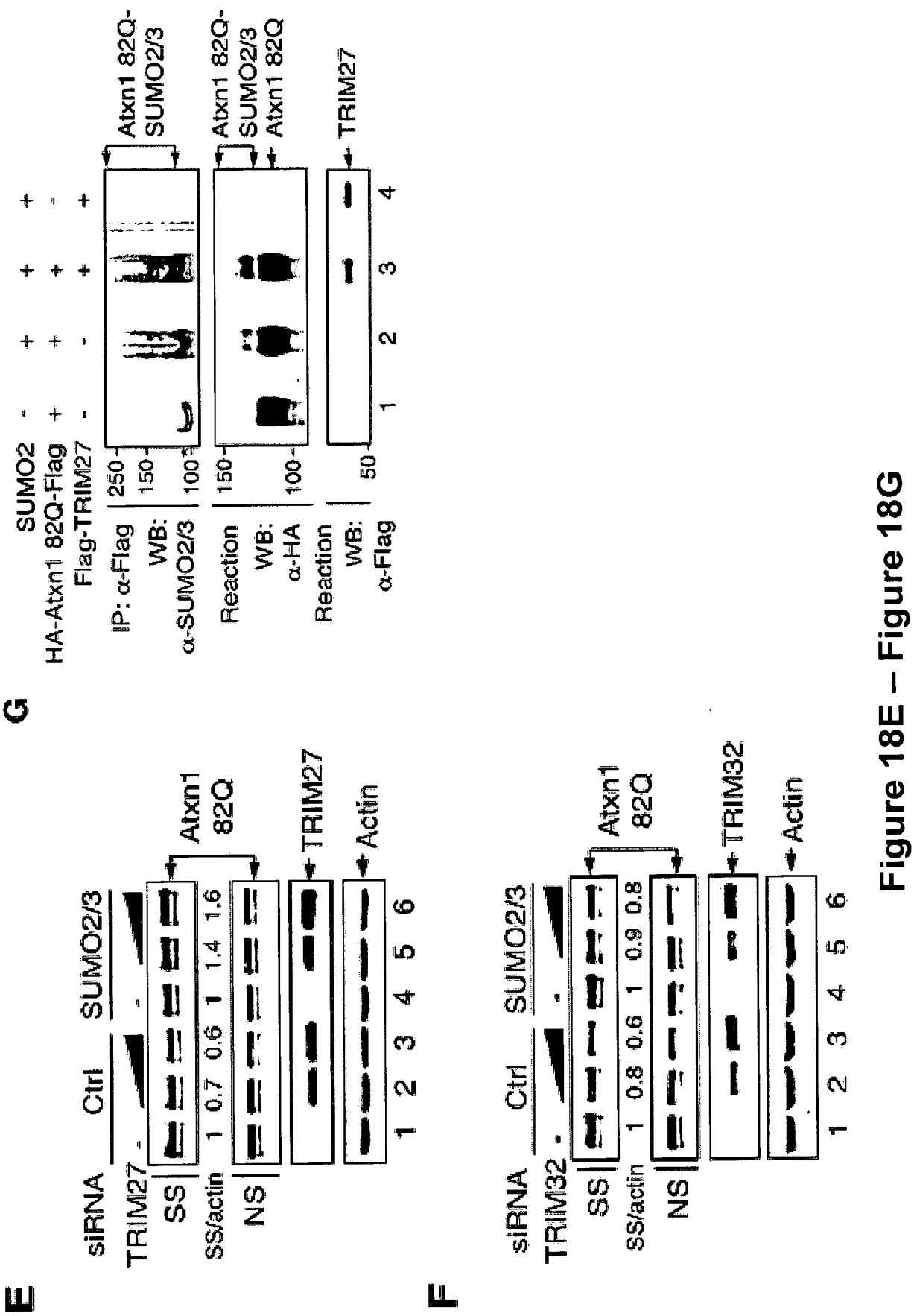


Figure 18E – Figure 18G

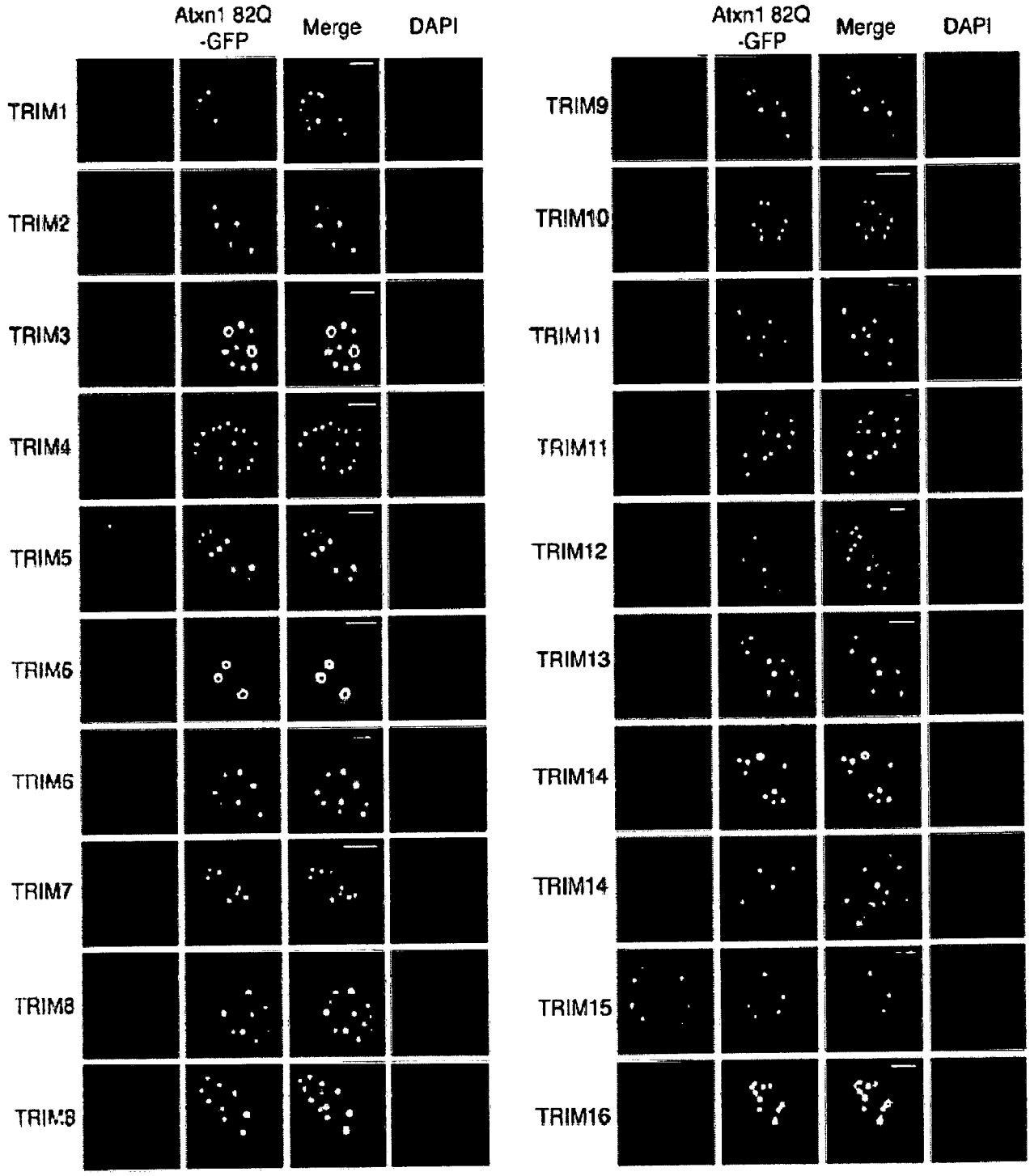

Atxn1 82Q -GFP
Merge
DAPI
TRIM1
TRIM2
TRIM3
TRIM4
TRIM5
TRIM6
TRIM6
TRIM7
TRIM8
TRIM8
Atxn1 82Q -GFP
Merge
DAPI
TRIM9
TRIM10
TRIM11
TRIM11
TRIM12
TRIM13
TRIM14
TRIM14
TRIM15
TRIM16

Figure 19A

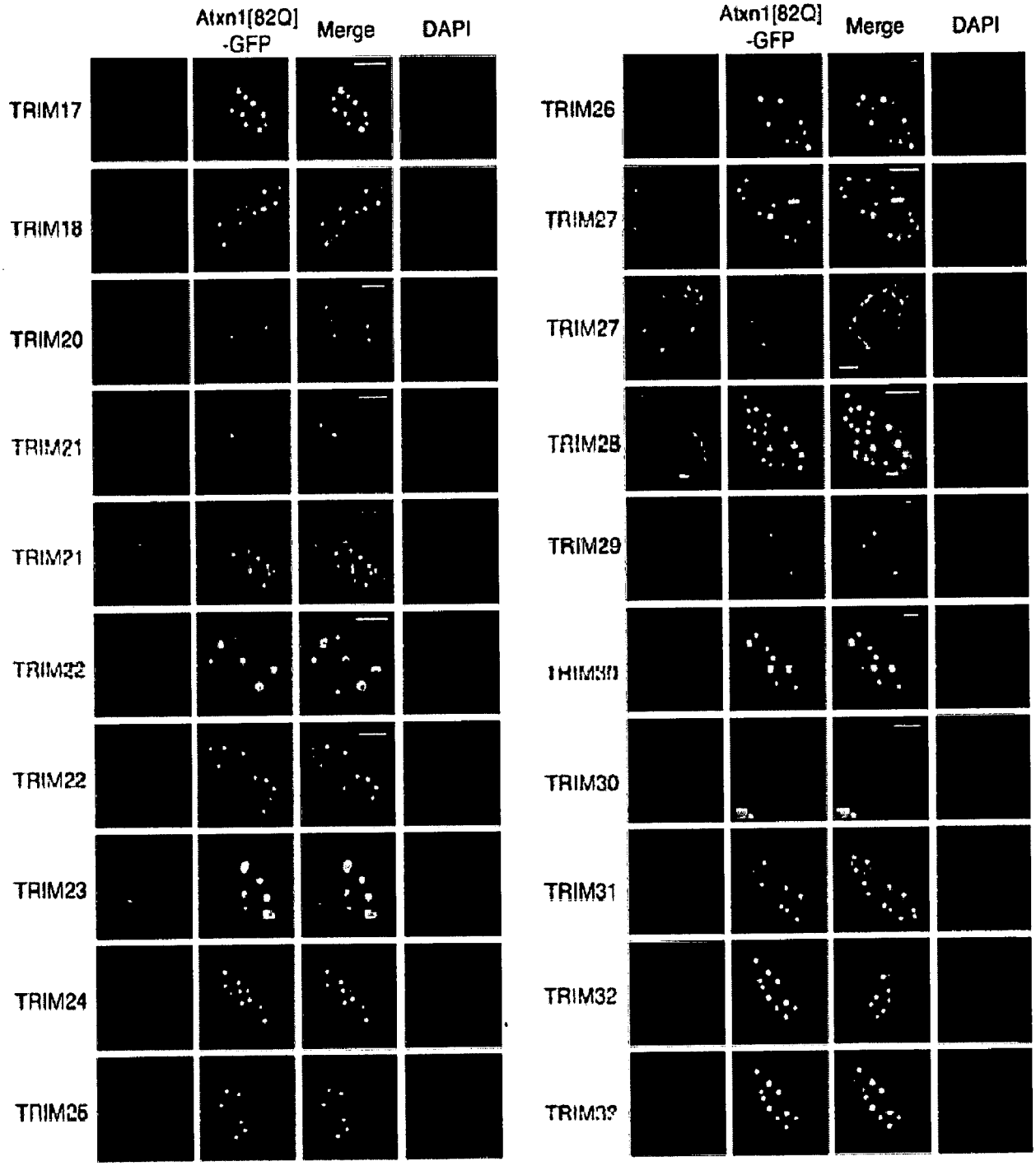

Atxn1[82Q]
-GFP
Merge
DAPI
TRIM17
TRIM18
TRIM20
TRIM21
TRIM21
TRIM22
TRIM22
TRIM23
TRIM24
TRIM25
Atxn1[82Q]
-GFP
Merge
DAPI
TRIM26
TRIM27
TRIM27
TRIM28
TRIM29
TRIM30
TRIM30
TRIM31
TRIM32

Figure 19B

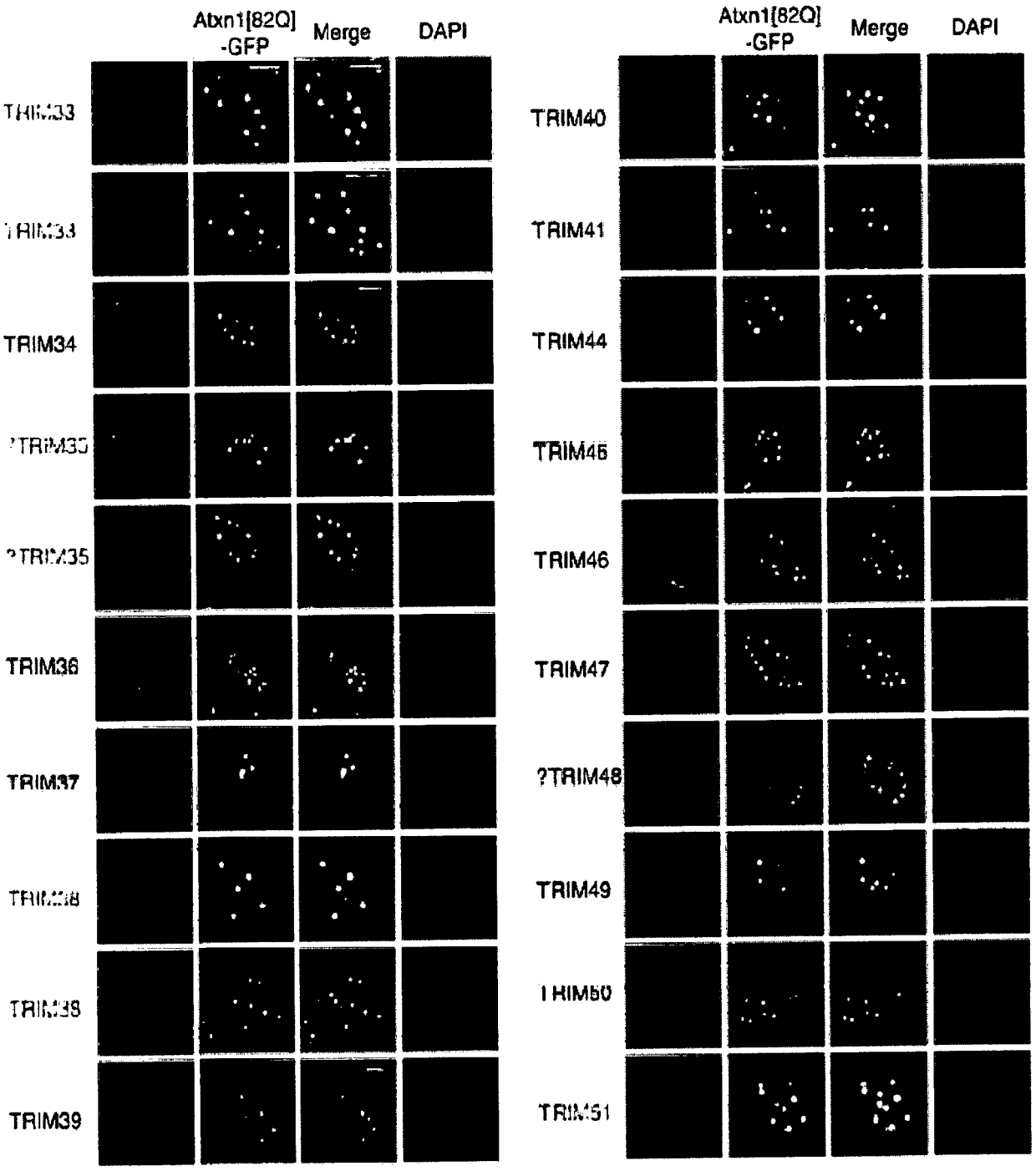

Atxn1[82Q]
-GFP
Merge
DAPI
TRIM33
TRIM34
TRIM35
TRIM36
TRIM37
TRIM38
TRIM39
TRIM40
TRIM41
TRIM44
TRIM45
TRIM46
TRIM47
TRIM49

Figure 19C

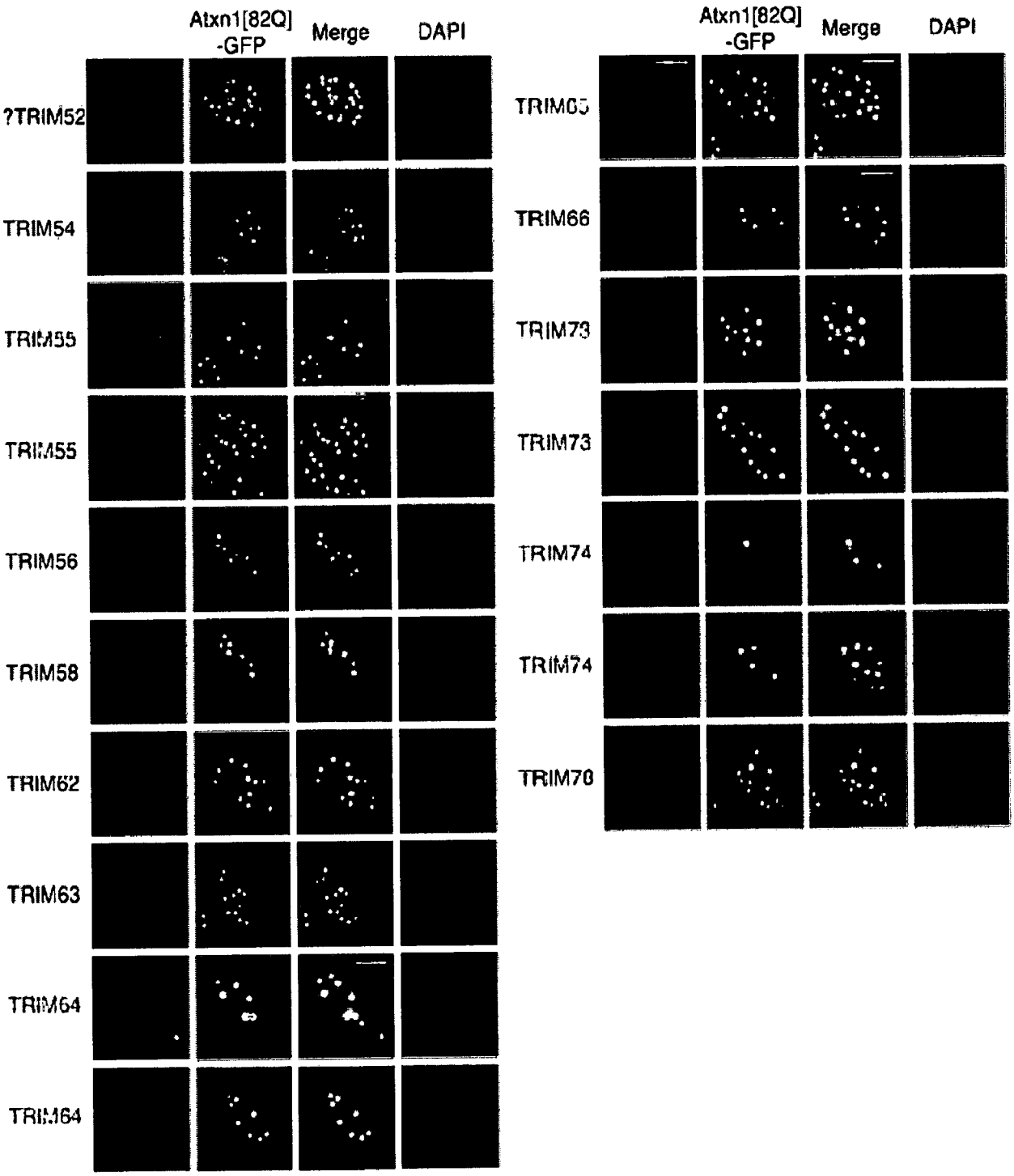

Atxn1[82Q]
-GFP
Merge
DAPI
?TRIM52
TRIM54
TRIM55
TRIM55
TRIM56
TRIM58
TRIM62
TRIM63
TRIM64
TRIM64
Atxn1[82Q]
-GFP
Merge
DAPI
TRIM65
TRIM66
TRIM73
TRIM73
TRIM74
TRIM74
TRIM76

Figure 19D

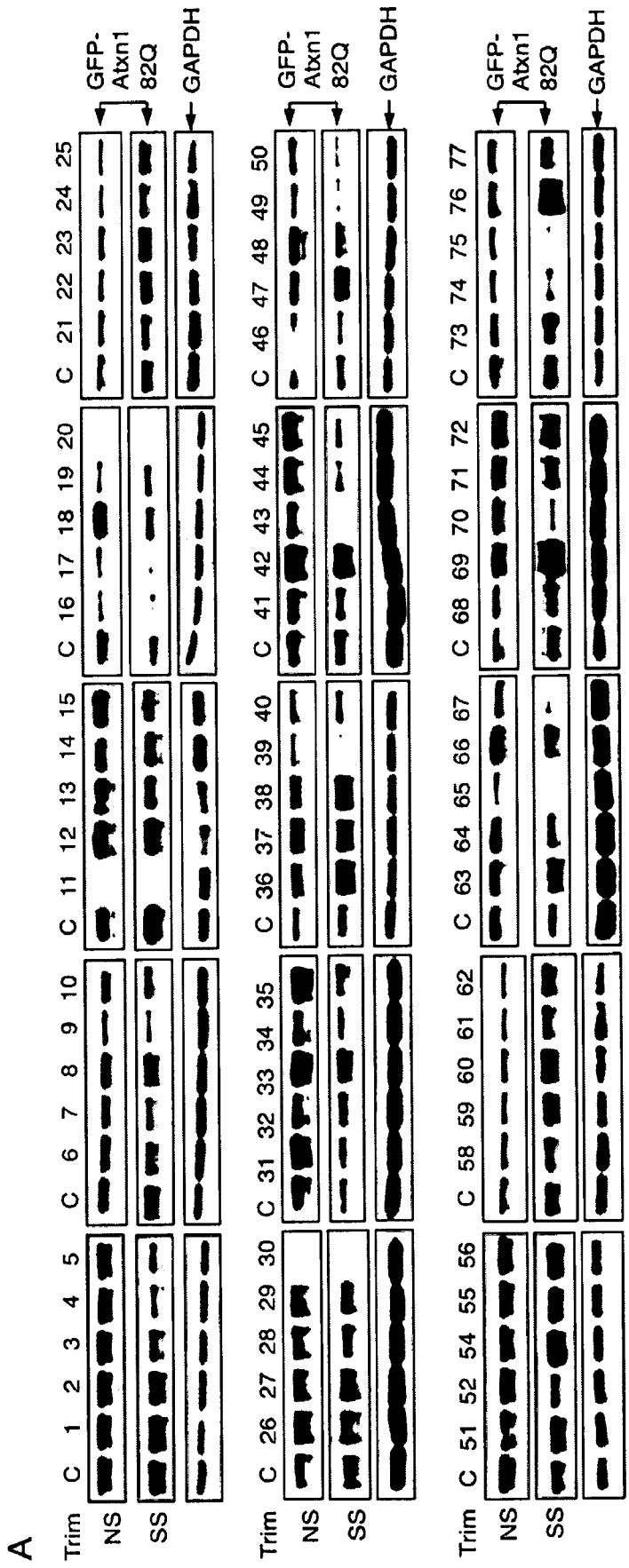

A
Trim
NS
SS
C 1 2 3 4 5 C 6 7 8 9 10 C 11 12 13 14 15 C 16 17 18 19 20 C 21 22 23 24 25
C 26 27 28 29 30 C 31 32 33 34 35 C 36 37 38 39 40 C 41 42 43 44 45 C 46 47 48 49 50
C 51 52 54 55 56 C 58 59 60 61 62 C 63 64 65 66 67 C 68 69 70 71 72 C 73 74 75 76 77
GFP-Atxn1 82Q
GAPDH

Figure 20A

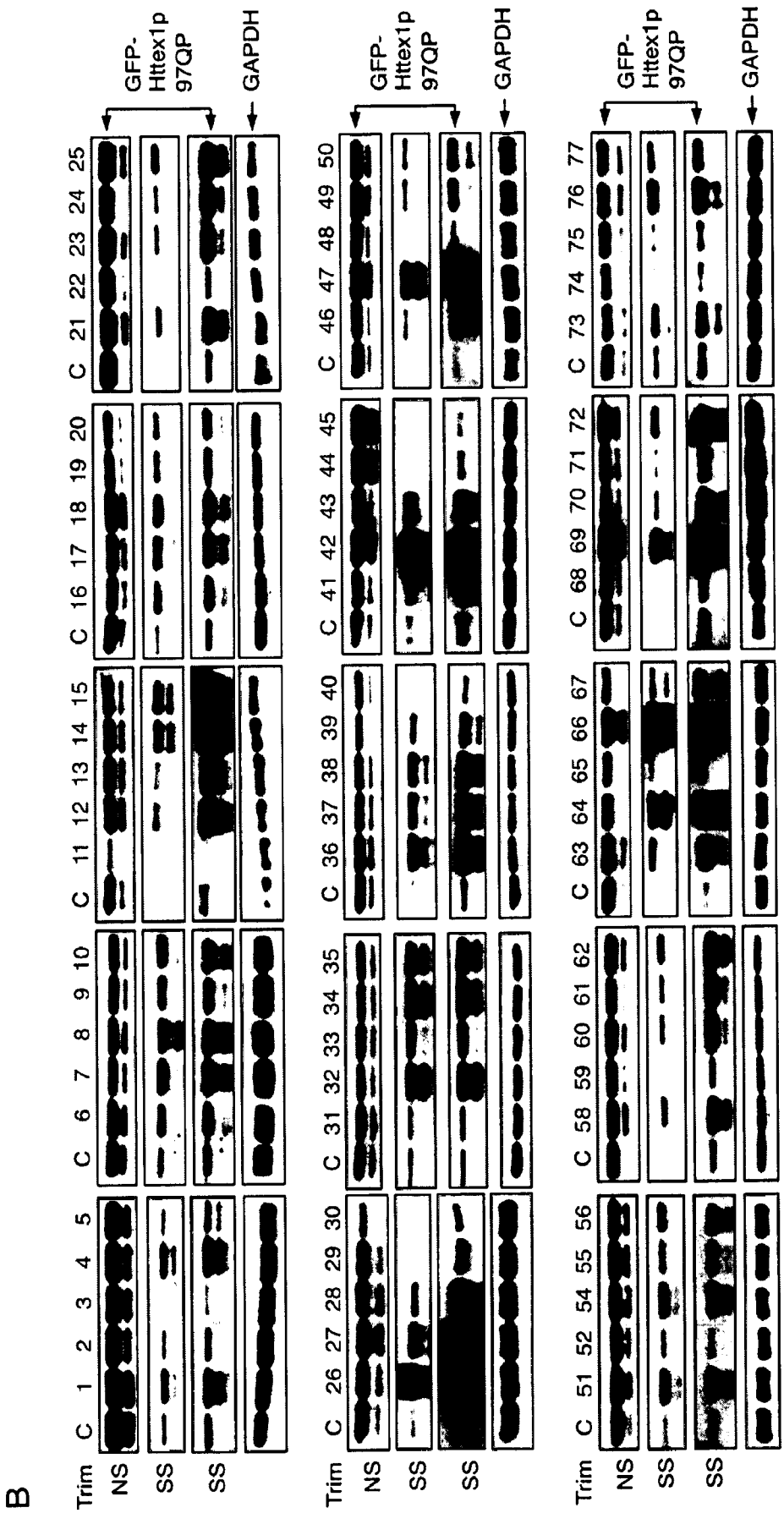

B
Trim
NS
SS
SS
C 1 2 3 4 5 C 6 7 8 9 10 C 11 12 13 14 15 C 16 17 18 19 20 C 21 22 23 24 25
C 26 27 28 29 30 C 31 32 33 34 35 C 36 37 38 39 40 C 41 42 43 44 45 C 46 47 48 49 50
C 51 52 54 55 56 C 58 59 60 61 62 C 63 64 65 66 67 C 68 69 70 71 72 C 73 74 75 76 77
GFP-Httex1p 97QP
GAPDH

Figure 20B

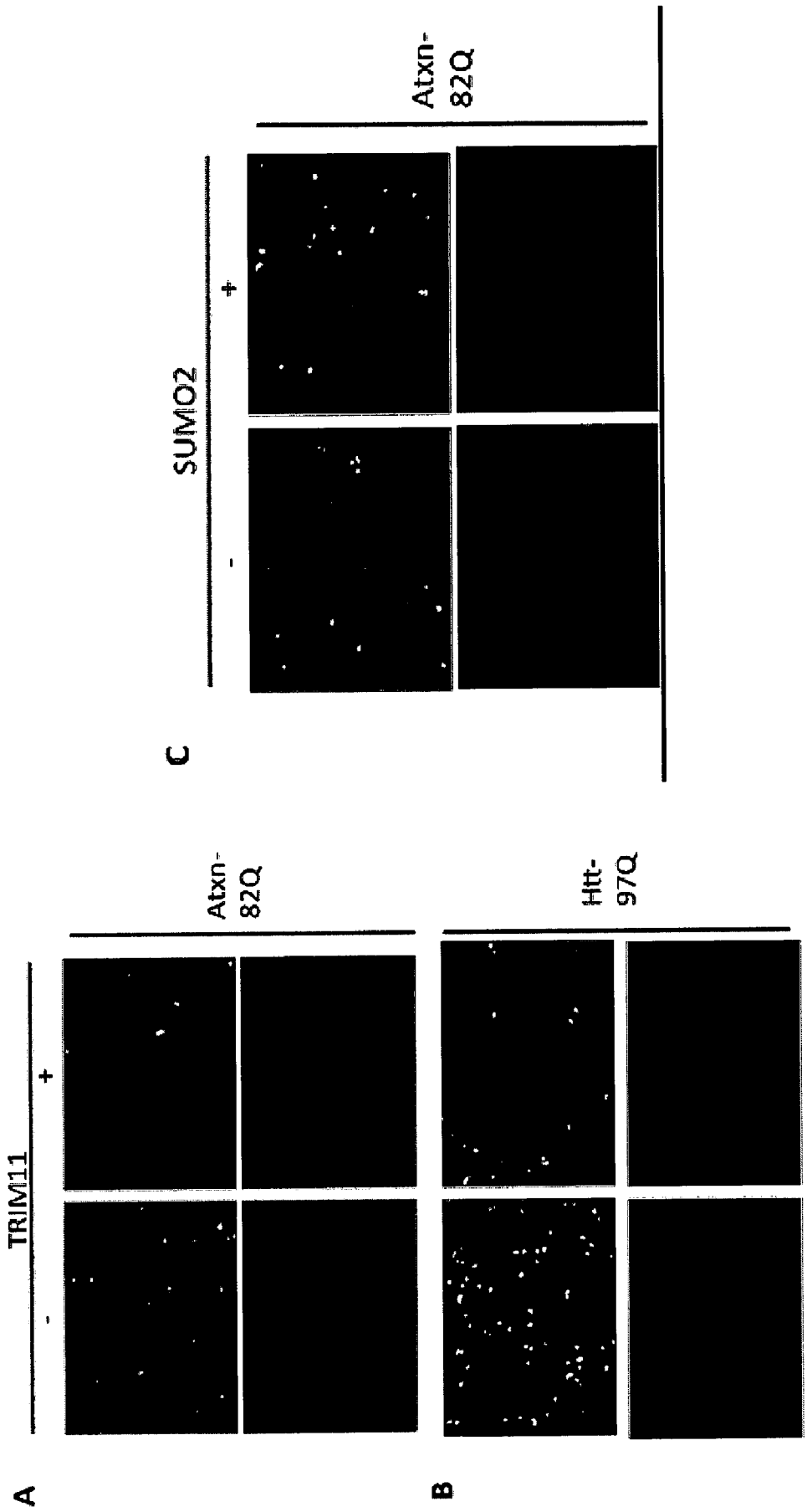


Figure 21A – Figure 21C

6His Tag TAT peptide HA tag

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDRWGSKLGYGRKKRRQRRRGGSTMSGYPYDVPDYAGS

TRIM11 (human) (SEQ ID NO: 22)

MAAPDLSTNL QEEATCAICL DYFTDPVMTD CGHNFCRECI RRCWGQPEGP YACPECRELS
PQRNLRPNRP LAKMAEMARR LHPPSPVPQG VCPAHREPLA AFCGDELRLL CAACERSGEH
WAHRVRPLQD AAEDLKAKLE KSLEHLRKQM QDALLFQAQA DETCVLWQKM VESQRQNVLG
EFERLRRLLA EEEQQLLQRL EEEELEVLPR LREGAAHLGQ QSAHLAELIA ELEGRCQLPA
LGLLQDIKDA LRRVQDVKLQ PPEVVPMELR TVCRVPGLVE TLRRFRGDVT LDPDTANPEL
ILSEDRRSVQ RGDLRQALPD SPERFDPGPC VLGQERFTSG RHYWEVEVGD RTSWALGVCR
ENVNRKEKGE LSAGNGFWIL VFLGSYYNSS ERALAPLRDP PRRVGIFLDY EAGHLSFYSA
TDGSLLFIFP EIPFSGTLRP LFSPLSSSPT PMTICRPKGG SGDTLAPQ

Figure 22

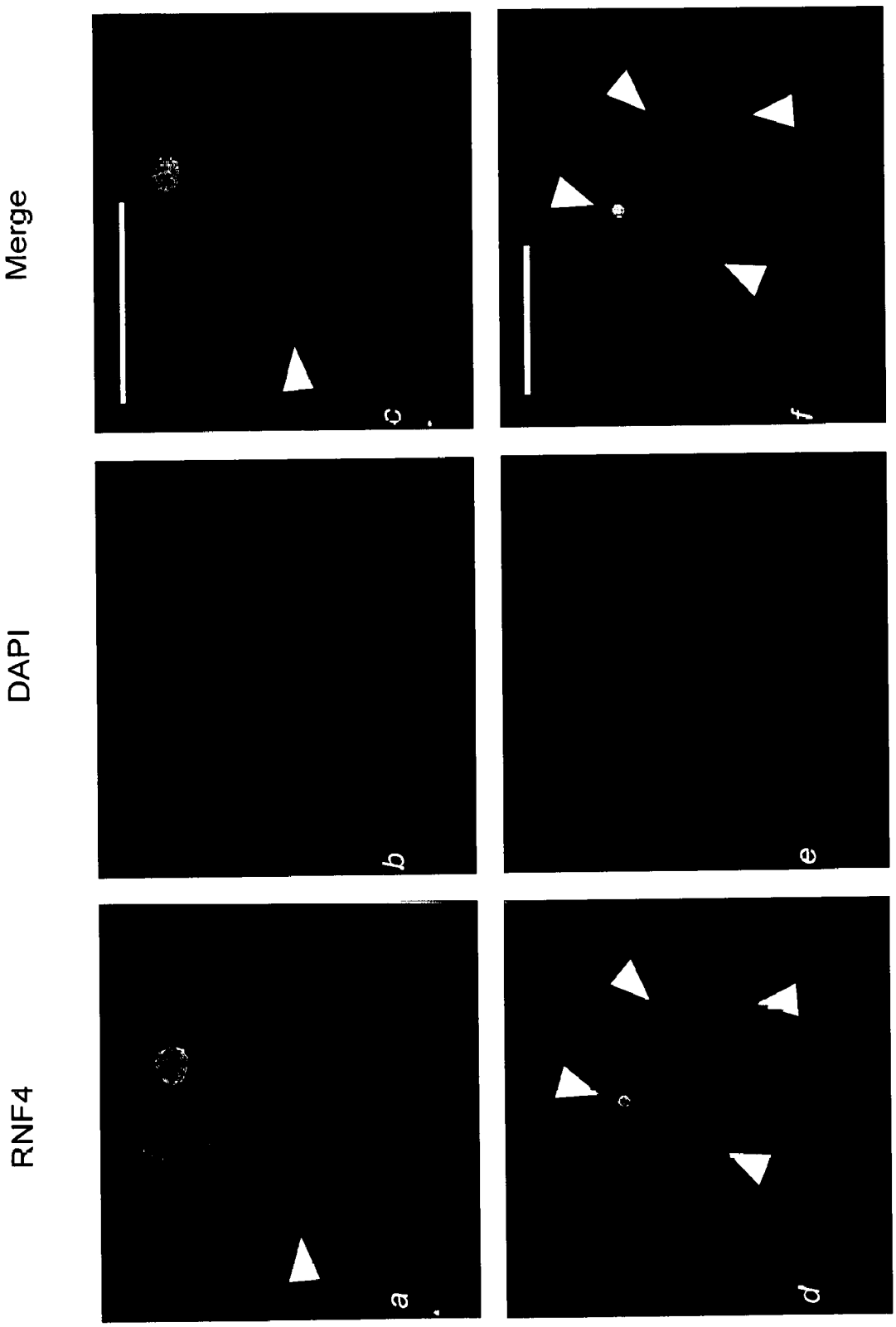


Figure 23

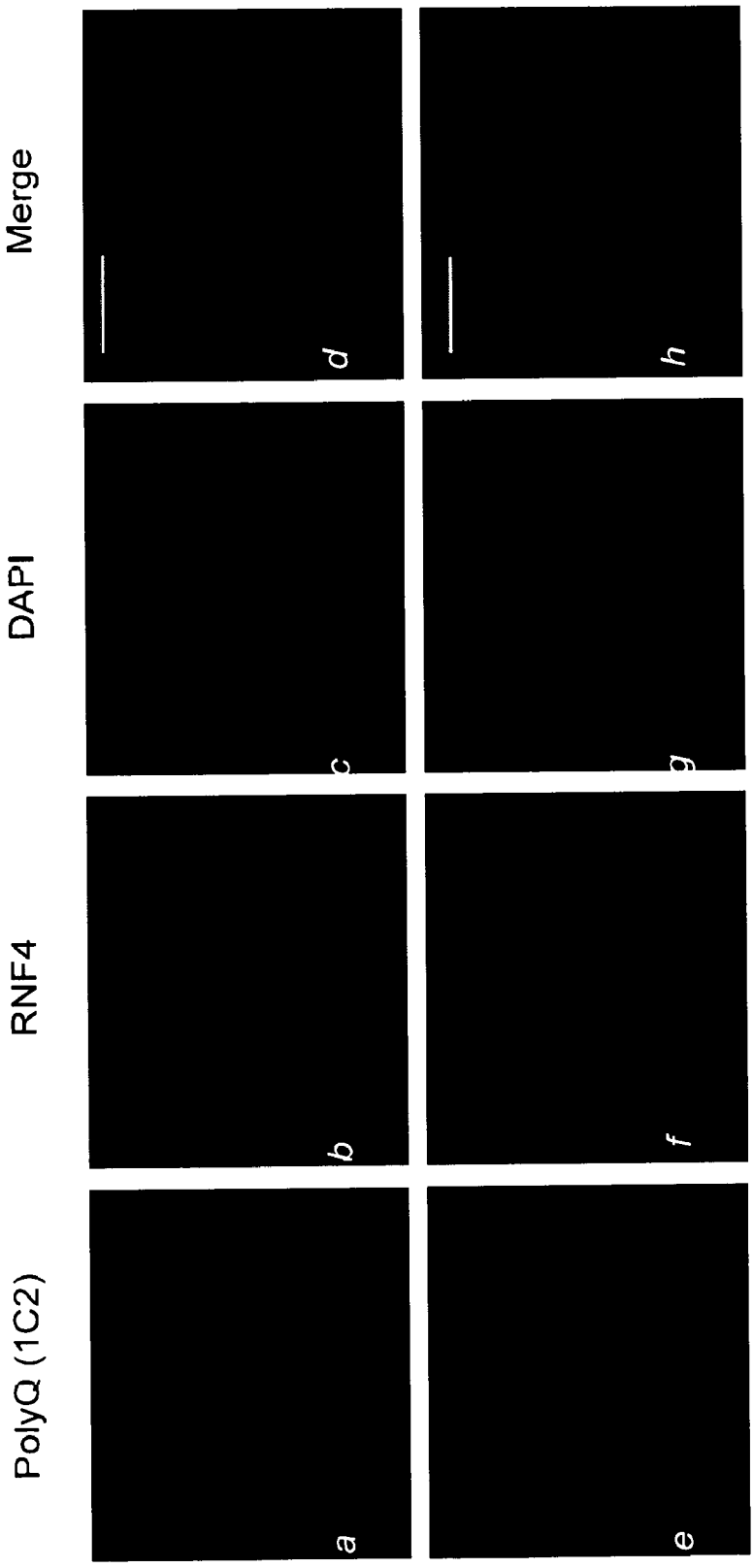


Figure 24

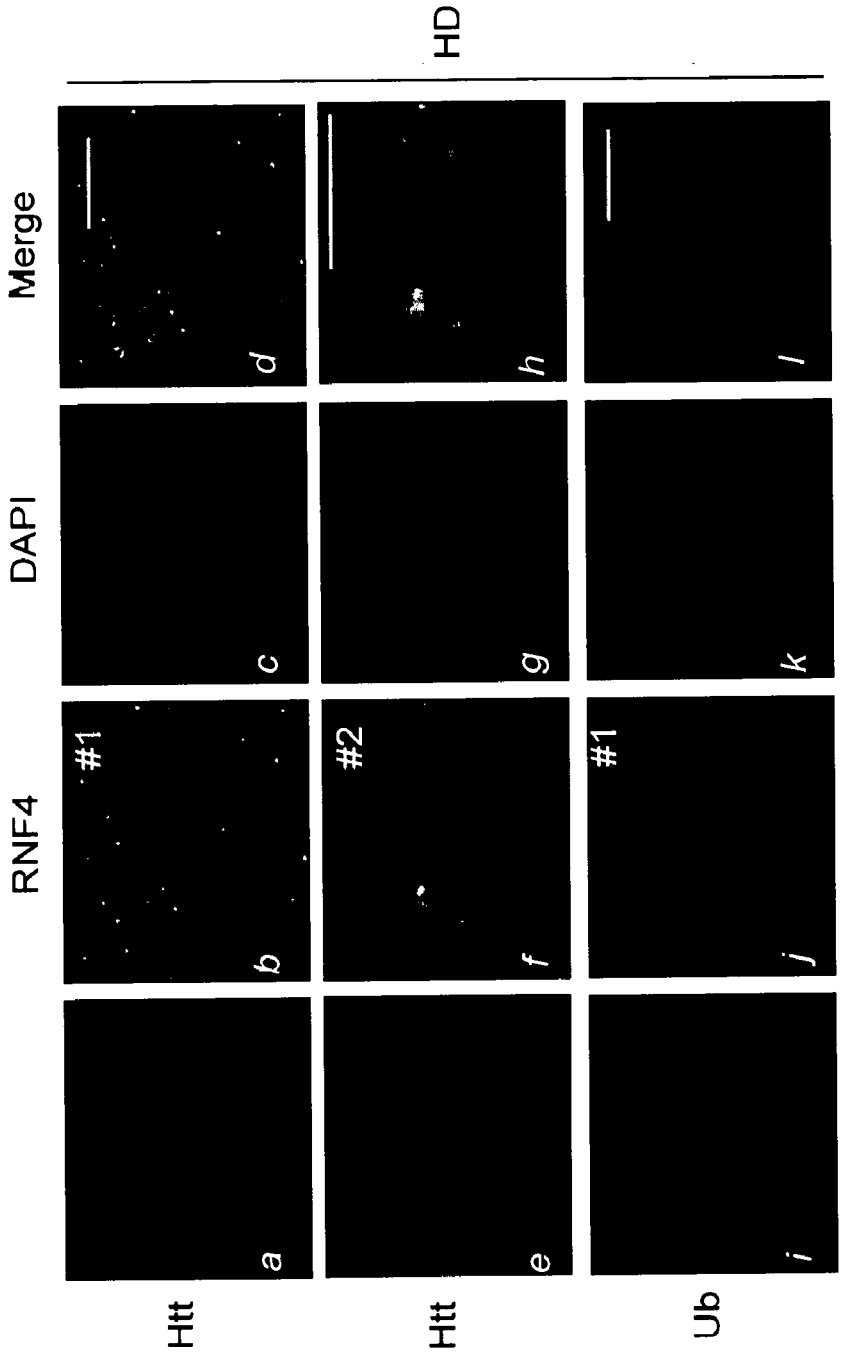


Figure 25

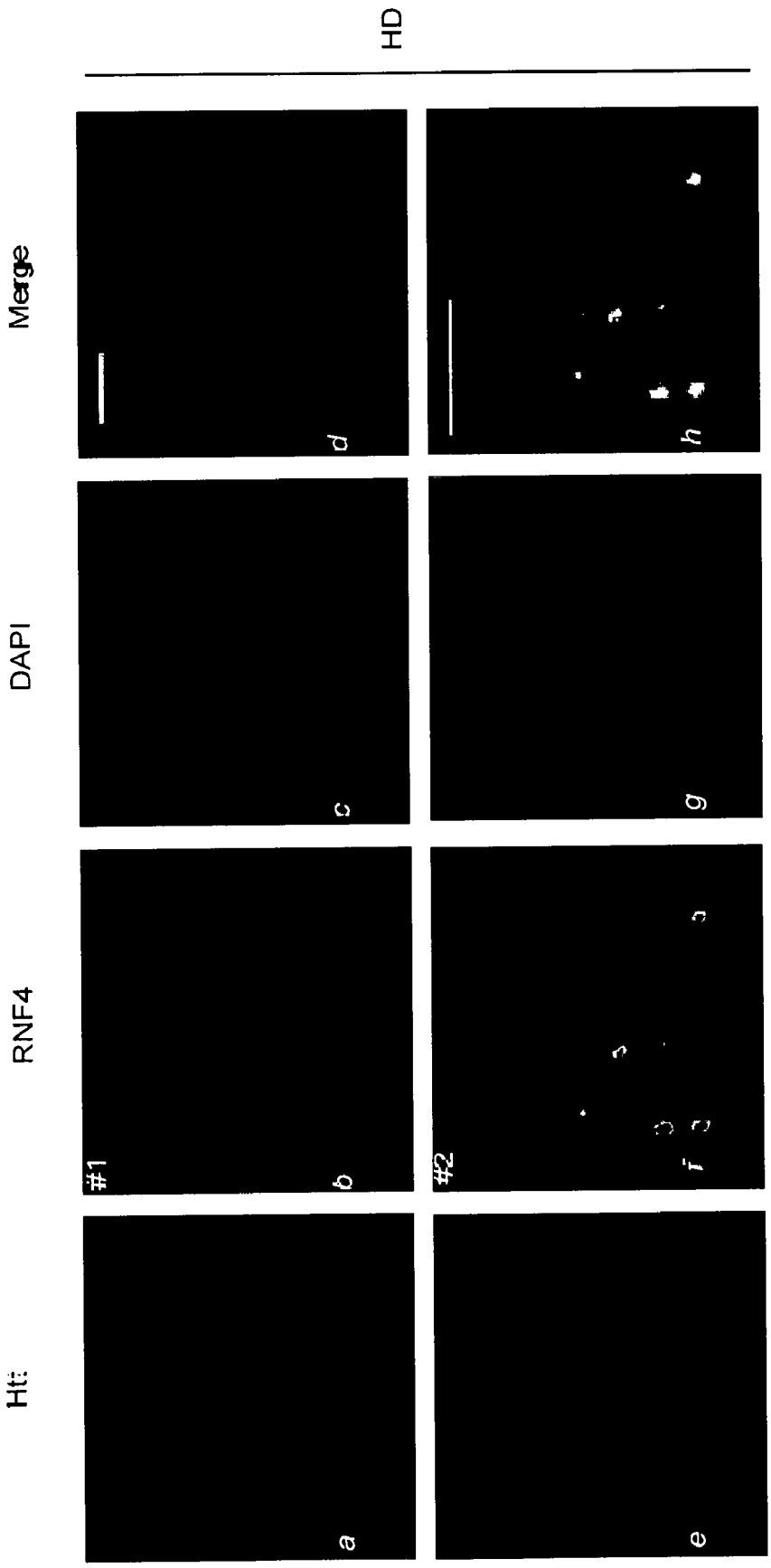


Figure 26

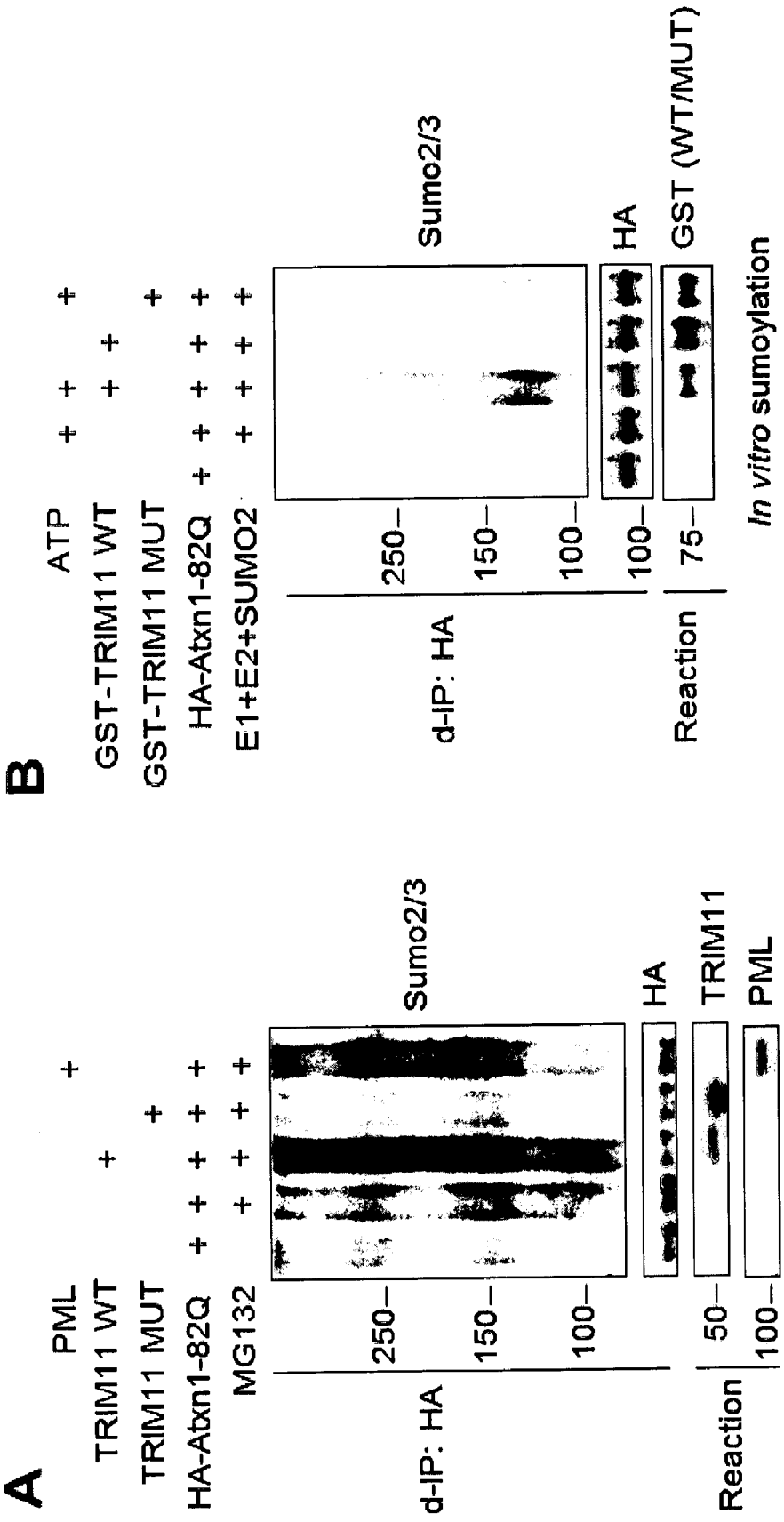


Figure 27A – Figure 27B

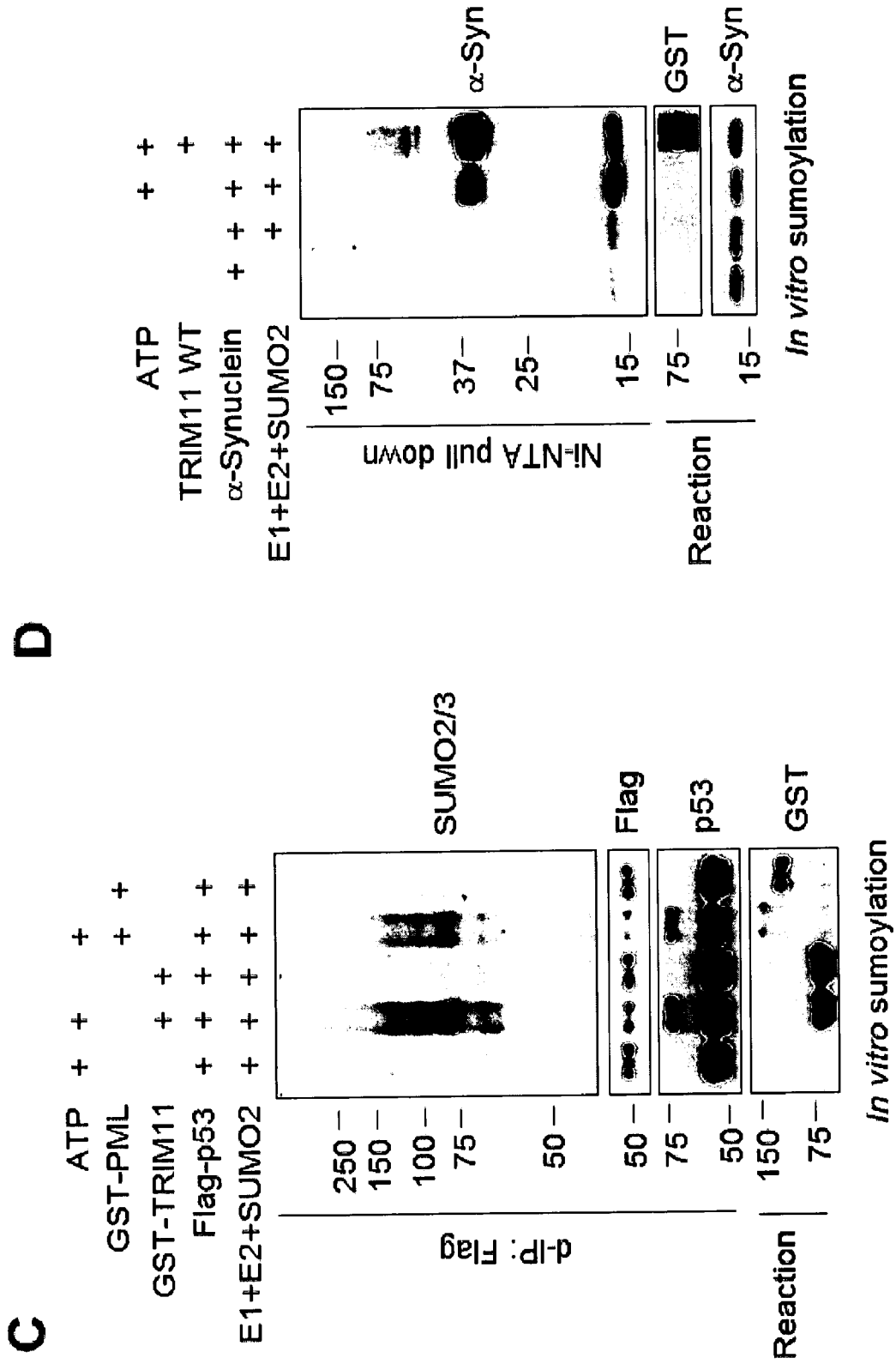


Figure 27C – Figure 27D

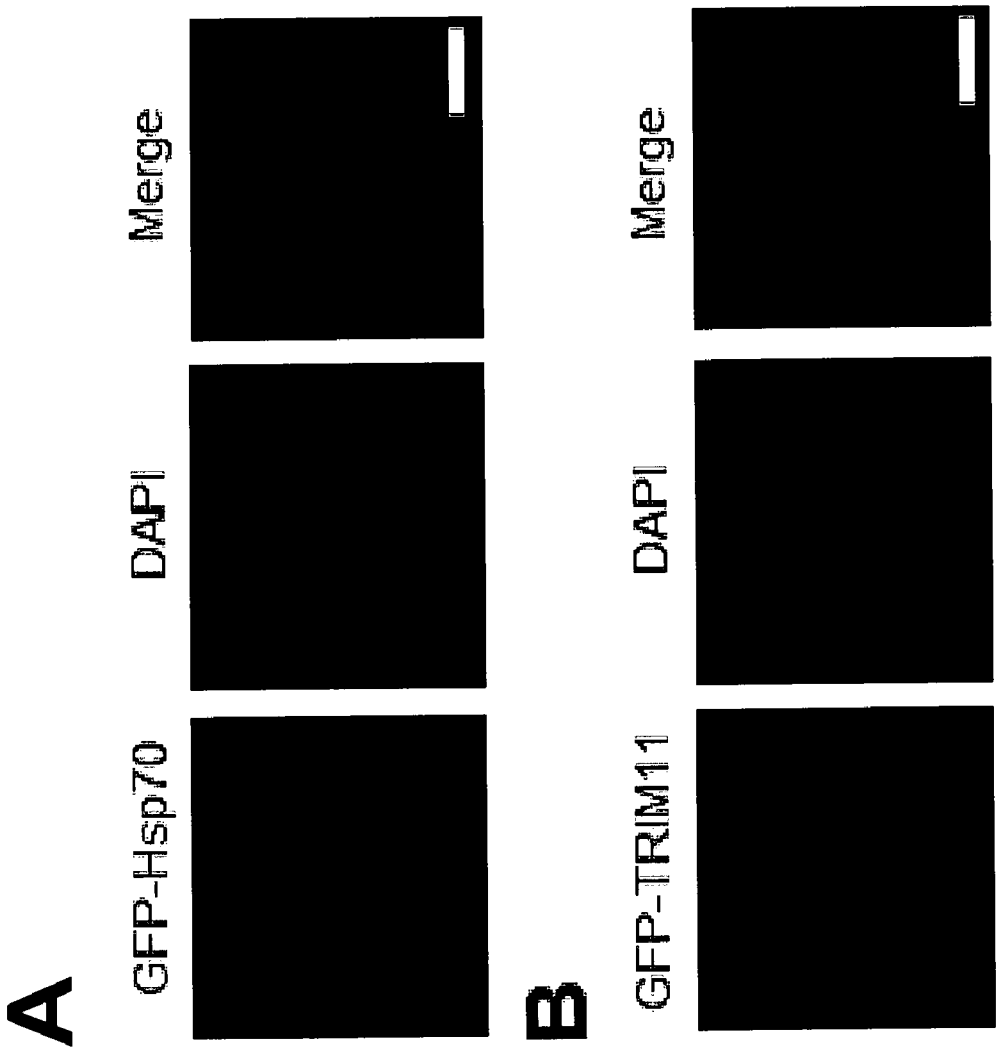


Figure 28A – Figure 28B

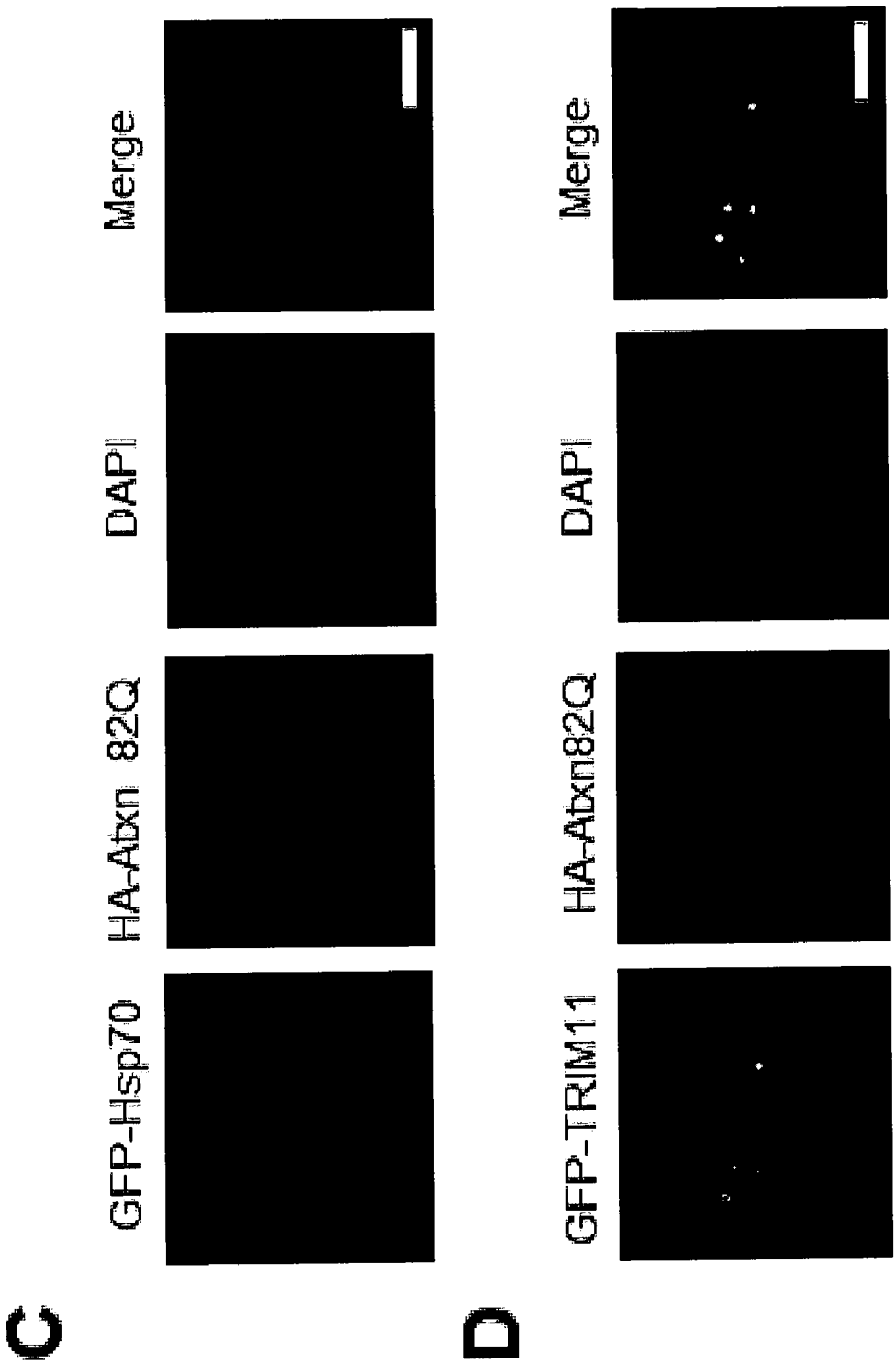


Figure 28C – Figure 28D

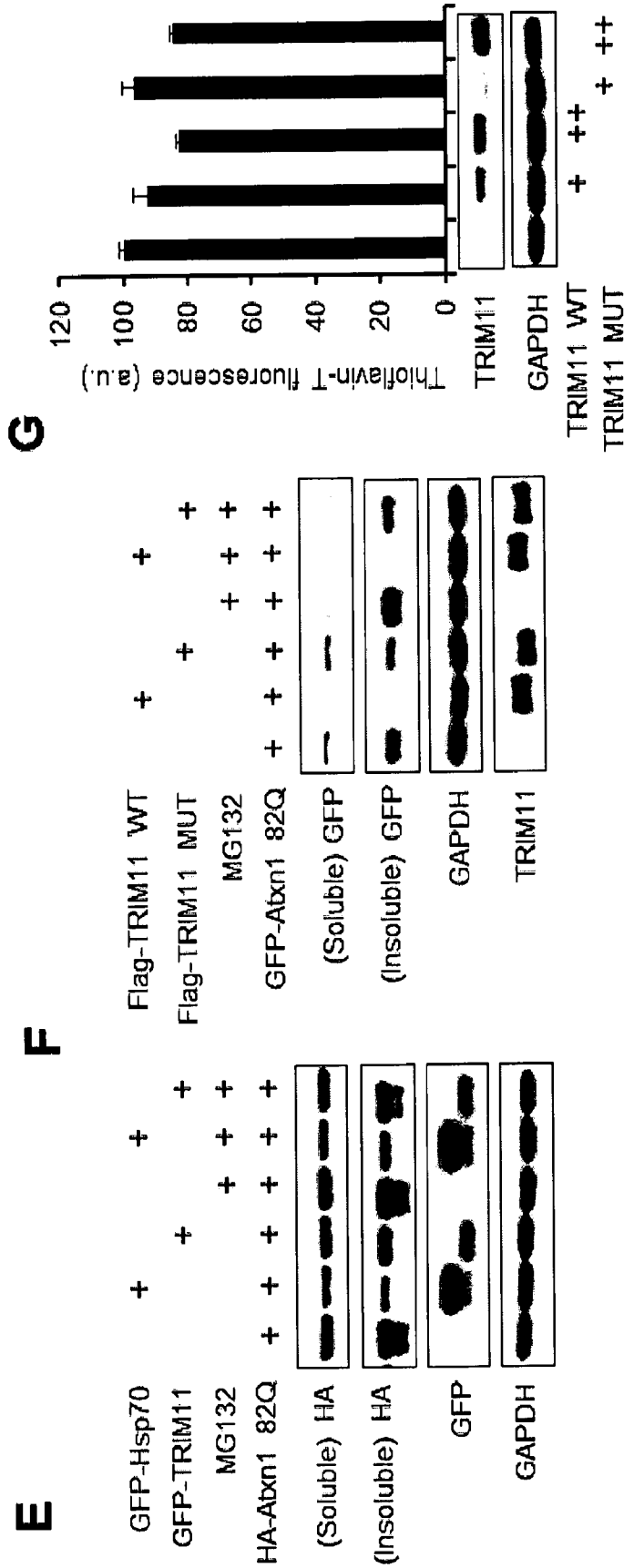


Figure 28E – Figure 28F

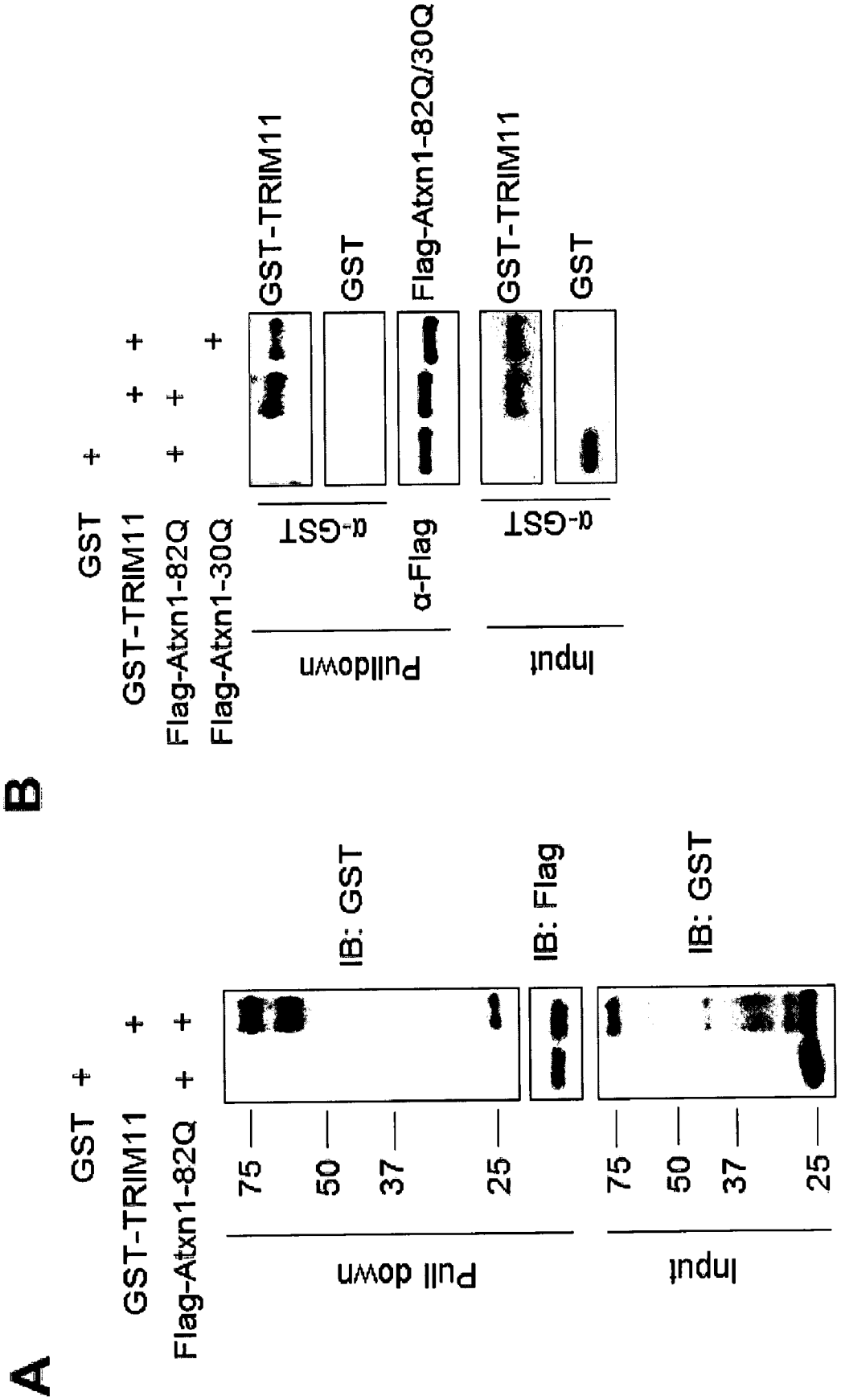


Figure 29A – Figure 29B

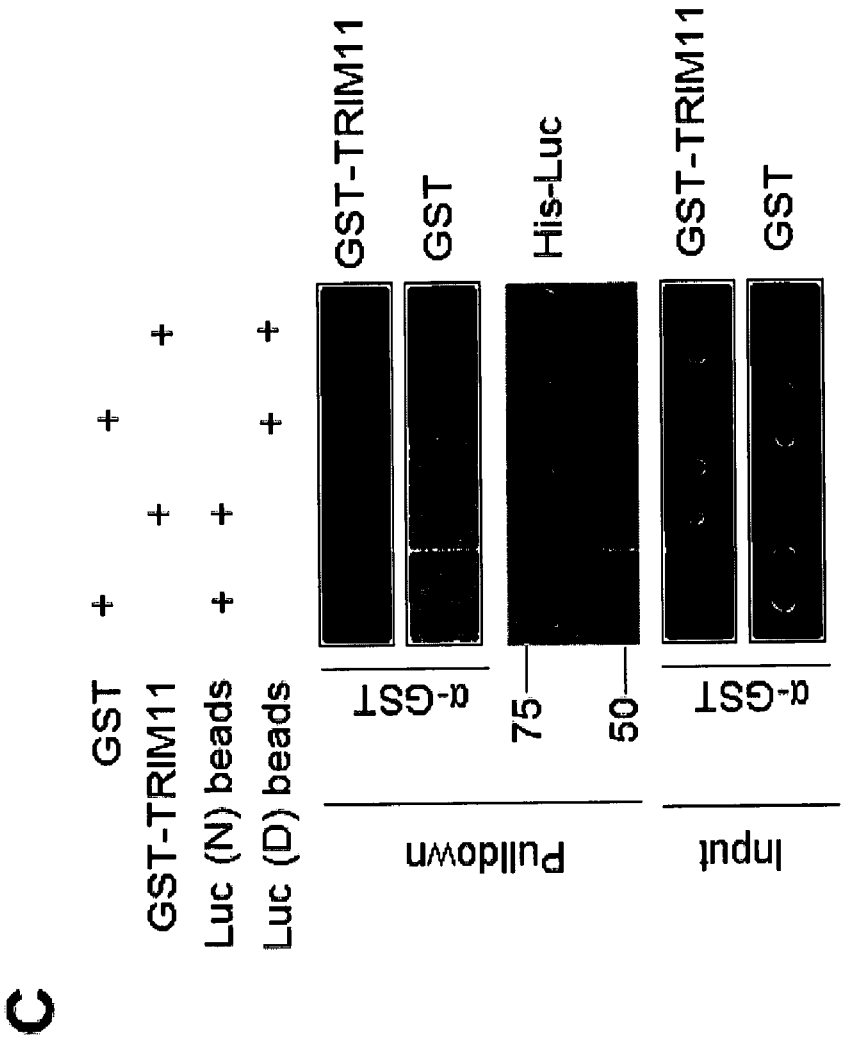

C
GST
GST-TRIM11
Luc (N) beads
Luc (D) beads
Pulldown
Input
α-GST
α-GST
75
50
GST-TRIM11
GST
His-Luc
GST-TRIM11
GST

Figure 29C

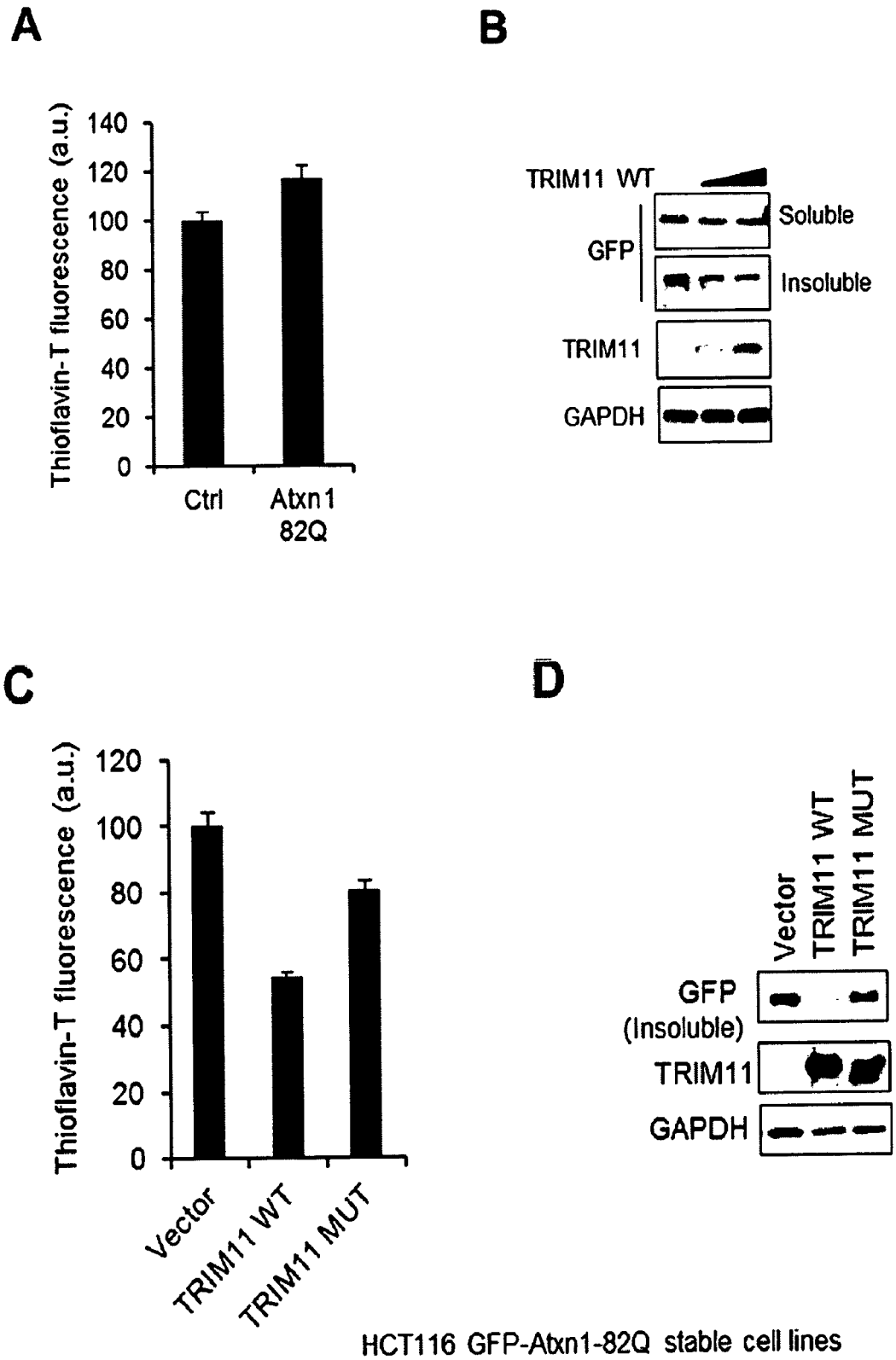


Figure 30A – Figure 30D

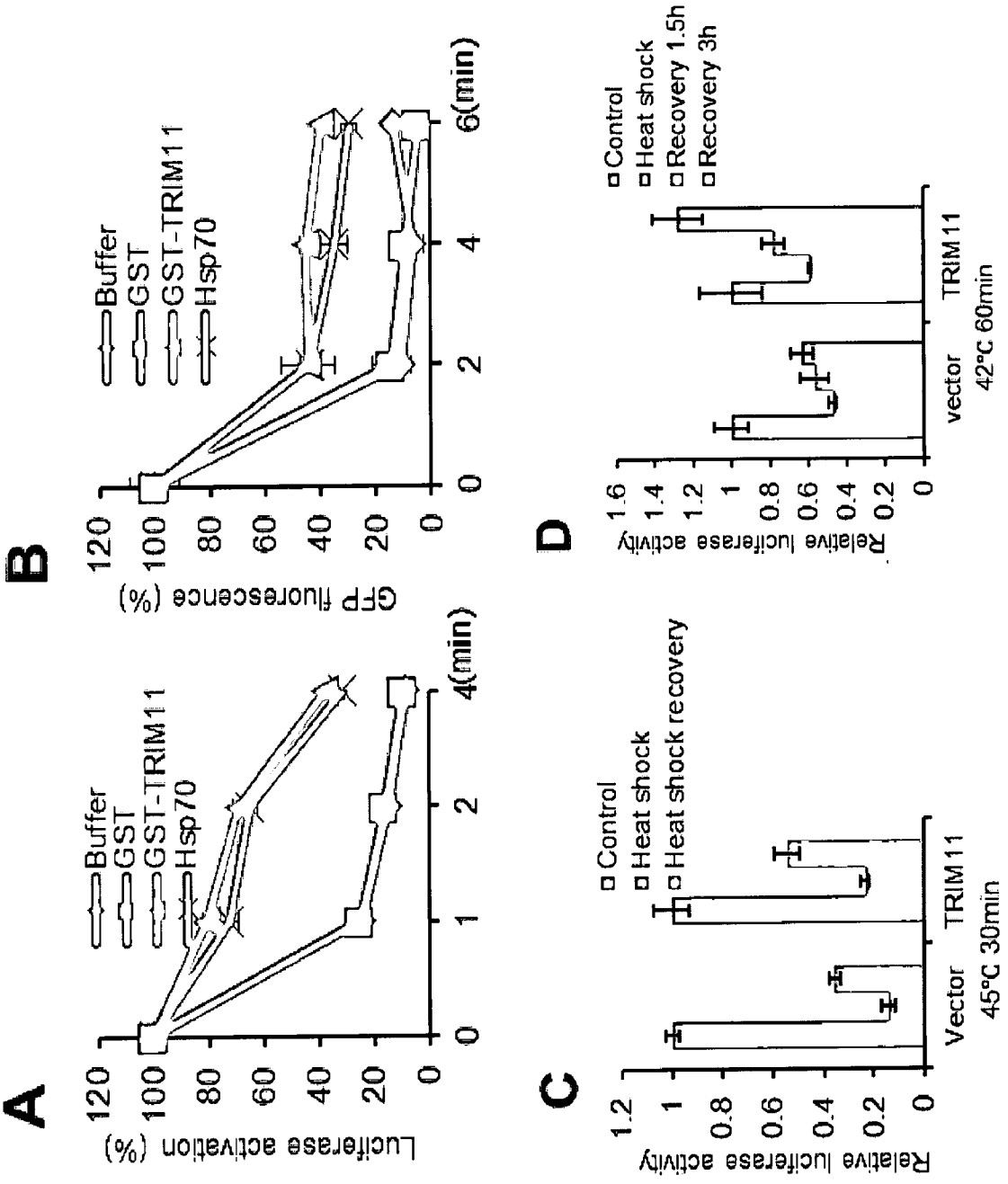


Figure 31A – Figure 31D

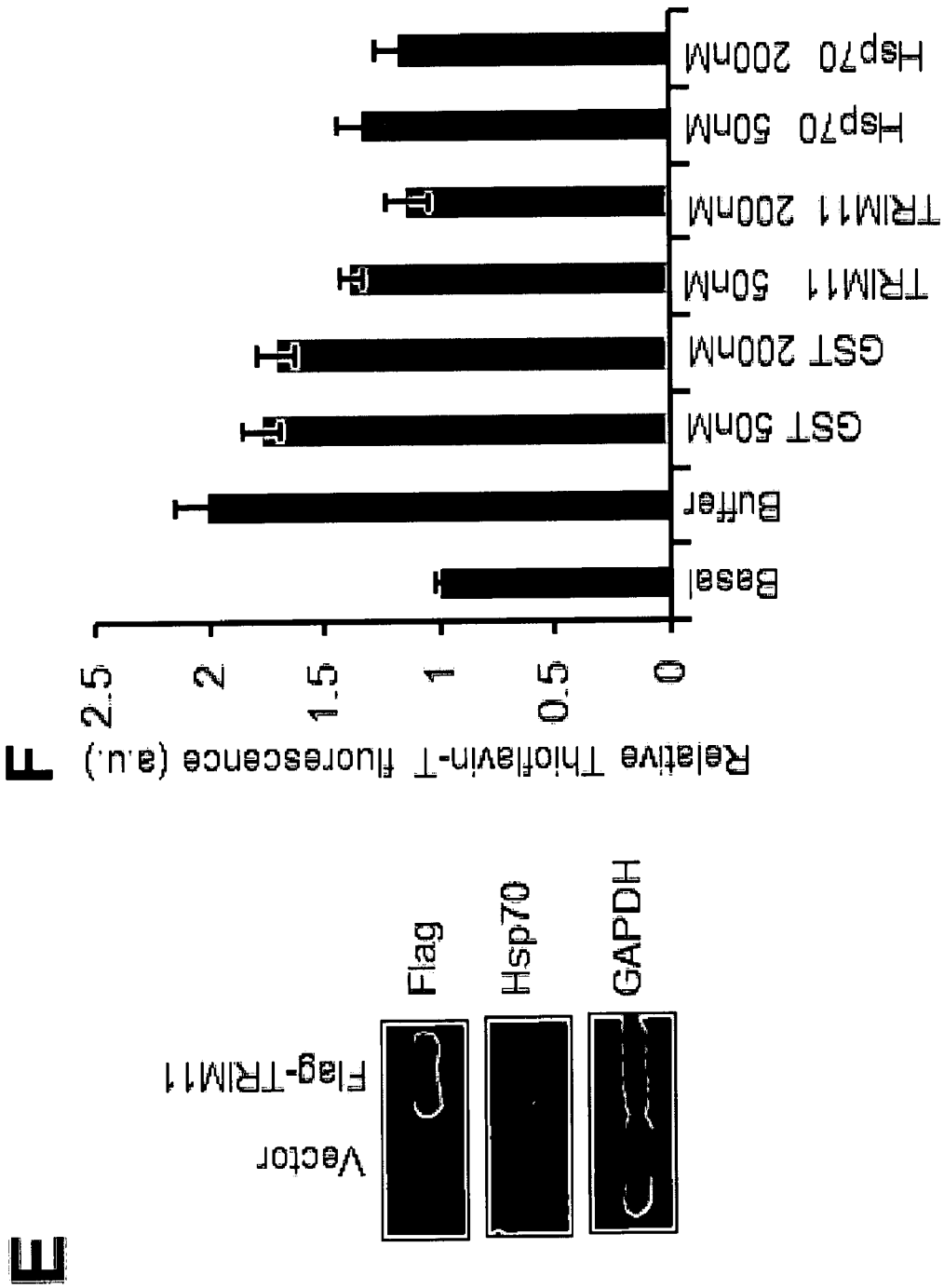


Figure 31E – Figure 31F

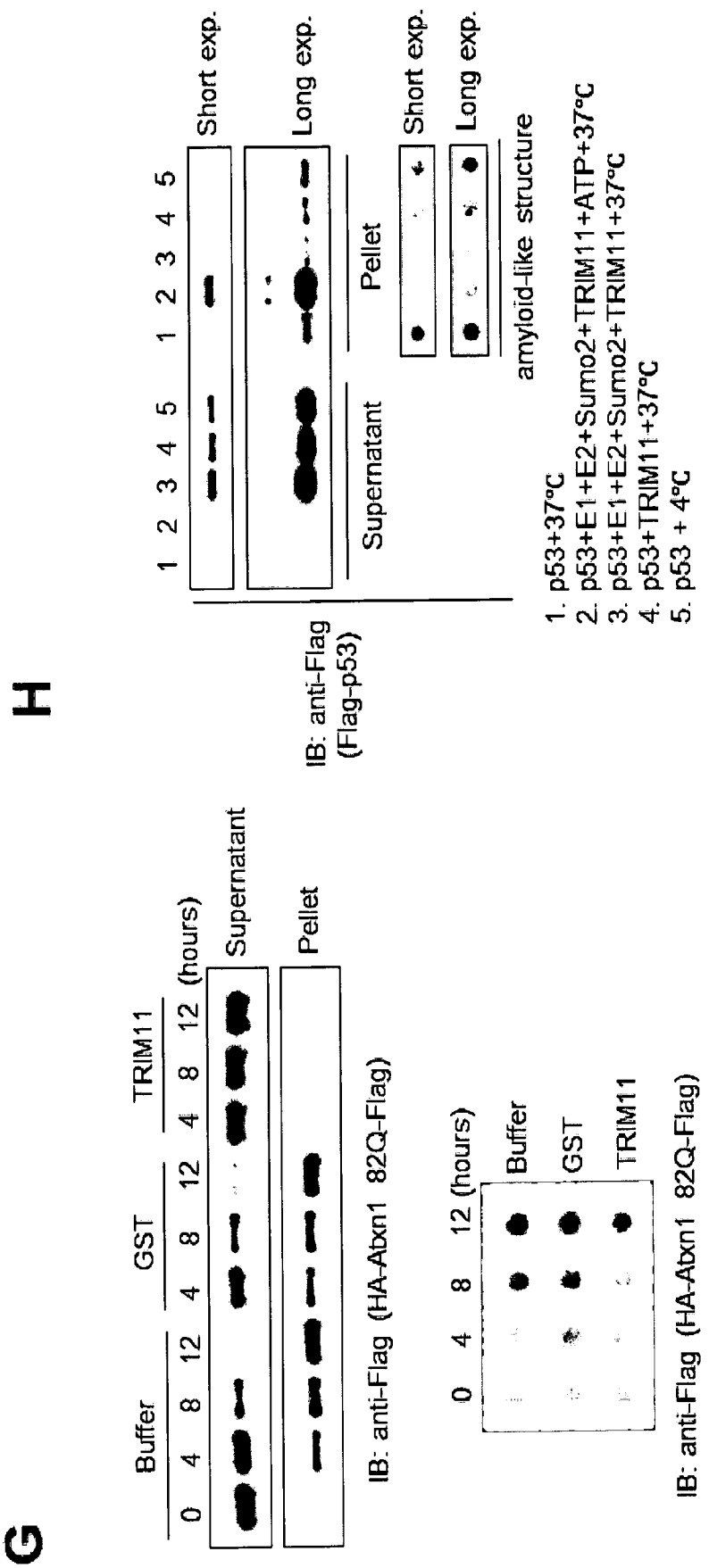


Figure 31G – Figure 31H

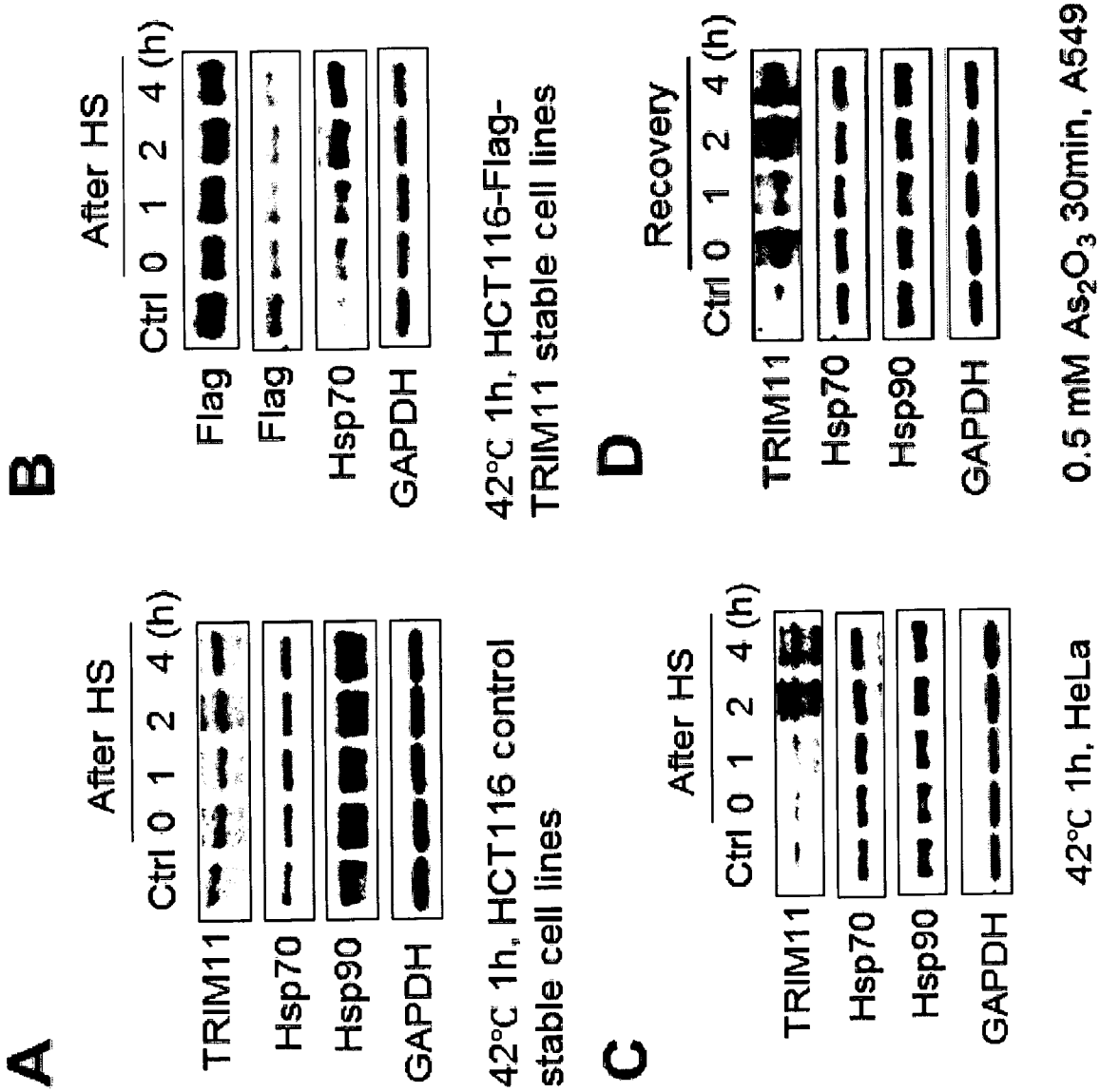


Figure 32A – Figure 32D

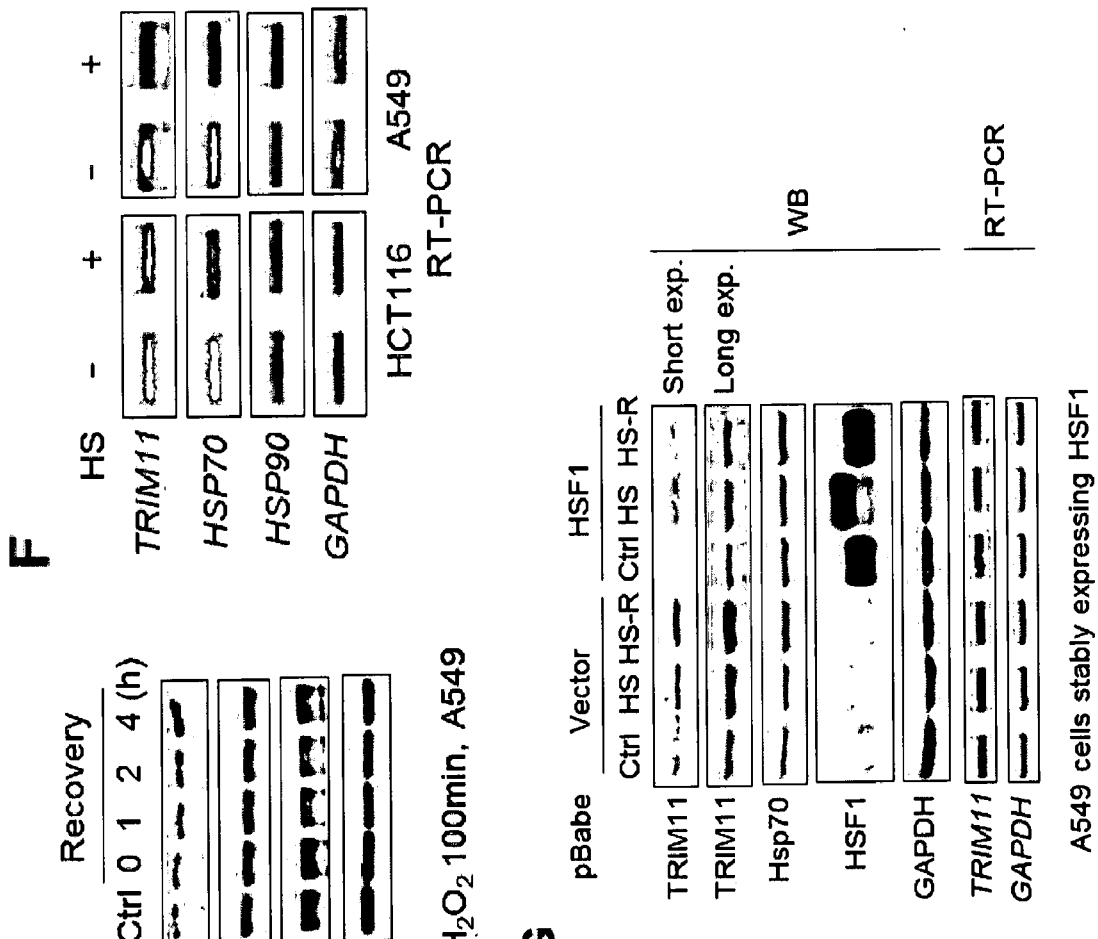


Figure 32E – Figure 32G

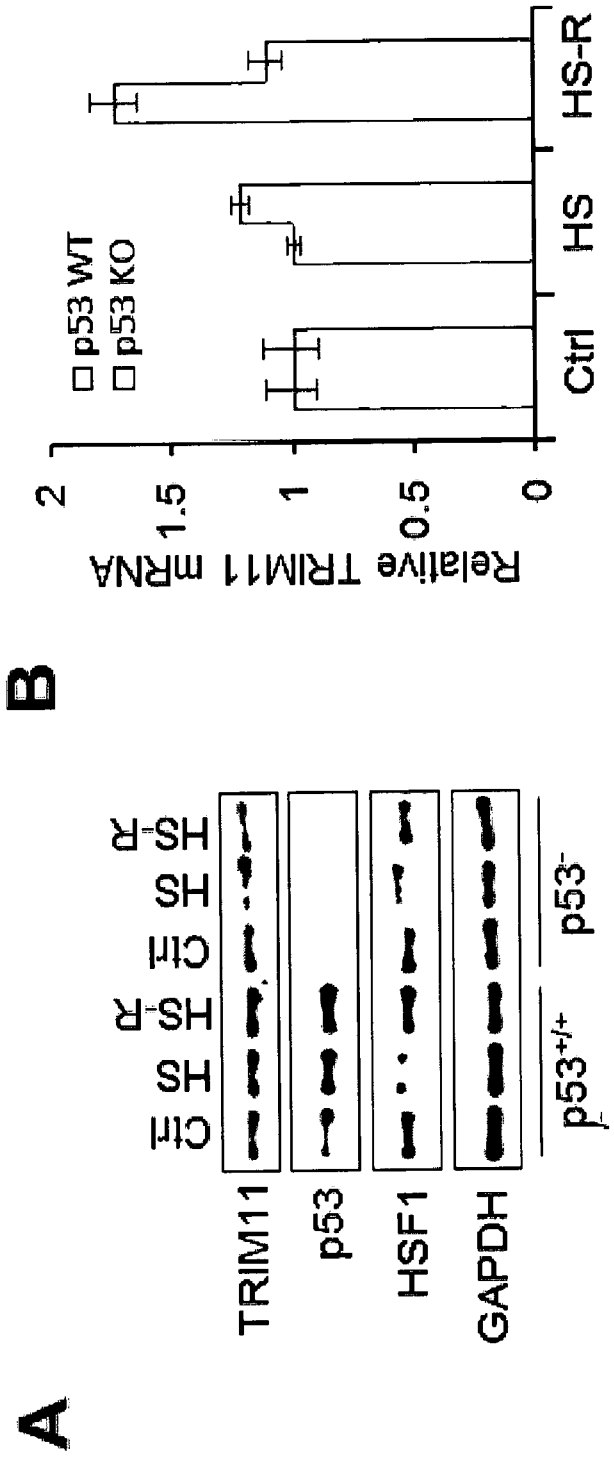


Figure 33A – Figure 33B

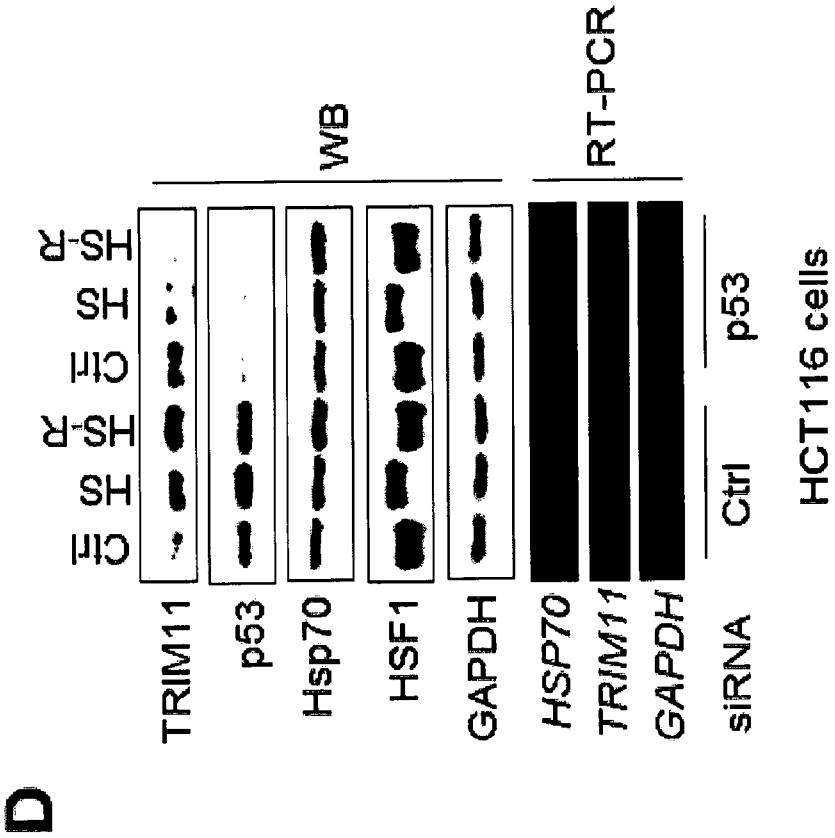


Figure 33C – Figure 33D

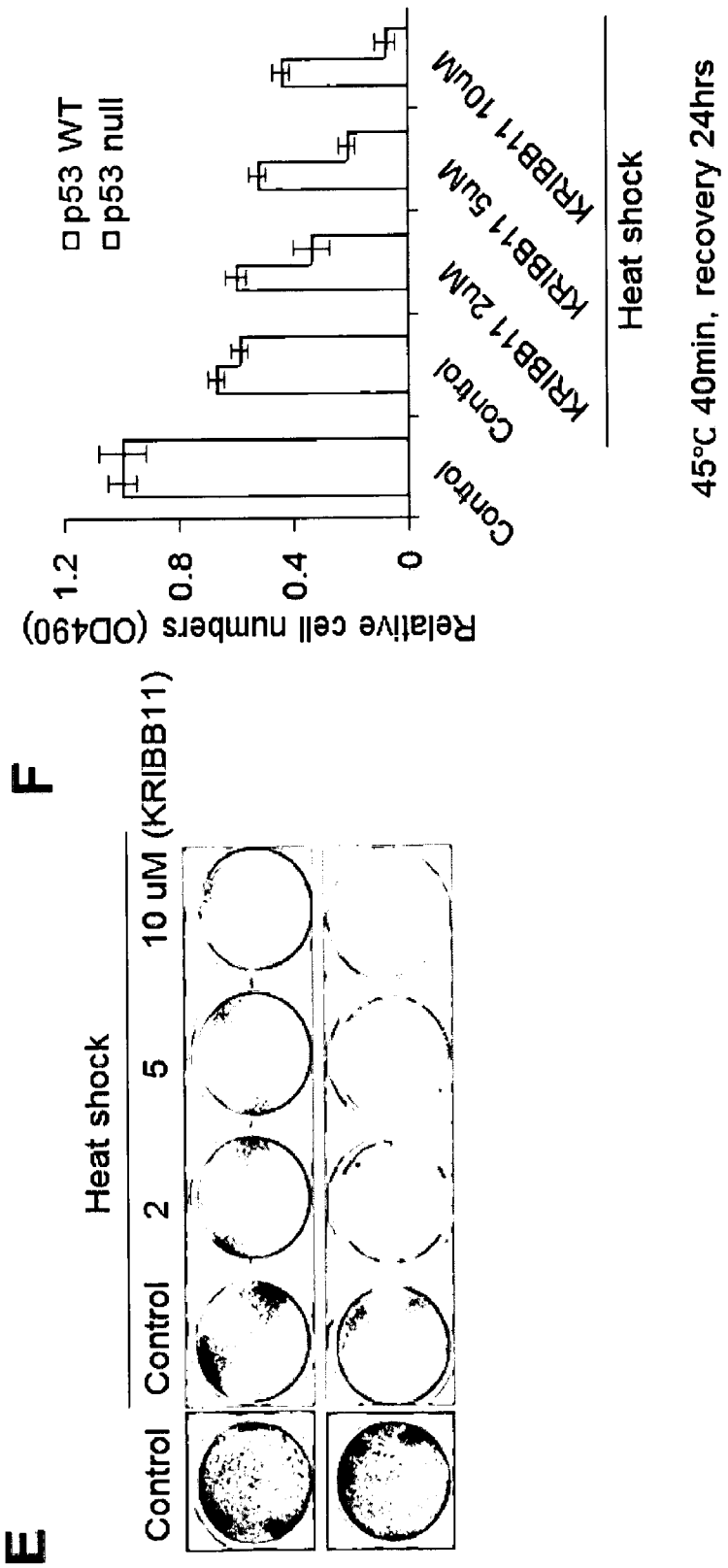


Figure 33E – Figure 33F

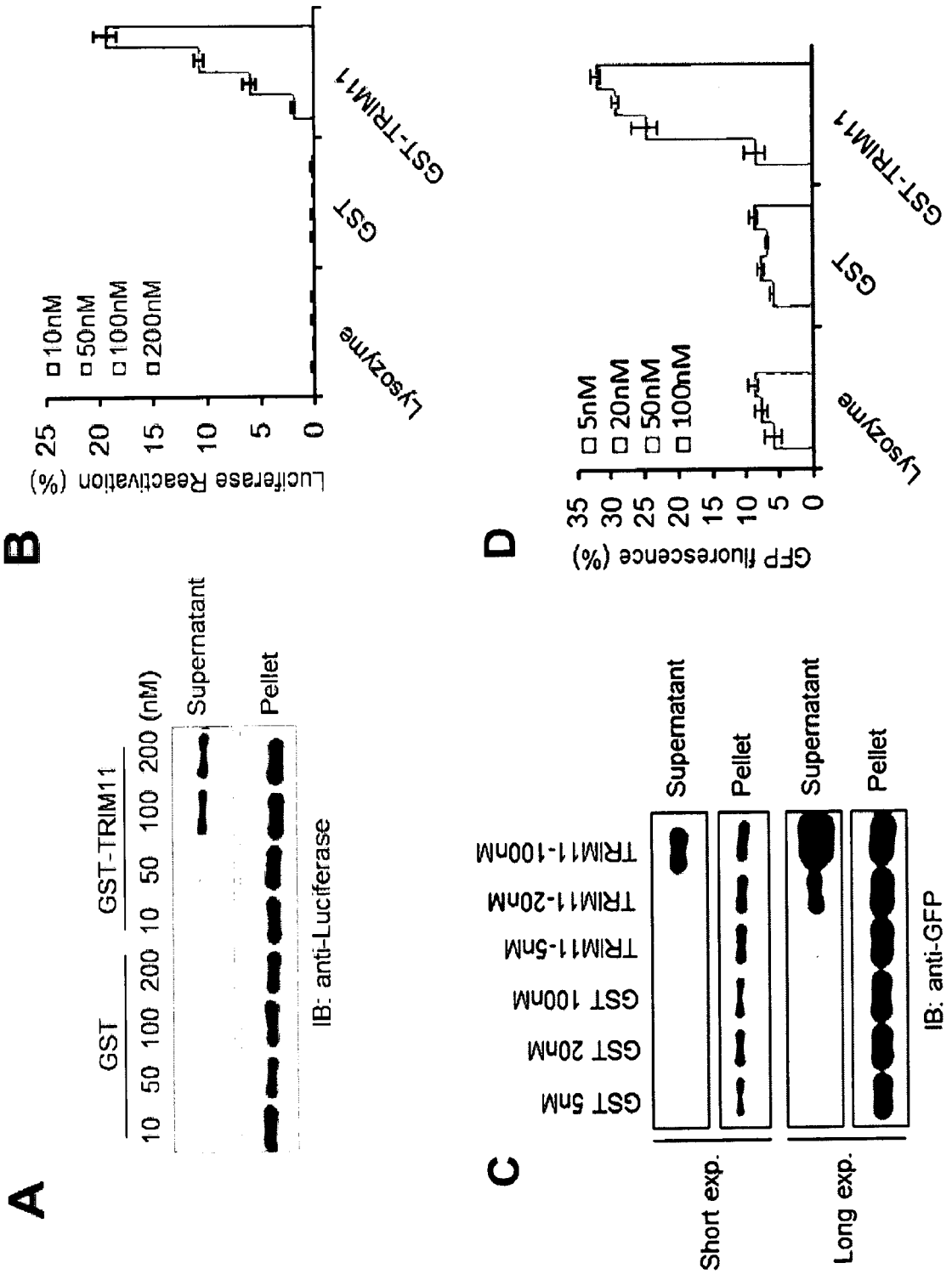


Figure 34A – Figure 34D

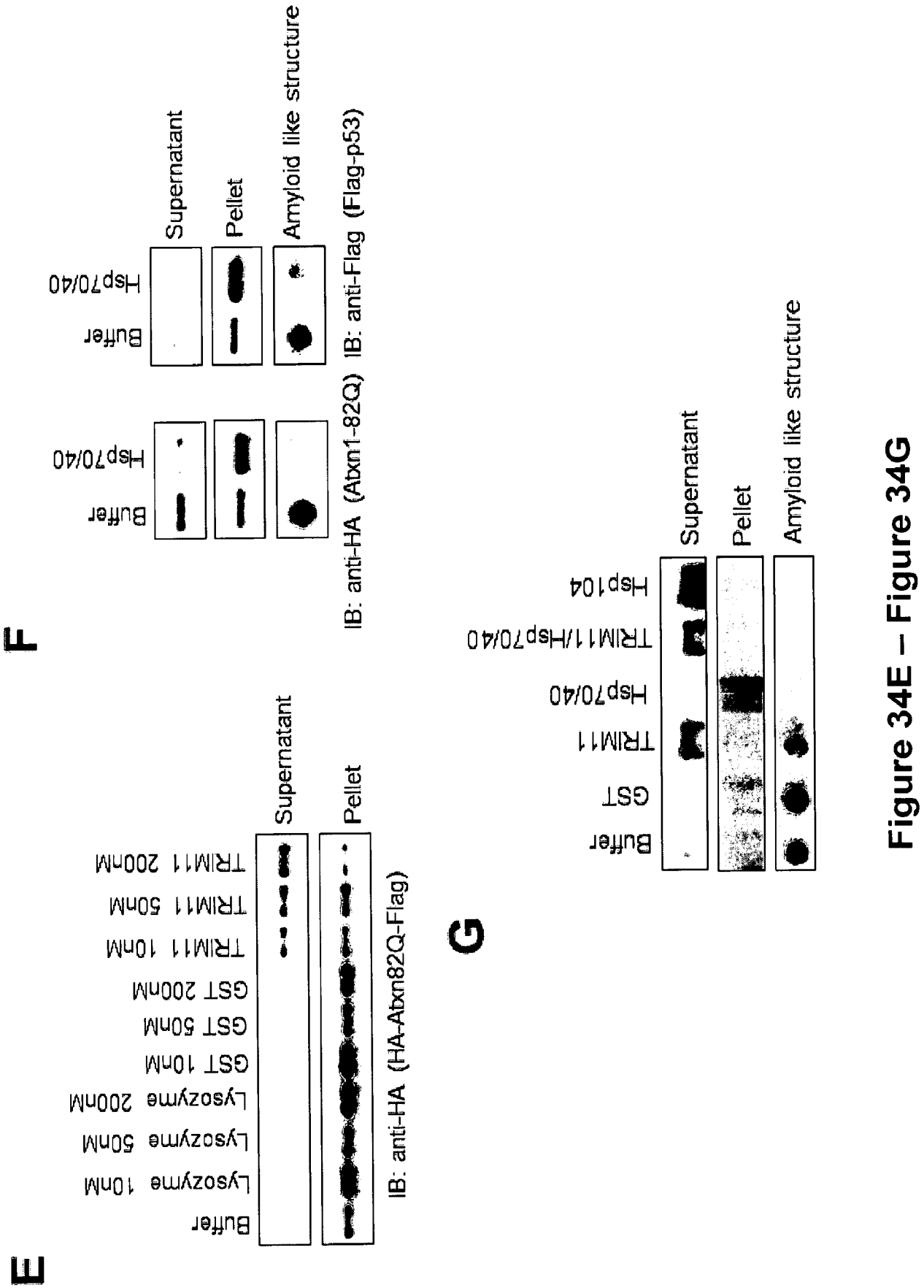


Figure 34E – Figure 34G

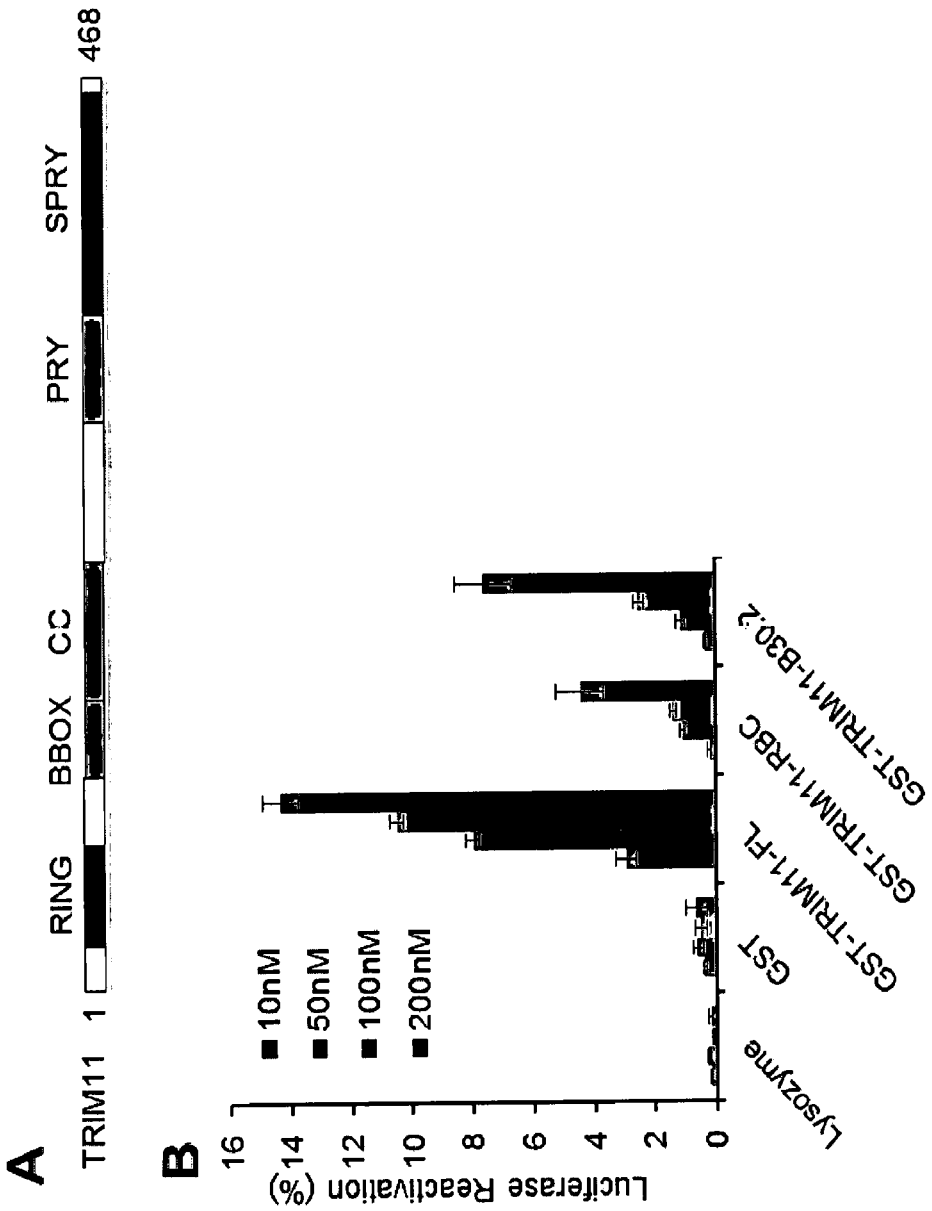
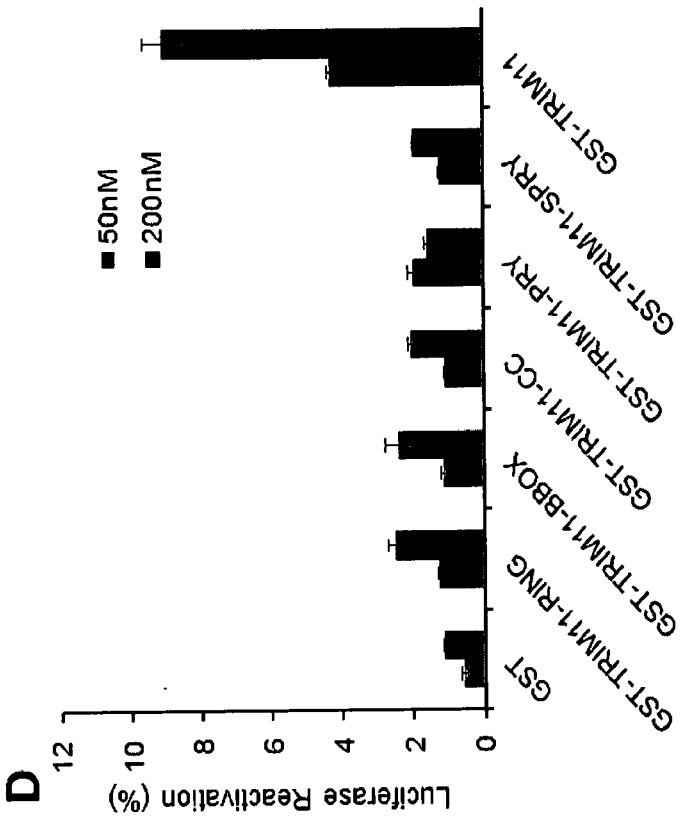


Figure 35A – Figure 35B



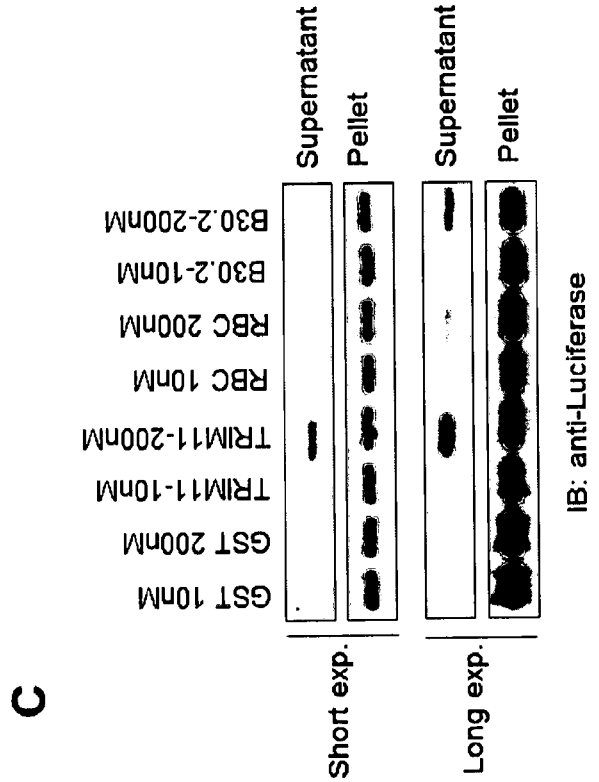


Figure 35C – Figure 35D

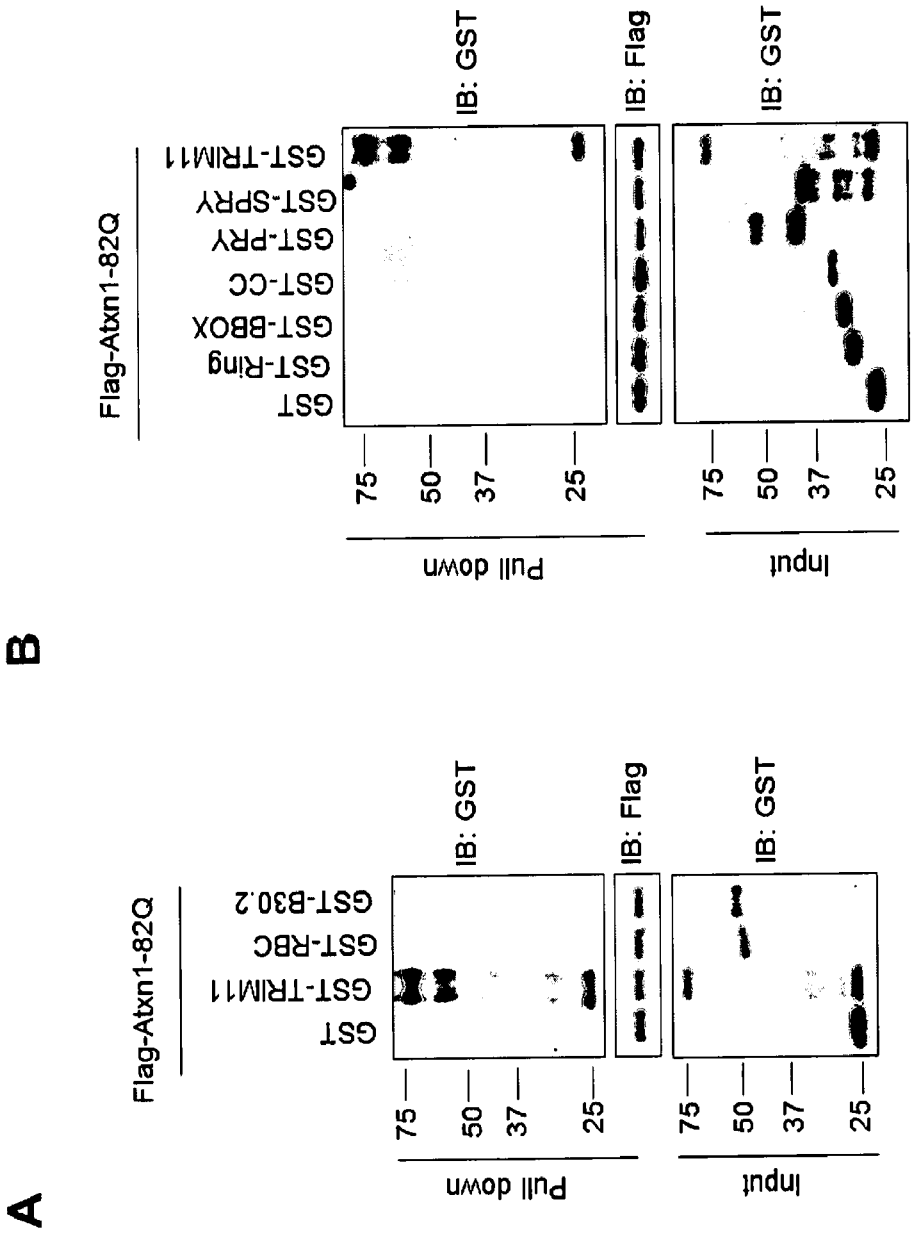


Figure 36A – Figure 36B

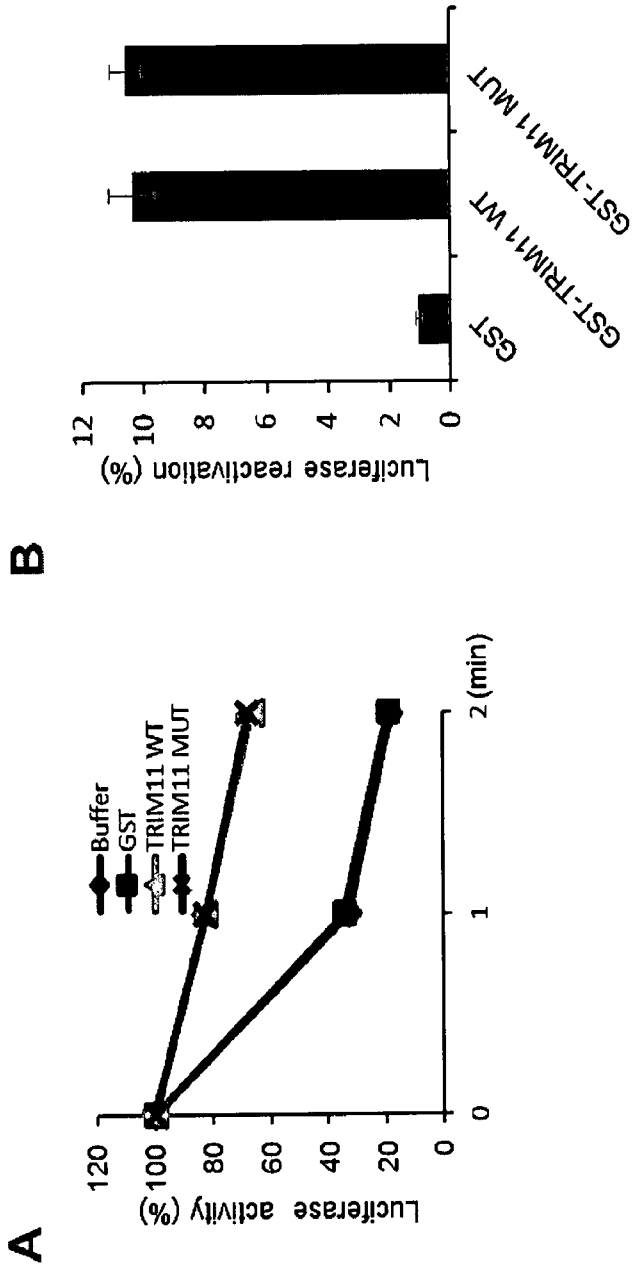


Figure 37A – Figure 37B

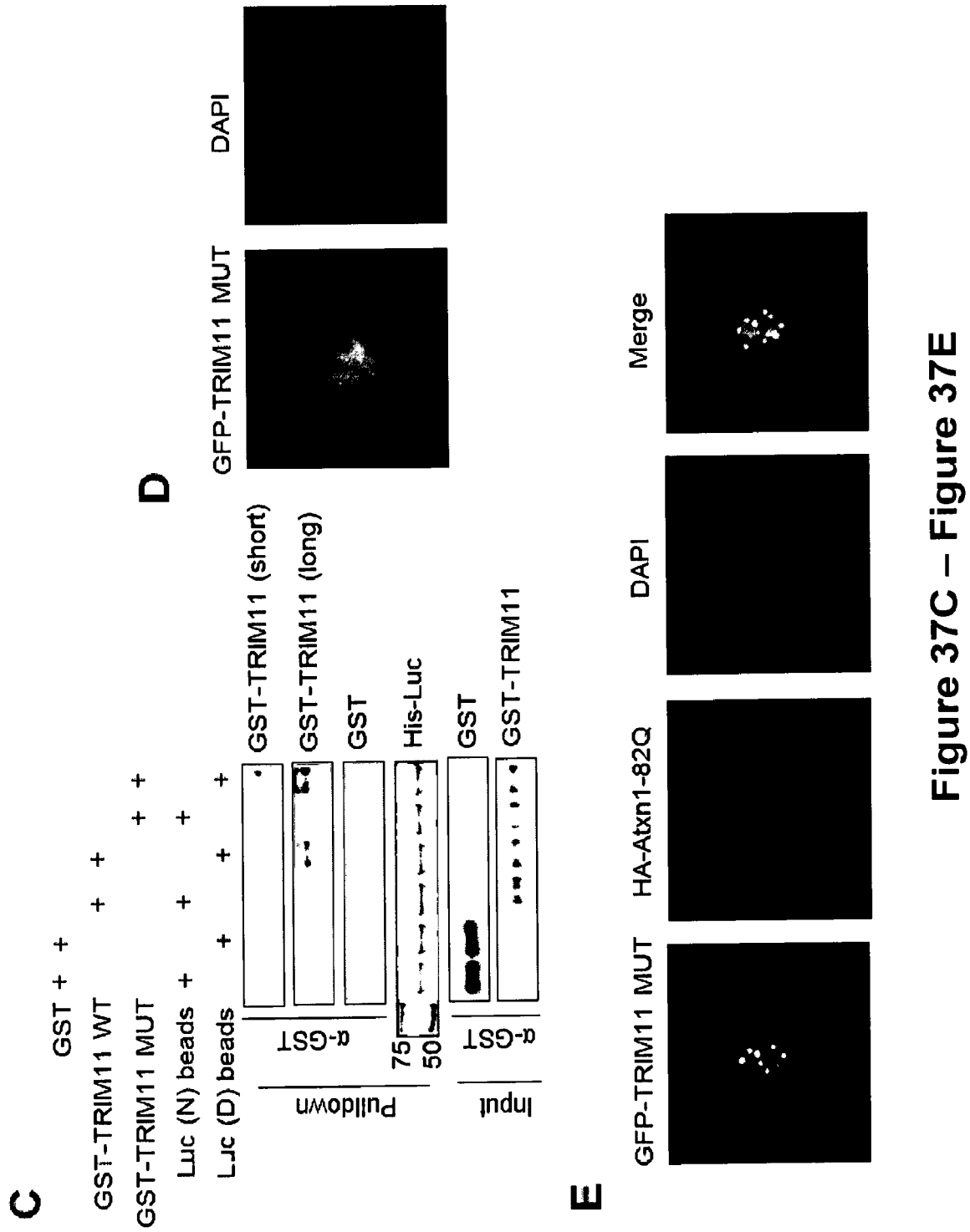


Figure 37C – Figure 37E

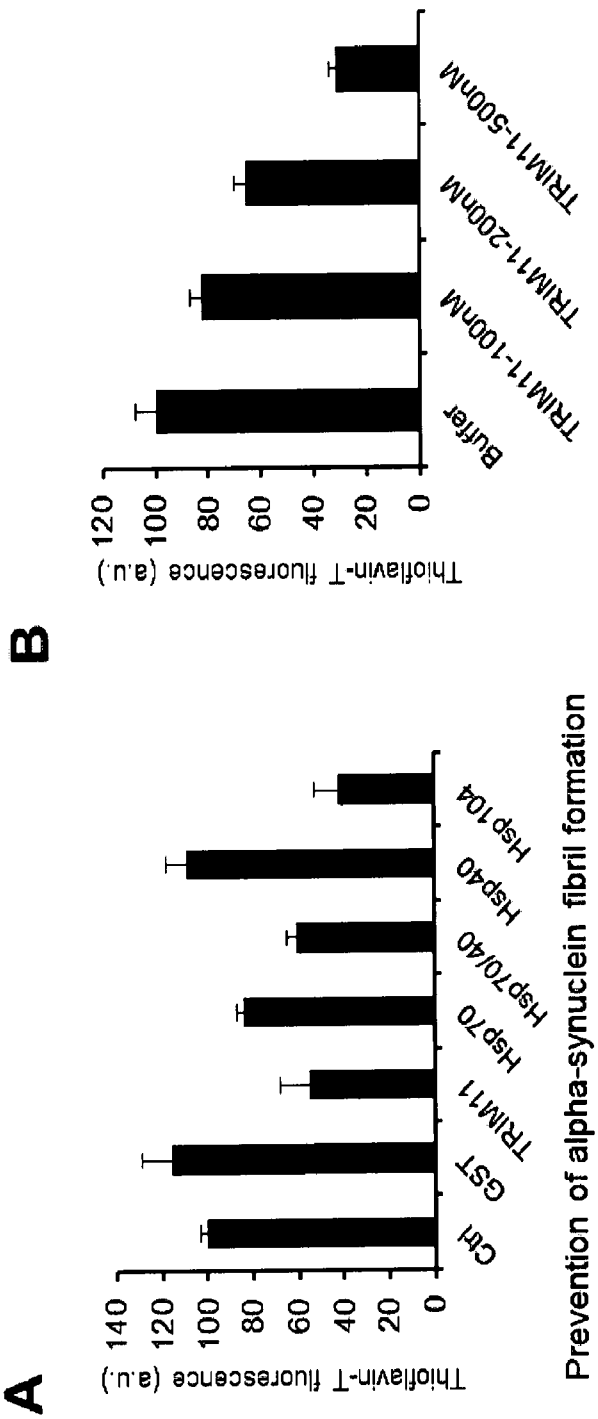


Figure 38A – Figure 38B

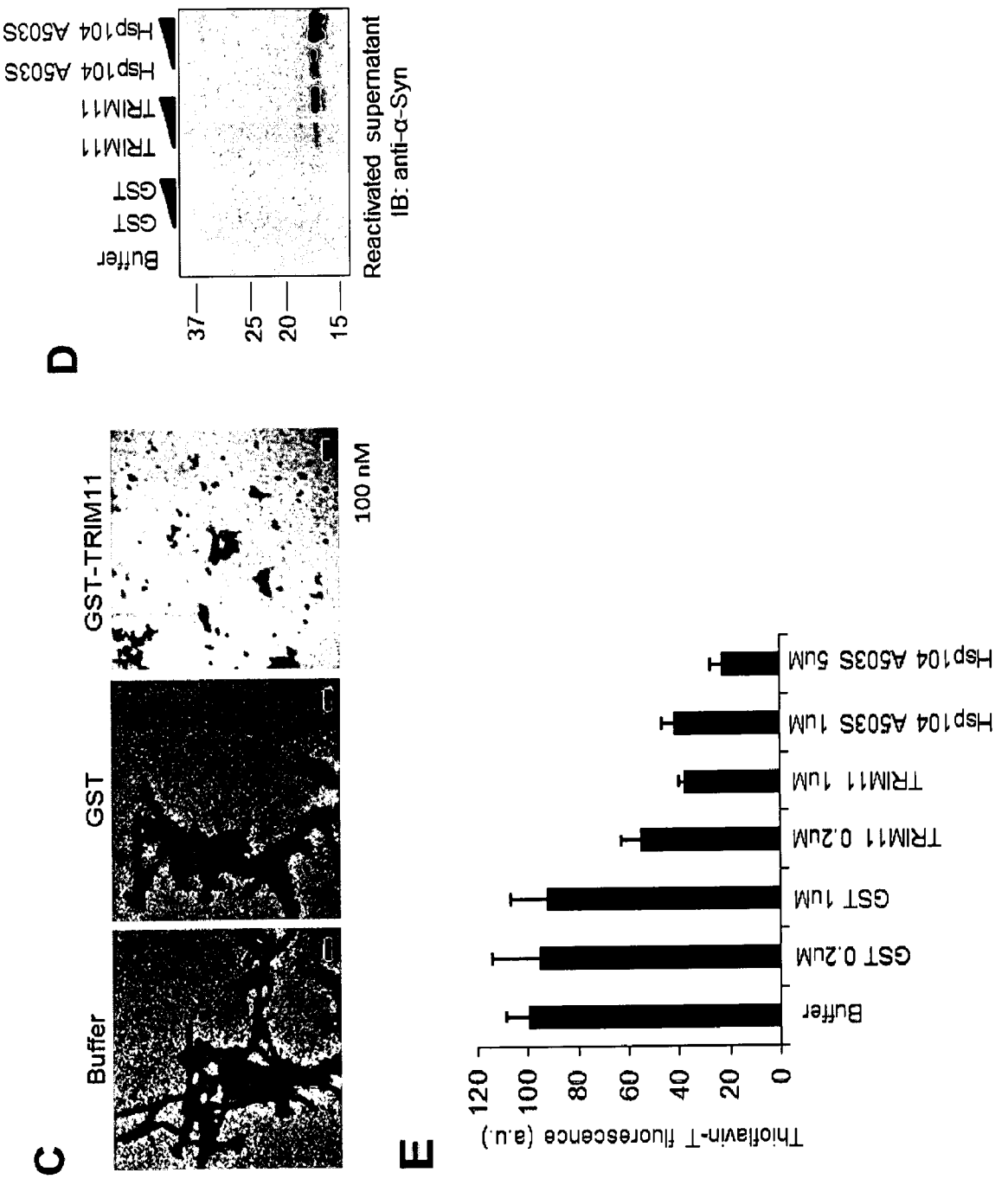


Figure 38C – Figure 38E

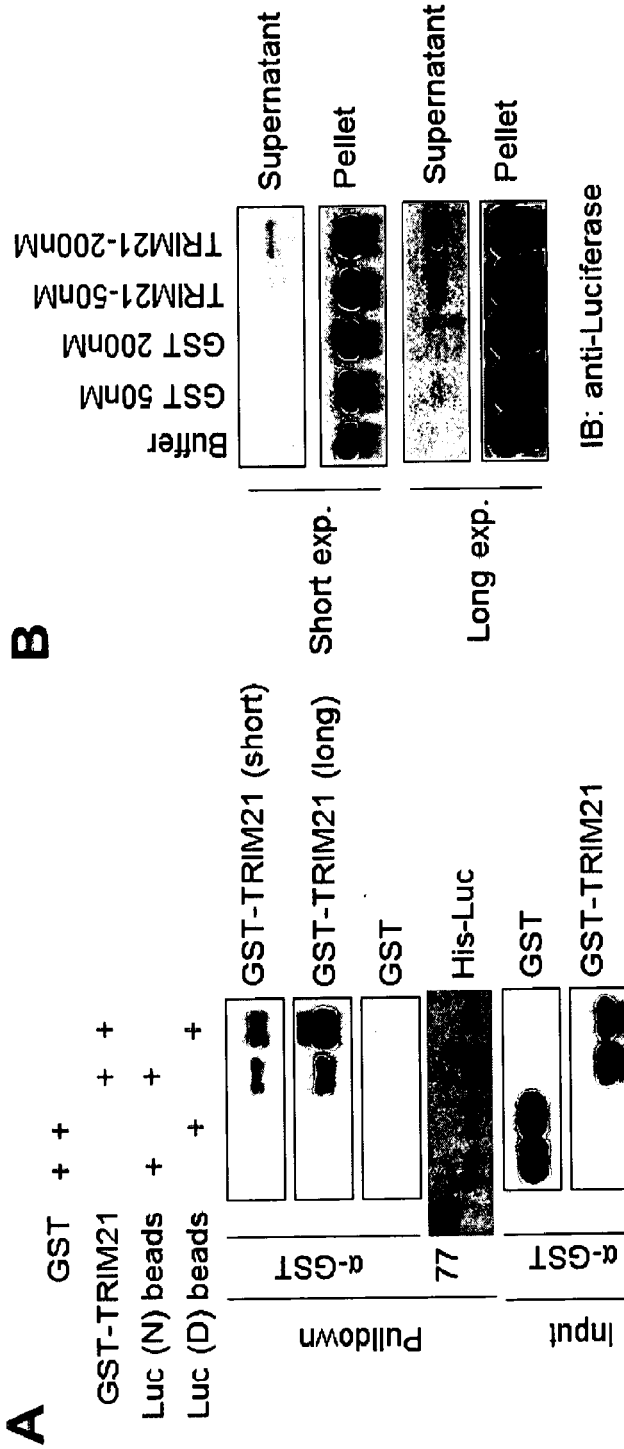


Figure 39A – Figure 39B

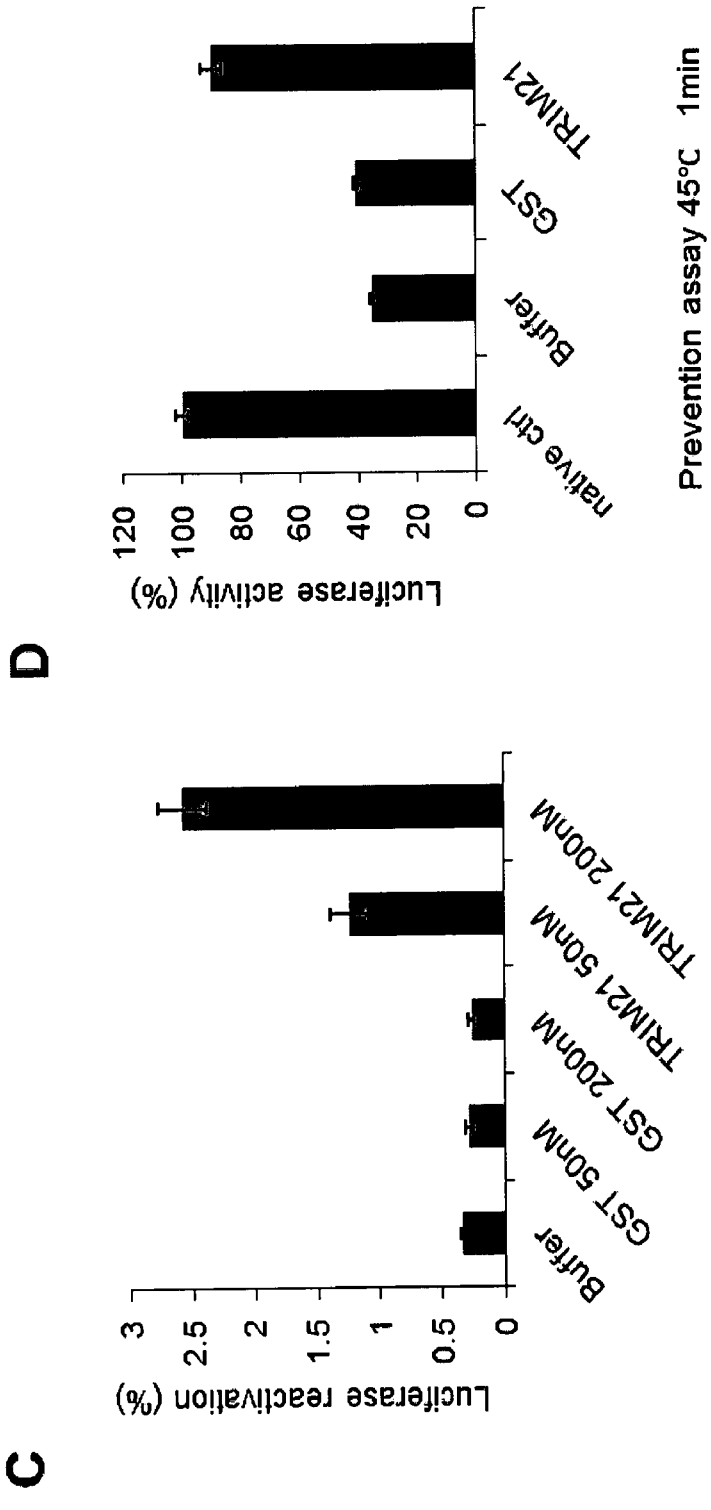
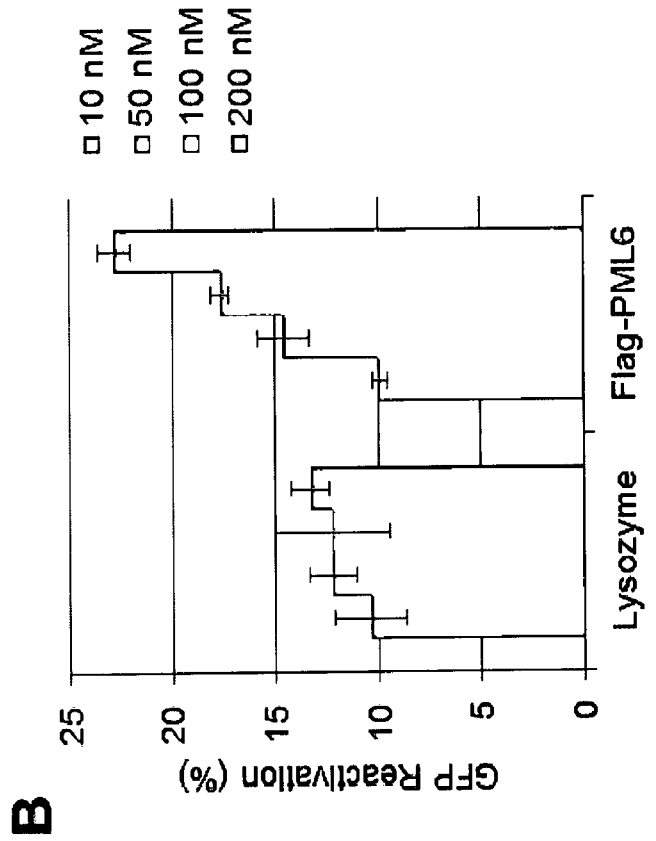


Figure 39C – Figure 39D



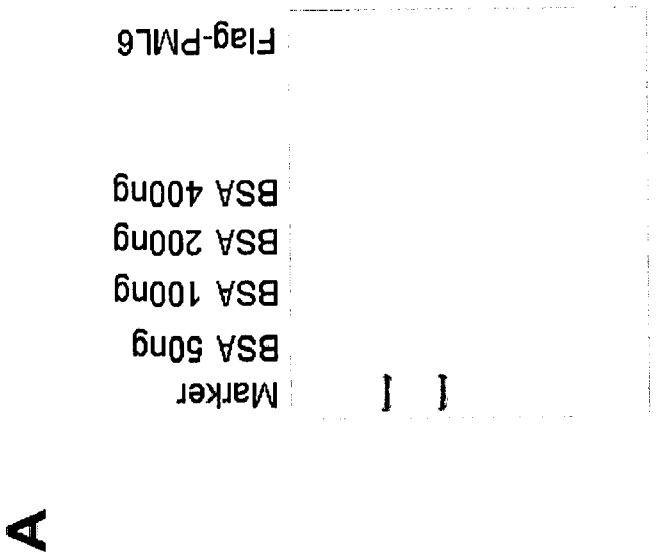


Figure 40A – Figure 40B

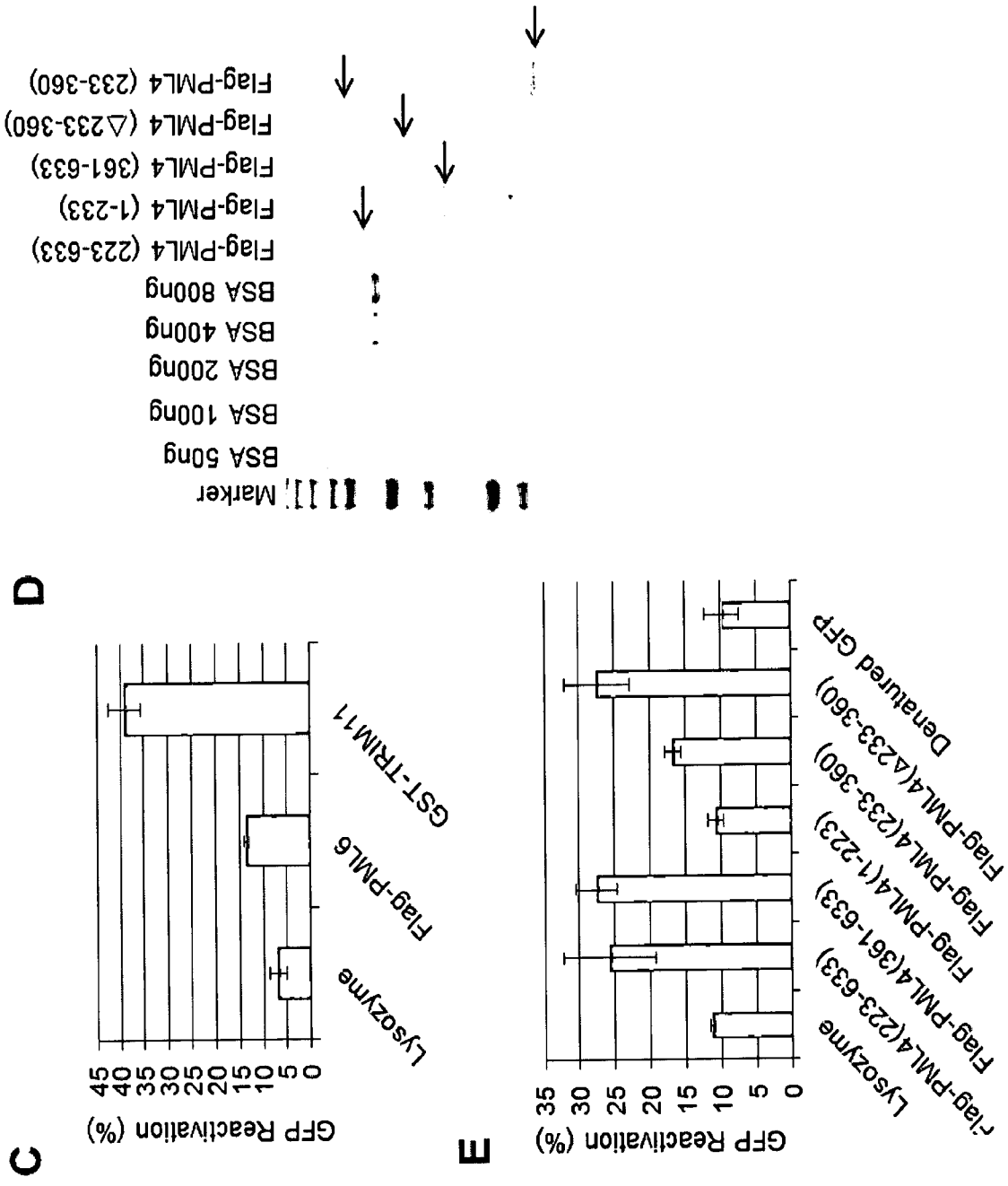
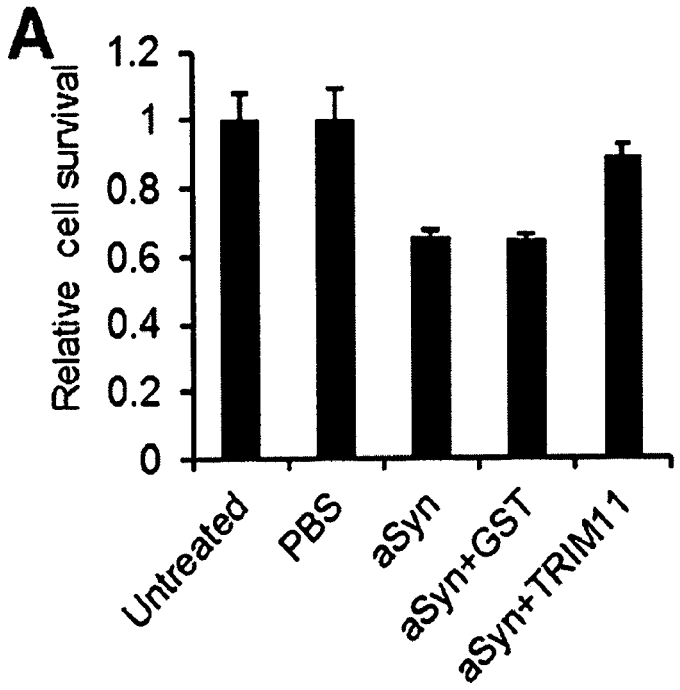


Figure 40C – Figure 40E



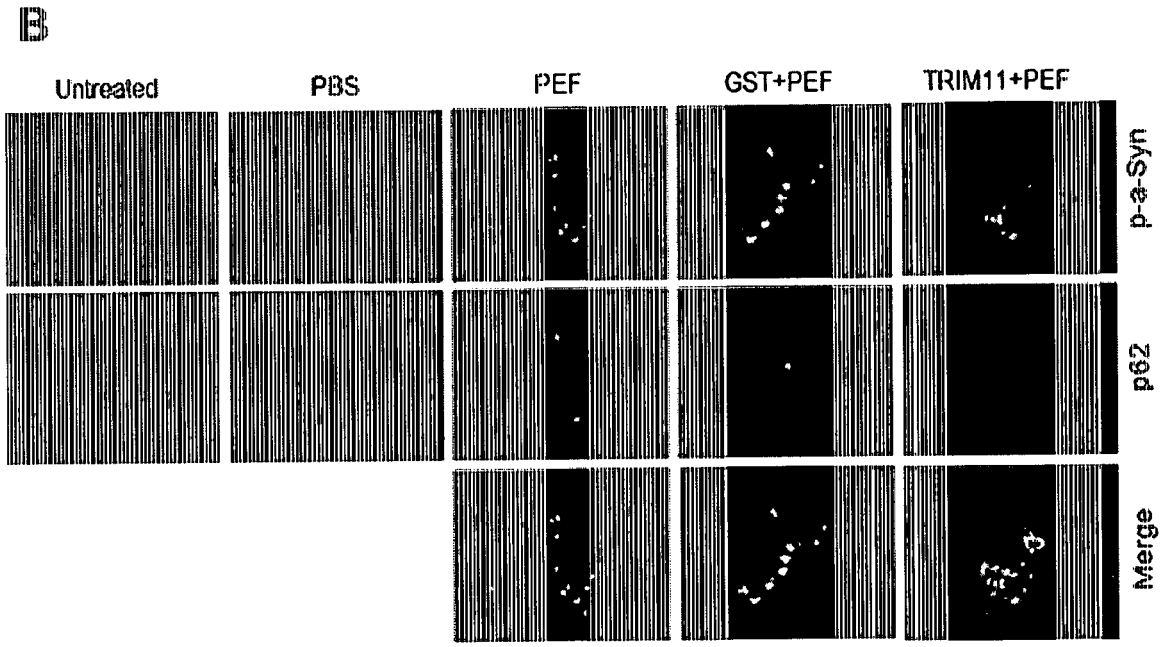


Figure 41A – Figure 41B

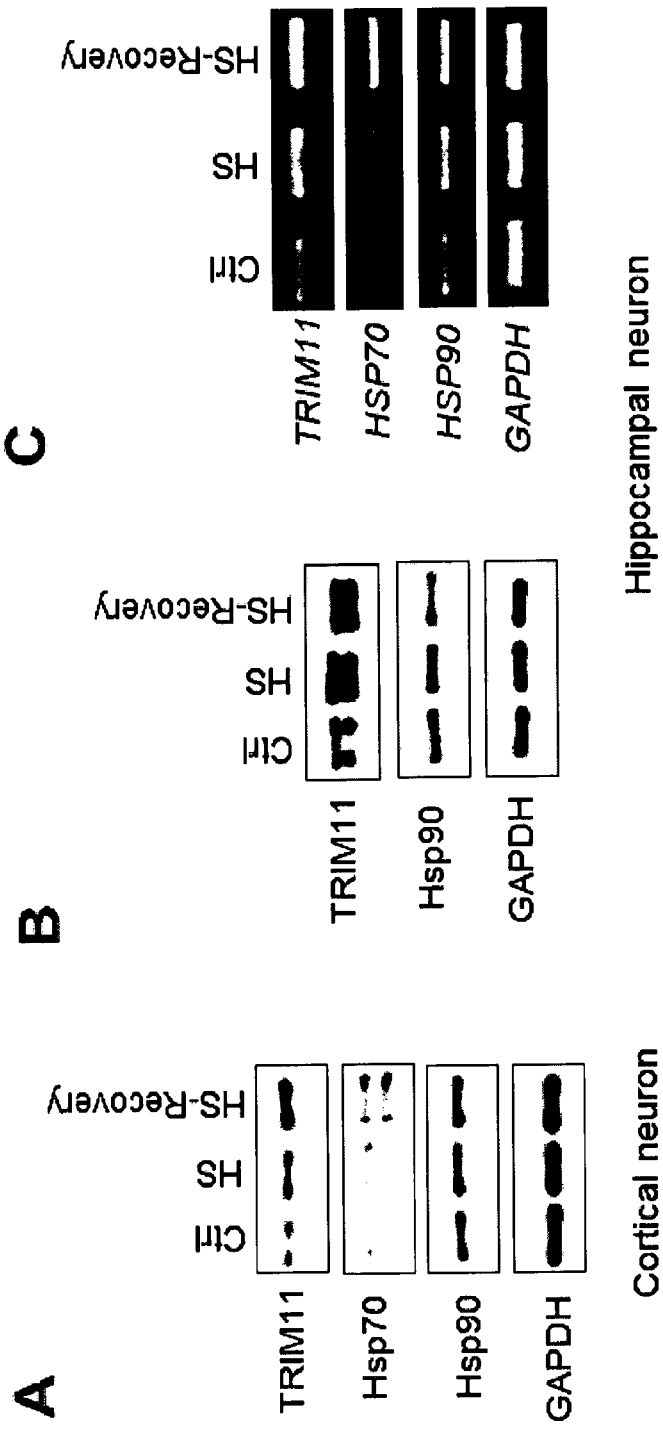


Figure 42A – Figure 42C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/034751

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 11/00; A61P 25/28; G01N 33/566; G01N 33/68 (2016.01)
CPC - A61K 47/48169; G01N 33/6845; G01N 33/6896; G01N 2333/4703; G01N 2333/90 (2016.08)
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)
IPC - A61P 11/00; A61P 25/28; G01N 33/566; G01N 33/68
CPC - A61K 47/48169; G01N 33/6845; G01N 33/6896; G01N 2333/4703; G01N 2333/90; G01N 2333/9015
G01N 2440/00; G01N 2500

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/7.1; 435/375; 436/501; 530/350 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar.
Search terms used: TRIM19, PML, RNF4, STUbl, ULS, E3-S, SUMO, targeted, aggregation, accumulation, folding, misfolding, conformation, modulate, increase, activity, induction, expression, protein, peptide, amyloidosis, CPP, penetrating, cystic fibrosis, CFTR, p53

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHORT et al. "Interferon β induces clearance of mutant ataxin 7 and improves locomotion in SCA7 knock-in mice," Brain, 21 March 2013 (21.03.2013), Vol. 136, Pt. 6, Pgs. 1732-45. entire document	1-4, 9, 10, 13-16, 21, 22, 25-28, 30-32
Y		5-8, 11, 12, 17-20, 23, 24, 29, 33
X	AHNER et al. "Small heat shock proteins target mutant cystic fibrosis transmembrane conductance regulator for degradation via a small ubiquitin-like modifier-dependent pathway," Mol Biol Cell, 14 November 2012 (14.11.2012), Vol. 24, No. 2, Pgs. 74-84. entire document	34-41
X	URANO et al. "TRIM44 interacts with and stabilizes terf, a TRIM ubiquitin E3 ligase," Biochem Biophys Res Commun, 07 April 2009 (07.04.2009), Vol. 383, No. 2, Pgs. 263-8. entire document	42-44
Y		5-8, 17-20
Y	WO 2014/086835 A1 (RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG) 12 June 2014 (12.06.2014) entire document	6, 7, 18, 19
Y	US 2010/0263062 A1 (DILLIN et al) 14 October 2010 (14.10.2010) entire document	11, 23
Y	HAUPT et al. "Loss of PML cooperates with mutant p53 to drive more aggressive cancers in a gender-dependent manner," Cell Cycle, 01 June 2013 (01.06.2013), Vol. 12, No. 11, Pgs. 1722-31. entire document	12, 24
Y	LUKACS et al. "Conformational maturation of CFTR but not its mutant counterpart (delta F508) occurs in the endoplasmic reticulum and requires ATP," EMBO J. 15 December 1994 (15.12.1994), Vol. 13, No. 24, Pgs. 6076-86. entire document	29, 33

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" 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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" 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
"X" 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
"Y" 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

Date of the actual completion of the international search	Date of mailing of the international search report
03 August 2016	09 SEP 2016

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P O Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/034751

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0208455 A1 (JANER et al) 20 August 2009 (20.08.2009) entire document	1-44

摘要

本發明涉及用於促進錯誤折疊的蛋白質和蛋白質聚集體的降解的組合物和方法。該組合物和方法可以用於治療與錯誤折疊的蛋白質或者蛋白質聚集體有關的病症。在某些情況下，該組合物和方法涉及一種或多種TRIM蛋白或者一種或多種STUbL的調節劑。