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(71) Applicant: STC.UNM [US/US]; 801 University Blvd. SE,
Ste 101, Albuquerque, NM 87106 (US).(72) Inventors: DERETIC, Vojo, P.; 45 Calle Cienega, Placitas,
NM 87043 (US). CHAUHAN, Santosh; 3901 Montgomery Blvd NE,
Apt#1104, Albuquerque, NM 87109 (US). NIMURA, Tomonori; 10700 Academy Rd. NE, Apt
2318, Albuquerque, NM 87111 (US).(74) Agent: COLEMAN, Henry, D.; Cosud Intellectual Property
Solutions, P.C., 714 Colorado Avenue, Bridgeport, CT
06605-1601 (US).

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(54) Title: IRGM AND PRECISION AUTOPHAGY CONTROLS FOR ANTIMICROBIAL AND INFLAMMATORY DISEASE STATES AND METHODS OF DETECTION OF AUTOPHAGY

(57) Abstract: The present invention relates to the discovery that IRGM, encoded by a uniquely human gene which confers risk for inflammatory diseases, affects autophagy through a hitherto unknown mechanism. The present invention shows that IRGM controls autophagy and that IRGM modulators, in particular, double-stranded RNA, including poly I:C, poly-UG (polyUGUGU) and poly-CLC and muramyl dipeptide and related analogs of same, including N-acetyl muramyl-L-alanyl-D-isoglutamine (DMP) and numerous other compounds as identified herein, which may be used alone, in combination, or in combination with alternative autophagy modulators and additional bioactive agents to provide effective therapies for a number of diseases, including cancer, bacterial infections and inflammatory diseases, especially including tuberculosis infections and Crohn's disease, among others. The present invention is also directed to compositions and methods for treating inflammatory or autophagy-related diseases including diseases which cause excessive inflammation in patients.



IRGM and Precision Autophagy Controls for Antimicrobial and Inflammatory Disease States and Methods of Detection of Autophagy

Related Applications and Grant Support

This application claims the benefit of priority of provisional applications US62/121,232, filed 26 February 2015, entitled "IRGM Controls the Core Autophagy Machinery to Conduct Antimicrobial Defense and Modulate Inflammatory Disease States" and US62/165,357, filed May 22, 2015, entitled "Methods for Regulating Inflammation By Precision Autophagy", the entire contents of each of these applications is incorporated by reference in its entirety herein.

This invention was made with government support under grant nos. AI04229 and AI111935, awarded by National Institutes of Health and grant no. ULTR000041, awarded by the National Center for Advancing Translation Sciences. The government has certain rights in the invention.

Field of the Invention

The present invention relates to the discovery that IRGM, encoded by a uniquely human gene which confers risk for inflammatory diseases, affects autophagy through a hitherto unknown mechanism. The present invention shows that IRGM controls autophagy and that IRGM modulators, in particular, double-stranded RNA, including poly I:C, poly-UG (polyUGUGU) and polyICLC and muramyl dipeptide and related analogs of same, including N-acetyl muramyl-L-alanyl-D-isoglutamine (DMP) and numerous other compounds as identified herein, which may be used alone, in combination, or in combination with alternative autophagy modulators and/or additional bioactive agents to provide effective therapies for a number of diseases, including cancer, bacterial infections and inflammatory diseases, especially including tuberculosis infections and Crohn's disease, among others.

The present invention is also directed to compositions and methods for treating inflammatory or autophagy-related diseases including diseases which cause excessive inflammation in patients. The approach taken to the treatment of these disease states and conditions which cause excessive inflammation is referred to as precision autophagy. This method utilizes interferon, especially interferon-gamma (IFN-gamma), pegylated interferon (PEG-IFN) and related compounds and/or certain TRIM proteins or variants thereof having at

least 90% sequence identity as described herein, in particular, TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:197), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302), TRIM75 (SEQ ID NO:338) and mixtures thereof, preferably TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:36), TRIM 20 (SEQ ID NO:96), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297), and mixtures thereof to treat extreme inflammation associated with disease states that cause excessive inflammation. Methods and pharmaceutical compositions are disclosed herein.

Background of the Invention

Autophagy is a cellular homeostatic mechanism with broad roles in human health and disease (Mizushima et al., 2008). Autophagy is at the intersection of metabolic (Rabinowitz and White, 2010; Settembre and Ballabio, 2014) and antimicrobial processes (Deretic et al., 2013; Ma et al., 2013). Thus, the system responds to a range of inputs such as starvation (Chauhan et al., 2013; Efeyan et al., 2013; Mihaylova and Shaw, 2011), lysosomal disruption (Settembre and Ballabio, 2014), endogenous danger associated molecular patterns and microbial products commonly referred to as pathogen-associated molecular patterns (PAMPS) (Deretic et al., 2013; Ma et al., 2013). Autophagic responses to PAMPS lead to direct antimicrobial action through a process termed xenophagy (Gomes and Dikic, 2014; Levine, 2005) and control of inflammation and other immune processes (Deretic et al., 2013).

Among the better-established links between autophagy and human diseases are the genetic polymorphisms in ATG16L1 and IRGM conferring risk for Crohn's disease (CD), an intestinal inflammatory disorder (Consortium, 2007; Craddock et al., 2010; Murthy et al., 2014). The human population polymorphisms in IRGM have been linked to autophagy (Consortium, 2007; Craddock et al., 2010) and to its effector outputs including antimicrobial defense (Brest et al., 2011; McCarroll et al., 2008). In keeping with its autophagy-mediated

antimicrobial role, IRGM is additionally a genetic risk factor for tuberculosis in different human populations (Bahari et al., 2012; Che et al., 2010; Intemann et al., 2009; King et al., 2011; Song et al., 2014) and may afford protection in leprosy (Yang et al., 2014). However, the molecular mechanism of IRGM's function in autophagy has remained a mystery.

IRGM has no homologs among the Atg genes in yeast, which makes it difficult to assign to it an autophagy-specific function; instead, IRGM has been considered to affect autophagy indirectly (Singh et al., 2006). A complicating factor in understanding the exact function of IRGM is that it is distinctly a human gene (Bekpen et al., 2010). Its orthologs are present only in African great apes and *Homo sapiens* but active alleles are absent in ancestral evolutionary lineages leading up to them (Bekpen et al., 2009). The mouse genome encodes a large family of immunity related GTPase (21 IRG genes) compared to a single gene (IRGM) in humans; furthermore, all murine IRGs encode ca. 40-kDa proteins that are much larger than the human IRGM (21 kDa). The prevailing view of the murine IRGs is that they have predominantly non-autophagy functions (Choi et al., 2014; Zhao et al., 2008). Thus the significant information gathered in the murine systems may have limited import on how the human IRGM works.

Given the significance of IRGM in human populations and the notoriously high prevalence of diseases such as CD and tuberculosis, it is surprising that IRGM's mechanism of action in autophagy remains unknown. Here we report that unexpectedly, IRGM physically interacts with key autophagy regulators, ULK1, Beclin 1, ATG14L and ATG16L1. We also show that, remarkably, IRGM links inputs from PAMP sensors by making molecular complexes with NOD2, another genetic risk factor in CD (Eckmann and Karin, 2005; Hugot et al., 2001; Ogura et al., 2001). The formation of NOD2-IRGM complex is stimulated in response to PAMPs, whereas increased association of NOD2 with IRGM promotes IRGM-directed assembly of autophagy regulators. IRGM undergoes post-translational modifications that stabilize components of the core autophagic machinery, and mutant IRGM protein that cannot direct these modifications is disabled for its role in autophagic defense against invasive bacteria.

Therapies to modulate autophagy are entering clinical trials but methods of monitoring whether drugs modulate autophagy in patients during such treatment are currently unavailable, but badly needed. In one aspect, the present invention addresses that need.

Brief Description of the Invention

IRGM, encoded by a uniquely human gene conferring risk for inflammatory diseases, affects autophagy through a hitherto unknown mechanism. The present invention is directed to showing that IRGM controls autophagy. IRGM interacts with ULK1 and Beclin 1 and promotes their coassembly into molecular complexes. IRGM stabilizes ULK1 and affects the stability of Beclin 1-interactors thus governing the composition of autophagy initiation complexes. We further show that IRGM interacts with pattern recognition receptors including NOD2. IRGM, NOD2 and ATG16L1, all of which are Crohn's disease risk factors and form a molecular complex to modulate autophagic responses to microbial products. NOD2 enhances K63-linked polyubiquitination of IRGM, which is required for interactions of IRGM with the core autophagy factors and for bacterial clearance. Thus, IRGM plays a direct role in organizing the core autophagy machinery to endow it with antimicrobial functions.

In one embodiment, the present invention relates to the use of IRGM modulators for the treatment of disease, in particular, bacterial infections and inflammatory diseases, most notably tuberculosis and Crohn's disease amongst a number of others. The compounds which are useful as modulators of IRGM include the double stranded RNA compounds, including poly I:C, poly-UG (poly UGUGU) and poly ICLC, among others, and muramyl dipeptide and its analogs and derivatives as otherwise disclosed herein.

In one embodiment, the present invention provides a method of modulating autophagy in a biological system, in particular a patient or subject. In this aspect of the invention, a compound identified herein as an IRGM modulator (which can be an inhibitor or agonist of IRGM and/or its pathway(s)), is presented to the biological system, including administration to a patient or subject in need, in order to modulate autophagy and effect a favorable result in the biological system, often a patient or subject. The resulting modulation may be monitored or applied in the biological system to effect a favorable result, including the inhibition, treatment and/or prevention of cancer, including metastasis of cancer, or the inhibition, treatment (including the amelioration of symptoms) and/or prevention of one or more disease states or conditions in which the modulation, especially including upregulation or inhibition of autophagy provides a favorable result in numerous disease states and/or

conditions including neurodegeneration (including, for example, Alzheimer's disease, Parkinson's disease; other ataxias), chronic inflammatory diseases (including, for example, inflammatory bowel disease, including Crohn's disease, rheumatoid arthritis, lupus, multiple sclerosis, chronic obstructive pulmonary disease/COPD, pulmonary fibrosis, cystic fibrosis, Sjogren's disease), diabetes and metabolic syndrome, muscle degeneration and atrophy, frailty in aging, stroke and spinal cord injury, arteriosclerosis, infectious diseases, especially bacterial infections such as tuberculosis, viral infections (HIV I and II, HBV, HCV, including secondary disease states or conditions associated with infectious diseases, including AIDS) and tuberculosis, among others. The common principle of this embodiment of the invention is that compounds which modulate IRGM, are outstanding autophagy modulators (i.e., inhibitors or activators of autophagy), depending upon the disease state, condition or symptom to be treated, may cure, prevent (including reducing the likelihood of), improve prognosis, ameliorate symptoms and/or improve the quality of the patient's or subject's life. In addition, in the therapeutic aspects of the invention, the administration of an autophagy modulator (i.e., one or more IRGM modulators alone or in combination with an additional autophagy modulator and/or an additional bioactive agent) may prolong the life of the patient, as well as improve the quality of life in the aging patient or subject.

In one embodiment the method of treating an autophagy-mediated disease state or condition comprising administering at least one dsRNA or a muramyl dipeptide analog or derivative (collective referred to as "IRGM modulators"), optionally in combination with at least one additional autophagy modulator and/or bioactive agent to a patient in need. In this method at least one IRGM modulator as described above, alone or in combination with an additional autophagy modulator, such as an autophagy modulator selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon and nortriptyline, tetrachlorisophthalonitrile and phenylmercuric acetate, pharmaceutically acceptable salts thereof and mixtures thereof, alone, optionally in further combination with at least one additional bioactive agent, optionally in combination with a pharmaceutically acceptable carrier, additive or excipient, may be administered to a patient or subject in need to treat an autophagy-mediated disease state and/or condition. It is noted that flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine,

memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline and their pharmaceutically acceptable salts show activity as agonists or inducers of autophagy in the treatment of an autophagy-mediated disease, tetrachlorisophthalonitrile, phenylmercuric acetate and their pharmaceutically acceptable salts, find use as antagonists or inhibitors of autophagy. All of these compounds will find use as modulators of autophagy in the various autophagy-mediated disease states and conditions described herein, with the agonists being preferred in most disease states other than cancer and in the case of the treatment of cancer, the inhibitors described above are preferred, alone or in combination with an autophagy agonist as described above and/or an additional anticancer agent as otherwise described herein.

Pharmaceutical compositions according to the present invention comprise an effective amount of at least one IRGM modulator as described herein in combination with an autophagy modulator selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphen citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, tetrachlorisophthalonitrile, phenylmercuric acetate and their pharmaceutically acceptable salts, optionally in combination with a pharmaceutically acceptable carrier, additive and/or excipient and further optionally, in combination with at least one additional bioactive agent (e.g., an anticancer agent, antibiotic, anti-tuberculosis agent, antiviral agent such as an anti-HIV agent, anti-HBV agent or anti-HCV agent, etc.), preferably at least one anticancer agent as otherwise disclosed herein or at least one additional autophagy modulator as otherwise described herein. In the present invention, an additional autophagy modulator (autostatin) may be selected from the group consisting of may be combined with an additional autophagy modulator selected from the group consisting of benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandrosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurazone, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clocortolone, doxycycline, pirlindole

mesylate, doxazosin, depropine, nocodazole, scopolamine, oxybenzone, halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene and mixtures thereof.

In still another embodiment, the invention provides a method of treating a subject who has been infected with tuberculosis (e.g. *M. tuberculosis*) or who is at risk of such infection, the method comprising administering to the subject a pharmaceutically effective amount of a IRGM modulator as described hereinafter. In another embodiment, the invention provides a method of treating Crohn's disease comprising administering to a patient in need a pharmaceutically effective amount of a IRGM modulator as described hereinafter.

The present invention provides methods of treating inflammatory or autophagy-related diseases. Autophagy is a eukaryotic intracellular pathway that carries out key aspects of cytoplasmic homeostasis. Autophagy has many biological effects that include immunological processes and inflammation, and one aspect is regulation of activation inflammasome activity. We disclose the methods to regulate disease-causing excessive inflammation by one form of selective autophagy named precision autophagy. This method provide therapeutic options for inflammatory or autophagy-related diseases by modulating precision autophagy. Several forms of precision autophagy could be induced by compounds, such as IFN-gamma, or related compounds. The present invention could be used to upregulate autophagy, for example in the case of disease states such as tuberculosis and other disease states where an upregulation of autophagy would be beneficial for disease treatment. This therapy could be effected by administering an effective amount of one or more TRIM proteins as otherwise described herein to a patient in need, the result being the upregulation of autophagy and the treatment of a disease state and/or condition which is mediated through autophagy (an autophagy-mediated disease). In other instances, the present invention could be used to regulate (i.e. down-regulate) some forms of precision autophagy, and precision autophagy in turn modulate several forms of inflammation, such as inflammasome or type I interferon response in order to bring the autophagy response back in to balance. The targeting disorders for precision autophagy down-regulation include autophagy-related diseases or inflammatory diseases, including autoimmune diseases, infectious diseases, cardiovascular diseases, and metabolic diseases including diabetes mellitus. For example, the inflammatory response is essential to human beings, however, excessive inflammatory response is a lethal condition seen in several diseases in different stages, including autoimmune diseases and

acute viral/bacterial infection. The inventors have found that the excessive inflammation associated with these disease states and/or conditions could be regulated by precision autophagy, including the administration of siRNAs as described herein which specifically inhibit one or more TRIM proteins as otherwise described herein. In addition, the inventors find that certain disease states could benefit from an initial upregulation of autophagy which could benefit the disease treatment, followed by down-regulation of autophagy during the course of therapy for the disease state and/or condition in order to reduce an excessive autophagy response.

Thus, the present invention utilizes certain preferred precision autophagy modulators to treat disease states and conditions which cause excessive inflammation and particularly seen in a number of disease states, especially including inflammatory diseases as otherwise described herein, autoimmune diseases, infectious diseases (generally, after an initial period of beneficial upregulation of autophagy), cardiovascular diseases and metabolic diseases, including diabetes mellitus. These precision autophagy modulators may include interferons such as interferon gamma (IFN-gamma) and pegylated interferon (PEG-IFN), as well as the preferred TRIM (tripartite motif containing) proteins or variants exhibiting 90% sequence identity to the TRIM proteins, preferably TRIM proteins selected from at least one TRIM protein selected from the group consisting of TRIM1, TRIM3, TRIM8, TRIM10, TRIM13, TRIM17, TRIM19, TRIM20, TRIM21, TRIM22, TRIM38, TRIM 41, TRIM43, TRIM44, TRIM45, TRIM46, TRIM54, TRIM55, TRIM56, TRIM58, TRIM59, TRIM60, TRIM65, TRIM66 and TRIM75 with TRIM 1, TRIM 8, TRIM 20, TRIM 21, TRIM 22, TRIM 56 and TRIM 65 and mixtures thereof being preferred as autophagy upregulators.

The present invention relates to a method of treating excessive inflammation in inflammatory diseases, autoimmune diseases, infectious diseases, cardiovascular diseases and metabolic diseases in a patient in need thereof comprising administering to said patient an effective amount of a precision autophagy modulator selected from the group consisting of an interferon, including interferon gamma (IFN-gamma) and pegylated interferon (PEG-IFN) and at least one TRIM protein (including a TRIM protein variant), preferably a TRIM protein selected from the group consisting of TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:197), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID

NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302), TRIM75 (SEQ ID NO:338) and mixtures thereof, preferably TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:36), TRIM 20 (SEQ ID NO:96), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297), and mixtures thereof, optionally in combination with an additional autophagy modulator (including an alternative TRIM protein) and/or an additional bioactive agent. In certain instances, it may be beneficial to down-regulate autophagy and inhibit TRIM protein response in order to reduce an excessive autophagy response through the use of one or more siRNA as described herein which specifically inhibits one or more TRIM protein. Additional autophagy modulators for use in the present invention include, for example, flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphenecitrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, tetrachlorisophthalonitrile and phenylmercuric acetate, benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandrosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurantoin, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clocortolone, doxycycline, pirlindole mesylate, doxazosin, depropion, nocodazole, scopolamine, oxybenzone, halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene, pharmaceutically acceptable salts thereof and mixtures thereof, alternative TRIM proteins or variants exhibiting 90% sequence identity to the TRIM proteins, including, but are not limited to, TRIM2 (SEQ ID NO:6), TRIM 4 (SEQ ID NO:16), TRIM5 (TRIM5 α) (SEQ ID NO:21), TRIM6 (SEQ ID NO:26), TRIM7 (SEQ ID NO:31), TRIM9 (SEQ ID NO:41), TRIM11 (SEQ ID NO:51), TRIM14 (SEQ ID NO:61), TRIM15 (SEQ ID NO:66), TRIM16 (SEQ ID NO:71), TRIM18 (SEQ ID NO:86), TRIM23 (SEQ ID NO:111), TRIM24 (SEQ ID NO:116), TRIM25 (SEQ ID NO:121), TRIM27 (SEQ ID NO:126), TRIM28 (SEQ ID NO:131), TRIM29 (SEQ ID

NO:136), TRIM30, TRIM 31 (SEQ ID NO:141), TRIM32 (SEQ ID NO:146), TRIM33 (SEQ ID NO:151), TRIM34 (SEQ ID NO:156), TRIM35 (SEQ ID NO:161), TRIM36 (SEQ ID NO:166), TRIM37 (SEQ ID NO:167), TRIM39 (SEQ ID NO:177), TRIM40 (SEQ ID NO:182), TRIM42 (SEQ ID NO:192), TRIM47 (SEQ ID NO:217), TRIM48 (SEQ ID NO:222), TRIM49 (SEQ ID NO:227), TRIM50 (SEQ ID NO:232), TRIM51 (SEQ ID NO:237), TRIM55 (SEQ ID NO:252), TRIM68 (SEQ ID NO:312), TRIM72 (SEQ ID NO:323), TRIM73 (SEQ ID NO:328), TRIM74 (SEQ ID NO:333), TRIM76 (SEQ ID NO:343), and mixtures thereof, with TRIM2 (SEQ ID NO:6), TRIM5 (SEQ ID NO:21), TRIM6 (SEQ ID NO:26), TRIM11 (SEQ ID NO:51), TRIM23 (SEQ ID NO:111), TRIM27 (SEQ ID NO:126), TRIM28 (SEQ ID NO:131), TRIM31 (SEQ ID NO:141), TRIM 32 (SEQ ID NO:146), TRIM33 (SEQ ID NO:151), TRIM42 (SEQ ID NO:192), TRIM49 (SEQ ID NO:227), TRIM50 (SEQ ID NO:232), TRIM51 (SEQ ID NO:237), TRIM68 (SEQ ID NO:312), TRIM72 (SEQ ID NO:323), TRIM73 (SEQ ID NO:328), TRIM74 (SEQ ID NO:333) and TRIM (SEQ ID NO:343) being preferred. Neutral lipids such as lipids selected from the group consisting of triglycerides, diglycerides, monoglycerides, glycolated mono- or diacylglycerides, dolichol, polyprenol, polyprenal or very long chain fatty acids may also be administered in combination with the precision autophagy modulators according to the present invention to increase lipid storage and enhance the therapeutic effect of autophagy modulators used to treat excessive inflammation as otherwise disclosed herein. Additional bioactive agents as otherwise described herein may be administered in combination with the one or more of the above precision autophagy modulators and optionally, additional modulators and bioactive agents as otherwise described herein. .

Pharmaceutical compositions according to the present invention comprise an effective amount of interferon, including interferon gamma (IFN-gamma) and pegylated interferon (PEG-IFN) in combination with at least one TRIM protein or a variant thereof, preferably a TRIM protein selected from the group consisting of TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:197), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID

NO:302), TRIM75 (SEQ ID NO:338) and mixtures thereof, preferably TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:36), TRIM 20 (SEQ ID NO:96), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297) and mixtures thereof, optionally in combination with an additional autophagy modulator (including an alternative TRIM protein as otherwise described herein) and/or an additional bioactive agent as otherwise described herein in combination with a pharmaceutically acceptable carrier, additive or excipient. Compositions comprising an effective amount of at least one TRIM protein or a variant thereof, preferably a TRIM protein selected from the group consisting of TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:197), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302), TRIM75 (SEQ ID NO:338) and mixtures thereof, with TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:36), TRIM 20 (SEQ ID NO:96), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297), and mixtures thereof being preferred in combination with at least one additional bioactive agent, including an autophagy modulator as otherwise described herein including an alternative TRIM protein. In addition, neutral lipids such as lipids selected from the group consisting of triglycerides, diglycerides, monoglycerides, glycolated mono- or diacylglycerides, dolichol, polyprenol, polyprenol or very long chain fatty acids may also be included in the pharmaceutical compositions according to the present invention in combination with the precision autophagy modulators according to the present invention to increase lipid storage and enhance the therapeutic effect of autophagy modulators used to treat excessive inflammation as otherwise disclosed herein

Methods of treating a disease state and/or condition with precision autophagy in a patient or subject in need (where upregulation of autophagy is desirable) comprise administering to said patient an effective amount of at least one compound selected from the group consisting of interferon gamma (IFN-gamma), pegylated interferon (PEG-IFN) and at least one TRIM protein or a variant thereof, preferably a TRIM protein selected from the group consisting of TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID

NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:197), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302) , TRIM75 (SEQ ID NO:338) and mixtures thereof, preferably TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:36), TRIM 20 (SEQ ID NO:96), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297) and mixtures thereof, optionally in combination with an additional autophagy modulator (including an alternative TRIM protein) and/or an additional bioactive agent. In these methods, neutral lipids such as lipids selected from the group consisting of triglycerides, diglycerides, monoglycerides, glycolated mono- or diacylglycerides, dolichol, polyprenol, polyprenal or very long chain fatty acids may also be administered in combination with the precision autophagy modulators according to the present invention to increase lipid storage and enhance the therapeutic effect of autophagy modulators used to treat excessive inflammation as otherwise disclosed herein. The present methods apply to a number of disease states and/or conditions which are mediated through autophagy and which often can result in an excessive autophagy response. In certain preferred aspects, the administration of TRIM20, alone or in combination with an additional autophagy modulator and/or bioactive agent as otherwise described herein is useful for upregulating autophagy and treating disease through modulation (up-regulation) of autophagy. This approach is especially useful in the case of certain disease states and/or conditions, especially microbial infections such as bacterial and viral infections where upregulation of TRIM proteins, especially TRIM20 is useful in inhibiting early stages of disease, especially viral and bacterial infections, including early stage tuberculosis (note that in later stage tuberculosis it may be preferable to down-regulate the autophagy response and inhibit the TRIM proteins by administering a TRIM protein inhibitor, especially including a siRNA). In this embodiment, a TRIM protein, especially including TRIM20 may be administered alone or in combination with interferon-gamma (IFN-gamma), pegylated interferon (PEG-IFN) and/or an additional autophagy modulator and/or an additional bioactive agent in order to treat a disease state and/or condition which is mediated through autophagy (an autophagy-mediated disease state and/or condition).

Methods according to the present invention also include down-regulating autophagy where an inflammatory response is elevated (in autoimmune disease, inflammatory diseases and in later stage disease states such as viral and/or bacterial infections, especially including tuberculosis, among others, the method comprising administering an inhibitor of a TRIM protein (including TRIM21) as otherwise set forth herein, especially siRNA which is an inhibitor of a TRIM protein. In preferred aspects, a siRNA inhibitor of TRIM21 is particularly useful in treating these disease states, especially including tuberculosis at any time during a tuberculosis infection. In other embodiments, a siRNA inhibitor of TRIM20 is administered at a later stage of tuberculosis in order to enhance the therapy of the disease state by reducing and/or the impact of autophagy.

Brief Description of the Figures

FIGURE 1 IRGM shows that IRGM activates AMPK signaling and interacts with core autophagy machinery. (A) Lysates from HT-29 colon epithelial cells transfected with control and IRGM siRNA were subjected to Western blotting with antibodies to phospho-AMPK (Thr-172), AMPK, IRGM and actin. (B) Levels of phospho-AMPK (Thr-172) and phospho-Beclin 1 (Ser-93/96) in lysates from HEK293T cells co-expressing Flag-Beclin 1 and GFP or GFP-IRGM. (C) Levels of active phospho-ULK1 (Ser-555 and Ser-317) in lysates of HEK293T cells co-expressing Myc-ULK1 and either GFP or GFP-IRGM. Numbers beneath bands in B, C, quantification of phosphorylated proteins relative to the total abundance of the same protein. (D) Co-immunoprecipitation (Co-IP) analysis of interaction between IRGM and endogenous ULK1 and AMBRA1 in HEK293T lysates of cells expressing GFP or GFP-IRGM. (E) Top, confocal microscopy images of HEK293T cells expressing IRGM-V5 and Myc-ULK1 subjected to starvation for 2 h. Arrowheads, co-localization. Bottom, fluorescence intensity line tracing. (F) Co-IP analysis in lysates of HEK293T cells expressing indicated proteins. (G) Confocal microscopy images of HEK293T cells transiently expressing V5-IRGM and Flag-Beclin1 subjected to starvation for 2 h. Details as for panel E. (H) Lysates of HEK293T cells expressing GFP or GFP-IRGM with Myc-ULK1 subjected to immunoprecipitation with anti-GFP and blots probed with phospho-ULK1 Ser-317 or Ser-757 antibodies. (I) Lysates of cells expressing Myc-ULK1, Flag-Beclin-1 and increasing concentrations of GFP-IRGM subjected to immunoprecipitation with anti-Flag; blots probed as indicated. (J) HEK293T cell lysates co-expressing GFP-IRGM and Flag-Beclin 1 subjected to Western blotting with antibody to phospho-Beclin 1 (Ser-15) and antibodies as

indicated. (K) Co-IP analysis of Flag-IRGM and endogenous ATG14. (L, M) Mapping of Beclin 1 regions interacting with IRGM. (L) Lysates of HEK293T cells co-expressing GFP-IRGM and Flag-Beclin 1 variants in panel M were subjected to immunoprecipitation with anti-Flag and blots probed as indicated. (M) Beclin 1 domain organization indicating its interacting proteins along with deletion constructs used in Co-IP analysis in panel L. (N) Co-IP analysis of the effects of IRGM overexpression on the interaction of Beclin 1 with its regulatory proteins. Lysates of HEK293T cells co-expressing GFP-IRGM and Flag-Beclin 1 were subjected to immunoprecipitation with anti-Flag and blots probed as indicated. (O) Model of IRGM-dependent autophagy induction based on the results obtained in FIGURE 1 IRGM and FIGURE S1 IRGM. See also FIGURE S1 IRGM.

FIGURE 2 IRGM shows that IRGM is required for stable levels of the autophagy initiation proteins. (A,C,E) U937 cells transfected with control or IRGM siRNAs, untreated or treated with LPS (500 ng/ml for 4 h) were lysed and subjected to Western blotting with antibody to (A) ULK1, (C) ATG14L, AMBRA1 and ATG5, and (E) ATG16L1. IRGM knock down efficiency and quantifications are shown in Supplementary FIGURE S2 IRGM A,B. (B,D,F) Left, confocal images of U937 cells transfected with control or IRGM siRNA treated with LPS (500 ng/ml for 4 h), Immunofluorescence analysis was performed with (B) phospho-ULK1 (Ser-317), (D) ATG5, and (F) ATG16L1. Graphs, means $\pm$ SD (corrected total cell fluorescence of cells; > 30 cells from 5 fields measured using Image J). *, $p < 0.05$ (Student's unpaired t test). (G) Lysates from HEK293T cells expressing GFP or GFP-IRGM were subjected to immunoprecipitation with anti-GFP and blot probed with indicated antibodies. (H) Schematic of ATG16L1 domain structure indicating IRGM interacting regions mapped in panels I. (I) Lysates of HEK293T cells co-expressing GFP-IRGM and the indicated Flag-ATG16L1 variants in panel H were subjected to immunoprecipitation with anti-Flag and blots probed as indicated. Results, representative of three independent experiments. See also FIGURE S2 IRGM

FIGURE 3 IRGM shows that IRGM is required for PAMPs induced autophagy. (A) Abundance of IRGM mRNA (relative to GAPDH) in THP-1 cells (control or infected with invasive *E. coli* LF82) determined by quantitative real-time PCR (qRT-PCR). (B) Effect of LPS (30 min) or (C) MDP exposure (16 h) on IRGM mRNA levels in U937 cells. Gene expression (qRT-PCR) was normalized relative to GAPDH. Data, means $\pm$ SD ($n > 3$); *, $p < 0.05$ (Student's unpaired t test). (D) Schematic summary of the physiological signals

activating *IRGM* expression based on data in panels A-C and in FIGURE S3A-H. (E, F) Left, Western blot analysis of LC3-II abundance in U937 cells transfected with control or *IRGM* siRNA: (E) treated or not with LPS (500 ng/ml; 4 h); (F) treated or not with MDP (5 μ g/ml for 8 h). Right, densitometric analysis of Western blots using ImageJ software. (G, H) Left, confocal images of LC3 puncta in LPS treated (500 ng/ml; 4 h) (G) or MDP-treated (5 μ g/ml; 8 h), (H) U937 cells transfected with control or *IRGM* siRNA. Graphs (right of panels G and H), represent mean corrected total cell fluorescence $\pm$ SE (25-35 cells from 10-15 fields measured using ImageJ. *, $p < 0.05$ (ANOVA). (I) Analysis of endogenous interactions (Co-IP) using THP-1 lysates infected with invasive *E. coli* LF82 (1 h) or stimulated with LPS (2 μ g/ml, 2 h) or MDP (10 μ g/ml, 2 h). Lysates were subjected to immunoprecipitation with *IRGM* antibody or control IgG and probed as indicated. (J) Schematic summary of the results obtained in FIGURE 3E-I. See also FIGURE S3 *IRGM*.

FIGURE 4 *IRGM* shows that *IRGM* interacts and co-localizes with ATG16L1 and NOD2. (A, B) Co-IP analysis of endogenous (A) or overexpressed (B) *IRGM*, with NOD2 and ATG16L1 in (A) starved HT29 cells and (B) HEK293T cells. (C) Top, confocal microscopy images of HEK293T cells transiently expressing GFP-*IRGM* and Flag-NOD2. Bottom, fluorescence intensity line tracing corresponding to dashed line. (D) Schematic of NOD2 domain organization along with deletion constructs used in Co-IP analysis in panel E. (E) Left panel, lysates of HEK293T cells co-expressing GFP-*IRGM* and the Flag-NOD2 variants shown in panel D subjected to immunoprecipitation with anti-Flag and blot probed with antibodies as indicated. Right panel, densitometric analysis of Western blots (IP blot/Input blot). (F) Flag tag pull-down assays performed with affinity purified NOD2 variants from 293T cell lysates and purified recombinant GST-*IRGM* shown in the schematic (left panel). (G) Top, confocal microscopy images showing co-localization of GFP-*IRGM* and Flag-NOD2 and Rhodamine-MDP in HEK293T cells. Bottom, fluorescence intensity line tracing corresponding to red line. (H) Effect of MDP (10 μ g/ml, 8 h) on GFP-*IRGM* and Flag-NOD2 interactions in HCT116 cells. (I) Model of *IRGM*-NOD2 interactions. See also FIGURE S4 *IRGM*.

FIGURE 5 *IRGM* show s that NOD2 promotes K63-linked polyubiquitination of *IRGM*, enhancing its interactions with autophagy initiation factors. (A-C) Effects of NOD2 expression on *IRGM* self-association (A), and *IRGM*'s interaction with Beclin 1 (B) or with ULK1 (C) in HEK293T cells. (D, E) Analysis of *IRGM* ubiquitination in HEK293T cells.

Cells co-expressing GFP or GFP-IRGM and (D) HA-tagged Ubiquitin C or (E) HA-tagged Ubiquitin C mutated for all lysines except lysine 48 (HA-K48) or Lysine 63 (HA-K63) and Flag-NOD2 were subjected to immunoprecipitation with GFP antibody and blots probed with indicated antibodies. Blot in (E) was processed to remove irrelevant lanes (dashed vertical line). (F) Cells co-expressing GFP-IRGM, HA-K63 and Flag-NOD2 deletion variants as in Figure 4D were subjected to immunoprecipitation analysis with anti-GFP and blot probed with indicated antibodies. (G) Cells co-expressing GFP or GFP-IRGM or GFP-IRGM-K^{mut} (IRGM variant with all lysine residues mutated to alanine) and HA-K63 were subjected to immunoprecipitation analysis with anti-GFP and blot was probed with indicated antibodies. Blot was processed (dashed vertical line) to remove irrelevant lanes. (H) Lysates of cells co-expressing GFP or GFP-IRGM or GFP-IRGM-K^{mut} and Flag-IRGM were subjected to immunoprecipitation with anti-GFP and blot probed with indicated antibodies. (I) Lysates of cells expressing GFP or GFP-IRGM or GFP-IRGM-K^{mut} were subjected to immunoprecipitation with anti-GFP and blots probed with indicated antibodies. Results representative of three independent experiments. See also FIGURE S5 IRGM.

FIGURE 6 IRGM shows that ubiquitination of IRGM is required for NOD2 degradation and ULK1 stability. (A) Effects of IRGM expression on NOD2 levels in transfected HEK293T cells. Data, means $\pm$ SE; *, $p < 0.05$ (Student's unpaired t test). (B) Lysates of HEK293T cell co-expressing GFP or GFP-IRGM and Flag-NOD2, untreated/treated with Bafilomycin A1 (100 nM for 8 h) were subjected to Western blotting. (C) Lysates of cells co-expressing Flag-NOD2 and GFP, GFP-IRGM, or GFP-IRGM-K^{mut} were subjected to Western blotting. (D, E) Lysates from HEK293T cells co-expressing Myc-ULK1 and either GFP or increasing amounts of GFP-IRGM were subjected to Western blotting as in (D) with the relative abundance of Myc-ULK1 shown in (E). Blot was processed (dashed vertical line) to remove irrelevant lanes. (F) HEK293T cells transfected with plasmids encoding GFP, GFP-IRGM, or GFP-IRGM-K^{mut} and either Myc-ULK1 or Flag-Beclin 1 were lysed and subjected to Western blotting. Data from densitometric analyses of Western blots (B, C, E), means $\pm$ SE, $n=3$ *, $p < 0.05$ (ANOVA). (G) Depiction of the role of IRGM ubiquitination in NOD2 degradation and ULK1 stabilization. See also FIGURE S6 IRGM.

FIGURE 7 IRGM shows that ubiquitination of IRGM is important for preventing inflammation. (A) Effect of IRGM (WT and K^{mut}) expression with and without NOD2 on the

nuclear localization of NF- κ B-p65 in HeLa cells upon *E. coli* LF82 infection. (B) Graph, mean % cells with NF κ B-p65 nuclear localization (from 10 microscopic fields) $\pm$ SD; *, $p < 0.05$ (ANOVA). (C) Effect of *E. coli* infection on IL-1 β mRNA expression in THP-1 cells subjected to IRGM knockdown (qRT-PCR normalized to GAPDH). Data, means $\pm$ SD ($n > 3$); *, $p < 0.05$ (ANOVA). (D, E, F) Lysates of cells co-expressing either GFP or GFP-IRGM and (D) Flag-NOD1, (E) Flag-Rig-I, or (F) Flag-TLR3, subjected to immunoprecipitation with anti-GFP (D, E) or anti-Flag (F); blots were probed with indicated antibodies. (G) Effect of FLAG-tagged NOD1, RIG-I, or TLR3 expression on IRGM ubiquitination (K63-linked) in HEK293T cells. (H) Model of IRGM-mediated xenophagy. IRGM expression is induced by physiological cues including starvation, microbes, or microbial products (PAMPs). IRGM protein increases the abundance of active AMPK, which subsequently promotes autophagy by activating ULK1 and Beclin 1. Not only does IRGM amplify this fundamental autophagy signaling but it also assembles the core autophagy machinery. Association of IRGM with NOD2, which is enhanced in the presence of MDP, promotes IRGM ubiquitination and the assembly of autophagy initiation factors. Together, these molecular events promote antimicrobial autophagy and suppress excessive inflammatory responses. See also FIGURE S7 IRGM.

FIGURE S1 IRGM, related to **FIGURE 1 IRGM** shows that IRGM interacts with core autophagy machinery. (A) Left panel, Western blotting with lysates of bafilomycin (100 nM, 2 hr) treated or untreated HCT116 cells expressing GFP or GFP-IRGM. Right panel, densitometric analysis of Western blots. (B) Co-IP analysis with HEK293T cell co-expressing either GFP or GFP-IRGM and Myc-ULK1. (C-F) Co-IP experiment with HEK293T cell expressing GFP or GFP-IRGM (C, D, F) and Myc-AMBRA1 (E) were subjected to Western blotting with indicated antibodies.

FIGURE S2 IRGM, related to **FIGURE 2 IRGM** shows that IRGM stabilizes core autophagy machinery. (A) Graph showing the knockdown efficiency of *IRGM* in U937 monocytic cells. (B) Graph showing the densitometric analysis of Western blots in FIGURE 2 IRGM 2 A, C, E. Result shown are mean $\pm$ S.D of three independent experiments. *, $p > 0.05$. (C) U937 monocyte cells transfected with control or *IRGM* siRNA, untreated or treated with LPS (500 ng/ml for 4 h) were lysed and subjected to Western blotting with indicated antibodies.

FIGURE S3 IRGM, related to **FIGURE 3 IRGM** shows that starvation induces IRGM expression through AMPK. (A, B) Analysis of *IRGM* expression in several cell lines by quantitative real-time PCR (qRT-PCR). PBMC-Peripheral blood mononuclear cell (C, D) Starvation induces *IRGM* expression in several cell lines and notably in (D) HT-29 cells (~20 fold). RNA isolated from fed and starved cells were subjected to qRT-PCR. (E) Western blot from fed and starved HT-29 cells lysates showing induction of IRGM and LC3B. (F, G) AMPK is required for starvation induced *IRGM* expression in HT-29 cells. (F) qRT-PCR from RNA isolated from fed or starved HT-29 cells, treated with increasing concentration of compoundC (20, 40, 80, 160 μ M). CompoundC is potent inhibitor of AMPK. (G) Knocking down AMPK α 2 blunted starvation induced *IRGM* expression. Inset, Western blotting showing AMPK α 2 knock down efficiency. (H) RNA isolated from U937 cells treated with IFN γ were subjected to qRT-PCR. (I) Graph showing knockdown efficiency of *IRGM* in U937 monocytic cells.

FIGURE S4 IRGM, related to **FIGURE 4 IRGM** shows that IRGM interacts and co-localizes with ATG16L1 and NOD2. (A) Endogenous IRGM interact with NOD2 and ATG16L1 in starved HT-29 cells. Co-IP analysis using IRGM antibody and Western blotting with indicated antibodies. (B) Lysates from cells expressing GFP-IRGM and Flag-NOD2 were subjected to immunoprecipitation with anti-GFP and blots were probed with antibodies as indicated. (C) Representative confocal images of HEK293T cells expressing GFP-IRGM alone or with NOD2 (D, E) HEK293T cells expressing GFP-IRGM (D) or GFP-IRGM and Flag-NOD2 (E) were subjected to immunofluorescence with Tom 20 (mitochondrial marker) antibody. Bottom, co-localization profile measurement along straight line using LSM 510 software.

FIGURE S5 IRGM, related to **FIGURE 5 IRGM** shows that NOD2 enhances ubiquitination of IRGM. (A) HEK293T cell lysates expressing the indicated set of proteins were subjected to immunoprecipitation with Flag antibody and Western blotted with antibodies as indicated. (B) HEK293T cell lysates co-expressing GFP-IRGM alone or along with NOD2 were subjected to immunoprecipitation with GFP antibody and Western blotted with antibody to GFP. (C) HEK293T cell lysates co-expressing IRGM-V5 and HA-K63 were subjected to immunoprecipitation with V5 antibody and Western blotted with antibody to HA. (D) Analysis of effect of NOD2 on IRGM/IRGM^{mut}- Beclin 1 interaction by Co-IP experiment.

FIGURE S6 IRGM, related to **FIGURE 7 IRGM** shows that ubiquitination of IRGM is important for its anti-inflammatory function. (A) Starvation reduces intracellular replication of invasive *E. coli* LF82 in HEK 293T cells. Results are expressed as mean $\pm$ standard error of colony-forming units (cfu) per ml per 10⁴ live cells. *, $p < 0.05$. (B) Representative confocal images of GFP-IRGM transfected HEK293T cells infected with invasive *E. coli* LF82 (red, LPS antibody). (C) Analysis of NF κ B-p65 nuclear translocation following LF82 infection in HeLa cells expressing GFP or GFP-IRGM or GFP-IRGM-Kmut and/or Flag-NOD2. (D) Graph showing the knock down efficiency of *IRGM* in LF82 infected THP-1 cells (E) Lysates of cells co-expressing control vector or Flag-IRGM and GFP-TLR4 were subjected to immunoprecipitation with anti-Flag and blots were probed with indicated antibodies.

FIGURE 1 PRECISION shows that TRIM proteins regulate IFN- γ -induced autophagy. (A) THP-1 cells were subjected to TRIM knockdown, treated with 1,000 U/mL IFN- γ for 4 h, and high content (HC) analysis was performed using a Cellomics HCS scanner (epifluorescence) and iDEV software. HC (magenta, endogenous LC3B immunofluorescence [IF]; blue, nuclei stained with Hoechst). Mask overlay, software-defined objects (primary objects, cell outlines; internal secondary objects, LC3 puncta). (B) Average count of LC3 puncta per cell from cells treated as in (A) (Data from two 96-well plates with identical siRNA arrangements; the corresponding data are shown in Supplementary figure 1C). Encircled are INF- γ -treated wells (right) and wells with vehicle controls (bottom left). TRIM knockdowns that reduced LC3 puncta readout in both two experiments by 3 SDs (horizontal dot lines) from the average of IFN- γ -treated controls (horizontal solid line) are indicated by corresponding TRIM numbers (open circle). TRIMs that were chosen in follow-up experiments in Figure 1C are also indicated with number. (C) Similar to (B), except that THP-1 cells were subjected to specific TRIM or scrambled (Scr; control) knockdown, and were analyzed in more than quadruplicate manner. (D) Model of TRIMs-mediated IFN- γ -induced autophagy based on the results obtained in Figure 1 and Figure S1 thus far. (E) THP-1 cells were treated with TRIM20 or Scr siRNAs, treated with or without IFN- γ for 4h in the presence of bafilomycin A1, and LC3-II conversion was determined by immunoblots. (F) HeLa cells were transfected with GFP or GFP-TRIM20, and HC analysis performed. Data, means $\pm$ SE, $n \geq 3$, * $P < 0.05$; $^{\dagger}P \geq 0.05$ (ANOVA). Scale bars, 5 μ m.

FIGURE 2 PRECISION shows that TRIM20 interacts with ULK1 and Beclin 1. (A,B) Co-immunoprecipitation analysis of GFP-TRIM20 (T20) with (A) Myc-ULK1 or (B) Flag-Beclin 1 in HEK293 cells extracts. IP, immunoprecipitation; WB, western blot. (C) Confocal microscopy of HeLa cells co-expressing mCherry-TRIM20 with GFP-ULK1. Line tracing corresponds to arrow. White outline, cell boundary defined by background fluorescence. Scale bars, 10 μ m. (D) Co-immunoprecipitation analysis of TRIM20 complexes with p-ULK1 (Ser-317) in HEK293 cells. (E) TRIM20 domains and deletion constructs used. Dotted lines, deleted regions. (F) Co-immunoprecipitation analysis of interactions between deletion variants of TRIM20 (as GFP fusions) with Myc-ULK1 in HEK293 cells. (G) GST pull-down analysis of radiolabeled Myc-ULK1 with GST-tagged deletion variants of TRIM20. Top, autoradiogram of pull-down products. Bottom, Coomassie Brilliant Blue (CBB)-stained SDS-polyacrylamide gel with GST-deletion variants of TRIM20. Data representative of three or more experiments.

FIGURE 3 PRECISION shows that TRIM20 assembles ULK1 and Beclin 1 in a complex and interacts with ATG16L1. (A) TRIM20 domains and deletion constructs used. (B) Co-immunoprecipitation analysis of interaction between deletion variants of TRIM20 (as GFP fusions; asterisks denote fusion products on the bottom blot) with Flag-Beclin 1 in HEK293 cells. (C) Co-immunoprecipitation analysis of ULK1 in Beclin 1 complexes in the presence and absence of TRIM20 from HEK293T cell lysates. (D) Co-immunoprecipitation analysis of GFP-TRIM20 with endogenous ATG16L1. (E) TRIM20 domains and deletion constructs used. (F) Co-immunoprecipitation analysis of interaction between deletion variants of TRIM20 with Flag-ATG16L1 in HEK293 cells. (G) ATG16L1 domains and deletion constructs used. (H) Co-immunoprecipitation analysis of interactions between deletion variants of Flag-ATG16L1 and GFP-TRIM20 in HEK293 cells. (I) Model of TRIM20-dependent autophagy induction based on Figure 2, 3, and Supplementary Figure 2. Data representative of three or more experiments.

FIGURE 4 PRECISION shows that TRIM20 interacts with mammalian Atg8 paralogs (mAtg8s). (A) GST pull-down analysis of interactions between radiolabeled Myc-TRIM20 and GST-tagged mAtg8s. Top, autoradiogram of pull-down products. Bottom, CBB-stained SDS-polyacrylamide gel with GST-mAtg8s. (B) TRIM20 domains and deletion constructs used. (C) GST pull-down analysis of binding between radiolabeled Myc-TRIM20 deletion

variants and GST-GABARAP and GST-LC3A. (D) Identification of GABARAP interacting regions on TRIM20 by peptide array. Three series of TRIM20 peptides (regions of primary sequence staggered by 3 amino acid residues), with either three or four positive consecutive binding signals, were identified. The peptide sequences corresponding to the positive binding signals (encompassed spots; defined as Region I, II, III) were mutated as described, and were subjected to the GST pull-down experiments in (E) and Figure S2F. (E) GST pull-down analysis of interaction between radiolabeled Myc-TRIM20 triple mutants and GST-GABARAP. Data representative of three or more experiments.

FIGURE 5 PRECISION shows that TRIM20 degrades inflammasome components through autophagy. (A) Levels of NLRP3 were determined in lysates from THP-1 cells subjected to TRIM20 or Scr siRNA were activated with 1,000 U/mL IFN- γ for 3 h, and 2.5 μ g/mL LPS for 2 h (for optimal TRIM20 expression; Supplementary Figure S3B). RI, relative intensity. (B) Levels of NLRP3 were determined from THP-1 subjected to TRIM20 or control knockdown and treated or not with bafilomycin A1 (Baf A₁). (C) The abundance of NLRP3 protein was determined from THP-1 cells subjected to TRIM20 or control knockdown and exposed to *Escherichia coli* strain LF82 and IFN- γ in the presence or absence of bafilomycin. (D) The abundance of NLRP3 protein was determined from primary human MDMs subjected to TRIM20 or control knockdown and exposed to LPS and IFN- γ in the presence or absence of bafilomycin. (E) Levels of NLRP3 were determined from THP-1 cells subjected to ULK1, Beclin 1, or Scr siRNA were treated with IFN- γ and LPS. (F-H) Levels of NLRP3 (F), NLRP1 (G), or pro-caspase 1 (H) were determined in cells expressing GFP or GFP-TRIM20 following autophagy induction (EBSS, 3 h) in the presence or absence of bafilomycin A1. Data, means $\pm$ SE, $n \geq 3$, * $P < 0.05$, $^{\dagger}P \geq 0.05$ (ANOVA).

FIGURE 6 PRECISION shows that ULK1 is recruited to NLRP3 complexes by wild type TRIM20 but not by FMF disease-associated TRIM20 mutants. (A) Co-immunoprecipitation analysis of ULK1 in NLRP3 complexes in HEK293T cells expressing GFP-TRIM20 or GFP alone. (B) The effect of NLRP3 expression on the presence of phospho-ULK1 in TRIM20 complexes. Lysates from HEK293 cells transiently expressing Myc-ULK1, GFP-TRIM20 (or GFP alone), and Flag-NLRP3 (or not) were immunoprecipitated with anti-GFP and immunoblots were probed as indicated. (C) Model of TRIM20's function in autophagy as a regulator-receptor: TRIM20 assembles autophagy machinery (ULK1, Beclin 1, ATG16L1,

mAtg8s) and recognizes substrates (NLRP3, pro-caspase 1 and NLRP1) delivering them for autophagic degradation. The recognition of substrate enriches active p-ULK1 on the TRIM20 platform. (D) FLICA-positive cells were quantified using THP-1 cells that had been subjected to knockdown of TRIM20, treated with IFN- γ , and then treated with or without LPS (2 h) and nigericin (10 min), and stained for active caspase-1 (with FLICA); >150 cells per experiment were analyzed for quantification. (E) The levels of IL-1 β were determined from supernatants of THP-1 cells that had been subjected to knockdown of ULK1 or TRIM20, treated with IFN- γ and LPS, and stimulated with nigericin for 30 min. (F) Predominant FMF-associated point mutations of TRIM20 reside in the PRY/SPRY domain. (G) Levels of NLRP3 were determined from lysates of HEK293 cell expressing GFP-TRIM20 (wild type or FMF-associated variants) or GFP and induced for autophagy by starvation in EBSS for 3 h. (H) Effects of FMF-associated variants on ULK1 presence in TRIM20 complexes. HEK293 cells were transiently transfected with Myc-ULK1, and either GFP-TRIM20 (wild type or FMF-associated variants) or GFP alone. Lysates were immunoprecipitated with anti-GFP, and immunoblots were probed as indicated. Numbers indicate relative intensity of the indicated band. (I) Model of FMF-associated mutation in NLRP3 degradation. The presence of NLRP3 promote phosphorylation of ULK1 in TRIM20 complex, leading to autophagic degradation of NLRP3. TRIM20 mutants harbored less ULK1 and phospho-ULK1, which results in less autophagic activity and less degradation of inflammasome components. Asterisks denote common FMF-associated point mutations in TRIM20. Data, means $\pm$ SE, $n \geq 3$, $*P < 0.05$, (ANOVA).

FIGURE 7 PRECISION shows that TRIM21 interacts with autophagy regulators and effectors. (A and B) Co-immunoprecipitation analyses of GFP-TRIM21 (T21) with (A) Myc-ULK1 and (B) Flag-Beclin 1 in HEK293 cells extracts. (C) GST pull-down analysis of binding between radiolabeled Myc-TRIM21 and GST-mAtg8s. Top, autoradiogram of pull-down products. Bottom, CBB-stained SDS-polyacrylamide gel with GST-mAtg8s. (D) TRIM21 domains and deletion constructs used. (E) GST pull-down analysis of binding between radiolabeled Myc-TRIM21 deletion mutants and GST-GABARAP and GST-p62. Asterisks and squares denote presence or absence of Myc-TRIM21, respectively. (F) p62 domains and deletion constructs used. (G) GST pull-down analysis of interaction between radiolabeled Myc-TRIM21 and GST-tagged p62. Data representative of three or more experiments.

FIGURE 8 PRECISION shows that TRIM21 promotes autophagic degradation of IRF3 dimers and attenuates type I interferon production. (A) Confocal microscopy of HeLa cells co-expressing mCherry-TRIM21, Flag-IRF3, and GFP-LC3B in the presence of bafilomycin A1. White outline, cell boundary. Arrows indicate the colocalization. (B) Confocal microscopy of HEK293 cells co-expressing mCherry-TRIM21, Flag-IRF3, and GFP-ULK1. (C) Co-immunoprecipitation analysis of IRF3-ULK1 complexes in the presence and absence of TRIM21. Lysates from HEK293 cells transiently expressing Myc-ULK1, Flag-IRF3, and either GFP-TRIM20 or GFP were immunoprecipitated with anti-Myc, and immunoblots were probed as indicated. (D) Levels of dimerized IRF3 were assessed by native PAGE from THP-1 cells subjected to TRIM21 or control knockdown, and stimulated for 12h by herring testis DNA (HT-DNA) transfected into the cells in the presence of 200 U/mL IFN- γ . (E) The effect of autophagy inhibition with bafilomycin on TRIM21-dependent IRF3 dimer degradation in THP-1 cells. (F) The effect of TRIM21 knockdown on IFN- β mRNA levels following stimulation of THP-1 cells with IFN- γ and HT-DNA. (G) Model of TRIMs' roles in regulation of inflammation by precision autophagy. TRIM20 targets the inflammasome components for autophagic degradation, whereas TRIM21 targets IRF3, to suppress inflammasome activity and type I IFN response, respectively. TRIM20 and TRIM21, both of whose expression response to IFN- γ , directly bind their respective cargo, cooperate in IFN- γ induction of autophagy (dashed line), and recruit autophagic machinery to execute degradation. Scale bars, 10 μ m. Data, means $\pm$ SE, $n \geq 3$, $*P < 0.05$, $^{\dagger}P \geq 0.05$ (ANOVA).

FIGURE S1 PRECISION shows that TRIM proteins regulate IFN- γ -induced autophagy. (A and B) High content image analysis of LC3 puncta in (A) THP-1 cells or (B) human MDM cells treated with IFN- γ for 4 h. HC and mask overlays are as in Fig 1. (C) Screen data from Figure 1B showing average $\pm$ range. (D) Knockdown efficacy of TRIMs were determined by RT-PCR. (E and F) THP-1 cells were treated with (E) escalating doses of IFN- γ for 4 h or (F) 1,000 U/ml of IFN- γ for indicated times, and TRIM20 mRNA levels were determined by quantitative RT-PCR. Values are standardized to (E) no IFN- γ control or (F) 0 h time point. (G) THP-1 cells were subjected to TRIM20 or scrambled siRNA, treated with IFN- γ for 4 h, and HC analysis performed. (H) Knockdown of TRIM20 mRNA levels was examined by quantitative RT-PCR. Values are standardized to control (Scr, scrambled; no IFN- γ). (I) LC3-II conversion in HEK293 cells transfected with GFP-TRIM20 (T20) or GFP. Data, means $\pm$

SE, $n \geq 3$ experiments, except panel (C). Scale bars, 5 μm . * $P < 0.05$, $^{\dagger}P \geq 0.05$ (t test in (B) or ANOVA (other panels)).

FIGURE S2 PRECISION shows that TRIM20 interacts with ULK1, Beclin 1, and mAtg8s. (A,B) Co-immunoprecipitation analysis of GFP-TRIM20 with endogenous (A) ULK1 or (B) Beclin 1 in HEK293 cells extracts. (C) Beclin 1 domains and deletion constructs used. (D) Co-immunoprecipitation analysis of interactions between deletion variants of Flag-Beclin 1 (asterisks and squares in the top blot denote presence or absence of Flag-Beclin 1, respectively) and GFP-TRIM20 in HEK293 cells. (E) Confocal microscopy of HEK293 cells co-expressing mCherry-TRIM20 with GFP-GABARAP. Line tracings correspond to arrows. (F) Confocal microscopy of HeLa cells co-expressing mCherry-TRIM20 with GFP-LC3B in the presence of bafilomycin A1. Line tracing corresponds to arrows. (G) GST pull-down analysis of interaction between radiolabeled Myc-TRIM20 harboring single or double mutants (corresponding to Figure 4D) and GST-GABARAP. Data representative of three independent experiments. Scale bars, (E) 5 μm (2 μm for inset) and (F) 10 μm .

FIGURE S3 PRECISION shows that TRIM20 degrades NLRP3 through autophagy. (A) Co-immunoprecipitation analysis of deletion variants of TRIM20 (as GFP fusions; asterisks denote fusion products on the bottom blot) with NLRP3 in HEK293 cells. (B) THP-1 cells were treated with IFN- γ for 3 h, additionally treated with 2.5 $\mu\text{g/ml}$ of LPS for indicated periods, and TRIM20 mRNA levels were determined by quantitative PCR. Values are standardized to IFN- γ -untreated control. (C and D) Levels of NLRP3 were determined in lysates from THP-1 cells subjected to TRIM20 or Scr siRNA were (C) untreated either IFN- γ or LPS, or (D) LPS alone for 2h. (E) THP-1 cells were treated with 1.0 $\mu\text{g/ml}$ of LPS for 3h, and levels of NLRP3 in lysate were determined by immunoblots. (F) Knockdown efficacies of ULK1 and Beclin 1 by siRNA were examined by quantitative RT-PCR. (G and H) Levels of GFP-TRIM20 were determined in cells co-expressing (G) with or (H) without NLRP3 following autophagy induction (EBSS, 3 h) in the presence or absence of bafilomycin A1. (I) Co-immunoprecipitation analysis of ULK1 in NLRP3 protein complexes in the presence and absence of TRIM20 knockdown. Lysates from THP-1 cell subjected to each knockdown and treatment of 200 U/mL IFN- γ for 3h and additional LPS (1.0 $\mu\text{g/ml}$) treatment 2h, were immunoprecipitated with anti-NLRP3, and immunoblots were probed as indicated. (J) Co-immunoprecipitation analysis of AMPK in GFP-TRIM20 complexes in HEK293T cells

expressing Myc-ULK1 and Flag-NLRP3 (or not). Data, means $\pm$ SE, $n \geq 3$ experiments, $*P < 0.05$, $^{\dagger}P \geq 0.05$ (C, t-test; D, ANOVA).

FIGURE S4 PRECISION shows the effects of TRIM20 on inflammasome activity and FMF-associated variants of TRIM20 decrease number of TRIM20 and LC3 puncta. (A) LDH release of supernatants in Figure 6D. (B and C) Supernatants were harvested from THP-1 cells that had been subjected to double knockdown as indicated, treated with IFN- γ and LPS, additionally stimulated with nigericin (20 μ M) for 30 min, and levels of IL-1 β and LDH release were measured. (D) Knockdown efficacy of NLRP3 by siRNA was examined by immunoblotting. (E) Confocal microscopy of THP-1 cells that had been subjected to knockdown of TRIM20, treated with IFN- γ , and then treated with or without LPS (2h) and nigericin (10min), and stained for active caspase-1 (with FLICA) and nucleus (TO-PRO-3). Arrowheads, FLICA-positive puncta; asterisk, cell; white outline, cell boundary. (F) Confocal microscopy of GFP-TRIM20 (wild type or FMF-associated variants) or GFP in HEK293 cells. (G) HC image (epifluorescence) analysis of TRIM20 puncta in HeLa cells expressing GFP-TRIM20 (wild type or FMF-associated variants) or GFP. (H) HC image analysis of LC3 puncta in HeLa cells expressing GFP-TRIM20 (wild type or triple mutant TRIM20). Data, means $\pm$ SE, $n \geq 3$ experiments, $*P < 0.05$, $^{\dagger}P \geq 0.05$ (t test or ANOVA). Scale bar, 5 μ m.

FIGURE S5 PRECISION shows that TRIM21 affects the level of dimerized IRF3 in HIV1 infection. (A) THP-1 cells were treated with 1,000 U/ml of IFN- γ for indicated times, and TRIM21 mRNA levels were determined by quantitative RT-PCR. (B) Co-immunoprecipitation analysis of GFP-TRIM20 with Flag-TRIM21 in HEK293 cells extracts. (C) Knockdown efficacy of TRIM21 level was examined by immunoblotting. (D) Levels of dimerized IRF3 were assessed by native PAGE from THP-1 cells subjected to TRIM21 or control knockdown, untreated with IFN- γ , and transfected with herring testis DNA (HT-DNA). (E and F) THP-1 cells subjected to TRIM21 or control knockdown were infected with a single-round infection HIV1 virus in the presence of 200 U/mL IFN- γ for 20h, and (E) the levels of dimerized IRF3 or (F) mRNA levels of IFN- β were determined. (G) Model of TRIM21's dual function in autophagy as a regulator-receptor: TRIM21 assembles autophagy machinery (ULK1, Beclin 1, and mAtg8s) and recognizes substrates (dimerized IRF3) delivering them for autophagic degradation to suppress type I IFN response and inflammation. Dashed outlines (ULK1 and Beclin 1), domain binding location not mapped;

solid outline for mAtg8 (GABARAP) reflects mapping data. (H) The effect of TRIM21 knockdown on IFN- β mRNA levels following stimulation of THP-1 cells with 1,000 U/ml IFN- γ for 3h and then with 2.5 μ g/mL LPS for 2h. Data, means $\pm$ SE, $n \geq 3$ experiments, $*P < 0.05$ (ANOVA).

Detailed Description of the Invention

It is noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the,” include plural referents unless expressly and unequivocally limited to one referent. Thus, for example, reference to “a compound” includes two or more different compound. As used herein, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or other items that can be added to the listed items.

The term “compound” or “agent”, as used herein, unless otherwise indicated, refers to any specific chemical compound disclosed herein and includes tautomers, regioisomers, geometric isomers as applicable, and also where applicable, optical isomers (e.g. enantiomers) thereof, as well as pharmaceutically acceptable salts thereof. Within its use in context, the term compound generally refers to a single compound, but also may include other compounds such as stereoisomers, regioisomers and/or optical isomers (including racemic mixtures) as well as specific enantiomers or enantiomerically enriched mixtures of disclosed compounds as well as diastereomers and epimers, where applicable in context. The term also refers, in context to prodrug forms of compounds which have been modified to facilitate the administration and delivery of compounds to a site of activity.

The term “patient” or “subject” is used throughout the specification within context to describe an animal, generally a mammal, including a domesticated mammal including a farm animal (dog, cat, horse, cow, pig, sheep, goat, etc.) and preferably a human, to whom treatment, including prophylactic treatment (prophylaxis), with the methods and compositions according to the present invention is provided. For treatment of those conditions or disease states which are specific for a specific animal such as a human patient, the term patient refers to that specific animal, often a human.

The terms “effective” or “pharmaceutically effective” are used herein, unless otherwise indicated, to describe an amount of a compound or composition which, in context, is used to produce or affect an intended result, for example the modulation of autophagy within the context of a particular treatment or alternatively, the effect of a bioactive agent which is coadministered with the autophagy modulator (autotoxin) in the treatment of disease.

The terms “treat”, “treating”, and “treatment”, etc., as used herein, refer to any action providing a benefit to a patient at risk for or afflicted by an autophagy mediated disease state or condition as otherwise described herein. The benefit may be in curing the disease state or condition, inhibition its progression, or ameliorating, lessening or suppressing one or more symptom of an autophagy mediated disease state or condition, as well as inhibiting or reducing excessive autophagy. Treatment, as used herein, encompasses both prophylactic and therapeutic treatment.

As used herein, the term “autophagy mediated disease state or condition” (which term may include the term “IRGM modulated disease” as a subset) refers to a disease state or condition that results from disruption in autophagy or cellular self-digestion and wherein IRGM or its pathway and/or the TRIM proteins and their pathways are involved in the disease state or condition. Autophagy is a cellular pathway involved in protein and organelle degradation, and has a large number of connections to human disease. Autophagic dysfunction is associated with cancer, neurodegeneration, microbial infection and ageing, among numerous other disease states and/or conditions. Although autophagy plays a principal role as a protective process for the cell, it also plays a role in cell death. Disease states and/or conditions which are mediated through autophagy (which refers to the fact that the disease state or condition may manifest itself as a function of the increase or decrease in autophagy in the patient or subject to be treated and treatment requires administration of an inhibitor or agonist of autophagy in the patient or subject) include, for example, cancer, including metastasis of cancer, lysosomal storage diseases (discussed hereinbelow), neurodegeneration (including, for example, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease; other ataxias), immune response (T cell maturation, B cell and T cell homeostasis, counters damaging inflammation) and chronic inflammatory diseases (may promote excessive cytokines when autophagy is defective), including, for example, inflammatory bowel disease, including Crohn’s disease, rheumatoid arthritis, lupus, multiple

sclerosis, chronic obstructive pulmonary disease/COPD, pulmonary fibrosis, cystic fibrosis, Sjogren's disease; hyperglycemic disorders, diabetes (I and II), affecting lipid metabolism islet function and/or structure, excessive autophagy may lead to pancreatic β -cell death and related hyperglycemic disorders, including severe insulin resistance, hyperinsulinemia, insulin-resistant diabetes (e.g. Mendenhall's Syndrome, Werner Syndrome, leprechaunism, and lipoatrophic diabetes) and dyslipidemia (e.g. hyperlipidemia as expressed by obese subjects, elevated low-density lipoprotein (LDL), depressed high-density lipoprotein (HDL), and elevated triglycerides) and metabolic syndrome, liver disease (excessive autophagic removal of cellular entities- endoplasmic reticulum), renal disease (apoptosis in plaques, glomerular disease), cardiovascular disease (especially including ischemia, stroke, pressure overload and complications during reperfusion), muscle degeneration and atrophy, symptoms of aging (including amelioration or the delay in onset or severity or frequency of aging-related symptoms and chronic conditions including muscle atrophy, frailty, metabolic disorders, low grade inflammation, atherosclerosis and associated conditions such as cardiac and neurological both central and peripheral manifestations including stroke, age-associated dementia and sporadic form of Alzheimer's disease, pre-cancerous states, and psychiatric conditions including depression), stroke and spinal cord injury, arteriosclerosis, infectious diseases (microbial infections, removes microbes, provides a protective inflammatory response to microbial products, limits adaptation of autophagy of host by microbe for enhancement of microbial growth, regulation of innate immunity) including bacterial, especially including *M. tuberculosis*, fungal, cellular and viral (including secondary disease states or conditions associated with infectious diseases), including HIV I and II, hepatitis B and C, AIDS and tuberculosis, among others, development (including erythrocyte differentiation), embryogenesis/fertility/infertility (embryo implantation and neonate survival after termination of transplacental supply of nutrients, removal of dead cells during programmed cell death) and ageing (increased autophagy leads to the removal of damaged organelles or aggregated macromolecules to increase health and prolong life, but increased levels of autophagy in children/young adults may lead to muscle and organ wasting resulting in ageing/progeria).

The term "lysosomal storage disorder" refers to a disease state or condition that results from a defect in lysosomal storage. These disease states or conditions generally occur when the lysosome malfunctions. Lysosomal storage disorders are caused by lysosomal dysfunction usually as a consequence of deficiency of a single enzyme required for

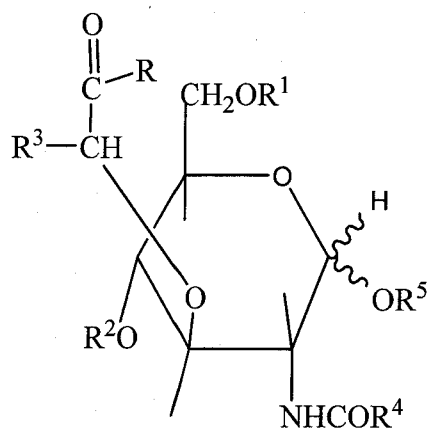
the metabolism of lipids, glycoproteins or mucopolysaccharides. The incidence of lysosomal storage disorder (collectively) occurs at an incidence of about about 1:5,000 - 1:10,000. The lysosome is commonly referred to as the cell's recycling center because it processes unwanted material into substances that the cell can utilize. Lysosomes break down this unwanted matter via high specialized enzymes. Lysosomal disorders generally are triggered when a particular enzyme exists in too small an amount or is missing altogether. When this happens, substances accumulate in the cell. In other words, when the lysosome doesn't function normally, excess products destined for breakdown and recycling are stored in the cell. Lysosomal storage disorders are genetic diseases, but these may be treated using autophagy modulators (autostatins) as described herein. All of these diseases share a common biochemical characteristic, i.e., that all lysosomal disorders originate from an abnormal accumulation of substances inside the lysosome. Lysosomal storage diseases mostly affect children who often die as a consequence at an early stage of life, many within a few months or years of birth. Many other children die of this disease following years of suffering from various symptoms of their particular disorder.

Examples of lysosomal storage diseases include, for example, activator deficiency/GM2 gangliosidosis, alpha-mannosidosis, aspartylglucosaminuria, cholesteryl ester storage disease, chronic hexosaminidase A deficiency, cystinosis, Danon disease, Fabry disease, Farber disease, fucosidosis, galactosialidosis, Gaucher Disease (Types I, II and III), GM1 Gangliosidosis, including infantile, late infantile/juvenile and adult/chronic), Hunter syndrome (MPS II), I-Cell disease/Mucopolysaccharidosis II, Infantile Free Sialic Acid Storage Disease (ISSD), Juvenile Hexosaminidase A Deficiency, Krabbe disease, Lysosomal acid lipase deficiency, Metachromatic Leukodystrophy, Hurler syndrome, Scheie syndrome, Hurler-Scheie syndrome, Sanfilippo syndrome, Morquio Type A and B, Maroteaux-Lamy, Sly syndrome, mucopolysaccharidosis, multiple sulfate deficiency, Niemann-Pick disease, Neuronal ceroid lipofuscinoses, CLN6 disease, Jansky-Bielschowsky disease, Pompe disease, pycnodysostosis, Sandhoff disease, Schindler disease, Tay-Sachs and Wolman disease, among others.

The term "modulator of autophagy", "regulator of autophagy" or "autophagy modulator" is used to refer to a compound or composition which modulates IRGM "ARGM modulator") or its pathway or TRIM proteins and their pathways ("precision autophagy modulators" or "TRIM protein modulators") and has an influence on treating diseases which

are modulated through those mechanisms. IRGM modulators pursuant to the present invention include double stranded RNA (dsRNA), in particular poly I:C, poly U-G (UGUGU) and modified dsRNA such as poly ICLC (poly I;C modified with lysine and carboxymethyl cellulose) and muramyl peptides or muramyl dipeptides as disclosed herein. TRIM protein modulators include interferon gamma, pegylated interferon or preferably, any one or more of the TRIM proteins otherwise disclosed herein or an inhibitor of a TRIM protein such as a siRNA which specifically inhibits one or more TRIM proteins.

The term “muramyl peptide” or “muramyl dipeptide” include compounds according to the chemical structure:



wherein:

R^1 represents a hydrogen atom or a C_1 - C_{22} acyl group;

R^2 represents a hydrogen atom or a C_1 - C_{22} acyl group;

R^3 represents a hydrogen atom or a C_1 - C_6 alkyl group;

R^4 represents a C_1 - C_{21} alkyl group or a C_5 or C_{10} aryl group;

R^5 represents a hydrogen atom; and

R represents the residue of an amino acid or a linear peptide built up of from 2 to 6 amino acid residues, at least one of the residues being optionally substituted with a lipophilic group including muramyl dipeptide and desmethylmuramyl dipeptide.

Preferred acyl groups for R^1 and R^2 are C_1 - C_5 acyl groups such as acetyl; it will be appreciated that the carbon count in the acyl group does not include the carbonyl moiety. Preferred alkyl groups for R^3 are C_1 - C_4 alkyl groups such as methyl and ethyl. Preferred

alkyl groups for R₄ and C₁ -C₆ alkyl groups, particularly C₁ -C₄ alkyl groups, such as methyl or ethyl; phenyl is a preferred aryl group. R preferably represents a mono-, di- or tri-peptide, more often a dipeptide. The proximal peptide residue (or the only peptide residue, if there is only one) is preferably that of an L-amino acid.

Examples include:

L-alanyl

L-valyl

L-leucyl

L-isoleucyl

L-a-aminobutyryl

L-seryl

L-threonyl

L-tryptophanyl

L-lysyl

L-ornithyl

L-arginyl

L-histidyl

L-glutamyl

L-glutaminy

L-methionyl

L-cysteinyl

L-phenylalanyl

L-tyrosyl

L-aspartyl

L-asparaginy

L-prolyl

L-hydroxyprolyl

L-alanyl is preferred, as is L-threonyl.

The next amino acid from the proximal end of the peptide is preferably of the D-configuration. It is preferably acidic and may be D-glutamic or D-aspartic acid or a mono-, di- or mixed C₁-C₂₂ (preferably C₁-C₅) alkyl ester, amide or C₁ -C₄ alkyl amide thereof. (The expression "mixed" is illustrated when one carboxyl group is amidated and the other

esterified. D-isoglutamine and D-glutamate are preferred. A third amino acid residue from the proximal end of the chain, if there is one, is preferably of the L-configuration, as indicated above in relation to the proximal amino acid residue. L-alanyl and L-lysyl are preferred.

The amino acid residue or linear peptide is optionally substituted with at least one lipophilic group. The lipophilic group may be a C₁₀-C₂₂ acyl group such as stearoyl or a di-(C₁₀-C₂₂ acyl)-sn-glycero-3'-hydroxyphosphoryloxy group wherein for example each of the C₁₀-C₂₂ acyl groups can be a palmitoyl group. The lipophilic group may alternatively (or in addition, as more than one substitution may be present) be a C₁-C₁₀ ester group, such as a C₂-C₆ ester group: an acetyl group or a butyl ester are examples.

Examples of muramyl dipeptides within the scope of general formula I include: muroctasin, otherwise known as MDP-Lys (L18) (N²-(N-acetylmuramyl-L-alanyl-D-isoglutaminyl)-N⁶-stearoyl-L-lysine); MTP-PE (N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-hydroxyphosphoryloxy)ethylamide, monosodium); murabutide (N-acetylmuramyl-L-alanyl-D-glutamine-aN-butyl ester); and t-MDP (N-acetylmuramyl-L-threonyl-D-isoglutamine).

The preparation of these and other compounds pursuant to the present invention is disclosed in EPA-15 0021367, USA-4317771, EPA-0025495, Lefrancier, et al, *J. Med. Chem.*, 25 87 (1982), as well as methods generally known in the art. Patent publications which give details of the preparations of muramyl peptide compounds generally include BEA-0834753, BEA-0834754, BEA-0847103, BEA-0849214, DEA-2710455, DEA-2922533, DEA-2747379, DE-A-2912865, FR-A-2355505, FRA-2358159, FRA-2375249, EP-A-0004512, EP-A-0002677, JP-A-54063016, JP-25 A-54073729, JPA-55019236, US-A-4082735 and US-A-4082736, among others. The preparation of prototype muramyl dipeptide is disclosed in DE-A-2450355 and USA-4235771.) All the documents referred to in this specification are incorporated herein by reference.

Other useful compounds are disclosed in WO96/01645 (the structures of these compounds may be found in the published PCT application and include the following compounds, among others:

GMDP;
GMDP-LL;
GMDP-Obu;
GMDO-Lys;
GMDB-Lys(St);
GMDBA-Lys(St);
GMDPA(OBzl)₂;
MeGMDP;
(GMDP)₂;
(GMDPA)₂;
(GMDPLys)₂;
[GMDP-Lys(St)]₂;
GMDP-Ad;
GMDP-tuftsins E;
GMDP-tuftsins A;
GMDP-tuftsins lipophilic;
GMDP-bursins;
GMDP-thymogen I;
GMDP-thymogen II;
GMDP-thymogen III;
Thr-MDP

The term “TRIM protein” or “tripartite motif containing protein” is used to describe a TRIM protein or variant thereof as otherwise disclosed herein which is integral to an autophagy response and may be integral as part of an upregulation of autophagy (TRIM20, etc.) or down-regulation of autophagy (TRIM21, etc.). Many TRIM proteins are induced by interferons, which are important components of resistance to pathogens and a number of TRIM proteins are known to be required for the restriction of infection by lentiviruses. In instances where a patient or subject is interferon deficient, the administration of TRIM proteins alone or in combination with interferon gamma and/or pegylated interferon may assist in treating disease, especially infections such as viral infections or bacterial infections, especially *Mycobacterium* infections such as *M. tuberculosis* infections. TRIM proteins are involved in pathogen-recognition and by regulation of transcriptional pathways in host defence. Numerous TRIM proteins may be used in the present invention as otherwise

described herein. Sequences of these proteins are included as are the accession numbers for identifying these proteins. TRIM proteins are known in the art. TRIM proteins which are useful and preferred in the present invention include the human full length TRIM proteins (TRIM1-75) as otherwise described herein. The sequences of TRIM proteins 1-75 as shown as well as polypeptide variants which have at least about a 90% sequence identity, and preferably at least about 95% sequence identify (about 96%, about 97%, about 98% and about 99% sequence identify) to the wild type polypeptide sequences of homo sapien TRIM proteins 1-75 are useful in the present invention. These sequences are set forth in the attached table on pages 92-116 just before the presentation of the claims. Note that TRIM proteins or polypeptide variants thereof or a pharmaceutically acceptable salt thereof may be used in the present invention. All 75 TRIM proteins as identified herein may be used in the present invention, although the preferred TRIM proteins have been identified and are more often used to modulate autophagy (either up-regulation or down-reglation) in order to favorably effect an intended outcome. SEQ ID NOs for TRIM proteins 1-75 and siRNA TRIM protein inhibitors are set forth in the table on pages 92-116 of the present application just before the claims.

In addition to TRIM proteins which find use in the present invention (pharmaceutical compositions comprising these proteins may be administered to patients in order to regulate (up- or down-regulate autophagy), inhibitors of these proteins, especially including small inhibitory RNAs or small interfering RNAs (siRNAs) may also be used to impact autophagy and treat disease states and/or conditions which are mediated through autophagy. A number of siRNAs can be used to inhibit any one or more of the TRIM proteins pursuant to the present invention. Exemplary siRNAs are presented herein in the table just before the claims. Thus, siRNAs which can be used in the present invention include the siRNAs according to the specific sequences indicated in the attached table, as well as oligos which are plus/minus up to five nucleotide units upstream or downstream of the identified siRNAs. Additional variants of these variants include those with 90% sequence identity to the siRNAs set forth in the table on pages 92-116 or variants that exhibit polymorphism to the disclosed siRNAs. These siRNAs range in size from about 9-10 nucleotide units up to about 29-30 nucleotide units, with 19-23 nucleotide units being preferred. Preferably, these siRNAs are the specific siRNAs which are disclosed in the table on pages 92-116 hereof or siRNAs which contain up to five nucleotide units more upstream and/or downstream to the disclosed siRNAs.

The term “modulator of autophagy”, “regulator of autophagy” or “autostatin” is used to refer to a compound which functions as an agonist (inducer or up-regulator) or antagonist (inhibitor or down-regulator) of autophagy and are unrelated to the IRGM modulators, interferons, TRIM proteins or TRIM protein inhibitors (e.g. siRNAs as disclosed herein). These modulators may be used in combination with an IRGM modulator and/or a TRIM protein, interferon or siRNA inhibitor in methods and compositions pursuant to the present invention. Depending upon the disease state or condition, autophagy may be upregulated (and require inhibition of autophagy for therapeutic intervention) or down-regulated (and require upregulation of autophagy for therapeutic intervention). In most instances, in the case of cancer treatment with a modulator of autophagy as otherwise described herein, the autophagy modulator is often an antagonist of autophagy. In the case of cancer, the antagonist (inhibitor) of autophagy may be used alone or combined with an agonist of autophagy

The following compounds have been identified as autophagy modulators according to the present invention and can be used in combination with an IRGM modulator or Trim protein as disclosed herein in the treatment of an autophagy mediated disease state or condition as otherwise described herein. It is noted that an inhibitor of autophagy is utilized where the disease state or condition is mediated through upregulation or an increase in autophagy which causes the disease state or condition and an agonist of autophagy is utilized where the disease state or condition is mediated through downregulation or a decrease in autophagy. The following compounds have been identified as autophagy modulators (autotaxins) in autophagy assays according to the present invention: flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon and nortriptyline, tetrachlorisophthalonitrile, phenylmercuric acetate and pharmaceutically acceptable salts thereof. It is noted that flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline and their pharmaceutically acceptable salts show activity as agonists or inducers of autophagy in the treatment of an autophagy-mediated disease, whereas tetrachlorisophthalonitrile, phenylmercuric acetate and their pharmaceutically acceptable salts, find use as antagonists or inhibitors of autophagy. All of these compounds will find use

as modulators of autophagy in the various autophagy-mediated disease states and conditions described herein, with the agonists being preferred in most disease states other than cancer (although inhibitors may also be used alone, or preferably in combination with the agonists) and in the case of the treatment of cancer, the inhibitors described above are preferred, alone or in combination with an autophagy agonist as described above and/or an additional anticancer agent as otherwise described herein.

Other compounds which may be used in combination with the IRGM modulators and/or TRIM proteins and/or siRNAs as otherwise described herein either alone or in combination with the autophagy modulators which are described above, include for example, other “additional autophagy modulators” or “additional autostatins” which are known in the art. These can be combined with one or more of the autophagy modulators which are disclosed above to provide novel pharmaceutical compositions and/or methods of treating autophagy mediated disease states and conditions which are otherwise described herein. These additional autophagy modulators including benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandrosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurantoin, iopanoic acid, naftopidil, Methimazole, Trimeprazine, Ethoxyquin, Clotacortolone, Doxycycline, Pirlindole mesylate, Doxazosin, Deptropine, Nocodazole, Scopolamine, Oxybenzone, Halcinonide, Oxybutynin, Miconazole, Clomipramine, Cyproheptadine, Doxepin, Dyclonine, Salbutamol, Flavoxate, Amoxapine, Fenofibrate, Pimethixene and mixtures thereof.

The following compounds have been identified as autophagy modulators according to the present invention and can be used in the treatment of an autophagy mediated disease state or condition as otherwise described herein. These include interferon, especially interferon-gamma (IFN-gamma), pegylated interferon (PEG-IFN) and related compounds and certain TRIM proteins and variants thereof, including TRIM1, TRIM3, TRIM8, TRIM10, TRIM13, TRIM17, TRIM19, TRIM20, TRIM21, TRIM22, TRIM38, TRIM 41, TRIM43, TRIM44,

TRIM45, TRIM46, TRIM54, TRIM55, TRIM56, TRIM58, TRIM59, TRIM60, TRIM65, TRIM66 and TRIM75 with TRIM 1, TRIM 8, TRIM 20, TRIM 21, TRIM 22, TRIM 56 and TRIM 65 and mixtures thereof and preferably, TRIM 1, TRIM 8, TRIM 20, TRIM 21, TRIM 22, TRIM 56, TRIM 65 and mixtures thereof. The following compounds have been identified as autophagy modulators which may be used in combination with the above-identified autophagy agents. These agents include, for example flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon and nortriptyline, tetrachlorisophthalonitrile, phenylmercuric acetate and pharmaceutically acceptable salts thereof. It is noted that flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandrosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nifedipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurantoin, iopanoic acid, naftopidil, Methimazole, Trimeprazine, Ethoxyquin, Clotrimazole, Doxycycline, Pirlindole mesylate, Doxazosin, Deptropine, Nocodazole, Scopolamine, Oxybenzone, Halcinonide, Oxybutynin, Miconazole, Clomipramine, Cyproheptadine, Doxepin, Dyclonine, Salbutamol, Flavoxate, Amoxapine, Fenofibrate, Pimethixene, and mixtures thereof. Additional autophagy agents include alternative TRIM proteins or variants thereof, such as, but not limited to, TRIM5 α , TRIM6, TRIM10, TRIM17, TRIM41, TRIM55, TRIM72, TRIM76, TRIM2, TRIM23, TRIM26, TRIM28, TRIM31, TRIM 32, TRIM33, TRIM38, TRIM42, TRIM44, TRIM45, TRIM49, TRIM50, TRIM51, TRIM58, TRIM59, TRIM68, TRIM73, TRIM74 and TRIM76 and mixtures thereof. Neutral lipids such as lipids selected from the group consisting of triglycerides, diglycerides, monoglycerides, glycolated mono- or diacylglycerides, dolichol, polyprenol, polyprenal or very long chain fatty acids and mixtures thereof and their pharmaceutically acceptable salts may also be included for use in the present invention either alone or preferably in combination with one or more TRIM protein. All of

these compounds will find use as modulators of autophagy in the various autophagy-mediated disease states and conditions described herein.

The term “co-administration” or “combination therapy” is used to describe a therapy in which at least two active compounds in effective amounts are used to treat an autophagy mediated disease state or condition as otherwise described herein, either at the same time or within dosing or administration schedules defined further herein or ascertainable by those of ordinary skill in the art. Although the term co-administration preferably includes the administration of two active compounds to the patient at the same time, it is not necessary that the compounds be administered to the patient at the same time, although effective amounts of the individual compounds will be present in the patient at the same time. In addition, in certain embodiments, co-administration will refer to the fact that two compounds are administered at significantly different times, but the effects of the two compounds are present at the same time. Thus, the term co-administration includes an administration in which one active agent (especially an autophagy modulator) is administered at approximately the same time (contemporaneously), or from about one to several minutes to about 24 hours or more than the other bioactive agent coadministered with the autophagy modulator. The additional bioactive agent may be any bioactive agent, but is generally selected from an additional autophagy mediated compound as described herein, an additional anticancer agent, or another agent, such as a mTOR inhibitor such as pp242, rapamycin, envirolimus, everolimus or cidaforollimus, among others including epigallocatechin gallate (EGCG), caffeine, curcumin or resveratrol (which mTOR inhibitors find particular use as enhancers of autophagy using the compounds disclosed herein and in addition, in the treatment of cancer with an autophagy modulator (inhibitor) as described herein, including in combination with tetrachlorisophthalonitrile, phenylmercuric acetate and their pharmaceutically acceptable salts, which are inhibitors of autophagy. It is noted that in the case of the treatment of cancer, the use of an autophagy inhibitor is preferred, alone or in combination with an autophagy inducer (agonist) as otherwise described herein and/or a mTOR inhibitor as described above. In certain embodiments, an mTOR inhibitor selected from the group consisting of pp242, rapamycin, envirolimus, everolimus, cidaforollimus, epigallocatechin gallate (EGCG), caffeine, curcumin, resveratrol and mixtures thereof may be combined with at least one agent selected from the group consisting of digoxin, xylazine, hexetidine and sertindole, the combination of such agents being effective as autophagy modulators in combination.

The term “cancer” is used throughout the specification to refer to the pathological process that results in the formation and growth of a cancerous or malignant neoplasm, i.e., abnormal tissue that grows by cellular proliferation, often more rapidly than normal and continues to grow after the stimuli that initiated the new growth cease. Malignant neoplasms show partial or complete lack of structural organization and functional coordination with the normal tissue and most invade surrounding tissues, metastasize to several sites, and are likely to recur after attempted removal and to cause the death of the patient unless adequately treated.

As used herein, the term neoplasia is used to describe all cancerous disease states and embraces or encompasses the pathological process associated with malignant hematogenous, ascitic and solid tumors. Representative cancers include, for example, stomach, colon, rectal, liver, pancreatic, lung, breast, cervix uteri, corpus uteri, ovary, prostate, testis, bladder, renal, brain/CNS, head and neck, throat, Hodgkin’s disease, non-Hodgkin’s lymphoma, multiple myeloma, leukemia, melanoma, non-melanoma skin cancer (especially basal cell carcinoma or squamous cell carcinoma), acute lymphocytic leukemia, acute myelogenous leukemia, Ewing’s sarcoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, Wilms’ tumor, neuroblastoma, hairy cell leukemia, mouth/pharynx, oesophagus, larynx, kidney cancer and lymphoma, among others, which may be treated by one or more compounds according to the present invention. In certain aspects, the cancer which is treated is lung cancer, breast cancer, ovarian cancer and/or prostate cancer.

The term “tumor” is used to describe a malignant or benign growth or tumefacent.

The term “additional anti-cancer compound”, “additional anti-cancer drug” or “additional anti-cancer agent” is used to describe any compound (including its derivatives) which may be used to treat cancer. The “additional anti-cancer compound”, “additional anti-cancer drug” or “additional anti-cancer agent” can be an anticancer agent which is distinguishable from a CIAE-inducing anticancer ingredient such as a taxane, vinca alkaloid and/or radiation sensitizing agent otherwise used as chemotherapy/cancer therapy agents herein. In many instances, the co-administration of another anti-cancer compound according to the present invention results in a synergistic anti-cancer effect. Exemplary anti-cancer compounds for co-administration with formulations according to the present invention

include anti-metabolites agents which are broadly characterized as antimetabolites, inhibitors of topoisomerase I and II, alkylating agents and microtubule inhibitors (e.g., taxol), as well as tyrosine kinase inhibitors (e.g., surafenib), EGF kinase inhibitors (e.g., tarceva or erlotinib) and tyrosine kinase inhibitors or ABL kinase inhibitors (e.g. imatinib).

Anti-cancer compounds for co-administration include, for example, Aldesleukin; Alemtuzumab; alitretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; Asparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan intravenous; busulfan oral; calusterone; capecitabine; carboplatin; carmustine; carmustine with Polifeprosan 20 Implant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cytarabine; cytarabine liposomal; dacarbazine; dactinomycin; actinomycin D; Darbepoetin alfa; daunorubicin liposomal; daunorubicin, daunomycin; Denileukin difitox, dexrazoxane; docetaxel; doxorubicin; doxorubicin liposomal; Dromostanolone propionate; Elliott's B Solution; epirubicin; Epoetin alfa estramustine; etoposide phosphate; etoposide (VP-16); exemestane; Filgrastim; floxuridine (intraarterial); fludarabine; fluorouracil (5-FU); fulvestrant; gemtuzumab ozogamicin; gleevec (imatinib); goserelin acetate; hydroxyurea; Ibritumomab Tiuxetan; idarubicin; ifosfamide; imatinib mesylate; Interferon alfa-2a; Interferon alfa-2b; irinotecan; letrozole; leucovorin; levamisole; lomustine (CCNU); meclorethamine (nitrogen mustard); megestrol acetate; melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane; mitoxantrone; nandrolone phenpropionate; Nofetumomab; LOddC; Oprelvekin; oxaliplatin; paclitaxel; pamidronate; pegademase; Pegasparase; Pegfilgrastim; pentostatin; pipobroman; plicamycin; mithramycin; porfimer sodium; procarbazine; quinacrine; Rasburicase; Rituximab; Sargramostim; streptozocin; surafenib; talbuvudine (LDT); talc; tamoxifen; tarceva (erlotinib); temozolomide; teniposide (VM-26); testolactone; thioguanine (6-TG); thiotepa; topotecan; toremifene; Tositumomab; Trastuzumab; tretinoin (ATRA); Uracil Mustard; valrubicin; valtorcitabine (monoval LDC); vinblastine; vinorelbine; zoledronate; and mixtures thereof, among others.

Co-administration of one of the formulations of the invention with another anticancer agent will often result in a synergistic enhancement of the anticancer activity of the other anticancer agent, an unexpected result. One or more of the present formulations comprising an IRGM modulator optionally in combination with an autophagy modulator (autostatin) as described herein may also be co-administered with another bioactive agent (e.g., antiviral

agent, antihyperproliferative disease agent, agents which treat chronic inflammatory disease, among others as otherwise described herein).

The term “antiviral agent” refers to an agent which may be used in combination with autophagy modulators (autostatins) as otherwise described herein to treat viral infections, especially including HIV infections, HBV infections and/or HCV infections. Exemplary anti-HIV agents include, for example, nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, fusion inhibitors, among others, exemplary compounds of which may include, for example, 3TC (Lamivudine), AZT (Zidovudine), (-)-FTC, ddI (Didanosine), ddC (zalcitabine), abacavir (ABC), tenofovir (PMPA), D-D4FC (Reverset), D4T (Stavudine), Racivir, L-FddC, L-FD4C, NVP (Nevirapine), DLV (Delavirdine), EFV (Efavirenz), SQVM (Saquinavir mesylate), RTV (Ritonavir), IDV (Indinavir), SQV (Saquinavir), NFV (Nelfinavir), APV (Amprenavir), LPV (Lopinavir), fusion inhibitors such as T20, among others, fuseon and mixtures thereof, including anti-HIV compounds presently in clinical trials or in development. Exemplary anti-HBV agents include, for example, hepsera (adefovir dipivoxil), lamivudine, entecavir, telbivudine, tenofovir, emtricitabine, clevudine, valtorecitabine, amdoxovir, pradefovir, racivir, BAM 205, nitazoxanide, UT 231-B, Bay 41-4109, EHT899, zadaxin (thymosin alpha-1) and mixtures thereof. Anti-HCV agents include, for example, interferon, pegylated interferon, ribavirin, NM 283, VX-950 (telaprevir), SCH 50304, TMC435, VX-500, BX-813, SCH503034, R1626, ITMN-191 (R7227), R7128, PF-868554, TT033, CGH-759, GI 5005, MK-7009, SIRNA-034, MK-0608, A-837093, GS 9190, ACH-1095, GSK625433, TG4040 (MVA-HCV), A-831, F351, NS5A, NS4B, ANA598, A-689, GNI-104, IDX102, ADX184, GL59728, GL60667, PSI-7851, TLR9 Agonist, PHX1766, SP-30 and mixtures thereof.

An “inflammation-associated metabolic disorder” includes, but is not limited to, lung diseases, hyperglycemic disorders including diabetes and disorders resulting from insulin resistance, such as Type I and Type II diabetes, as well as severe insulin resistance, hyperinsulinemia, and dyslipidemia or a lipid-related metabolic disorder (e.g. hyperlipidemia (e.g., as expressed by obese subjects), elevated low-density lipoprotein (LDL), depressed high-density lipoprotein (HDL), and elevated triglycerides) and insulin-resistant diabetes, such as Mendenhall's Syndrome, Werner Syndrome, leprechaunism, and lipotrophic diabetes, renal disorders, such as acute and chronic renal insufficiency, end-stage chronic

renal failure, glomerulonephritis, interstitial nephritis, pyelonephritis, glomerulosclerosis, e.g., Kimmelstiel-Wilson in diabetic patients and kidney failure after kidney transplantation, obesity, GH-deficiency, GH resistance, Turner's syndrome, Laron's syndrome, short stature, increased fat mass-to-lean ratios, immunodeficiencies including decreased CD4⁺ T cell counts and decreased immune tolerance or chemotherapy-induced tissue damage, bone marrow transplantation, diseases or insufficiencies of cardiac structure or function such as heart dysfunctions and congestive heart failure, neuronal, neurological, or neuromuscular disorders, e.g., diseases of the central nervous system including Alzheimer's disease, or Parkinson's disease or multiple sclerosis, and diseases of the peripheral nervous system and musculature including peripheral neuropathy, muscular dystrophy, or myotonic dystrophy, and catabolic states, including those associated with wasting caused by any condition, including, e.g., mental health condition (e.g., anorexia nervosa), trauma or wounding or infection such as with a bacterium or human virus such as HIV, wounds, skin disorders, gut structure and function that need restoration, and so forth.

An “inflammation-associated metabolic disorder” also includes a cancer and an “infectious disease” as defined herein, as well as disorders of bone or cartilage growth in children, including short stature, and in children and adults disorders of cartilage and bone in children and adults, including arthritis and osteoporosis. An “inflammation-associated metabolic disorder” includes a combination of two or more of the above disorders (e.g., osteoporosis that is a sequela of a catabolic state). Specific disorders of particular interest targeted for treatment herein are diabetes and obesity, heart dysfunctions, kidney disorders, neurological disorders, bone disorders, whole body growth disorders, and immunological disorders.

In one embodiment, “inflammation-associated metabolic disorder” includes: central obesity, dyslipidemia including particularly hypertriglyceridemia, low HDL cholesterol, small dense LDL particles and postprandial lipemia; glucose intolerance such as impaired fasting glucose; insulin resistance and hypertension, and diabetes. The term “diabetes” is used to describe diabetes mellitus type I or type II. The present invention relates to a method for improving renal function and symptoms, conditions and disease states which occur secondary to impaired renal function in patients or subjects with diabetes as otherwise described herein. It is noted that in diabetes mellitus type I and II, renal function is impaired from collagen deposits, and not from cysts in the other disease states treated by the present invention.

Mycobacterial infections often manifest as diseases such as tuberculosis. Human infections caused by *mycobacteria* have been widespread since ancient times, and tuberculosis remains a leading cause of death today. Although the incidence of the disease declined, in parallel with advancing standards of living, since the mid-nineteenth century, mycobacterial diseases still constitute a leading cause of morbidity and mortality in countries with limited medical resources. Additionally, mycobacterial diseases can cause overwhelming, disseminated disease in immunocompromised patients. In spite of the efforts of numerous health organizations worldwide, the eradication of mycobacterial diseases has never been achieved, nor is eradication imminent. Nearly one third of the world's population is infected with mycobacterium tuberculosis complex, commonly referred to as tuberculosis (TB), with approximately 8 million new cases, and two to three million deaths attributable to TB yearly. Tuberculosis (TB) is the cause of the largest number of human deaths attributable to a single etiologic agent (see Dye et al., J. Am. Med. Association, 282, 677-686, (1999); and 2000 WHO/OMS Press Release).

Mycobacteria other than *M. tuberculosis* are increasingly found in opportunistic infections that plague the AIDS patient. Organisms from the *M. avium*-intracellulare complex (MAC), especially serotypes four and eight, account for 68% of the mycobacterial isolates from AIDS patients. Enormous numbers of MAC are found (up to 10^{10} acid-fast bacilli per gram of tissue), and consequently, the prognosis for the infected AIDS patient is poor.

In many countries the only measure for TB control has been vaccination with *M. bovis* bacille Calmette-Guerin (BCG). The overall vaccine efficacy of BCG against TB, however, is about 50% with extreme variations ranging from 0% to 80% between different field trials. The widespread emergence of multiple drug-resistant *M. tuberculosis* strains is also a concern.

M. tuberculosis belongs to the group of intracellular bacteria that replicate within the phagosomal vacuoles of resting macrophages, thus protection against TB depends on T cell-mediated immunity. Several studies in mice and humans, however, have shown that *Mycobacteria* stimulate antigen-specific, major histocompatibility complex (MHC) class II- or class I-restricted CD4 and CD8 T cells, respectively. The important role of MHC class I-restricted CD8 T cells was convincingly demonstrated by the failure of $\beta 2$ -microglobulin deficient mice to control experimental *M. tuberculosis* infection.

As used herein, the term "tuberculosis" comprises disease states usually associated with infections caused by mycobacteria species comprising *M. tuberculosis* complex. The term "tuberculosis" is also often associated with mycobacterial infections caused by mycobacteria other than *M. tuberculosis*. Other mycobacterial species include *M. avium-intracellulare*, *M. kansarii*, *M. fortuitum*, *M. chelonae*, *M. leprae*, *M. africanum*, and *M. microti*, *M. avium paratuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. marinum*, *M. ulcerans*.

An "infectious disease" includes but is limited to those caused by bacterial, mycological, parasitic, and viral agents. Examples of such infectious agents include the following: *staphylococcus*, *streptococcaceae*, *neisseriaaceae*, *cocci*, *enterobacteriaceae*, *pseudomonadaceae*, *vibrionaceae*, *campylobacter*, *pasteurellaceae*, *bordetella*, *francisella*, *brucella*, *legionellaceae*, *bacteroidaceae*, gram-negative *bacilli*, *clostridium*, *corynebacterium*, *propionibacterium*, gram-positive *bacilli*, anthrax, *actinomyces*, *nocardia*, *mycobacterium*, *treponema*, *borrelia*, *leptospira*, *mycoplasma*, *ureaplasma*, *rickettsia*, *chlamydiae*, systemic mycoses, opportunistic mycoses, protozoa, nematodes, trematodes, cestodes, adenoviruses, herpesviruses, poxviruses, papovaviruses, hepatitis viruses (B and C, among others), orthomyxoviruses, paramyxoviruses, coronaviruses, picornaviruses, reoviruses, togaviruses, flaviviruses, bunyaviridae, rhabdoviruses, human immunodeficiency virus (I and II) and retroviruses.

In certain embodiments, an "infectious disease" is selected from the group consisting of tuberculosis, leprosy, Crohn's Disease, acquired immunodeficiency syndrome, Lyme disease, cat-scratch disease, Rocky Mountain spotted fever and influenza or a viral infection selected from HIV (I and/or II), hepatitis B virus (HBV) or hepatitis C virus (HCV).

While not being limited by way of theory, it is believed that autophagy-mediated disease states which evidence upregulated autophagy and upregulated TRIM proteins include inflammatory disease states and autoimmune disease states as otherwise described herein. These disease states and/or conditions may benefit from the inhibition of TRIM proteins where there is evidence that autophagy is up-regulated and needs to be brought back into balance in order to facilitate healing of the disease state and/or condition. In these disease states, the inhibition of TRIM proteins, including inhibiting TRIM proteins by administration

of small interfering RNAs (siRNAs) which inhibit the synthesis of the TRIM protein to be reduced in order to down regulate autophagy may be useful. This approach may provide beneficial treatment in a large number of disease states and conditions where upregulation of autophagy is responsible for the disease state and/or exacerbating the disease state. In other disease states, in particular, bacterial and viral infections, especially tuberculosis and in some instances of cancer, autophagy is often down-regulated and may benefit from the upregulation of autophagy through the administration of one or more TRIM proteins (especially TRIM20) alone or in combination with interferon-gamma, pegylated interferon and/or one more more additional autophagy agents including alternative TRIM proteins as otherwise disclosed herein.

According to various embodiments, the compounds according to the present invention may be used for treatment or prevention purposes in the form of a pharmaceutical composition. This pharmaceutical composition may comprise one or more of an active ingredient as described herein.

As indicated, the pharmaceutical composition may also comprise a pharmaceutically acceptable excipient, additive or inert carrier. The pharmaceutically acceptable excipient, additive or inert carrier may be in a form chosen from a solid, semi-solid, and liquid. The pharmaceutically acceptable excipient or additive may be chosen from a starch, crystalline cellulose, sodium starch glycolate, polyvinylpyrrolidone, polyvinylpolypyrrolidone, sodium acetate, magnesium stearate, sodium laurylsulfate, sucrose, gelatin, silicic acid, polyethylene glycol, water, alcohol, propylene glycol, vegetable oil, corn oil, peanut oil, olive oil, surfactants, lubricants, disintegrating agents, preservative agents, flavoring agents, pigments, and other conventional additives. The pharmaceutical composition may be formulated by admixing the active with a pharmaceutically acceptable excipient or additive.

The pharmaceutical composition may be in a form chosen from sterile isotonic aqueous solutions, pills, drops, pastes, cream, spray (including aerosols), capsules, tablets, sugar coating tablets, granules, suppositories, liquid, lotion, suspension, emulsion, ointment, gel, and the like. Administration route may be chosen from subcutaneous, intravenous, intestinal, parenteral, oral, buccal, sublingual, nasal, intramuscular, transcutaneous, transdermal, intranasal, intratracheal, intrathecal, pulmonary, intraperitoneal, and topical, among others. The pharmaceutical compositions may be immediate release,

sustained/controlled release, or a combination of immediate release and sustained/controlled release depending upon the compound(s) to be delivered, the compound(s), if any, to be coadministered, as well as the disease state and/or condition to be treated with the pharmaceutical composition. A pharmaceutical composition may be formulated with differing compartments or layers in order to facilitate effective administration of any variety consistent with good pharmaceutical practice.

The subject or patient may be chosen from, for example, a human, a mammal such as domesticated animal, or other animal. The subject may have one or more of the disease states, conditions or symptoms associated with autophagy as otherwise described herein.

The compounds according to the present invention may be administered in an effective amount to treat or reduce the likelihood of an autophagy-mediated disease and/or condition as well one or more symptoms associated with the disease state or condition. One of ordinary skill in the art would be readily able to determine an effective amount of active ingredient by taking into consideration several variables including, but not limited to, the animal subject, age, sex, weight, site of the disease state or condition in the patient, previous medical history, other medications, etc.

For example, the dose of an active ingredient which is useful in the treatment of an autophagy mediated disease state, condition and/or symptom for a human patient is that which is an effective amount and may range from as little as 100 μg or even less to at least about 500 mg up to a gram or more, which may be administered in a manner consistent with the delivery of the drug and the disease state or condition to be treated. In the case of oral administration, active is generally administered from one to four times or more daily. Transdermal patches or other topical administration may administer drugs continuously, one or more times a day or less frequently than daily, depending upon the absorptivity of the active and delivery to the patient's skin. Of course, in certain instances where parenteral administration represents a favorable treatment option, intramuscular administration or slow IV drip may be used to administer active. The amount of active ingredient which is administered to a human patient preferably ranges from about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 7.5 mg/kg, about 0.25 mg/kg to about 6 mg/kg., about 1.25 to about 5.7 mg/kg.

The dose of a compound according to the present invention may be administered at the first signs of the onset of an autophagy mediated disease state, condition or symptom. For example, the dose may be administered for the purpose of lung or heart function and/or treating or reducing the likelihood of any one or more of the disease states or conditions which become manifest during an inflammation-associated metabolic disorder or tuberculosis or associated disease states or conditions, including pain, high blood pressure, renal failure, or lung failure. The dose of active ingredient may be administered at the first sign of relevant symptoms prior to diagnosis, but in anticipation of the disease or disorder or in anticipation of decreased bodily function or any one or more of the other symptoms or secondary disease states or conditions associated with an autophagy mediated disorder to condition.

Synthesis of TRIM proteins according to the present invention may be performed by the routineer skilled in the art and may be provided by engineering polynucleotide sequences corresponding to the amino acid sequences of the TRIM proteins into plasmids for expression, transfecting the plasmids into both eukaryotic and/or prokaryotic cells and accumulating protein from the growth of the cells containing the plasmids. Alternatively, the proteins may be readily synthesized by standard, well-known peptide synthesis methods, including solid phase synthesis.

The following examples are provided to further describe the present invention. The examples, while descriptive of the present invention, are not to be construed as limiting the present invention.

EXAMPLES

First Set- IRGM Examples

Antibodies, plasmids, and siRNA

Antibodies were from Cell Signaling (AMPK, AMPK Thr-172, ULK1, ULK1 p-Ser 317, p-Ser 757, p-Ser555, NOD2, Beclin 1 p-Ser-93/96 and ATG5), MBL international corp. (ATG16L1, ATG14L, Rubicon and UVRAG), Abcam (GFP, IRGM, LPS, TRAF6 and BCL2), Sigma (LC3B, Flag), Millipore (V5 tag and HA tag), Abbiotec (Beclin 1 p-Ser15) and Novus biological (AMBRA1). GFP-tagged IRGM expression plasmid (GFP-IRGMd) was described previously (Singh et al., 2010). GFP-IRGM-K^{mut} was generated from GFP-IRGMd plasmid by replacing wild type IRGMd gene with synthetic mutated IRGMd gene

(GeneScript) with all lysine residues mutated to arginine. Flag-IRGM and IRGM-V5 were generated by Gateway cloning (Life technologies). HA-UbiquitinC, HA-UbiquitinC-K63 (all lysine mutated except K63, Plasmid 17606), HA-UbiquitinC-K48 (all lysine mutated except K48, Plasmid 17605), Flag-TLR3 (Plasmid 13084) and YFP-TLR4 (Plasmid 13018) were from Addgene. Flag-NOD2 and variants were from Dr. Thomas Kufer (University of Cologne, Germany). Flag-ATG16L1 and variants were from Dr. Ramnik Xavier (Massachusetts General Hospital, Boston). Flag-TRAF6 was from Dr. Edward Harhaj (Johns Hopkins School of Medicine, US). IRGM siRNA, TRAF6 siRNA, AMPK α 2 siRNA were from Dharmacon (siGENOME SMART pool).

Autophagy induction

U937 cells were treated with LPS (500 ng/ml) for 4 h or by transfecting MDP (5 μ g/ml) with calcium phosphate for 8 h. For induction of autophagy by starvation, cells were cultured in EBSS.

Protein interactions analyses

For co-immunoprecipitation assays, the cells were lysed using NP-40 buffer containing protease inhibitor cocktail and PMSF. Lysates were incubated with antibody for 2 h followed by incubation with proteinG Dynabeads (Life technologies) for 2 h. Beads were washed for four times with 1XPBS and then boiled with SDS-PAGE buffer for analysis of interacting protein by Immunoblotting. Immunoblots were quantified using Image J software.

Microscopy analyses and quantification

Immunofluorescence was performed as described earlier (Kyei et al., 2009). For quantification of puncta, images from different fields were captured and analyzed. For quantification of total cell fluorescence, image J was used as described previously (Chauhan et al., 2013).

Gene expression analysis

Total RNA was isolated from cell culture using Trizol as per the manufacturer's instruction (Invitrogen). For quantitative real-time PCR: TURBO DNA-free kit (Ambion) was used to remove contaminating residual DNA; cDNA was prepared using the high

capacity cDNA reverse transcription kit as per the manufacturer's instruction (Applied Biosystem). Taqman probes (Applied Biosystem) and realtime PCR master mixes (Applied Biosystem) were used for real-time PCR as per the manufacturer's instruction. Data were normalized using GAPDH.

Bacterial survival analyses

AIEC LF82 survival assay was performed as described previously (Lapaquette et al., 2009). HEK293T cells were infected with AIEC LF82 of MOI of 1:20 for 3 h. Cells were treated with gentamycin (100 µg/ml) for 1 h followed by incubation in fresh media for 2 h. Cells were lysed and surviving bacteria quantified by plating and determining colony forming units.

Cytokine and NF-κB responses

For NFκB-p65 nuclear localization assay, HeLa cells were plated on cover slips a day before infection. Cells were infected with AIEC LF82 strain at MOI of 1:20 for 2 h followed by washings with PBS and fixing the cells with 4% paraformaldehyde. Immunofluorescence imaging was performed as described earlier (Kyei et al., 2009). Cells were visualized using a laser confocal microscope and images were captured using LSM510 software. For IL-1β measurement, IL-1β transcription was determined using qRT-PCR in THP-1 cells.

Results (IRGM Examples) All Figures for this Section are Labeled As Figures IRGM for the attached figures

IRGM activates the core regulators of autophagy

Prior work has indicated that IRGM affects autophagy through processes influencing mitochondrial function, including mitochondrial fission and membrane potential collapse (Singh et al., 2010). Similar changes in mitochondrial function often lead to AMPK activation (Romanello et al., 2010; Turkieh et al., 2014). Thus, we tested the activation status of AMPK. A knockdown of IRGM reduced the total amounts of AMPK in both control or starved cells (Figure 1A) and decreased the levels of the activated form of AMPK

phosphorylated at Thr-172 (Figure 1A). Overexpression of IRGM increased levels of Thr-172 phosphorylated AMPK (Figure 1B).

AMPK has been previously shown to induce autophagy by directly phosphorylating ULK1 (Egan et al., 2011; Kim et al., 2011) and Beclin 1 (Kim et al., 2013). When we tested the phosphorylation status of ULK1 and Beclin 1, we observed that the expression of IRGM, which caused induction of autophagy (Figure S1A), enhanced phosphorylation at activating sites of Beclin 1 at Ser93/96 (Kim et al., 2013), and ULK1 at Ser-555 (Egan et al., 2011) and at Ser-317 (Kim et al., 2011) (Figure 1B,C).

IRGM assembles the core regulatory machinery for autophagy

The entire signaling cascade described above could explain how IRGM induces autophagy, e.g. by its effects on AMPK and activation of downstream autophagy regulators. However, IRGM showed a further, more direct role by interacting with the key regulators of autophagy. We found that IRGM co-immunoprecipitated and co-localized with both endogenous and overexpressed ULK1 and Beclin 1 (Figure 1D-G and S1B-C) but not with AMPK (Figure S1D). IRGM complexes with ULK1 were enriched for the activated, AMPK-dependent Ser-317, form of ULK1 relative to the inhibitory, mTOR-dependent, Ser-757 form (Figure 1H). Furthermore, expression of IRGM enriched ULK1 in the immunoprecipitated Beclin 1 complexes (Figure 1I and S1G). In keeping with this, cells overexpressing IRGM also showed increased Beclin 1 Ser-15 phosphorylation, the phosphorylated form of Beclin 1 dependent on ULK1 activity (Kim et al., 2013) (Figure 1J).

IRGM determines the composition of the Beclin 1 complex

We found that IRGM complexes also included autophagy-enhancing Beclin1 interactors, AMBRA1 (Figure 1D and S1E), ATG14L (Figure 1K) and UVRAG (Figure S1F) but not the autophagy inhibitory factor Rubicon (Figure S1F) (Fimia et al., 2007; Itakura et al., 2008; Matsunaga et al., 2009). Next, we mapped Beclin 1 regions required for interaction with IRGM (Figure 1M). IRGM interacted with two Beclin 1 regions: (i) BH3-containing 1-125 N-terminal portion, and (ii) a segment encompassing CCD and ECD, whereas it did not bind to the intervening CCD domain alone (Figure 1L,M).

Incidentally, two Beclin1 negative regulators Bcl-2 and Rubicon bind respectively to the regions spanning Beclin 1's BH3 domain and Beclin 1's CCD and ECD domains,

whereas ATG14L, a factor enabling Beclin 1 to activate the initiation complex (Kim et al., 2013), binds to the CCD domain of Beclin 1 (Sun et al., 2008). This domain occupancy on Beclin1 is compatible with simultaneous binding of IRGM and ATG14L and exclusion of autophagy negative regulators. When IRGM was overexpressed, it dis-enriched Rubicon and Bcl-2 from Beclin 1 and enriched ATG14L in Beclin 1 complexes (Figure 1N).

The above data indicate that IRGM forms protein complexes with the central regulators of autophagy and activates Beclin 1 by displacing its negative regulators (Figure 1O, Right). This, taken together with IRGM's ability to sponsor the phosphorylation cascade that activates ULK1 and Beclin 1, shows how IRGM promotes autophagy (Figure 1O, Left).

IRGM affects levels of autophagy regulators

As observed with AMPK (Figure 1A), IRGM affected the levels of a number of other autophagy regulators. IRGM knockdown in U937 monocytic cells (Figure S2A) reduced total amount of ULK1 (Figure 2A,B, Figure S2B), ATG14L (Figure 2C, Figure S2B), and AMBRA1 (Figure 2C, Figure S2B). In contrast to the above suite of autophagy regulators, Beclin 1 was not affected (Figure S2C). In addition to Beclin 1, IRGM did not alter cytoplasmic levels of ATG5-ATG12 conjugates (Figure 2C). However, the physical organization of ATG5-ATG12 was affected, since the numbers of its puncta, revealed by ATG5 immunofluorescence, were reduced upon IRGM knockdown (Figure 2D). ATG5 puncta formation is governed by ATG16L1 (Mizushima, 2003). We thus looked at the effects of IRGM on ATG16L1 levels and observed that they were reduced in IRGM knockdown cells (Figure 2E,F). This prompted us to test whether IRGM might interact with ATG16L1. IRGM was in complexes with endogenous Atg16L1 (Figure 2G). Further domain mapping showed that IRGM primarily interacted with the WD repeats region of ATG16L1 (Figure 2H,I). The residual weak interaction between IRGM and ATG16L1 outside of the WD repeats (construct ATG16L1(1-341)) was not due to FIP200, previously shown to bridge ATG16L1 with ULK1 (Gammoh et al., 2013) since interaction was not reduced upon FIP200 knockdown, and if anything was slightly increased (Figure S2D). In summary, in addition to directing the assembly of key autophagy-specific regulators, IRGM also stabilizes them. Furthermore IRGM interacts with and stabilizes ATG16L1, a component of the ATG5-Atg12/ATG16L1 E3 complex, which governs LC3 conjugation and autophagosome formation (Mizushima, 2003).

Expression of IRGM and its assembly with autophagy factors responds to microbial signals

Infection with CD-associated adhesive invasive *Escherichia coli* (AIEC) LF82 (Lapaquette et al., 2010) or treatment with LPS or muramyl dipeptide (MDP) induced *IRGM* expression in U937 cells (Figure 3A-C). The induction of *IRGM* was similar to other physiological inducers of autophagy: starvation and IFN- γ (Gutierrez et al., 2004) which acted in a cell type-dependent manner, and, in the case of starvation, showed AMPK dependence (Figure 3D, Figure S3A-I). When autophagy was induced by LPS (Figure S3J) or MDP (Figure S3K) (Cooney et al., 2010), a knockdown of *IRGM* (Figure S3L) precluded LC3B-II conversion and LC3B puncta formation in response to these stimuli (Figure 3E-H). Thus, *IRGM* is required for autophagy elicited by microbial products.

In experiments with endogenous proteins, we could not detect interactions of *IRGM* with ULK1 and ATG16L1 under basal conditions (Figure 3I, untreated lane). However, when a monocytic cell line (THP-1) was infected with *E. coli* LF82, immunoprecipitates of endogenous *IRGM* contained ULK1 and ATG16L1 (Figure 3I, AIEC lane). Similar effects were observed with MDP and LPS (Figure 3I). Of note, MDP (a NOD2-cogate ligand) was a stronger promoter of these effects than LPS. In contrast to ULK1 and ATG16L1, which showed interactions with endogenous *IRGM* only in samples from cells infected or treated with MDP or LPS, AMBRA1 showed association with endogenous *IRGM* even under basal conditions (Figure 3I). Thus, exposure of cells to microbes or their products affects *IRGM* expression and also influences interactions with the autophagic apparatus (Figure 3J).

Three Crohn's disease risk factors, NOD2, IRGM, and ATG16L1 interact

A known receptor for MDP is NOD2, a risk factor for familial CD (Ogura et al., 2001). Furthermore, ATG16L1, harboring an important CD-associated polymorphism (Consortium, 2007), interacts with NOD2 (Cooney et al., 2010; Travassos et al., 2010). Hence, we wondered whether *IRGM*, a third genetic CD risk factor (incidentally co-discovered with ATG16L1) (Consortium, 2007), is a part of this complex. Endogenous and overexpressed *IRGM* immunoprecipitates contained both NOD2 and ATG16L1 (Figure 4A, B). *IRGM* increased interactions between NOD2 and ATG16L1 (Figure S4A). In contrast, co-expression of NOD2 did not affect *IRGM*-ATG16L1 interactions (Figure S4B), suggesting that *IRGM* is important for promoting the assembly of the tri-partite complex.

Morphologically, NOD2 co-expression changed IRGM intracellular distribution from diffuse cytosolic to punctate (Figure S4C). A subset of these profiles colocalized with mitochondrial markers (Tom20; Figure S4D, E), in keeping with a partial NOD2 colocalization with mitochondrial antiviral signaling protein MAVS (Sabbah et al., 2009), and the previously reported partial IRGM localization to mitochondria (Singh et al., 2010).

All three factors, IRGM, ATG16L1, and NOD2 co-localized in co-transfected cells (Figure 4C). Mapping of interaction domains revealed that association of IRGM with NOD2 is likely a regulated event. A region containing the two CARD domains of NOD2 was required for IRGM interaction (Figure 4D,E). A deletion of the LRR domains in NOD2 enhanced interactions between IRGM and NOD2 (Figure 4D,E). The LRR domain region is known to be inhibitory to the previously established NOD2 activities (Tanabe et al., 2004) by keeping NOD2 in a closed conformation until it is activated through stimuli such as MDP (Tanabe et al., 2004). IRGM and NOD2 interaction was confirmed by proximity ligation assay (PLA; Figure S4F), which reports direct protein-protein interactions *in situ*. Positive PLA readouts of direct *in situ* interactions between proteins appear as fluorescent dots, the products of *in situ* PCR that generates a fluorescent product physically attached to antibodies against the two proteins that are <16 nm (FRET distance) apart. A deletion of the CARD domains in NOD2 reduced the NOD2-IRGM PLA signal (Figure S4F), in keeping with the importance of CARDS for the interactions between IRGM and NOD2. We carried out additional interaction experiments with purified GST-IRGM protein (Singh et al., 2010), prepared from insect cells (Figure S4G, isoform d, used in all experiments in this work), and Flag-NOD2 (full length and its variants Δ CARD and Δ LRR) prepared from 293T overexpressing cells. The results show that IRGM interacts with full length NOD2 and Δ LRR NOD2, but not with Δ CARD NOD2 (Figure 4F). These findings demonstrate that the NOD2 CARD domain is key for interactions with IRGM.

Fluorescently labeled MDP co-localized with NOD2 and IRGM in the cells (Figure 4G). In the presence of MDP, interactions between IRGM and NOD2 were enhanced (Figure 4H). These findings are consistent with the inhibitory action of LRRs in the resting state of NOD2, and with the observation that following activation with MDP, NOD2 becomes available for interactions with IRGM (Figure 4I). In summary, IRGM, NOD2, and ATG16L1

form a complex, with IRGM-NOD2 assembly being controlled by MDP, thus rendering the IRGM autophagy-promoting system responsive to microbial products.

NOD2 enhances IRGM interactions with ULK1 and Beclin 1

NOD2 affected IRGM quaternary structure. Co-expression of NOD2 and IRGM induced IRGM oligomerization within protein complexes (Figure 5A). NOD2 furthermore promoted interactions between IRGM and ULK1 as well as between IRGM and Beclin 1 (Figure 5B,C). Incidentally, NOD2 was also found in complexes with ULK1 (Figure S5A). IRGM co-expression increased ULK1-NOD2 complexes (Figure S5A). Thus, NOD2 modulates IRGM interactions with ULK1 and Beclin 1, in contrast to the above-described (Figure S4B) absence of NOD2 effects on IRGM-ATG16L1 complex formation. Based on these and above observations, IRGM is a pivotal organizer of the core parts of the autophagy initiation machinery (ULK1/Beclin1 and ATG16L1) along with NOD2.

Polyubiquitination of IRGM promotes its assembly with ULK1 and Beclin 1

In the co-immunoprecipitation experiments of NOD2 with IRGM, we observed the presence of multiple GFP-IRGM bands (Figure S5B). NOD2 is known to promote ubiquitination of several target proteins (Abbott et al., 2007; Hasegawa et al., 2008). We tested whether IRGM was ubiquitinated and observed that it can be polyubiquitinated whereas NOD2 enhanced IRGM ubiquitination (Figure 5D IRGM). To determine which ubiquitination linkage was involved, we co-expressed GFP-IRGM with two HA-tagged ubiquitin variants, one that can be ubiquitinated only at K63 and another one that can be ubiquitinated only at K48. The IRGM ubiquitination showed a much stronger signal with the HA-Ub-K63 (Figure 5E). Endogenous IRGM as well as a construct with a tag smaller than GFP (V5 tag; IRGM-V5) were K63 polyubiquitinated (Figure S5C IRGM, S5D IRGM). The K63 ubiquitination of IRGM was strongly enhanced in the presence of NOD2 (Figure 5E IRGM). Overexpression or downregulation of TRAF6, an E3 ligase known to work in concert within the NOD2 pathway (Abbott et al., 2007; Yang et al., 2007) increased or decreased IRGM ubiquitination (Figure S5E and S5F) suggesting a role for TRAF6 in IRGM ubiquitination. However, TRAF6 knockdown destabilized NOD2, so it was not possible to conclude that TRAF6 was the only E3 ligase responsible for IRGM ubiquitination. Next, we mapped which of the NOD2 domains are necessary for effective ubiquitination of IRGM, and found that deletion of the CARD domain in NOD2 prevented IRGM ubiquitination, consistent with IRGM's ability to bind to that region of NOD2 (Figure 5F). Moreover, when

the CARD domain of NOD2 was expressed alone, it enhanced IRGM K63 ubiquitination (Figure 5F).

Mutation of either individual or small clusters of K (Lys) residues in IRGM did not prevent K-63 linkage ubiquitination of IRGM in the presence of NOD2 (Figure S5G). In the absence of NOD2, the low level ubiquitination (see Figure 5E) of the same series of K mutants of IRGM also persisted (Figure S5H IRGM). A similar phenomenon, i.e. an absence of a dominant ubiquitination residue, has been described for several proteins including p53 (Chan et al., 2006) and cyclins (Fung et al., 2005). Paradoxically, mutation of the K-23/K-27 cluster in IRGM, enhanced K-63 linkage ubiquitination (Figure S5H IRGM); it nevertheless reduced K-48 linked ubiquitination (Figure S5I) suggesting that K-23/K-27 cluster may be a dominant K-48 ubiquitination site, and that its elimination enhances K-63 ubiquitination of IRGM. Thus, multiple K residues in IRGM are K63-ubiquitinated. When we mutated all twelve lysine residues in IRGM (IRGM-K^{mut}; K residues converted to R), the GFP-IRGM fusion lost ubiquitination capacity (Figure 5G). Nevertheless, GFP-IRGM-K^{mut} still bound ATG16L1 equally well as the wild type IRGM (Figure 5I). In contrast to its unaltered association with ATG16L1, GFP-IRGM-K^{mut} showed a reduced ability to oligomerize within protein complexes (revealed by using IRGM with two different tags; Figure 5H) and displayed diminished capacity for interactions with ULK1, Beclin 1 and AMBRA1 (Figure 5I). In addition, NOD2 could not increase Beclin 1-IRGM-K^{mut} interactions, although NOD2 increased Beclin 1 interactions with wild type IRGM (Figure S5J IRGM). Thus, polyubiquitination of IRGM is important for the assembly of the core regulatory machinery centered on ULK1 and Beclin 1, and this modification of IRGM is under the control by NOD2.

Polyubiquitinated IRGM inversely controls NOD2 and ULK1 protein levels

We observed that co-expression of GFP-IRGM had an effect on NOD2 protein amount, by diminishing its levels relative to control (Figure 6A IRGM). IRGM promoted NOD2 degradation, which was partially blocked by bafilomycin A1, commonly used to inhibit autolysosomal degradation (Figure 6B IRGM). The IRGM-K^{mut} variant of IRGM displayed a decreased ability to commit NOD2 for degradation (Figure 6C IRGM). In contrast to the destabilizing effects of IRGM on NOD2, expression of IRGM increased co-expressed myc-ULK1 in a dose-dependent manner (Figure 6D IRGM). The total amount of

ULK1 was not increased when the IRGM-K^{mut} variant was co-expressed (Figure 6E IRGM). This effect was ULK1-specific, since Beclin 1 levels were not affected when IRGM vs IRGM-K^{mut} were compared, corroborating with a related finding that IRGM did not affect Beclin 1 stability (Figure S3B IRGM). Thus, polyubiquitinated IRGM protects ULK1 and promotes degradation of NOD2 (Figure 6F IRGM). This represents a negative feedback regulatory loop, which induces autophagy but at the same time limits NOD2's ability to continue unabated stimulation of this process (Figure 6G IRGM).

IRGM affects antimicrobial and inflammatory outputs and interfaces with several innate immunity systems

IRGM has been shown to control intracellular bacteria (Brest et al., 2011; McCarroll et al., 2008)(Singh et al., 2006). Using a model system of transfected epithelial cells previously developed by others (Brest et al., 2011; Lapaquette et al., 2010) for monitoring autophagic handling of invasive bacteria, we tested how IRGM-K^{mut}, the mutant form of IRGM disabled for ubiquitination and examined for its effects in molecular relationships above, affected a subset of IRGM's immune outputs. Co-expression of NOD2 with IRGM-K^{mut} resulted in increased NF- κ B p65 nuclear translocation in response to *E. coli* LF82 (a CD isolate of adherent invasive *E. coli*) (Lapaquette et al., 2010) relative to NOD2 co-expression with IRGM wild type (Figure 7A,B, Figure S6A). Consistent with this observation, a monocytic cell line THP-1 infected with *E. coli* LF82 showed elevated pro-inflammatory response (increased IL-1 β transcription) when IRGM was knocked down (Figure 7C IRGM, Figure S6B IRGM). The increased NF- κ B response with IRGM-K^{mut} (Figure 7A,B IRGM) was mirrored in the effects of expressing IRGM or IRGM-K^{mut} on bacterial survival, reflected in the diminished ability of IRGM-K^{mut} to control *E. coli* LF82 (Figure S6C). Although IRGM expression on its own enhanced bacterial elimination, this was increased by co-expression with NOD2, an effect that was diminished when IRGM-K^{mut} was employed (Figure S6C IRGM). Although the overall magnitude of the effects on bacterial killing was subtle, it was in keeping with the known limitations of the system (Brest et al., 2011; Lapaquette et al., 2010) as reflected in its maximum output (upon starvation induction) of bacterial control by autophagy (Figure S6D). Based on the above experiments with IRGM-K^{mut}, the properties of IRGM that are essential for the assembly of the core autophagy machinery affect its antimicrobial and inflammatory outputs.

The inventors also tested localization of IRGM relative to the CD isolate *E. coli* LF82 (Lapaquette et al., 2010). We observed that without the co-expression of NOD2, IRGM had a diffuse cytosolic localization even when the cells were infected with bacteria (Figure S7A IRGM). However, when NOD2 was co-expressed with GFP-IRGM, IRGM was recruited to the invading bacteria (Figure S7B IRGM), in keeping the previously observed recruitment of ATG16L1 and NOD2 to bacterial entry sites (Travassos et al., 2010). While studying IRGM interacting partners, we observed a further ability of IRGM to engage other pattern recognition receptors (PRRs), such as NOD1, RIG-I, and TLR3 (Figure 7D-F). In contrast, IRGM did not interact with TLR4 (Figure S7C IRGM). Similarly to NOD2, NOD1, RIG-I, and TLR3 induced IRGM ubiquitination (Figure 7G IRGM). In conclusion, not only does IRGM assemble the core autophagy machinery to control innate immune responses to NOD2 agonists, but IRGM potentially has a broader repertoire of interactors among the PRR systems.

DISCUSSION (IRGM Examples)

In these examples, the inventors have shown that human IRGM, hitherto believed to have indirect effects on autophagy, directly governs the assembly of the principal autophagy regulators. Furthermore, it physically links the microbial sensors, including NOD2, to the core autophagic apparatus. This solves the long-standing puzzle regarding how IRGM works, and places it mechanistically at the center of action in autophagic responses to microbes. IRGM assembles ULK1 and Beclin 1 in their activated forms to promote autophagy. Of relevance for how these proteins become activated is that IRGM also stimulates AMPK by stabilizing it in its Thr-172 phosphorylated form, which is required for AMPK activation (Mihaylova and Shaw, 2011). This is likely due to effects of IRGM on mitochondria (Singh et al., 2010), which activates AMPK (Romanello et al., 2010; Turkieh et al., 2014), and may involve specific kinases upstream of AMPK including TAK1 (Criollo et al., 2011) and CAMKK β (Hoyer-Hansen et al., 2007) that have been shown to phosphorylate AMPK at Thr-172 (Mihaylova and Shaw, 2011) and activate autophagy (Criollo et al., 2011; Hoyer-Hansen et al., 2007). The stabilization of phospho-Thr-172 AMPK likely contributes to AMPK-dependent phosphorylation and activation of ULK1 (Egan et al., 2011; Kim et al., 2011) and Beclin 1 (Kim et al., 2013). Consistent with this, IRGM increases total activated ULK1 phosphorylated at Ser-317 and Ser-555 by AMPK (Egan et al., 2011; Kim et al., 2011), and the activated form of Beclin 1 that is phosphorylated at Ser-15 by ULK1 (Kim et

al., 2013) and at Ser-93 and Ser-96 by AMPK (Kim et al., 2013). IRGM has a second effect on autophagic regulators by assembling the activated ULK1 with Beclin 1. Thus, IRGM promotes phosphorylation cascade of key autophagy regulators and assembles them into autophagy initiation complexes (Figure 7J).

Of interest is that IRGM increases levels of a number of autophagy regulators (ULK1, ATG14L, AMBRA1, and ATGL1) but does not affect the stability of others (Beclin 1 and the ATG5-ATG12 complex). The apparent absence of effects on Beclin 1 stability may be explained by the bulk of Beclin 1 being predominantly in non-autophagy related hVPS34 complexes whereas ATG14L-associated Beclin 1 represents a minority of Beclin 1 species in the cell (Kim et al., 2013). IRGM also has an effect on NOD2 levels. However, IRGM reduces NOD2 levels, in contrast to IRGM-dependent stabilization of autophagy regulators. We interpret this dichotomy as a part of the well tuned circuitry in response to microbial challenge: whereas autophagy is activated as an antimicrobial effector mechanism, the stimulatory inputs into the system mediated by NOD2 are downregulated lest the system overcommits, which in turn may result in detrimental consequences for the host. PAMP (e.g. MDP) tolerance is an important mechanism to avoid septic shock, which is in part achieved by NOD2 degradation (Zurek et al., 2012).

It has been previously shown that ATG16L1 and NOD2 interact (Cooney et al., 2010; Travassos et al., 2010). This has placed two of the Crohn's disease-genetic risk factors together, but has left the role of IRGM unexplained. The data presented here show that IRGM is in complexes with ATG16L1 and NOD2 and that IRGM enhances assembly of Atg16L1 with NOD2. Moreover, IRGM affects the stability of each of the components of this complex. Although bringing ATG16L1 to the bacterial entry site marked by NOD2 is a previously known important step (Travassos et al., 2010), how this links up with the core autophagy regulators including ULK1 and Beclin 1 has not been addressed in prior studies. In this work we show that IRGM plays that bridging role by stimulating phosphorylation and activation of key autophagy regulators and placing them together with ATG16L1 (Figure 7J). This point is not trivial, as for example it has not been easy to connect the two seemingly separate systems of autophagy initiation: ULK1-Beclin1 complexes vs. LC3-II conjugation and localized autophagosomal membrane build up. Only recently a part of this key issue has been solved for conventional (non-immunological) autophagy by showing that ATG16L1 and WIPI2 directly interact (Dooley et al., 2014), with WIPI2 recognizing the lipid modification

products of the Beclin 1-directed hVPS34 activity. We propose here that IRGM acts with a similar purpose by bridging ULK1-Beclin 1 complexes with the autophagy conjugation machinery, as shown here for ATG16L1. This can additionally explain why ATG5 is found in IRGM complexes (Gregoire et al., 2011).

Ubiquitination has been implicated in autophagy in several ways primarily in targeting of substrates for autophagic elimination (Stolz et al., 2014). However, the role of K63-linked polyubiquitination has also begun to be appreciated as a mechanism for stabilization of large autophagy-initiating complexes (Nazio et al., 2013; Shi and Kehrl, 2010). Polyubiquitination of IRGM and its role in autophagy (Figure 7J IRGM) does not play a role in targeting substrates for autophagy; instead, it stabilizes multi-protein autophagy initiation complexes. The ubiquitination of IRGM is under the control by NOD2. NOD2 enhances association of ubiquitination-competent IRGM with ULK1 and Beclin 1, whereas NOD2 has no similar effect on the ubiquitination-null mutant of IRGM (IRGM-K^{mut}). Importantly, IRGM-K^{mut} retains certain activities: it maintains the ability to bind ATG16L1 equally well as the ubiquitination-competent IRGM.

IRGM gene expression is cell-type dependent and responds to both starvation and microbial products. *IRGM* is particularly inducible in cells (intestinal epithelial cells and macrophages) derived from tissues affected in diseases where IRGM has been implicated as a genetic risk factor: CD and tuberculosis (Consortium, 2007; Craddock et al., 2010; Intemann et al., 2009). PAMPs induce autophagy in macrophages through IRGM linking the PAMP detection by NOD2 with the autophagic machinery activation (Figure 7J IRGM). IRGM controls not just initiation of autophagy but may also affect its maturation. IRGM complexes include UVRAG, a regulator of autophagic maturation (Itakura et al., 2008). IRGM displaces Rubicon, known to inhibit maturation complexes (Matsunaga et al., 2009). Thus, IRGM controls several points along the autophagy pathway and contributes to efficient xenophagy. In conclusion, IRGM orchestrates antimicrobial autophagic responses. We have shown here how IRGM does that and what are the exact molecular processes that IRGM controls. This explains the hitherto mysterious role of IRGM in autophagy, places it at the center of molecular complexes controlling and executing autophagy, and molecularly connects biological inputs with autophagic outputs. Finally, our findings indicate that IRGM links up not only with NOD2 but also with several other PRRs, such as NOD1, RIG-I and TLR3. Thus, IRGM and possibly its distant IRG homologs in other vertebrates may act as

transmission modules between a selective sub-repertoire of innate immune responses and the autophagy machinery.

FURTHER EXAMPLES

Second Set- Precision Autophagy Examples- All Figures for this Section are Labeled As FIGURE PRECISION for the attached figures

MATERIAL AND METHODS

Cells, plasmids, siRNA, and transfection

THP-1, HeLa and HEK293T cells were from ATCC. Human peripheral blood monocytes were from StemCell Technologies or from healthy individual donors, and cultured as described previously (Gutierrez et al., 2004). THP-1 cells were differentiated with PMA (50nM) for overnight before use. Full-length human TRIM20 was synthesized and TRIM21 was purchased from DNASU, and both were cloned by PCR into pDONR221. The TRIMs mutants were generated by site-directed mutagenesis and confirmed by sequencing. pENTR or pDONR221 vectors were generated by BP cloning and expression vectors were made by the LR reaction (Gateway; Invitrogen). Other plasmids used were Beclin 1 and its deletion mutants (from B. Levine), ULK1 (from S. Tooze), ATG16L1 and its deletion mutants (from R. Xavier), pCI-Caspase 1 (from K. Fitzgerald), IRF3 (DNASU), pUNO1-hNLRP3a and pUNO1-hNLRP1 (Invivogen). siRNAs were from Dharmacon, and were delivered to cells by either RNAiMax (Lifetechnologies) or nucleoporation (Amaxa). Plasmid transfections were performed by either calcium phosphate or nucleoporation (Amaxa). Herring testis (HT)-DNA (Sigma) was transfected as described previously (Gao et al., 2013).

Bacterial and viral infection

For infection studies, *Escherichia coli* strain LF82 (Lapaquette et al., 2010) was infected at MOI of 1:20. Single-cycle infection HIV-1 viruses were generated as previously described (Mandell et al., 2014), were infected to undifferentiated THP-1 cells (Gao et al., 2013).

Antibodies and reagents

Antibodies used were: Flag (Sigma), HA (Roche), LC3 (Sigma), AMPK, ULK1 p-Ser 317 and p-Ser 555 (Cell signalling), NLRP1 (Cell signaling), NLRP3 (Adipogen), Caspase-1

and ULK1 (Santa Cruz), and GFP, IRF3, Myc and Actin (Abcam). To determine autophagic activity by immunoblotting, cells were cultured in the presence of bafilomycin A1, and lysates were subjected to immunoblotting as described previously (Mizushima et al., 2010). The reagents used were Ultrapure LPS (Invivogen), IFN- γ (PeproTech), Cytotoxic LDH assay (Promega), TO-PRO-3 Iodide (lifetechnologies). Immunoblotting, immunostaining were conducted as described (Kyei et al., 2009). FAM-YVAD-FMK stainings (FLICA, ImmunoChemistry Technologies) were performed according to the manufacture's instruction.

IL-1 β measurement

For IL-1 β secretion, THP-1 cells that had been subjected to the differentiation with PMA (50nM) for overnight, were treated with 2.5 μ g/mL LPS for 2h, and then treated with nigericin (20 μ M) for 30 min. IL-1 β measurements were performed using HEK-Blue IL-1 β Cells (Invivogen).

TRIM family screen

THP-1 cells were cultured in 96-well plates containing SMARTpool siRNA (Dharmacon), RNAiMax (Lifetechnologies), and PMA. Culture media were changed after overnight incubation, and forty-eight hours after plating, cells were treated with IFN- γ or vehicle for 4hr, and then fixed and stained to detect endogeneous LC3 (Alexa Fluor 488 as a flurochrome) and nuclei (Hoechst 33342). Plates with cells were subjected to high content analysis for image acquisition and data processing. Two separate siRNA screen for induced autophagy were carried out with the cutoff (>3 SDs change relative to the mean of stimulated control) for hits.

High content image analysis

High content imaging and analysis was performed using a Cellomics V^{TI} HCS scanner and iDEV software (ThermoScientific). Automated epifluorescence image collection was carried out until a minimum of 500 cells per well per siRNA knockdown per plate was acquired. Epifluorescence images were machine analyzed using present scanning parameters and object mask definitions. Hoechst 33342 staining were used to automatically detect cellular outlines based on background staining of the cytoplasm, and the mean count of LC3 puncta per cell was determined. Autophagy induction with IFN- γ resulted in a Z' value of 0.87.

High content analysis of puncta in subpopulations of transfected cells

HeLa and THP-1 cells were transfected with plasmids or siRNA, and cultured in full media for overnight (plasmids) or 48h (siRNA). Cells were then fixed and stained to detect, LC3 (Alexa Fluor 488 or 568 as fluorochromes), GFP, and nuclei. High content imaging and analysis was performed using a Cellomics V^{TI} HCS scanner and iDEV software (ThermoScientific). >200 cells were analyzed in more than quadruplicate manner using a 20x objective at room temperature. Hoechst 33342 staining were used to automatically detect cellular outlines based on background Hoechst staining, and the mean total count or area of punctate of LC3, or TRIM20 per cell was determined. For sub-population analyses, cells that have above the threshold of the background fluorescence were gated as successfully transfected ones.

Fluorescence confocal microscope image acquisition

Fluorescence confocal microscopy was carried out as described previously (Kyei et al., 2009). In brief, Images were acquired using a Zeiss META microscope equipped with a 63x/1.4 NA oil objective, LSM META camera and AIM software (Zeiss) at room temperature. Fluorochromes associated with secondary antibodies were Alexa Fluor 488, 568, or 647. The images were adjusted for brightness and contrast using ImageJ.

IRF3 dimerization assay and quantitative RT-PCR.

Detection of IRF3 dimerization was performed by native polyacrylamide gel electrophoresis (PAGE) as previously described (Takahasi et al., 2003). Quantitative RT-PCR was performed as previously described (Kimura et al., 2013) using the following primer sets: *ULK1*, (AGATGTTCCAGCACCGTGAG, AATGCACAGCTTGCACTTGG); *BECN1*, (GGAGAACCTCAGCCGAAGAC, ACGTTGAGCTGAGTGTCCAG); *ACTIN*, (GGGCATGGGTCAGAAGGATT, TCGATGGGGTACTTCAGGGT); *TRIM1*, (AAGAATGTGACGAGTTGGTAGAG, ATGAGGACTGTTGACCGTTC); *TRIM5*, (CATGCCTCACTGCAAACCAC, GGTAAGTATCCGGCACACA); *TRIM8*, (ATCCTGATGGACAGGACCCA, CTCCTTCTTGCCACTTCGT); *TRIM16*, (GTAAGCCCACGAACACAAATG, TCCAGCCCTGAAACTTCTATTC); *TRIM20*, (CTGAGTCAGGAGCACCAAGG, GCTGCTCCTCCCCTGATTTT); *TRIM21*, (CAGTCTCGGAAACACCGTGA, AATGCCACCTGGAGCTTCTC); *TRIM22*, (CTCGACCTGCTTATCCGTATTT, CTCAGCACAAGGGCTACTATG); *TRIM28*, (CCATACTGTGCGCTCTACTG, GGTTTCATGCTTGTGTACGTTG); *TRIM56*,

(TTCTTCGTCAATGGGCTGCT, AAGTCATCGGCACAGTCCAG); and *TRIM65*, (GATCTACCTGAACTTGCCTCTG, GAGGAGGGAGGAATCTGTCT). For *IFN- β* and *GAPDH*, Taqman probes and real-time PCR master mixes were used.

Co-immunoprecipitation and GST pull-down

Co-immunoprecipitations were performed as previously described (Kyei et al., 2009) with slight modification. In brief, cells were lysed with NP-40 buffer (lifetechnologies) containing 1mM PMSF and protease inhibitor cocktail (Roche) for 45 min, followed by centrifugation. Supernatants were incubated for 2 h with antibodies at 4°C. The immune complexes were captured with Dynabeads (lifetechnologies). Immunoprecipitates were washed three times with PBS, eluted with Laemmli SDS-PAGE sample buffer, and subjected to immunoblots analysis.

GST and GST-tagged proteins were expressed in *Escherichia coli* BL21 (DE3) or SoluBL21 (Amsbio). GST and GST-fusion proteins were purified and immobilized on glutathione-coupled sepharose beads (Amersham Bioscience, Glutathione-sepharose 4 Fast Flow) and pull-down assays with in vitro translated [³⁵S]-labeled proteins were done as described previously (Pankiv et al., 2007). The [³⁵S] labeled proteins were produced using the TNT T7 Quick Coupled Transcription/Translation System (Promega) in the presence of [35 S] L-methionine. The proteins were eluted from washed beads by boiling for 5 min in SDS-PAGE gel loading buffer, separated by SDS-PAGE, and radiolabeled proteins detected in a Fujifilm bioimaging analyzer BAS-5000 (Fuji).

Peptide array overlay assay

Peptide arrays were synthesized on cellulose membrane using a MultiPep automated peptide synthesizer (INTAVIS Bioanalytical Instruments AG, Germany) as described previously (Kramer et al., 1996). Interaction analyses between peptide and recombinant protein were probed by overlaying the membranes with recombinant protein, and bound proteins were detected with HRP-conjugated anti-GST antibody (clone RPN1236; GE Healthcare).

Statistical analyses

Either a two-tailed Student's t test or ANOVA were used. Statistical significance was defined as $P < 0.05$.

Cell culture

Cell lines were maintained and primary human peripheral blood-monocyte-derived macrophages were isolated and maintained as described (Gutierrez et al., 2004).

Transfections

Plasmid transfections in HEK293T were performed using ProFection Mammalian Transfection System from Promega; siRNAs were delivered to cells by nucleoporation (Amaxa).

Microscopy analyses and quantification

Immunofluorescence was performed as described earlier (Kyei et al., 2009). For quantification of puncta, images from different fields were captured and analyzed. For quantification of total cell fluorescence, image J was used as described previously (Chauhan et al., 2013).

Gene expression analysis

Total RNA was isolated from cell culture using Trizol as per the manufacturer's instruction (Invitrogen). For quantitative real-time PCR: TURBO DNA-free kit (Ambion) was used to remove contaminating residual DNA; cDNA was prepared using the high capacity cDNA reverse transcription kit as per the manufacturer's instruction (Applied Biosystem). Taqman probes (Applied Biosystem) and real-time PCR master mixes (Applied Biosystem) were used for real-time PCR as per the manufacturer's instruction. Data were normalized using GAPDH.

Cytokine and NF- κ B responses

For NF κ B-p65 nuclear localization assay, HeLa cells were plated on cover slips a day before infection. Cells were infected with AIEC LF82 strain at MOI of 1:20 for 2 h followed by washings with PBS and fixing the cells with 4% paraformaldehyde. Cells were visualized using a laser confocal microscope and images were captured using LSM510 software. For IL-1 β measurement, IL-1 β transcription was determined using qRT-PCR in THP-1 cells.

Bacterial survival analyses

AIEC LF82 survival assay was performed as described previously (Lapaquette et al., 2010). HEK293T cells were infected with AIEC LF82 of MOI of 1:20 for 3 h. Cells were

treated with gentamycin (100 µg/ml) for 1 h followed by incubation in fresh media for 2 h. Cells were lysed and surviving bacteria quantified by plating and determining colony forming units.

Proximity ligation assay (PLA)

HEK293T cells transiently expressing the plasmid constructs were fixed and PLA (Soderberg et al., 2006) performed according to the manufacturer's protocol (Olink Bioscience). Samples were then imaged and analyzed by high content microscopy using a CellomicsArrayScan (Thermo Scientific) with images analyzed using pre-set parameters for cell and PLA puncta identification within iDev software (Thermo Scientific). The average total area of red PLA puncta was determined per cell for a minimum of 500 GFP-IRGM positive cells.

Flag pull-down assay

Lysates of HEK293T cells transiently expressing the Flag-NOD2 constructs were incubated with anti-Flag magnetic beads (Sigma) for 2 h. Beads were washed thoroughly (5X) to remove unbound contaminants. The collected beads were incubated with purified recombinant proteins (GST or GST-IRGMd (Singh et al., 2010)) for 2 h and then washed again (5X). The beads were boiled in SDS-PAGE buffer and subjected to Western blotting

IFN- γ induces autophagy (Fabri et al., 2011; Gutierrez et al., 2004; Inbal et al., 2002) and influences cytokine networks and polarization of immune systems (Ghezzi and Dinarello, 1988; Mishra et al., 2013; Schroder and Tschopp, 2010), whereas TRIMs are involved in immune responses (Kawai and Akira, 2011) and, through an assortment of proposed mechanisms affect autophagy (Barde et al., 2013; Khan et al., 2014; Mandell et al., 2014; Niida et al., 2010; Pineda et al., 2015; Pizon et al., 2013; Tomar et al., 2012; Yang et al., 2013). IFN- γ can induce expression of a subset of TRIMs (Carthagen et al., 2009). We wondered whether TRIMs might be contributing mediators to autophagy induction by IFN- γ . We employed an image-based high content (HC) analysis of LC3 puncta (Fig. 1A) to screen for effects of TRIM knockdowns on IFN- γ -induced autophagy in human myelomonocytic cells. IFN- γ induced autophagy in THP-1 (Fig. 1A), also showing dose dependence (Fig. S1A PRECISION), and in primary human macrophages (Fig. S1B). For standardization, we used THP-1 cells for the screen (Fig. 1B and Fig. S1C). Out of the 70 human TRIMs tested,

knockdowns of 24 different TRIMs reduced endogenous LC3 puncta per cell under IFN- γ treatment (Fig. 1B, open circles; Fig. S1C shows average $\pm$ range values from two independent screens). We followed this up by individual knockdowns of a subset of 6 positive and 4 neutral TRIMs from the screen (Fig. 1C). All 6 TRIMs that were positive hits from the screen, TRIM1, TRIM8, TRIM20, TRIM21, TRIM22, and TRIM65 (knockdowns were evaluated in Fig. S1D), were required for optimal induction of autophagy by IFN- γ (Figure 1C). Of the neutral TRIMs, TRIM56 that was marginally positive in the screen, showed a borderline but statistically significant effect (Fig. 1C). Thus, TRIMs contribute to autophagy induction in response to IFN- γ (Fig. 1D).

TRIM20 induces autophagy

The inventors focused on TRIM20 as a TRIM strongly induced by IFN- γ (Carthagen et al., 2009; Chae et al., 2011). We confirmed that TRIM20 expression was responsive to IFN- γ in our system and tested its kinetics and dose-response (Fig. S1E, F). The inventors next used HC analysis to establish in a dose response setting that TRIM20 was required for IFN- γ -induced autophagy (Fig. S1G, H). This was confirmed in immunoblot assays of LC3 lipidation in the presence of bafilomycin A1, an inhibitor of autophagic flux (LC3-II conversion; Fig. 1E). Mirroring these findings, overexpression of GFP-TRIM20 increased LC3 puncta (Fig. 1F), and enhanced LC3-II conversion in immunoblots (Fig. S1I); as expected, the LC3-II band was revealed only in bafilomycin A1-treated cells, which protects it from degradation through autophagic flux. These results indicate that activation of autophagy by IFN- γ depends on TRIM20 and that elevated expression of TRIM20, a TRIM whose transcription is known to be strongly activated by IFN- γ (Carthagen et al., 2009; Chae et al., 2011), induces autophagy.

TRIM20 interacts with ULK1, Beclin 1 and ATG16L1

The inventors next examined how TRIM20 induced autophagy. Autophagy requires ULK1 and Beclin 1, both of which play pivotal roles in autophagy initiation in mammalian cells (He and Levine, 2010; Mizushima et al., 2011). We detected GFP-TRIM20 in immunoprecipitates with co-expressed Myc-ULK1 and Flag-Beclin 1 (Fig. 2A and B) and with endogenous ULK1 and Beclin 1 (Fig. S2A and B). TRIM20 puncta colocalized with ULK1 in the cytoplasm (Fig. 2C). Induction of autophagy depends on a phosphorylation cascade, which includes activation of ULK1 by phosphorylation at Ser-317 (Kim et al., 2011). Active p-ULK1 (Ser-317) co-immunoprecipitated with TRIM20 (Fig. 2D). We next mapped ULK1-binding regions within TRIM20 (Fig. 2E). Like the majority of TRIMs (Kawai and Akira, 2011; Reymond et al., 2001), TRIM20 has B box, CCD and PRY/SPRY domains, but lacks an E3 ligase RING domain, and is uniquely endowed with a pyrin (PYD) domain. TRIM20 constructs lacking PYD and PRY/SPRY domains still bound ULK1 in immunoprecipitation assays (Fig. 2F). Direct binding between TRIM20 and ULK1 was established in GST pull-down experiments (Fig. 2G). Both *in vivo* and *in vitro* experiments pointed to the middle portion (including B-box and CCD) of TRIM20 as being critical for association with ULK1, whereas the N-terminal PYD and the C-terminal SPRY domains were dispensable (Fig. 2E).

Beclin 1 showed a more complex domain-requirement for inclusion in TRIM20 complexes, with either the middle portion (including B-box and CCD) or the C-terminal region (PRY/SPRY) displaying an independent capacity to bring down Beclin 1 in immunoprecipitates (Fig. 3A and B). We also examined Beclin 1 for regions required for the ability to co-immunoprecipitate with TRIM20 (Fig. S2C and D). Two Beclin 1 regions appeared to be required: the first one between BH3 and CCD and the second one overlapping with the ECD domain of Beclin 1 (Fig. S2C and D). Furthermore, in the presence of TRIM20, the immunoprecipitated Beclin 1 complexes were enriched for ULK1 (Fig. 3C). Thus, TRIM20 can interact simultaneously with multiple autophagy factors and serves as a platform for co-assembly of ULK1 and Beclin1.

The inventors also found that TRIM20 co-immunoprecipitated with ATG16L1 (Fig. 3D). TRIM20 displayed a complex domain requirement for inclusion in ATG16L1 complexes, with either the middle portion (including B-box and CCD) or the C-terminal region (PRY/SPRY) showing an independent capacity to bring down ATG16L1 in immunoprecipitates (Fig. 3E and F). TRIM20 primarily interacted with the WD repeat of

ATG16L1 (Fig. 3, G and H). Thus, the TRIM20 platform (Fig. 3I) contains other autophagy regulators, such as ATG16L1, a component of the autophagy E3-like complex that regulates LC3 conjugation and autophagosome formation (Mizushima et al., 2003).

TRIM20 interacts with a subset of mammalian Atg8 paralogs

The inventors examined whether TRIM20 possessed the ability to interact with mammalian Atg8 paralogs (mAtg8s), factors required for autophagosomal membrane formation (Mizushima et al., 2011). Although no binding was detected with LC3B, the commonly used marker for autophagic membrane (Kabeya et al., 2000), GST pull-down experiments revealed interactions of TRIM20 with GABARAP and GABARAPL1, and to a lesser extent with LC3A, LC3C, and GABARAPL2 (Fig. 4A). GABARAP colocalized with TRIM20 (Fig. S2E). Albeit TRIM20 did not directly interact with LC3B, mCherry-TRIM20 profiles were closely juxtaposed to conventional LC3-positive puncta (Fig. S2F). The region of TRIM20 (Fig. 4B) responsible for the interaction with mAtg8s, GABARAP and LC3A, was mapped. A TRIM20 deletion construct spanning residues 375-595 retained capacity to bind GABARAP or LC3A (Fig. 4B and C). To delimit further the TRIM20 sequence required for mAtg8s binding we used GST-GABARAP as bait in a binding assay with an array of TRIM20 peptides (Fig. 4D). Three series of TRIM20 peptides (regions of primary sequence staggered by 3 amino acid residues), with either three or four positive consecutive binding signals, were identified (Fig. 4D). The most upstream region (397-ICSLSHQEH-404; Region I) did not contain a recognizable LIR motif, whereas Region II (470-YYFLEQQEHFFVSLEDVG-498) and Region III (523-SEWELLQD-530) contained potential LIR motifs (Birgisdottir et al., 2013). In follow-up mutational analyses, no single or double alterations of the Regions I-III abrogated GABARAP binding (Fig. S2G). Only when all three regions (I, II and III) were mutated, did this cause loss of GABARAP binding (Fig. 4E and Fig. S2G). Thus, all three regions contribute to the binding of TRIM20 to mAtg8s. Collectively the above findings and experiments described in previous sections demonstrate that TRIM20 assembles both the key regulators of autophagy (ULK1, Beclin 1, ATG16L1) and a subset of effector factors (mAtg8s).

TRIM20 is a receptor for selective autophagy of inflammasome components

TRIM20, encoded by the *MEFV* gene, is a risk locus for familial Mediterranean fever (FMF) French FMF Consortium, 1997, The International FMF Consortium, 1997. TRIM20

has 305 FMF-associated variants website fmf.igh.cnrs.fr/ISSAID/infevers/, with frequent mutations in its PRY/SPRY domain (Masters et al., 2009). The PYD domain of TRIM20 has been the primary focus of interest in inflammasome regulation due to its potential to bind the cognate PYD domain of ASC (Schroder and Tschopp, 2010). However, it has been reported that the PRY/SPRY domain, located at the other end of TRIM20, recognizes and binds to NLRP3 (Papin et al., 2007). The latter relationship has remained obscure despite the frequency of mutations in the PRY/SPRY domain (Masters et al., 2009). We explored the significance of the interactions between the TRIM20 PRY/SPRY domain and NLRP3 in the context of the above recognized function of TRIM20 in autophagy. The full length TRIM20 and a TRIM20 construct containing only the PRY/SPRY domain both interacted with NLRP3 (Fig. S3A). A knockdown of TRIM20 spared NLRP3 from degradation in cells treated with IFN- γ and LPS (Fig. 5A; Fig. S3B and C). When cells were treated with LPS alone, a knockdown of TRIM20 had no effect on NLRP3 levels (Fig. S3D), albeit LPS alone increased NLRP3 levels (Fig. S3E) as expected (Bauernfeind et al., 2009), in keeping with TRIM20 acting to transduce the effects of IFN- γ . Bafilomycin A1 (an inhibitor of autophagic degradation) protected NLRP3, whereas TRIM20 knockdown increased amounts of NLRP3 and eliminated the protective effects of bafilomycin A1 (Fig. 5B). Similar effects were observed with THP-1 cells exposed to pathogens (adherent-invasive *Escherichia coli* LF82 (Lapaquette et al., 2010)) and with primary human peripheral blood monocyte-derived macrophages (MDMs) treated as above (Fig. 5C and D).

Degradation of NLRP3 depended on ULK1 and Beclin 1, establishing that disposal of NLRP3 was through autophagy (Fig. 5E; and Fig. S3F). Conversely, expression of TRIM20 decreased levels of co-expressed NLRP3 (Fig. 5F). The destabilizing effect of TRIM20 overexpression on NLRP3 levels was suppressed by bafilomycin A1 (Fig. 5F). Additionally, TRIM20 was protected by bafilomycin A1 from degradation in the presence of NLRP3 (Fig. S3G and H), indicating that TRIM20 is degraded along with the delivery of its substrate to autolysosomal compartments.

In addition to NLRP3, other inflammasome components, pro-caspase 1 (Chae et al., 2006; Papin et al., 2007) and NLRP1 (Papin et al., 2007), have been previously shown to interact with the PRY/SPRY domain of TRIM20. When pro-caspase 1 and NLRP1 were co-expressed with TRIM20, they too were subject to degradation inhibitable by bafilomycin A1

(Fig. 5, G and H). These data show that TRIM20 acts as an autophagy receptor for degradation of inflammasome components and that TRIM20 is responsible for delivery of NLRP3 and other tested inflammasome components for autophagic degradation.

Presence of target substrate potentiates assembly of activated autophagic components on the TRIM20 platform

The inventors tested whether the availability of substrate, NLRP3, influenced TRIM20 assembly with ULK1. Although ULK1 was enriched in NLRP3 immunoprecipitates when cells expressed TRIM20 (Fig. 6A) this was reduced when cells were subjected to TRIM20 knockdown (Fig. S3I), the presence of NLRP3 did not affect levels of total ULK1 in TRIM20 immunoprecipitates (Fig. 6B). However, the presence of NLRP3 increased the amount of active p-ULK1 (Ser-317 and Ser-555) (Egan et al., 2011; Kim et al., 2013) associated with TRIM20 (Fig. 6B). Because these two sites of ULK1 are phosphorylated by AMPK (Egan et al., 2011; Kim et al., 2011), we tested whether AMPK is recruited to the TRIM20 complex. AMPK was found in TRIM20 complexes with or without NLRP3 (Fig. S3J). These data indicate that modulation of TRIM20 action, in the presence of its cognate autophagic target, is reflected in ULK1 phosphorylation state and not in ULK1 or AMPK levels. These and above data suggest a model in which not only does TRIM20 organize autophagic machinery by serving as a platform for the assembly of ULK1, Beclin 1, ATG16L1, and mAtg8s, but it also recognizes autophagic substrates via its PRY/SPRY domain, and that this substrate recognition enriches ULK1 in its activated state on the TRIM20 platform (Fig. 6C).

Disease-associated mutations in TRIM20 diminish its autophagic potency

A physiologically relevant consequence of TRIM20 mutations in FMF is excessive IL-1 β production (Chae et al., 2011; Meinzer et al., 2011; Omenetti et al., 2014). In patients (Omenetti et al., 2014), albeit not in murine systems (Chae et al., 2011), this is dependent on NLRP3 in the context of TRIM20 mutations. A knockdown of ULK1 or TRIM20 elevated IL-1 β responses (Fig. 6E; specifically for IL-1 β since LDH release was unaffected, S4B). When the cells knocked down for either ULK1 or TRIM20 were also subjected to knockdowns of NLRP3, the latter normalized IL-1 β expression (Fig. S4C-E). When cells were subjected to inflammasome activation with LPS and nigericin, FLICA staining (based on a fluorogenic probe FAM-YVAD-FMK for detection of *in situ* caspase 1 activity)

revealed active caspase-1 puncta, as reported previously (Broz et al., 2010). The number of FLICA-positive cells increased when cells were subjected to a TRIM20 knockdown (Fig. 6D and S4A). Thus, TRIM20 suppresses caspase-1 activation and IL-1 β production.

We then tested whether the disease-causing variants of TRIM20 affected autophagy and clearance of inflammasome components. We chose the three most frequent variants found in FMF patients (Masters et al., 2009), M680I, M694V and V726A (Fig. 6F). Compound (double or triple) mutant variants of TRIM20 formed fewer TRIM20 puncta (Fig. S4F and G). Whereas expression of wild type TRIM20 resulted in degradation of NLRP3, overexpression of TRIM20 single (M694V), double (M680I and M694V) and triple (M680I, M694V and V726A) mutants showed diminished degradation of NLRP3 (Fig. 6G).

Furthermore, protein complexes with the M694V, double (M680I+M694V), and triple (M680I+M694V+V726A) TRIM20 mutants harbored less ULK1, a trend that was paralleled by phospho-ULK1 levels (Fig. 6H). Consistent with this, there were fewer LC3 puncta per cell induced through expression of the triple mutant TRIM20 (M680I+M694V+V726A) than by the wild type TRIM20 (Fig. S4H). Thus, the disease-associated mutations in the PRY/SPRY domain of TRIM20 perturb ULK1 recruitment and autophagic degradation of NLRP3 and hence may contribute to the inflammatory phenotype associated with FMF mutations (Fig. 6I).

TRIM21 interacts with autophagy factors

The IFN- γ screen with TRIM family of proteins yielded additional hits beside TRIM20 (Fig. 1B), several of which were validated in follow-up analyses (Fig. 1C). Among these was TRIM21 (also known as Ro52/SSA associated with Sjögren syndrome), which is transiently induced by IFN- γ (Fig. S5A). Incidentally, TRIM20 and 21 could be co-immunoprecipitated (Fig. S5B). The IFN- γ induction of TRIM21 expression was in agreement with previous reports (Carthagena et al., 2009; Espinosa et al., 2009). TRIM21 has an acknowledged role in regulating type I interferon responses (Espinosa et al., 2009; Higgs et al., 2008; McEwan et al., 2013; Yoshimi et al., 2009; Zhang et al., 2013). In one mechanism, TRIM21 has been reported to cause IKK β degradation most likely through autophagy, based on its 3-methyladenine protection and LC3 localization (Niida et al., 2010). Based on our detailed studies with TRIM20 described above, we wondered whether TRIM21 might also act as a platform for assembly of autophagic regulatory factors. Indeed, TRIM21 bound both regulators, ULK1 and Beclin 1 (Fig. 7A and B), and a subset of mAtg8s, most

prominently GABARAP (Fig. 7C). GABARAP binding to TRIM21 did not require the SPRY domain of TRIM21 (Fig. 7D and E). Unlike TRIM20, which does not bind Sequestosome1/p62 (p62) (Mandell et al., 2014), a well-known autophagic receptor (Birgisdottir et al., 2013), TRIM21 did bind p62 (Fig. 7F and G). The TRIM21-binding region within p62 was delimited to the residues 170–256 of p62 (Fig. 7F and G). The regions of TRIM21 binding p62 excluded its SPRY domain (Fig. 7D and E). Thus, TRIM21 interacts with multiple regulators and effectors of autophagy.

TRIM21 is a regulator-receptor for autophagic degradation of activated IRF3

TRIM21 is known to interact with the transcription factor IRF3 through its SPRY domain (Higgs et al., 2008). It has been proposed that TRIM21 can suppress type I IFN response (Espinosa et al., 2009; Higgs et al., 2008; Yoshimi et al., 2009; Zhang et al., 2013), albeit an activation effect (McEwan et al., 2013) has also been reported. The proposed mechanism for negative regulation of IRF3 is mainly focused on proteasomal degradation of IRF3 (Higgs et al., 2008; Saitoh et al., 2006). However, autophagy is also known to play a suppressive role on type I IFN (Deretic et al., 2015; Jounai et al., 2007; Mathew et al., 2014; Saitoh et al., 2009). We thus wondered if TRIM21 could cause autophagic degradation of IRF3, analogous to what we observed with TRIM20 and NLRP3. IRF3 colocalized with TRIM21 in LC3-positive dots (Fig. 8A). Furthermore, IRF3⁺ TRIM21⁺ profiles were also ULK1 positive (Fig. 8B). Moreover, IRF3 was found in protein complexes with ULK1 when TRIM21 was present (Fig. 8C).

Cytosolic DNA (during viral infection, e.g. with HIV) induces type I interferon response through endogenous second messenger (cyclic GMP-AMP) by utilizing its adaptor protein STING that results in IRF3 dimerization/activation (Gao et al., 2013). It is the dimerized form of IRF3 that activates type I IFN responses (Takahashi et al., 2003). A knockdown of TRIM21 increased levels of IRF3 dimers in IFN- γ -treated cells stimulated with double stranded DNA (HT-DNA) transfected into the cells (Fig. 8D) but not in cells treated with HT-DNA alone, i.e. in the absence of IFN- γ (Fig. S5D), in keeping with the role of TRIM21 in acting as an effector of IFN- γ . A knockdown of TRIM21 also increased IRF3 dimers in cells infected with a single-cycle infection HIV-1 virus under conditions when cells were treated with INF- γ (Fig. S5E). Bafilomycin A1 protected dimerized IRF3 from degradation; this protection was no longer apparent in cells knocked down for TRIM21 (Fig.

8E), indicating that dimerized IRF3 was routed for autophagic degradation by TRIM21. As a physiologically relevant consequence, knockdown of TRIM21 resulted in increased levels of IFN- β expression after DNA transfection or infection with HIV-1 (Fig. 8F; and Fig. S5G). These data show that TRIM21 acts as a platform for IRF3 degradation, connecting it with the autophagic regulators (ULK1) and effectors (mAtg8s)(Fig. S5I). A knockdown of TRIM21 resulted in increased levels of IFN- β response to LPS (Fig. S5H), in keeping with the proposed autophagic targeting of IKK β (Niida et al., 2010)) within a parallel pathway to IRF3-dependent activation of type I interferon responses.

Collectively, the present findings show that multiple TRIMs participate in autophagic response to IFN- γ . Specifically, TRIM20 and TRIM21 organize autophagic apparatus to degrade their cognate targets and downregulate responses via inflammasome/IL-1 β and IRF3/type I IFN (Fig. 8G). Tapering of such responses may be essential to prevent excessive inflammation.

Discussion Precision Autophagy

The inventors' findings show that a subset of TRIMs act as receptors and regulators for selective autophagy targeting components of the inflammasome and type I interferon response systems. TRIM20 recognizes the inflammasome components, NLRP1, NLRP3, and pro-caspase 1, and leads to their autophagic degradation. A similar principle is at work with TRIM21, which targets activated (dimerized) IRF3 for autophagy. Not only do TRIM20 and TRIM21 directly bind their respective cargo, but they also recruit autophagic machinery thus coordinating target recognition with assembly of the autophagic apparatus and initiation of autophagy. These studies increase the repertoire of currently known autophagic receptors (Birgisdottir et al., 2013; Johansen and Lamark, 2011), and expand the target-receptor role of TRIMs in autophagy, previously indicted only for TRIM5 α (Mandell et al., 2014). Thus, direct target recognition and assembly of autophagic machinery to conduct a process referred to as precision autophagy (Deretic et al., 2015) is a more general feature of the TRIM family of proteins.

The recognition of cognate targets by TRIM20 and TRIM21 is reminiscent of direct retroviral capsid recognition by TRIM5 α (Stremlau et al., 2006) (Stremlau et al., 2006),

which, as recently shown (Mandell et al., 2014) leads to autophagic degradation of HIV. The principles of precision autophagy (Deretic et al., 2015) may differ fundamentally from targeting of a variety of ubiquitinated cargo earmarked for autophagy by ubiquitin-binding receptors (Stolz et al., 2014). Incidentally, TRIM20 does not possess the RING E3 ubiquitin ligase domain, and does not bind p62 (Mandell et al., 2014). The absence of a RING domain and absence of binding to p62 underscores the ubiquitin-independent nature of target recognition by TRIM20. However, engagement of other Sequestosome 1-like receptors, a class (Deretic et al., 2013) of ubiquitin and galectin recognizing receptors (Gomes and Dikic, 2014; Randow and Youle, 2014) may not be ruled out, as well as a non-targeting role for ubiquitination in stabilizing autophagy initiation complexes (Chauhan et al., 2015; Nazio et al., 2013; Shi and Kehrl, 2010). Furthermore, inclusion of additional cytoplasmic material along with specific targets during TRIM-directed autophagy may not be ruled out.

Importantly, these findings indicate that substrate recognition by TRIM20 also directs precision autophagy machinery assembled by TRIM20. Thus, in their role in autophagy, TRIM20 and TRIM21 act not only as receptors for autophagy but also as platforms for assembly of regulators (ULK1, Beclin 1) and effectors (mAtg8s; p62 in the case of TRIM21), into initiation complexes. The presence in TRIM20 complexes of ATG16L1 may reflect direct association or reinforcement of indirect links between ULK1 and ATG16L1 (Gammoh et al., 2013; Nishimura et al., 2013). Other TRIMs may function similarly, as observed with TRIM5 α and preliminarily with TRIM6, TRIM17, TRIM22, TRIM49, and TRIM55 (Mandell et al., 2014). The concept of platforms for assembly of autophagic machinery in mammalian cells also extends to generic, starvation induced autophagy, which utilizes exocyst components specifically endowed with Exo84 (Bodemann et al., 2011). However, TRIM engagement with autophagy may entail other mechanisms, as for example TRIM28 has multiple (both positive and negative) proposed mechanisms of action (Barde et al., 2013; Pineda et al., 2015; Yang et al., 2013), whereas the mechanism of autophagy induction for TRIM13 in response to the ER stress has not been fully delineated (Tomar et al., 2012) although it shows a relationship with p62 and DFCP, an ER-derived autophagy precursor compartment termed omegasome (Axe et al., 2008).

A further major biological finding reported here is that TRIMs are mediators of IFN- γ induced autophagy. The engagement of multiple TRIMs revealed in our screen should not be

surprising, as multiple systems can trigger INF- γ -induced autophagy, such as the previously described DAPK phosphorylation of Beclin 1 (Inbal et al., 2002; Zalckvar et al., 2009) and immunity related GTPases (IRG)-dependent induction of autophagy (Gutierrez et al., 2004), which has recently been shown to act through a co-assembly of ULK1 and Beclin 1 (Chauhan et al., 2015). Additional upstream mechanisms may be controlled by TRIMs detected in our screen, as in the case of TRIM8, which is known to be inducible by IFN- γ (Toniato et al., 2002). TRIM8 activates TAK1 (Li et al., 2011), which is proposed to occur through K63 polyubiquitination. TAK1, in turn, activates AMPK-dependent autophagy (Criollo et al., 2011; Herrero-Martin et al., 2009; Kanayama et al., 2004) by phosphorylating AMPK (Xie et al., 2006). Hence, TRIM8 affects upstream pathways known to activate autophagy. This may explain why TRIM8 was identified as a hit in our IFN- γ -dependent autophagy induction screen. Furthermore, it is likely that TRIMs, known to hetero-oligomerize (Bell et al., 2012) as supported by our observations with TRIM20 and TRIM21, cooperate in IFN- γ induction of autophagy.

The finding that TRIM20 is a mediator of IFN- γ suppression of inflammasome activation provides a mechanism for this important IFN- γ effect in prevention of excessive inflammasome activation and associated pathology in infectious and autoimmune diseases (Minguela et al., 2007; Nandi and Behar, 2011), for which a satisfactory definition has been lacking albeit indirect mechanisms have been proposed (Mishra et al., 2013). The TRIM20-dependent direct recognition and autophagic degradation of the inflammasome components NLRP3, pro-caspase 1, and NLRP1, differs from the previous reports of indirect effect on inflammasome activation via mitophagy (Nakahira et al., 2011; Zhou et al., 2011), and is more akin to the proposed autophagic degradation of AIM2, a sensory component of the DNA-reactive specialized inflammasome, albeit AIM2 has been proposed to be eliminated by ubiquitin-tag recognizing receptor (Shi et al., 2012). We furthermore demonstrated that FMF disease-associated mutations in the PRY/SPRY domain of TRIM20 (Masters et al., 2009), alter the capacity of TRIM20 to direct autophagic degradation of inflammasome components. These mutations reduced the binding of ULK1, thus explaining in part how the common mutations associated with FMF work. We propose that IFN- γ -TRIM20-autophagy axis normally suppress excessive inflammasome and IL-1 β activation, and that this ability is blunted by common disease-associated TRIM20 polymorphisms occurring in FMF.

The reported TRIM21-dependent suppression of type I IFN activation by autophagic degradation of IRF3 dimers mirrors the action of TRIM20 in suppressing inflammasome activation. TRIM21, an autoantigen associated with Sjögren syndrome and systemic lupus erythematosus, suppresses type I IFN response (Espinosa et al., 2009; Higgs et al., 2008; Yoshimi et al., 2009; Zhang et al., 2013), albeit this has been ascribed to proteasomal degradation of IRF3 (Higgs et al., 2008) and IRF7 (Higgs et al., 2010). Nevertheless, type I IFN can also be activated by NF- κ B, and autophagy has been implicated in degradation of the upstream NF- κ B activating kinase, IKK β (Niida et al., 2010). The TRIM21-directed autophagic degradation of activated IRF3 shown here complements the action of TRIM21 on NF- κ B (Niida et al., 2010). Although the mechanism is not fully known, activation of type I IFN system is one major feature of Sjögren syndrome and systemic lupus erythematosus (Banchereau and Pascual, 2006). We thus raise the possibility that perturbations of IFN- γ -TRIM21-autophagy axis may cause activation of type I IFN in autoimmune diseases. The inventors' findings reported here broaden the concept of TRIMs acting as autophagic receptors and as platforms for assembly of autophagy initiation complexes. Our findings also link cargo recognition by a TRIM, acting as an autophagic receptor, with the function of the same TRIM in the assembly of autophagic machinery triggering the execution of autophagy of a very specific cytoplasmic targets. This brand of autophagy, termed precision autophagy, is guided by TRIMs and has important biological functions. For example, the TRIM20- and TRIM21-precision autophagy uncovered here balances key innate immunity responses, potentially serving as a guardian against excessive inflammation, which in turn may cause pathology during autoimmune processes or in infections causing cytokine storms. We propose that the large family of TRIMs with 70 members in humans endows cells with a precision in deploying autophagy.

References (IRGM)

- Abbott, D.W., Yang, Y., Hutti, J.E., Madhavarapu, S., Kelliher, M.A., and Cantley, L.C. (2007). Coordinated regulation of Toll-like receptor and NOD2 signaling by K63-linked polyubiquitin chains. *Mol Cell Biol* 27, 6012-6025.
- Bekpen, C., Marques-Bonet, T., Alkan, C., Antonacci, F., Leogrande, M.B., Ventura, M., Kidd, J.M., Siswara, P., Howard, J.C., and Eichler, E.E. (2009). Death and resurrection of the human IRGM gene. *PLoS Genet* 5, e1000403.

- Brest, P., Lapaquette, P., Souidi, M., Lebrigand, K., Cesaro, A., Vouret-Craviari, V., Mari, B., Barbry, P., Mosnier, J.F., Hebuterne, X., *et al.* (2011). A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn's disease. *Nat Genet* 43, 242-245.
- Chan, W.M., Mak, M.C., Fung, T.K., Lau, A., Siu, W.Y., and Poon, R.Y. (2006). Ubiquitination of p53 at multiple sites in the DNA-binding domain. *Mol Cancer Res* 4, 15-25.
- Choi, J., Park, S., Biering, S.B., Selleck, E., Liu, C.Y., Zhang, X., Fujita, N., Saitoh, T., Akira, S., Yoshimori, T., *et al.* (2014). The parasitophorous vacuole membrane of *Toxoplasma gondii* is targeted for disruption by ubiquitin-like conjugation systems of autophagy. *Immunity* 40, 924-935.
- Consortium (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447, 661-678.
- Cooney, R., Baker, J., Brain, O., Danis, B., Pichulik, T., Allan, P., Ferguson, D.J., Campbell, B.J., Jewell, D., and Simmons, A. (2010). NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 16, 90-97.
- Craddock, N., Hurles, M.E., Cardin, N., Pearson, R.D., Plagnol, V., Robson, S., Vukcevic, D., Barnes, C., Conrad, D.F., Giannoulatou, E., *et al.* (2010). Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature* 464, 713-720.
- Criollo, A., Niso-Santano, M., Malik, S.A., Michaud, M., Morselli, E., Marino, G., Lachkar, S., Arkhipenko, A.V., Harper, F., Pierron, G., *et al.* (2011). Inhibition of autophagy by TAB2 and TAB3. *The EMBO journal* 30, 4908-4920.
- Deretic, V., Kimura, T., Timmins, G., Moseley, P., Chauhan, S., and Mandell, M. (2015). Immunologic manifestations of autophagy. *The Journal of Clinical Investigation* 125, 75-84.
- Dooley, H.C., Razi, M., Polson, H.E., Girardin, S.E., Wilson, M.I., and Tooze, S.A. (2014). WIPI2 Links LC3 Conjugation with PI3P, Autophagosome Formation, and Pathogen Clearance by Recruiting Atg12-5-16L1. *Molecular cell* 55, 238-252.
- Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., *et al.* (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331, 456-461.

- Fimia, G.M., Stoykova, A., Romagnoli, A., Giunta, L., Di Bartolomeo, S., Nardacci, R., Corazzari, M., Fuoco, C., Ucar, A., Schwartz, P., *et al.* (2007). Ambra1 regulates autophagy and development of the nervous system. *Nature* 447, 1121-1125.
- Fung, T.K., Yam, C.H., and Poon, R.Y. (2005). The N-terminal regulatory domain of cyclin A contains redundant ubiquitination targeting sequences and acceptor sites. *Cell Cycle* 4, 1411-1420.
- Gammoh, N., Florey, O., Overholtzer, M., and Jiang, X. (2013). Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex-dependent and -independent autophagy. *Nat Struct Mol Biol* 20, 144-149.
- Gregoire, I.P., Richetta, C., Meyniel-Schicklin, L., Borel, S., Pradezynski, F., Diaz, O., Deloire, A., Azocar, O., Baguet, J., Le Breton, M., *et al.* (2011). IRGM is a common target of RNA viruses that subvert the autophagy network. *PLoS pathogens* 7, e1002422.
- Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 119, 753-766.
- Hasegawa, M., Fujimoto, Y., Lucas, P.C., Nakano, H., Fukase, K., Nunez, G., and Inohara, N. (2008). A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. *EMBO J* 27, 373-383.
- Hoyer-Hansen, M., Bastholm, L., Szyniarowski, P., Campanella, M., Szabadkai, G., Farkas, T., Bianchi, K., Fehrenbacher, N., Elling, F., Rizzuto, R., *et al.* (2007). Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Molecular cell* 25, 193-205.
- Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M., *et al.* (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411, 599-603.
- Intemann, C.D., Thye, T., Niemann, S., Browne, E.N., Amanua Chinbuah, M., Enimil, A., Gyapong, J., Osei, I., Owusu-Dabo, E., Helm, S., *et al.* (2009). Autophagy gene variant IRGM -261T contributes to protection from tuberculosis caused by Mycobacterium tuberculosis but not by M. africanum strains. *PLoS Pathog* 5, e1000577.
- Itakura, E., Kishi, C., Inoue, K., and Mizushima, N. (2008). Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* 19, 5360-5372.

- Kim, J., Kim, Y.C., Fang, C., Russell, R.C., Kim, J.H., Fan, W., Liu, R., Zhong, Q., and Guan, K.L. (2013). Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* 152, 290-303.
- Kim, J., Kundu, M., Viollet, B., and Guan, K.L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature cell biology* 13, 132-141.
- Lapaquette, P., Glasser, A.L., Huett, A., Xavier, R.J., and Darfeuille-Michaud, A. (2010). Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 12, 99-113.
- Levine, B., Mizushima, N., and Virgin, H.W. (2011). Autophagy in immunity and inflammation. *Nature* 469, 323-335.
- Matsunaga, K., Saitoh, T., Tabata, K., Omori, H., Satoh, T., Kurotori, N., Maejima, I., Shirahama-Noda, K., Ichimura, T., Isobe, T., *et al.* (2009). Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol* 11, 385-396.
- McCarroll, S.A., Huett, A., Kuballa, P., Chilewski, S.D., Landry, A., Goyette, P., Zody, M.C., Hall, J.L., Brant, S.R., Cho, J.H., *et al.* (2008). Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet* 40, 1107-1112.
- Mihaylova, M.M., and Shaw, R.J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nature cell biology* 13, 1016-1023.
- Mizushima, N. (2003). Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *Journal of cell science* 116, 1679-1688.
- Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451, 1069-1075.
- Nazio, F., Strappazzon, F., Antonioli, M., Bielli, P., Cianfanelli, V., Bordi, M., Gretzmeier, C., Dengjel, J., Piacentini, M., Fimia, G.M., *et al.* (2013). mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nature cell biology* 15, 406-416.
- Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., *et al.* (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603-606.

- Romanello, V., Guadagnin, E., Gomes, L., Roder, I., Sandri, C., Petersen, Y., Milan, G., Masiero, E., Del Piccolo, P., Foretz, M., *et al.* (2010). Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J* 29, 1774-1785.
- Sabbah, A., Chang, T.H., Harnack, R., Frohlich, V., Tominaga, K., Dube, P.H., Xiang, Y., and Bose, S. (2009). Activation of innate immune antiviral responses by Nod2. *Nat Immunol* 10, 1073-1080.
- Shi, C.S., and Kehrl, J.H. (2010). TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal* 3, ra42.
- Singh, S.B., Davis, A.S., Taylor, G.A., and Deretic, V. (2006). Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 313, 1438-1441.
- Singh, S.B., Ornatowski, W., Vergne, I., Naylor, J., Delgado, M., Roberts, E., Ponpuak, M., Master, S., Pilli, M., White, E., *et al.* (2010). Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 12, 1154-1165.
- Song, J.H., Kim, S.Y., Chung, K.S., Moon, C.M., Kim, S.W., Kim, E.Y., Jung, J.Y., Park, M.S., Kim, Y.S., Kim, S.K., *et al.* (2014). Association between genetic variants in the IRGM gene and tuberculosis in a Korean population. *Infection* 42, 655-660.
- Stolz, A., Ernst, A., and Dikic, I. (2014). Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol* 16, 495-501.
- Sun, Q., Fan, W., Chen, K., Ding, X., Chen, S., and Zhong, Q. (2008). Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proceedings of the National Academy of Sciences of the United States of America* 105, 19211-19216.
- Tanabe, T., Chamaillard, M., Ogura, Y., Zhu, L., Qiu, S., Masumoto, J., Ghosh, P., Moran, A., Predergast, M.M., Tromp, G., *et al.* (2004). Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J* 23, 1587-1597.
- Travassos, L.H., Carneiro, L.A., Ramjeet, M., Hussey, S., Kim, Y.G., Magalhaes, J.G., Yuan, L., Soares, F., Chea, E., Le Bourhis, L., *et al.* (2010). Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nature immunology* 11, 55-62.
- Turkieh, A., Caubere, C., Barutaut, M., Desmoulin, F., Harmancey, R., Galinier, M., Berry, M., Dambrin, C., Polidori, C., Casteilla, L., *et al.* (2014). Apolipoprotein O is mitochondrial and promotes lipotoxicity in heart. *J Clin Invest* 124, 2277-2286.

- Yang, D., Chen, J., Zhang, L., Cha, Z., Han, S., Shi, W., Ding, R., Ma, L., Xiao, H., Shi, C., *et al.* (2014). Mycobacterium leprae Upregulates IRGM Expression in Monocytes and Monocyte-Derived Macrophages. *Inflammation* 37, 1028-1034.
- Yang, Y., Yin, C., Pandey, A., Abbott, D., Sasseti, C., and Kelliher, M.A. (2007). NOD2 pathway activation by MDP or Mycobacterium tuberculosis infection involves the stable polyubiquitination of Rip2. *The Journal of biological chemistry* 282, 36223-36229.
- Zurek, B., Schoultz, I., Neerincx, A., Napolitano, L.M., Birkner, K., Bennek, E., Sellge, G., Lerm, M., Meroni, G., Soderholm, J.D., *et al.* (2012). TRIM27 negatively regulates NOD2 by ubiquitination and proteasomal degradation. *PloS one* 7, e41255.

Supplemental References (IRGM)

- Chauhan, S., Goodwin, J.G., Manyam, G., Wang, J., Kamat, A.M., and Boyd, D.D. (2013). ZKSCAN3 is a master transcriptional repressor of autophagy. *Molecular cell* 50, 16-28.
- Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 119, 753-766.
- Kyei, G.B., Dinkins, C., Davis, A.S., Roberts, E., Singh, S.B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., *et al.* (2009). Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *The Journal of cell biology* 186, 255-268.
- Lapaquette, P., Glasser, A.L., Huett, A., Xavier, R.J., and Darfeuille-Michaud, A. (2010). Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 12, 99-113.
- Singh, S.B., Ornatowski, W., Vergne, I., Naylor, J., Delgado, M., Roberts, E., Ponpuak, M., Master, S., Pilli, M., White, E., *et al.* (2010). Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 12, 1154-1165.
- Soderberg, O., Gullberg, M., Jarvius, M., Ridderstrale, K., Leuchowius, K.J., Jarvius, J., Wester, K., Hydbring, P., Bahram, F., Larsson, L.G., *et al.* (2006). Direct observation of individual endogenous protein complexes in situ by proximity ligation. *Nature methods* 3, 995-1000.

References (Precision Autophagy)

- Axe, E.L., S.A. Walker, M. Manifava, P. Chandra, H.L. Roderick, A. Habermann, G. Griffiths, and N.T. Ktistakis. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol.* 182:685-701.
- Banchereau, J., and V. Pascual. 2006. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity.* 25:383-392.
- Barde, I., B. Rauwel, R.M. Marin-Florez, A. Corsinotti, E. Laurenti, S. Verp, S. Offner, J. Marquis, A. Kapopoulou, J. Vanicek, and D. Trono. 2013. A KRAB/KAP1-miRNA cascade regulates erythropoiesis through stage-specific control of mitophagy. *Science.* 340:350-353.
- Bauernfeind, F.G., G. Horvath, A. Stutz, E.S. Alnemri, K. MacDonald, D. Speert, T. Fernandes-Alnemri, J. Wu, B.G. Monks, K.A. Fitzgerald, V. Hornung, and E. Latz. 2009. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J Immunol.* 183:787-791.
- Bell, J.L., A. Malyukova, J.K. Holien, J. Koach, M.W. Parker, M. Kavallaris, G.M. Marshall, and B.B. Cheung. 2012. TRIM16 acts as an E3 ubiquitin ligase and can heterodimerize with other TRIM family members. *PLoS One.* 7:e37470.
- Birgisdottir, A.B., T. Lamark, and T. Johansen. 2013. The LIR motif - crucial for selective autophagy. *Journal of cell science.* 126:3237-3247.
- Bodemann, B.O., A. Orvedahl, T. Cheng, R.R. Ram, Y.H. Ou, E. Formstecher, M. Maiti, C.C. Hazelett, E.M. Wauson, M. Balakireva, J.H. Camonis, C. Yeaman, B. Levine, and M.A. White. 2011. RalB and the Exocyst Mediate the Cellular Starvation Response by Direct Activation of Autophagosome Assembly. *Cell.* 144:253-267.
- Broz, P., J. von Moltke, J.W. Jones, R.E. Vance, and D.M. Monack. 2010. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe.* 8:471-483.
- Carthagen, L., A. Bergamaschi, J.M. Luna, A. David, P.D. Uchil, F. Margottin-Goguet, W. Mothes, U. Hazan, C. Transy, G. Pancino, and S. Nisole. 2009. Human TRIM gene expression in response to interferons. *PLoS One.* 4:e4894.

- Chae, J.J., Y.H. Cho, G.S. Lee, J. Cheng, P.P. Liu, L. Feigenbaum, S.I. Katz, and D.L. Kastner. 2011. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1 β activation and severe autoinflammation in mice. *Immunity*. 34:755-768.
- Chae, J.J., G. Wood, S.L. Masters, K. Richard, G. Park, B.J. Smith, and D.L. Kastner. 2006. The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1 β production. *Proc Natl Acad Sci U S A*. 103:9982-9987.
- Chan, E.Y., and S.A. Tooze. 2009. Evolution of Atg1 function and regulation. *Autophagy*. 5:758-765.
- Chauhan, S., M.A. Mandell, and V. Deretic. 2015. IRGM Governs the Core Autophagy Machinery to Conduct Antimicrobial Defense. *Molecular cell*. 58:507-521.
- Criollo, A., M. Niso-Santano, S.A. Malik, M. Michaud, E. Morselli, G. Marino, S. Lachkar, A.V. Arkhipenko, F. Harper, G. Pierron, J.C. Rain, J. Ninomiya-Tsuji, J.M. Fuentes, S. Lavandero, L. Galluzzi, M.C. Maiuri, and G. Kroemer. 2011. Inhibition of autophagy by TAB2 and TAB3. *The EMBO journal*. 30:4908-4920.
- Deretic, V., T. Kimura, G. Timmins, P. Moseley, S. Chauhan, and M. Mandell. 2015. Immunologic manifestations of autophagy. *J Clin Invest*. 125:75-84.
- Deretic, V., T. Saitoh, and S. Akira. 2013. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol*. 13:722-737.
- Egan, D.F., D.B. Shackelford, M.M. Mihaylova, S. Gelino, R.A. Kohnz, W. Mair, D.S. Vasquez, A. Joshi, D.M. Gwinn, R. Taylor, J.M. Asara, J. Fitzpatrick, A. Dillin, B. Viollet, M. Kundu, M. Hansen, and R.J. Shaw. 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*. 331:456-461.
- Espinosa, A., V. Dardalhon, S. Brauner, A. Ambrosi, R. Higgs, F.J. Quintana, M. Sjostrand, M.L. Eloranta, J. Ni Gabhann, O. Winqvist, B. Sundelin, C.A. Jefferies, B. Rozell, V.K. Kuchroo, and M. Wahren-Herlenius. 2009. Loss of the lupus autoantigen Ro52/Trim21 induces tissue inflammation and systemic autoimmunity by dysregulating the IL-23-Th17 pathway. *J Exp Med*. 206:1661-1671.
- Fabri, M., S. Stenger, D.M. Shin, J.M. Yuk, P.T. Liu, S. Realegeno, H.M. Lee, S.R. Krutzik, M. Schenk, P.A. Sieling, R. Teles, D. Montoya, S.S. Iyer, H. Bruns, D.M. Lewinsohn, B.W. Hollis, M. Hewison, J.S. Adams, A. Steinmeyer, U. Zugel, G. Cheng, E.K. Jo, B.R. Bloom, and R.L. Modlin. 2011. Vitamin D is required for IFN- γ -mediated

antimicrobial activity of human macrophages. *Science translational medicine*. 3:104ra102.

- Frake, R.A., T. Ricketts, F.M. Menzies, and D.C. Rubinsztein. 2015. Autophagy and neurodegeneration. *The Journal of Clinical Investigation*. 125:65-74.
- French_FMF_Consortium. 1997. A candidate gene for familial Mediterranean fever. *Nature genetics*. 17:25-31.
- Gammoh, N., O. Florey, M. Overholtzer, and X. Jiang. 2013. Interaction between FIP200 and ATG16L1 distinguishes ULK1 complex-dependent and -independent autophagy. *Nature structural & molecular biology*. 20:144-149.
- Gao, D., J. Wu, Y.T. Wu, F. Du, C. Aroh, N. Yan, L. Sun, and Z.J. Chen. 2013. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science*. 341:903-906.
- Ghezzi, P., and C.A. Dinarello. 1988. IL-1 induces IL-1. III. Specific inhibition of IL-1 production by IFN-gamma. *J Immunol*. 140:4238-4244.
- Gomes, L.C., and I. Dikic. 2014. Autophagy in antimicrobial immunity. *Molecular cell*. 54:224-233.
- Gutierrez, M.G., S.S. Master, S.B. Singh, G.A. Taylor, M.I. Colombo, and V. Deretic. 2004. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell*. 119:753-766.
- He, C., and B. Levine. 2010. The Beclin 1 interactome. *Current opinion in cell biology*. 22:140-149.
- Herrero-Martin, G., M. Hoyer-Hansen, C. Garcia-Garcia, C. Fumarola, T. Farkas, A. Lopez-Rivas, and M. Jaattela. 2009. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. *The EMBO journal*. 28:677-685.
- Higgs, R., E. Lazzari, C. Wynne, J. Ni Gabhann, A. Espinosa, M. Wahren-Herlenius, and C.A. Jefferies. 2010. Self protection from anti-viral responses--Ro52 promotes degradation of the transcription factor IRF7 downstream of the viral Toll-Like receptors. *PLoS One*. 5:e11776.
- Higgs, R., J. Ni Gabhann, N. Ben Larbi, E.P. Breen, K.A. Fitzgerald, and C.A. Jefferies. 2008. The E3 ubiquitin ligase Ro52 negatively regulates IFN-beta production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. *J Immunol*. 181:1780-1786.

- Inbal, B., S. Bialik, I. Sabanay, G. Shani, and A. Kimchi. 2002. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. *J Cell Biol.* 157:455-468.
- Johansen, T., and T. Lamark. 2011. Selective autophagy mediated by autophagic adapter proteins. *Autophagy.* 7:279-296.
- Jounai, N., F. Takeshita, K. Kobiyama, A. Sawano, A. Miyawaki, K.Q. Xin, K.J. Ishii, T. Kawai, S. Akira, K. Suzuki, and K. Okuda. 2007. The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc Natl Acad Sci U S A.* 104:14050-14055.
- Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, and T. Yoshimori. 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *The EMBO journal.* 19:5720-5728.
- Kanayama, A., R.B. Seth, L. Sun, C.K. Ea, M. Hong, A. Shaito, Y.H. Chiu, L. Deng, and Z.J. Chen. 2004. TAB2 and TAB3 activate the NF-kappaB pathway through binding to polyubiquitin chains. *Molecular cell.* 15:535-548.
- Kawai, T., and S. Akira. 2011. Regulation of innate immune signalling pathways by the tripartite motif (TRIM) family proteins. *EMBO molecular medicine.* 3:513-527.
- Kenific, C.M., and J. Debnath. 2015. Cellular and metabolic functions for autophagy in cancer cells. *Trends Cell Biol.* 25:37-45.
- Khan, M.M., S. Strack, F. Wild, A. Hanashima, A. Gasch, K. Brohm, M. Reischl, S. Carnio, D. Labeit, M. Sandri, S. Labeit, and R. Rudolf. 2014. Role of autophagy, SQSTM1, SH3GLB1, and TRIM63 in the turnover of nicotinic acetylcholine receptors. *Autophagy.* 10:123-136.
- Kim, J., Y.C. Kim, C. Fang, R.C. Russell, J.H. Kim, W. Fan, R. Liu, Q. Zhong, and K.L. Guan. 2013. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell.* 152:290-303.
- Kim, J., M. Kundu, B. Viollet, and K.L. Guan. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* 13:132-141.
- Kimura, T., A. Takahashi, Y. Takabatake, T. Namba, T. Yamamoto, J.Y. Kaimori, I. Matsui, H. Kitamura, F. Niimura, T. Matsusaka, T. Soga, H. Rakugi, and Y. Isaka. 2013. Autophagy protects kidney proximal tubule epithelial cells from mitochondrial metabolic stress. *Autophagy.* 9:1876-1886.
- Konno, H., K. Konno, and G.N. Barber. 2013. Cyclic Dinucleotides Trigger ULK1 (ATG1) Phosphorylation of STING to Prevent Sustained Innate Immune Signaling. *Cell.*

- Kramer, R.M., E.F. Roberts, S.L. Um, A.G. Borsch-Haubold, S.P. Watson, M.J. Fisher, and J.A. Jakubowski. 1996. p38 mitogen-activated protein kinase phosphorylates cytosolic phospholipase A2 (cPLA2) in thrombin-stimulated platelets. Evidence that proline-directed phosphorylation is not required for mobilization of arachidonic acid by cPLA2. *J Biol Chem.* 271:27723-27729.
- Kroemer, G. 2015. Autophagy: a druggable process that is deregulated in aging and human disease. *The Journal of Clinical Investigation.* 125:1-4.
- Kyei, G.B., C. Dinkins, A.S. Davis, E. Roberts, S.B. Singh, C. Dong, L. Wu, E. Kominami, T. Ueno, A. Yamamoto, M. Federico, A. Panganiban, I. Vergne, and V. Deretic. 2009. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol.* 186:255-268.
- Lapaquette, P., A.L. Glasser, A. Huett, R.J. Xavier, and A. Darfeuille-Michaud. 2010. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cellular microbiology.* 12:99-113.
- Li, Q., J. Yan, A.P. Mao, C. Li, Y. Ran, H.B. Shu, and Y.Y. Wang. 2011. Tripartite motif 8 (TRIM8) modulates TNFalpha- and IL-1beta-triggered NF-kappaB activation by targeting TAK1 for K63-linked polyubiquitination. *Proc Natl Acad Sci U S A.* 108:19341-19346.
- Liang, Q., G.J. Seo, Y.J. Choi, M.J. Kwak, J. Ge, M.A. Rodgers, M. Shi, B.J. Leslie, K.P. Hopfner, T. Ha, B.H. Oh, and J.U. Jung. 2014. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe.* 15:228-238.
- Liang, X.H., S. Jackson, M. Seaman, K. Brown, B. Kempkes, H. Hibshoosh, and B. Levine. 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature.* 402:672-676.
- Ma, Y., L. Galluzzi, L. Zitvogel, and G. Kroemer. 2013. Autophagy and cellular immune responses. *Immunity.* 39:211-227.
- Maejima, I., A. Takahashi, H. Omori, T. Kimura, Y. Takabatake, T. Saitoh, A. Yamamoto, M. Hamasaki, T. Noda, Y. Isaka, and T. Yoshimori. 2013. Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury. *The EMBO journal.* 32:2336-2347.
- Mandell, M.A., A. Jain, J. Arko-Mensah, S. Chauhan, T. Kimura, C. Dinkins, G. Silvestri, J. Munch, F. Kirchhoff, A. Simonsen, Y. Wei, B. Levine, T. Johansen, and V. Deretic.

2014. TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. *Dev Cell*. 30:394-409.
- Masters, S.L., A. Simon, I. Aksentijevich, and D.L. Kastner. 2009. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Annual review of immunology*. 27:621-668.
- Mathew, R., S. Khor, S.R. Hackett, J.D. Rabinowitz, D.H. Perlman, and E. White. 2014. Functional Role of Autophagy-Mediated Proteome Remodeling in Cell Survival Signaling and Innate Immunity. *Molecular cell*.
- McEwan, W.A., J.C. Tam, R.E. Watkinson, S.R. Bidgood, D.L. Mallery, and L.C. James. 2013. Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. *Nature immunology*. 14:327-336.
- Meinzer, U., P. Quartier, J.F. Alexandra, V. Hentgen, F. Retornaz, and I. Kone-Paut. 2011. Interleukin-1 targeting drugs in familial Mediterranean fever: a case series and a review of the literature. *Seminars in arthritis and rheumatism*. 41:265-271.
- Minguela, A., S. Pastor, W. Mi, J.A. Richardson, and E.S. Ward. 2007. Feedback regulation of murine autoimmunity via dominant anti-inflammatory effects of interferon gamma. *J Immunol*. 178:134-144.
- Mishra, B.B., V.A. Rathinam, G.W. Martens, A.J. Martinot, H. Kornfeld, K.A. Fitzgerald, and C.M. Sasseti. 2013. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1beta. *Nature immunology*. 14:52-60.
- Mizushima, N., A. Kuma, Y. Kobayashi, A. Yamamoto, M. Matsubae, T. Takao, T. Natsume, Y. Ohsumi, and T. Yoshimori. 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *Journal of cell science*. 116:1679-1688.
- Mizushima, N., T. Yoshimori, and B. Levine. 2010. Methods in mammalian autophagy research. *Cell*. 140:313-326.
- Mizushima, N., T. Yoshimori, and Y. Ohsumi. 2011. The role of atg proteins in autophagosome formation. *Annual review of cell and developmental biology*. 27:107-132.
- Nakahira, K., J.A. Haspel, V.A. Rathinam, S.J. Lee, T. Dolinay, H.C. Lam, J.A. Englert, M. Rabinovitch, M. Cernadas, H.P. Kim, K.A. Fitzgerald, S.W. Ryter, and A.M. Choi. 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nature immunology*. 12:222-230.

- Nandi, B., and S.M. Behar. 2011. Regulation of neutrophils by interferon-gamma limits lung inflammation during tuberculosis infection. *J Exp Med.* 208:2251-2262.
- Nazio, F., F. Strappazzon, M. Antonioli, P. Bielli, V. Cianfanelli, M. Bordi, C. Gretzmeier, J. Dengjel, M. Piacentini, G.M. Fimia, and F. Cecconi. 2013. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol.* 15:406-416.
- Niida, M., M. Tanaka, and T. Kamitani. 2010. Downregulation of active IKK beta by Ro52-mediated autophagy. *Mol Immunol.* 47:2378-2387.
- Nishimura, T., T. Kaizuka, K. Cadwell, M.H. Sahani, T. Saitoh, S. Akira, H.W. Virgin, and N. Mizushima. 2013. FIP200 regulates targeting of Atg16L1 to the isolation membrane. *EMBO Rep.* 14:284-291.
- Omenetti, A., S. Carta, L. Delfino, A. Martini, M. Gattorno, and A. Rubartelli. 2014. Increased NLRP3-dependent interleukin 1beta secretion in patients with familial Mediterranean fever: correlation with MEFV genotype. *Annals of the rheumatic diseases.* 73:462-469.
- Pankiv, S., T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, and T. Johansen. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem.* 282:24131-24145.
- Papin, S., S. Cuenin, L. Agostini, F. Martinon, S. Werner, H.D. Beer, C. Grutter, M. Grutter, and J. Tschopp. 2007. The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. *Cell Death Differ.* 14:1457-1466.
- Pineda, C.T., S. Ramanathan, K. Fon Tacer, J.L. Weon, M.B. Potts, Y.H. Ou, M.A. White, and P.R. Potts. 2015. Degradation of AMPK by a Cancer-Specific Ubiquitin Ligase. *Cell.* 160:715-728.
- Pizon, V., S. Rybina, F. Gerbal, F. Delort, P. Vicart, G. Baldacci, and E. Karsenti. 2013. MURF2B, a novel LC3-binding protein, participates with MURF2A in the switch between autophagy and ubiquitin proteasome system during differentiation of C2C12 muscle cells. *PLoS One.* 8:e76140.
- Rabinowitz, J.D., and E. White. 2010. Autophagy and metabolism. *Science.* 330:1344-1348.
- Randow, F., and R.J. Youle. 2014. Self and Nonself: How Autophagy Targets Mitochondria and Bacteria. *Cell Host Microbe.* 15:403-411.

- Reymond, A., G. Meroni, A. Fantozzi, G. Merla, S. Cairo, L. Luzi, D. Riganelli, E. Zanaria, S. Messali, S. Cainarca, A. Guffanti, S. Minucci, P.G. Pelicci, and A. Ballabio. 2001. The tripartite motif family identifies cell compartments. *The EMBO journal*. 20:2140-2151.
- Rogov, V., V. Dotsch, T. Johansen, and V. Kirkin. 2014. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Molecular cell*. 53:167-178.
- Rubinsztein, D.C., P. Codogno, and B. Levine. 2012. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nature reviews. Drug discovery*. 11:709-730.
- Russell, R.C., Y. Tian, H. Yuan, H.W. Park, Y.Y. Chang, J. Kim, H. Kim, T.P. Neufeld, A. Dillin, and K.L. Guan. 2013. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol*. 15:741-750.
- Saitoh, T., N. Fujita, T. Hayashi, K. Takahara, T. Satoh, H. Lee, K. Matsunaga, S. Kageyama, H. Omori, T. Noda, N. Yamamoto, T. Kawai, K. Ishii, O. Takeuchi, T. Yoshimori, and S. Akira. 2009. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *Proc Natl Acad Sci U S A*. 106:20842-20846.
- Saitoh, T., N. Fujita, M.H. Jang, S. Uematsu, B.G. Yang, T. Satoh, H. Omori, T. Noda, N. Yamamoto, M. Komatsu, K. Tanaka, T. Kawai, T. Tsujimura, O. Takeuchi, T. Yoshimori, and S. Akira. 2008. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature*. 456:264-268.
- Saitoh, T., A. Tun-Kyi, A. Ryo, M. Yamamoto, G. Finn, T. Fujita, S. Akira, N. Yamamoto, K.P. Lu, and S. Yamaoka. 2006. Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1. *Nature immunology*. 7:598-605.
- Schroder, K., and J. Tschopp. 2010. The inflammasomes. *Cell*. 140:821-832.
- Shi, C.S., and J.H. Kehrl. 2010. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal*. 3:ra42.
- Shi, C.S., K. Shenderov, N.N. Huang, J. Kabat, M. Abu-Asab, K.A. Fitzgerald, A. Sher, and J.H. Kehrl. 2012. Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. *Nature immunology*. 13:255-263.
- Simonsen, A., and S.A. Tooze. 2009. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol*. 186:773-782.
- Stolz, A., A. Ernst, and I. Dikic. 2014. Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol*. 16:495-501.

- Stremlau, M., M. Perron, M. Lee, Y. Li, B. Song, H. Javanbakht, F. Diaz-Griffero, D.J. Anderson, W.I. Sundquist, and J. Sodroski. 2006. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc Natl Acad Sci U S A*. 103:5514-5519.
- Takahasi, K., N.N. Suzuki, M. Horiuchi, M. Mori, W. Suhara, Y. Okabe, Y. Fukuhara, H. Terasawa, S. Akira, T. Fujita, and F. Inagaki. 2003. X-ray crystal structure of IRF-3 and its functional implications. *Nature structural biology*. 10:922-927.
- Tal, M.C., M. Sasai, H.K. Lee, B. Yordy, G.S. Shadel, and A. Iwasaki. 2009. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc Natl Acad Sci U S A*. 106:2770-2775.
- The International FMF Consortium. 1997. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell*. 90:797-807.
- Tomar, D., R. Singh, A.K. Singh, C.D. Pandya, and R. Singh. 2012. TRIM13 regulates ER stress induced autophagy and clonogenic ability of the cells. *Biochimica et biophysica acta*. 1823:316-326.
- Toniato, E., X.P. Chen, J. Losman, V. Flati, L. Donahue, and P. Rothman. 2002. TRIM8/GERP RING finger protein interacts with SOCS-1. *J Biol Chem*. 277:37315-37322.
- Weidberg, H., T. Shpilka, E. Shvets, A. Abada, F. Shimron, and Z. Elazar. 2011. LC3 and GATE-16 N termini mediate membrane fusion processes required for autophagosome biogenesis. *Developmental cell*. 20:444-454.
- Xie, M., D. Zhang, J.R. Dyck, Y. Li, H. Zhang, M. Morishima, D.L. Mann, G.E. Taffet, A. Baldini, D.S. Khoury, and M.D. Schneider. 2006. A pivotal role for endogenous TGF-beta-activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. *Proc Natl Acad Sci U S A*. 103:17378-17383.
- Yang, Y., W. Fiskus, B. Yong, P. Atadja, Y. Takahashi, T.K. Pandita, H.G. Wang, and K.N. Bhalla. 2013. Acetylated hsp70 and KAP1-mediated Vps34 SUMOylation is required for autophagosome creation in autophagy. *Proc Natl Acad Sci U S A*. 110:6841-6846.
- Yoshimi, R., T.H. Chang, H. Wang, T. Atsumi, H.C. Morse, 3rd, and K. Ozato. 2009. Gene disruption study reveals a nonredundant role for TRIM21/Ro52 in NF-kappaB-dependent cytokine expression in fibroblasts. *J Immunol*. 182:7527-7538.
- Youle, R.J., and D.P. Narendra. 2011. Mechanisms of mitophagy. *Nature reviews. Molecular cell biology*. 12:9-14.

- Zalckvar, E., H. Berissi, L. Mizrachy, Y. Idelchuk, I. Koren, M. Eisenstein, H. Sabanay, R. Pinkas-Kramarski, and A. Kimchi. 2009. DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-X(L) and induction of autophagy. *EMBO Rep.*
- Zhang, Z., M. Bao, N. Lu, L. Weng, B. Yuan, and Y.J. Liu. 2013. The E3 ubiquitin ligase TRIM21 negatively regulates the innate immune response to intracellular double-stranded DNA. *Nature immunology.* 14:172-178.
- Zhou, R., A.S. Yazdi, P. Menu, and J. Tschopp. 2011. A role for mitochondria in NLRP3 inflammasome activation. *Nature.* 469:221-225.

Supplemental References (Precision Autophagy)

- Chauhan, S., Goodwin, J.G., Manyam, G., Wang, J., Kamat, A.M., and Boyd, D.D. (2013). ZKSCAN3 is a master transcriptional repressor of autophagy. *Molecular cell* 50, 16-28.
- Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 119, 753-766.
- Kyei, G.B., Dinkins, C., Davis, A.S., Roberts, E., Singh, S.B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., *et al.* (2009). Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *The Journal of cell biology* 186, 255-268.
- Lapaquette, P., Glasser, A.L., Huett, A., Xavier, R.J., and Darfeuille-Michaud, A. (2010). Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol* 12, 99-113.
- Singh, S.B., Ornatowski, W., Vergne, I., Naylor, J., Delgado, M., Roberts, E., Ponpuak, M., Master, S., Pilli, M., White, E., *et al.* (2010). Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol* 12, 1154-1165.
- Soderberg, O., Gullberg, M., Jarvius, M., Ridderstrale, K., Leuchowius, K.J., Jarvius, J., Wester, K., Hydbring, P., Bahram, F., Larsson, L.G., *et al.* (2006). Direct observation of individual endogenous protein complexes in situ by proximity ligation. *Nature methods* 3, 995-1000.

TRIM #	Accession #	Sequence	SiRNA
TRIM1	NM_012216	MGESPASVVLNASGGLFSLKMETLESELTCPICLIEFEDPLLLP CAHSLCFSAHRILVSSCSSGESIEPITAFQCPTCRYVISLHNRGLDGLKRNVTLQN IDRFOKASVSGPNPSPESRRERTYRPTTAMSSERIAACFCEQDPPRDVAVKTCITCEVS YDRCRLRATHPNKKPFTSHRLVEPVDPDTHLRGITCLDHENEKVMYCVSDDQLICALC KLVGRHRDHQVASLNDREFEKLKQTEMLNLTNLVKNRSELENQMAKLIQICQVEVNTA MHEAKLMEECDELVEIIQQRKQIAVAKIKETKVMKRLKLAQQVANCRCLESTVTLIN QAEHILKENDQARFLQSAKNAIARVAMATASSQVLIPIDNFNDAFENFALDFSREKKL LEGDLTLTAPNPPSIREELCTASHDTITVHWISDDEFSISSYELQYTIIFTGQANFISK SWCSWGLWPEIRKCKEAVSCSRLAGAPRGLYNSVDWMIVPNIKQNHYYTVHGLQSGTR YIFIVKAINQAGSRNSEPTRLKTNQPFKLDPKMTHKKLIKISNDGLQMEKDESSLKKS HTPERFSGTGCYGAAGNIFIDSGCHYWEVVMGSSWTWYAIAYKSAKNEWIGKNASS WVFSRCNSNFVVRHNNKEMLVDPHLLKRLGLVLLDYDNNMLSFYDPANSLHLHTFDVT FILPVCPTFTIWNKSLMILSGLPAPDFIDYPERQECNCRPQESPYPVSGMKTCH SEQ ID NO: 1	GAUGAAAGCUCUCUAAAGA SEQ ID NO: 2 GAACAAAUCCCUAAUGAUC SEQ ID NO: 3 GUAGACAGCUGGAUGAUUG SEQ ID NO: 4 CAAAUCAGCUCCAAAGAAU SEQ ID NO: 5
TRIM2	NM_015271	MHRSGRYGTQQQAGSKTAGPPCQWSRMASEGTNIPSPVVRQID KQFLICISICLERYKNPKVLPCLHTFCERCLQNYIPAHSLTLSCPVCRQTSILPEKQVA ALQNNFFITNLMVLRQTPGSAEESILETVTAAAGKPLSCPNDHGNVMEFYCQSC ETAMCRECTEGEHAETVPLKDVVEQHKASLQVQLDAVNKRLPEIDSALQFISEIHH QLTNQKASIVDDIHSTFDELQKTLNVRKSVLLMELEVNYGLKHKVLQSLDTLLQGE SIKSCSNFTAQALNHGTETEVLLVKKQMSKELNELADQDFPLHPRENDQLDFIVETEG LKSIHNLGTILTITNAVASETVATGEGLRQTIIGQPMSTVITTKDKDGLCKTGNAYL TAEI STPDGSAVADGEILDNKNGTYEFLYTVQKEGDFTLISRLYDQHIRGSPFKLVIR SADVSPTEGVRKRVKSPGSGHVQKAVKRPASMYSTGKRKENPIEDDLIFRVGTGR NKGEFTNLQGVAASTNGKILIAASNQCQVIFSDNGQFKSRFGIRGSRPGQLQRPQTV AVHPSGDI IADYDNKWVSI FSSDGKFKTKIGSGKLMGPKGVSDRNGHIIIVDNKAC CVFIFQPNQKIVTRFSGRNGDRQFAGPHFAAVNSNNEIIITDFHNHVKVFNQEGEF MLKFGSNGEGNQFNAPTGVAVDSNGNIIIVADWGNRIQVFDGSGSFLSYINTSADPL YGPQGLALTS DGHVVVADSGNHCFKRYRYLQ SEQ ID NO: 6	GAAGGACCCUAUGAGUUU SEQ ID NO: 7 GGAAGGAGAAUUCAGUUUG SEQ ID NO: 8 GGAUUGUGAUGGAAUUUUU SEQ ID NO: 9 CAACCAAUGUGUGCAGAU SEQ ID NO: 10
TRIM3	NM_006458	MAKREDSGPEVQPMQKFLVCSICLDRYQCPKVLPCILHTFCER CLQNYIPAQSLTILSCPVCRTQTSILPEQGVSAALQNNFFISSLMEAMQAPDGAHPEDP HPLSVVAGRPLSCPNEGHTMEFYCEACETAMCGECRAGEHREHGTVLLRDVVEQHK ALQRLQEA VRGRLPOLSAALVGGISQQLQERKAEALAQISAAFEDEALQQRKQA LVSDLETICGAKQKVLQSLDTLRQGEHIGSSCSFAEQALRLGSAPEVLLVRKHMRE RLAALAAQAFPERPHENAQLLEVLVDGLRRLSVNLGALLTTSATAHETVATGEGLRQ ALVGQPASLTVTTKDKGRLVRTGSAELRAEITGPDGTRLPPVPVVDHKNGTVELVYTA RTEGELLVLLYQGPVVRGSPFRVRLRPGDLPPSPDDVKRRVKSPGGPGSHVRQKAV	GGAAGGACGAGGAGUUUUU SEQ ID NO: 12 GAAAGGACAACCCAAUUGA SEQ ID NO: 13

TRIM4	NM_033017	RRPSSMYSTGGKRDNPIDELVFRVSGRGREKGEFTNLQGVSAASSGRIVVADSNQ CIQVFSNEGQFKFRFGVGRSPGQLQRPVAVDTNGDIIIVADYDNRWVSIFSPEGKF KTKIGAGRLMGPKGAVDRNGHIIIVDNKSCCVFTFQPNGKLVGRFGGRGATDRHFAG PHEVAVNNKNEIIVTDFHNSVKVYSADGEFLFKFGSHGEGNGQFNAPTGVAVDSNGN IIIVADWGNRIQVFDSSGSFSLSYINTSAEPLYGPQGLALTSDGHVVVADAGNHCFKAY RYLQ SEQ ID NO: 11	CCACAAGAAUGGCACAUAA SEQ ID NO: 14
		MEAEEDIEELTCPICLDYFDQDPVSIIEGHNFCRGCLHRNWAPGG GPFPCPECRHPSAPAAALRPNWALARLITEKTQRRRLGPVPPGLCGRHWEPIRLFCEDDQ RPVCLVCRESEHQTHAMAPIDEAFESYRTGNFDIHVDEWKRRLLIRLLLYHFKEEKL LKSQRLVAKMKVMHLQDVEVKNATQWKDKIKSQMRISTEFSKLNFLVEEEDLFL QRLNKEEETKKLNENTLKLNTIASLKKLILEVGEKSQAPTELLQNPKEVLTRSE IQDVNYSLEAVKVTVCQIPLMKEMLKRFQAVNLAEDTAHPKLVFSQEGRYVKNNTAS ASSWPVFSSAWNYFAGWRNPQKTAVERFQHLPCVLGKNVFTSGKHYYEVESSRDSLEV AVGVCREDVMGITDRSKMSPDVGIWAIYWSAAGYWPPLIGFPPTQQEPALHRVGYYL DRGTGNVSFYSAVDGVHLHTFSCSSVSRLRPFFWLSPLASLVIIPVTDK SEQ ID NO: 16	CCAAGUGGCUGUAAACCUA SEQ ID NO: 17 GAAGACAGUGGCCAGAUAA SEQ ID NO: 18 GAAGUUGAGAGUAGAGAUAA SEQ ID NO: 19 CAAACUACGCUUCAUUGA SEQ ID NO: 20 GCAGAAAGUUGAUCAUUGU SEQ ID NO: 22 GAGAGUAGCUGCCCUGUGU SEQ ID NO: 23 GGAAUCCUGGUUAAUGUAA SEQ ID NO: 24 UUACCAAGCCUGAGAACAA SEQ ID NO: 25
TRIM5	NM_033034	MASGILVNVEEVTCPICILELLTQPLSLDCGHSFCQACLTANHK KSMLDKGESSCPVCRIISYQENIRPNRHVANIIVEKLREVKLSPEGQKVDHCAHGEKL LLFCQEDGKVICWLERSQEHGHTFLTEEVAREYQVKLQAALEMLRQKQEAEELE ADIREEKASWKTQIQYDKTNVLADFEQLRDILDWEESNELQNEKEEEDILKSLTNSE TEMVQQTQSLRELISDLEHRLQGSVMELLQGVGVIKRTENVTLKKPETFPKNQRRVF RAPDLKGMLEVFRELTDVRRYWVDVTVAPNNISCAVISEDKRVSSPKPQIIYGARGT RYQTFVNFNYCTGILGSQISITSGKHYYEVDVSKKTAWILGVCAGFPDAMCNIEKNEN YQPKYGYWVIGLEEGVKCSAFQDSSFTSPVPIVPLSVIICPDRVGVFLDYEAETVS FFNITNHGFLIYKFSHCSFSQPVFPYLNPRKCGVPMTL CSPSS SEQ ID NO: 21	UAAAGAAGCUGAAGAACGA SEQ ID NO: 27 CUACAAAGCUGAGAAAGUAA SEQ ID NO: 28 GGACCUACAUUCUCUUUCA SEQ ID NO: 29 CCACUACUCUUUGUCCAUA SEQ ID NO: 30
TRIM6	NM_058166	MTSPVLVDIREEVTCPICILELLTEPLSIDCGHSFCQACITPNGR ESVIGQEGERSCPVCQTSYQPNLRPNRHANIVRRLREVLPGLKQKLVKADHGE KLQLFQEDGKVICWLERSQEHGHTFLVEEVAQYQEKFSKLLKNEEQEAEEK LTAFIREKTSWKNQMEPERCQIQTEFNQLRNILDRVEQRELKLEQEEKGLRIIEE AENDLVHQTQSLRELISDLERRCQGSTMELLQDVSDVTERSEFWTLRKPEALPTKLS MFRAPDLKRLRVCRELTDVQSYWVDVTLPHTANLNLVAKNRRQVRFVGAQVSGPS CLEKHYDCSVLGSQHSFSGKHYYEVDVAKKTAWILGVCNSLGPFTSFNFHFAQNHSA SRYQPQSGYWVIGLQHNHEYRAYEDSSPSSLLSMTVPVRRVGVFLDYEAGTVSYFNV NHGFPIYTFSKYYFPTTLCPYFNPCNCVPMTLRRPSS SEQ ID NO: 26	

TRIM7	NM_203294	MAAEQEKVGAFFQALRAFLVEQEGRLIGRIEELSREVAQKQENEN LAQLGVEITQLSKLSSQIQETAQKPDLDLQEFKSTLSRCSNVPGPKPTTVSSEMKNK VWNVSLKTFVLKGMKKFKEDLRGELEKEEKVELTLDPTDANPRILISLDLKGVRIGE RAQDLPHNPCRFDNTNRVILASCGFSSGRHWEVEVSGKDGWAFGVARSVRRKGLTPF TPEEGVWALQINGGQYWAVTSPERSPLSCGHLRVRVALDLEVGAVSFYAVEDMRHLY TFRVNFQERVFFLFSVCSTGTYLRIWP SEQ ID NO: 31	GAAGGGUGGCAGUGGGCUA SEQ ID NO: 32 GCUCUAAACAACACACAGA SEQ ID NO: 33 CAAAUAGCUCUGACGCGA SEQ ID NO: 34 CAUCCUGACCAAUGCGACA SEQ ID NO: 35 GCAAGAUUCUCUGUCUGUUC SEQ ID NO: 37 GGAUGAAAUCCGGAAGAU SEQ ID NO: 38 GGACAACUGUACUGUUCU SEQ ID NO: 39 GAACACCAAGUCUGUGAAA SEQ ID NO: 40 CCACAGGUCUCAUGGAGUA SEQ ID NO: 42 GCUUGGAGGUGAUUAAGGA SEQ ID NO: 43 CAACGGCGUCCGCGUGUUU SEQ ID NO: 44 AAACAGGAGUCAGCCCGUA SEQ ID NO: 45
		MAENWKNCFEEELICPICLHVFEVPQLPCKHNFRCGICGEAWA KDSGLVRCPECNQAYNQKPGLEKNLKLTNIVEKFNALHVEKPPAALHCVFCRRGPPLP AQKVLRCCEAPCCQSHVQTHLQQPSTARGHLIVAEADVRAMSCPQHNAIRLYHCEAEQ VAVCYCCYYSGAHQHGSVCDVEIRNEIRKMLMKQDRLEREQDIEDQLYKLESDK RLVEEKVNQLKEEVRLQYEKLHQLLDEDLRQTVEVLDKAQAKFCSENAQAALHLGERM QEAKKLIGSLQLLFDKTEDVSFMKNTKSVKIIMDRQTCTSSLSPTKIGHNLSKLFL NEVAKKEQLRKMLEGPFSTPVFLQSVPLPCGVSSSGAEKRKHSTAFPEASFLETS SGPVGGQYGAAGTASGEGSQPLGPCSSTQHLVALPGAQPVHSSPVFPSPYNGS AAQQPMLPQYGGRKILVCSVDNICYSSVANHGHPYPRSGHFPWTVPSQEYSHPLPP TPSPVQSLPSLAVRDWLDASQQPGHQDFYRVYGPSTKHYYVTS SEQ ID NO: 36	
		MEEMEELKCPVCGSFYREPIILPCSHNLQACARNILVQTPES ESPQSHRAAGSGVSDYDLDLDMSLYSEADSGYSGGFASAPTPCQKSPNGVRVF PPAMPPATHLSPALAPVRNISCITCPQCHRSLIIDRGLRGFPKNRVLEGVIDRYQQ SKAAALCQICEKAPKEATVCEQCDVFCDCPLRCHPPRGPLAKHRLVPPAQGRVS RRLSPRKVSTCTDHELENHSMYCVQCKMPVCYQCLEEGKHSHEVKALGAMWKLHKSQ LSQALNGLSDRAKEAKEFLVQLRNMVQOIQENSVEFEACLVAQCDALIDALNRRKAQL LARVNKEHEHKLKVVRDQISHCTVKLRQTTGLMEYCLEVIKENDPSGFLQISDALIRR VHLTEDQWKGTLTPRMTDFDLSDNSPLLQSIHQLDVQVKASSPVPAATPILQLEE CCTHNNSATLSWKQPPLSTVPADGYILELDDGNGGQFREYVVGKETMCTVDGLHFNST YNARVKAFNKTGVSYSKTLVLQTSVAVAWFAFDPGSAHSDIILSNDNLTVTCSSYDDR VVLGKTGFSKGIHYWELTVDRYDNHDPDPAFGVARMVDMKDMGLKDDKAWAMYVDNRR SWFMHNNSHNTREGITKGATIGVLLIDNRKNLTFFINDEQQGPFAFDNVEGLFFPA VSLNRNVQVTLHTGLPVPDFYSSRASIA SEQ ID NO: 41	
		MASAAVTSLADEVNCPICQGTTLREPVTIDCGHNFACRACLTRYC EIPGPDLEESPTCPLCKEPFRPGSFRPNWQLANVVENIERQLVSTLGLGEEDVCQEH GEKIYFFCEDDEMQLCVVCREAGEHATHMTMRFLEDAAPYREQIHKCLKLKEREEI QEIQSRENKRMQVLLTQVSTKRQQVISEFAHLRKFLEEQQSILLAQLESQDGDILRQR	GAGAGGAGAUUCAAGAAAU SEQ ID NO: 47 CAGAAGCACUCUAUAAGA SEQ ID NO: 48
TRIM8	NM_030912		
TRIM9	NM_015163		
TRIM10	NM_052828		

TRIM11	NM_145214	DEFDLLVAGEICRFSALIEELEEKNERPARELLTDIRSTLIRCETRKCRKPVAVSP GQRI RDPQALPLQREMKMFLEKLCFELDYEPAHISLDPTQSHPKLLISEDHQAQF SYKWQNSPDNPQRFDRATCVLAHTGITGGRHTWVMARVPGDSCCQFCSPSPVLGTE VAA SEQ ID NO: 46	GGGAACAAUCCAUAAGUG SEQ ID NO: 49 GCUUGAGUUGGACUAUGA SEQ ID NO: 50
		MAAPDLSTNLQEEATCAICLDYFTDPVMTDCGHNFRCRRCRW GQPEGYACPECRELS PQRLNRPNRPLAKMAEMARRLHPPSPVPGVC PAHREPLAAF CGDELRLCAACERSGEHWAHRVRPLQDAAEDLKAKLEKSLHLRKQMDALLFQAQA DETCVLWQKMVESQRQNVIGEFERLRLLAEEEEQQLLQRLLEEELEVLPRLEGAHL GQSAHLAEILAELEGRCQLPALGLIQDIKDARRVDVKLQPPPEVVPMELRVCRVP GLVETLRFRGDVTLDPDTANPELILSEDRRSVQRGDLRQALPDSPERFDPGCVLGQ ERFTSGRHYWEVEVGDRTSWALGVCRENVRKEKELGELSAGNGFWILVFLGSYNSSE ALAPLRDPPRRVGIFLDYEAGHLSFYSATDGSLLFIPEIPFSGTLRPLFSPLSSPT PMTICRPKGGGDTLAPQ SEQ ID NO: 51	GGACAUCUCUCUUCUAACA SEQ ID NO: 52 GGGAGAACGUGAACAGGAA SEQ ID NO: 53 GAGCUGAUCCUGUCUGAAG SEQ ID NO: 54 UCACUGCUAUUCAUCUUUC SEQ ID NO: 55
TRIM13	NM_005798	MELLEEDLTCPICCSLFDPRVLPCHSHNFCCKCLEGILEGSVRN SLWRPAPFKCPTCRKETSATGINSLOVNYSLKIGIVEKYNKIKISPKMPVCCKHLGQPL NIFCLTDMQLICGICATRGHTKHVFCSEDAYAQERDAFESLQSFETWRRGDALSR LDLTETSKRKSLLTKDSKVKKEFEKLQHTLDQKKNEILSDFETMKLAVMQAYDPE INKLNTILOEQMAFNIAEAFKDVSEPIVFLQMQEFREKIKIVKETPLPPSNLPASP LMKNFDTSQWEDIKLVVDKLSLPQDTGTFSKIPWSFYKFLILLILLGLVIVFGPTM FLEWSLFDLDTATWKGLSNFSSYLTKTADFIEQSVFYWEQVTDGFFIFNERFKNFTLV VLNNVAEFVCKYKLL SEQ ID NO: 56	GAGGAAAUCCCUACAGUUA SEQ ID NO: 57 UGAACAAUGUGGCAGAAU SEQ ID NO: 58 GACACUGGCACAUUCAUUA SEQ ID NO: 59 UAACAUUGCUGAGGCUUUC SEQ ID NO: 60
		MAGAAATGSRTPGRSELVEGCGWRCPEHGDRVAELFCRRRCRCVC ALCPVLGAHRGHPVGLALEAAVHVQKLSQELKQLAIKKQOHIDNITQIEDATEKLKA NAESKTLWLGKFTTELRLLLDEEALAKKFDKNTQLTLQVYREQADSCREQLDIMND LSNRVWSISOEPDPVQRLQAYTATEQEMQQMSLGELCHPVPPLSFEPVKSFFKGLVEA VESTLQTPLDIRLKEINQCLSDPSSTKPGTLLKTSPSPERSLLIKYARTPTLDPDTM HARLRLSADRLTVRCGLLGLSGPVPVLRFDALWQVLARDCFATGRHYWEVDVQEAAG WWVGAAYASLRRRGASAAAARLGCNRQSWCLKRYDLEYWAFHDGQRSRLRPRDDLRLG VFLDYEAGVLAFYDVTGGMSHLHTFRATFQEPLYPALRLWEGAISIPRLP SEQ ID NO: 61	CAACAUAACCCAGAUAGAA SEQ ID NO: 62 UCCAGAGGCUUCAGGCAUA SEQ ID NO: 63 GCUAAUGCAGAGUCAAGUA SEQ ID NO: 64 CAGAUUACUUCUAGACGAA SEQ ID NO: 65
TRIM15	NM_033229	MPATPSLKVVHELPACTLCAGPLEDAVTIPCGHTFCRLCLPALS QMGAAQSSGKILLCLPLCQEEEAETPMAPVPLGLGTYCEEHGEKIYFFCENDAEFLC	CAGCAGAUUUGGAAGGAGA SEQ ID NO: 67

TRIM16	NM_006470	VFCREGPTHQAHTVGFLDEAIQPYRDLRLSRLEALSTERDEIEDVKCEDQKIQVLIT QIESKKHQVETAFERLQOELEQRCILLARLELEQOIWKERDEYITKVSEEVTRLGA QVKELEKCCQPASELLQDVRVNQSRCEMKTFSPEAISPDLVKKIRDFHRKILTLPE MMRMFSENLAHLEIDSGVITLDPQTASRSLVSEDRKSVRYTRQKKSPLPDSPLRFDG LPAVLGFPGFSSGRHRWQDLQLGDGGCTVGAGEGVRKRGEMGLSAEDGVWAVIIS HQQCWASTSPGTDLPLSEIPRGVRVALDYEAGQVTLHNAQTQEPITFTTASFSGKVFP FFAVWKKGSLTLKG SEQ ID NO: 66	CGGAGAGAGAGAGAUUGA SEQ ID NO: 68
		MAELDLMAPGLPRATAQPPAPLSPDSGSPDSGSASPVEED VGSSEKLGRETEEQSDSAEQDPAGEGKEVLCDFCLDDTRRVKAVKSLTCMVNYCE EHLQPHQVNIKLQSHLLTEPVKDNWRYCPAHSPLSAFCCPDQCCICQDCCQEHSGH TIVSLDAARRDKAEALQCTQLDLERKLKLNENAI SRLQANQKSVLVSSEVKAVAEHQ FGELLAARKAQANVLFLEEKEQAALSOANGIKAHLEYRSAEMEKSKQELERMAAIS NTVQFLEEYCKFKNTEDITFPSVYVGLKDKLSGIRKVTITESTVHLIQLLENYKKKLQE FSKEEYDIRTQVSAVQRYWTSKPEPSTREQFLQYAYDITFDPDTHAKYLRLEEN RKVTNTTPWEHPYPDLPSRFLHWRQVLSQQSLYLHRYFFEVEIFGAGTYVGLTCKGID RKGEERNSCISGNNFWSLQWNGKEFTAWYSDMETPLKAGPFRRLGVYIDFPGGILSF YGVEYDTMTLVHKFACKFSEPVYAAFWSLKKENAIRIVDLGEEPEKPAPSLVGTP SEQ ID NO: 71	GGGAUGAAUAUACACAAA SEQ ID NO: 69
		MEVELARKLQEEATCSICLDYFTDPVMTTCGHNFRCACIQLSW EKARGKKGRKRKGSFPCECREMSPQRNLLPNRLLTKVAEMAQQHPGLQKQDLQCEH HEPLKLFQCKDQSPICVVCRESREHRLHRLVPAEEAVQYKLEEDMEYLRQITRT GNLQAREEQSLAEWQGVKERRERIVLEFEKMNLYLVEEQRLLQALETEEEETASRL RESVACLDROGHSLLELLQLLEERSTQGPLQMLQDMKEPLSRKNNSVQCPEVAPPTR PRTVCRVPGQIEVLRGFLEDVVPDATSAYPYLLLYESRQRRYLGSSEGSFGFCSDRF VAYPCAVGQTAFSSGRHYWEVGMNITGDALWALGVCRDNVSRKDRVPKCPENGFWVVO LSKGTLYLSTFSALTTPVMLMEPPSHMGIFLDFEAGEVSFYSVSDGSHLHTYSQATFPF PLQPFCLGAPKSGQMVISTVTMMVK SEQ ID NO: 81	GGUGUGAGAGAGACUUU SEQ ID NO: 70
		MOFCELLAAVRKAQANVLFLEEKEQAALSOANGIKAHLEYRSA EMEKSKQELETMAAISNTVQFLEEYCKFKNTEDITFPSVYIGLKDLSGIRKVTITEST VHLIQLLENYKKKLQEFSEKEEYDIRTQVSAIVQRYWTSKPEPSTREQFLQYVHDIT FDPDTHAKYLRLEENRKVTNTTPWEHPYPDLPSRFLHWRQVLSQQSLYLHRYFFEVE IFGAGTYVGLTCKGIDQKGEERSSCISGNNFWSLQWNGKEFTAWYSDMETPLKAGPF WRLGVYIDFPGGILSFYGYEYDSMTLVHKFACKFSEPVYAAFWSLKKENAIRIVDLGE EPEKPAPSLVGTP SEQ ID NO: 76	GAGGAGAGAGAGAGAUUGA SEQ ID NO: 77
		MEVELARKLQEEATCSICLDYFTDPVMTTCGHNFRCACIQLSW EKARGKKGRKRKGSFPCECREMSPQRNLLPNRLLTKVAEMAQQHPGLQKQDLQCEH HEPLKLFQCKDQSPICVVCRESREHRLHRLVPAEEAVQYKLEEDMEYLRQITRT GNLQAREEQSLAEWQGVKERRERIVLEFEKMNLYLVEEQRLLQALETEEEETASRL RESVACLDROGHSLLELLQLLEERSTQGPLQMLQDMKEPLSRKNNSVQCPEVAPPTR PRTVCRVPGQIEVLRGFLEDVVPDATSAYPYLLLYESRQRRYLGSSEGSFGFCSDRF VAYPCAVGQTAFSSGRHYWEVGMNITGDALWALGVCRDNVSRKDRVPKCPENGFWVVO LSKGTLYLSTFSALTTPVMLMEPPSHMGIFLDFEAGEVSFYSVSDGSHLHTYSQATFPF PLQPFCLGAPKSGQMVISTVTMMVK SEQ ID NO: 81	GAGGAGAGAGAGAGAUUGA SEQ ID NO: 78
TRIM17	NM_001024940*	MEVELARKLQEEATCSICLDYFTDPVMTTCGHNFRCACIQLSW EKARGKKGRKRKGSFPCECREMSPQRNLLPNRLLTKVAEMAQQHPGLQKQDLQCEH HEPLKLFQCKDQSPICVVCRESREHRLHRLVPAEEAVQYKLEEDMEYLRQITRT GNLQAREEQSLAEWQGVKERRERIVLEFEKMNLYLVEEQRLLQALETEEEETASRL RESVACLDROGHSLLELLQLLEERSTQGPLQMLQDMKEPLSRKNNSVQCPEVAPPTR PRTVCRVPGQIEVLRGFLEDVVPDATSAYPYLLLYESRQRRYLGSSEGSFGFCSDRF VAYPCAVGQTAFSSGRHYWEVGMNITGDALWALGVCRDNVSRKDRVPKCPENGFWVVO LSKGTLYLSTFSALTTPVMLMEPPSHMGIFLDFEAGEVSFYSVSDGSHLHTYSQATFPF PLQPFCLGAPKSGQMVISTVTMMVK SEQ ID NO: 81	GAGGAGAGAGAGAGAUUGA SEQ ID NO: 79
		MEVELARKLQEEATCSICLDYFTDPVMTTCGHNFRCACIQLSW EKARGKKGRKRKGSFPCECREMSPQRNLLPNRLLTKVAEMAQQHPGLQKQDLQCEH HEPLKLFQCKDQSPICVVCRESREHRLHRLVPAEEAVQYKLEEDMEYLRQITRT GNLQAREEQSLAEWQGVKERRERIVLEFEKMNLYLVEEQRLLQALETEEEETASRL RESVACLDROGHSLLELLQLLEERSTQGPLQMLQDMKEPLSRKNNSVQCPEVAPPTR PRTVCRVPGQIEVLRGFLEDVVPDATSAYPYLLLYESRQRRYLGSSEGSFGFCSDRF VAYPCAVGQTAFSSGRHYWEVGMNITGDALWALGVCRDNVSRKDRVPKCPENGFWVVO LSKGTLYLSTFSALTTPVMLMEPPSHMGIFLDFEAGEVSFYSVSDGSHLHTYSQATFPF PLQPFCLGAPKSGQMVISTVTMMVK SEQ ID NO: 81	AGGAUAAACUCUCGGGCAU SEQ ID NO: 80
		MEVELARKLQEEATCSICLDYFTDPVMTTCGHNFRCACIQLSW EKARGKKGRKRKGSFPCECREMSPQRNLLPNRLLTKVAEMAQQHPGLQKQDLQCEH HEPLKLFQCKDQSPICVVCRESREHRLHRLVPAEEAVQYKLEEDMEYLRQITRT GNLQAREEQSLAEWQGVKERRERIVLEFEKMNLYLVEEQRLLQALETEEEETASRL RESVACLDROGHSLLELLQLLEERSTQGPLQMLQDMKEPLSRKNNSVQCPEVAPPTR PRTVCRVPGQIEVLRGFLEDVVPDATSAYPYLLLYESRQRRYLGSSEGSFGFCSDRF VAYPCAVGQTAFSSGRHYWEVGMNITGDALWALGVCRDNVSRKDRVPKCPENGFWVVO LSKGTLYLSTFSALTTPVMLMEPPSHMGIFLDFEAGEVSFYSVSDGSHLHTYSQATFPF PLQPFCLGAPKSGQMVISTVTMMVK SEQ ID NO: 81	GAGGAGAGAGAGAGAUUGA SEQ ID NO: 85

TRIM18	NM_033290	METLESELTCICLELFEDPLLLPCAHSCLFCNCAHRILVSHCAT NESVESITAFQCPTCRHVITLSQRGLDGLKRNVTLQNIIDRFQKASVSGPNPSETRR ERAFDANTMTSAEKVLCQFCDDQDPAQDAVKTCTVCEVSYCDECLKATHPNKKPFTGHR LIEPIPDSHIRGLMCLEHEDEKVMYCVTDQDLICALCKLVGRHRDHQVAALSERYDK LKQNLSENLTNLIKRNTELETLIAKLIQTQHVENVASRQEAKLTEECDLLIEIIQQR RQIIGTKIKEGVMRLKLAQQIANCKQCIERSASLISQAEHSLKENDHARFLQTAKN ITERVSMATASSQVLIPEINLNDTFTFALDFSREKKLLECLDYLITAPNPPTIREELC TASYDTITVHWTSDDEFVSVELQYTIFTGQANVSVLCSNADSMMIVPNIKQNHYT HGLQSGTKYIFMVKAINQAGSRSEPGKLTNSQPFKLDPKSAHRKLKVSHDNLITVER DESSKKSHTPERFTSQSGYGVAGNVFIDSGRHYWEVVISGSTWYAIGLAYKSAPKHE WIGKNSASWALCRCNNWVVRHNSKEIPIEPAPHLRRVGILLDDYDNGSIAFYDAINSI HLYTFDVAFAQPVCPFTFTVWNKCLTIITGLPIPDHLDCTEQLP SEQ ID NO: 86	CAGCAAAGACGACAGAUUA SEQ ID NO: 87 GCUGAUAGCUGGAUGAUAG SEQ ID NO: 88 GAACAAGUGUCUGACGAUUA SEQ ID NO: 89 AGAAGAAACUGCUAGAAUG SEQ ID NO: 90
TRIM19	NM_033247	MEPAPARSPRQDDPARPQEPMTPTPETPSEGRQPSPPSPPTER APASEEEFQFLRCQQCAEAKCPKLLPCLHTLCSGCLEASGMQCPICQAPWPLGADTP ALDNVFFESLQRLSVYRQIVDAQAVCTRCKESADFWCFECEQLLCAKCFEAHQWFLK HEARPLAELRNQSVREFLDGTRKTNNIFCSNPNHRTPTLTSIYCRGCSKPLCCSCALL DSSHSELKCDISAEIQORQEELDAMTQALQEODSAFQAVHAQMAAVGQGLRARAETE ELIRERVQVVAHVRAQERELLEAVDARYQDYEEEMASRLGRIDAVLQIRITGSALVQ RMKCYASDQEVLDHMGFLRQALCRLRQEEPQSLQAAVRTDGFDEFKVLQDLSSCITQ GKDAAVSKKASPEAASTPRDPIDVDLLPPPAHALTGPAQSSTH SEQ ID NO: 91	GGGGAAGAUGCAGCUGUA SEQ ID NO: 92 GCAAAGAGUCGGCGGACUU SEQ ID NO: 93 GCGCUGGUCAGAGGAUGA SEQ ID NO: 94 CCGAUGGCUUCGACGAGUU SEQ ID NO: 95
TRIM20	NM_000243	MAKTPSDHLLSTLEELVPYDFEFKFKLQNTSVQKEHSRIPRSQ IQRARPVKMATLLVITYGEEYAVQTLTQVLRAINQRLLAELHRAAIQEYSTQENGTD DSAASSSLGENKPRSLKTPDHPEGNEGNGPRPYGGGAASLRCSQPEAGRLSRKPLSK RREKASEGLDAQGKPRTRSPALPGGRSPGPCRALEGGQAEVRLRRNASSAGRLQGLAG GAPGQKECRPFVYLPSCGMRPRSLEVTISTGEKAPANPEILLTLEEKTAANLDSATE PRARPTPDGGASADLKEGPNPEHSVTGRPPDTAASPRCHAQEGDPVDTCTVRDSCSF PEAVSGHPQASGSRSPGCRQDSHERKSPGSLSPQLPQCKRHLKQVQLLFCEDHDE PICLICSLSQEHQHRVRPIEEVALEHKKIKQLEHLKLRKSQEEQSRSYGEEKAVS FLKQTEALKQVRQKLEQVYFLEQQEHFFVASLEDVGMVQGIKRAYDTRVSDIAL LDALIGELEAKECQSEWELLQDIGDILHRAKTVPVPEKWTTPQEIKQIKILLHQKSEF VEKSTKYFSETLRSEMEMFNVPDELIGAQAHAVNVILDAETAYPNLIFSDDLKSVRLGN KWERLPDGPQRFDSCLIVLGSPSFLSGRRYWEVEVGDKTAWILGACKTISRKGNMTL SPENGYWVWIMMKENEYQASSVPPTRLLIKEPPKRVGIFVDYRVGSI SFYNVTARSHI YTFASCSFSGPLQPIFSPGTRDGGKNTAPLTICPVGGQGPD SEQ ID NO: 96	GACCACUCCUCAAGAGUA SEQ ID NO: 97 GAGAAUGGCUACUGGGUGG SEQ ID NO: 98 GCCCGCAAAUCCAGAAUU SEQ ID NO: 99 GCAUAUGACACCCCGGUAU SEQ ID NO: 100

TRIM21	NM_003141	MASAA RLTMWEEVTCPICLDPFVPEVPSIECGHSFCQECISQVG KGGSVCPVCRQRFLLKNLRPNRQLANMVNNLKEISQEA REGTQGERCAVHGERLHLF CEKDGKALCWVCAQSRKHRDHAMVPLEEAAQEQYQEKLOVALGELRRKQELAEKLEVEI AIKRADWKKTVEQKSRIHAEFVQKNFLVEEQRLQLELEKDEREQLRILGEKEAKL AQQSOALQELISELDRRCHSSALELLQEVIIVLERSESNLKDLDITSPELRSVCHVP GLKMLRTCAVHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNEERFDSYPMVLGA QHFGSGKHYWEVDVTGKEAWDLGVCRDVSRRKGHFLSSKSGFTWILWNKQKYEAGT YPQTPLHLQVPPCQVGFILDYEAGMVSFYNTDGHSLIYSFSECAFTGLRPFPSPGF NDGGKNTAPLTLCPLNIGSQGSTDY SEQ ID NO: 101	UCUCAGAGCUAGAUCCGAAAG SEQ ID NO: 102 GAGCAUACCUGGAAAUAGAA SEQ ID NO: 103 GGUGAUAAUUGUCCUGGAA SEQ ID NO: 104 AAGAGUGGCUUCUGGACAA SEQ ID NO: 105
TRIM22	NM_006074	MDFSVKVDIEKEVTCPICLELLTEPLSLDCGHSFCQACITAKIK ESVII SRGESSCPVCQTRFQGNLRPNRHLANI VERVEKVMSPQEGQKRDVCEHHGK KLQIFCKEDGKVICWVCELSQEHQGHQTFRINEVVEKCEQEKLOVALQRLIKEDQEA EK LEDDIRQERTAWKNYIQIERQKILKGFNEMRVILDNEEQRELQKLEEGEVNVDNLAA ATDQLVQQRQDASTLISDLQRLRGSSVEMLDQVIDVMKRSESWTLKPKSVSKKLKS VFRVPDLGMLQVLKELTDVQY YWVDVMLNPGSATSNAISVDQVQKTVRTCTFKNS NPCDFSAGVFGCYFSSGKYWEVDVSGKI AWILGVHSKISSINKRKSSGFAFDPSV NYSKVYSRYRPQYGYWVIGLQNTCEYNAFEDSSSDPKVLTLMFVPPCRIGVFLDYE AGIVSFFNVTNHGALIYKFSGCRFSRPAYPFYFNPNCLVPMTVCPSPSS SEQ ID NO: 106	GUAGCACCUGCACAUUUA SEQ ID NO: 107 CACCAACAUAUCCGCAUAA SEQ ID NO: 108 CCAGAUUAGACCUCUAUA SEQ ID NO: 109 AGAAUUAUAUCCAGAUCCA SEQ ID NO: 110
TRIM23	NM_001656	MATLVNKL GAGVDSGRQSGRTAVVKVLECGVCEDVFSLQGDK VPRLLCGHTVCHDCLTRLPLHGRAIRCPFDRQVTDLGDSGVWGLKKNFALLELLERL QNGPIGQYGAAEESIGISGESIIRCDDEAHLASVYCTVCATHLCECSQVTHSTKTL AKHRRVPLADKPHEKTMCSQHQVHAIEFVCLCEGCQTSPLMCCVCKEYGKHQGHKHSV LEPEANQIRASILDMAHCIRTFTEEISDYSRKLVGIVQHIEGGEQIVEDGIGMAHTEH VPGTAENARSCIRAYFYDLHETLCRQEEEMALSVDAHVREKLIWLRQQQEDMTILLSE VSAACLHCEKTLQDDDCRVVLAKQEI TRLLETLOKQQQFTEVADHIQLDASIPVTFT KDNRVHIGPKMEIRVVTGLDGAGKTTILFKLKQDEFMQPIPTIGFNVTVEYKNLKF TIWDVGGKHLRPLWKHYLNTQAVFVVDSSHRDRI SEAHSELAKLLTEKELRDALL LIFANKQDVAGALSVEEITELL SLHLKCCGRSWYIQGCDARS GMGLYEGLDWLSRQLV AAGVLDVA SEQ ID NO: 111	GAAGAAGGUUGUCAACUA SEQ ID NO: 112 UCACAAGCAUUCAGUAUUG SEQ ID NO: 113 GCAAAGUUGUUAACGGAAA SEQ ID NO: 114 GGAGAGAGCAUCAUUCGUU SEQ ID NO: 115
TRIM24	NM_003852	MEVAVEKAVAAAAAASAAAGGPSAAPSGENEAESRQGPDSERG GEAARLNLDTCAVCHQNIQSRAPKLLPLCLHSFCQRCPLPAPQRYLMLPAPMLGSAETP PPVPAPGSPVSGSSPFATQVGVI RCPVCSECAERHIIDNFFVKDTTEVPSSTVEKSN	GAACAUACCACGACAAGCA SEQ ID NO: 117

QVCTSCEDNAEANGFCVECEWLCKTCIRAHQRVKFTKDHTVRQKEEVSPEAVGVT SQ RPVFCPFHKKEQLKLYCETCDKLTCDRCQLLEHKEHRYQFIEEAFQNKQKVIIDTLITK LMEKTKYIKFTGNQIQNRRIEVENQKQVEQDIKVAIFTLMVEINKKGKALLHQLLES AKDRMKLMQOQOQEVAGLSKQLEHVMHFSKWAVSSGSSTALLYSKRLITYRLRHLRA RCDASPVNTNTIQFHCDPSFWAQNIIINLSLVIEDKESQPMKQNPVVEQNSQPPSG LSSNQLSKFTQISLAQLRLQHMQQQPPRLINFNHSPKPNPGLPPHPPQQLRYPP NQNIQRAIKPNPLQMAFLAQQAQKQWQISSGQGTPTTNTSSTSSPTTITSAAGYD GKAFGSPMIDLSSPVGGSYNLPSLDIDCSSTIMLDNIVRKDTNIDHGQPRPPSNRTV QSPNSSVPSPGLAGPVTMTSVHPPIRSPSASSVSGRSGSSSKPAGADSTHKVPVVM LEPIRIKQENSGPPENYDFPWIIVKQESDEESRPQANYPRLSILTSLLNSSQSSTSE ETVLRSDAPDSTGDQGLHQDNSSNGKSEWLDPSQKSLHVGETRKEDDPNEDWCVC QNGGELLCCCEKCPKVFHLSCHVPTLTNFPSEWICTFCRDLKPEVEYDCDAPSHNSE KKKTEGLVKLTPIDKRKCERLLLLFLYCHEMSLAFQDPVPLTVPDYKIIKNPMDLSTI KKRLQEDYSMYSKPEDFVADFRLLIFQNCAEFNEPDSEVANAGIKLENYFEELLKNLYP EKRFKPEFRNESEDNKFSDDDDDFVQPRKKRLKSI EERQLLK	AGACUUAUCUAAACACAGAA SEQ ID NO: 118 CUUUAGUAAUCGAGAUAA SEQ ID NO: 119 CUUUUAGCAAACGACUGA SEQ ID NO: 120
TRIM25 NM_005082	MAELCPIAEELSCSICLEPFKEPVTTPCGHNF CGSCLNETWAVQ GSPYLCPCQCRAVYQARPQLHKNTVLCNVVEQFLQADLAREPPADVWTPPARASAPSN AQVACDHCLKEAAVKTCLVCMASFCQEHLPQHFDSQAFQDHPLOPPVRDLRRKCSQH NRLREFFCPEHSECICHICLVEHKTCSPASLSQASADLEATLRHKLTVMYSQINGASR ALDDVRNRQOQDVRMTANRKVEQLQOQYTEMKALLDASETTSTRKIKEEKRVSNSKFD IYQILLKKKSEIQTLKEEIEQSLTKRDEFEFLEKASKLRGISTKPVYIPEVELNHKLI KGIHQSTIDLKNELKQICIGRLQEPPTSSGDPGEHDPASTHKSTRPVKKVSKEEKSKK PPVPALPSKLPTFGAPEQLVDLQAGLEAAAKATSSHPNSTSLKAKVLETFLAKSRP ELLEYYIKVILDYNTAHNKVALSECYTVASVAEMPQNYRPHQRFYCSQVGLHCHYK KGIHYWEVELQKNNFCGVGICYGSMNRQGPESRLGRNSASWCVEWFNTKISAWHNNVE KTL PSTKATRVGVLLNCDHGFVIFFAVADKVHLMYKFRVDFTEALYPAFWVFSAGATL SICSPK SEQ ID NO: 121 GACCGCAGCUGACAAGAA SEQ ID NO: 122 CAACUAAACUGUCAUGUAC SEQ ID NO: 123 CAACAAGAAUACACGGAAA SEQ ID NO: 124 GCGGAUGACUGCAAACAGA SEQ ID NO: 125

TRIM26	NM_003449* *variant 1	MATSAPLRSLSEEVTCISICLDYLRDPVTIDCGHVFCRSCITDVR PISGSRPVCPLCKKPFKKNIRPVWQLASLVENIERLKVDKGRQPGEVIREQQDAKLC ERHREKLHYECEDDGGKLLCVMCRESREHPRHTAVLMEKAAQPHREKILNLHLSTLRDR DKIQCFQAKGEADILAAALKKIQDQRYIVAEFEQGHQFLREREHLLLEQLAKLEQELT EGREKFKSRGVGELARLALVISELEKAAQPAALMQDTRDFLNRPRKKFWVGKPIA RVVKKKTGEFSDKLLSLQRLREFQCKLLRLDLEYKTVSVTLDPQASGYLQLSSEDMKC VYTSLYKSAYLHPQQFDCPEPGLGSKGFTWGVYWEVEVEREGWSEDEEEGDEEEEG EEEEEEAAGYGDGYDDWETDEDEESLGDEEEEEEEEEEEVLESCVMGVARDSVKRRK DLSLRPEDGVWALRLSSGIWANTSPEAELFPALRPRRVGIALDYEGGTVTFTNAESQ ELIYTFATFTTRRLVPFLWLKWPGRLLLRP SEQ ID NO: 348	GAGCAGGGCUGAAAGAAUC SEQ ID NO: 127 UAGAGAGGCUCAGUUAUA SEQ ID NO: 128 GCUGAACUCUUGAGCCUAA SEQ ID NO: 129 GAAGAUUGUUUGGAGUUU SEQ ID NO: 130
TRIM27	NM_006510	MASGSVAECLQQETTCTPCVCLQYFAEPPMMLDCGHNICCACLARCW GTAETNVSCPQCRETFFQRMHMRPNRHIANVTQLVKQLRTERPSGPGGEMGVCEKHREP LKLYCEEDQMPICVVCDRSREHRGHSVPLEEAVEGFKEQIQNQLDHLKRVKDLKKRR RAQGEQARAELLSLTQMEREKIVWEFEQLYHSLKEHEYRLARLEELDIAIYNSINGA ITQFSCNISHLSSLIAQLEEKQQPTRELLQDIDGTLSTRAERIRIPEPWITPPDLQEK IHIFAQKCLFLTESLKQFTEKMQSDMEKIQELREAQLYSVDTVLDPTAYPSLILSDN LRQVRYSLQDDLPDNPENFNLFPVGLGSPCFIAGRHYWEVEVGDKAKWTIGVCEDSV CRKGGVTSAPQNGFWAVSLWYKEYWALTSPTMALPLRTPLRVGFILDYDAGEVSFY NVTERCHTFTFSHATFCGPVRPYFSLSYSGGKSAAPLIICPMGIDGFGSHVGNHGS METSP SEQ ID NO: 126	GACCAAACCUUGUCUUAUG SEQ ID NO: 132 GAUGAUCCCUACUCAAGUG SEQ ID NO: 133 GCGAUCUCUGGUUAUGUGCAA SEQ ID NO: 134 AGAAUUAUUUCAUGCGUGA SEQ ID NO: 135
TRIM28	NM_005762	MAASAAAAAASASGSPGEGSAGGEKRRSTAPSAASASA SAAASSPAGGGAELLELHCGVCRRERLRPERPRLLPCLHSACSACLGPAAPAAANS SGDGAAGDGTVDPCVCKQCFKSDIVENYFMRDSGSKAATDAQDANQCCTSCEDNA PATSYCECSEPLCETCVAHQVRVYTKDHTVRSTGPAKSRDGERTVYCNVHKHEPLV LFCECDTLTCRDCQLNAHKDHQYQFLEDAVRNQRKLLASLVKRLGDKHATLQKSTKE VRSSIRQVSDVQKRVQVDVKMAILQIMKELNKRGRVLVNDQKVTEGQERLERQHWI MTKIQKHQEHILRFASWALESDNNTALLSKKLIYFQLHRAIKMIVDPVEPHGEMKFQ WDLNWTKSAAEAFKIVAERPNTSTGPAPMAPPRAPGLSKQSGSSQPMVEQEGYG FGSGDDPYSSAEPHVGVKRSRSGEVSGLMRKVPRVSLERLRLDLTADSQPPVFKV FPGSTTEDYNLIVIERGAAAAATGQPGTAPAGTAPAGPLAGMAIVKEEETEAAIGAPP TATEGPETKPVLMALAEPGAEGPRLASPSGSTSSGLEVVAPEGTAPGGGPGTLDSDS ATICRVCKPQDGLVMCNQCEFCFHLDCPLALQDVPGEWSCSLCHVLPDLKEEDGSL SLDGADSTGVWAKLSPANQRKCEVLLALFCHEPCRPLHQLATDSTFSLDQPGGTLDL TLIRARLQEKLSPPYSSPQEFADQVGRMFKQFNKLTEDKADVQSIIGLQRFETRMNE AFGDTKFSAVLVEPPPMSLPGAGLSSQELSGGPGDGP	

TRIM29	NM_012101	SEQ ID NO: 131 MEAADASRNGSSPEARARSPPSGSLENGTKADGKDAKTIN GHGEEAAEGKSLGALKPGEGRSALFAGNEWRRPIIQVESGDDKNSNYFSMDSMEGK RSPYAGLQLGAAKKPPVTFAEKGELRKSIFSESRKPTVSIMEGETRRNSYPRADTGL FSRKSGSEEVLCDSICIGNKQKAVKSLVCQASFCEHLKPHLEGAARFDHQLLEPIR DFEARKCPVHGKTMELFCQTDQTCICYLCMFQEHKNHSTVTVEEAKAEKETELSLQKE QLQLKIEIEDEAEKQKEKDRIKSFTTNEKAILQNFRLDVRDLEKQKEEVRAALEQ REQDAVDQVKVIMDALDERAKVLHEDKQTRQLHSISDSVLFLOEFGALMSNYSLPPP LPTYHVLLLEGELGQSLGNFKDDLLNVCMRHVEKMKADLSRNFIERNHMENGGDHRY VNNYTNFSGEWSAPDTMKRYSMYLTPKGVRTSYQPSPPGRFTKETTKNFNLYGT KGNYSRWWEYSSSIQNSDNDLPVVQSSSFLKGYPSLMRSQSPKAPQPTWKSQKQT MLSHYRPFYVNGNGIGSNEAP SEQ ID NO: 136 MASGQFVNKLQEEVICPICLDILQKPVTIDCGHNFCLKCITQIG ETSCGFFKCPCKTSVRKNAIRFNSLLRNIVEKIQALQASEVQSKRKEATCPRHQEMF HYFCEDDGKFLCFVCRESKDHKSHNSVLIIEAAQNYQGIQEQIQLQKEKETVQVK AQGVHRVDVFTDQVEHEKQRIILTEFELLHQVLEEKNFLLSRIYWLHEGTEAGKHVY ASTEPQLNDLKKLVDSLTKQNMPPRLLEDIKVILCRSEEFQFLNPTVPVLELEKKL SEAKSRHDSITGSLKKFKDQLQADRKKNDRFFKSMKNKNDKMSWGLLKNNHMKMNKTS EPGSSSAGGRTTSGPPNHHSSAPSHSLFRASSAGKVTFPVCLLASIDEISGQGASSQD TKTFDVALSEELHAALSEWLTAIRAWFCEVPSS SEQ ID NO: 141 MAAAAASHLNLDAIREVLECPICMESFTEEQLRPKLLHCGHTIC RQCLEKLLASSINGVRCPCFSKITRITSLTQTDNLTVLKIIDTAGLSEAVGLLMCRS CGRRLPRQFCRSCGLVLCPEPCREADHPGHCCTLPVKEAAEERRRDFGEKLTRELML GELQRRKAALLEGVSKDLQARYKAVLQEYGHERRVQDELARSRKFFTGSLAEVEKSNS QVVEEQSYLLNIAEVQAVSRCDFYFLAKIQADVALLEETADEEEPELTASLPRELTQ DVELLKVGHVGPLOIGQAVKKPRTVNVEDSWAMEATASAASTSVTFREMDMSPEEVVA SPRASPAKQRGPEAASNIQQCLFLKKMGAKGSTPGMFNLPVSLYVTSQGEVLVADRG YRIQVFTRKGFLEIRRRSPSGIDSFVLSFLGADLPNLTPLSVAMNCQGLIGVTDSDYN SLKVYTLDGHCVACHRSQLSKPWGITALPSGQFVTVTVDEGGKLCWCTVDRSGSVKYS CLCSAVRPKFVTCDAEGTVYFTQGLGLNLENRQNEHLEGGSIGSVGPDGQLGRQIS HFFSENEDFRCIAGMCDVARGDLIVADSSRKEILHFPKGGGYSVLIREGLTCPVGIAL TPKGQLLLVDCWDHCIKIYSYHLRRYSTP SEQ ID NO: 146	GCAGGAAUUUGGUGCAUUG SEQ ID NO: 137 GAUCAUGGAGUCUCUGGAU SEQ ID NO: 138 GAAGAGAUACUCCAUGUAC SEQ ID NO: 139 CCAGAAGAAUUUCAACAAU SEQ ID NO: 140 CGAAGAAGCUGCCCCAGAAU SEQ ID NO: 142 GGAGAAGAAUUUCCUGCUA SEQ ID NO: 143 GAUGAGAUUCUGGUGCAAG SEQ ID NO: 144 GAGCCACAGUUGAACCGAUC SEQ ID NO: 145 GAUCAGGGGUGGUGCAAAUA SEQ ID NO: 147 GCAUAGCCCCUAACUCCUAA SEQ ID NO: 148 GAGCUGUGGUUUGGUGUUA SEQ ID NO: 149 GUGAAGUACUAGUCGCUGA SEQ ID NO: 150
TRIM31	NM_007028		
TRIM32	NM_001099679		

TRIM33	NM_033020	MAENKGGGEAEISGGGSGSAPVTAGAAAPAAQAEAPPLTAVLVE EEEEEGGRAGAEAGGAGDDGGVAAAASGSAQAASSPAASVGTGVAGAVSTPAPAPA SAPAPGPSAGPPPPASLLDTCVACQQLSRREAEPKLLPCLHSLRCLPEPERQ LSVPIPGGNGDIQQVGIVRCVCRQECRQIDLVDNYFVKDTSEAPSSSDEKSEQVCT SCEDNASAVGFCVEGGEWLCKTCEIAHQVRVKTCDHLIRKKEDVSESVGASGQRPVFC PVHQEQKLKFCETCDRLTCRDCQLLEHKEHRYQFLEEAFFQKQKGAITENLLAKLLEKK NYVHFAATQVQNRKIKEVNETNKRVEQEIKVAITFLINEINKKSKSLLOQLENNVTKERQ MKLLQQQNDITGLSRQVKHVMFTNWAIASGSTALLYSKRLITFQLRHILKARCDPV PAANGAIRFHCDPTFWAKNVVNLGNLVIESKPAPGYTPNVVVGQVPPGTHHSKTPGQ INLAQLRLQHMQQQVYAQKHQQLQOMRMQPPAPVPTTTTQQHPRQAAPQMLQQQP PRLISVQTMQRGNMNCGAFQAHQMLAQNAARIIPGIPRHSQPQYSMMQPHIQRQHSNP GHAGPFPVSVHNTTINPTSTTATMANANRGPTSPSVTAIELIPSVTNPENLPSLPD IPPIQLEDAGSSSLDNLISRYISGSHLPQPTSTMNPSPGPSALSPGSSGLSNSHTPV RPPSTSTGSRGCGSGRTAEKTSLSFKSDQVKVQEPGTEDEICFSFGGVKQEKTE DGRSACMLSSPESSLTPLSTNLHLESELDALASLENHVKIEPADMNECKQSGLS LVNGKSPIRSLMHSARIIGDGNKDDDPNEDWCAVCQNGGDLCCCEKCPKVFHITCH VPTLLSFPDGDWICTFCRDIGKPEVEYDCDNLQHSKKGKTAQGLSPVDQRKCEKLLLY LYCHELSIEFQEPVPASIPNYKYIKKPMDDLSTVKKKIQKHSQHYQIPDDFVADVRL IFKNCEFRNEADSEVAQAGKAVALLYFEDKLTIELYSRDTFAPLPEFEQEEEDDGEVTEDS DEDFIQPRRKRLKSDERPVIK SEQ ID NO: 151	GGACAAACCAUUAUAGUAA SEQ ID NO: 152 GCAAGCGACUGAUUACUUU SEQ ID NO: 153 UGAAACAUGUGAUAGAUUG SEQ ID NO: 154 GUGAUAAUUUGCAACAUAG SEQ ID NO: 155
TRIM34	NM_130390	MASKILLNVQEEVTCPICLELLTEPLSLDCGHSCLRACITVSNK EAVTSMGGKSSCPVCGISYSEFHLQANQHLANIVERLKEVKLSPDNGKKRDLCDHHE KLLLFCKEDRKVICWLCEERSQEHGHTVLTTEEVFKECQEKIQAIVLKRLLKEEEFAFK LEADIRREKTSWKYQVQTERQRIQTEFDQLRSILNNEEQRELQRLKEEKKTLIDKFAE AEDELVQKQLVRELISDVECRSQWSTWELLQDMSGIMKWCVVVARSGACEL SEQ ID NO: 156	GAAAAGAAGACGCGUGGAUA SEQ ID NO: 157 GGAGGAAGUAUUAAGGAA SEQ ID NO: 158 UGUCGGAGUCAGUGGUCAA SEQ ID NO: 159 AAUUCUUGCUUAACGUACA SEQ ID NO: 160 GACCUGCGCACCAACCACA SEQ ID NO: 162 ACAAGGAGCUGCUGUGCUG SEQ ID NO: 163 CCACUGCCCAGUGUGCAA SEQ ID NO: 164 GUGCAGCCGGUGAAGGACA
TRIM35	NM_171982	MERSPDVSPGPSRSFKEELLCAVCYDPFRDAVTLRCGHNFCRGC VSRWEVQVSPTCPVKCKDRASPADLRNTHTLNNLVEKLLREEAEGARWTSYRFSRVCR LHRGQLSLFCLEDKELLCCSCQADPRHQGHVRVQVKTADHDFRAKCRNMEHALREKAK AFWAMRRSYEAIKHNQVEAAWLEGRIRQEFDKLREFLRVEEQAILDAMAEETRQKQL LADEKMKQLTEETEVLAEIERLOMEMKEDDVSLMKHKSRRRLCTMEPEPVQPGM LIDVCKYLSLQYRVWKKMLASVESVFFSDPNTAAGWLSVSDDLTSTVTHGYRVQVE NPERFSSAPCLLGSRVFSQGSRAWELGGLQSWRVGVVRVQRDGAEGHSHSYHDT RSGFWYVCRTOQVEGDHCVTSDPATSPVLVLAIPRRLRVELECEEGELSFDYDAERHCHL YTFHARFGEVRPYFYLGGARGAGPPEPLRICPLHISVKEELD	

TRIM36	NM_018700* *variant 1	SEQ ID NO: 161	SEQ ID NO: 165
		MSESGEMSEFGYIMELIAKGKVTIKNIERELICPACKELFTHPL ILPCQHSICHKCVKELLILLTDDSFNDVGSNDNSQSSPRLRLPSPSMDKIDRINRPGWK RNSLTPTTVPFPCGCEHVDLGERGINGLFRNFTLETIVERYRQAARAATAIMCDLC KPPQESTKSCMDCSASYCNECFKIHHPWGTIKAQHEXVVGPTNFRPKILMCPHEHETE RINMYCELRRPVCCHLCKLGGNHANHRVTTMSSAYKTLKEKLSKDDIDYLLIGKESQVKS QISELNLLMKETECNGERAKEEAI THFEKLFEVLEERKSSVLKAIDSSKKLRLLDKFQT QMEYQGLLENNGLVGYAQEVLTQSCFVQTAQQLHLRIQKATESLKSFRPAAQTS FEDYVWNTSKQTELLGELSFFSSGIDVPEINEEQSKVYNNALINWHHPKDKADSYVL EYRKINRDDMSWNEIEVCGTSKIIQDLENSSTYAFRVAYKGSICSPCSRELILHTP PAPVFSFLFDEKCGYNNEHLLNLKRDRESRAGFNLLAAERIQVGYTSLDYIIGD TGITKGKHFWAFRVEPYSLVKGVASSDKLQEWLRSPRDAVSPRYEQDSDGHDGSED ACFDSSQPFTLVITIGMQKFFIPKSPSTSSNEPENRVLPMPTSIGIFLDCDKGKVDFYDM DQMKCLYERQVDCSHTLYPAFALMGSGGIQLEEPITAKYLEYQEDM	
		SEQ ID NO: 166	

TRIM37	NM_001005207	MDEQSVESIAEVFRFCFICMEKLRDARLCPHC SKLCCFSCIRRWL TEQRAQCPCPHCRAPLQRLREL VNCRWAEVVTQQLDTLQLCSLTKHEENKDKCENHHEKL SVFCWTCKKCI CHQCALWGMHGGHTFKPLAIEIYEQHVTKVNEEVAKLRRLMELISL VQEVERNVEAVRNAKDERVREIRNAVENMMIARLDTQLKNKLTILMGQKTSLTQETELL ESLLQVEVEHQLRSCSKSELISKSSEILMMFQQVHRKPMASFVTTVPVPPDFTSELVPSY DSATFVLENFSTLRQADPVYSPPLQVSGLCWRLKVYPDNGVGVYGLSVFLELSAG LPETSKYEYRVEMVHQSCNDPTKNIIRFASDFEVGECWGNRFFRLDLLANEGYLN QNDTVILRFQVRSPFTFFQKSRDQHWYITQLEAAQTSYIQQINNLERLTIELSRTQKS RDLSPPDNHLSPQNDDALETRAKKSACSDMLLEGGPTTASVREAKEDEDEEKTQNEED YHHELSGDGLDLVYEDEVNQLDGSSSSASSSTATSNTTEENDIDEETMSGENDVEYNN MELEEGELMEDAAAAAGPAGSSHG YVGS SRI SRTHLCSAATSSLLDIDPLILHLDD LKDRSS IENLWGLQPRPPASLLQPTASYSRKDKQKQQAAMWRVPSDLKMLKRLKTQM AEVRCMKT DVKNLTSEIKSSSAAASGDMQTSLSFADQAALAAACGTENSGRLQDLGMELL AKSSVANCYIRNSTNKKSNPKPARSSVAGSLSLRRAVDPPGENSRSKGDCQTLSEGSP GSSQSGSRHSSPRALIHGSI GDILPKTEDRQCKALDSDAVVAVFSGLP AVEKRRKMV TLGANAKGGHLEGLQMTDLENNSETGELQPVLP EGASAAPEEGMSSDSDIECDTENEE QEEHTSVGGFHDSFMVMTQPPDETHSSFPDGEQIGPEDLSFNFDENSGR SEQ ID NO: 167	GGACAUACCUUUUAAACCUU SEQ ID NO: 168 ACACACAGCUGAAGAAUAA SEQ ID NO: 169 GCAGACUGAUUUUGAA SEQ ID NO: 170 UACGAGAACUAGUAAAUUG SEQ ID NO: 171
TRIM38	NM_006365	MASTSTKKMEEATCSICLSLMTNPVSNCGHSYCHLCITDFF KNPSQKQLRQET FCCPQCRAPFHMDSLRPNKQLGSLIEALKETDQEMSCEEHGEQFHL FCEDEGQLICWRCERAPQHKGHTTALVEDVCQGYKEKQLQKAVTKLKQLEDRCTEQKLS TAMRITKWKEKVQIQRQKIRSDFKNLQCFLEHEEKSYLWRLKEEQQTL SRLRDEYAG LGLKSNELKSHILEEEKCQGSQAQKLLQNVNDTILSRWAVKLETSEAVSLELHMTMCNV SKLYFDVKKMLRSHQSVTLDPDTAHHELIILSEDRRQVTRGYTQENQDTSRRRTAFP CVLGCEGFTSGRRYFEVDVGEGTGWDLGVCMENVQRGTGMKQEPQSGFWTLRLCKKKG YVALTSPPTSLHLHEQPLLVGIFLDYEAGVVSFYNGTGTGCHIFTFPKASFSDTLRPYF QVYQYSPLFLPPGD SEQ ID NO: 172	GCGAAUAAACUAAAUUGAAA SEQ ID NO: 173 AGAAUUGCUGCAGAAUGU SEQ ID NO: 174 CAACUUGAAGACAGAUUA SEQ ID NO: 175 AGAUACAGCUCAUCAGAA SEQ ID NO: 176
TRIM39	NM_172016	MAETSLLEAGASAAATAALENLQVEASCSVCLEYLKEPVIIEC GHNFCKACITRWWEDELFPCPVCRTSRYSRLRPNRQLGSMVEIAKQLQAVKRRKIR DESLCPQHHEALSLFCYEDQEA VCLICAI SHTHRAHTVVPD DATQYKEKQLQKCLEP LEQKIQEITRCKSSEKKPGELKRLVESRRQQLREFEELHRLDEEQVLLSRLEEE EQDILQRLRENAAHLDKRRDLAHLAAEVEGKCLQSGFEMLKDVKSTLEKCEKVTME VTSVSI ELEKNFSNFPQYFALRKILKQLIADVTLDPETAHPNLVLSEDRKSVKFVET RLRDLPTPRRFTFYPCVLATEGFTSGRHYWEVEVGDKTHWAVGVCRDSVSRKGELTP LPETGYWRVRLWNGDKYAATTTPTPLHIKVKPKRVGIFLDYEAGTILSFYNVTD RSHI	GAAGGAACCUUGUCAUCAU SEQ ID NO: 178 UGACUUCAGUAUCCAUAGA SEQ ID NO: 179 GCUUCGAGUCCUUAAGGA SEQ ID NO: 180 AGGGUAAGGUUGCGAUUAU SEQ ID NO: 181

TRIM40	NM_138700	YTFDTFTTEKLWPLFYPIGIRAGRKNAAPLTIRPPTDWE SEQ ID NO: 177 MIPLQKDNQEEGVCPICQESLKEAVSTNCGHLFCRVCLTQHVEK ASAGVFCCPLCRKPCSEEVLTGYICPNHQKRVCFCEESRLLLCVCCLVSPHEHMSH HELT IENALSHYKERLNRRSRKLRKDIAELQRLKAQEKKLQALQQLGQLEHMPAEA ARILDISRAVTQRLSLVIDLERTAKELDTNTLKNAGDLLNRSAPQKLEVIYPQLEKGV SELLQPPQKL SEQ ID NO: 182	CCACAGAAAUAUAGAGGUUA SEQ ID NO: 183 GAGCAGACUUCUUAUGU SEQ ID NO: 184 UCAGAAGCCUGGUCAUUGA SEQ ID NO: 185 GGACGGCCAAAGGAUUAGA SEQ ID NO: 186 CAAGGAGACUUUCAUAGG SEQ ID NO: 188 CCAAUUGGUCCAGGUGAU SEQ ID NO: 189 GAGAUGAGUUAGAUCGGGA SEQ ID NO: 190 GGAUGAAGACUACGAGGGU SEQ ID NO: 191
TRIM41	NM_201627	MAAVAMTPNPVQTLQEEAVCAICLDYFTDPVSGCGHNFRCVVCV TQLWGGEDDEDRDELDRREEEDGEEEEVEAVGAGAGWDTPMRDEYEGDMEEEVEEEE EEGVFTSGMSRSSWDNMDYVWEEDEDEEDLDYLGDMEEEDLRGEDEDEEEVLEEV EEEDLDPVTPPLPPPPAPRRCTCPQCRKSFPRRSFRPNLQLANMVQVIRQMHTPGRG SRVTDQGICPKHQEALKLFCFVDEEAI CVVCRESRSHKQHSVVPLEEVVQYKAKLQG HVEPLRKHLEAVQMKAKEERRVTELKQMKSELA AVASEFGRLTRFLAEEQAGLERR LREMHEAQLGRAGAAAARLAEQAAQLSRLIAEAQERSQQGGLRLQLDIKETFNRCVEV QLQPEVWSPPCQPHSHDFLTDAI VRKMSRMFCQAARVDTLDPDTAHPALMLSPDR RGVRLAERRQEVADHPKRFSDCCVLGAQGFRRSGRHYWEEPKESPWPPAQPSLTYYVC PTDRPEFSFT SEQ ID NO: 187	GCAUACCAUCAUCACUUU SEQ ID NO: 193 CCAAUGAUCCCCAACUGUAA SEQ ID NO: 194 CAAGUUCUCUUUCCACAAU SEQ ID NO: 195 CAGAAUACGUGUUUAAAAGU SEQ ID NO: 196
TRIM42	NM_152616	METAMCVCCPCCTWQRCCPQLCSCLCKKFI FTSEFNCTCFPCPY KDERNCQFCHCTCSESPNCHWCCSWANDPNCCKCTTASSNLNICYYESRCCRNITIT FHKGRILRSIHTSSKTAIRTGSSDTQVDEVKSI PANSHLVNHLNCPMCSRLRLHSFMLP CNHSLCEKCLRLQLOKHAENVTEFFILICPVCDRSHCMPYSNKMQLPENYLHGRLLTKRY MQEHGYLKWFRDRSSGPILCQVCRNKRIAYKRCITCRNLNCLNCLKAFHSDVAMQDHV FVDTSAEEQDEKICIIHPPSSRI IEYCRNDNKLCTCFKFSFHNGHDTISLIDACSERA ASLFSIAIKFAKAVRYEIDNDLMEFNILKNSFKADKEAKRKEIRNGFLKRLRSILQEKKEK IIMEQIENLEVSROKELIEKYVYVVTMKVNMEDGLIAYSKEALKETGQVAFQLQSAKILV DQIEDGIQTTYRPDPQLRLHSINYVPLDFVELSSAIEHELFTGPKKVRSSGDSLSPPY PVHSETMIARKVTFSTHSLGNQHIYQRSSSMLSFNTDKKAKVGLFACGRAQSATPAK PTDGLTYWSAGADSQSVQNSSSFHNWYSFNDGSVKTGPIVIYQTLVYPRAKVYWT CPAEDVDSFEMEFEYEVITSPNNVQMELCGQIRDIMQONLELHNLTPNTEYVFKVRAI NDNGPGQWSDICKVVTDPDGHGKNRAKWLKLLKNIQSALQKHF SEQ ID NO: 192	CCAGAGAAGUUGGUGUCAA SEQ ID NO: 198
TRIM43	NM_138800	MSDSFSAFQKELTCVICLNLYLVDVPTICCGHSFCRPLCLSWE	

TRIM44	NM_017583	EAQSPANCPACREPSPKMDFKTNILLKNLVTIARKASLWQFLSSEKQICGTHRQTMM FCDMDKSILLCLCSNSQEHGAHKHHPIEEAAEEHREKLLKQMRILWKIKIENQRNLYE EGRTAFLWRGNVVLRAQMIIRNEYRKLHPVLHKEEKQHLERLNKEYQEIFQQLQRSWK MDQSKHLKEMYQELMEMCHKDPVELLDGLDIVARSESVLLHMPQVNPPELTAGPIT GLVYRLNRFVEISFHFVETNNIRLFEFVRSWMFRGPNLNSDRSDYFAAWGARVFSF GKHYWELDVNSCDWALGVCNNNSWIRKNSTMVNSEIDIFLLCLKVDNHNLLTTSFVF PHYIEKPLGRVGVFLDFESGVSFLNVTKSSLIWSYPAGSLTFPVRPFYTGHR SEQ ID NO: 197	GGACCAUAGGCAACAAA SEQ ID NO: 199
		MASGVGAFAFEELPHDGTCEDEPDEAPGAHEVCRECGFCYCRRH AEAHRQKFLSHHLAEYVHGSQAWTPPADGEGAGKEEAHVQEREIESEAGEESESE EESESESESESESESESESESESESESESESESESESESESESESESESESESESESE SEFDPEIEMEAEARVAKRKCDDHGLDLSTYCQEDRQLICVLCVPVIGAHQGHQLSTLDEA FEELRSKDSGGLKAAMIELVERLKFSSDPKVTDRDQMKMFIQQEFFKVKQKVIADDEEQK ALHLVDIQEAMATAHVTEILADIQSHMDRLMTQMAQAKEQLDTSNESAEFPKAEGDEEG PSGASEEEDT SEQ ID NO: 202	GAGGAAGUGUGCCGAGAAU SEQ ID NO: 203
		MSENRKPLLGVFSKLTSGTALNSGKTHCPCLCLGLFKAPRLLP LHTVCTTCELEPFVSVVDIRGGSDTSSEGSIFQELKPRSLQSIGILCPVCDQV LPMGGVKALITDHLAVNDVMELESIRGEGQGLVCDLCNDREVEKRCQTKANLCHFCQ AHRQKKTTHYTMVDLKDILKGYSGRIKPIICPVHPAFELRLFCFCDRQVQDCVVE HREHPCDFTSNVIHKGDSVWELLKGTQPHVEALEEALQIHIINSALQKRVEAVAD VRTFSEGYIKAIEEHRDKLLKQLEDIRAQKENSILQKQALEQLLADMRTGVEFTEHL LTSGSDLEILITKRVVVERLRLKLNKQYSTRPGVNDKIRFCPQEKAGQCRGYEITYGTI NTKEVDPAKCVLQGEDLHRAREKQTASTFTLLCKDAAGEIMGRGDNVQVAVVPKDKKD SPVRTMVQDNKDGTYYISYTPKEPGVYTVWVCIKEQHVQGSPTVMVRRKHRPHSGVF HCCTFCSSGGQKTARACGGTTPGGYLGCGHGHKGHPHWSGCCGKFNEKSECTWTG GQSAPRSLRLRTVAL SEQ ID NO: 207	GUCACCAUCUGGCCGAAUA SEQ ID NO: 204
TRIM45	NM_025188	MSENRKPLLGVFSKLTSGTALNSGKTHCPCLCLGLFKAPRLLP LHTVCTTCELEPFVSVVDIRGGSDTSSEGSIFQELKPRSLQSIGILCPVCDQV LPMGGVKALITDHLAVNDVMELESIRGEGQGLVCDLCNDREVEKRCQTKANLCHFCQ AHRQKKTTHYTMVDLKDILKGYSGRIKPIICPVHPAFELRLFCFCDRQVQDCVVE HREHPCDFTSNVIHKGDSVWELLKGTQPHVEALEEALQIHIINSALQKRVEAVAD VRTFSEGYIKAIEEHRDKLLKQLEDIRAQKENSILQKQALEQLLADMRTGVEFTEHL LTSGSDLEILITKRVVVERLRLKLNKQYSTRPGVNDKIRFCPQEKAGQCRGYEITYGTI NTKEVDPAKCVLQGEDLHRAREKQTASTFTLLCKDAAGEIMGRGDNVQVAVVPKDKKD SPVRTMVQDNKDGTYYISYTPKEPGVYTVWVCIKEQHVQGSPTVMVRRKHRPHSGVF HCCTFCSSGGQKTARACGGTTPGGYLGCGHGHKGHPHWSGCCGKFNEKSECTWTG GQSAPRSLRLRTVAL SEQ ID NO: 207	ACGAAGCCUUUGAAGAAU SEQ ID NO: 205
TRIM46	NM_025058	MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	GCUUGUGCUCGCCGAGUAA SEQ ID NO: 206
		MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	GCACCAUCUACAUUCCUA SEQ ID NO: 209
		MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	GUGCAGGCGCUGGCCAUUCA SEQ ID NO: 210
TRIM46	NM_025058	MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	GGGAGGAGACAAACGUUCAA SEQ ID NO: 211
		MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	UGACAUACAUCUGGGGAA SEQ ID NO: 213
		MAEGEDMQTFTSIMDALVRISTSMKNMEKELLCPVCQEMYKQPL VLPCTHNVCQACAREVLGQQGYIGHGGDPSEPTSPASTPSTRPRLSRRLPKPDRIL DRLLKSGFGTYPRKRKRGALHPQVIMFPCPACQGDVELGERGLAGLFRNLTLERVVY RQSVSVGGAILCQLCKPPPLEATKGTCTECRATFCNECFKLPHFWGTQKAQHEPTLPTL SFRPKGLMCPDHHKEEVTHYCKTCQRLVCQLCRVRRTHSGHKITPVLISAYQALKDKLTK SLTYIILGNQDVTQIQICELEEAHVHTEVSGQQAKEEVSQVRLGLGAVLEEKRASLLQA IEECQQRERLARLSAQIQEHRSLLDGSLVGYAQEVLKETDQPCFVQAAKQLHNRIARA	GGACAUACCCUGGGAGGAA SEQ ID NO: 214

TRIM47	NM_033452	TEALQTRPAASSFRHCQLDVGREMKLLTELNFLRVPEAPVIDTQRTFAYDQIFLCW RLPPHSPPAWHYTVEFRRTDVPAPQPGPTRWQRREEVGTSALENPDGTGSVYVLRVG CNKAGYGEYSEDVHLHTPPAPVLHFFLDSRWGASRERLAI SKDQRAVRSVPGLPLLLA ADRLLTGCHLSVDVVLGDVAVTQGRSYWACAVDPASVYLVKVGLESKLQESFQGAPD VISPRYDPDSGHDGAEDATVEASPPFAFLTIGMGKILLGSGASSNAGLTGRDGP TAG CTVPLPRLGICLDYERGRVSFLDAVSFRGLLECP LDCSGPVPAPFCFIGGGAVQLQE PVGTKPERKVITIGGFAKLD SEQ ID NO: 212	GC GAUAUACAGUGAAGAUGU SEQ ID NO: 215
		MDGSGPFSCPICLEPLREPVTLP CGHNFC LACLGALWPHRGASG AGGPGGAARCPLCQEPFPDGLQLRKNHTLSELLQLRQSGPGSGPGPAPALAPEPSAP SALPSVPEPSAPCAPEWPAGEEPVRC DACPEGAALPAALSCLSLASF CPAHLGPHE RSPALRGHRLVPLRLLEESLCPRHLRPLERYCRAERVCLCEACAAQEH RHGHELVPLE QERALQEAEOQSKVLSAVEDRMDDELGAGIAQSRRTVALIKSAAVAERERSRLFADAAA ALQGFQTQVLGFIEEGEAAMLGRSQD LRRQEEQRSLSRARQNLSQVPEADSVSFLQ ELLALRLALEDGCGPGPPPRELSFTKSSQAVRAVRDMLAVACVNQWEQLRPGGNEID GPQLDSEADAEQDLESTNLLSEAPRDYFLKFAYIVDLSDTDADKFLQFGTKGVK RVLCPINYPLSPTRFTHCEQVLGEGALDRGTYYWEVEIIEGWVSMGVMAEDFSPQEPY DRGRLGRNAHSCCLQWNGRSFSVWFHGLEAPLPHFPSP TVGVCL EYADRALAFYAVRD GKMSLLRRLKASRPRRGGIPASPIDPFQSR LDSHFAGLFTHRLKPAFFLESVD AHLQI GPLKKSCISVLKRR SEQ ID NO: 217	GUACGGGACGGCAAGAUGA SEQ ID NO: 218
TRIM48	NM_024114	MSRRIIVGTQRTQRMNSGISQVFORELTCTPICMNYFIDPVTI DCGHSFCRPCFYLNWQDIPILTQC FECIKTIQQRNLKTNIRLKKMASLARKASLWFL SSEEQMCGIHRET KMFCEVDRSLLCLLCSQQEHRYHRHCPAEWAEEHWEKLLKKM QSLWEKACENQRNLNVETTRISHWKAFGDILYRSESVLLHMPQPINLAIRAGPITGLR DRLNQF SEQ ID NO: 222	GAACCAAAAGGUGUCAAGAG SEQ ID NO: 219
			GCAUAUCCGUGUGAAGAG SEQ ID NO: 220
TRIM49	NM_020358	MNSGILQVFQGELICPLCMNYFIDPVTIDCGHSFCRPCFYLNWQ DIPFLVQCSECTKSTEQINLKTNIHLKKMASLARKVSLWFLSSEEQMCGTHRETKKI FCEVDRSLLCLLCSQQEHRYHRHPIEWAEEHREKLLQKMQSLWEKACENHRNLNV ETTRTRCWKDYVNLRL EAI RAEYQKMPAFHHEEKKHNL EMLKKKGKEIFHRLHLSKAK MAHRMEILRGMYEELNEMCHKDPVELLOAFGDI LHRSESVLLHMPQPINPELSAGPIT GLDRNLNQFRVHITLHHEEANN DIFLYEILRSMCI GCDHQDVYFTATPRSF LAWGVQ TFTSGKYWEVHVGD SWNWA FGVCNM YRKEKNQNEKIDGKAGLFLLCGVKNDIQCSLF TTSPLMLQYIPKPTSRVGLFLDCEAKTVSFVDVNQSSLIYTI PNCSFSPPLRPI FCCI	CAUCAAGAGUGCAGCCGUA SEQ ID NO: 221
			GCAUAAAGACAAUACAGCA SEQ ID NO: 223
TRIM49	NM_020358		CAGAGAAACCUGAAUGUGG SEQ ID NO: 224
			UGCUUGAAUGCAUAAAGA SEQ ID NO: 225
TRIM49	NM_020358		GAAGGCUUUGGAGACAUA SEQ ID NO: 226
			GAAGAAGCCCAACAUAUGAU SEQ ID NO: 228
TRIM49	NM_020358		GGAAGGAUUAUGUGAAUUU SEQ ID NO: 229
			GAACGAAAUUGGCCAUA AAA SEQ ID NO: 230
TRIM49	NM_020358		GAUCAGAAUGAGAAGAU SEQ ID NO: 231

TRIM50	NM_178125	HF SEQ ID NO: 227 MAWQVSLPELEDRLQCPICILEVFKEPLMLQCGHSHYCKGCLVLSLS CHLDAELRCVPVCRQAVDGSSSLPNVSLARVIEALRLPGDPEPKVCVHHNPLSLFCEK DQELICGLCGLLGSHQHPVTPVSTVSRMKEELAALISELKQEQKKVDELIAKLNN RTRIVNESDVFSWVIRREFQELHHLVDEEKARCLEGIGGTRGLVASLDMQLEQAQGT RERLAQAEVLEQFGNEDHHKFIKRFHSMASRAEMPQARPLEGAFSPISFKPGLHQAD IKLTWVKRLFRKVLPAPEPLKLDPAHAHPLLELSKGNVTVQCGLLAQRASQPERFDY STCVIASRGFCGRHYWEVVVGSKSDWRLGVIKGTASRKGLNRSPEHGVWLI GLKEG RVYEAFACPRVPLPVAGHPHRI GLYLHYEQGELTFFDADRPDDLRLPLYTFQADFQGKL YPILDTCHWHERGSNSLPMVLP PPSGPGPLSPEQPTKL SEQ ID NO: 232 MNSGILQVFQRALTCPICMNYFLDPVTIDCGHSFCRCPCLYLNWQ DTAVLAQCSECKKTTRQRNLTNDICLKNMAFIARKASLRQFLSSEEQICGMHRETKM FCEVDKSLCLPCSNSQEHNRNHIHCPIEWAAEERREELKKMQSLWEKACENLRNLNM ETTRTRCWKDYVSLRIEAI RAEYQKMPAFLHEEEQHHLERLRKEGEDI FQQLNESKAR MEHSRELLRGMIEDLKQCHKADVELLQAFGDI LHYESLLLQVSEPVNPELSAGPIT GLLDSLSGFRVDFTLQPERANSHIFLCGLRSMNVGCDPDDPDITGKSECFVLVWGAQ AFTSGKYYWEVHMGD SWNWA FGVCNNYWKERQNDKIDGEEGLFLLGCVKEDTHCSLF TTSPLVVQYVPRPTSTVGLFLDCEGRTVSFVDVDQSSLIYTI PNCSFSPPLRPIFCCS HF SEQ ID NO: 237 MAGYATTPSPMQTLQEEAVCAICLDYFKDPVSI SCGHNFRCGV TQLWSKEDEEDQNEEEDEWEDEEEA VGAMDGWDGSI REVL YRGNAD EELFQDDDDDD ELWLGDSGITNWDNVDMWDEEEEDQDY YLGGLRPDLRIDVYREEEILEAYDED EDEELYPDIHP PPSLPLPGQFTCPQCRKSFTRRSFRPNLQLANMVQIIRQMCPTPYRG NRSNDQGMCFKHQEA LKLFCEVDKEAICVWCRESRSHKHQHSVLPLEEVVQYEQEIKLE TTLVGILQIEQESIHSKAYNQ SEQ ID NO: 242 MNFTVGFKPLLGDASHMDNLEKQLICPICLEMFSKPVVILPCQH NLCKRCANDVFQASNPLWQSRGSTTVSSGGRFCRPSCRHEVLD RHGVYGLQRNLLVE NIIDIYKQESSRPLHSAEQHLMCEEHEEEKINIYCLSCVPTCSLCKVFGAHKDCV APLPTIYKRQKSELSDGIAMLVAGNDRVQAVITQMEEVQCITIEDNSRRQKQLLNQRF SLCAVLEERKGELLQALAREQEEKLQVRGLIRQYGDHLEASSKLVESAIQSMEEPQM ALYLQQA KELINKVGAMSKVELAGRPEPGYESMEQFTVRVEHVAEMLRTIDFQPGASG EEEEVAPDGEESAGPEEERPDGP	SEQ ID NO: 231 GGACCCGAUUCGUAUAUGA SEQ ID NO: 233 GGCUCUACCGUGCACUAUGA SEQ ID NO: 234 GCAACUCGCGUGCCCAUGGU SEQ ID NO: 235 UGCAGCCCUCAUCUCUGA SEQ ID NO: 236 GGAAGGAUUAUGUGAGUUU SEQ ID NO: 238 ACUUGGAAAGGCUGCGAAA SEQ ID NO: 239 AAGCAGAUGUGGAGCUACU SEQ ID NO: 240 GGACAGCCUCAGUGGAUUC SEQ ID NO: 241 GACCUGACCUGAGAAUUGA SEQ ID NO: 243 UGACCCAGCUGUGGAGUAA SEQ ID NO: 244 GGGACAACGUAGACUAUUA SEQ ID NO: 245 ACGAAGAGUUGUCCCAAGA SEQ ID NO: 246 GAGGAGGUGUGCCAGACUA SEQ ID NO: 248 GAACAUUAUCGACAUUUAUAC SEQ ID NO: 249 UCUACGGCCUGCAGCGAAA SEQ ID NO: 250 CAUAAAGAACUCGAGCGU
TRIM51	NM_032681		
TRIM52	NM_032765		
TRIM54	NM_187841		

TRIM55	NM_184087	SEQ ID NO: 247 MSASLNYSKFSKEQQTMDNLEKQLICPICLEMFTKPVILPCQH NLCKKASDIFQASNPYLPTRGTTMASGGRFCPSCRHEVLDHRGVYGLQRNLLVE NIIDIYKQESTRPEKKSQQPMCEEEHEERINICYLNCEVPTCSLCKVFGAHKDCQVAP LTHVFQRKSELSDGIAIILVGSNDRVQGVISQLEDCTCKTIEIGFEAPPLQGQAAAPAS GSGADSEPARHIFSFWSLNSLNE SEQ ID NO: 252	SEQ ID NO: 251 GCGCAUCUCUGAAUUAACA SEQ ID NO: 253 GAAUGUGCCAGUGAUUU SEQ ID NO: 254 GGUUGAACUCCCUAAAUGA SEQ ID NO: 255 CAUGAAGAGGAGCGCAUCA SEQ ID NO: 256 GGACUGGCCGAGACUUG SEQ ID NO: 258 GGUGUGGCCCUUCAAGA SEQ ID NO: 259 GCGGAGGCCUGGAGACAAG SEQ ID NO: 260 GAUAGAAGGGGCUACAUCU SEQ ID NO: 261
TRIM56	NM_030961	MVSHGSSPSLLEALSSDFFLACKICLEQLRAPKTLPCLIHTYCQDC LAQLADGGRRVRCPECRETVPVPPPEGVASFKNFFVNGLLDLVKARACGDLRAGKPACA LCPLVGGTSTGGPATARCLDCADDLCQACADGHRCTRQTHTRVVDLVGYRAGWYDEE ARERQAAQCQHPGEALRFLCQPCSQLLCRECRLDPLHDHPCPLPLAEAVRARRPPGLEG LLAGVDNNLVELEAARRVEKEALARLREQAARVGTQVEEAAGVLRALLAQKQEVILGQ LRAHVEAAEEFAARERLAEELEGREQVARAAAAFARRVLSLGREAFILSLEGAIAQRLRQ LQGCWPAPGPAPCLLPQLELHPGLLDKNCHLLRLSFEEOQQPKDGGKDGAGTQGGES QSRREDEPKTERQGGVQPOAGDGAQTPKEEKAQTTREEGAQTLEEDRAQTPHEDGGPQ PHRGGPNKKKKFKGRLLKSI SREPSALGNLDGSLLPRI FYCSPTRMPGDKRSP RITGLCPFGPREILVADEQNRAKRFSLNGDYKGTVPVPEGCSPCSVAALQSAVAFSA SARLYLINPNGEVQWRRALSLSQASHAVAALPSGDRVAVSVAGHVEVYNMEGSLATRF IPGKASRGLRALVFLTTSPQGHFVGSQNSVVICDGLGQVVGVEYKGPGLHGCQPG SVSVDKKGYIFLTREVNKVVILDPKGSLLIGDFLTAYHGLEKPRVTMTVDGRYLVVSL SNGTIHIFRVRSPDS SEQ ID NO: 257	SEQ ID NO: 262 GAAAGUCCUGCGCAUUG SEQ ID NO: 263 CUAUGAAGCCGGUGAAAUU SEQ ID NO: 264 GAUUGGAGUUUGAGAGCA SEQ ID NO: 265 GGAAGAGUUUGGAGGACGC SEQ ID NO: 266
TRIM58	NM_015431	MAWAPGERLREDARCPVCLDFLQEPVSVDCGHSFCLRCISEFC EKSDGAQGGVYACPCQCRGPFPRPSGFRPNRQLAGLIVESVRRLLGLGAGPGARRCARHGED LSRFCEEDEAALCWVCDAGPEHRTHTAPLQEAAGSYQVKLQMALELMRKELEDALTQ EANVGKKTIVWKEKVMQQRFRLEFEKHRGFLAQEEQRLRLEAEERATLQRLRES KSRLVQOSKALKELADELQERCQRPALGLLEGVRGVLRSKAVTRLEAFENIPMELKTA CCIPGRRELLRKQVDVKLDPATAPAHPSLLLTADLRSVQDGEPPWRDVPNNPERFDTWPC ILGLQSFSSGRHYWEVLVGEAGWGLGVCQDTLPKGETTPSPENGVWALWLLKNEY MVLASPSVPLLIQLESPRCIGIFLDYEAGEISFYNVTDGSIYITFNQLFSGLLRPYFFI CDATPLILPPTTIAGSGNWARSDHIDPASDVRDDHL SEQ ID NO: 262	GUACAGAUUUUGAAACAAA SEQ ID NO: 268
TRIM59	NM_173084	MHNFEELTCPICYISIFEDPRVLPCSHSTFCRNCLENILQASGNF YIWRPLRIPLKPCNCRSITEIAPTGIESLPVNFALRAI IEKYQQEDHPDIVTCPEHYR	

		QPLNVYCLLDKKLVCGHCLTIGQHHGHPIDDLQSAYLKEKDTTPQKLLLEQLTDTHWTDL THLIEKLKEQKSHSEKMIQGDKEAVLQYFKELNDTLEQKKKSLTALCDVGNLINQEY TPQIERMKEIREQOLELMALTISLQEEPLKFLKVDVDRQHVLKQRPPLPEVQPV IYPRVSKILKEEWSRTEIGQIKNVLPKWKISPKRMSCSWPGKDEKEVEFLKILNIV VTLSVILMSILFFNQHIITFLSEITLIWFSEASLSVYQSLNSLHKVKNILCHIFYL LKEFVWKIVSH SEQ ID NO: 267	CAACUGGCAUUGAAUCUUU SEQ ID NO: 269 GCACUAGGGCUAUUAUUG SEQ ID NO: 270 GAUGUUGGCAUUAUUA SEQ ID NO: 271
TRIM60	NM_152620	MEFVTAIVNLQEESSCPICLEYLKDPTVINCNGHNFCSRCLSVSW KDLDDTFFPCPVCRFCFPYKSFRRNPQLRNLTEIAKQLQIRRSKRKRQKENAMCEKHQ FLTLFCVKDLEILCTQCSFSTKHQHYICPIKKAASYHREILEGSLEPLRNNIERVEK VILQGSVELKKVEYKREEINSEFEQIRLFQNEQEMILRQIQDEEMNILAKLINE NLVELSDYVSTLKHLLREVEGKSVQSNLELLTQAKSMHHKYQNLKPELFSRLTKYG FSLPPQYSGLDRIIKPFQVDVILDNTAHPQLLVSEDRKAVRYERKKRNICYDPRRFY VCPAVLGSQRFSSGRHYWEVEVGNKPKWILGVCQDCLLRNWQDQPSVLGGFWAIGRYM KSGYVASGPKTTQLLPVVKPSKIGIFLDYELGDLSFYNMNDRSILYTFNDCFTTEAVWP YFYTGTDSEPLKICSVSDSER SEQ ID NO: 272	GAAAGAGAAUGCCAUUGUGU SEQ ID NO: 273 GGAUCUAGAUGAUACCUUU SEQ ID NO: 274 GGUCUAUUCUCUAUACUUU SEQ ID NO: 275 GCAUUGGGCGAUACAUGA SEQ ID NO: 276
TRIM61	NM_001012414	MEFVTAIALDLRAEASCPICLDYLDKDPVTISCGHNFCLSCIIMSW KDLHDSFPFCFCHFCPCPERKFI SNPQLGSLTEIAKQLQIRSKRRKRQEEKHVCKKHQ VLTFQCQKDLLELLCPRCSLSTDHQQHCVWP IKKAASYHRKKLEEFYNAPWKERVLEIEK VITMQTRKSLELKKMESPSVTRLECSTISAHFNRLRPGSSDSSASGS SEQ ID NO: 277	UCAGAAAGACCUAGAGCUU SEQ ID NO: 278 CUGGUAGUUUGACUGAAA SEQ ID NO: 279 UAGAAGCAUAAUACAGGUU SEQ ID NO: 280 GAGAGUGGGAACUAAUUG SEQ ID NO: 281 CUACAUGCUGAUGACAUG SEQ ID NO: 283 UCGGACGACUGCACC AUUG SEQ ID NO: 284 CGCCAAAGCGCUUCCGAUGU SEQ ID NO: 285 GGAUCAACACCGUCCGCAU SEQ ID NO: 286
TRIM62	NM_018207	MACSLKDELLCSICLSIYQDPVSLGCEHYFCRRCTEHWVRQEA QGARDCECRRRTFAEPALAPSLKLANIVERYSSFPDLAILNARRAARPCQAHDKVKLIF CLTDRAILLCFDEPALHEQHQTGIDDAFDELQRELKQALQDSEHEATEALQLL KRQLAETKSSTKSLRTTIGEAFAERLHRLRERQKAMLEELEADTARTLTDIEQKVQRY SQQLRKVQEGAQILQERLAETDRHTFLAGVASLSERLKGKIHEITNITYEDFPSTKYTG PLQYTIWKSFLQDIHPVPAALTDPGTAHQRLIISDDCTIVAYGNLHPQLQDSPKRF DVEVSVLGSEAFSSGVHYWEVVAEKTQWVIGLAHEAASRKSGIQIPSRGFYCI VMH DGNQYSACTEPWTRLNVRDKLDKVGFLDYDQGLLI FYNADMSWLYTFREKFPGLKIC SYFSPGQSHANGKNVQPLRINTVRI SEQ ID NO: 282	CUACAUGCUGAUGACAUG SEQ ID NO: 283 UCGGACGACUGCACC AUUG SEQ ID NO: 284 CGCCAAAGCGCUUCCGAUGU SEQ ID NO: 285 GGAUCAACACCGUCCGCAU SEQ ID NO: 286
TRIM63	NM_032588	MDYKSSLIQDGNPMENLEKQLICPICLEMTKPVVILPCQHNL RKCANDIFQAANPYWTSRGSSVSMGGRFCRPTCRHEVIMDRHGVGLQRNLLVENII	GGAAGAAGGACACCAGUAA SEQ ID NO: 288

TRIM64	NM_001136486	DIYKQECSSRPLQKGSHPMCKEHEDEKINITYCLTCEVPTCSMCKVFGIHKACEVAPLQ SVFQGGKTELNNCISMLVAGNDRVQTITQLEDSRRVTKENS HQVKEELS QKFDTLYA ILDEKKS ELLQRITQE QEKKLSFIEALIQYQEQLDKSTKLIVETAIQSLDEPGGATFL LTAKQLIKSIVEASKGCGKTEQGFENMDFFTLDEHIA DALRAIDFGTDEEEEFII EEEDQEEEEESTEGKEEGHQ SEQ ID NO: 287 MSDDDLQVFQNELICICVNYFIDPVTIDCGHSFCRPLCLCLCSE EGRAPMRCPSCKRISEKPNFTNWLKLLSSLARQTRPQNINSSDNICVLHEETKELF CEADKRLLCGPCSESEPEHMAHSHSPIGWAAEECREKLIKEMDYLWEINQETRNINQOE TRTFHSLKDYVSVRKRIITIQYQKMPIFLDEEEQRHLQALEREAEELFQQLQDSQVRM TQHLERMKDMYRELWETCHVPDVELLDVNRVSARTDLAQMKPQPVNPELTSWCITG VLDMLNFRVDSALSTEMIPCYISLSEDRVYVIFGDDHLSAPTDPPQGVDSFAVWGAQA FTSGKHYWEVDVTLSSNWILGVCQDSRTADANFVIDSDERFFLISSKRSNHYSLSSTNS PPLIQYVQRPLGQGVFLDYDNGSVSFFDVSKGSLIYGFPSPSSPLRPFPCFGCT SEQ ID NO: 292 MAAQLLEKLTCAICGLYQDPVTLPCGHNF CGACIRDWDRCG KACPECREFPDGAELRNVALSGVLEVVRAGPARDPGDPGPDPAARCPRHGRPL ELFCRTEGRCVSVCTVRECLHERALLDAERLKREQLRASLEVTQQATQAEQQLL ELRKQSSQIONSACILASWVSGKFSSLLQALEIQHTTALRSIEVAKTQALAAQARDEEQ RLRVHLEAVARHGCRIRELLEQVDEQTFLOESQLLPFPGLPLTFLQWDEDQQLGDL KQLLSRLCGLLLEEGSHPGAPAKPVDLAPVEAPGLAPVSTVCPLRRKLWQNYRNLIT FDPVSANRHLYLSRQDQVKHCRQSRGPGPGSFELMQVQCAQS FQAGHHYWEVRASD HSTLGVSYPLPRCLRGPHTDNIGRGPCSWGLCVQEDSLQAWHNGEAQRLPGVSGRL LGMDLDLASGCLTFYSLEPQTQPLTYTFHALFNQPLTPVFWLLEGRITLTLCHQPGAVFP LGPQEEVLS SEQ ID NO: 297	UCACUCAGCUGGAGGAUUC SEQ ID NO: 289 GAACAUGGACUUCUUUACU SEQ ID NO: 290 GGAAUCCCAUGGAGAACUU SEQ ID NO: 291 UCAGUAAGGAAGAGGAUAA SEQ ID NO: 293 UGUGGUUAUGAAUGGGAUA SEQ ID NO: 294 AAAGUUGGUUUCACGAUGA SEQ ID NO: 295 AAGCAUUCACCUCGCGCAA SEQ ID NO: 296 GCAGCCAGAUCCAGAACUC SEQ ID NO: 298 AGCCAAGCCUGUGGACUUA SEQ ID NO: 299 GUAGGACCCUGACCCUGUG SEQ ID NO: 300 UGGCAGAAUUAUCGCAUUC SEQ ID NO: 301
TRIM66	NM_014818	MARNCECKEKRAAHILCTYCNRWLCSCTEEHRHSPVPGGPF PRAQKSGPVNGPGDFTLYCPLHTQEV LKFCETCDMLTCHSCLVVEHKEHRCRHE EVLQNRMLLEGVTTQVAHKKSSLQTS AKIEDRIFEVKHQHKKVENQIKMAKVM LN ELNKQANGLIELEGITNERKRKLEQQLQSIMVLNRQFEHVQNFINWAVCSKTSVPFL FSKELIVFQMQRLLLETSCNTDPGSPWSIRFTWEPNFWTKQLASLGCTTTEGGQMSRAD APAYGGLQGSPPFYQSHQSPVAQQEALSHPSHKFQSPAVCSSSVCCSCHSPVSPSLKG QVPPPSIHPAHSFRQPPMVPQQGLSLQCSALLPREKELACSPHPKLLQPWLETQPP	CGGCAUUUAUACCAGAUUA SEQ ID NO: 303

		VEQESTSQRGLGQQLTSQPVCIIVPPQDVQQAHAQPTLQTPSIQVQFGHHQKLKLSHFQ QQPQQQLPPPPPLPHPPPPPLPPPPQPPHPLPPSQHLASSQHESPPGPACSQNMMDIM HHKFELEEMQKDELELLQAQPSLQLSQTKSPQHLQOTIVGQINIVRQAPVQSQSQ EETLQATDEPPASQSGKPALPLDKNTAAALPQASGEETPLSPVPDSTIQHSSPNVVR KHSTSLIMGFSNTLEMELSSTRLERPLEPQIQSVNLTAGAPQAVPSLLSAPPKMVS SLTSVQNAQMPSLTTSHLQTVPSLVHSTFQSMENLLSDSPQAMASLASDHPQAGPSLM SGHTQAVPSLATCPLQSIPIPVSDMQPETGSSSSSGRTSGSLCPRDGADPSLENALCKV KLEEPINLSVKKPPLAPVWSTSTALQYQNPKECENFEQGALELDAKENQSIKRAFNSE HKIPYVRLERLKI CAASSGEMPVFKLPQKNDQDGSFLIIIECGTESSSMSIKVSQDR LSEATQAPGLEGRKVTVTSLAGQRPPEVEGTSPPEHRLIPRTPGAQKGPAP IENEDF CAVCLNGGELLCCDRCPKVFHLSCHVPALLSPFGGEWVCTLCRSLTQPEMEYDCENAC YNQPGMRAS PGLSMYDQKKCEKLVLSLCCNNLSLFPHEPVSPLARHYQIIKRPMDLS IIRKLIQKKDPAHYTTPEEVVSDVRLMFWNCAKFNYPDSEVAEAGRCLEVFEGWLKE IYPEKRFAQPRQEDSDSEEVSSSESGCSTPQGFPPPYMQEGIQPKRRRRHMEMENERAKR MSFRLANSISQV SEQ ID NO: 302	GCACAGAGGAACACCGACA SEQ ID NO: 304 CCUUCAAUAGUGAGCAUAA SEQ ID NO: 305 UGUUUCAGAUAGCAGCGAUU SEQ ID NO: 306
TRIM67	NM_001004342	MEELKCPVCGSLFREPIILPCSHNVCLPCARTIAVQTPDGEQH LPQPLLLSRGSLQAGAAAAAASLEHDAAGPACGGAGGSAAGLGGAGGGGDHADKL SLYSETDSGYGYSTPSLKSPNGVRVLPMPVAPP GSSAAAAARGAACSSLSSSSSITCP QCHRSASLDHRGLRGFORNRLLEAIVQRYQQGRGAVPGTSAANAIAICQLCDRTTPEP AATLCEQCDVLYCSACQLKCHPSRGPFAKHLVQPPPPPPPAEAAAGPTGTAGGAPS GGGCKSPGGAGAGATGGSTARKFTCTPEHEMENYSMYCVSCRTPVCYLCLEEGRHAK HEVKPLGAMWKQHAQLSQALNGVSDKAKEAFLVQLKNILQOIQENGLDYEACLV QCDALVDALTRQAKALLTKVTKEHKLKMWWDQINHCITLKRQSTGLMEYCLEVIKE NDPSGFLQISDALIKRVQVSQEQWVKGALEPKVSAEFDLTLDSEPLIQAIIHQLDFIQM KCRVPPVPLLQLEKCCTRNNSVTIAWRMPPTTHSPVDGYILLEDDGAGGQFREVVYVK ETLCTIDGLHFNSTYNARVKAFNSSGVGPYKTVLQTSVAVWFTFDPNSGHRDIILS NDNQATATCSSYDDRVVLGTAAFSKGVHYWELHVDYDNHDPDPAGVARASVVKDMMLG KDDKAWAMYVDNNSRWFHMCNSHTNRTEGGVCKGATVGVLDDLKNKHTLTFFINGQQQG PTAFSHVDGVFMPALSLNRNVQVTLHTGLEVPTNLGRP KLSGN SEQ ID NO: 307	GGUAAGGAGACUUUGUGUA SEQ ID NO: 308 GCACAUUGAAGCUGCGUCA SEQ ID NO: 309 GAAGUGUCUGCGGAGUUU SEQ ID NO: 310 GAGAAUUGCUGCACCCGUA SEQ ID NO: 311
TRIM68	NM_018073	MDPTALVEAIVEEVACPICTFLREPMSIDCGHSFCHSCLSGLW EIPGESQNWGYTCPLCRAPVQPNLRLPNWQLANVEKVRLLRLHPGMGLKGDLCERHG EKLKMFCKEDVLMCEACSQSPEHAHSVPMEDVAWEYKWELEHLEHLLKKEFEAW KLEVGERKRTATWKIQVETRKQSIWFEFEKYQRLLEKKQPPHRLQGAEEVAAALASLQR EAAETMQKLELNHSELIQQSQVLWRMIAELKERSQRPVRWMLQDIQEVNRSKWSLQ QPEPISLELKTDCRVLGLREILKTYAADVRLDPDTAYSRLIVSEDRKRVHYGDTNQKL PDNPERFYRYNIVLGSQCISSSGRHYWEVEVGDRSEWGLGVCKQNVDRKEVVYLSPHYG	GAGAGAUCCUGAAGACUUA SEQ ID NO: 313 CAAGGAACCUUGCGGCCUAA SEQ ID NO: 314 GGGAAAAGCUGAAGAUGUU SEQ ID NO: 315

TRIM69	NM_182985* *variant a	FWVIRLRKNEYRAGTDEYPILSLPVPPRRVGI FVDYEAHDISFYNVTDGSHIFTFP RYPFPGRLLPYFSPCYSIGTNNNTAPLAICSLDGED SEQ ID NO: 312	GGAGGAUGAUUGCAGAGUU SEQ ID NO: 316
		MEVSTNPSSNIDPGDYVEMNDSITHLPKVVIIQDITMELHCLPLC NDWFRDPLMLSCGHNFCEACIQDFWRLQAKETFCPECKMLCQYNNCTFNPVLDKLVEK IKKLPLLLKGHPQCEPHGENIKLFSKPDGKLI CFQCKDARLSVQSKEFLQISDAVHFF TEELAIQQGQLETTIKELQTLRNMQKEAIAAHKENKLHLQOHVSMEFLKLHQLHLSKE KDILTELREEGKALNEEMELNLSQLQECLLAKDMLVSIQAKTEQQNSFDLKDITTL LHSLAQGMKVLATRELISRKLNLGQYKGP IQYMWVREMQDTLCPGLSPLTLDPKTAHP NLVLSKQTSVWHGDIKKIMPDDPERFDSVAVLGSRGFTSGKWYWEVEVAKTKWTV GVVRESIIRKGSCLPTPEQGFWLLRLRNQTDLKALDLPFSFLTTLNNLDKVGIIYLDYE GGQLSFYNAKTMTHIYTFSTNFMELIYPYFCPLNDGGGENKEPLHILHPQ SEQ ID NO: 317	GGAGGAGGUAGAGCGCUA SEQ ID NO: 319
TRIM71	NM_001039111	MASFPTDFQICLLCKEMCGSPAPLSSNSSASSSSQTSTSSGG GGGPGAAARRLHVLPCLHAFCRPCLEAHRLLPAAGGGAAGEPLKLRCPVCDQKVVLA AAGMDALPSSAFLLSNLLDAVVATADEPPPKNGRAGAPAGAGGHSNHRHHAHPRA SASAPPLPQAPQPPAPSPRSAPGPAASPSAILLRPHGSCSSDEGNAASSRCLDCQEH LCDNCVRAHQVRILT KDHYIERGPPGAAAAQQLGLGPPFPFPFISVFPRLG FCQHHDDDEVILHLYCDTCSVPICRECTMGRHGGHSFIYLOEALQDSRALTIQLLADAQQ GRQAIQLSIEQAQTVAEQVEMKAKVVQSEVKAVTARHKKALEERECELLWKVEKIRQV KAKSLYLOVEKLRQNLNKLLESTISAVQQVLEEGRALDILLARDRLAQVQELKTVRSL LQPDQEDRVMTTPDQALYLAIKSFGFVSSGAFAPLTATGDGLKRALQKGVASFTVI GYDHDGEPRLSGGDLMSAVVLGPDGNLFGAEVSDQNGTYVVSYPQLGEHLVSVTL CNQHIENSFPFKVVVKSGRSYVIGLPLGLSFGSESDGKLCRPWGVSDKEGYIIIVAD RSNNRIQVFKPCGAFHKKFGTLGSRPQGFDRPAGVACDASRRIVVADKDNHRIQIFTF EGQFLKFKGEKGTNGQFNYPWDVAVNSEGKILVSDTRNHRIQLFGPDGVFLNKYGF GALWKHFDSPRGVAFNHEGLVVTDFNNHRLVVIHPDCQASARFLGSEGTGNGQFLRPQ GVAVDQEGRIIVADSRNHRVQMFESNGSFLCKFGAQSGFGQMDRPSGIAITPDGMIV VVDFGNRLILVF SEQ ID NO: 318	AGAAAGUAGUGCUAGCCGA SEQ ID NO: 320
			CUUGGGAUGUGGCGGUGAA SEQ ID NO: 321
			CACCAAGGCCACAGCGCAU SEQ ID NO: 322
TRIM72	NM_001008274	MSAAPGLLHQELSCPLCLQLFDAPVTAECGHSFCRACIGRVAGE PAADGTVLCPCCAQAPTRPQALSTNLQARLVEGLAQVPQGHCEEHLDPLSIYCEQDRA LVCGVCASLGSRHRLLLPAAEAHARLKTQLPQKQLQOEACMRKEKSVAVLEHQLVE VEETVRQFRGAVGEOLGKMRVFLAALLEGSLDREAERVGEAGVALRRELGSLSYLEQ LRQMEKVLEEVADKPQTEFLMKYCLVTSRLQKILAESPPPARLDIQLPIISDDFKFQV WRKMFRLMPALEELTFDPSSAHPSLVSSSGRRVECEKQKAPPAGEDPRQFDKAVAV	CCACGCGCAUUGGCCUUUA SEQ ID NO: 324
			UCUCCGAGGCGGAGCACUA SEQ ID NO: 325
			GACAUCCAGCUGCCAAUUA SEQ ID NO: 326

TRIM73	NM_198924	VAHQQLSEGEHYWEVDVGDKPRWALGVIAAEAPRRGRHLHAVPSQGLWLLGLREGKILF AHVEAKEPRALRSPERRPTRIGLYLSFGDGLVSFYDASDADALVPLFAFHERLPRPVY PFFDVCWHDKGNAQPLLLVGPGEAFA SEQ ID NO: 323 MAWQVSLLELEDRLQCPICLEVFKESLMLQCGHSYCKGCLVLSLS YHLDTKVRCPMCQVVDGSSSLPNVSLAWVIEALRLPGDPEPKVCVHHRNPLSLFCEK DQELICGLCGLLGSHQHHPVTPVSTVCSRMKEELAAALFSELKQEQKKVDELLIAKLVKR RTRIVNESDVFSWVIRREFQELRHPVDEEKARCLEGGHTRGLVASLDMQLEQAQGT RERLAQAECVLEQFGNEDHHEFIWKFFHSMASR SEQ ID NO: 328	CGACAAAGCCGCAGACUGA SEQ ID NO: 327 GGACCCGAAUCGUCAAUGA SEQ ID NO: 329 CAAGGAGUCCCUAAUGCUA SEQ ID NO: 330 UCGCAGCCCUUCUCUCUGA SEQ ID NO: 331 AGUGUGUGCUGGAACAGUU SEQ ID NO: 332 GAAUGAGGACCACCAUGA SEQ ID NO: 334 GGACCCGAAUCGUCAAUGA SEQ ID NO: 335 CAAGGAGUCCCUAAUGCUA SEQ ID NO: 336 UCGCAGCCCUUCUCUCUGA SEQ ID NO: 337
TRIM74	NM_198853	MAWQVSLLELEDRLQCPICLEVFKESLMLQCGHSYCKGCLVLSLS YHLDTKVRCPMCQVVDGSSSLPNVSLAWVIEALRLPGDPEPKVCVHHRNPLSLFCEK DQELICGLCGLLGSHQHHPVTPVSTVCSRMKEELAAALFSELKQEQKKVDELLIAKLVKR RTRIVNESDVFSWVIRREFQELRHPVDEEKARCLEGGHTRGLVASLDMQLEQAQGT RERLAQAECVLEQFGNEDHHEFIWKFFHSMASR SEQ ID NO: 333	
TRIM75	A6NK02	MAVAAALTGLQAEAKCSICLDYLSDPVTIECGHNFCRSCIQQSWLDLQEL FPCPVCRHQCEGHFRSNTQLGRMIEIAKLLOSTKSNKRKQEEETTLCEKH NQPLSVFCKEDLMVLCPLCTQPPDHQGHVVRPIEKAAIHYYKRFCSYIQP LKKQLADLQKLIISTQSKKPLEIREMVENQRQELSSFEFHINQFLDREQQA VLSRLAEEKDNQKLSANITAFSNYSATLKSQLSKVVELSELSELELLS QIKIFYESENESSPSIFSIHLKRDGCSFPPQYSALQRIIKKFKVEIILD ETAHPNLIIVSEDKKRVFTTKRKQKVPFGPKRFTVKPVLGFPYFHSGRHF WEIEVGDKSEWAGIGICKDSLPTKARRPSSAQCECWRIELQDDGYHAPGAF PTPLLELVKARAIGIFLDYEMGEISFYNNMAEKSHICTFTDTFTGTLRPFYF YVGPDSPRLRICTGTVCE SEQ ID NO: 338	

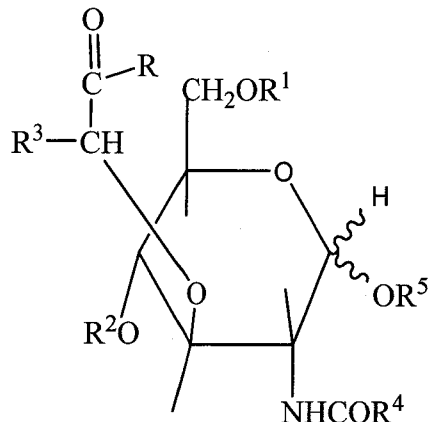
TRIM76	NM_153610	MASRDSNHAGESFLGSDGDEEATRELETEEESEGEDEETAASE EEPDSRLSDQDEEGKIKQEIYIISDPFSMTVQREDSGITWETNSSRSTPWAEEESQ TSGVCSREGSTVNSPPGNVSFIVDEVKKVRKRTHSKHGSPSLRRKGNRKRNSFESQD VPTNKKGSPLTSASQVLTTEKEKSYTGIDYKARKKKTTSNTPTTGAITYKEHKPLVLR PVYIGTVQYKIKMFNSVKEELIPLQFYGTLPKGYVKEIHYRKGKDASISLEPDLDNS GSNTVSKTRKLVQAQSIEDKVKEVFPWRGALSKGSESLTLMFSHEDQKKIYADSPINA TSALEHTVPSYSSSGRAEQIQLRHSQVPQPEDEAKPHEVEPPSVTPDTPATMFLR TTKEECELASPGTAASENDSSVSPSFANEVKEDVYSAHHSISLEAASPLAASTQDG LDPDQEQPDLTSIERAEPVSAKLTPTHPSVKEKEENMLEPSISLSEPLMLEEPEKEE IETSLPIAITPEPEDSNLVEEEIIVELDYPEPLVSEKFPFPHMSPEVEHKEEELILPL LAASSPEHVALSEEEEREIEASVSTGSAFVSEYSPQDLNHELQEQEGEPVPPSNVEAI AEHAVLSEEEENEFEAYSPAAAPTSESSLSPSTTEKTSSENQSPFLSTVTPEYMWLSGD EASESGCYTPDSTSAEYSPSLATKESLKKTIIDRKSPILILKGVSEYMIIPSEEKEDTG SFTPAVAPASEPSLSPSTTEKTSQSPPLPSTATSEHVVPSEGEDLGSEFTPDCLKI SKYAAPLNATQESQKKIINEASQFKPKGISEHTVLSVDGKEVIGPSSPDLVVASEHSF PPHTTENTSECQAPPLSATPSEYVWLDEEAVALERYTPSSTSAEFSFVPPYATPEAQ EEEIVHRSNLKGAASSPMNLSEEDQEDIGFPSPDSAFVSEFSFPPYATQEAKEKREFC DSPICLITSPSEHTILSDEDETEEAELFSPDSASQVSIPPFRISSETEKNELEPDSLITAV SAGYSCFSEADEEDIGSTAATPVSEQFSSQKQKAETFFLMSPLEDLSLPPSTDKSE KAEIKPEIPTTSTSVSEYLIILAQKQKQAYLEPESEDLIPSHLTSEVEKGEREASSV AAIPALPAQSSIVKEETKPASPHSVLPDSVPAIKKEQEPATAALTILKAADEQMALSKV RKEEIVPDSQEAATAHVSDQKMEPQPPNPVPESEMKYSVLPDMVDEPKKGVKPKLVNLV TSELEQRKLSKNEPEVIKPYSPLETSLSGPEALSVMEMKHDHSKITTTPIVLHSAS SGVEKQVEHGPPALAFSALSEEIKKEIEPSSSTTTASVTKLDSNLTTRAVKEEIPDSS LITPVDRPVLTKVKGELGSLPPLVTSADHSVLAEEDEKVAIKGASPIETSSKHLAW SEAEKEIKFDSLPSVSSIAEHSVLEVEAKEVKAGLPVICTSSQHSDDKSEEARVEDK QDLLFSTVCDSERLVSSQKKSIMSTSEVLEPEHELPLSLWGEIKKKETELPSSQNVSP ASKHII PKGKDEETASSPELENIASGLAPTLLILSDDKNKPAVEVSSTAQDFFPSEK QDVALAELSLPEKKDKPHQPLELPNAGSEFSSDLGRQSGSIGTKQAKSPI TETEDSV LEKGPaelRSREGKEENREL CASSTMPAISELSSLLREESQNEEIKFPSPKII SLESK EPPASVAEGGNPEEFQPTFSLKGLSEEVSHPADFKKGGNQEIGPLPPTGNLKAQVMG DILDKLSEETGHPNSSQVLSITEPSKIAPSDLLVEQKKTEKALHSDQTVKLPDVSTS SEDKQDLGIKQFSLMRENPLEQSKSFMTTKPADVKE TKMEEFFI SPK DENWMLGKPE NVAHQHEQRIAGSVQLDSSSNELRPGQLKAAVSSKDHTCEVRKQVLP HSAEESHLS QEAVSALDTSSGNTETLSSKSYSSSEEVKLAEEP KSLVLAGNVERNIAEGKEI HSLMES ESLLLEKANTELSWPSKEDSQEKIKLPPERFFQKPVSGLSVEQVKSETISSSVKTAHF PAEGVEPALGNEKEAHRSTPPFPEEKPLEESKMVQSKVIDDADEGKKPSPEVKIPTQR
		GCACAACAAUUGCAGUUUA SEQ ID NO: 344 GGAUAUCAAUGAAAGGUUG SEQ ID NO: 345 GGAAGGAGUUCUAUCACGA SEQ ID NO: 346 GAACUUCACUGGAUGUAGC SEQ ID NO: 347

KPISSIHAREPQSPESPEVTQNPPTQPKVAKPDLPEEKGGKGISSFKSWMSLFFGSS
 TPDNKAQEDLETPSPSVEKAVTVIDPEGTIPTNFNVAEKPADHSLSEVKLKTAD
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 TLKSRLEDEKVTPLKENKQKETHKTHKEEISTDSETDLSFIQPTIPSEEDYFEKYTLID
 YNISPDPEKQKAPQKLNVEEKLKSEVTEETISFPVSSVESALEHEYDLVKLDESFGP
 EKGHNILSHPETQSONSADRNVSKDTRDVSCKSPGMPLFEAEGLVLSRTQIFPTTIK
 VIDPEFLEEPPALAFLYKDLVEEAVGEKKKEETASEGDSVNSEASFPSRNSDTHDGT
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 KSLEEKGVYGESEVDHETVGNVAMQKAPITEDVRVATQKISYAVPFEDTHHVL
 RADEAGSHGNEVGNASPEVNLNVQVSPFEEFASGATHVQETSLEEPKILVPPPEPS
 EERLRNSPVQDEYEFTESLHNEVVPQDILSEELSSSESTPEDVLSQKESFEHISENEF
 ASEAEQSTPAEQKELGSEKKEEDQLSSEVVTEKAQKELKKSQIDTYCYTCKCPI SATD
 KVFGTHKDHVSTLDTAISAVKVQLAEFLENLQEKSLRIEAFVSEIESFFNTIEENCS
 KNEKRLEEQNEEMMKVLAQYDEKAQSFEEVKKKKWEFLHEQWVHFLQSMDTAKDTLE
 TIVREAEELDEAVFLTSFEEINERLLSAMESTASLEKMPAAFSLFEHYDDSSARSQDM
 LKQVAVPQPPRLEPQEPNSATSTTIAVYWSMNKEDVIDSFQVYCMEEPQDDQEVNELV
 EEYRLTVKESYCIFEDLEPDRCYQVWMAVNFTCGSLPSERAIFRTAPSTPVIRAE DC
 TVCWNTATIRWRPTTPEATETTYTIEYCRQHSPEGEGLRFSFGIKGLQKLVNLPNDNY
 FFYVRAINAFGTSEQSEAAIISTRGTRFLLIRETAHPALHISSSGTVISFGERRRLTE
 IPSVLGEELPSCGQHYWETTVTDCPAYRLGICSSAVQAGALQGETSWYMHCEPQR
 YTFFYSGIVSDVHVTERPARVGILLDYNQRLIFINAESEQLLFIIRHRFNEGVHPAF
 ALEKPGKCTLHLGIEPPDSVRHK

SEQ ID NO: 343

Claims:

1. A pharmaceutical composition comprising:
 - (a) an IRGM modulator in an effective amount and optionally one or more of the following:
 - (b) an autophagy modulator in an effective amount;
 - (c) a pharmaceutically-acceptable carrier, additive and/or excipient; and
 - (d) at least one additional bioactive agent,
 or a pharmaceutically acceptable salt of any one or more of the above agents thereof.
2. The composition according to claim 1 wherein said IRGM modulator is a double stranded RNA or a muramyl peptide or a pharmaceutically acceptable salt thereof.
3. The composition according to claim 2 wherein said double stranded RNA is poly I:C, poly U-G or poly ICLC, or a pharmaceutically acceptable salt thereof.
4. The composition according to claim 2 wherein said muramyl peptide is a compound



wherein:

- R^1 represents a hydrogen atom or a C_1 - C_{22} acyl group;
 R^2 represents a hydrogen atom or a C_1 - C_{22} acyl group;
 R^3 represents a hydrogen atom or a C_1 - C_6 alkyl group;
 R^4 represents a C_1 - C_{21} alkyl group or a C_5 or C_{10} aryl group;
 R^5 represents a hydrogen atom; and

R represents the residue of an amino acid or a linear peptide built up of from 2 to 6 amino acid residues, at least one of the residues being optionally substituted with a lipophilic group including muramyl dipeptide and desmethylmuramyl dipeptide.

5. The composition according to claim 2 wherein said muramyl peptide is
muroctasin;

MTP-PE;

murabutide;

t-MDP;

GMDP

GMDP-LL;

GMDP-Obu;

GMDO-Lys;

GMDB-Lys(St);

GMDBA-Lys(St);

GMDPA(OBzl)₂;

MeGMDP;

(GMDP)₂;

(GMDPA)₂;

(GMDPLys)₂;

[GMDP-Lys(St)]₂;

GMDP-Ad;

GMDP-tuftsins E;

GMDP-tuftsins A;

GMDP-tuftsins lipophilic;

GMDP-bursins;

GMDP-thymogen I;

GMDP-thymogen II;

GMDP-thymogen III;

Thr-MDP, and mixtures thereof.

6. The composition according to claim 1 wherein said autophagy modulator is selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine,

dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, tetrachlorisophthalonitrile, phenylmercuric acetate, benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurazone, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clocortolone, doxycycline, pirlindole mesylate, doxazosin, depropion, nocodazole, scopolamine, oxybenzone, halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene and mixtures thereof, or their pharmaceutically acceptable salts thereof and mixtures thereof.

7. The composition according to any of claims 1-6 wherein said autophagy modulator is selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphenes citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline pharmaceutically acceptable salts thereof and mixtures thereof.

8. The composition according to any of claims 1-7 wherein said autophagy modulator is selected from the group consisting of benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurazone, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clocortolone, doxycycline, pirlindole mesylate, doxazosin, depropion, nocodazole, scopolamine, oxybenzone,

halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene, pharmaceutically acceptable salts thereof and mixtures thereof..

9. The composition according to any of claims 1-8 wherein said additional bioactive agent is an antibiotic or an antiviral agent.
10. The composition according to claim 9 wherein said antibiotic is an anti-tuberculosis agent.
11. The composition according to claim 9 wherein said antiviral agent is an anti-HIV agent, an anti-HBV agent, anti-influenza agent, an anti-herpes agent or an anti-HCV agent.
12. The composition according to claim 11 wherein said antiviral agent is an anti-HIV agent is a nucleoside reverse transcriptase inhibitor (NRTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor, a fusion inhibitor or a mixture thereof.
13. The composition according to claim 11 wherein said anti-HIV agent is 3TC (Lamivudine), AZT (Zidovudine), (-)-FTC, ddI (Didanosine), ddC (zalcitabine), abacavir (ABC), tenofovir (PMPA), D-D4FC (Reverset), D4T (Stavudine), Racivir, L-FddC, L-FD4C, NVP (Nevirapine), DLV (Delavirdine), EFV (Efavirenz), SQVM (Saquinavir mesylate), RTV (Ritonavir), IDV (Indinavir), SQV (Saquinavir), NFV (Nelfinavir), APV (Amprenavir), LPV (Lopinavir), fusion inhibitors such as T20, among others, fuseon and mixtures thereof.
14. The composition according to any of claims 9-13 wherein said bioactive agent includes an anticancer agent.
15. The composition according to claim 14 wherein said anticancer agent is an antimetabolite, an inhibitor of topoisomerase I and/or II, an alkylating agent, a microtubule inhibitor, a tyrosine kinase inhibitor, an EGF kinase inhibitor or an ABL kinase inhibitor.
16. The composition according to claim 14 wherein said anticancer agent is Aldesleukin; Alemtuzumab; alitretinoin; allopurinol; altretamine; amifostine; anastrozole; arsenic trioxide; Asparaginase; BCG Live; bexarotene capsules; bexarotene gel; bleomycin; busulfan

intravenous; busulfan oral; calusterone; capecitabine; carboplatin; carmustine; carmustine with Polifeprosan 20 Implant; celecoxib; chlorambucil; cisplatin; cladribine; cyclophosphamide; cytarabine; cytarabine liposomal; dacarbazine; dactinomycin; actinomycin D; Darbepoetin alfa; daunorubicin liposomal; daunorubicin, daunomycin; Denileukin diftitox, dexrazoxane; docetaxel; doxorubicin; doxorubicin liposomal; Dromostanolone propionate; Elliott's B Solution; epirubicin; Epoetin alfa estramustine; etoposide phosphate; etoposide (VP-16); exemestane; Filgrastim; floxuridine (intraarterial); fludarabine; fluorouracil (5-FU); fulvestrant; gemtuzumab ozogamicin; gleevec (imatinib); goserelin acetate; hydroxyurea; Ibritumomab Tiuxetan; idarubicin; ifosfamide; imatinib mesylate; Interferon alfa-2a; Interferon alfa-2b; irinotecan; letrozole; leucovorin; levamisole; lomustine (CCNU); mecllorethamine (nitrogen mustard); megestrol acetate; melphalan (L-PAM); mercaptopurine (6-MP); mesna; methotrexate; methoxsalen; mitomycin C; mitotane; mitoxantrone; nandrolone phenpropionate; Nofetumomab; LOddC; Oprelvekin; oxaliplatin; paclitaxel; pamidronate; pegademase; Pegaspargase; Pegfilgrastim; pentostatin; pipobroman; plicamycin; mithramycin; porfimer sodium; procarbazine; quinacrine; Rasburicase; Rituximab; Sargramostim; streptozocin; surafenib; talbuvudine (LDT); talc; tamoxifen; tarceva (erlotinib); temozolomide; teniposide (VM-26); testolactone; thioguanine (6-TG); thiotepa; topotecan; toremifene; Tositumomab; Trastuzumab; tretinoin (ATRA); Uracil Mustard; valrubicin; valtorcitabine (monoval LDC); vinblastine; vinorelbine; zoledronate or a mixture thereof.

17. A method of treating an autophagy-mediated disease in a patient in need thereof comprising administering to said patient an effective amount of a composition according to any of claims 1-16.

18. The method according to claim 17 wherein said autophagy-mediated disease is cancer, lysosomal storage diseases, Alzheimer's disease, Parkinson's disease; a chronic inflammatory disease, Crohn's disease, diabetes I, diabetes II, metabolic syndrome, an inflammation-associated metabolic disorder, liver disease, renal disease, cardiovascular disease, muscle degeneration and atrophy, symptoms of aging (including the amelioration or the delay in onset or severity or frequency of aging-related symptoms and chronic conditions including muscle atrophy, frailty, metabolic disorders, low grade inflammation, atherosclerosis and associated conditions such as cardiac and neurological both central and peripheral manifestations including stroke, age-associated dementia and sporadic form of

Alzheimer's disease, pre-cancerous states, and psychiatric conditions including depression), spinal cord injury, infectious disease and developmental disease.

19. The method according to claim 17 wherein said autophagy-mediated disease is selected from the group consisting of Type I and Type II diabetes, severe insulin resistance, hyperinsulinemia, hyperlipidemia, obesity, insulin-resistant diabetes, Mendenhall's Syndrome, Werner Syndrome, leprechaunism, lipotrophic diabetes, acute and chronic renal insufficiency, end-stage chronic renal failure, glomerulonephritis, interstitial nephritis, pyelonephritis, glomerulosclerosis, GH-deficiency, GH resistance, Turner's syndrome, Laron's syndrome, short stature, increased fat mass-to-lean ratios, decreased CD4⁺ T cell counts and decreased immune tolerance, chemotherapy-induced tissue damage, congestive heart failure, Alzheimer's disease, Parkinson's disease, multiple sclerosis, Crohn's disease, peripheral neuropathy, muscular dystrophy, myotonic dystrophy, anorexia nervosa, a viral infection, and a bacterial infection.

20. The method according to claim 17 wherein said autophagy-mediated disease is selected from the group consisting of activator deficiency/GM2 gangliosidosis, alpha-mannosidosis, aspartylglucosaminuria, cholesteryl ester storage disease, chronic hexosaminidase A deficiency, cystinosis, Danon disease, Fabry disease, Farber disease, fucosidosis, galactosialidosis, Gaucher Disease (Types I, II and III), GM1 Gangliosidosis, including infantile, late infantile/juvenile and adult/chronic), Hunter syndrome (MPS II), I-Cell disease/Mucopolysaccharidosis II, Infantile Free Sialic Acid Storage Disease (ISSD), Juvenile Hexosaminidase A Deficiency, Krabbe disease, Lysosomal acid lipase deficiency, Metachromatic Leukodystrophy, Hurler syndrome, Scheie syndrome, Hurler-Scheie syndrome, Sanfilippo syndrome, Morquio Type A and B, Maroteaux-Lamy, Sly syndrome, mucopolysaccharidosis, multiple sulfate deficiency, Niemann-Pick disease, Neuronal ceroid lipofuscinoses, CLN6 disease, Jansky-Bielschowsky disease, Pompe disease, pycnodysostosis, Sandhoff disease, Schindler disease, Tay-Sachs or Wolman disease.

21. A method of treating of cancer in a patient in need, comprising administering to said patient an effective amount of a composition according to any of claims 1-16.

22. A method of treating of treating Crohn's disease or tuberculosis, the method comprising administering an effective amount of a composition according to any of claims 1-16.
23. A method of treating excessive inflammation associated with an autophagy-related diseases in a patient in need thereof, the method comprising administering an effective amount of a compound or composition selected from the group consisting of at least one TRIM protein or a variant thereof having at least 90% sequence identity to said TRIM protein, at least one inhibitor of a TRIM protein or a mixture thereof, and optionally at least one additional bioactive agent.
24. The method according to claim 23 wherein said TRIM protein is TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:97), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302) and TRIM75 (SEQ ID NO:338).
25. The method according to claim 23 wherein said TRIM protein is TRIM 1 (SEQ ID NO:1), TRIM 8 (SEQ ID NO:236), TRIM 20 (SEQ ID NO:20), TRIM 21 (SEQ ID NO:101), TRIM 22 (SEQ ID NO:106), TRIM 56 (SEQ ID NO:257), TRIM 65 (SEQ ID NO:297) or a mixture thereof.
26. The method according to any of claims 23-26 wherein said TRIM protein inhibitor is a siRNA of about 9-30 nucleotide units in length.
27. The method according to any of claims 23-26 wherein said autophagy-related disease is an inflammatory disease, an autoimmune disease, an infectious disease, a cardiovascular disease or a metabolic disease.

28. The method according to any of claims 23-27 wherein said autophagy-related disease is selected from the group consisting of a lysosomal storage disease, neurodegeneration, autoimmune diseases and chronic inflammatory diseases resulting in excessive inflammation, hyperglycemic disorders, liver disease, renal disease, cardiovascular disease, muscle degeneration and atrophy, symptoms of aging, pre-cancerous states, psychiatric conditions, stroke, spinal cord injury, arteriosclerosis and infectious diseases.

29. The method according to any of claims 23-26 wherein said autophagy-related disease is a lysosomal storage disease, Alzheimer's disease, Parkinson's disease, Huntington's disease, inflammatory bowel disease, rheumatoid arthritis, lupus, multiple sclerosis, chronic obstructive pulmonary disease/COPD, pulmonary fibrosis, cystic fibrosis, Sjogren's disease; hyperglycemic disorders, diabetes (I and II), affecting lipid metabolism islet function and/or structure, excessive autophagy may lead to pancreatic β -cell death and related hyperglycemic disorders, including severe insulin resistance, hyperinsulinemia, insulin-resistant diabetes (e.g. Mendenhall's Syndrome, Werner Syndrome, leprechaunism, and lipoatrophic diabetes) and dyslipidemia (e.g. hyperlipidemia as expressed by obese subjects, elevated low-density lipoprotein (LDL), depressed high-density lipoprotein (HDL), and elevated triglycerides) and metabolic syndrome, liver disease (excessive autophagic removal of cellular entities- endoplasmic reticulum), renal disease (apoptosis in plaques, glomerular disease), cardiovascular disease (especially including ischemia, stroke, pressure overload and complications during reperfusion), muscle degeneration and atrophy, symptoms of aging (including amelioration or the delay in onset or severity or frequency of aging-related symptoms and chronic conditions including muscle atrophy, frailty, metabolic disorders, low grade inflammation, atherosclerosis and associated conditions such as cardiac and neurological both central and peripheral manifestations including stroke, age-associated dementia and sporadic form of Alzheimer's disease, pre-cancerous states, and psychiatric conditions including depression), stroke and spinal cord injury, arteriosclerosis, infectious diseases (microbial infections, removes microbes, provides a protective inflammatory response to microbial products, limits adaptation of autophagy of host by microbe for enhancement of microbial growth, regulation of innate immunity) including bacterial, fungal, cellular and viral (including secondary disease states or conditions associated with infectious diseases, especially including hepatitis B and C and HIV I and II), including AIDS and tuberculosis.

30. The method according to any of claims 23-29 wherein said compound or composition is further co-administered with at least one additional bioactive agent.

31. The method according to claim 30 wherein said additional bioactive agent is interferon- γ or pegylated interferon.

32. The method according to claim 30 wherein said additional bioactive agent is at least one compound selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, tetrachlorisophthalonitrile and phenylmercuric acetate, benzethonium, niclosamide, monensin, bromperidol, levobunolol, dehydroisoandosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurazone, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clocortolone, doxycycline, pirlindole mesylate, doxazosin, depropylamine, nocodazole, scopolamine, oxybenzone, halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene, TRIM2 (SEQ ID NO:6), TRIM 4 (SEQ ID NO:16), TRIM5 (TRIM5 α) (SEQ ID NO:21), TRIM6 (SEQ ID NO:26), TRIM7 (SEQ ID NO:31), TRIM9 (SEQ ID NO:41), TRIM11 (SEQ ID NO:51), TRIM14 (SEQ ID NO:61), TRIM15 (SEQ ID NO:66), TRIM16 (SEQ ID NO:71), TRIM18 (SEQ ID NO:86), TRIM23 (SEQ ID NO:111), TRIM24 (SEQ ID NO:116), TRIM25 (SEQ ID NO:121), TRIM27 (SEQ ID NO:126), TRIM28 (SEQ ID NO:131), TRIM29 (SEQ ID NO:136), TRIM30, TRIM 31 (SEQ ID NO:141), TRIM32 (SEQ ID NO:146), TRIM33 (SEQ ID NO:151), TRIM34 (SEQ ID NO:156), TRIM35 (SEQ ID NO:161), TRIM36 (SEQ ID NO:166), TRIM37 (SEQ ID NO:167), TRIM39 (SEQ ID NO:177), TRIM40 (SEQ ID NO:182), TRIM42 (SEQ ID NO:192), TRIM47 (SEQ ID NO:217), TRIM48 (SEQ ID NO:222), TRIM49 (SEQ ID NO:227), TRIM50 (SEQ ID NO:232), TRIM51 (SEQ ID NO:237), TRIM55 (SEQ ID NO:252), TRIM68 (SEQ ID NO:312), TRIM72 (SEQ ID NO:323), TRIM73 (SEQ ID

NO:328), TRIM74 (SEQ ID NO:333), TRIM76 (SEQ ID NO:343), a triglyceride, a diglyceride, a monoglyceride, a glycolated mono- or diacylglyceride, dolichol, polyprenol, polyprenal, very long chain fatty acids or a pharmaceutically acceptable salt thereof.

33. The method according to claim 30 wherein said additional bioactive agent is selected from the group consisting of pp242, rapamycin, envirolimus, everolimus, cidaforollimus, epigallocatechin gallate (EGCG), caffeine, curcumin, resveratrol, digoxin, xylazine, hexetidine, sertindole and mixtures thereof.

34. The method according to claim 30 wherein said additional bioactive agent is an antiviral agent.

35. A pharmaceutical composition comprising an effective amount of at least one TRIM protein selected from the group consisting of TRIM1 (SEQ ID NO:1), TRIM3 (SEQ ID NO:11), TRIM8 (SEQ ID NO:36), TRIM10 (SEQ ID NO:46), TRIM13 (SEQ ID NO:56), TRIM17 (SEQ ID NO:81), TRIM19 (SEQ ID NO:91), TRIM20 (SEQ ID NO:96), TRIM21 (SEQ ID NO:101), TRIM22 (SEQ ID NO:106), TRIM38 (SEQ ID NO:172), TRIM 41 (SEQ ID NO:187), TRIM43 (SEQ ID NO:97), TRIM44 (SEQ ID NO:202), TRIM45 (SEQ ID NO:207), TRIM46 (SEQ ID NO:212), TRIM54 (SEQ ID NO:247), TRIM55 (SEQ ID NO:252), TRIM56 (SEQ ID NO:257), TRIM58 (SEQ ID NO:262), TRIM59 (SEQ ID NO:267), TRIM60 (SEQ ID NO:272), TRIM65 (SEQ ID NO:297), TRIM66 (SEQ ID NO:302) and TRIM75 (SEQ ID NO:338) or a pharmaceutically acceptable salt thereof in combination with an additional bioactive agent.

36. The composition according to claim 35 wherein said additional bioactive agent is interferon-gamma (IFN-gamma), pegylated interferon (PEG-IFN), a siRNA or a mixture thereof.

37. The composition according to claim 35 further including at least one compound selected from the group consisting of flubendazole, hexachlorophene, propidium iodide, bepridil, clomiphene citrate (Z,E), GBR 12909, propafenone, metixene, dipivefrin, fluvoxamine, dicyclomine, dimethisoquin, ticlopidine, memantine, bromhexine, norcyclobenzaprine, dipiperodon, nortriptyline, tetrachlorisophthalonitrile and phenylmercuric acetate, benzethonium, niclosamide, monensin, bromperidol, levobunolol,

dehydroisoandosterone 3-acetate, sertraline, tamoxifen, reserpine, hexachlorophene, dipyridamole, harmaline, prazosin, lidoflazine, thiethylperazine, dextromethorphan, desipramine, mebendazole, canrenone, chlorprothixene, maprotiline, homochlorcyclizine, loperamide, nicardipine, dexfenfluramine, nilvadipine, dosulepin, biperiden, denatonium, etomidate, toremifene, tomoxetine, clorgyline, zotepine, beta-escin, tridihexethyl, ceftazidime, methoxy-6-harmalan, melengestrol, albendazole, rimantadine, chlorpromazine, pergolide, cloperastine, prednicarbate, haloperidol, clotrimazole, nitrofurazone, iopanoic acid, naftopidil, methimazole, trimeprazine, ethoxyquin, clonidine, doxycycline, pirlindole mesylate, doxazosin, depropramine, nocodazole, scopolamine, oxybenzone, halcinonide, oxybutynin, miconazole, clomipramine, cyproheptadine, doxepin, dyclonine, salbutamol, flavoxate, amoxapine, fenofibrate, pimethixene, a TRIM protein selected from the group consisting of TRIM2 (SEQ ID NO:6), TRIM 4 (SEQ ID NO:16), TRIM5 (TRIM5 α) (SEQ ID NO:21), TRIM6 (SEQ ID NO:26), TRIM7 (SEQ ID NO:31), TRIM9 (SEQ ID NO:41), TRIM11 (SEQ ID NO:51), TRIM14 (SEQ ID NO:61), TRIM15 (SEQ ID NO:66), TRIM16 (SEQ ID NO:71), TRIM18 (SEQ ID NO:86), TRIM23 (SEQ ID NO:111), TRIM24 (SEQ ID NO:116), TRIM25 (SEQ ID NO:121), TRIM27 (SEQ ID NO:126), TRIM28 (SEQ ID NO:131), TRIM29 (SEQ ID NO:136), TRIM30, TRIM 31 (SEQ ID NO:141), TRIM32 (SEQ ID NO:146), TRIM33 (SEQ ID NO:151), TRIM34 (SEQ ID NO:156), TRIM35 (SEQ ID NO:161), TRIM36 (SEQ ID NO:166), TRIM37 (SEQ ID NO:167), TRIM39 (SEQ ID NO:177), TRIM40 (SEQ ID NO:182), TRIM42 (SEQ ID NO:192), TRIM47 (SEQ ID NO:217), TRIM48 (SEQ ID NO:222), TRIM49 (SEQ ID NO:227), TRIM50 (SEQ ID NO:232), TRIM51 (SEQ ID NO:237), TRIM55 (SEQ ID NO:252), TRIM68 (SEQ ID NO:312), TRIM72 (SEQ ID NO:323), TRIM73 (SEQ ID NO:328), TRIM74 (SEQ ID NO:333) and TRIM76 (SEQ ID NO:343), a triglyceride, a diglyceride, a monoglyceride, a glycolated mono- or diacylglyceride, dolichol, polyprenol, polyprenal, very long chain fatty acids or a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable salt thereof.

38. The composition according to any one of claims 35-37 wherein said composition includes at least one additional bioactive agent selected from the group consisting of pp242, rapamycin, envirolimus, everolimus, cidaforollimus, epigallocatechin gallate (EGCG), caffeine, curcumin, resveratrol, digoxin, xylazine, hexetidine, sertindole and mixtures thereof.

39. The composition according to any one of claims 35-38 wherein said composition further comprises an antiviral agent.
40. The composition according to claim 36 wherein said siRNA is a siRNA according to any one of the sequences set forth in the table on pages 92-116 hereof or an oligonucleotide which contains plus or minus up to 5 nucleotide units upstream or downstream of any of said siRNA sequences.
41. The composition according to claim 40 wherein said siRNA is between 19 and 23 nucleotide units.
42. The method according to claim 23 or 26 wherein said siRNA is a siRNA according to any one of the sequences set forth in the table on pages 92-116 hereof or an oligonucleotide which contains plus/minus up to 5 nucleotide units upstream or downstream of any of said siRNA sequences.
43. The method according to claim 42 wherein said siRNA is between 19 and 23 nucleotide units.

FIGURE 1 IRGM

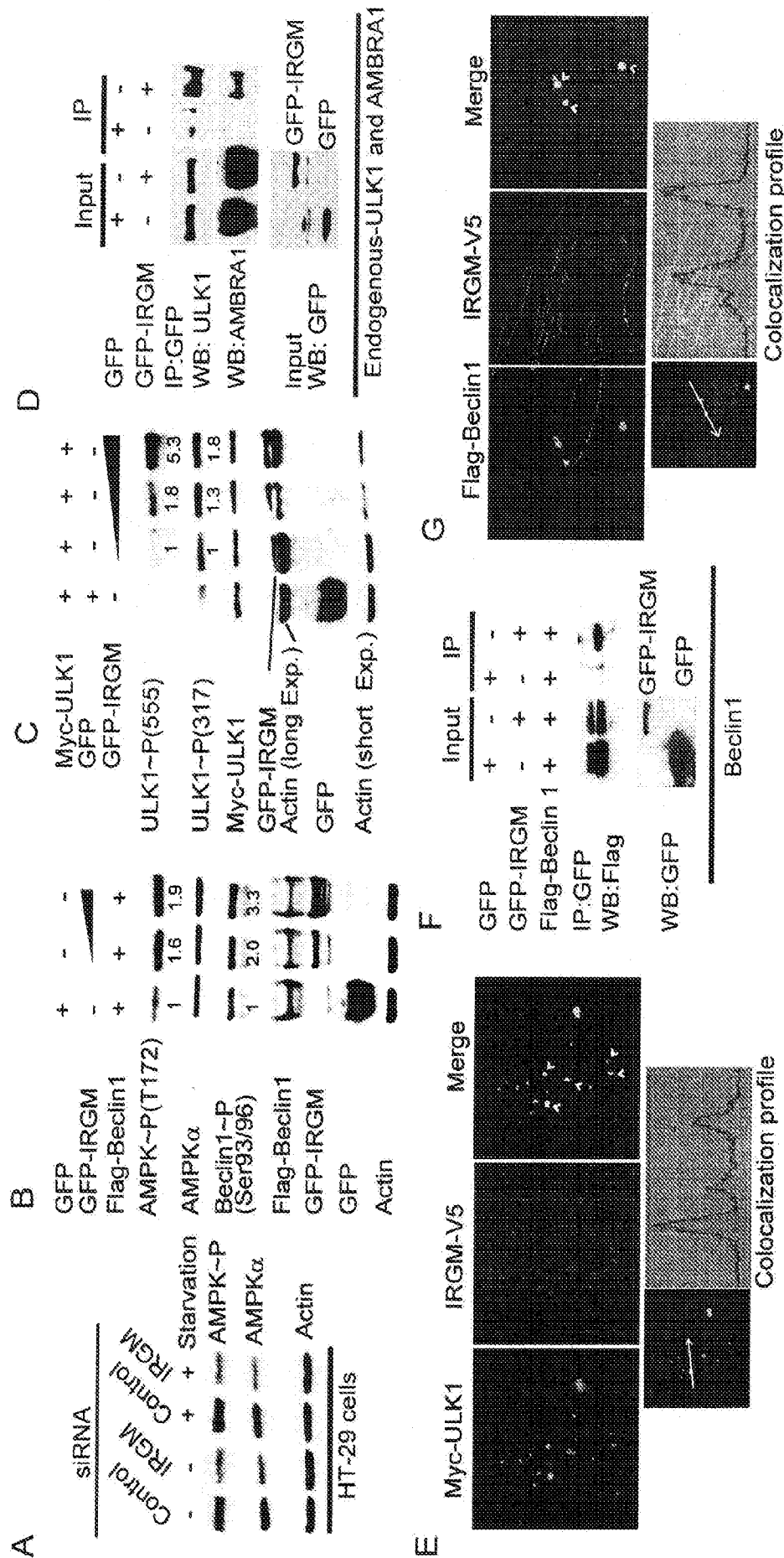


FIGURE 1 IRGM (CONT'D)

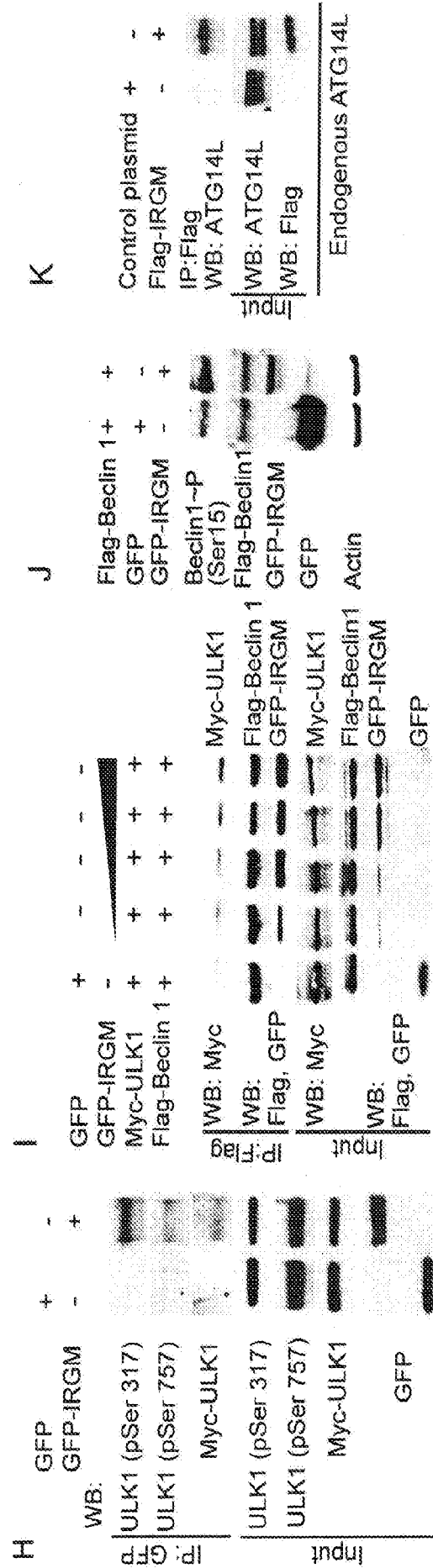


FIGURE 1 IRGM (CONT'D)

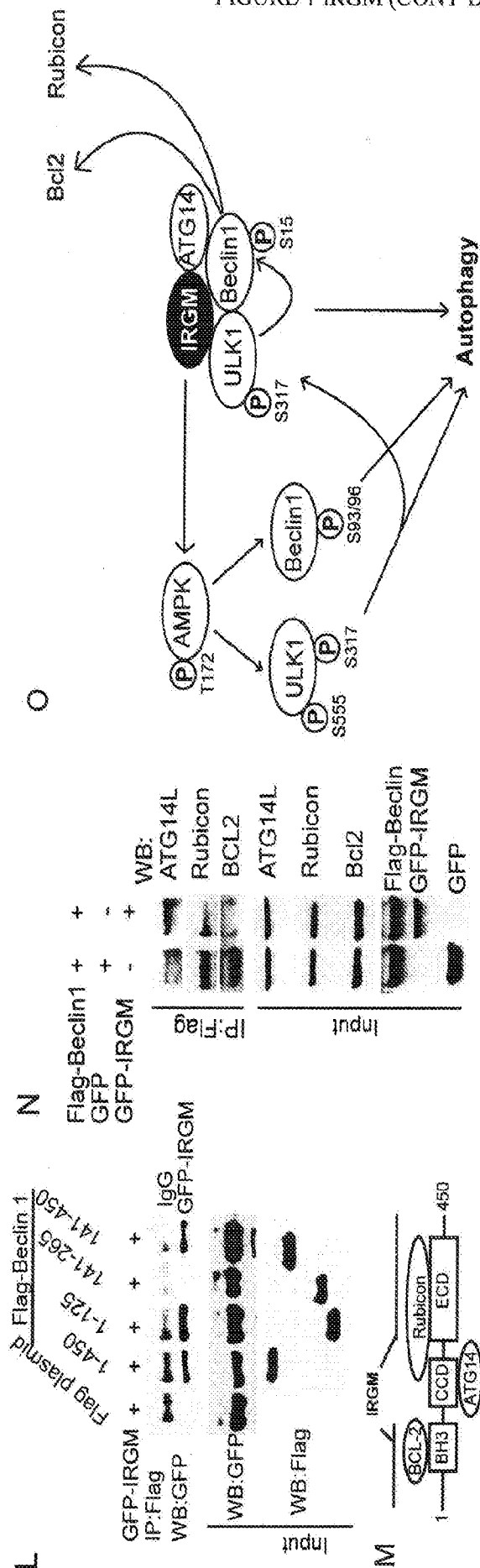


FIGURE 2 IRGM

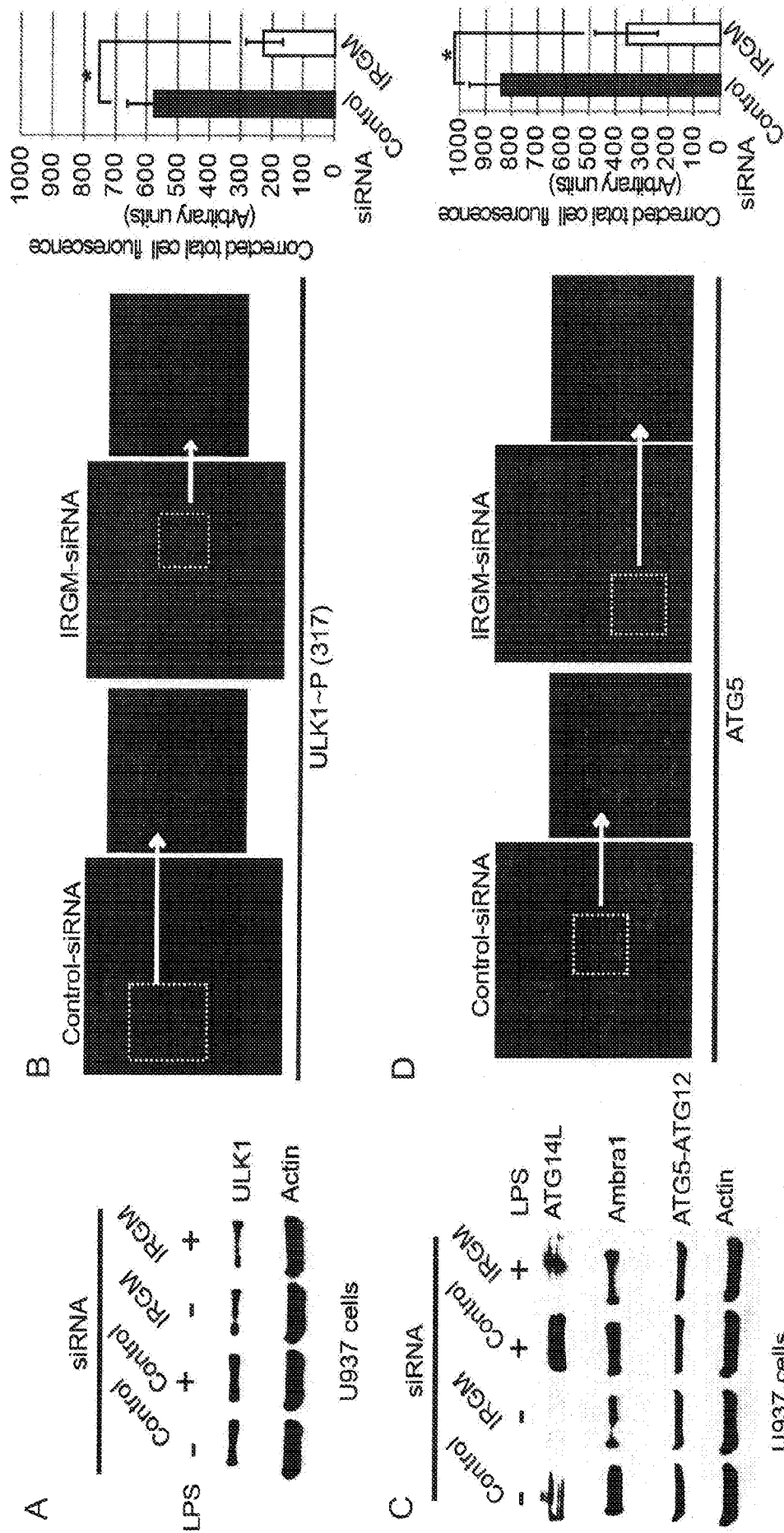


FIGURE 2 IRGM (CONT'D)

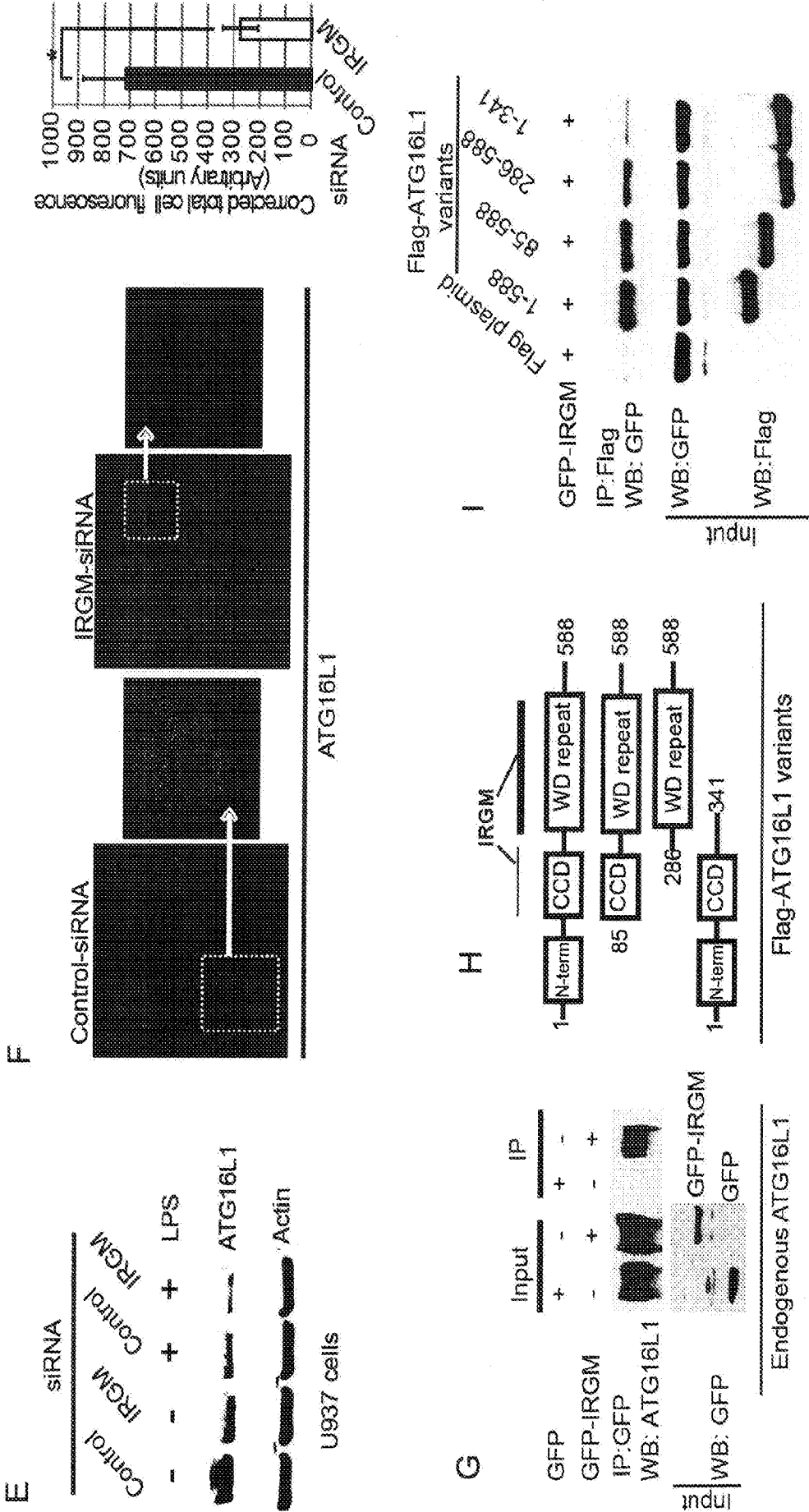
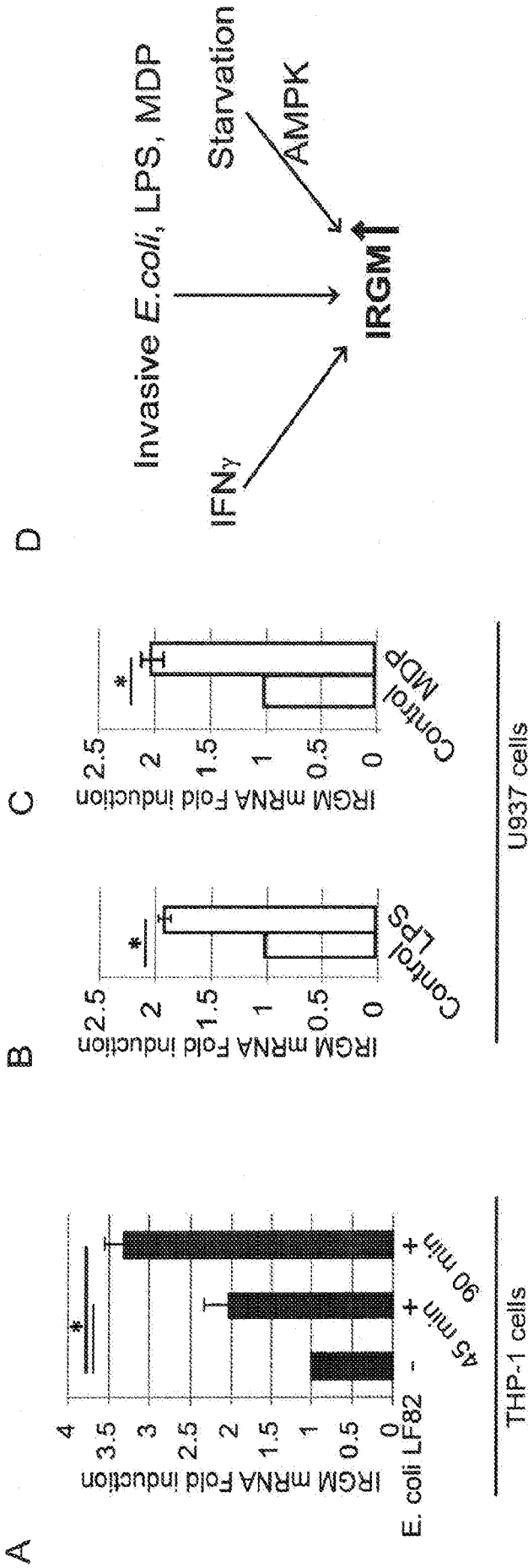


FIGURE 3 IRGM



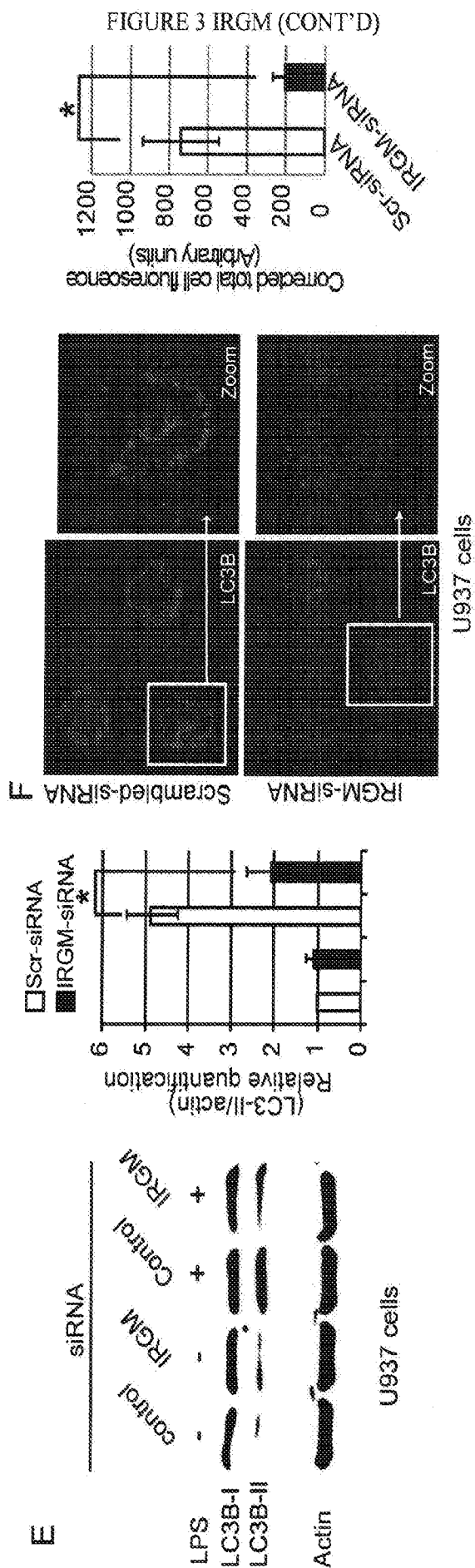


FIGURE 3 IRGM (CONT'D)

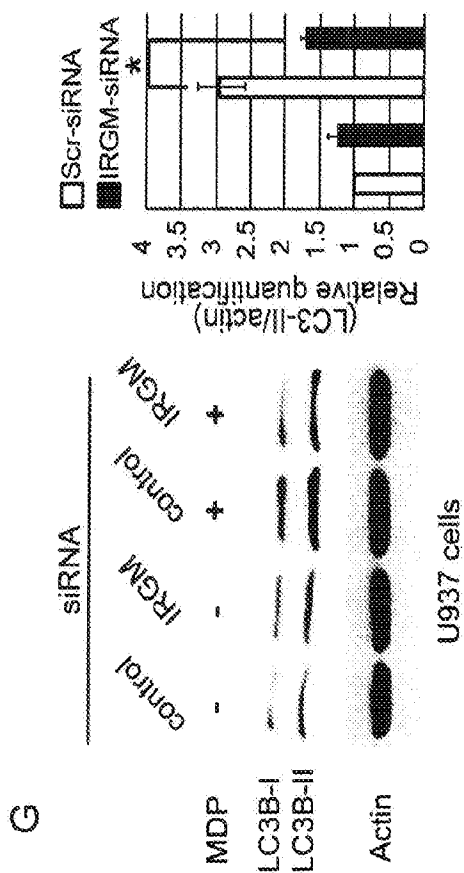
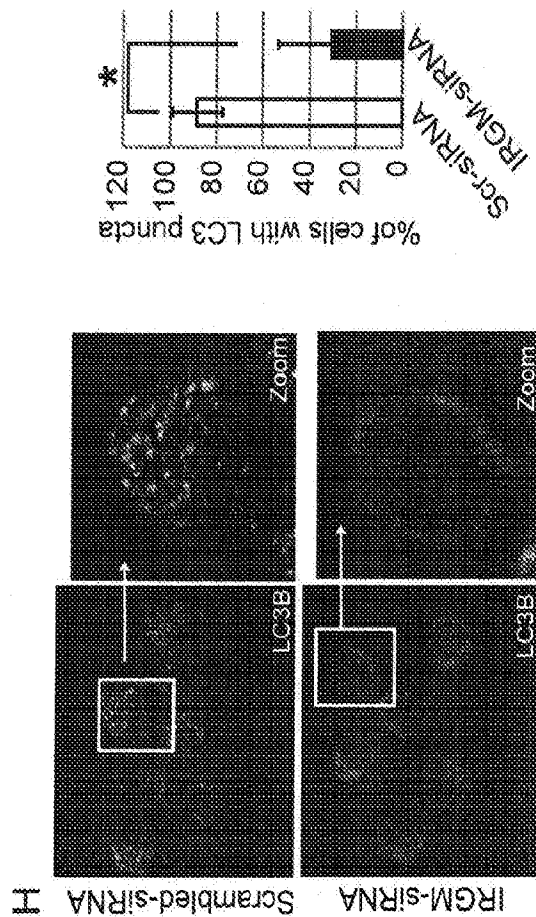


FIGURE 4 IRGM

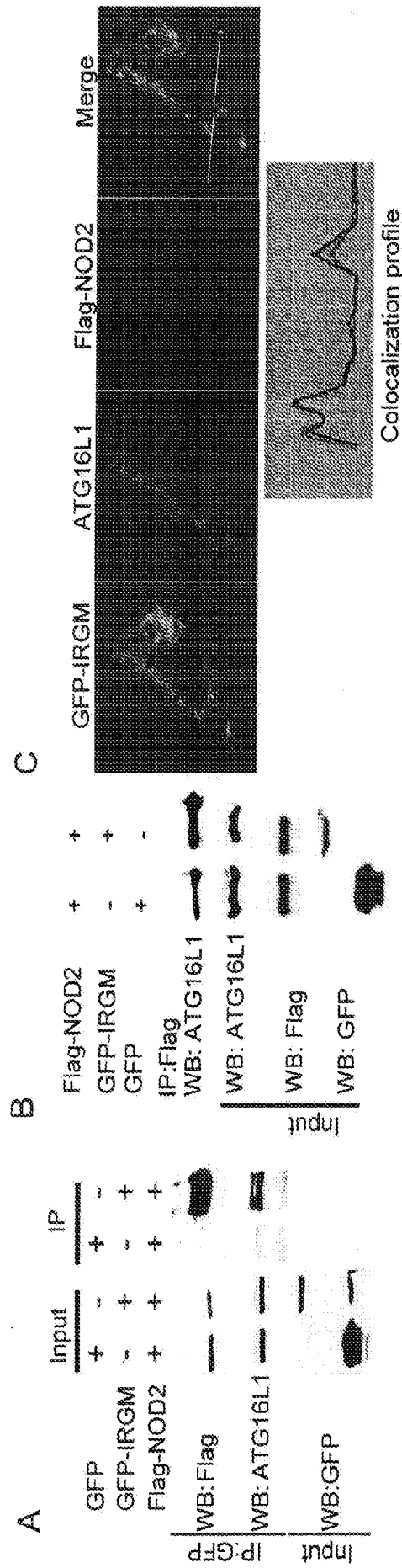


FIGURE 4 IRGM (CONT'D)

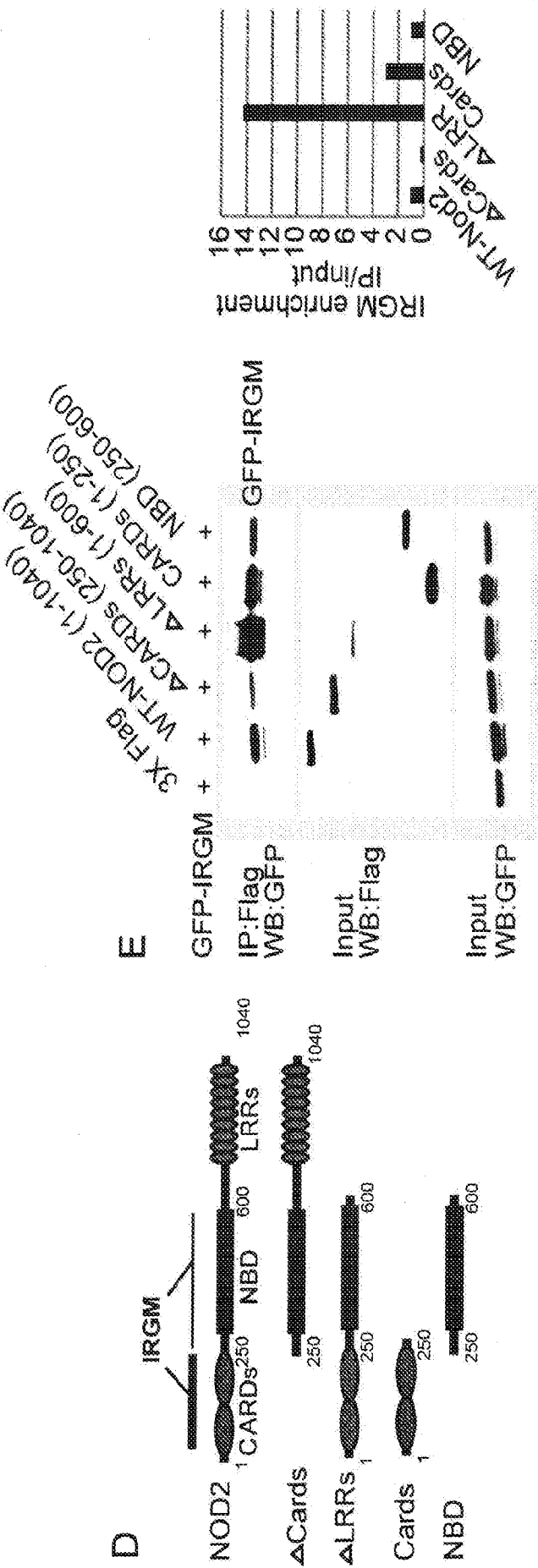


FIGURE 4 IRGM (CONT'D)

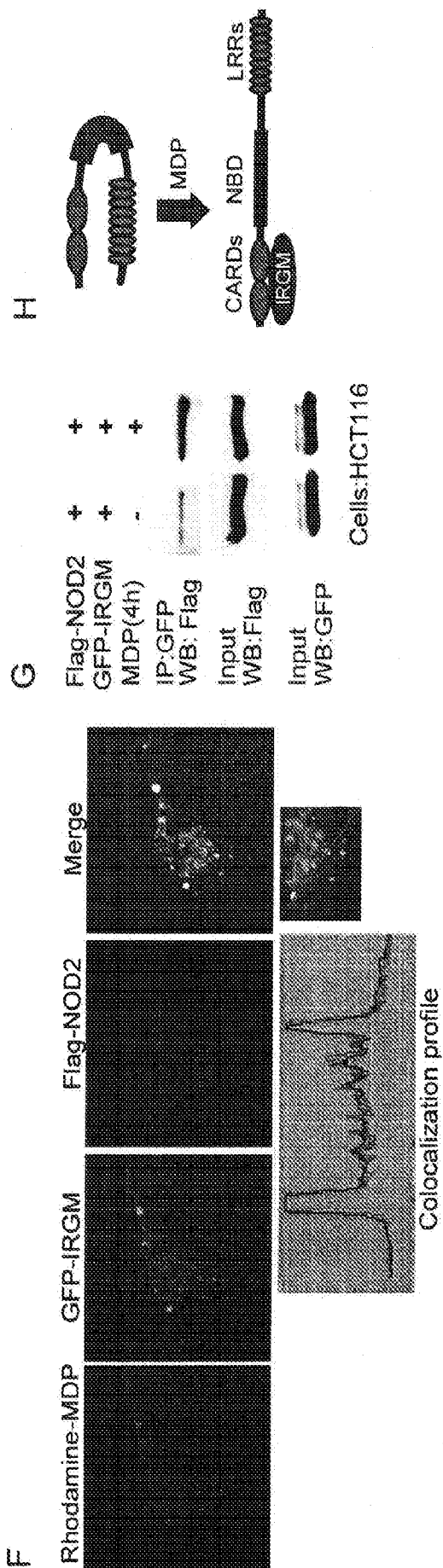


FIGURE 5 IRGM

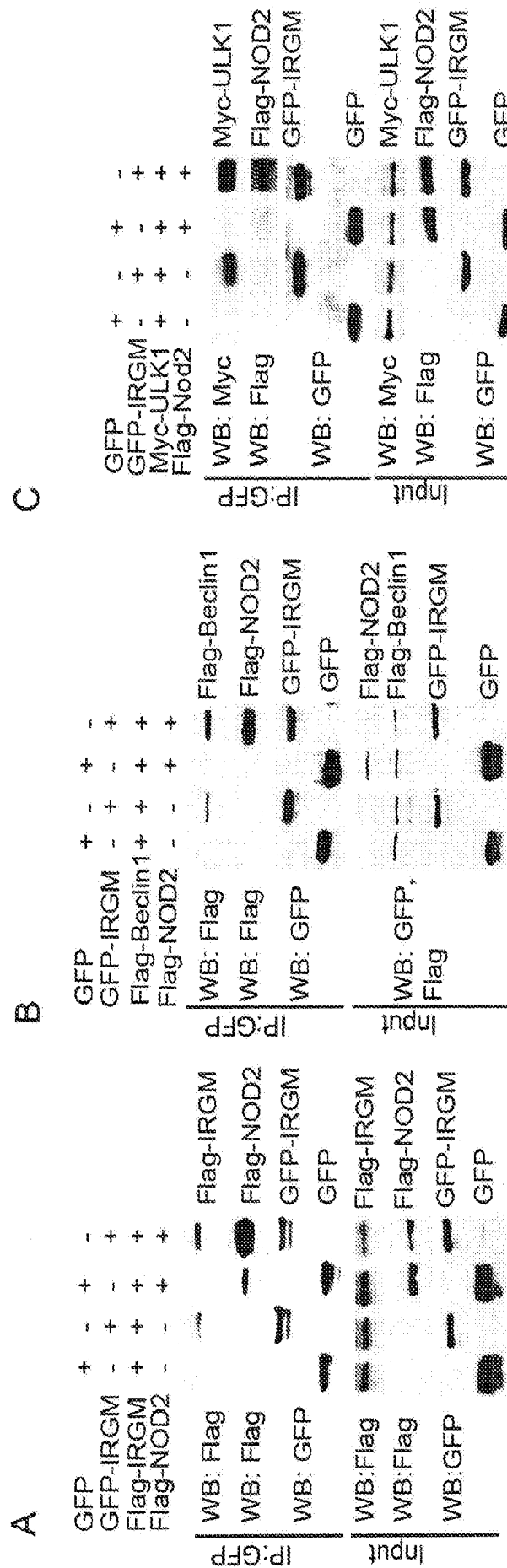


FIGURE 5 IRGM (CONT'D)

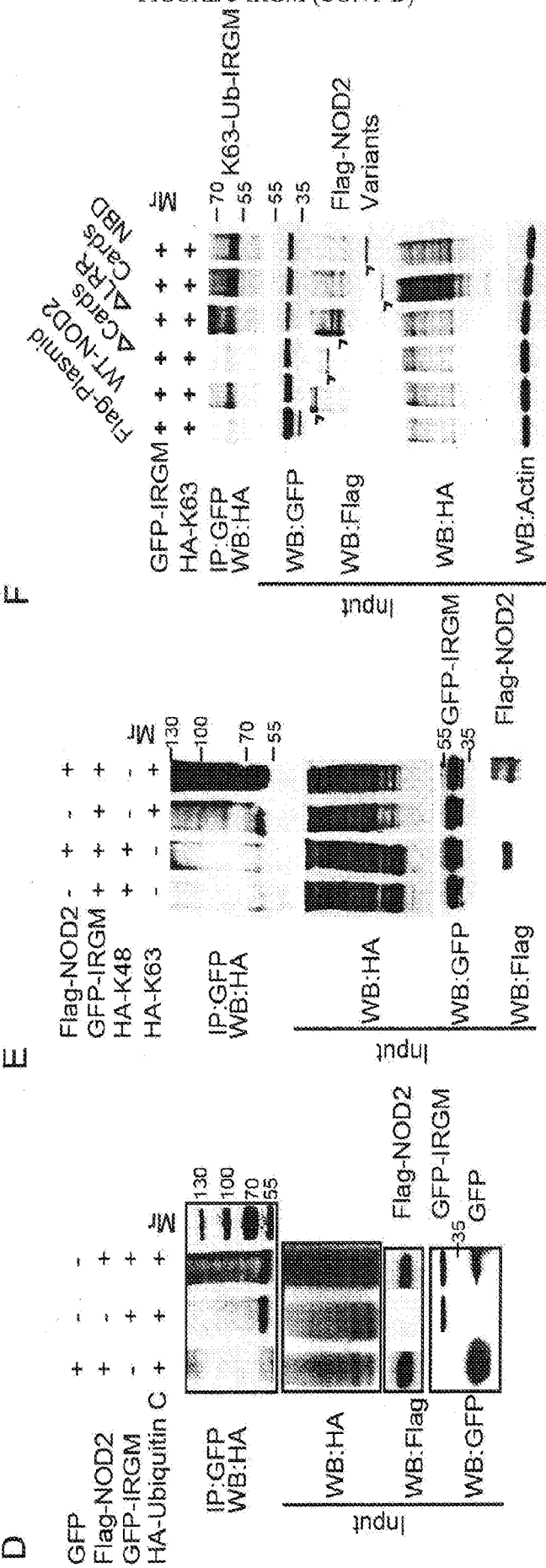


FIGURE 5 IRGM (CONT'D)

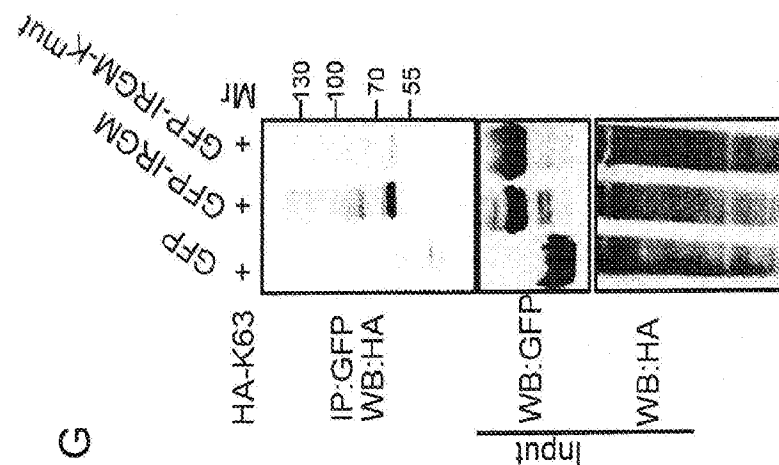
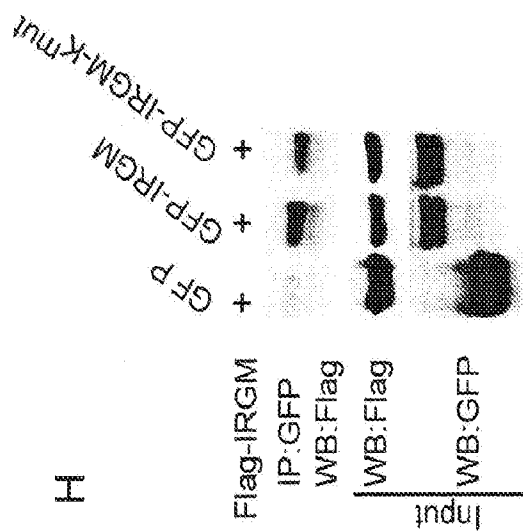
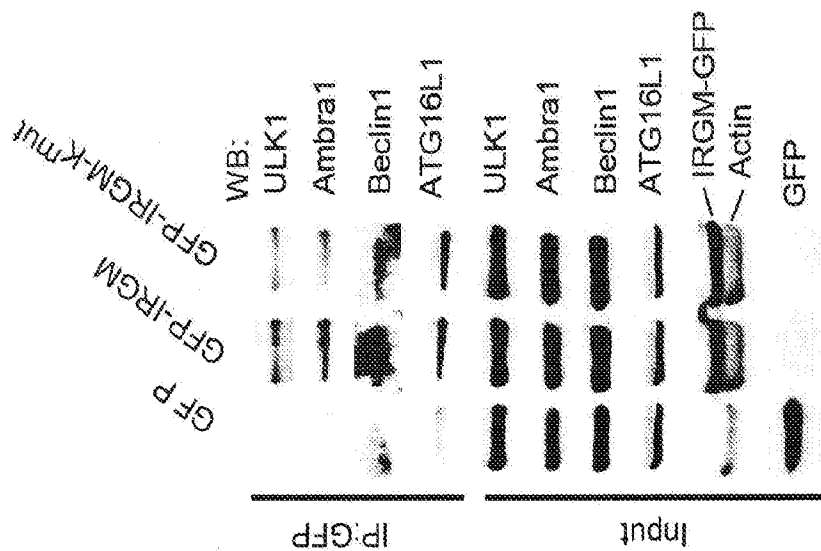
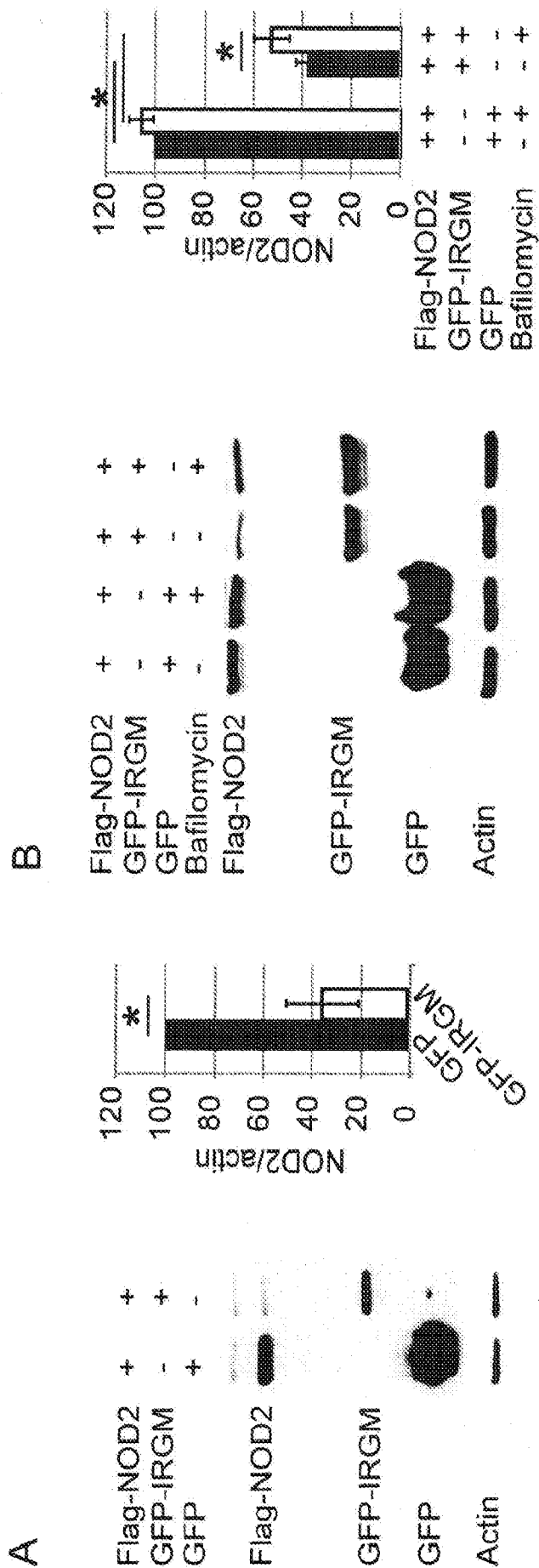


FIGURE 6 IRGM



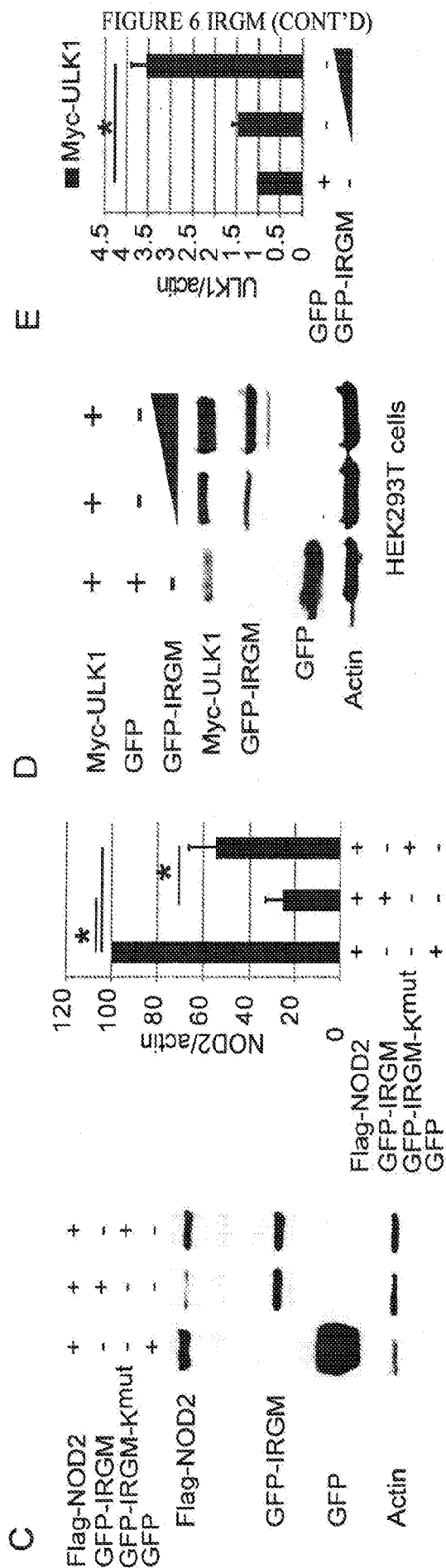
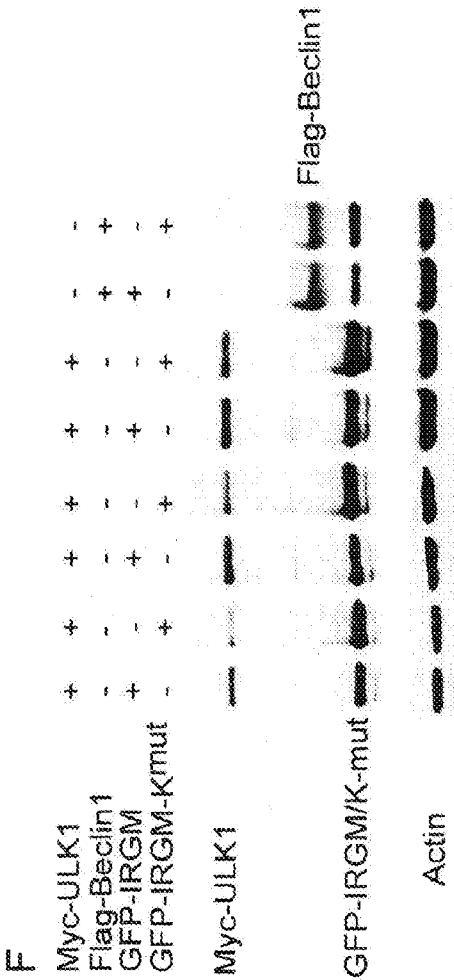
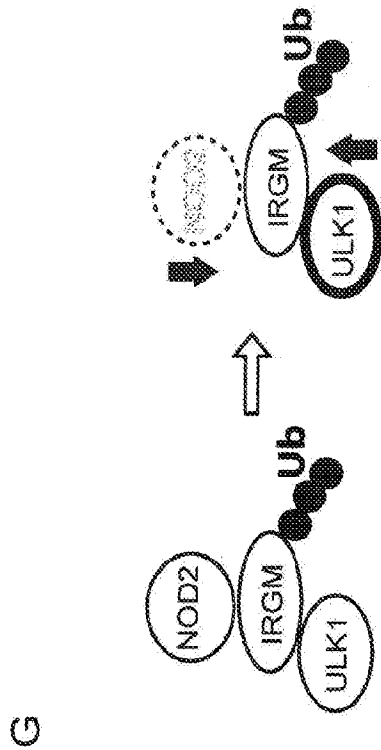


FIGURE 6 IRGM (CONT'D)



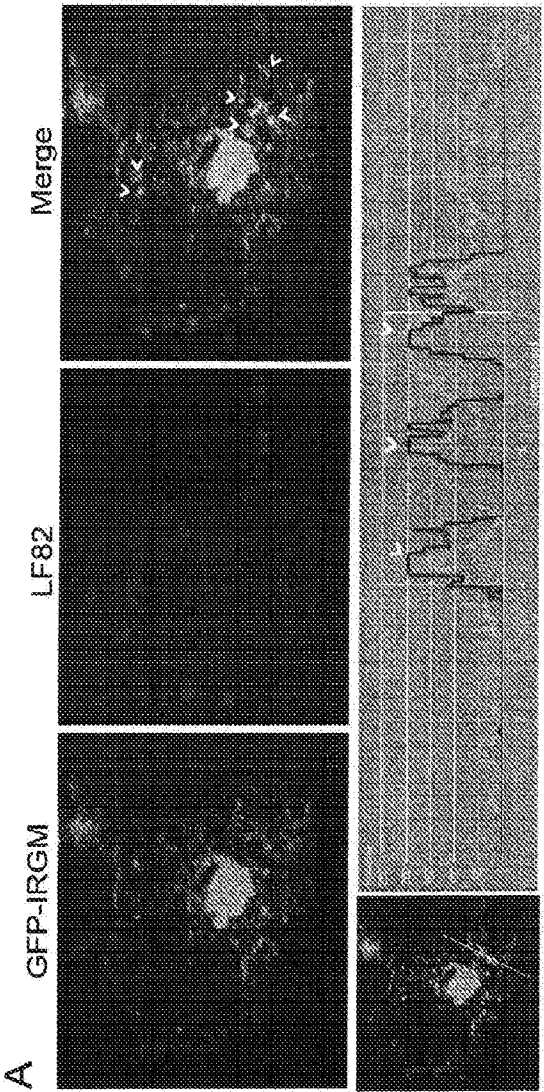
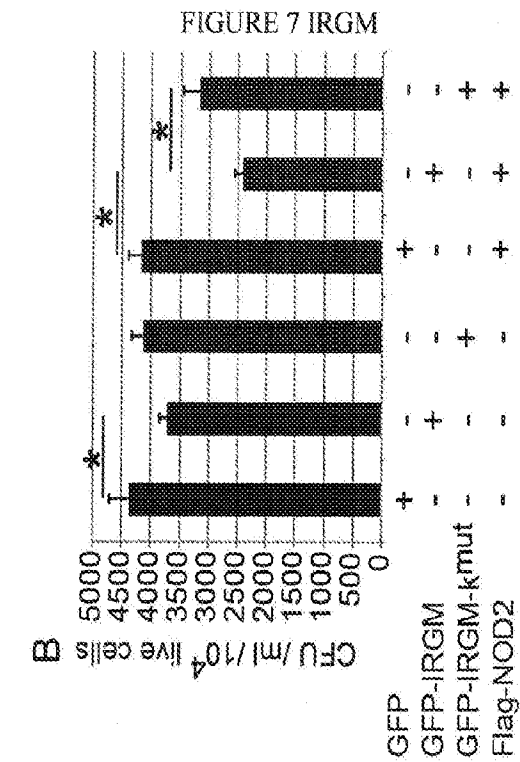


FIGURE 7 IRGM (CONT'D)

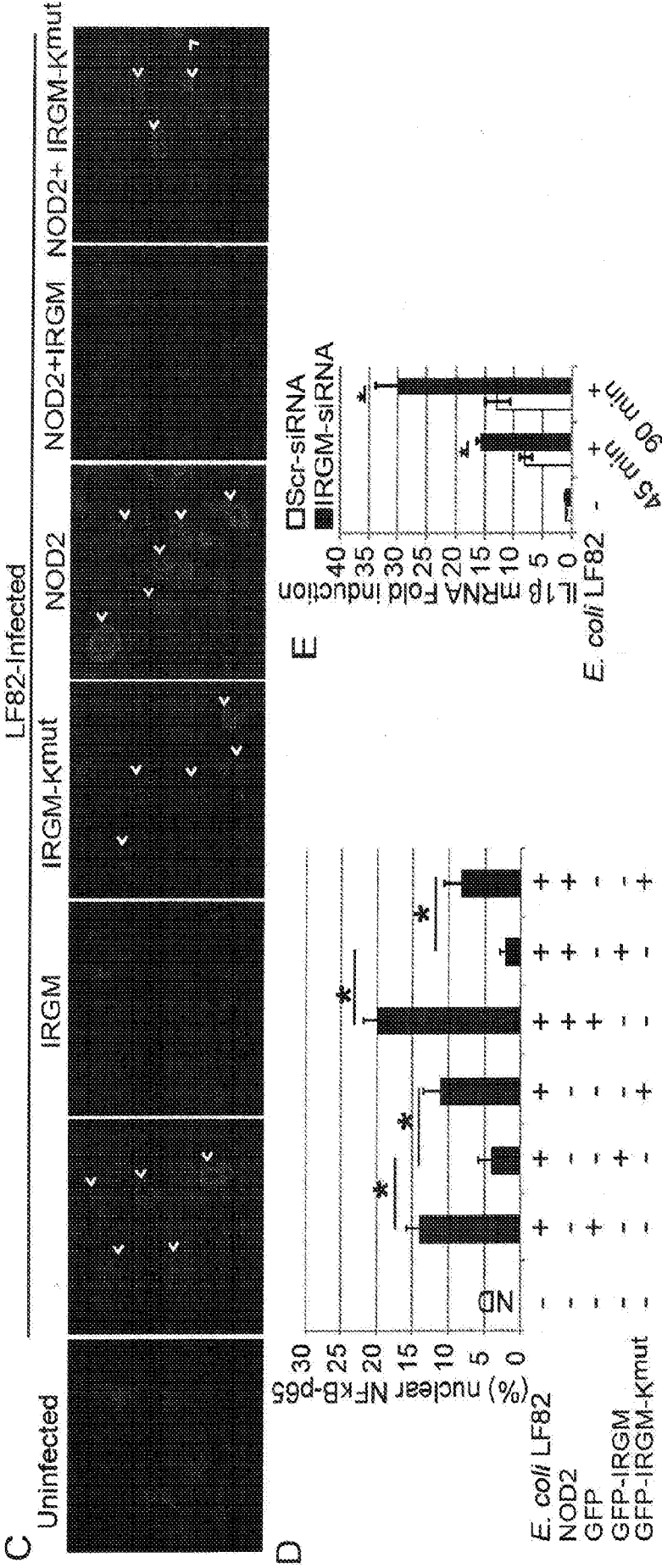


FIGURE 7 IRGM (CONT'D)

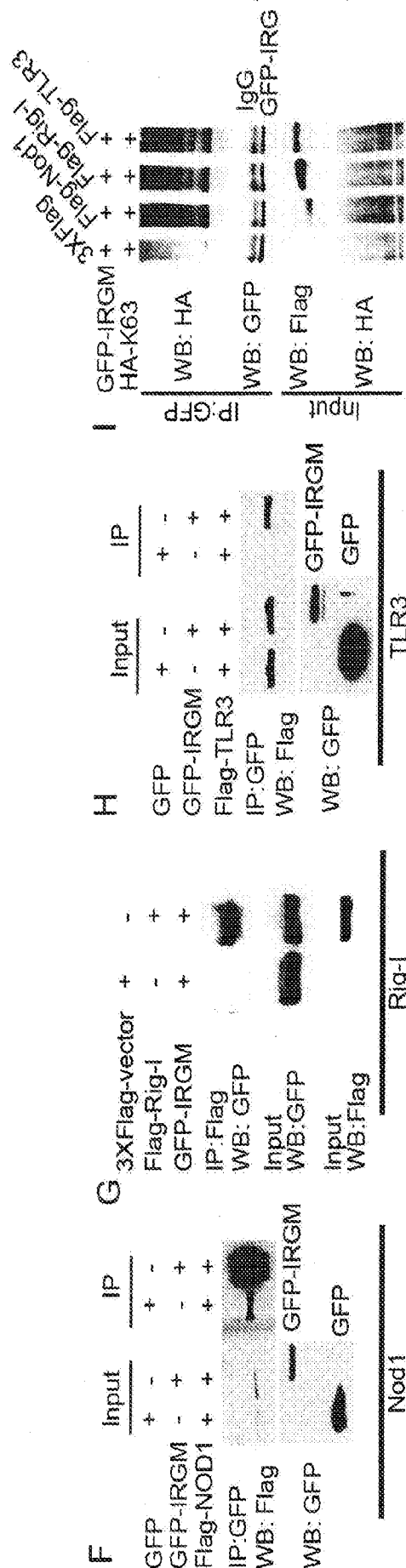


FIGURE 7 IRGM (CONT'D)

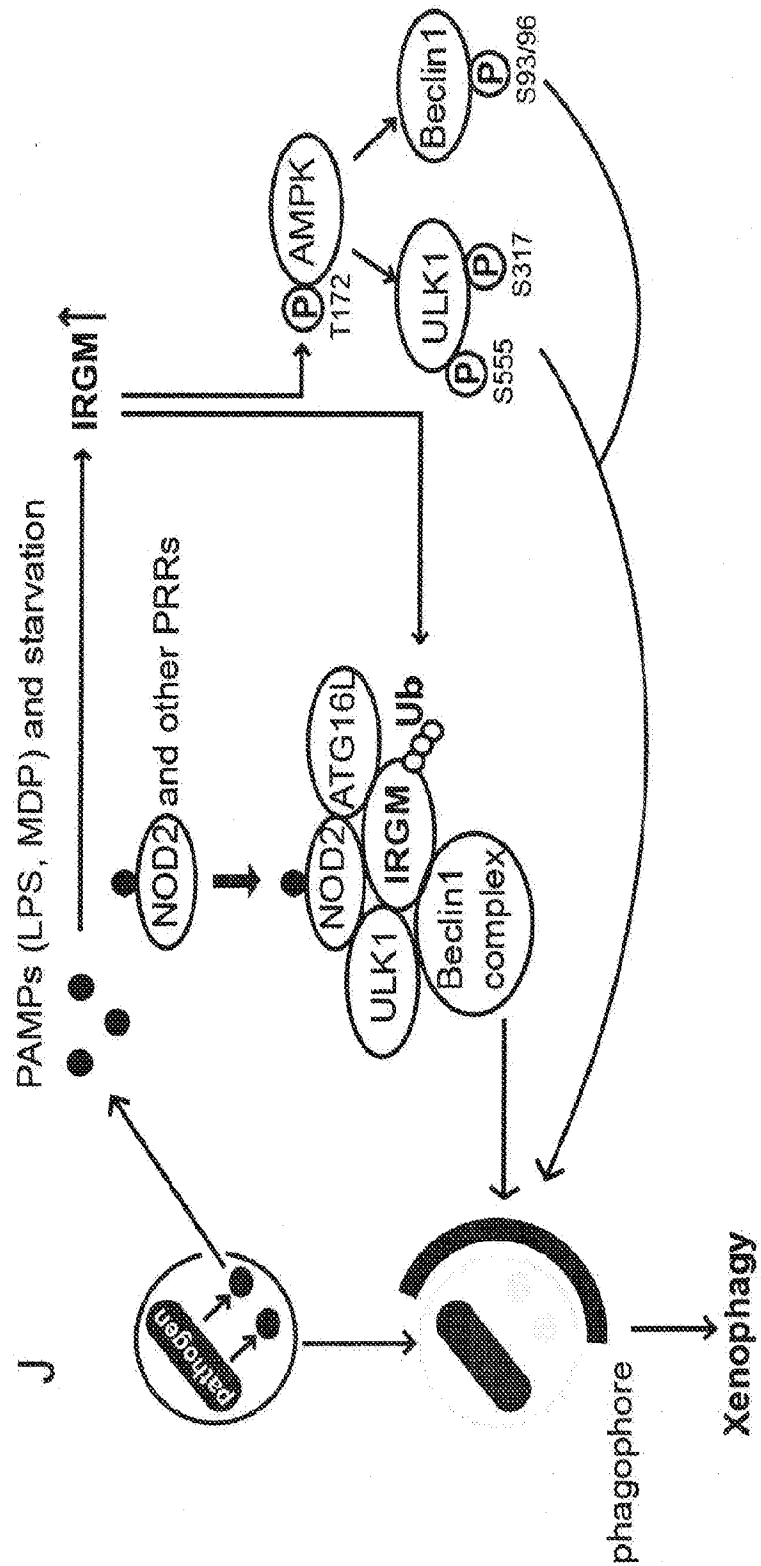


FIGURE S1 IRGM

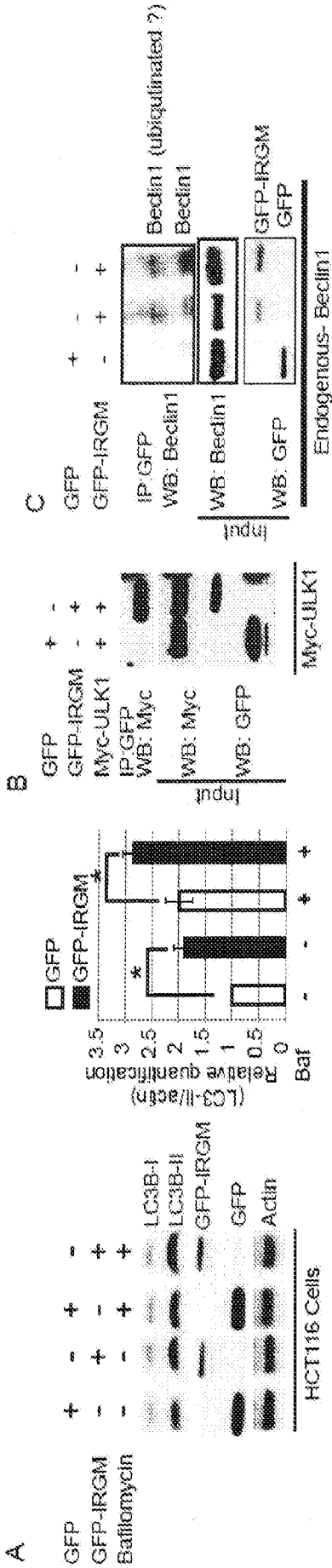


FIGURE S1 IRGM (CONT'D)

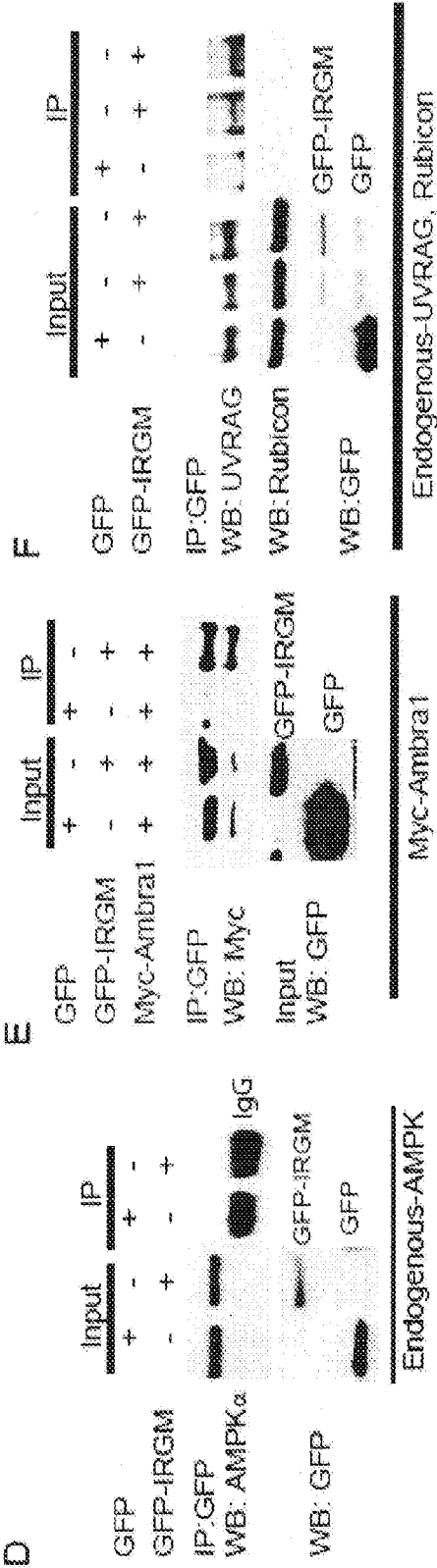


FIGURE S2 IRGM

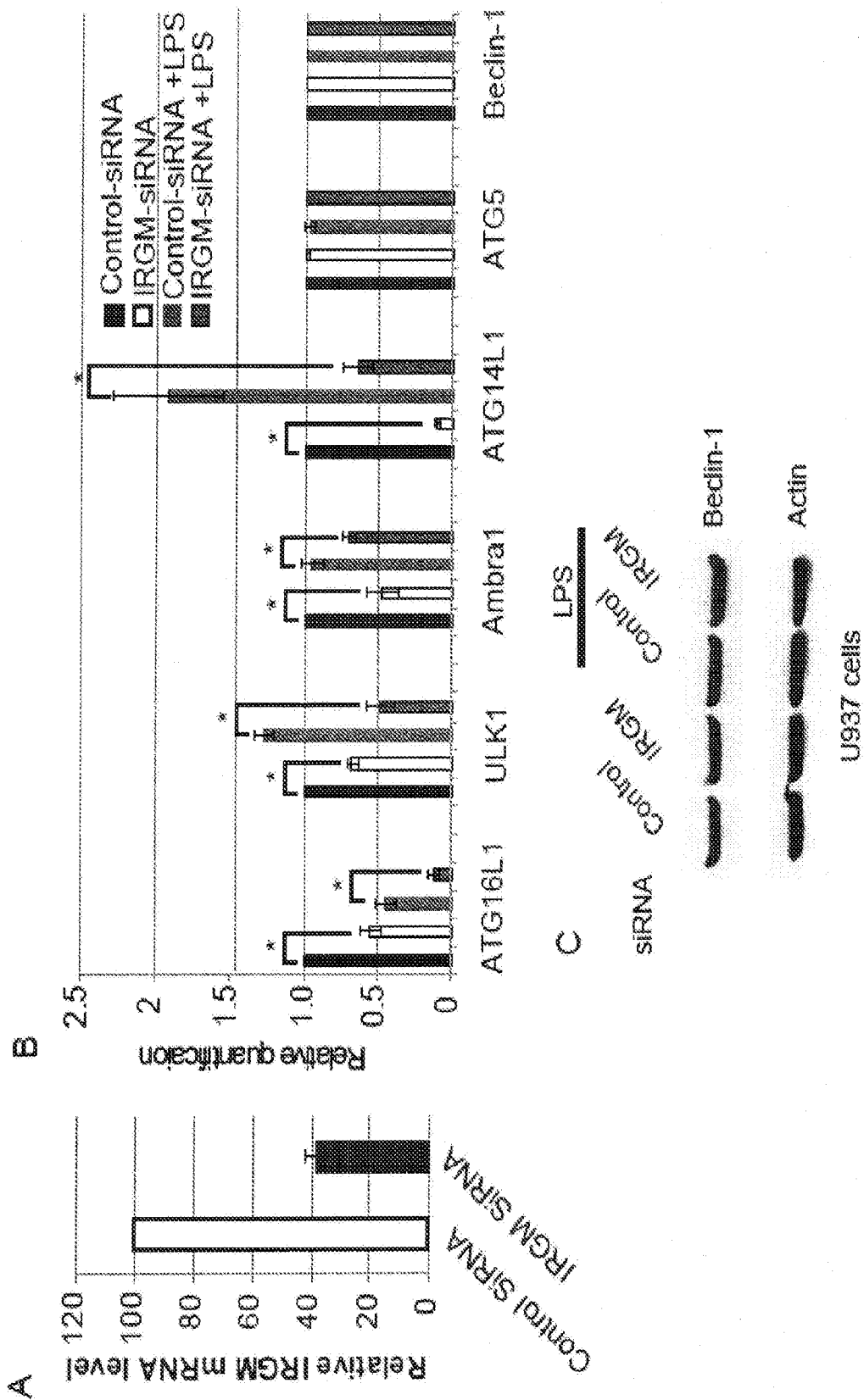


FIGURE S3 IRGM

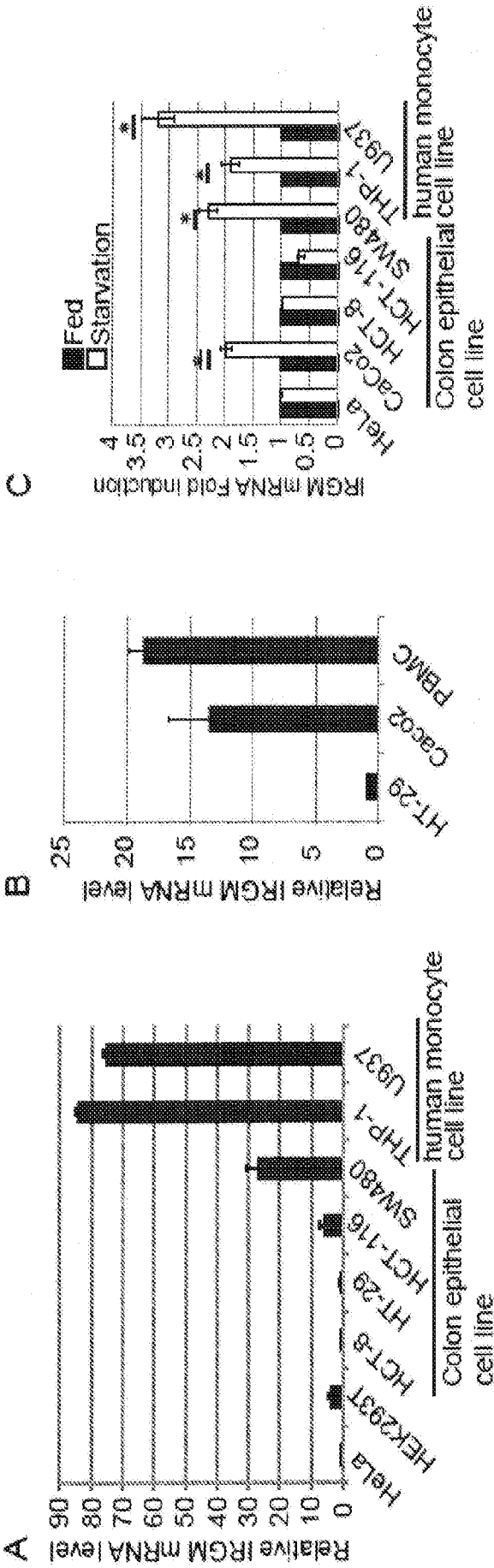
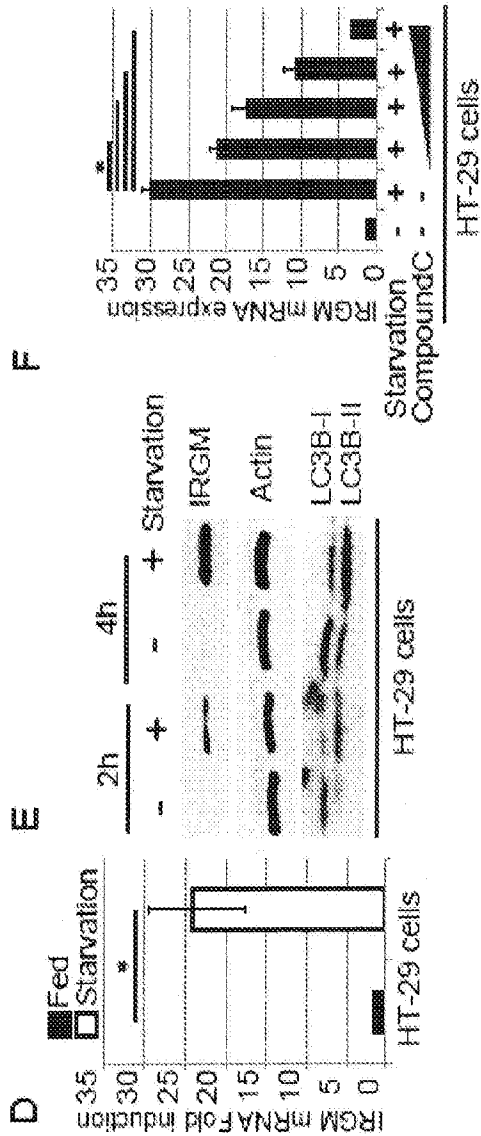


FIGURE S3 IRGM (CONT'D)



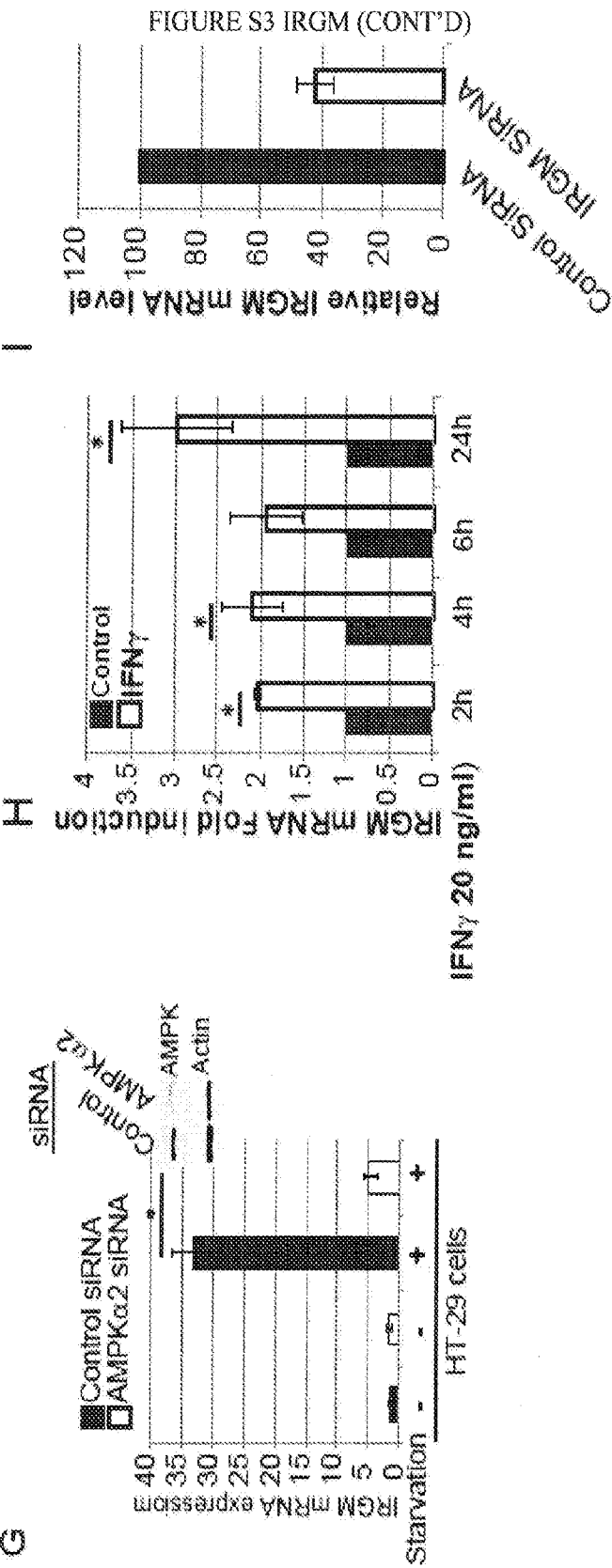


FIGURE S4 IRGM

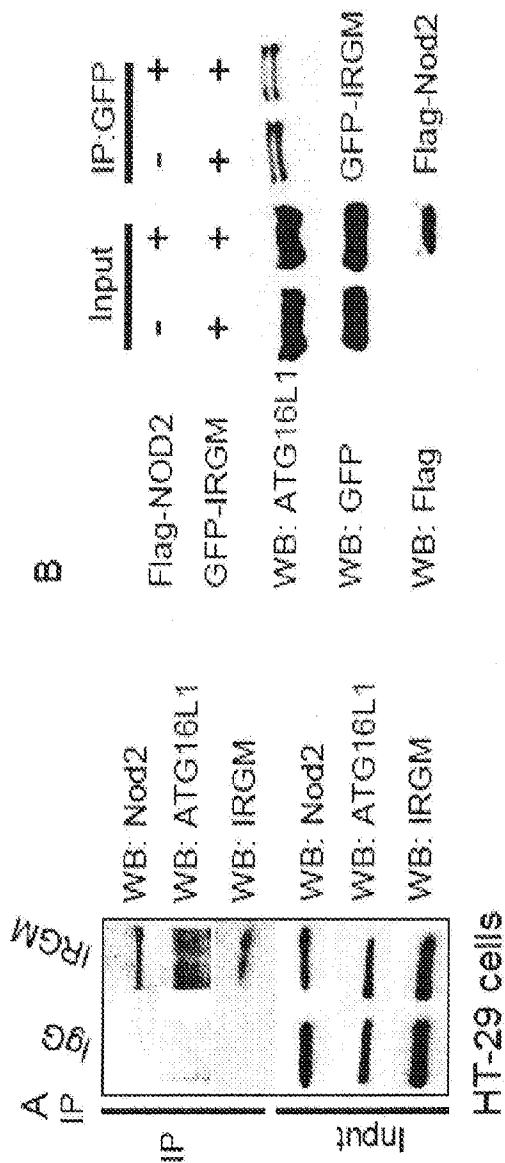


FIGURE S4 IRGM (CONT'D)

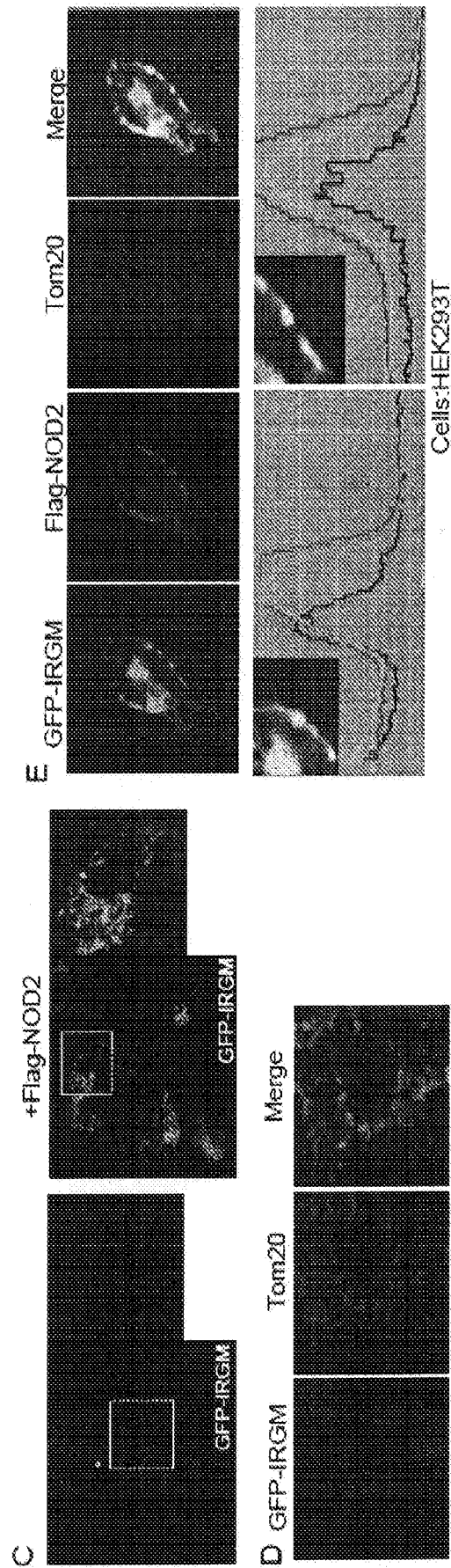


FIGURE S5 IRGM

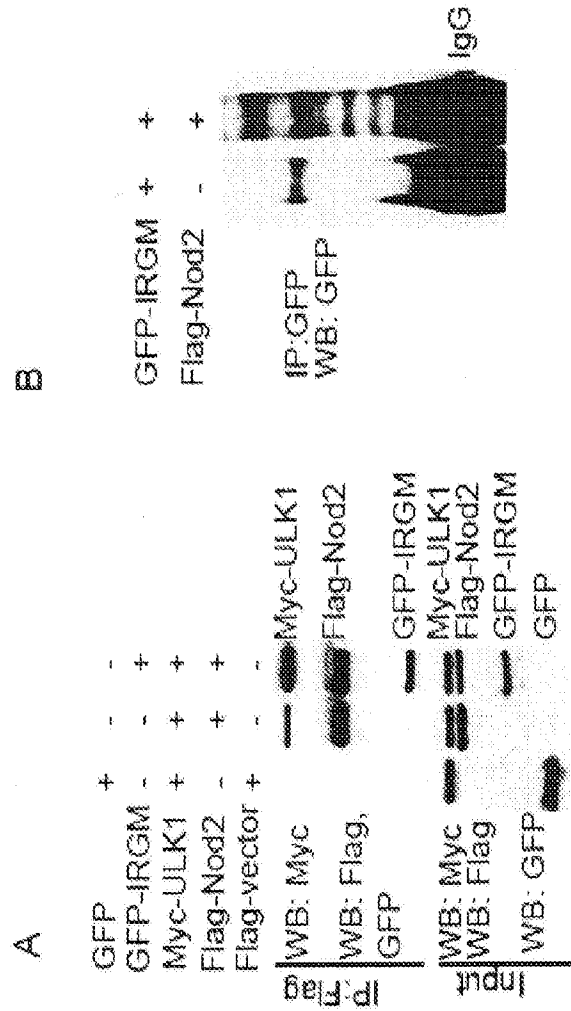


FIGURE S5 IRGM (CONT'D)

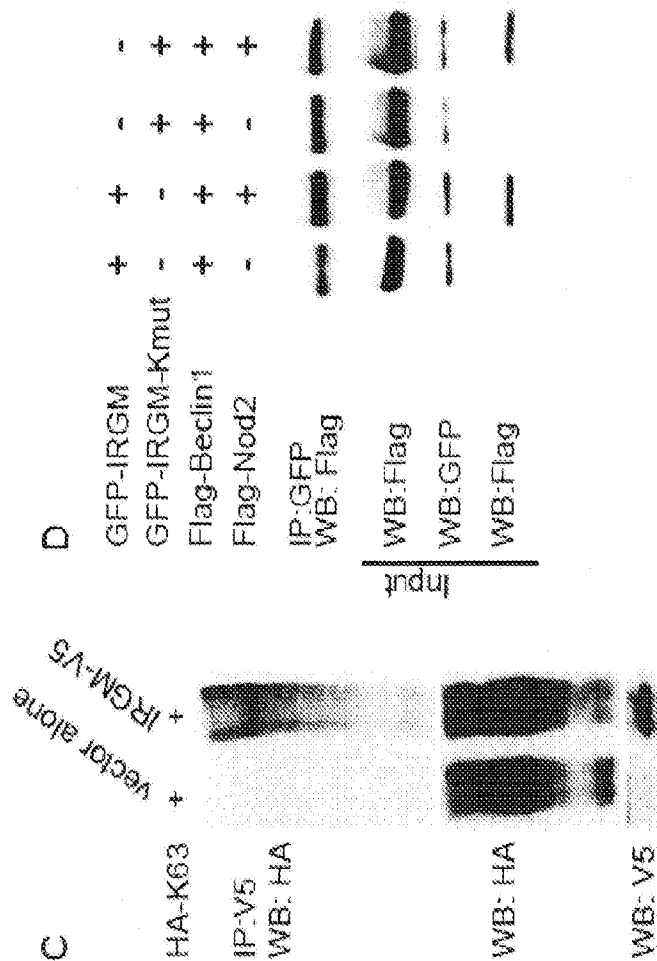


FIGURE S6 IRGM

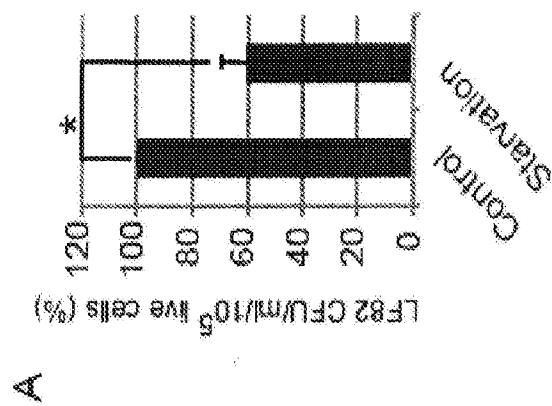
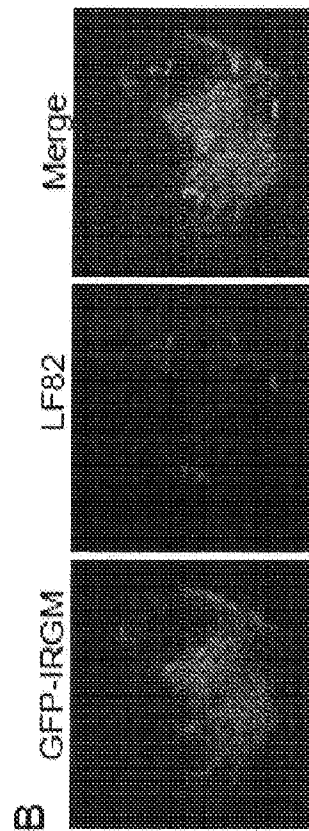


FIGURE S6 IRGM (CONT'D)



FIGURE 1 PRECISION

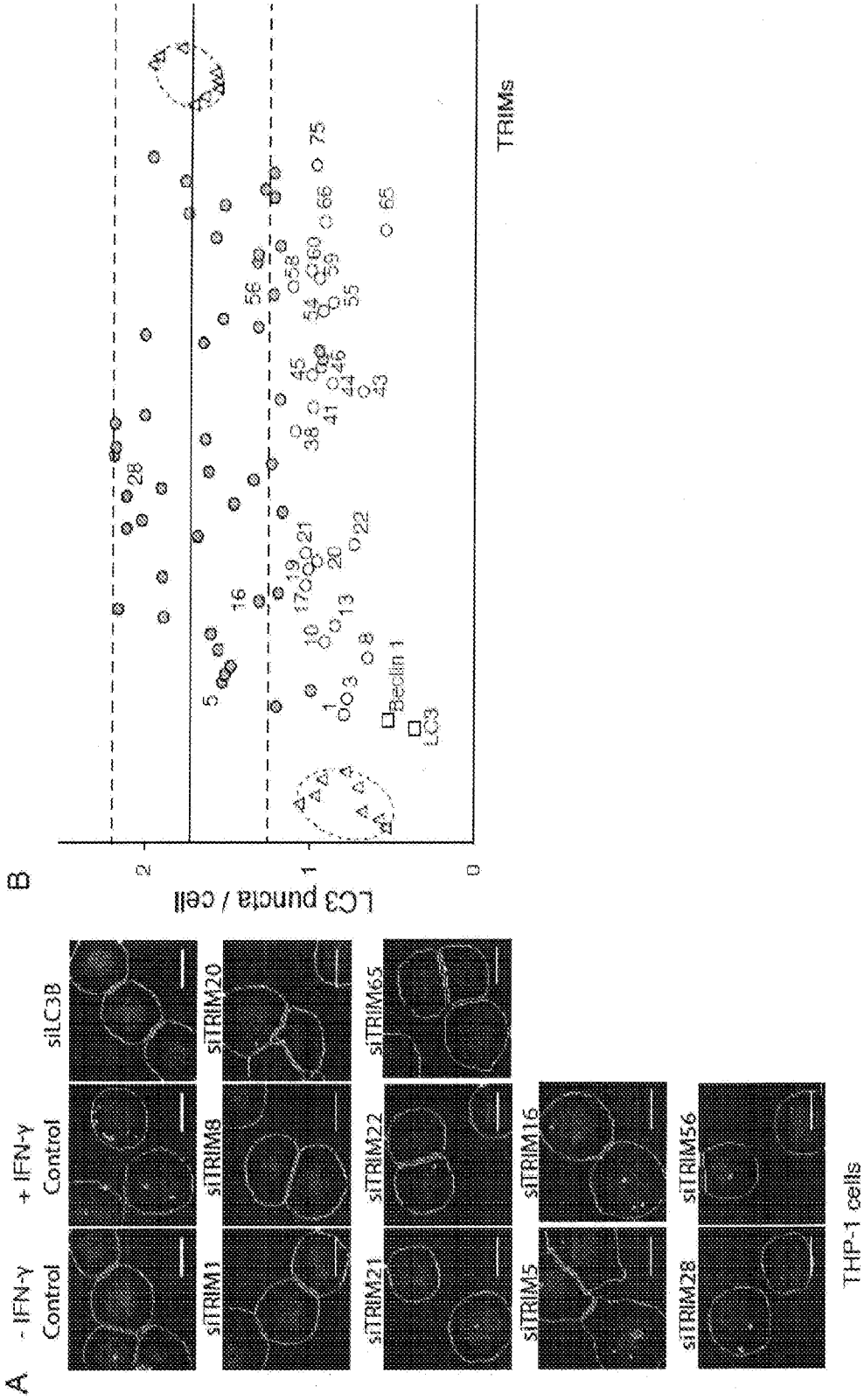


FIGURE 1 PRECISION (CONT'D)

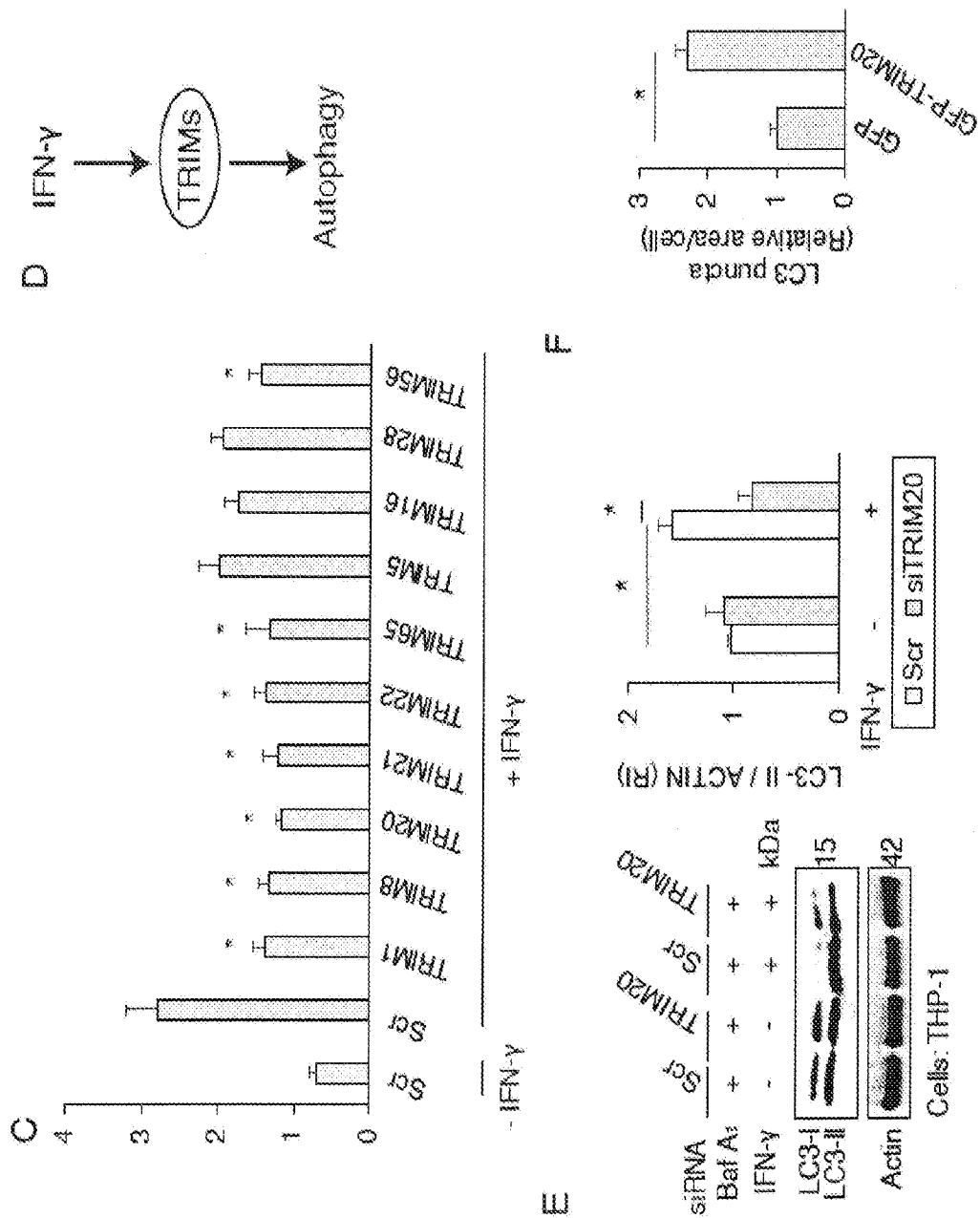


FIGURE 2 PRECISION

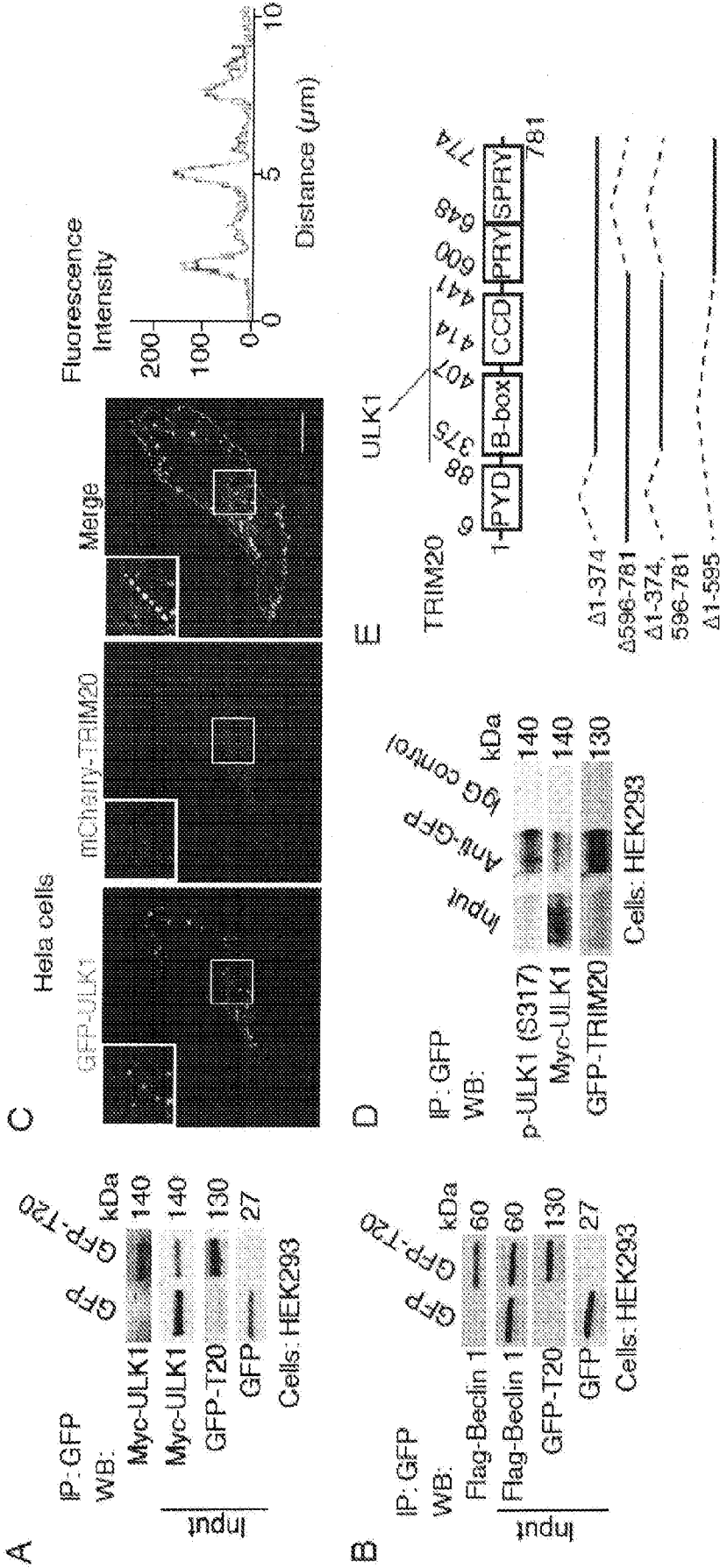


FIGURE 2 PRECISION (CONT'D)

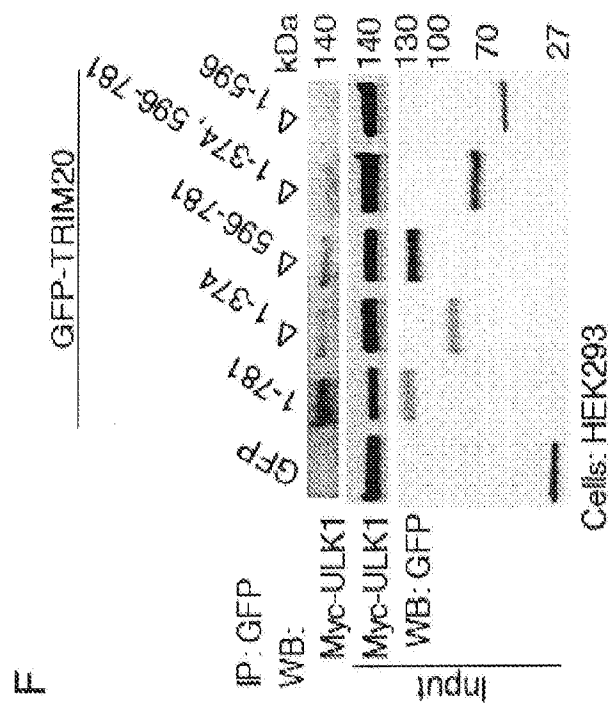
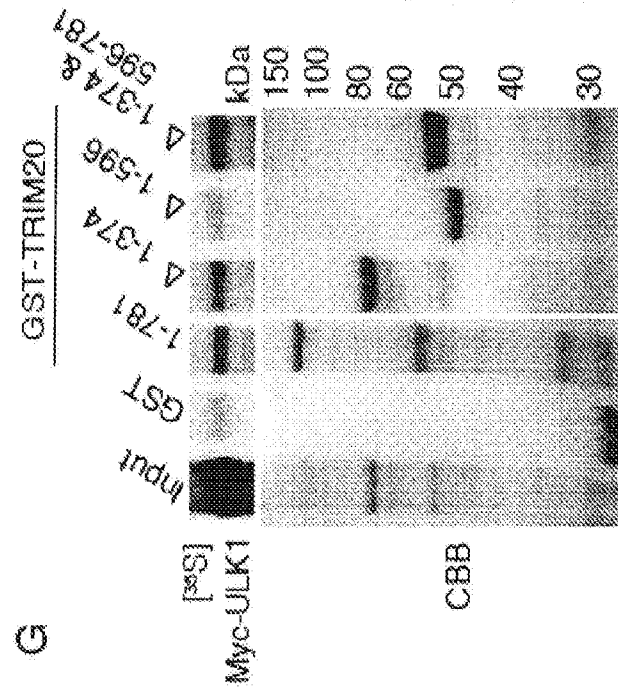


FIGURE 3 PRECISION

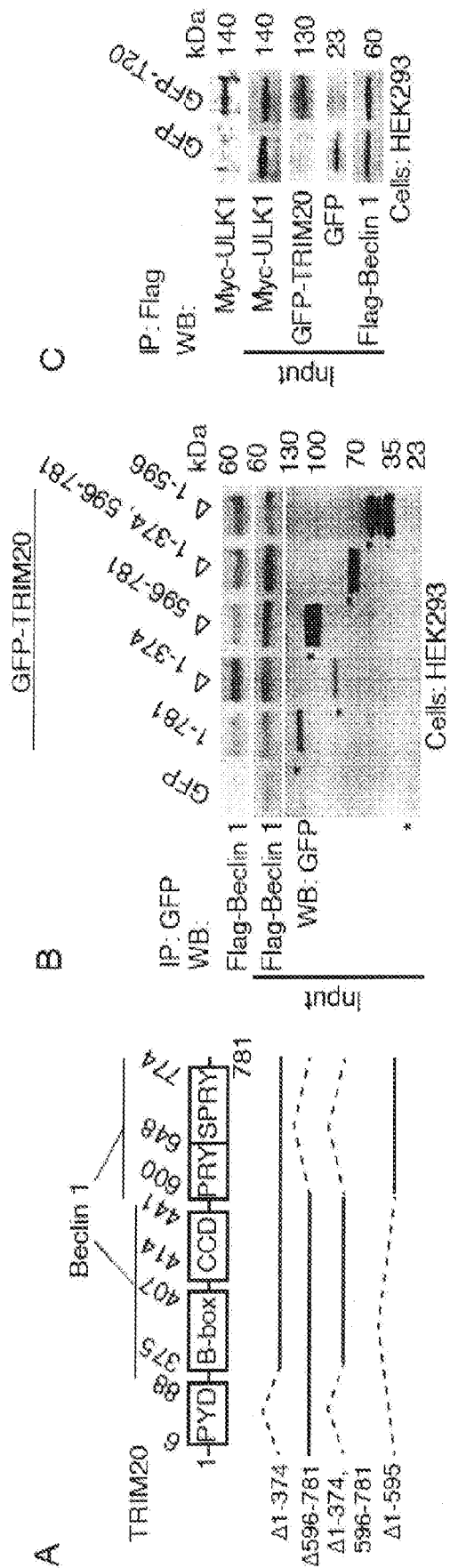


FIGURE 3 PRECISION (CONT'D)

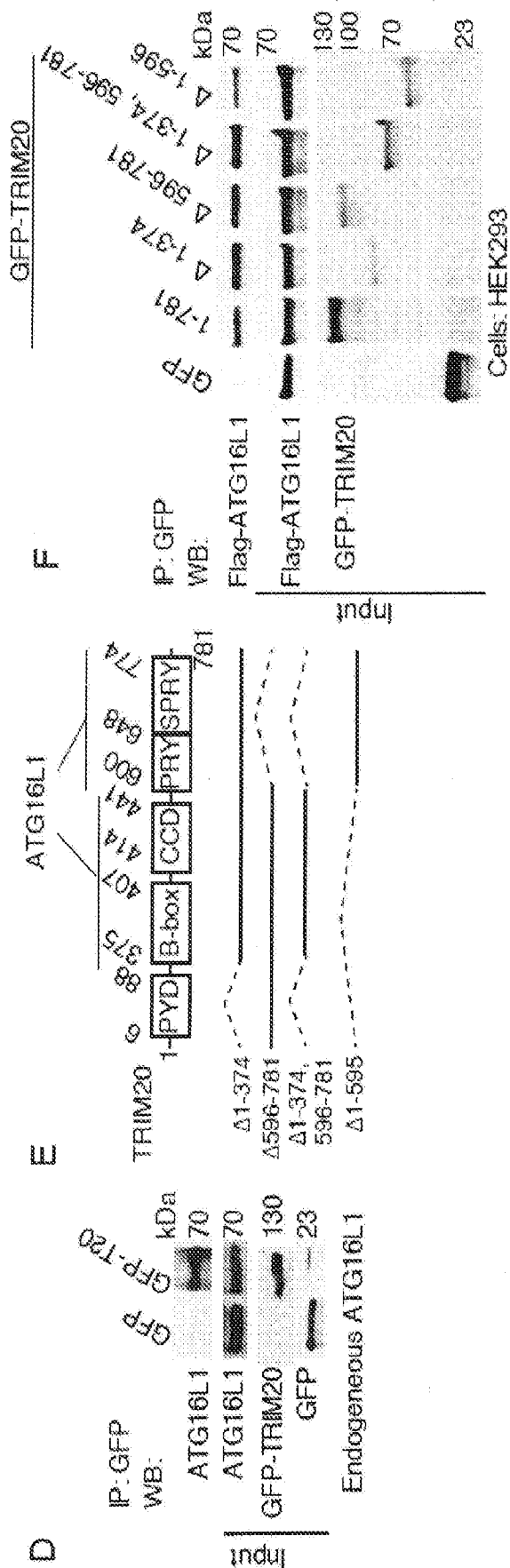


FIGURE 3 PRECISION (CONT'D)

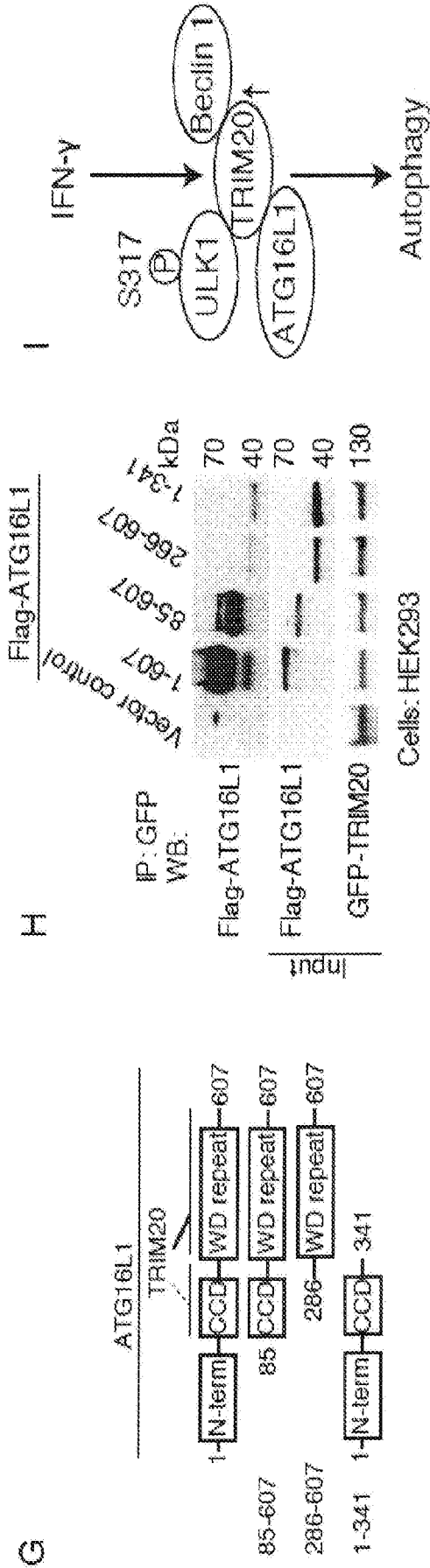
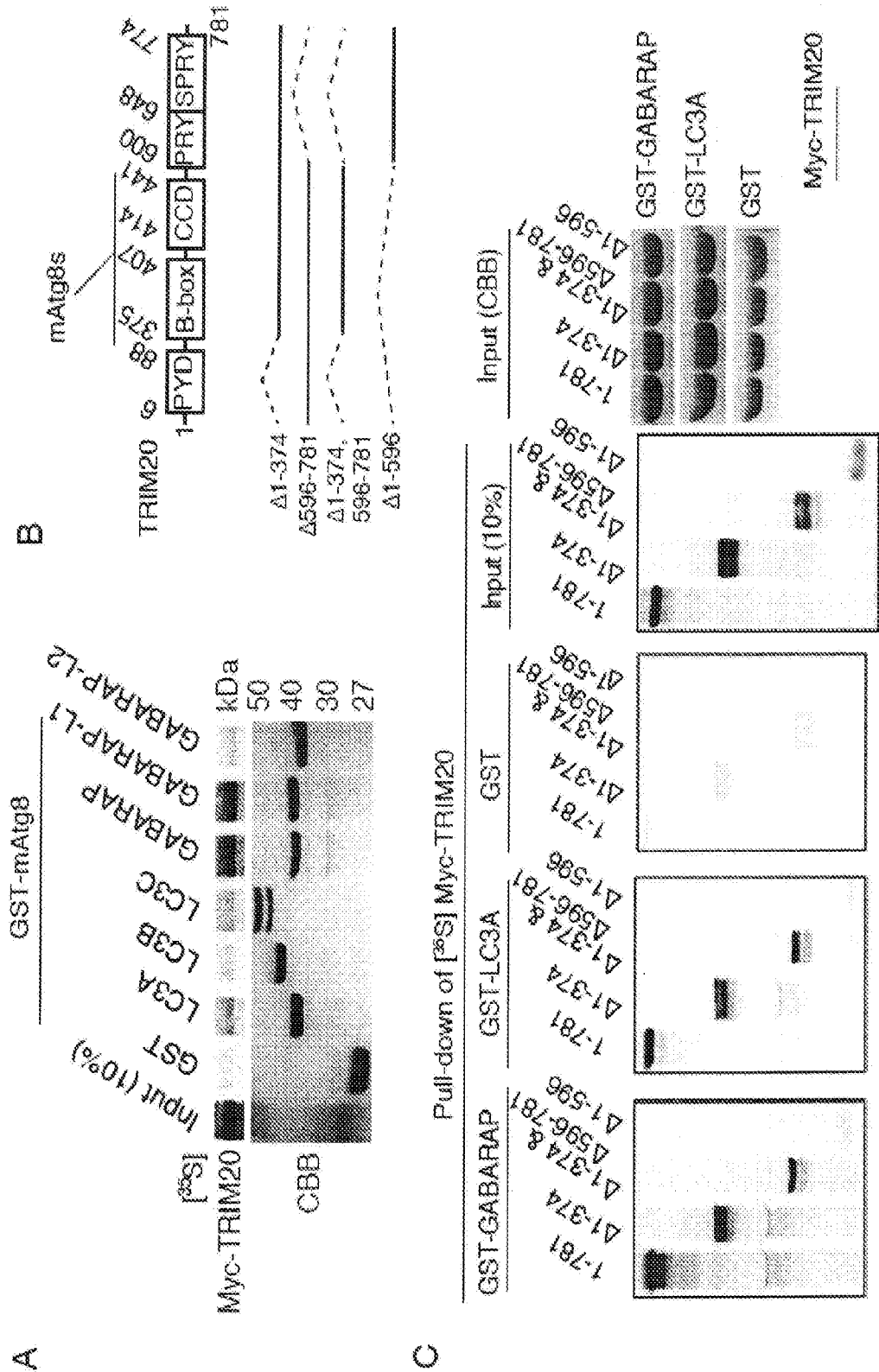


FIGURE 4 PRECISION



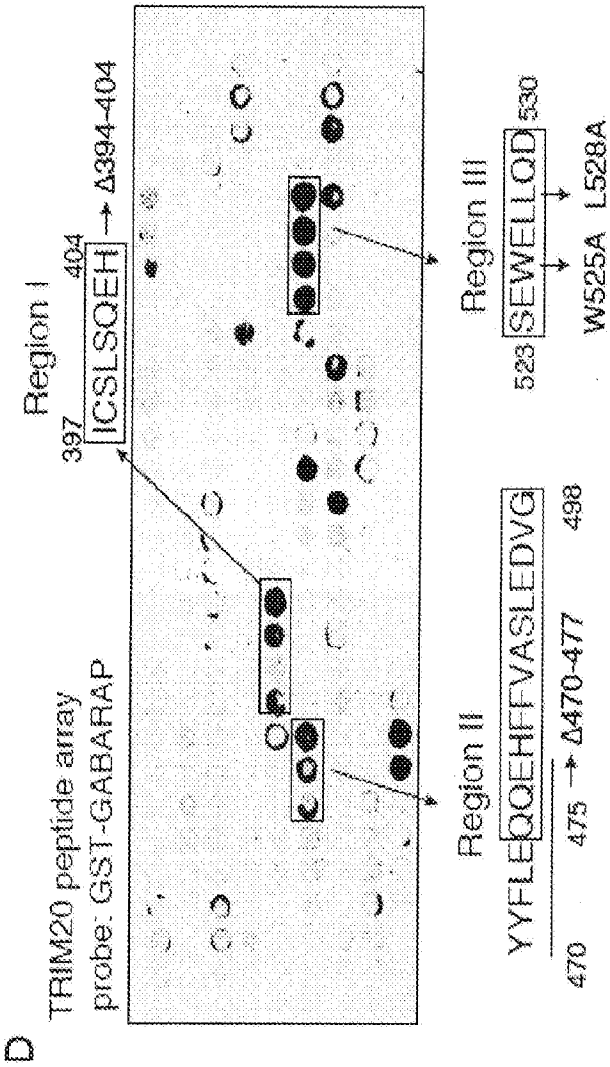
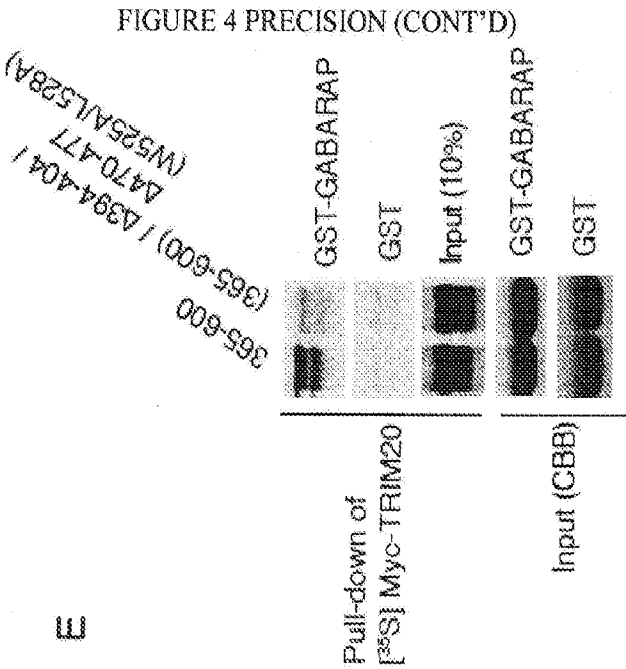


FIGURE 5 PRECISION

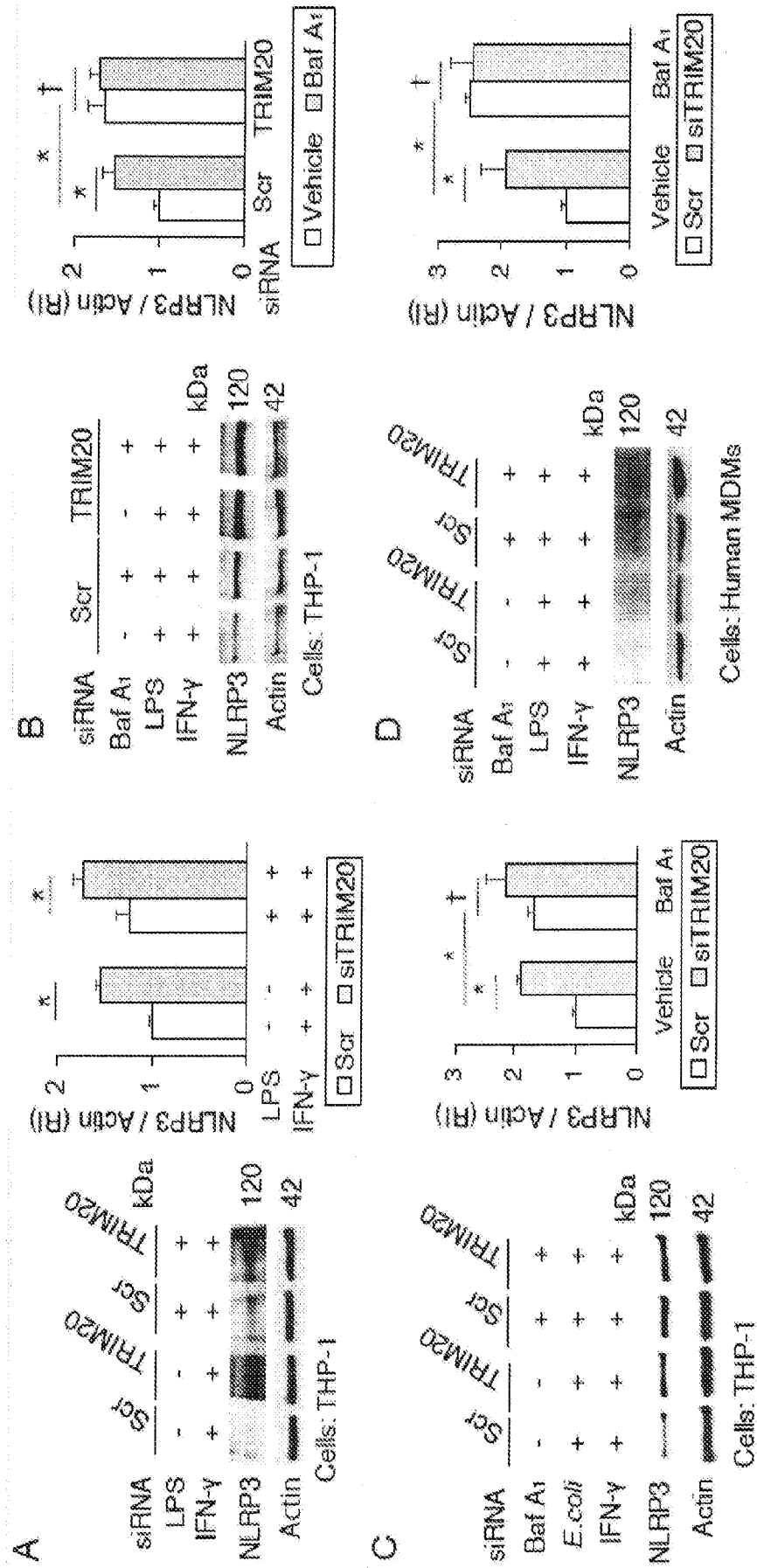


FIGURE 5 PRECISION (CONT'D)

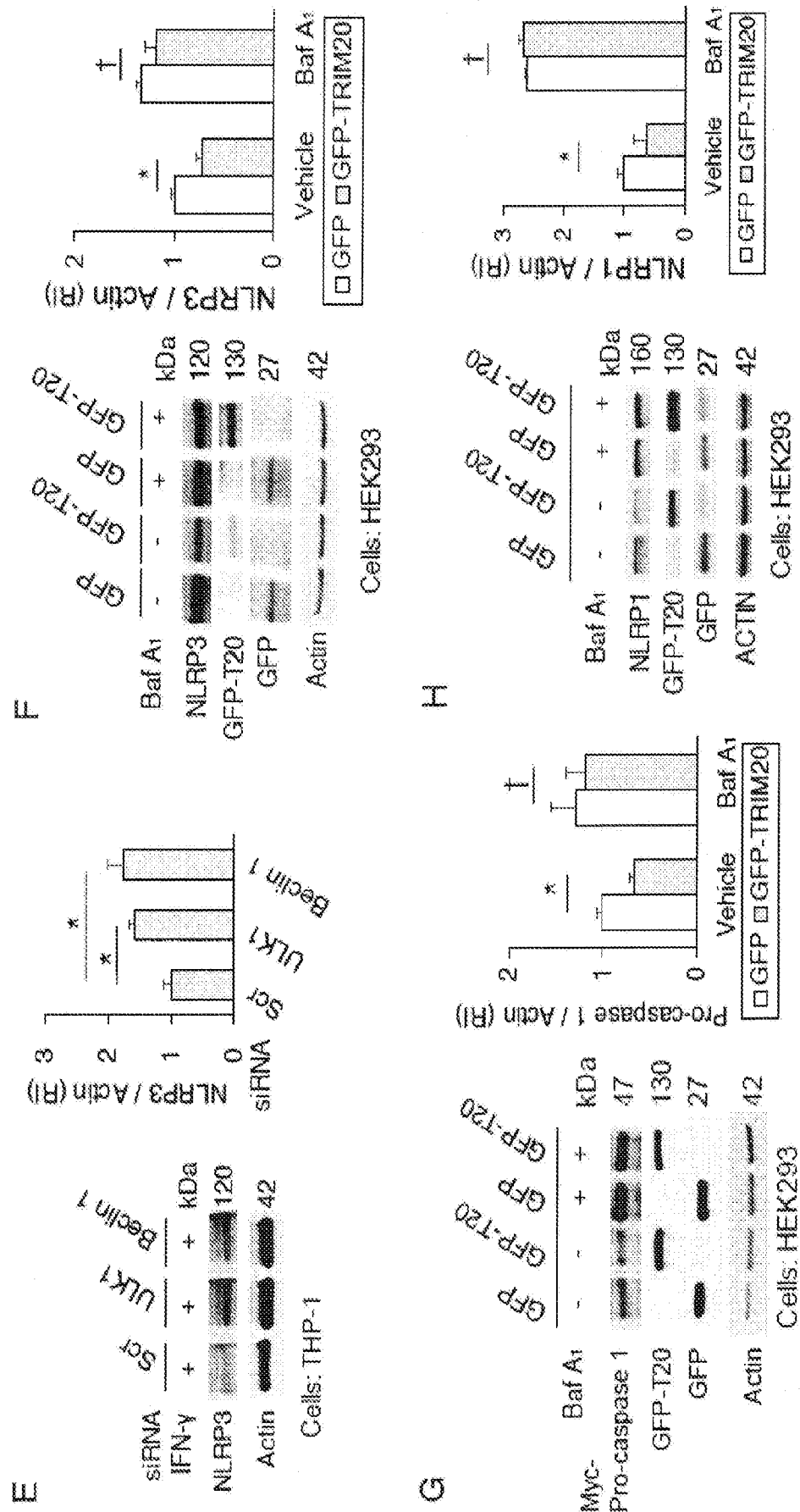


FIGURE 6 PRECISION

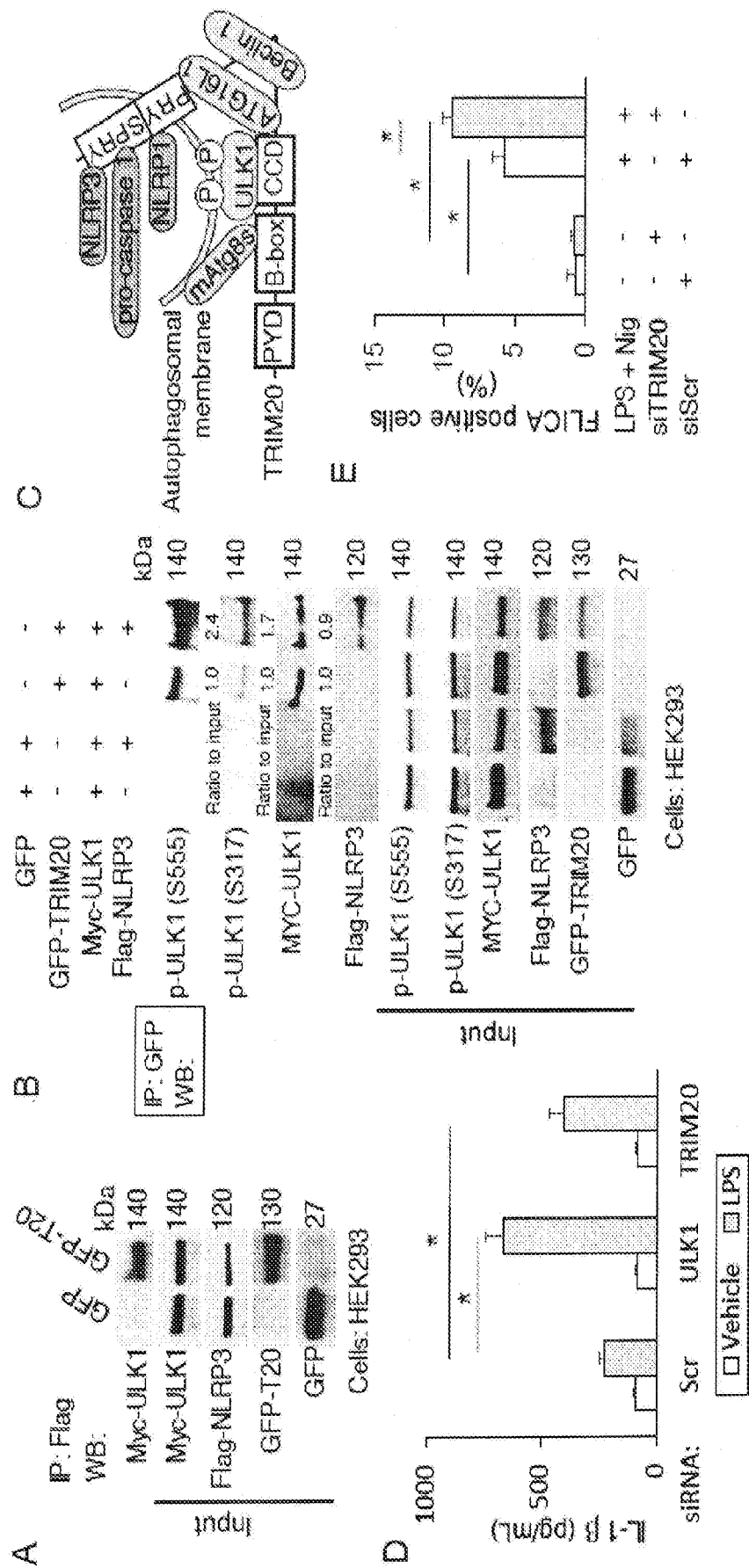


FIGURE 6 PRECISION (CONT'D)

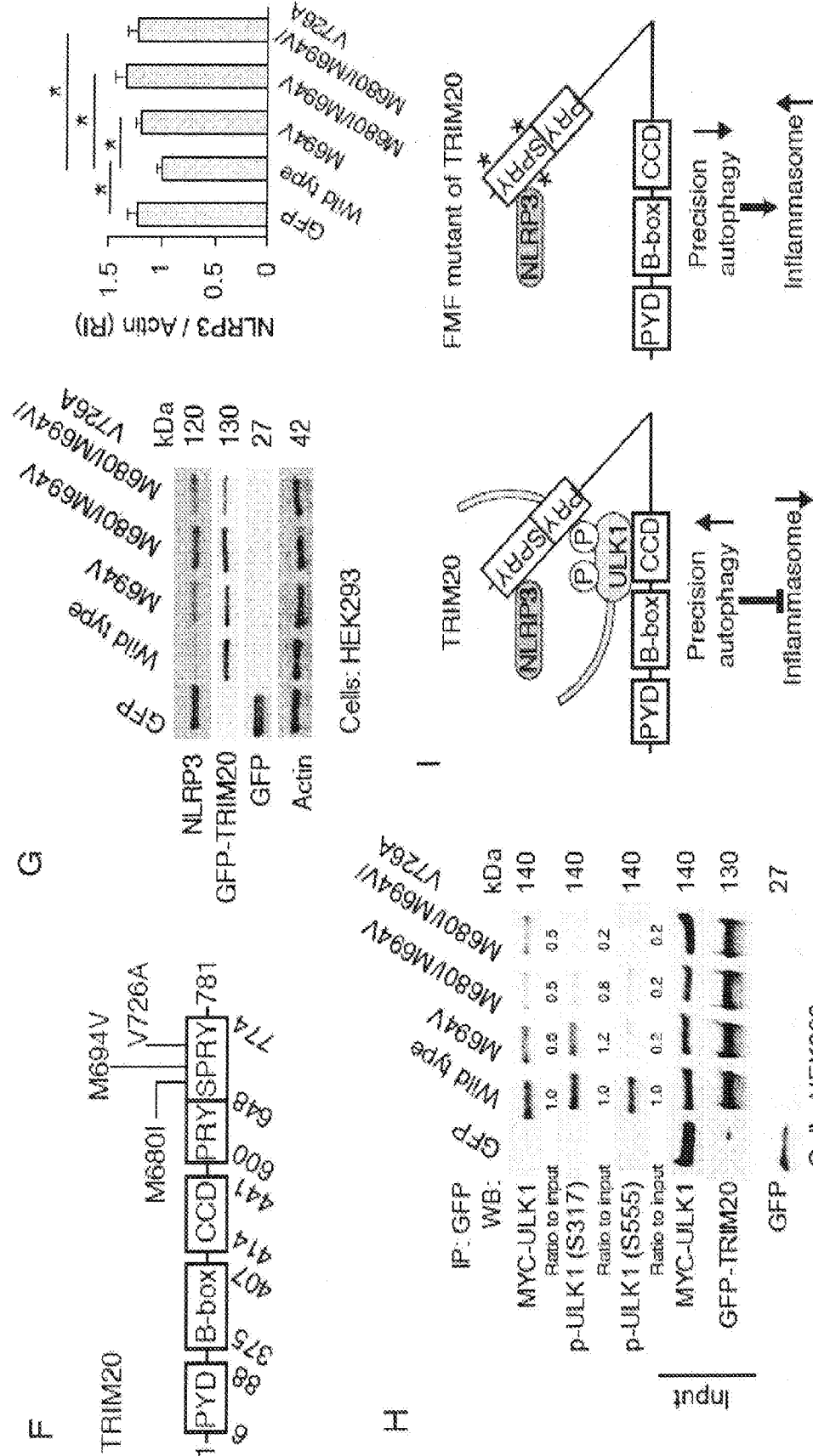


FIGURE 7 PRECISION

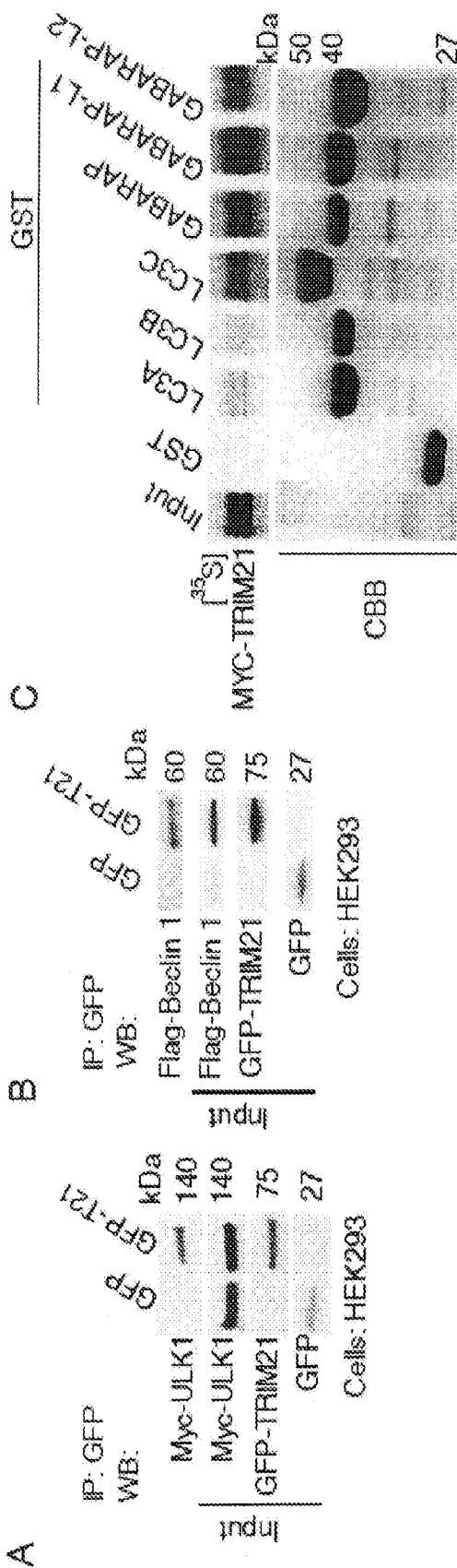


FIGURE 7 PRECISION (CONT'D)

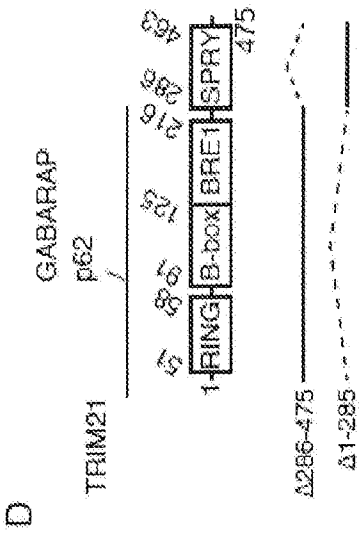
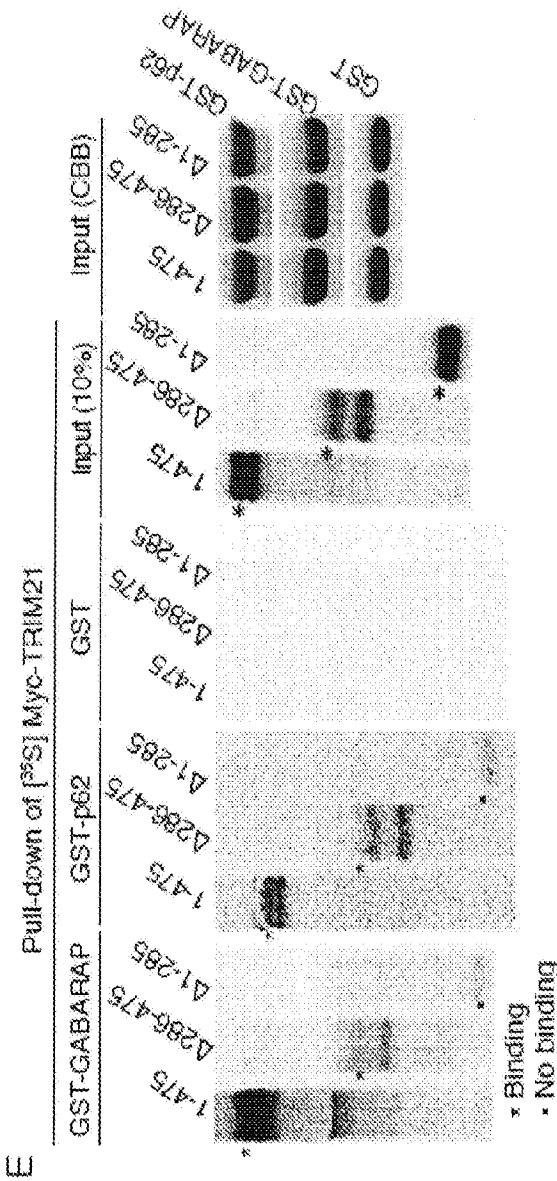


FIGURE 7 PRECISION (CONT'D)

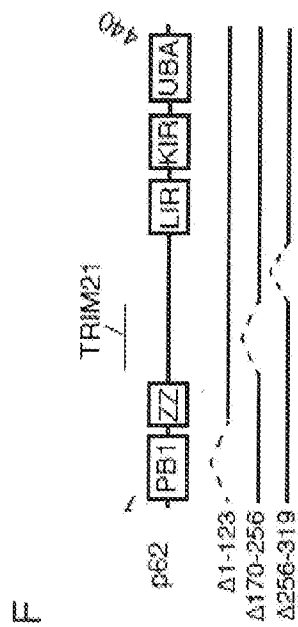
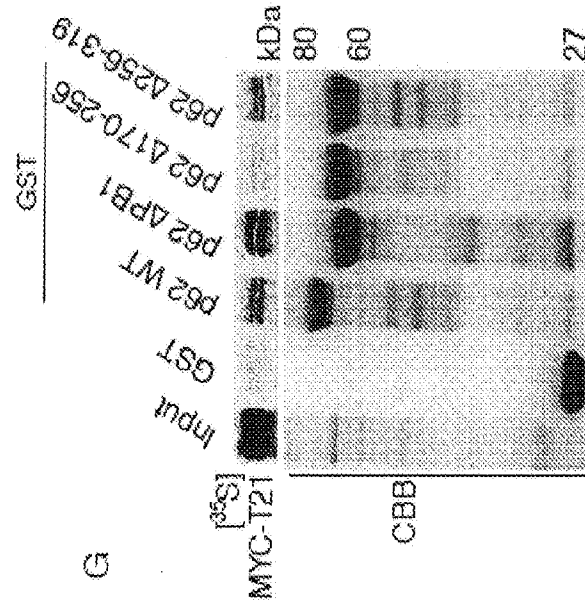
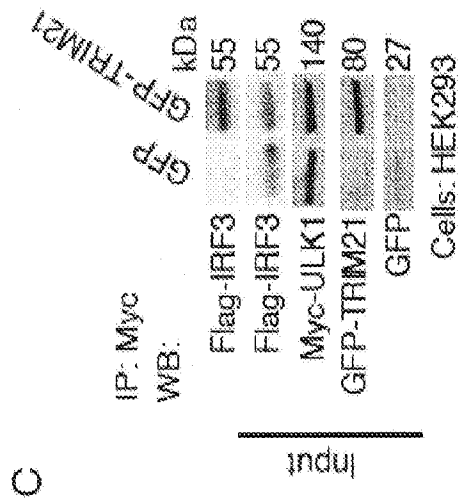


FIGURE 8 PRECISION



C

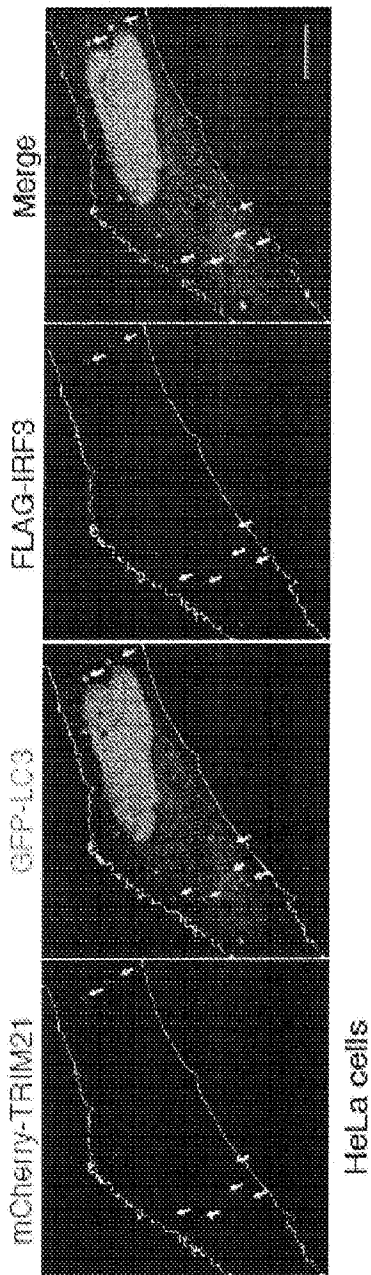


FIGURE 8 PRECISION (CONT'D)

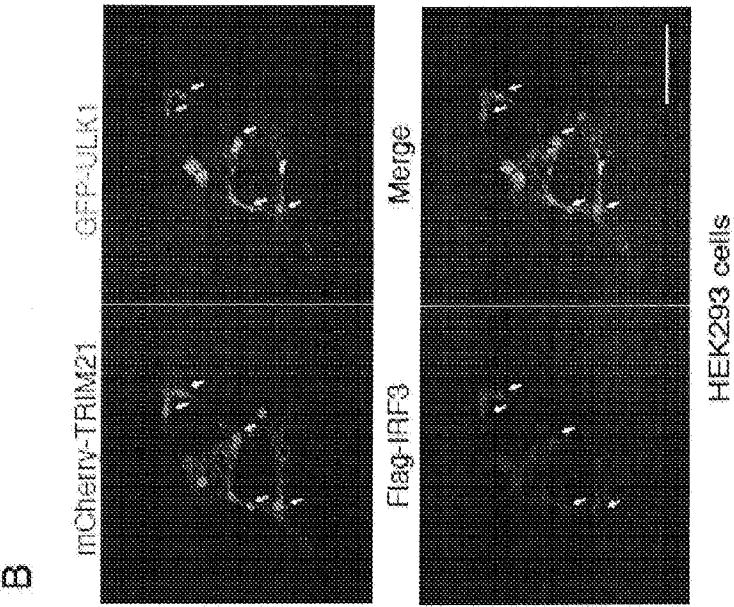
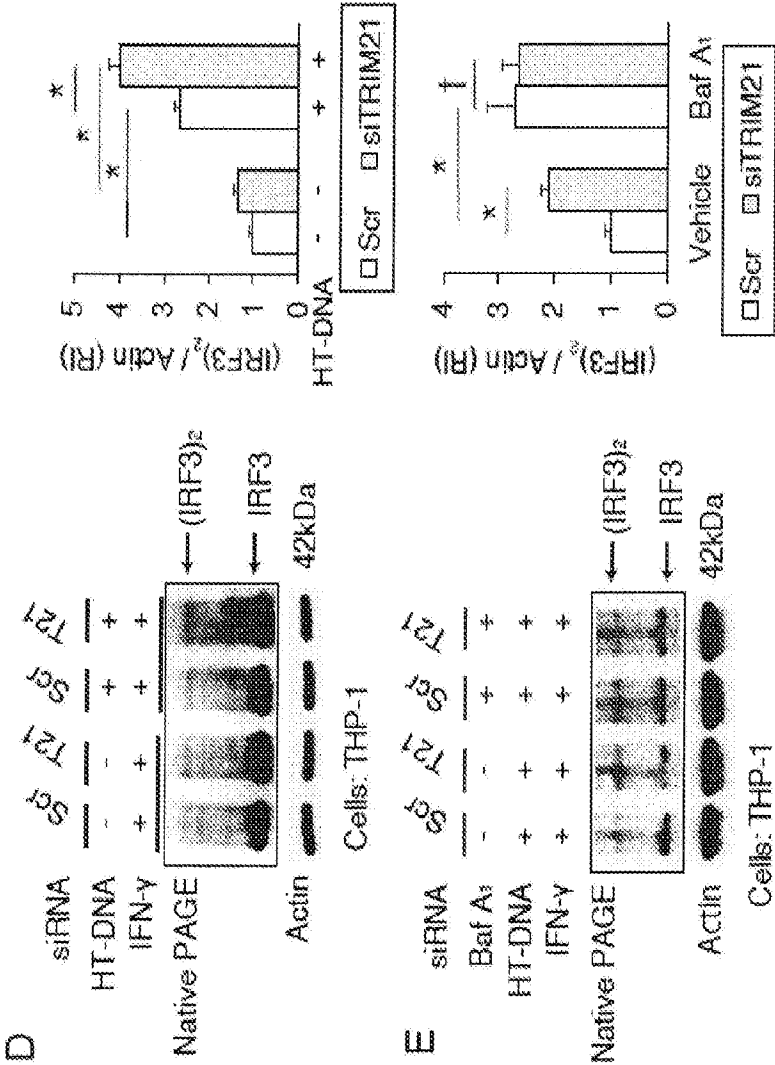


FIGURE 8 PRECISION (CONT'D)

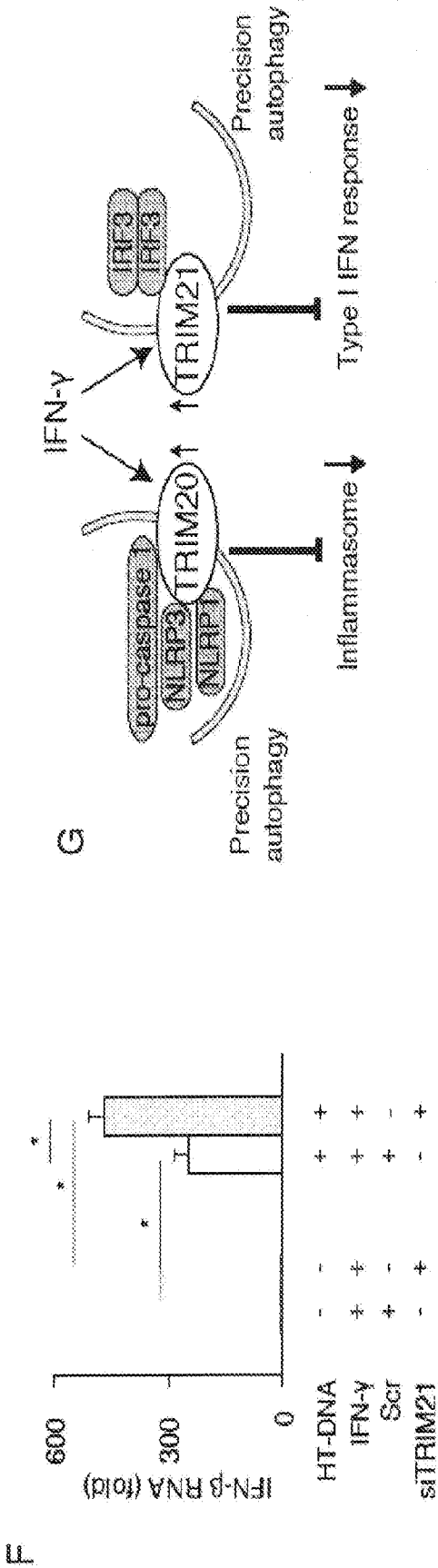


FIGURE S1 PRECISION

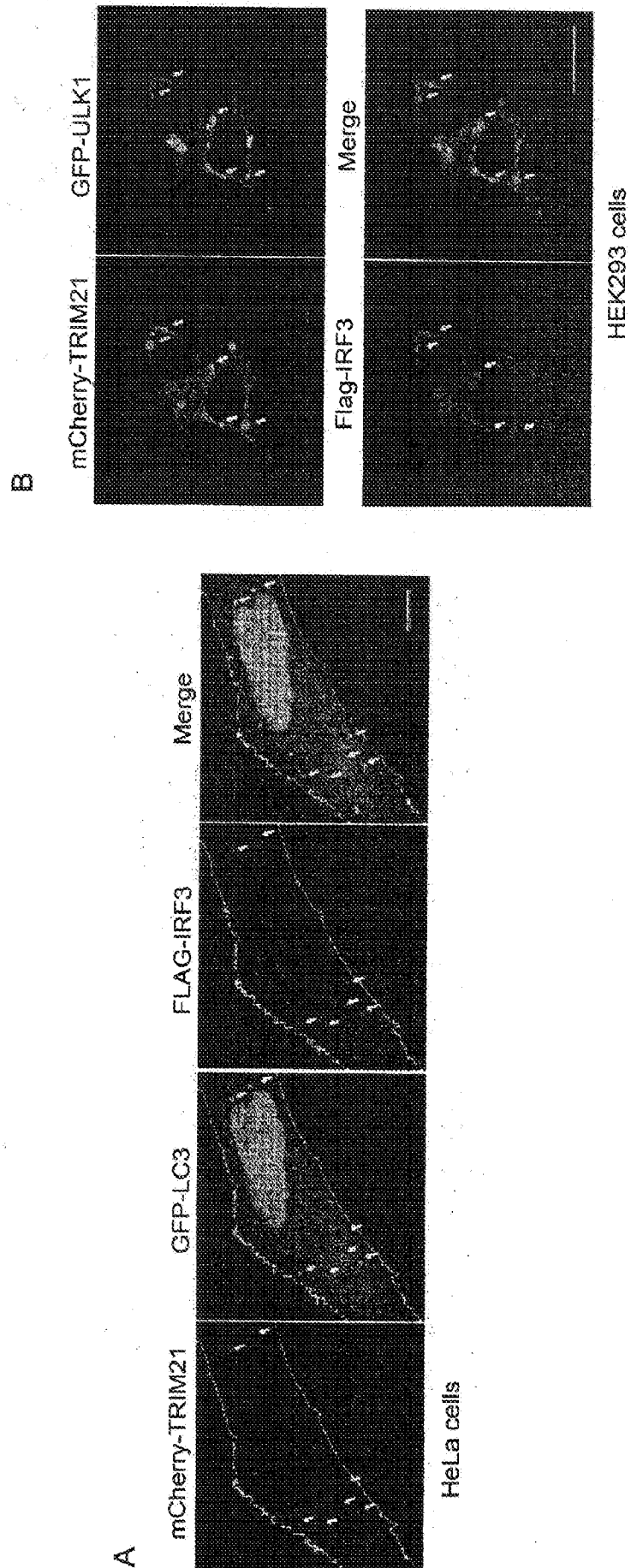


FIGURE S1 PRECISION (CONT'D)

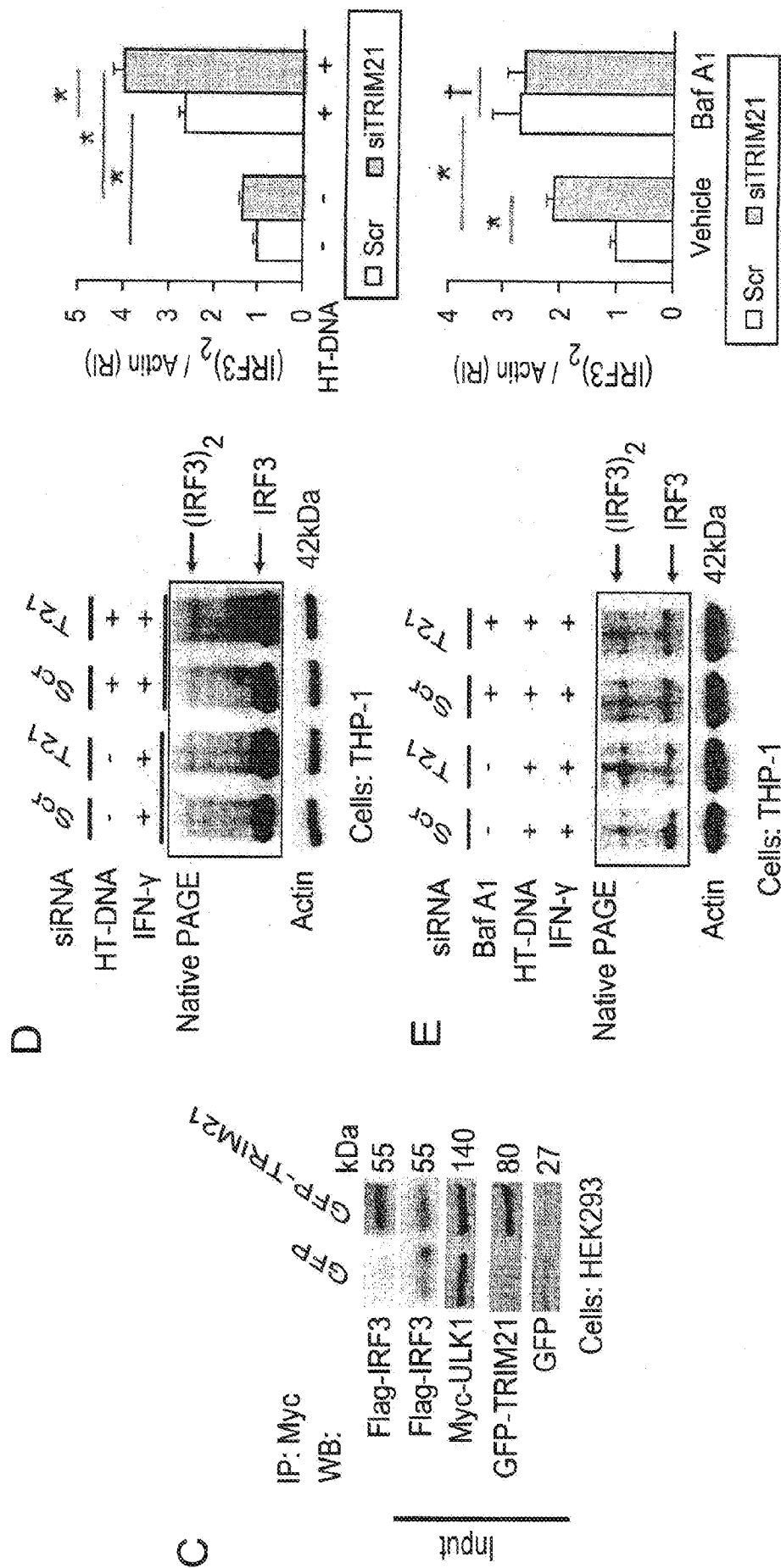


FIGURE S1 PRECISION (CONT'D)

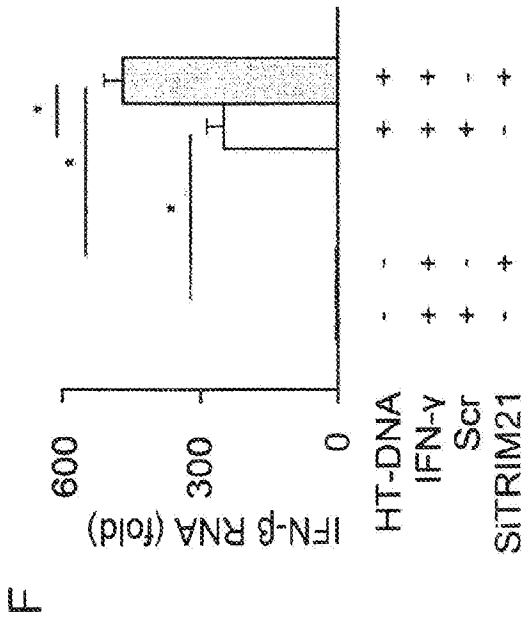
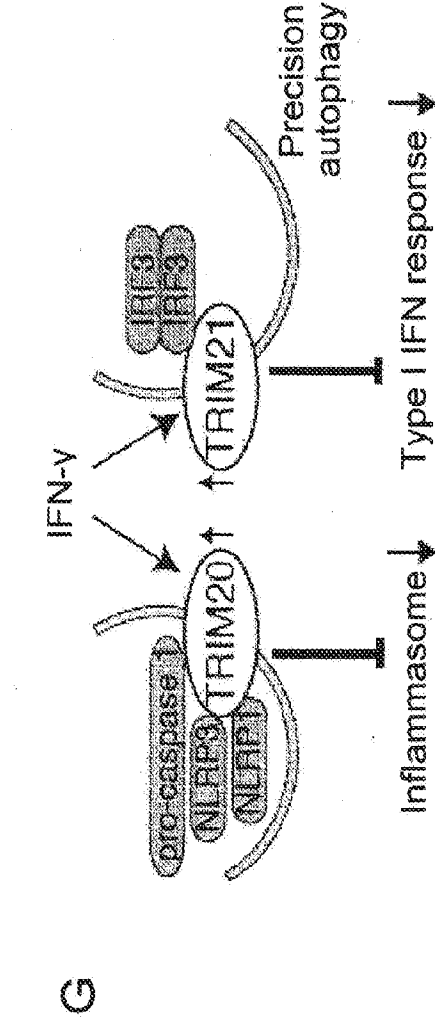


FIGURE S2 PRECISION

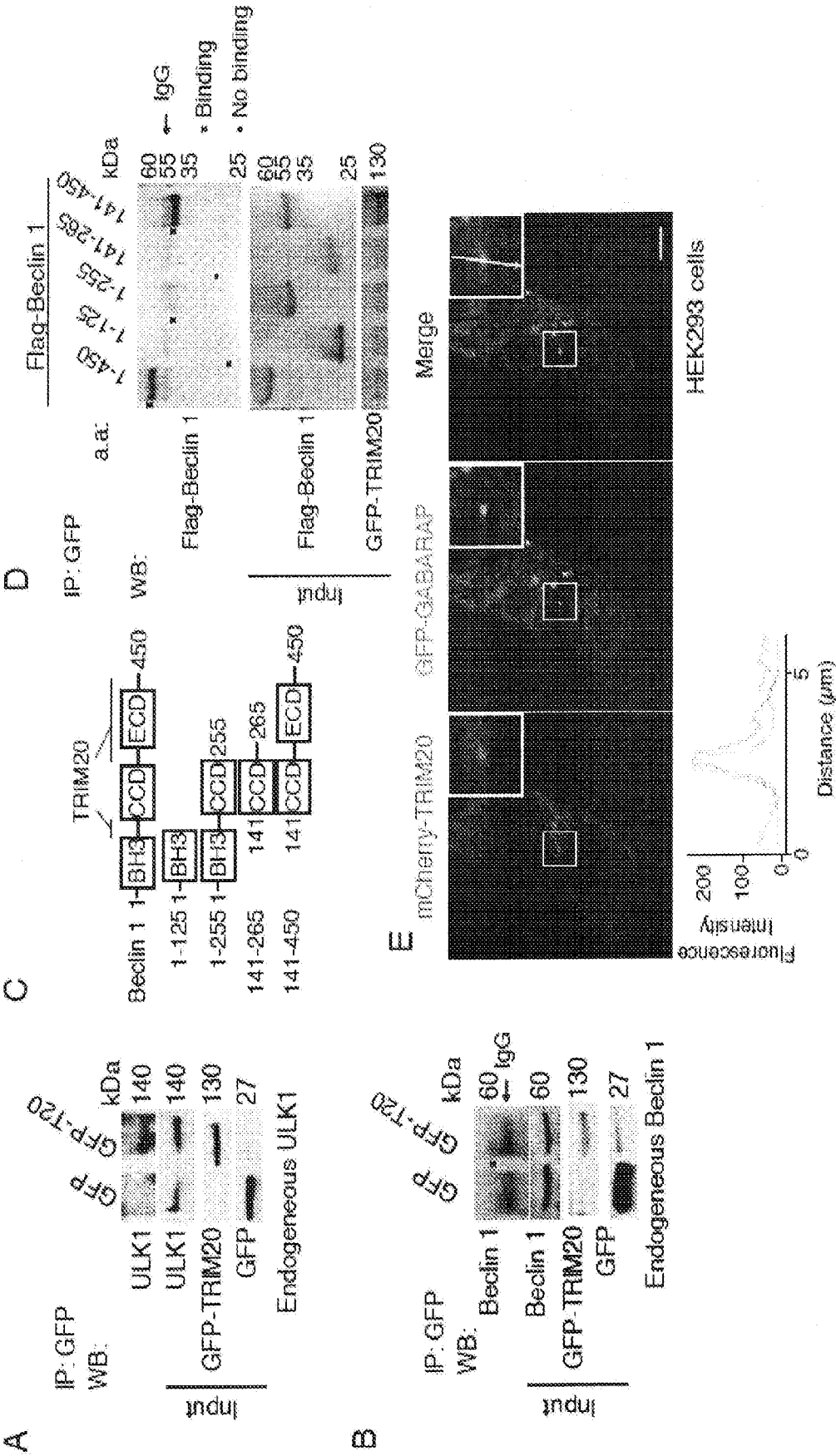


FIGURE S2 PRECISION (CONT'D)

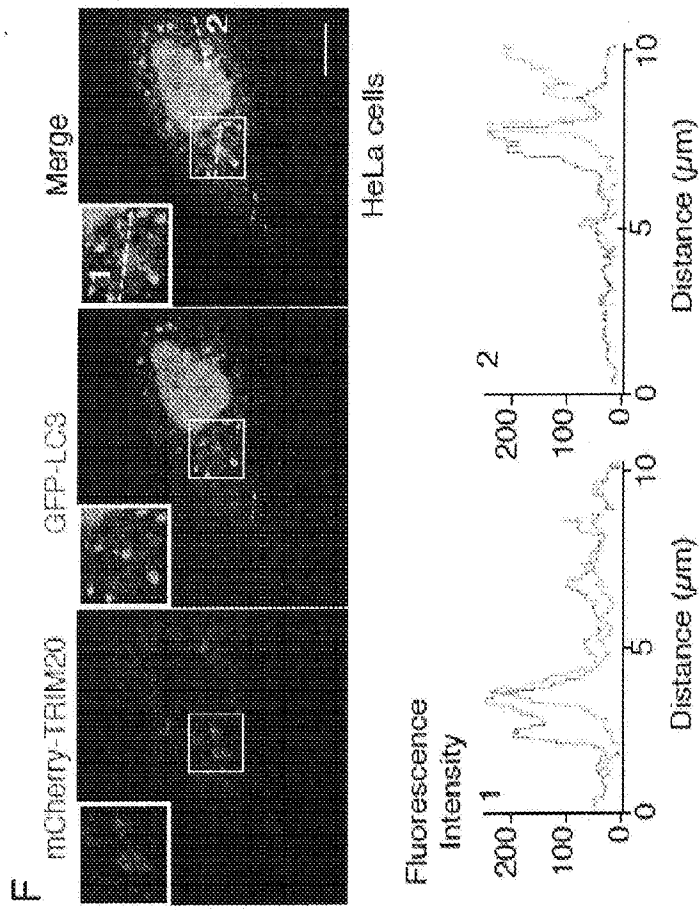
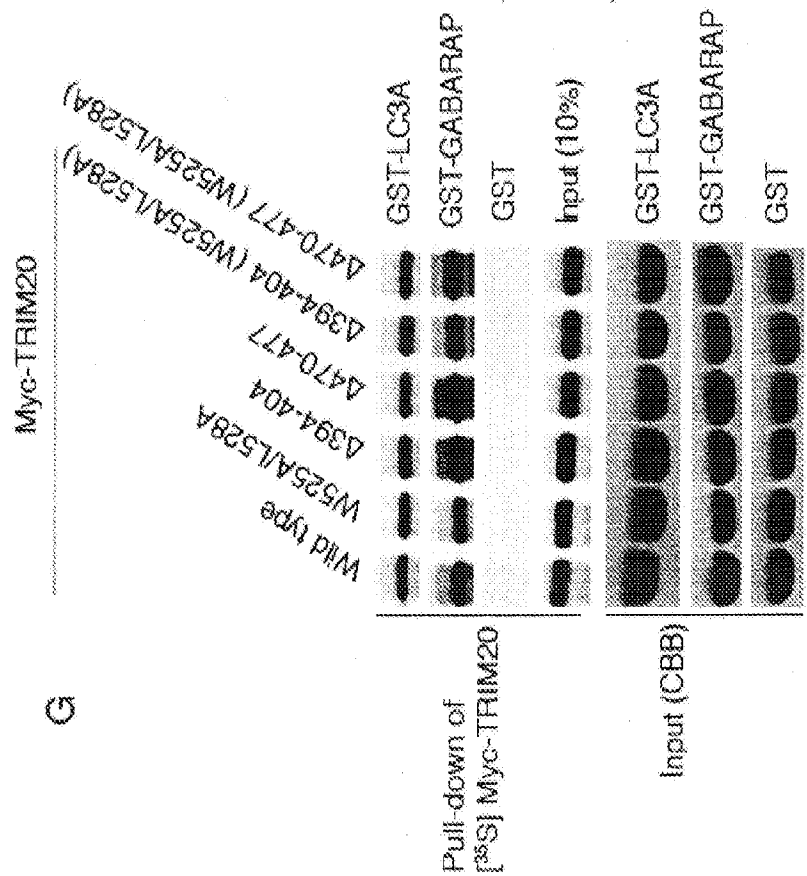


FIGURE S3 PRECISION

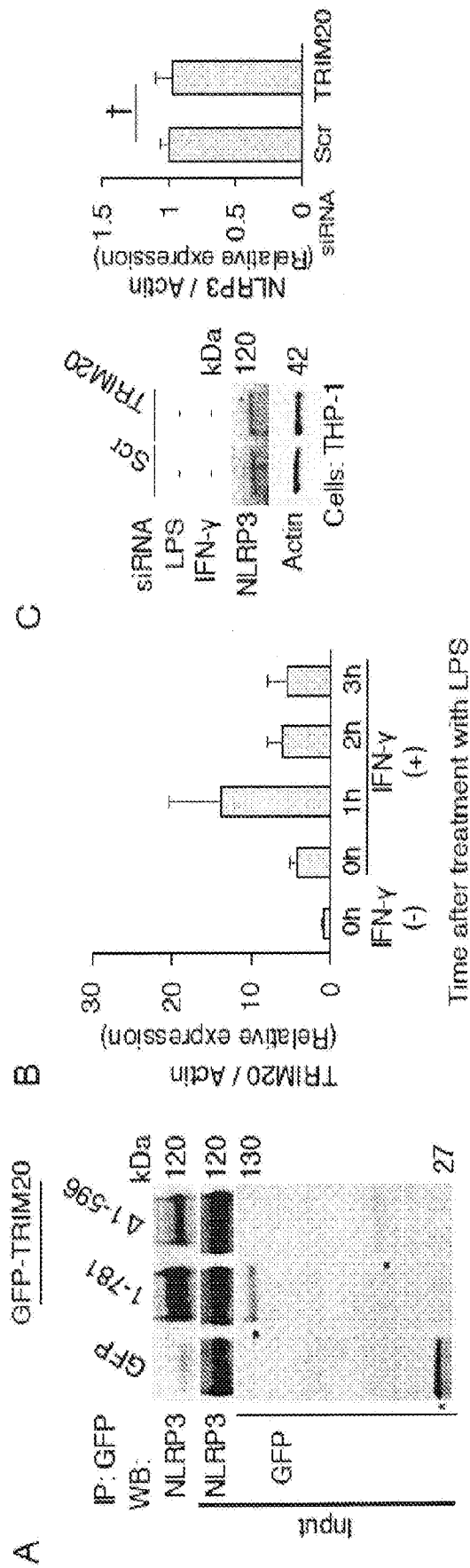


FIGURE S3. PRECISION (CONT'D)

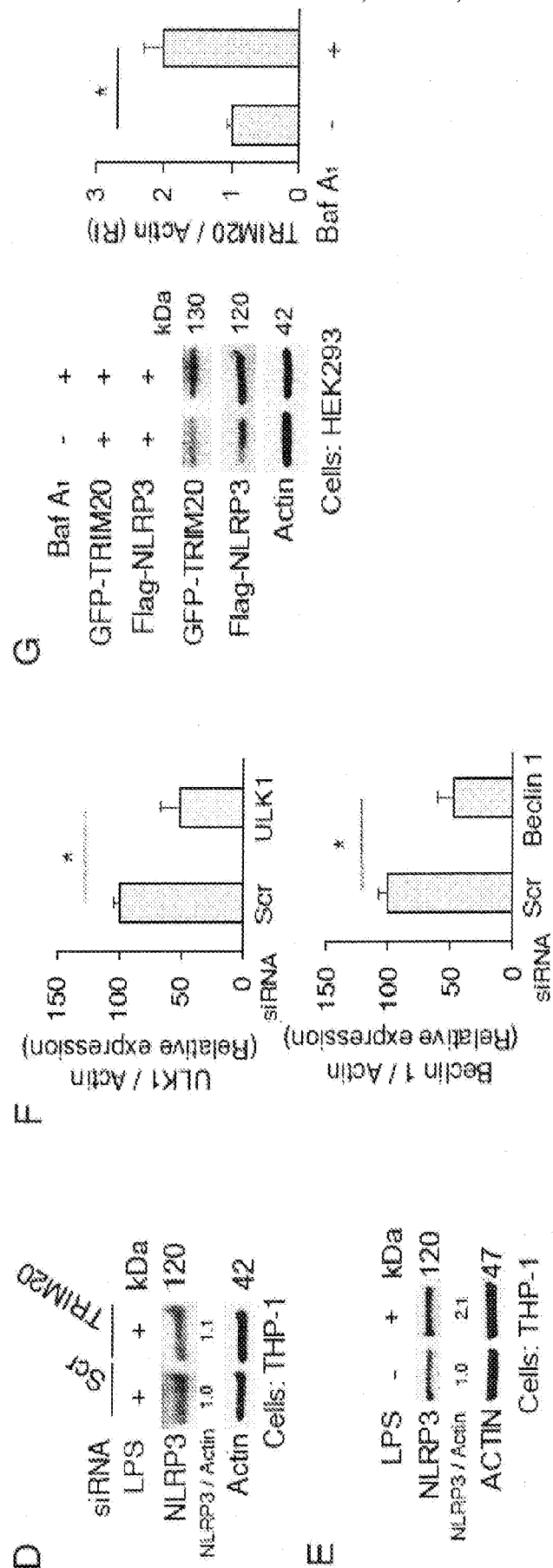


FIGURE S3 PRECISION (CONT'D)

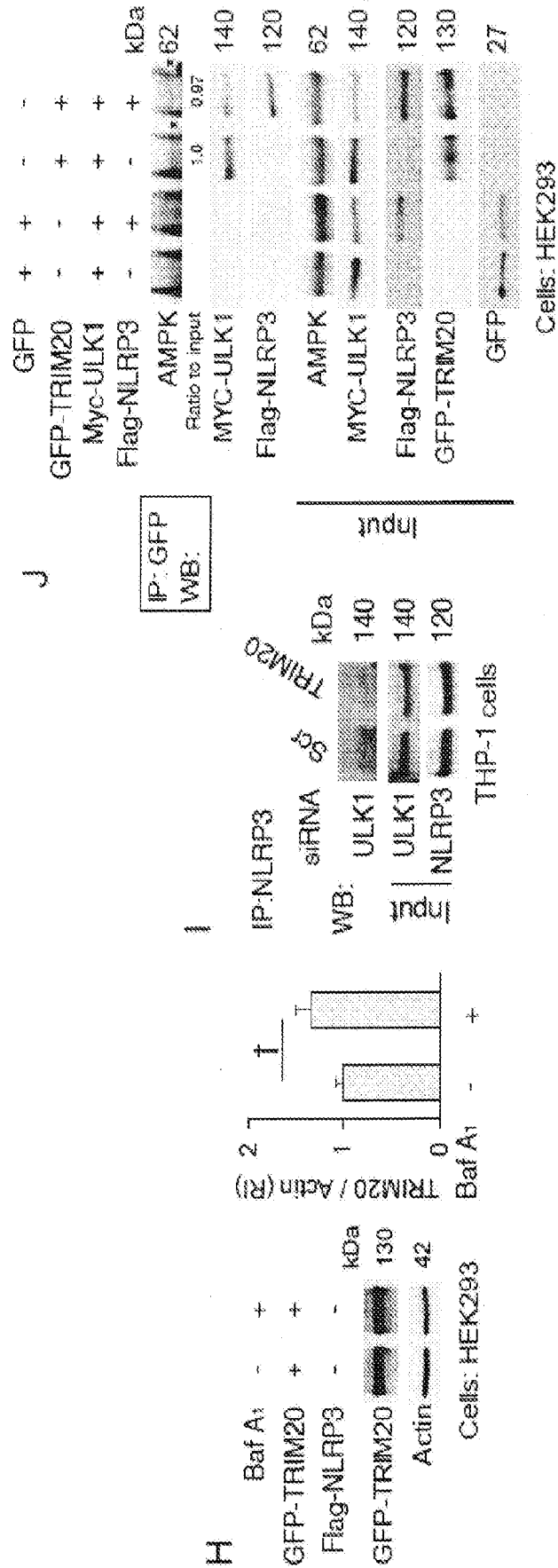


FIGURE S4 PRECISION

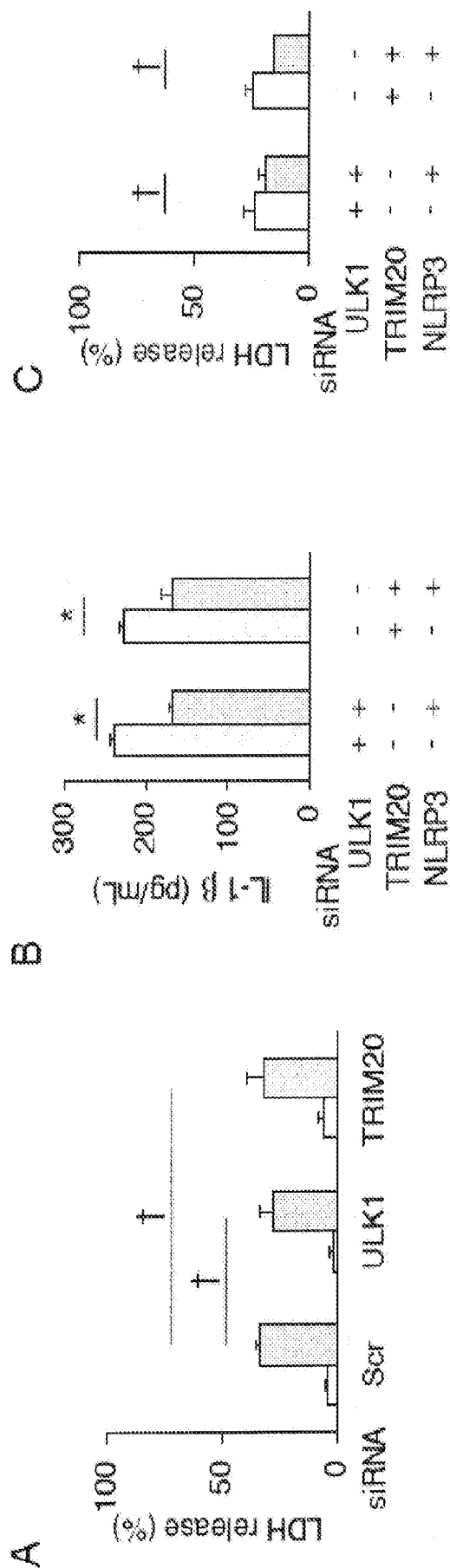


FIGURE S4 PRECISION (CONT'D)

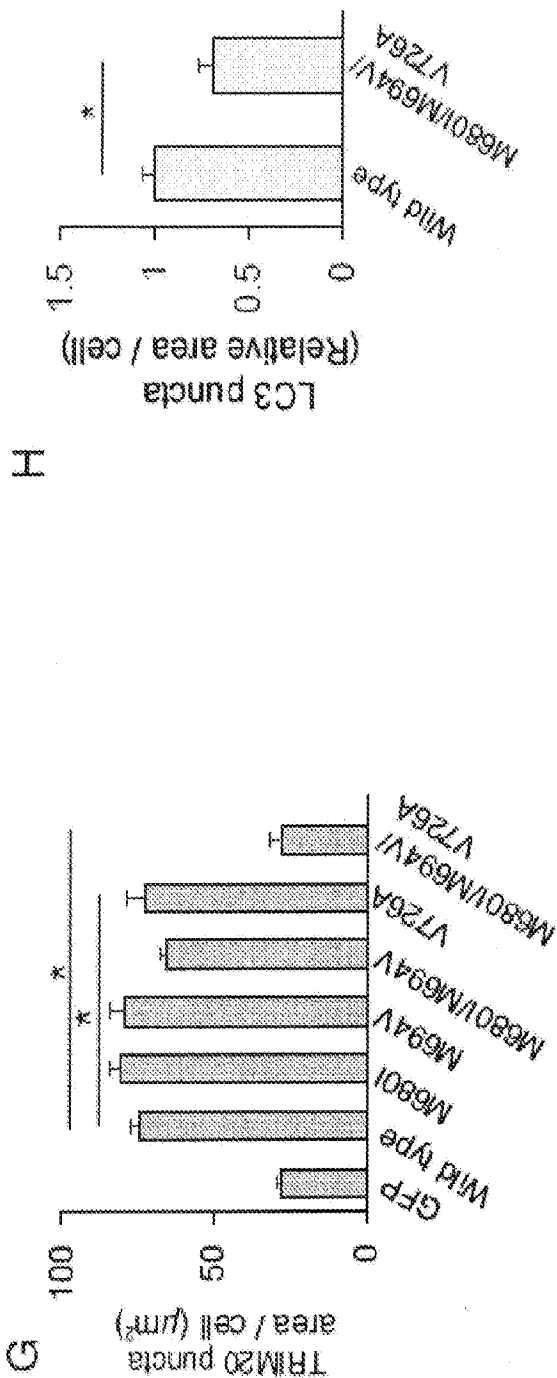


FIGURE S5 PRECISION

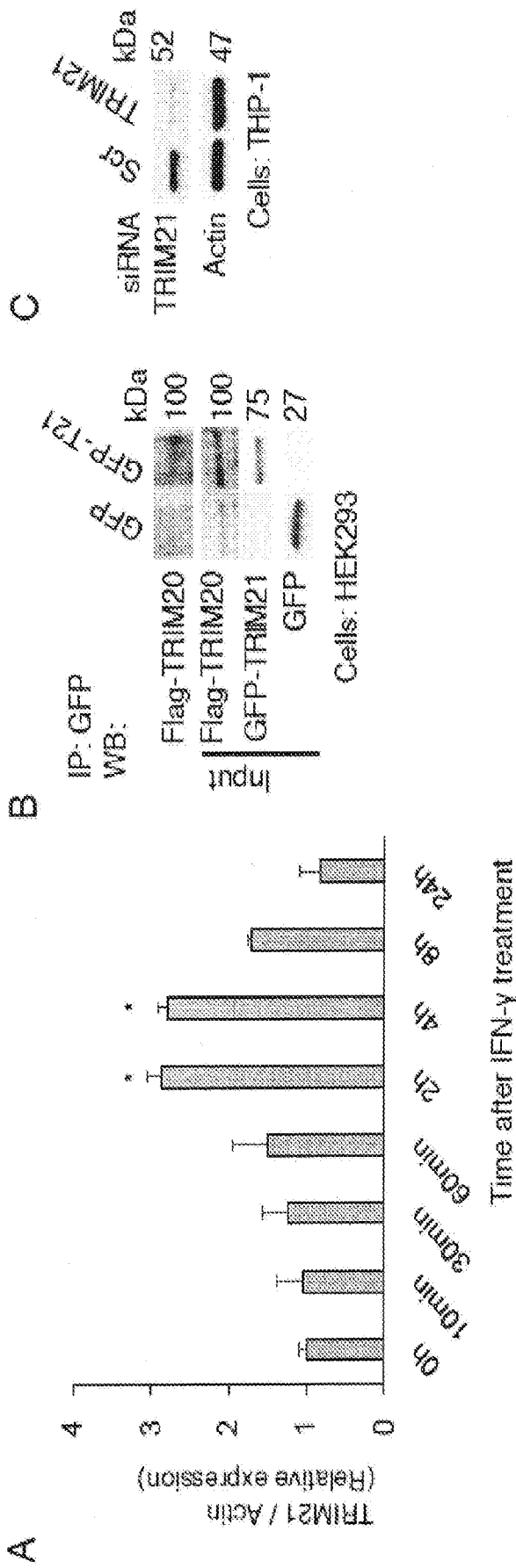


FIGURE S5 PRECISION (CONT'D)

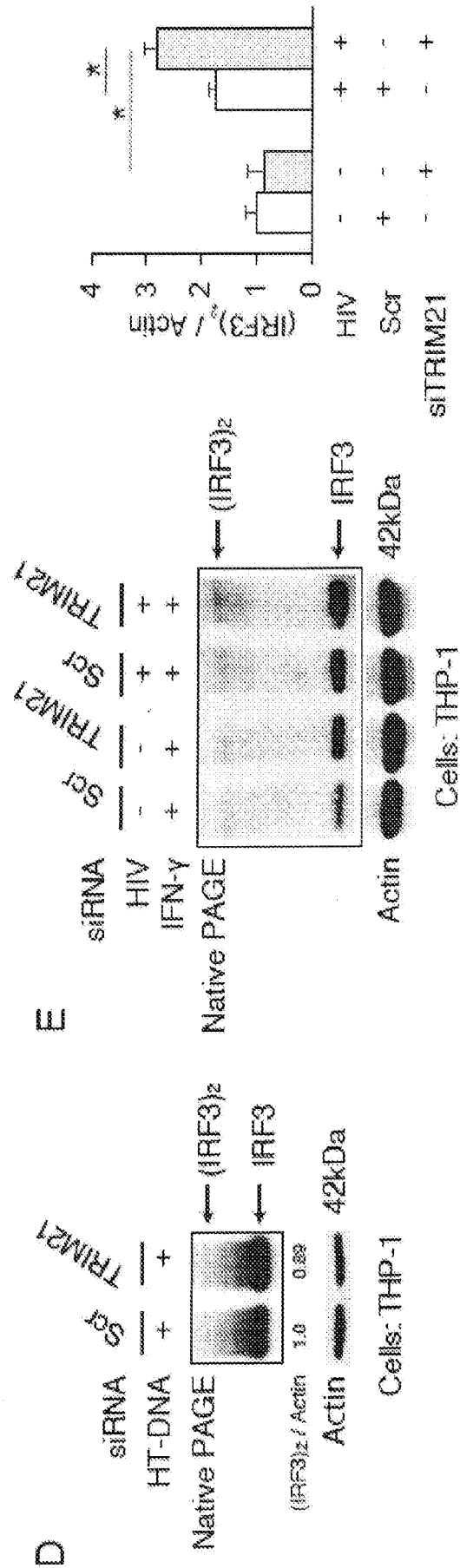
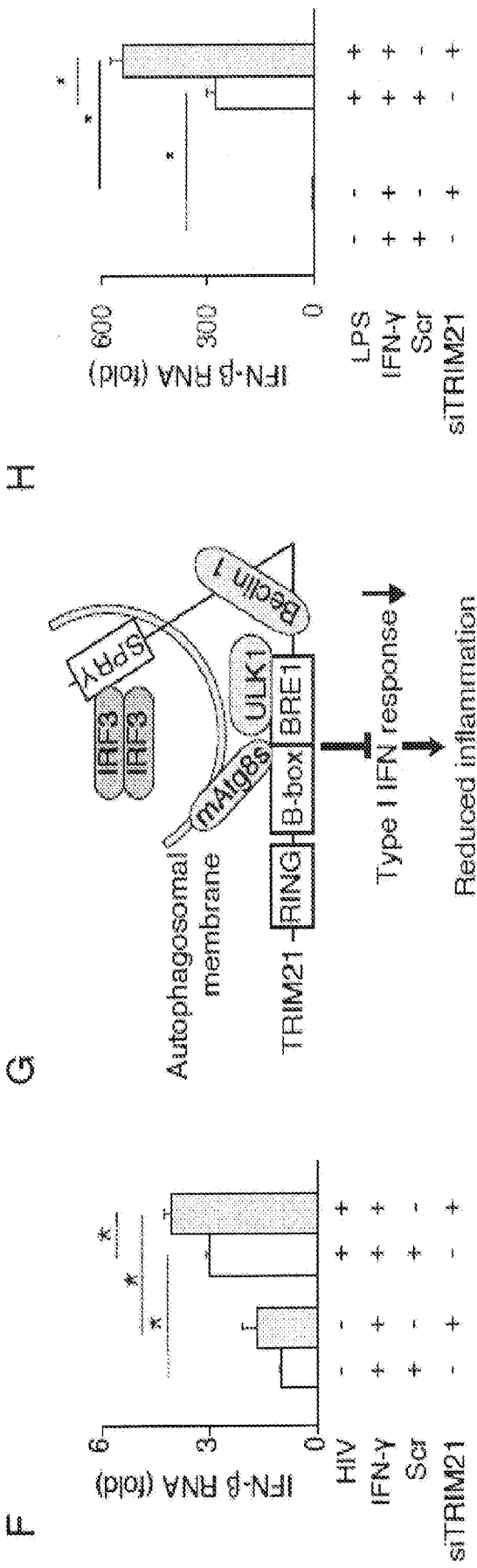


FIGURE S5 PRECISION (CONT'D)



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/019599

A. CLASSIFICATION OF SUBJECT MATTER (see extra sheet)		
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)		
A61K 31/713, 31/00, 38/17, A61P 25/16, 25/28, 31/06, 35/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
Esp@cenet, USPTO, PubMed, RUPAT, PatSearch (RUPTO internal), PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y A	WO 2014/200705 A1 (STC.UNM) 18.12.2014, abstract, claims	23, 27, 29 1-2, 6-7, 17-22 3-5, 24-26, 35-35, 40-43
Y	WO 2011/133879 A2 (UNIVERSITY OF MASSACHUSETTS et al.) 27.10.2011, abstract, claims	1-2, 6-7, 17-22
Y	LU X.C. et al. "Association between variants of the autophagy related gene-IRGM and susceptibility to Crohn's disease and ulcerative colitis: a meta-analysis", PLOS ONE., 2013 Nov 13; 8(11):e80602, p.1-13. doi: 10.1371/journal.pone.0080602	1-2, 6-7, 17-22
A	MANDELL M.A. et al. "TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction", Autophagy. 2014;10(12):2387-2388. doi: 10.4161/15548627.2014.984278	1-7, 17-27, 29, 35-38, 40-43
A	MANDELL M.A. et al. "TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition", Dev Cell. 2014 Aug 25;30(4):394-409. doi: 10.1016/j.devcel.2014.06.013, pp.1-13	1-7, 17-27, 29, 35-38, 40-43
☐ 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 document 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
29 June 2016 (29.06.2016)		14 July 2016 (14.07.2016)
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37		Authorized officer V. Kulikov Telephone No. 495 531 65 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/019599

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8-16, 28, 30-34, 39
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.

PCT/US 2016/019599

A61K 31/713 (2006.01)

A61K 31/00 (2006.01)

A61P 25/16 (2006.01)

A61P 25/28 (2006.01)

A61P 31/06 (2006.01)

A61P 35/00 (2006.01)

A61K 38/17 (2006.01)