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(54) **Title**: ANTAGONISTS OF BETA-CATENIN FOR PREVENTING AND/OR TREATING NEURODEGENERATIVE DISORDERS

(57) **Abstract**: The invention relates to antagonists of β-catenin for use in the prevention and/or treatment of neurodegenerative disorders, β-catenin has been found to specifically accumulate in Huntington's disease and this accumulation is toxic in striatal neuron expressing mutant huntingtin, the causal protein of Huntington's disease. Since β-catenin had been previously reported to accumulate in Alzheimer's disease, interfering with β-catenin accumulation in neuronal cells provides a therapeutic approach for the prevention and/or treatment of neurodegenerative disorders.

ANTAGONISTS OF BETA-CATENIN FOR PREVENTING AND/OR TREATING NEURODEGENERATIVE DISORDERS

The invention relates to antagonists of ß-catenin for use in the prevention and/or treatment of neurodegenerative disorders. ß-catenin has been found to specifically accumulate in Huntington's disease and this accumulation is toxic in striatal neurons expressing mutant huntingtin, the causal protein of Huntington's disease. Since ß-catenin had been previously reported to accumulate in Alzheimer's disease, interfering with ß-catenin accumulation in neuronal cells provides a therapeutic approach for the prevention and/or treatment of neurodegenerative disorders.

Huntington's disease (HD) is a devastating neurodegenerative disorder which is dominantly inherited and is characterized by choreiform movements, psychiatric and cognitive decline, and the graded loss of medium spiny projection neurons in the striatum and of cortical neurons. HD is initiated by an unstable CAG triplet repeat that lengthens a polyglutamine tract in the huntingtin (Htt) protein above ~37 residues, such that disease severity is increased with increased polyglutamine length. The expanded polyglutamine (poly(Q)) tract in Htt protein (polyQ-Htt) confers a novel gain of function on the mutant protein. Disease symptoms typically manifest in mid-life caused by mutant huntingtin with about 50 glutamines or in juvenile years caused by mutant huntingtin with more than ~55 glutamines. The pathogenic mechanisms that lead to disease are not fully understood but a pathological hallmark of HD is the formation of neuronal intracellular inclusions (NIIs) caused by aggregates of polyQ-Htt. These inclusions are also found in other triplet diseases, and in particular polyglutamine diseases. Although polyQ-Htt aggregates are apparently not directly associated with apoptosis induction, the aggregates would induce neuronal dysfunction, such as proteasome activity alteration. There is currently no treatment to delay or prevent death of the neurons and appearance or progression of the symptoms.

As of today, ten different neurodegenerative disorders caused by polyglutamine expanded tract have been identified. Although the genes involved in different polyglutamine diseases have little in common, the disorders they cause follow a strikingly similar course. Each disease is characterized by a progressive

degeneration of a distinct group of nerve cells. The major symptoms of these diseases are similar, although not identical, and usually affect people in midlife.

The Inventors have found that ß-catenin specifically accumulates in HD, as identified in various cell types that express polyQ-Htt, in *Drosophila* models of HD and in post-mortem samples from patients. They have further demonstrated that accumulation of ß-catenin is toxic in striatal neurons expressing polyQ-Htt as reducing ß-catenin levels is protective.

Divergent findings were reported as regards involvement of ß-catenin in Huntington's disease.

A comprehensive study of huntingtin protein interactors by high-throughput yeast two-hybrid screening and affinity pull down followed by mass spectrometry led to the identification of 234 high-confidence Htt-associated proteins, including ß-catenin (Kaltenbach et al. (2007). PLoS Genet 3(5): e82). An arbitrary set of 60 proteins was further selected to assay ability to modify a neurodegenerative phenotype in *Drosophila* induced by a 336 amino acid long Htt-fragment. 80% of the genes enhanced or suppressed the neurodegenerative phenotype induced in *Drosophila*, 45% (27) of the genes assayed being regarded as high-confidence modifiers. 18 of these 27 genes (i.e. 30% of the overall genes assayed) behaved as suppressors of the neurodegenerative phenotype, either by partial loss-of-function or by over-expression. Within these 18 genes, reduced levels of armadillo, the *Drosophila* homologue of ß-catenin, were reported to suppress the Htt-induced phenotype.

It had been previously demonstrated in genetic Huntington's disease mouse and striatal cell models that early events of the disease are associated with activation of the Akt pro-survival signaling pathway. Akt activation in mutant striatal cells was further found to be associated with inactivation of GSK3ß (glycogen synthase kinase 3 ß), via phosphorylation of GSK3ß at Ser9, resulting in increased ß-catenin stabilization and cytosolic concentration. ß-catenin is an essential transcriptional coactivator whose turnover and translocation to the nucleus is regulated by GSK3ß phosphorylation. However, this increased ß-catenin cytosolic concentration was regarded as a pro-survival response, as enhanced ß-catenin signalling was assumed to be protective from the effect of mutant hungtintin (Gines et al. (2003) J Biol Chem 278(50): 50514-22).

Furthermore, it was found that lithium treatment (LiCl) in Huntington's disease cell models reduces poly(Q) toxicity. This effect was associated with GSK-3ß inhibition as poly(Q) toxicity was also reduced by SB216763, a GSK-3ß inhibitor. GSK-3ß inhibition by LiCl and SB216763 resulted in increased ß-catenin dependent T-cell factor mediated transcription. ß-catenin levels were actually found to be reduced in cells expressing expanded polyQ repeats (Carmichael et al. (2002) J Biol Chem. 277(37):33791-8).

Therefore, ß-catenin levels have been found to be both increased and, conversely, decreased in cell and animal models of Huntington's disease. Additionally, although a partial loss-of-function mutation in armadillo (ß-catenin) was described as being associated with suppression of neurodegenerative phenotype in a *Drosophila* model of Huntington's disease, accumulation of ß-catenin was generally assumed to represent a protective response in the context of Huntington's disease.

Normally cytopasmic ß-catenin level is kept low through continuous ubiquitin-proteasome-mediated degradation of ß-catenin, which is regulated by a so-called "destruction complex". In this complex, Axin and adenomatous polyposis coli (APC) function as scaffold proteins that facilitate sequential phosphorylation of ß-catenin at the N-terminus by casein kinase Iα (CKIα; on Ser45) and GSK3ß (on Ser33, Ser37 and Thr41). Phosphorylated ß-catenin is then recognized by ß-TrCP (ß-Transducing repeat containing protein), a component of the E3 ubiquitin ligase complex, and targeted for ubiquitin-mediated proteosomal degradation.

The Inventors investigated the mechanisms by which mutant huntingtin leads to cytoplasmic ß-catenin accumulation by examining whether huntingtin could interact with and regulate the destruction complex. It was found that polyQ-huntingtin leads to the stabilization of ß-catenin by reducing its interaction with the destruction complex and ß-TrCP.

The Inventors further demonstrated that manipulating this pathway regulates mutant-htt-induced toxicity. In particular, overexpression of ß-TrCP exerts a neuroprotective effect in a neuronal model of HD which depends on ß-TrCP ability to bind the E3 ubiquitin ligase complex, SCF, as a mutant form of ß-TrCP that cannot bind SCF is not protective. Moreover, chimeric Fbox proteins that can only degrade ß-catenin had no effect on neuronal toxicity by themselves,

but significantly reduced polyQ-induced toxicity to levels comparable to the wild-type situation indicating that the neuroprotective properties of ß-TrCP in HD specifically depend on its capacity to degrade ß-catenin. Together these results indicate that pathways or compounds that reduce ß-catenin accumulation are of therapeutic interest for HD.

This was further confirmed *in vivo* in a relevant validated *Drosophila* model of HD. The toxic accumulation of ß-catenin could be rescued by the overexpression of ß-TrCP in HD flies. Indomethacin, an inhibitor of cyclooxygenase which was shown to downregulate ß-catenin mRNA and protein level in cells, was further assayed in a neuronal model of HD and in the *Drosophila* model of HD. It was observed that indomethacin treatment also reduced the level of ß-catenin in neurons, and the eye depigmentation phenotype observed in HD flies was completely abolished by the treatment. It was thus demonstrated that indomethacin rescues degeneration in cells and that this drug could be a suitable HD treatment.

Altogether, it is proposed that Htt could be a scaffold protein which facilitates ß-catenin interaction with ß-TrCP and association with the destruction complex. In HD context, association with the destruction complex would be altered due to the loss of ß-catenin / ß-TrCP interaction, leading to a cytoplasmic accumulation of ß-catenin. This model is in agreement with results showing weak binding of ß-catenin to ß-TrCP suggesting that other proteins may participate in stabilization of this interaction (Wu et al. (2003) Mol Cell. 11(6):1445-56).

More generally, these results support that antagonists of ß-catenin represent a possible therapeutic approach for the treatment of Huntington's disease and polyglutamine diseases.

Furthermore, increased ß-catenin levels were also found in Alzheimer's disease. More specifically, it was shown that presenilin 1 (PS1) deficiency, one of the major causes of Alzheimer's disease onset, results in elevated cytosolic ß-catenin levels suggesting that PS1 could be involved in targeting phosphorylated ß-catenin for degradation (Soriano et al. (2001) J Cell Biol. 152(4):785-94). Presenilin 1 was indeed further demonstrated to contribute to ß-catenin degradation by facilitating the stepwise phosphorylation of ß-catenin independently from the Axin destruction complex (Kang et al. (2002) Cell. 110(6):751-62).

Accordingly, huntingtin and presenilin 1 are both examples of proteins contributing to neurodegenerative disease onset which are found to be implicated in ß-catenin degradation in wild-type conditions.

It is thus proposed that impaired ß-catenin degradation, and resulting increased ß-catenin signalling, could be a general mechanism leading to neuron dysfunction in neurodegenerative diseases.

Methods of treatment

The invention thus relates to an antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder.

The invention also relates to an antagonist of ß-catenin for the manufacture of a medicament intended for preventing and/or treating a neurodegenerative disorder.

The invention further provides for a method of preventing and/or treating a neurodegenerative disorder which comprises administering a patient in need thereof with an antagonist of ß-catenin.

In particular, the antagonist of ß-catenin is intended to interfere with cytoplasmic accumulation of ß-catenin in a neuronal cell.

As used herein, the term “subject” or “patient” denotes a human or non-human mammal, such as a rodent, a feline, a canine, or a primate.

The prevention and/or treatment of a neurodegenerative disorder in a human patient is preferably contemplated.

In the context of the invention, the term "treating" or "treatment” is used herein to characterize a method or process that is aimed at (1) delaying or preventing the onset of a disorder or condition to which such term applies; (2) slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the disease state or condition to which such term applies; (3) alleviating or bringing about ameliorations of the symptoms of the disease state or condition to which such term applies; and/or (4) reversing or curing the disease state or condition to which such term applies. A treatment may be administered prior to the onset of the disease, for a prophylactic or preventive action. Alternatively or additionally, a treatment may be administered after initiation of the disease or condition, for a therapeutic action.

The neurodegenerative disorder may be selected from the group consisting of polyglutamine (polyQ) diseases, Alzheimer's disease, Parkinson's disease, prion diseases, multiple sclerosis, and amyotrophic lateral sclerosis.

Preferably the neurodegenerative disorder is a disorder in which neuronal toxicity is associated with cytoplasmic ß-catenin accumulation, in particular due to impaired ß-catenin degradation.

Prion diseases are also called "spongiform encephalopathies" and include Creutzfeld-Jacob Disease, Gerstmann-Straussler-Scheinker syndrome, Fatal familial Insomnia, Kuru, Alpers Syndrome, bovine spongiform encephalopathy, scrapie, transmissible mink encephalopathy and chronic wasting disease.

Preferably, the neurodegenerative disorder is a polyglutamine (polyQ) disease selected from the group consisting of Hungtinton's disease, Kennedy disease, Haw River syndrome, Spinocerebellar ataxia type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3, Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 7, Spinocerebellar ataxia type 12 and Spinocerebellar ataxia type 17.

Still preferably, the neurodegenerative disorder is Hungtinton's disease.

The genes involved in the different polyglutamine diseases and the numbers of glutamine repeat usually recognized as associated with onset of the disease are reported in Table 1.

Table 1 below identifies the causative proteins implicated in these polyglutamine diseases.

Table 1: polyglutamine diseases

Disease	Gene name	Chromosomal location	Pattern of inheritance	Protein	Normal repeat length	Disease repeat length
Spinobulbar muscular atrophy (Kennedy disease)	*AR*	Xq13–21	X-linked recessive	androgen receptor (AR)	11-33	40–62
Huntington disease	*HD*	4p16.3	autosomal dominant	huntingtin	9–34	35–240
Dentatorubral-pallidoluysian atrophy (Haw River syndrome)	*DRPLA*	12p13.31	autosomal dominant	atrophin-1	7-23	49–75
Spinocerebellar ataxia type 1	*SCA1*	6p23	autosomal dominant	ataxin-1	6–44	39–83
Spinocerebellar ataxia type 2	*SCA2*	12q24.1	autosomal dominant	ataxin-2	14–31	33–64
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	*SCA3*	14q32.1	autosomal dominant	ataxin-3	12–40	54–86
Spinocerebellar ataxia type 6	*SCA6*	19p13	autosomal dominant	$a1_A$-voltage-dependent calcium channel subunit	4–20	20–31
Spinocerebellar ataxia type 7	*SCA7*	3p12–13	autosomal dominant	ataxin-7	4–27	37–>200
Spinocerebellar ataxia type 12	*SCA12*	5q31-33	autosomal dominant	Protein phosphatase 2 regulatory subunit B	<29	66-93
Spinocerebellar ataxia type 17	*SCA17*	6q27	autosomal dominant	TATA binding protein	25–42	45–63

Antagonists of ß-catenin

As used herein, an “antagonist of ß-catenin” denotes an agent according to the present invention (i.e., molecule) which inhibits or blocks ß-catenin activity, in particular in a neuronal cell. The antagonist of ß-catenin may inhibit ß-catenin expression (i.e. ß-catenin gene transcription and/or translation) and/or ß-catenin functional activity. Preferably, the antagonist is a direct antagonist of ß-catenin. By

"direct antagonist" of ß-catenin is meant an antagonist of ß-catenin that exerts its antagonist effect through direct physical interaction with ß-catenin protein or nucleic acid.

ß-catenin activity is determined by the amount of ß-catenin in the cytoplasm. Non-phosphorylated ß-catenin escaping ß-TrCP recognition and subsequent degradation is translocated into the nucleus where it forms a complex with a transcription factor of the T-cell factor (TCF)/lymphocyte enhancer factor (LEF) family and induces expression of Wtn target genes including c-myc and cyclin D1. Human transcription factors of the TCF/LEF family include TCF1 (also called TCF7), TCF3 (also called TCF7L1), TCF4 (also called TCF7L2), and LEF1.

ß-catenin stimulates expression of Wtn target genes by recruiting various co-activators, including CBP (CREB-binding protein) and p300.

The Wtn/ ß-catenin pathway has been reviewed for instance by Takemaru et al. (2008, Handb Exp Pharmacol. (186):261-84).

The ß-catenin antagonist may inhibit ß-catenin expression, preferably in a brain cell.

The ß-catenin antagonist may also interfere with cytoplasmic accumulation of ß-catenin, preferably in a brain cell. In particular, said ß-catenin antagonist may increase ß-catenin degradation, especially by the Axin degradation complex. The ß-catenin antagonist may also induce ß-catenin relocalization at the cell membrane.

Alternatively, the ß-catenin antagonist may interferes with ß-catenin binding to a transcription factor of the T-cell factor (TCF)/lymphocyte enhancer factor (LEF) family or to a ß-catenin co-activator.

The ß-catenin antagonists according to the invention are capable of inhibiting or eliminating the functional activity of ß-catenin *in vivo* and/or *in vitro*. The antagonist may inhibit the functional activity of ß-catenin by at least about 10%, preferably by at least about 30%, preferably by at least about 50%, preferably by at least about 70, 75 or 80%, still preferably by 85, 90, 95, or 100%.

The one skilled in the art may readily assess if a candidate molecule is an antagonist of ß-catenin according to known methods.

For instance, it can be assayed whether a candidate molecule decreases expression of ß-catenin or increases degradation of ß-catenin by qualitatively or

quantitatively assaying variation of ß-catenin levels in a cell expressing ß-catenin which was contacted with said candidate molecule, as compared with levels of ß-catenin levels observed in said cell under the same conditions but in the absence of the candidate molecule. ß-catenin levels may be determined by any conventional method, such as antibody-based methods like Westen blot, immunoprecipitation, or ELISA.

It can also be assayed whether a candidate molecule interferes with ß-catenin binding to TCF/LEF for instance using a TCF/LEF Reporter kit, such as commercially available through SABiosciences. Use may be made of a cell transfected with a TCF/LEF-responsive reporter gene construct which encodes a reporter gene under the control of a minimal CMV promoter and tandem repeats of the TCF/LEF transcriptional response element (TRE). Detection of the signal associated with the expression of the reporter gene is indicative of ß-catenin binding with TCF/LEF resulting in activation of TRE. Accordingly, a decrease in, or absence of, reporter gene signal when the cell is contacted with a candidate molecule is indicative of a candidate molecule interferes with ß-catenin binding to TCF/LEF.

Alternatively, as screen base on surface Plasmon resonance studies, such as described by Lepourcelet et al. (2004. Cancer Cell. 5(1):91-102), may be used to identify antagonist of ß-catenin/TCF interaction.

The above methods are non limitative examples of methods which can be used in the frame of the invention to determine if a candidate molecule is a ß-catenin antagonist.

The ß-catenin antagonist may for instance be a peptide or polypeptide, such as an antibody or aptamer directed against ß-catenin; a nucleic acid molecule which reduces or prevents the expression of ß-catenin, such as an aptamer, a ß-catenin anti-sense oligonucleotide, a ß-catenin interfering RNA (iRNA) such as siRNA, or a ribozyme; or a small molecule inhibitor of ß-catenin activity.

As used herein, “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also

antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In particular the antibody may be monoclonal (recombinant or produced by hybridoma), humanized, and/or a fragment such as Fv, Fab, $F(ab')_2$, Fd, dAb, dsFv, scFv, $sc(Fv)_2$.

Aptamers are a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity. Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., Science, 1990, 249(4968):505-10. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., Clin. Chem., 1999, 45(9):1628-50. Peptide aptamers consists of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., Nature, 1996,380, 548-50).

In particular, the ß-catenin antagonist may be an antibody or aptamer directed against ß-catenin and which blocks interaction of ß-catenin with TCF/LEF.

Antisense oligonucleotides usually comprise a single-stranded polynucleotide sequence (either RNA or DNA) capable of binding to target mRNA (antisense oligonucleotide) or DNA (sense oligonucleotide) sequences. Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block or inhibit protein expression by one of several means, including enhanced degradation of the mRNA by RNAse H, inhibition of splicing, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of proteins.

Suitable ß-catenin antisense oligonucleotides comprise fragments of the targeted polynucleotide sequence encoding ß-catenin. Such a fragment generally comprises at least about 14 nucleotides, typically from about 14 to about 30 nucleotides. The ability to derive an antisense oligonucleotide, based upon a nucleic acid sequence encoding a given protein is described in, for example, Stein

and Cohen (Cancer Res., 1988, 48:2659), and van der Krol et al. (BioTechniques, 1988, 6:958).

Antisense oligonucleotides comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable in vivo (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L)-lysine. Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense oligonucleotides may be introduced into a cell containing the target nucleic acid by any gene transfer method, including, for example, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or lipofection, or by using gene transfer vectors such as Epstein-Barr virus or adenovirus.

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleic acid by formation of a conjugate with a ligand molecule such as growth factors, other cytokines, or other ligands that bind to cell surface receptors.

Additional methods for preventing expression of ß-catenin include RNA interference (RNAi). RNAi is carried out through the use of an interfering RNA (iRNA) which is capable of down-regulating the expression of the targeted protein. As used herein, the term “iRNA” encompasses small interfering RNA (siRNA), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), micro-RNA (miRNA) and short hairpin RNA (shRNA) molecules, specific for pre-mRNA or mRNA of said ß-catenin. Short hairpin RNAs (shRNA) will be cleaved by the cellular machinery into siRNA specific for pre-mRNA or mRNA of said receptors or said ligands (Paddison et al., Genes & Development, 16: 948-958, 2002).

RNA interference, designate a phenomenon by which dsRNA specifically suppress expression of a target gene at post-translational level.

In normal conditions, RNA interference is initiated by double-stranded RNA molecules (dsRNA) of several thousands of base pair length. In vivo, dsRNA introduced into a cell is cleaved into a mixture of short dsRNA molecules called siRNA. The enzyme that catalyzes the cleavage, Dicer, is an endo-RNase that contains RNase III domains (Bernstein et al., Nature, 409: 363-366, 2001). In mammalian cells, the siRNAs produced by Dicer are 21-23 bp in length, with a 19 or 20 nucleotides duplex sequence, two-nucleotide 3' overhangs and 5'-triphosphate extremities (Elbashir et al., Nature, 411: 494-498, 2001; Elbashir et al., Genes. Dev. 15(2): 188-200, 2001; Zamore et al., Cell, 101(1): 25-33, 2000). A number of patents and patent applications have described, in general terms, the use of siRNA molecules to inhibit gene expression, for example, WO 99/32619, US 20040053876, US 20040102408 and WO 2004/007718.

siRNA are usually designed against a region 50-100 nucleotides downstream the translation initiator codon, whereas 5'UTR (untranslated region) and 3'UTR are usually avoided. The chosen siRNA target sequence should be subjected to a BLAST search against EST database to ensure that the only desired gene is targeted. Various products are commercially available to aid in the preparation and use of siRNA. In a preferred embodiment, the RNAi molecule is a siRNA of at least about 15-50 nucleotides in length, preferably about 20-30 base nucleotides.

RNAi can comprise naturally occurring RNA, synthetic RNA, or recombinantly produced RNA, as well as altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end of the molecule or to one or more internal nucleotides of the RNAi, including modifications that make the RNAi resistant to nuclease digestion.

RNAi may be administered in free (naked) form or by the use of delivery systems that enhance stability and/or targeting, e.g., liposomes, or incorporated into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, bioadhesive microspheres, or proteinaceous vectors (WO

00/53722), or in combination with a cationic peptide (US 2007275923). They may also be administered in the form of their precursors or encoding DNAs.

RNA interference (RNAi) produced by the introduction of specific small interfering RNA (siRNA), as described, for example in Bosher et al., Nature Cell Biol 2, E31-E36 (2000). ß-catenin duplexed siRNA which may be used in the frame of the invention have already been described by Gu et al. (2008 Mol Cell Biol. 28(16):4963-74).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of ß-catenin mRNA sequences are thereby useful within the scope of the present invention.

In particular, the antagonist may be selected from the group consisting of a ß-catenin antisense oligonucleotide, a ß-catenin siRNA, a ß-catenin ribozyme, an antibody or aptamer directed against ß-catenin and which blocks interaction of ß-catenin with TCF/LEF, a vector containing a β-TrCP encoding sequence, a F-box chimera, and small molecule inhibitors of ß-catenin binding to TCF/LEF or a ß-catenin co-activator.

Small molecule inhibitors, such as PKF115-854, CGP049090, and ICG-001, have been reported to disrupt ß-catenin/TCF complex (Lepourcelet et al. 2004. Cancer Cell. 5(1):91-102).

A F-box chimeric protein, or F-box chimera, may also be used to achieve very specific degradation of ß-catenin. The F-box motif of ß-TrCP is known to assign ß-catenin for degradation. F-box chimeric proteins may consist in a F-box protein in which the WD40 domain is replaced by TCF4 or E-cadherin binding domain to ß-catenin (Cong et al. (2003) BMC Mol Biol. 4:10; Liu et al. (2004) Biochem Biophys Res Commun. 313(4):1023-9) or in a F-box protein fused to 14aa repeats of APC protein (Su et al. (2003) Proc Natl Acad Sci U S A. 100(22):12729-34). These chimeric protein very specifically recognize cytoplasmic and nuclear ß-catenin, thereby enhancing specific ß-catenin degradation (Su et al. (2003) Proc Natl Acad Sci U S A. 100(22):12729-34, 2003; Liu et al. (2004)

Biochem Biophys Res Commun. 313(4):1023-9). Reference is made to the review by Dihlmann and von Knebel Doeberitz (2005) Int J Cancer. 113(4):515-24.

Increased ß-catenin degradation may also be obtained by over-expressing ß-TrCP, for instance by delivery a vector, such as a virus, containing a ß-TrCP encoding sequence.

According to an embodiment, the ß-catenin antagonist is elected from the group consisting of PKF115-854, CGP049090, and ICG-001.

Preferably, the ß-catenin antagonist is selected from the group consisting of a ß-catenin antisense oligonucleotide, a ß-catenin siRNA, an antibody or aptamer directed against ß-catenin and which blocks interaction of ß-catenin with TCF/LEF, a vector containing a β-TrCP encoding sequence, and a F-box chimera.

Other active substances

An active substance may be used in combination with said antagonist of ß-catenin activity, either by simultaneous administration (e.g. when the antagonist of ß-catenin activity and the at least one other active substance are formulated in a same pharmaceutical composition), or sequential administration ((e.g. when the antagonist of ß-catenin activity and the at least one other active substance are administered separately in time).

The other active substance may also be a ß-catenin antagonist but not necessarily. It may be an active substance which may be used for the prevention and/or treatment of the neurodegenerative disorder intended to be prevented/treated, but which acts by mechanisms not mediated by ß-catenin, or not only mediated by ß-catenin.

In particular, the active substance may be selected from the group consisting of non-steroidal antiinflammatory drugs (NSAIDs), inhibitors of cyclooxygenase (COX) enzymes, a Histone deacetylase (HDAC) inhibitor, imatinib or a salt or ester thereof, endostatin, vitamin A and retinoids, vitamin D, differentiation-inducing factors (DIFs), lithium, curcumin, and flavonoids.

NSAIDs are widely prescribed for the treatment of pain, fever and inflammation. Their effects are largely attributed to the inhibition of cyclooxygenase COX-1 and/or COX-2.

NSAIDs have also been reported to inhibit the Wnt/ß-catenin signaling pathway. For instance indomethacin would repress ß-catenin transcription and induce ß-catenin protein degradation whereas aspirin (acetyl salicylic acid) would stabilize ß-catenin in its inactive phosphorylated form, thereby preventing its function as a co-transcription factor. Nitric oxide releasing aspirin (NO-ASA) is suggested to affect ß-catenin signalling by disrupting the ß-catenin/TCF complex in the nucleus. Sulindac and sulindac metabolites would induce ß-catenin degradation. The mechanisms of action of these NSAIDs on the Wnt/ß-catenin pathway have been reviewed by Dihlmann and von Knebel Doeberitz (2005. Int J Cancer. 113(4):515-24). Accordingly, NSAIDs which may be used in the frame of the invention may be for instance indomethacin, sulindac, sulindac metabolite, naproxen, ketoprofen, ibuprofen, acetyl salicylic acid, NO-acetyl salicylic acid, celecoxib, and rofecoxib.

The active substance may be for instance a histone deacetylase (HDAC) inhibitor used in combination another type of ß-catenin antagonist.

HDAC6 has been shown to deacetylate ß-catenin on Lys49, thereby preventing ß-catenin phosphorylation on Lys45 and subsequent degradation by the destruction complex.

Beside this direct effect of HDAC inhibitors on ß-catenin activity, recent studies have identified an altered microtubule (MT)-dependent transport of organelles in HD. It was previously demonstrated that htt associates with molecular motors and activates the MT-dependent transport of vesicles containing brain-derived neurotrophic factor (BDNF). Wild-type (WT) htt enhances the velocity of the vesicles and reduces the amount of time they spend not moving (pausing time). In HD, in which the htt contains the polyQ expansion, the intracellular transport of BDNF-containing vesicles is altered, resulting in reduced trophic support of neurons and their death.

Histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), NaPB and resveratrol have been suggested to protect neurons in HD by promoting the intracellular transport of BDNF through the inhibition of HDAC6 and subsequent tubulin acetylation. Furthermore, HDAC inhibitors were suggested more generally to find use for the treatment of

neurodegenerative diseases associated with altered intracellular transport such as Alzheimer's disease (Dompierre et al., 2007).

Accordingly, the other active substance may be a HDAC inhibitor preferably where the neurodegenerative disorder is HD or Alzheimer's disease.

Where it is contemplated to induce ß-TrCP overexpression to restore ß-catenin degradation, the other active substance may be, e.g., a calcineurin inhibitor, such as FK506.

Endostatin in known to induce ß-catenin degradation by the proteasome.

Glivec® (imatinib mesylate) downregulates ß-catenin signalling, probably by inhibiting tyrosine-phosphorylation of ß-catenin.

Vitamin A and its metabolites (retinoids), as well as vitamin D, differentiation-inducing factors (DIFs), lithium, curcumin, and flavonoids, have been reported to inhibit the Wnt/ ß-catenin pathway (for a review see e.g. Takahashi-Yanaga and Sasaguri (2009) J Pharmacol Sci. 109(2):179-83).

In particular, the active substance to be used in combination with the antagonist of ß-catenin activity may therefore be selected from the group consisting of indomethacin, sulindac, sulindac metabolite, naproxen, ketoprofen, ibuprofen, acetyl salicylic acid, NO-acetyl salicylic acid, celecoxib, rofecoxib, and suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), and NaPB

Screening methods

The results disclosed herein strongly suggest that Htt would be a scaffold protein which facilitates ß-catenin interaction with ß-TrCP and association with the destruction complex. This scaffold function would be impaired in polyQ-Htt thereby resulting in loss of ß-catenin / ß-TrCP interaction.

Accordingly, it is proposed that molecules capable of restoring normal interaction of polyQ-Htt with ß-catenin could prevent ß-catenin accumulation and neuronal death in Huntington's disease, e.g. by inducing post-translational modifications of polyQ-Htt.

Therefore, another object of the invention is a method for screening molecules likely to prevent and/or treat Huntington's disease, which method comprises:

a) contacting a candidate molecule with a cell expressing polyglutamine-huntingtin (polyQ-Htt) and ß-catenin; and

b) detecting if :

i) polyQ-Htt interacts with ß-catenin in the presence of said candidate molecule wherein, detection of an interaction between polyQ-Htt and ß-catenin in the presence of said candidate molecule is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

ii) ß-catenin cellular level is decreased in the presence of said candidate molecule as compared with ß-catenin cellular level in the absence of said candidate molecule, wherein decreased ß-catenin cellular level is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

iii) ß-catenin localization at the cell membrane is increased in the presence of said candidate molecule as compared with ß-catenin localization at the cell membrane in the absence of said candidate molecule, wherein increased ß-catenin localization at the cell membrane is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

iv) ß-catenin degradation is increased in the presence of said candidate molecule as compared with ß-catenin degradation in the absence of said candidate molecule, wherein increased ß-catenin degradation is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease.

The cell may have been transformed to express polyglutamine-huntingtin (polyQ-Htt).

The cell may further express the proteins constitutive of the axin destruction complex. In such a case, it may be also assessed whether the candidate molecule is capable of restoring ß-catenin degradation by the destruction complex.

Methods are known to the one skilled in the art which are suitable to determine interaction between two proteins. Reference may be made for instance to Biacore, fluorescence-based such as FRET (fluorescence resonance energy transfer), or bioluminescence-based such as BRET (Bioluminescence Resonance Energy Transfer).

Use may be made of flurorescence techniques and phenotypic screening methods to measure ß-catenin accumulation and/or degradation and/or specific

localization within the cell (plasma membrane) as a read-out upon expression of polyQ-htt in cells and/or neurons.

The invention will be further illustrated in view of the following figures and examples.

FIGURES

Fig. 1. ß-catenin accumulates in HD. (*A* and *B*) ß-catenin accumulates in cells expressing polyQ-huntingtin. MDCK (*A*) and HEK (*B*) cells are transfected with htt-480-17Q or htt-480-68Q constructs or empty vector (pcDNA) and analyzed by immunoblotting for the presence of ß-catenin, α-tubulin and huntingtin. Quantification of 4 independent experiments in MDCK shows a statistically significant increase in ß-catenin level. (*C*) Armadillo is stabilized in a *Drosophila* model of HD. UAS-htt^{548aa}-Q0 and UAS-htt^{548aa}-Q128 flies are crossed with *w;gmr-*Gal4;UAS-LacZ. Armadillo (arm) levels are analyzed in the progeny by immunoblotting. Quantification of 4 experiments reveals a strong increase of armadillo expression. (*D*) ß-catenin is upregulated in HD brains. Protein extracts are prepared from *post-mortem* striatum of CT (samples 1 and 2) and HD individuals (HD grade 3, sample 3; HD grade 4, sample 4) and analyzed by immunoblotting for ß-catenin and ß-actin. Quantification of the Western Blot shows a statistically significant increase in the protein level of ß-catenin in HD samples compared with control samples. Results are expressed as a ratio between ß-catenin and α-tubulinor actin intensity. (*E*) PolyQ-huntingtin induces ß-catenin relocalization. HeLa cells are transfected with ß-catenin-GFP and htt-480-17Q, htt-480-68Q or the empty vector (pcDNA), fixed and immunostained for huntingtin and GFP. (*F*) Quantitative analysis of ß-catenin localization expressed as a ratio of cytoplasm/nuclear intensities normalized to the control situation (pcDNA). (*G*) Preventing accumulation of ß-catenin reduces toxicity in neurons. Striatal neurons were nucleofected with siRNA directed against ß-catenin (si-ß-catenin) or scramble RNA and 48 h later transfected with htt-480-17Q or htt-480-68Q. Data from 4 independent experiments demonstrate a protective effect of ß-catenin downregulation towards polyQ-huntingtin-induced toxicity. (NS, not significant;

*P<0.05; **P<0.01; ***P<0.001; see supporting information for detailed statistical analyses and number of measures).

Fig. 2. Accumulation of ß-catenin is toxic in HD. (A-E) ß-catenin is upregulated in CAG140 knock-in mice and HD brains. (A) Cortical extracts from eight month old wild-type (WT, samples 1 and 2) and CAG140 (CAG140, samples 1 to 3) knock-in mice are analyzed by immunoblotting for the presence of ß-catenin, GFAP, bIII-tubulin and a-tubulin used as a loading control. Quantification shows an increase in ß-catenin levels in HD. (B) Cortical extracts from 22 month old wild-type (WT, samples 1 to 4) and CAG140 (CAG140, samples 1 to 3) knock-in mice are analyzed by immunoblotting for the presence of ß-catenin, its phosphorylated forms at serines 33-37-41 (P33-37-41) and 41-45 (P41-45) and a-tubulin. (C) Quantification shows that ß-catenin accumulates under its phosphorylated form. (D) Protein extracts are prepared from *post-mortem* striatum of control (CT samples 1 and 2) and HD individuals (HD grade 3, sample 3; HD grade 4, sample 4) and analyzed by immunoblotting for ß-catenin, its phosphorylated forms at serines 33-37-41 (P33-37-41) and 41-45 (P41-45), and ß-actin (actin). (E) Quantification of the Western Blot shows an increase in the protein level of phosphorylated ß-catenin in HD samples compared with control samples. (A-E) Values refer to band densities. Results are expressed as ratios between ß-catenin and a-tubulin or ß-actin intensity. (F, G) PolyQ huntingtin does not regulate downstream targets of ß-catenin. Total RNA is extracted from cortices of 22 month old wild-type (WT) and CAG140 knock-in mice. The mRNA levels of c-myc (F) and axin2 (G) are determined by quantitative PCR analysis. (NS, not significant; *P<0.05; **P<0.01).

Fig. 3. Huntingtin associates with the ß-catenin destruction complex. (A) Huntingtin and ß-TrCP interact in a polyQ independent manner. Primary culture of cortical neurons from wild-type mice (+/+) or $Hdh^{Q111/Q111}$ knock-in mice (Q/Q) are electroporated with HA-tagged ß-TrCP. ß-TrCP immunoprecipitation (HA antibody; IP HA) experiments show an interaction of ß-TrCP with endogenous huntingtin (htt). No difference in the interaction is observed between wild-type and polyQ-huntingtin. Immunoprecipitation with mouse IgG (IP IgG) is used as a control. (B) Similar results are obtained doing the converse immunoprecipitation (4C8; IP htt) on whole brain extracts from wild-type mice (+/+) or $Hdh^{Q111/Q111}$ knock-in mice

(Q/Q). Anti-GFP antibody is used to perform the control immunoprecipitation (IP GFP). (C) Huntingtin interacts with axin and this binding is not modified by the polyQ expansion as revealed by a myc immunoprecipitation (IP myc). HEK 293 cells are transfected with htt-480 constructs or the corresponding empty vector (pcDNA) and myc-axin.

Fig. 4. PolyQ-huntingtin decreases ß-catenin binding to the destruction complex. (A-C) The presence of an abnormal polyQ expansion in huntingtin alters the binding of huntingtin to ß-catenin. (A) HEK 293 cells are transfected with YFP-fused htt-1301 variants and the corresponding empty vector (control, CT). A 4C8 immunoprecipitation (IP htt) shows a decreased interaction of huntingtin (htt) with ß-catenin in polyQ-huntingtin compared to wild-type huntingtin expressing cells. (B, C) A 4C8 immunoprecipitation (IP htt) from cortical extracts from wild-type mice (+/+) or $Hdh^{Q111/Q111}$ knock-in mice (Q/Q) demonstrates a decreased interaction of ß-catenin with polyQ-huntingtin compared to wild-type huntingtin. Similar results are obtained doing the converse immunoprecipitation (IP ß-catenin). Immunoprecipitations with mouse IgG (IP IgG) are used as controls. The graphs represent the quantitative assessment of the band densities of the co-immunoprecipitated versus immunoprecipitated proteins. (*P<0.05; **P<0.01).

Fig. 5. (A, B) ß-catenin binding to axin is impaired in polyQ context. HEK 293 cells are transfected with myc-tagged axin and htt-480-17Q or htt-480-68Q constructs or empty vector (pcDNA). Immunoprecipitation experiments using myc (A, IP myc) or ß-catenin antibodies (B, IP ß-catenin) reveal a strong decrease of ß-catenin interaction with axin in presence of polyQ-huntingtin compared to wild-type huntingtin. (C) PolyQ-huntingtin modifies ß-catenin interaction with ß-TrCP. ß-catenin immunoprecipitation (IP ß-catenin) in HEK 293 cells expressing myc-ß-TrCP and a fragment of huntingtin (htt-480-17Q or -68Q) shows a defect of ß-catenin and ß-TrCP binding when huntingtin encompasses an abnormal polyQ expansion. The graphs represent the quantitative assessment of the band densities of the co-immunoprecipitated versus immunoprecipitated proteins. (*P<0.05; **P<0.01).

Fig. 6. Preventing accumulation of ß-catenin reduces polyQ-huntingtin induced toxicity in striatal neurons. Wild-type huntingtin (htt-480-17Q) and polyQ-huntingtin (htt-480-68Q) are co-transfected with ß-TrCP, the dominant negative

form ß-TrCPΔF or the empty vector (pCS2) in striatal neurons. Data from six independent experiments reveal that ß-TrCP significantly reduces polyQ-huntingtin-induced cell death (*A*), whereas ß-TrCPΔF has the opposite effect (*B*). (*C*) Neuronal cell death was assessed in primary culture of striatal neurons transfected with htt constructs (htt-480-17Q or 68Q) and chimeric F-box fusion proteins, Fbox-ßBD^{Tcf4} or Fbox-ßBD^{Ecad}). (D) Striatal neurons are electroporated with siRNA directed against ß-catenin (si-ß-catenin) or scramble RNA and 48 h later transfected with htt-480-17Q or htt-480-68Q. Data from 4 independent experiments demonstrate a protective effect of ß-catenin downregulation towards polyQ-huntingtin-induced toxicity. Immunoblotting using anti-ß-catenin and anti-α-tubulin antibodies reveals a decrease in ß-catenin levels in the si-ß-catenin treated neurons compared to scramble situation. Values refer to band densities. Results are expressed as a ratio between ß-catenin and a-tubulin intensity. (E) Striatal neurons are electroporated with ß-catenin or the corresponding empty vector (pCMV). Data from 4 independent experiments demonstrate a toxic effect of ß-catenin expression. (NS, not significant; *P<0.05; **P<0.01; ***P<0.001).

Fig. 7. Slimb, the *Drosophila* homologue of ß-TrCP, reduces polyQ-induced toxicity and ß-catenin level in a *Drosophila* model of HD. (*A*) Left panel: Slimb regulates polyQ-huntingtin-induced eye depigmentation. The following crosses are analyzed : *w*;*gmr*-Gal4-UAS-GFP with *w*;+;UAS-LacZ or with *w*;+;UAS-Slimb as controls; UAS-htt^{548aa}-Q0 with *w*;*gmr*-Gal4;UAS-LacZ and UAS-htt^{548aa}-Q128 with *w*;*gmr*-Gal4;UAS-LacZ or *w*;*gmr*-Gal4;UAS-Slimb. The progeny (females) is analyzed at 10 days for eye pigmentation. Eye-specific polyQ-huntingtin expression leads to a complete loss of pigmentation (compare line 4 to line 3). Expression of UAS-Slimb transgene in those flies rescue the toxicity (line 5). The graph represents the percentage of flies with more than 50% of eye depigmentation. Right panels: Visualization of ommatidial morphology using scanning electron microscopy. PolyQ-huntingtin expression induces a strong desorganisation of ommatidia that is compensated by expression of slimb. (*B*) Slimb reverses ß-catenin accumulation in polyQ-huntingtin expressing *Drosophila*. UAS-htt^{548aa}-Q0 and UAS-Htt^{548aa}-Q128 flies are crossed with *w*;*gmr*-Gal4;UAS-LacZ or *w*;*gmr*-Gal4;UAS-Slimb. Immunoblotting analysis of the progeny shows a loss of ß-catenin accumulation in htt-548-Q128 flies expressing Slimb as

compared to lacZ. Quantification of 4 independent experiments shows an important rescue of ß-catenin level when slimb is expressed. Results are expressed as a ratio between armadillo and α-tubulinsignal intensity and are normalized to control (htt-Q0 X *gmr*-LacZ). (NS, not significant; *P<0.05; **P<0.01; ***P<0.001; see supporting information for detailed statistical analyses and number of measures).

Fig. 8. Indomethacin reduces htt-polyQ-induced toxicity in neurons and in *Drosophila.* (*A*) Indomethacin treatment of striatal neurons reduces the level of ß-catenin as revealed by immunoblotting. (*B*) Primary cultures of striatal neurons are transfected with htt-480-17Q or htt-480-68Q and treated with indomethacin (indoM, 600µM) or 1% DMSO for 24 hours. Indomethacin treatment results in a statistically significant decrease of polyQ-htt-induced neuronal death. (*C*) Wild-type htt-548-Q0 and polyQ htt-548-Q128 flies are feed with indomethacin. Expressing mutant polyQ-huntingtin in developing eyes leads to adults with a loss of eye pigmentation. Indomethacin rescues the loss of eye pigmentation induced by mutant huntingtin in the developing eyes of flies in a statistically significant manner. (**P<0.01; ***P<0.001; see supporting information for detailed statistical analyses and number of measures).

Fig. 9. Indomethacin reduces polyQ-huntingtin-induced toxicity in neurons and in *Drosophila*. (A) Armadillo accumulation in polyQ-huntingtin expressing *Drosophila* is blocked by indomethacin. (*gmr*-Gal4,UAS-GFP/UAS-htt^{548aa}-Q0) and (*gmr*-Gal4,UAS-GFP/UAS-htt^{548aa}-Q128) flies are treated with indomethacin or ethanol (CT) and analyzed by immunoblotting for armadillo level (arm). (B) The lifespan of polyQ-huntingtin flies is significantly prolonged by indomethacin. *Elav*-Gal4/UAS-htt^{548aa}-Q0 and *elav*-Gal4/UAS-htt^{548aa}-Q128 flies are raised on standard media containing indomethacin or ethanol (CT). Lifespan of the progeny is analyzed. (NS, not significant; **P<0.01; ***P<0.001).

EXAMPLES

Example 1: Materials and methods

Statistical analyses. Statview 4.5 software (SAS Institute, Cary, NC) was used for statistical analyses. All data herein described were performed in triplicate.

Data are expressed as means ± SEM. Statistical analyses may be found in the supporting information.

DNA constructs, siRNA and antibodies. Constructs encoding ß-galactosidase, ß-catenin-enhanced green fluorescent protein (eGFP), Myc- or hemagglutinin (HA)-tagged ß-TrCP, inactive HA-ß-TrCPΔF and FLAG-Fbox-ßBD^{Tcf4} and FLAG-Fbox-ßBD^{Ecad} were described elsewhere (Delmas et al., 2007; Margottin et al., 1998, Liu, 2004). The wild-type and polyQ-huntingtin constructs htt-480-17Q, htt-480-68Q, YFP-htt-1301-17Q and YFP-htt-1301-73Q were previously described (Anne et al., 2007; Saudou et al., 1998). The siRNA sequence targeting rat ß-catenin was already described (Gu et al., 2008). The scramble RNA (scRNA, control; Eurogentec, Seraing, Belgium) used had a unique sequence which did not match with any sequence in the genome of interest. The following antibodies were used: mouse monoclonal anti-htt (clone 1HU-4C8; (Trottier et al., 1995), WB 1:5000, IF 1:200), anti-ß-catenin (BD bioscience, San Jose, CA, WB 1:5000), anti-α-tubulin(clone DM1A, Sigma, St. Louis, MO, WB, 1:3000), anti-ß-galactosidase (Promega, Madison, WI, IF, 1:100), anti-ß-TrCP (clone 1B1D2; Zymed, San Fransisco, CA, WB:1:500) , anti-ß-actin (Sigma, St. Louis, MO, WB, 1:5000), anti-GFP (Roche, Mannheim, Germany), anti-Armadillo (clone N27A1, ascites, Developmental Studies Hybridoma Bank, Iowa city, IA, WB 1:500) and anti-mouse IgG (Upstate, Charlottesville, VA, USA), polyclonal rabbit anti-GFP (Millipore, Bedford, MA, IF 1:200) and monoclonal Rat anti-HA (clone 3F10, Roche, Mannheim, Germany, WB 1:5000). Anti-mouse and anti-rabbit secondary antibodies conjugated to AlexaFluor-488, AlexaFluor-555 were purchased from Molecular Probes (Invitrogen, Breda, The Netherlands). Anti-mouse and anti-rat secondary antibodies conjugated to HRP were purchased respectively from Jackson Immunoresearch Laboratories (West Grove, PA) and Beckman coulter (Fullerton, CA). Indomethacin was purchased from Sigma (Sigma, St. Louis).

Cell culture, transfection. Primary cultures of striatal neurons were prepared from embryonic day 17 Sprague Dawley rats (Saudou et al., 1998) and transfected at 4 days *in vitro* by a modified calcium phosphate technique (Xia et al., 1996) or by lipofectamine (Invitrogen, Carlsbad, CA). Primary cultures of cortical neurons were prepared from knock-in wild-type *Hdh*$^{Q7/Q7}$ and mutant

Hdh$^{Q111/Q111}$ mice (Wheeler et al., 2002; Wheeler et al., 2000) at embryonic day 14. When indicated, neurons were electroporated the day of plating with the rat neuron Nucleofector kit (Amaxa Biosystems, Cologne, Germany). Human embryonic kidney 293 (HEK293), Madin-Darby canine kidney (MDCK) and human epithelial HeLa cells were cultured in DMEM supplemented with 10% bovine calf serum (BCS) and 6 mM L-glutamine. HEK cells were transfected by the calcium phosphate technique whereas MDCK and HeLa cells were transfected by lipofectamine (Invitrogen, Carlsbad, CA).

Cell extracts, immunobloting and immunoprecipitation experiments. HEK and MDCK cells were lyzed 48 h after transfection, in Triton X-100 buffer (pH 7.4; 160 mM NaCl; 50 mM Hepes; 2.5 mM $MgCl_2$; 1.5 mM $CaCl_2$; 2.5 mM KCl; 1% Triton; 1 mM PMSF; 2 µg/ml Pepstatin; 2 µg/ml leupeptin; 2 µg/ml Aprotinin; 1 mM Iodoacetamide; 2 mM DTT; and 1 mM orthovanadate) and centrifuged at 11,000 x *g* (10 min; 4°C). Proteins (1–20 µg) were loaded onto SDS-PAGE and subjected to Western blot analysis.

For immunoprecipitation with anti-huntingtin (4C8) antibody, HEK transfected cells or brain from knock-in wild-type *Hdh*$^{Q7/Q7}$ and mutant *Hdh*$^{Q111/Q111}$ mice (Wheeler et al., 2002; Wheeler et al., 2000) were lyzed in 1% Triton X-100 buffer containing protease and phosphatase inhibitors and centrifuged at 16,000 g for 15 min. Lysates (500 µg at 1 µg/µl) were precleared 1 h at 4°C with 50 µl of a 50% solution of protein G beads. Extracts were incubated for 1 h at 4°C with 2 µl of anti-huntingtin (4C8) antibody or 4 µl of anti-GFP prebound with 50 µl of a 50% solution of protein G sepharose beads (Sigma). Beads were washed three times with Triton X-100 buffer. For immunoprecipitation with anti-HA antibody, primary culture of cortical neurons from KI mice were lysed in 1% Triton X-100 buffer containing protease and phosphatase inhibitors and centrifuged at 16,000 g for 15 min. Lysates (300 µg at 1 µg/µl) were incubated 1 h at 4°C with 2 µl of anti-HA antibody or 8µl of mouse-IgG and 2 h with 40 µl of protein G sepharose beads (50 % solution). Beads were washed three times with Triton X-100 buffer. Bound proteins were eluted with sodium dodecyl sulfate (SDS) loading buffer and resolved by SDS–PAGE (polyacrylamide gel electrophoresis) and subjected to immunoblotting analysis. For immunoprecipitation with anti-ß-catenin antibody, HEK cells were lyzed in 0.5% Triton X-100 buffer (pH 7.5, 150mM NaCl, 20mM

Tris, 2mM EDTA, 10mM NaF, 10mM NaPPi, 50mM KH_2PO_4, 0,5% Triton) containing protease and phosphatase inhibitors and centrifuged at 16,000 g for 15 min. Lysates (400 µg at 1 µg/µl) were precleared 1 h at 4°C with 25 µl of a 50% solution of protein G beads. Extracts were incubated overnight at 4°C with 2 µl of anti-ß-catenin antibody or same amount of anti-IgG and 1 h with 40 µl of protein G sepharose beads (50 % solution). Beads were washed three times with lysis buffer. Analysis of interaction between ß-catenin and axin or htt using anti-Myc antibody were done respectively in 0.5% Triton X-100 buffer and in 0.1% Triton X-100 buffer (pH 7.5, 150mM NaCl, 20mM Tris, 400µM EDTA, 10mM NaF, 10mM NaPPi, 0.1% Triton). Lysates (400 µg at 1 µg/µl) were precleared 1 h at 4°C with 25 µl of a 50% solution of protein G beads. Extracts were then incubated for 1 hour at 4°C with 2 µl of anti-myc antibody and 1 h with 40 µl of protein G sepharose beads (50 % solution). Beads were finally washed three times with lysis buffer.

Mouse tissues

Hdh$^{Q111/Q111}$ and CAG140 knock-in mice have been previously described (Wheeler *et al*, 2000). To collect brain samples, mice were deeply anesthetized in a CO_2 chamber, and their cortices were dissected out on ice and rapidly frozen using CO_2 pellets. All experimental procedures were performed in strict accordance with the recommendations of the European Community (86/609/EEC) and the French National Committee (87/848) for care (*Hdh*$^{Q111/Q111}$ mice) and in accordance with the US Public Health Service Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UCLA (CAG140 knock-in mice).

Brain tissues. Tissues were obtained from the Harvard Brain Tissue Resource Center (HBTRC; Belmont, MA): two controls (samples 1–2), one HD grade 3 (HD3, sample 3), and one grade 4 (HD4, sample 4) patients. Samples correspond respectively to brain numbers 4741, 4744, 4797 and 4680 as numbered by HBTRC. Samples were homogenized in NP-40 lysis buffer and cleared by centrifugation at 6000 x *g* (15 min; 4°C). Western blot analysis was performed on 10 µg of total extracts.

Immunofluorescence experiments. HeLa cells were grown on uncoated glass coverslips and transfected with ß-catenin-eGFP and htt-480-17Q, htt-480-68Q or empty vector (pcDNA) (ratio 7:1) for 48 h. Cells were fixed with 4%

paraformaldehyde (PFA) for 20 min and incubated with anti-GFP (rabbit, 1:200) and anti-Htt (4C8; 1:200) antibodies. Pictures were collected using a 3D microscope (Gauthier et al., 2004) or a confocal Leica SP5. Projections, and analyses were generated using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2009). For ß-catenin-GFP quantifications, a home-built macro was used (A. Chapelle and O. Anezo). GFP average intensity was measured in a 10 pixel wide square in the nucleus and in the cytoplasm. The background was also evaluated by measuring the intensity outside the cell. This value was subtracted to cytoplasm and nucleus intensities. We analyzed the ratio between cytoplasm and nucleus intensity. A minimum of 250 cells per condition was scored.

Drosophila Stocks and experiments. UAS-LacZ (on III) and *gmr*-Gal4 (on II) and *gmr*-Gal4-UAS-GFP (on II) strains were obtained from F. Maschat; UAS-Htt^{548aa}-Q0 and UAS-Htt^{548aa}-128Q from J. Troy Littleton (Lee et al., 2004) and UAS-Slimb (on III) from B. Limbourg-Bouchon (Grima et al., 2002). Using those stocks, we generated the following homozygous strains: *w*;*gmr*-Gal4;UAS-LacZ and *w*;*gmr*-Gal4;UAS-Slimb. All flies were raised on standard medium at 25°C.

For rescue experiments, we crossed either virgin *w*; UAS-Htt^{548aa}-Q0 or *w*; UAS-Htt^{548aa}-Q128 with *w*;*gmr*-Gal4;UAS-Slimb or *w*;*gmr*-Gal4;UAS-LacZ males. As control, virgins *w*;*gmr*-Gal4-UAS-GFP were crossed with UAS-LacZ and UAS-Slimb males. All crosses were done at the same time and under the same condition: flies were allowed to mate for 3 days at 25°C, and then the eggs were transferred to 29°C. Phenotypes were analyzed between 10 and 12 days after adults eclosion, when the eye depigmentation is maximal for UAS-Htt^{548aa}-128Q X *w*;*gmr*-Gal4;UAS-LacZ crosses. As a rescue, we considered fly with over than 50% of remaining pigmentation. For analysis of ommatidial morphology, flies were frozen on dry ice, and kept at -80°C until used. Before use, all samples were dehydrated and coated with gold/palladium. Structure of ommatidia was then analyzed by scanning electron microscopy. For Western Blotting experiments, crosses were performed at 25°C and one day old adult were collected. *Drosophila* were frozen in dry ice, heads were isolated and homogenized in lysis buffer (25 mM Tris-HCl, 5 mM EDTA, 250 mM NaCl and 1% triton supplemented with

protease and phosphatases inhibitors cocktail (Sigma, Steinhelm, Germany)), let for 20 min on ice and centrifuged at 11,000 x *g* for 20 min at 4°C. Equal amount of proteins were loaded onto SDS-PAGE and subjected to Western blot analysis.

For indomethacin rescue experiment virgins *w*;*gmr*-Gal4-UAS-GFP were crossed with UAS-Htt^{548aa}-Q0 or *w*; UAS-Htt^{548aa}-Q128 males on standard drosophila food containing either indomethacin (250mg/L) or EtOH as control. Flies were allowed to mate for 3 days at 25°C, and then the eggs were transferred to 29°C. Adult were isolated everyday and transferred to a fresh tube every other day. Phenotypic analyses were conducted as described above.

Measurement of neuronal survival. Three days after plating, primary cultures of striatal neurons were transfected by a modified calcium phosphate technique (Saudou et al., 1998) with the same amount of wild-type or polyQ-huntingtin and various ß-TrCP plasmids. Cytomegalovirus-ß-galactosidase plasmid (10:1 ratio) was also added to identify the transfected cells. Forskolin (10 µM; Sigma) and IBMX (100 µM; Sigma) were added to the cultures 2 h after transfection. One day after transfection, cells were fixed with 4% paraformaldehyde (PFA) for 20 min and immunostained with anti-htt 4C8 and anti-ß-galactosidase antibodies. When stated, neurons were electroporated the day of plating with si-ß-catenin or scramble RNA and, 2 days after electroporation, transfected by lipofectamine (Invitrogen, Carlsbad, CA) with the different huntingtin constructs. For indomethacin experiments, cells were transfected with lipofectamine 2 days after plating. Conditioned media containing either indomethacin or DMSO was added to cells 6 hours after transfection for 24 hours. Cells were fixed and immunostained with anti-huntingtin 4C8 antibody 1 day after transfection. 4C8-positive neurons of similar intensities were scored under fluorescence microscopy in a blinded manner. Neuronal degeneration was assessed by neurite loss and nuclear shrinkage (Anne et al., 2007). Cell death was expressed as a fold increase in neuronal cell death relative to the death induced by the htt-480-17Q construct. Each graph represents four to six independent experiments performed in duplicate. Each bar in a given graph corresponds to the scoring of approximately 2000 neurons. Data were submitted to complete statistical analysis.

Real-time RT-PCR. Dissected frozen sample (cortices) from CAG140 and wild-type mice were lysed in TRIzol (Invitrogen Corp.). Total RNA was extracted, and samples were retrotranscribed using the First-Strand cDNA Synthesis Kit (Invitrogen). cDNAs were then diluted 1:10 and submitted to RT-PCR (iQ SYBR Green Supermix; Bio-Rad) with the following c-myc (5′-CACCAGCAGCGACTCTGAA-3′ and 5′- GCCCGACTCCGACCTCTTG-3′) and axin2 (5′-GATTCCCCTTTGACCAGGTGG-3′ and 5′-CCATTACAAGCAAACCAGAAGT-3′) oligonucleotides. ß-actin gene was used as an internal control and quantified with the following oligonucleotides: 5′-AGGTGACAGCATTGCTTCTG-3′ and 5′-GCTGCCTCAACACCTCAAC-3′. Results were analyzed using the ICycler apparatus (Bio-Rad).

Example 2: ß-catenin accumulates in HD.

To analyze the effect of polyQ-huntingtin on ß-catenin, we expressed N-terminal fragments of huntingtin that contain the first 480 amino acids with 17Q (wild-type, htt-480-17Q) or 68Q (mutant, polyQ, htt-480-68Q) in MDCK and HEK 293 cells and determined the level of ß-catenin by immunoblotting (Fig. 1*A* and *B*). Whereas wild-type huntingtin had no effect on ß-catenin expression, expression of the htt-480-68Q fragment resulted in a significant increase in total ß-catenin in MDCK and HEK 293 cells.

We next wondered whether this accumulation of ß-catenin occured *in vivo*. We used a *Drosophila* model, where the N-terminal 548 amino acids of human huntingtin containing 0 (htt-548-Q0) or 128 (htt-548-Q128) glutamines, is inserted into the genome under the control of upstream activator sequences (UAS) that are recognized by the Gal4 transcription activator (Lee et al., 2004). This model recapitulates different hallmarks of HD (Lee et al., 2004). Htt-548-Q0 and htt-548-Q128 forms of huntingtin were expressed by using the eye-specific *gmr*-Gal4 driver, and the amount of armadillo (arm), the *Drosophila* homologue of ß-catenin, was analysed by immunobloting (Fig. 1*C*). We found that armadillo levels were two fold increased in the htt-548-Q128 flies compared to htt-548-Q0 flies.

We next analyzed the levels of ß-catenin in CAG140 knock-in HD mice. This mouse line carries a CAG expansion inserted into the endogenous mouse huntingtin gene. As revealed by immunoblotting, ß-catenin levels were significantly

higher in cortical extracts from 8 month old CAG140 mice compared to cortical extracts from wild-type littermates (Fig. 2A). As previously described, at this stage, brain extracts did not show profound modifications of the neuronal marker bIII-tubulin or the glial marker glial fibrillary acidic protein (GFAP). Similar observations were made in the striata of both CAG140 mice and *Hdh*$^{Q111/Q111}$ mice, a different knock-in model of HD. We also performed immunohistochemical staining of brain sections from 12 month old CAG140 mice. We found ß-catenin almost exclusively in the neuropil in the striatum. ß-catenin staining appeared to surround NeuN staining. In the thalamus, ß-catenin was nuclear. This localization agrees with a previous study and negates the possibility that ß-catenin accumulation only results from neuronal loss or gliosis.

To confirm the physiological relevance of this accumulation in HD, we assessed the level of ß-catenin in *post-mortem* striatal samples from grade 3 and 4 HD patients by Western Blot analyses (Fig. 1*D*) and observed a dramatic increase of ß-catenin in HD patients compared to control individuals. Together, these results establish that ß-catenin accumulates in cellular and *Drosophila* models of HD as well as in *post-mortem* samples from patients.

It has been well demonstrated that when not degraded, ß-catenin accumulates in the cytoplasm and then diffuses to the nucleus. We thus examined whether polyQ-huntingtin could influence the intracellular localization of ß-catenin (Fig. 1*E*). For this purpose, we investigated the subcellular localisation of GFP-tagged ß-catenin constructs in HeLa cells expressing either empty vector (pcDNA) or huntingtin constructs (htt-480-17Q or -68Q). As expected the overexpressed ß-catenin localizes mainly to the nucleus in control cells and in cells expressing wild-type huntingtin. Surprisingly, GFP-ß-catenin localized both in the cytoplasm and in the nucleus in cells transfected with htt-480-68Q (Fig. 1*E*). Moreover the ratio between cytoplasmic and nuclear ß-catenin was significantly increased in cells expressing polyQ-huntingtin as compared to control or wild-type huntingtin expressing cells (Fig. 1*F*). Together these results show that mutant huntingtin overexpression results in ß-catenin accumulation in the cytoplasm.

Example 3: Accumulation of ß-catenin is toxic in HD.

To establish whether the accumulation of ß-catenin observed in HD could promote neuronal toxicity, we tested whether reduction of ß-catenin expression could influence the polyQ-induced toxicity by studying a neuronal model of HD that recapitulates several features of the disease (Saudou et al., 1998). Primary culture of striatal neurons, were first electroporated with a siRNA directed against ß-catenin or with a scramble RNA and then transfected with htt-480-17Q or htt-480-68Q constructs (Fig. 1*G*). As expected, the 480-68Q fragment induced a statistically significant increase in neuronal death compared with the htt-480-17Q construct under control condition (scramble RNA) (Fig. 1*G*). Interestingly, downregulation of ß-catenin level completely inhibited polyQ-huntingtin-induced toxicity, suggesting that the abnormal accumulation of ß-catenin in HD is toxic.

Example 4: The ß-catenin which accumulates is phosphorylated and this accumulation does not result in transcriptional activation

Phosphorylation of ß-catenin by CKI and GSK3-b is a prerequisite step to its ubiquitination and degradation by the proteasome. We investigated the phosphorylation status of ß-catenin that accumulates in HD with specific antibodies recognizing forms of ß-catenin phosphorylated either at positions 41 and 45 or at positions 33, 37, 41 (Fig. 2B and D). Phosphorylated ß-catenin was elevated in brain extracts from 22 month old CAG140 mice and in *post-mortem* striatal samples from HD patients (Fig. 2B to E). Similar observations were made in 8 month old CAG140 mice (data not shown). Therefore, the stabilization of ß-catenin in HD occurs subsequent to its phosphorylation by CKI and GSK3-b.

Upon Wnt signaling, ß-catenin accumulates in the cytoplasm and translocates to the nucleus where it acts as a transcriptional co-activator to modulate the expression of target genes such as those encoding c-myc and axin2. We analyzed the levels of c-myc (Fig. 2F) and axin2 (Fig. 2G) transcripts by quantitative real-time RT-PCR in cortical extracts from 22 month old CAG140 knock-in mice. We did not find changes in axin2 or c-myc gene expression in HD mice as compared to their wild-type littermates. We conclude that polyQ-huntingtin-induced ß-catenin accumulation does not result in transcriptional

activation. This agrees with our observation that ß-catenin accumulates under its phophorylated form.

Example 5: Huntingtin associates with the ß-catenin destruction complex

As ß-catenin was found to accumulate in HD, we investigated whether huntingtin interacted with the ß-TrCP and axin components of the destruction complex, and the possible consequence of the abnormal polyQ expansion in mutant huntingtin. The F-box protein ß-TrCP is the substrate-recognizing subunit of the $SCF^{ß\text{-}TrCP}$ E3 ubiquitin ligase complex, which is required for ß-catenin degradation, and axin is a core component of the destruction complex. We used primary cultures of striatal neurons from wild-type mice or $Hdh^{Q111/Q111}$ knock-in mice, which have a CAG expansion inserted into the endogenous mouse huntingtin gene (Wheeler *et al*, 2000). We electroporated these neurons with HA-tagged ß-TrCP and carried out immunoprecipitation experiments with an anti-HA antibody. HA-ß-TrCP bound to endogenous huntingtin (Fig. 3A). Moreover, an endogenous interaction between these proteins occurred, as demonstrated by experiments in which huntingtin was immunoprecipitated from wild-type and $Hdh^{Q111/Q111}$ mouse brain extracts (Fig. 3B). The polyQ expansion did not affect the interaction of huntingtin with ß-TrCP, as no obvious differences were observed in HA and huntingtin immunoprecipitation experiments in wild-type and $Hdh^{Q111/Q111}$ conditions (Fig. 3A and B).

We next used an anti-myc antibody to study the interaction between huntingtin and axin in HEK 293 cells cotransfected with a myc-tagged axin construct and fragments of huntingtin (htt-480-17Q or -68Q) or an empty vector (pcDNA). Huntingtin interacted with axin and this interaction was not modulated by the polyQ expansion (Fig. 3C). In conclusion, huntingtin interacts with the ß-catenin destruction complex, but this interaction is unmodified by the presence of mutated huntingtin.

Example 6: ß-catenin binding to the destruction complex is altered in HD

We next analyzed huntingtin binding to ß-catenin. We transfected HEK 293 cells with constructs encoding 1301-amino acid N-terminal fragments of huntingtin containing 17 (wild-type, htt-1301-17Q) or 73 (polyQ, htt-1301-73Q) glutamines. These 1301-amino acid N-terminal fragments, like the htt-480-17Q or htt-480-68Q fragments, reproduce key features of HD (Anne *et al*, 2007; Saudou *et al*, 1998). Similarly to the htt-480-17Q fragment, the htt-1301-17Q fragment bound ß-catenin (Fig. 4A). Furthermore, ß-catenin did not interact with ataxin 3 and 7, whose mutations -abnormal polyQ expansion- are the cause of spinal and cerebellar ataxias. Therefore, the first 480 amino acids of huntingtin are sufficient for the huntingtin/ß-catenin interaction and this interaction is specific to huntingtin.

We also tested the influence of the presence of the polyQ tract and found that the co-immunoprecipitated ß-catenin was decreased in cells transfected with htt-1301-73Q (Fig. 4A). We then showed that endogenous huntingtin and ß-catenin interacted by performing a huntingtin immunoprecipitation from wild-type mouse cortical extracts (Fig. 4B). This association was decreased when huntingtin contained an abnormal polyQ expansion in $Hdh^{Q111/Q111}$ cortical extracts. When performing the converse immunoprecipitation experiment using an anti-ß-catenin antibody (Fig. 4C), we consistently observed that ß-catenin bound huntingtin in a polyQ-dependent manner.

Given this modification of the polyQ-huntingtin/ß-catenin interaction, we investigated the possible effect of the polyQ expansion on the recruitment of ß-catenin to the destruction complex. We thus analyzed the interaction between ß-catenin and axin in wild-type and polyQ-huntingtin conditions. We performed immunoprecipitation experiments with anti-myc antibodies on extracts from HEK 293 cells expressing myc-tagged axin and htt-480-17Q or htt-480-68Q constructs (Fig. 5A). The binding of ß-catenin to axin was significantly decreased in the polyQ context consistent with the impairment of ß-catenin binding to the degradation complex in HD. Similar results were obtained in the converse experiment, using anti-ß-catenin antibody (Fig. 5B). We also asked whether mutant huntingtin influences the interaction between ß-catenin and ß-TrCP. We performed ß-catenin immunoprecipitation experiments with HEK 293 cells expressing myc-ß-TrCP and

wild-type or polyQ-huntingtin constructs, and found that the interaction between ß-catenin and ß-TrCP was impaired by the presence of mutant huntingtin (Fig. 5C).

Thus, the decreased interaction between ß-catenin and polyQ-huntingtin reduces the binding of ß-catenin to the core of the destruction complex and to the subunit with the ubiquitination activity.

Example 7: ß-TrCP but not its dominant negative form protects striatal neurons from polyQ-htt- induced cell death.

As ß-catenin accumulates in HD and its degradation complex is impaired, we next investigated whether ß-TrCP possessed neuroprotective properties in HD. To this end, we evaluated the percentage of neuronal cell death in primary cultures of rat striatal neurons transfected with the 480-17Q and 480-68Q plasmids in the presence of a construct expressing HA-tagged ß-TrCP or a HA-tagged dominant negative form lacking the F-Box (ß-TrCPΔF). ß-TrCPΔF is an inactive form of ß-TrCP that cannot bind to the SCF complex subsequently blocking the degradation of phosphorylated ß-catenin (Hart et al., 1999; Latres et al., 1999). Interestingly, ß-TrCP decreased neuronal death induced by the htt-480-68Q fragment of huntingtin to levels comparable to the wild-type situation (Fig. 6*A*). However ß-TrCPΔF, completely failed to rescue polyQ-huntingtin-induced neuronal death (Fig. 6*B*). These findings show that ß-TrCP exerts a neuroprotective effect in a cellular model of HD and this depends on its ability to bind the E3 ubiquitin ligase complex SCF.

Example 8: ß-TrCP rescues neuronal cell death through the degradation of ß-catenin.

To unequivocally address whether ß-TrCP rescue polyQ-huntingtin-induced toxicity through the degradation of ß-catenin, we analysed whether Fbox proteins that can only degrade ß-catenin had neuroprotective properties in our cellular model of HD. We co-transfected primary cultures of striatal neurons with htt-480-17Q or htt-480-68Q constructs and with FLAG-tagged chimeric F-box fusion proteins, Fbox-ßBD^{Tcf4} or Fbox-ßBD^{Ecad}. In the latter constructs, the WD40-repeat of ß-TrCP was replaced with the ß-catenin binding domain from Tcf4 or E-cadherin (Liu et al., 2004). Fbox-ßBD^{Tcf4} and Fbox-ßBD^{Ecad} drastically increase ß-

catenin ubiquitination and turnover (Liu et al., 2004). We found that, whereas those chimeric Fbox proteins had no effect on neuronal toxicity by themselves, they significantly reduced polyQ-induced toxicity to levels comparable to the wild-type situation (Fig. 6*C*). We conclude that the neuroprotective properties of ß-TrCP in HD specifically depend on its capacity to degrade ß-catenin.

Example 9: Decreasing ß-catenin levels by RNAi-mediated silencing is protective in HD

As ß-TrCP exerts neuroprotective effects in HD by degrading ß-catenin, we directly tested the effect on polyQ-induced toxicity of reducing ß-catenin levels. Primary cultures of striatal neurons were electroporated with an siRNA directed against ß-catenin or with a scramble RNA and were then transfected with htt-480-17Q or htt-480-68Q constructs (Fig. 6D). Transfection with the htt-480-68Q construct resulted in significantly higher levels of neuronal death than transfection with the htt-480-17Q construct under control conditions (scramble RNA) (Fig. 6D). Thus, the downregulation of ß-catenin levels completely abolished the deleterious effects of polyQ-huntingtin.

Conversely, we addressed whether an abnormal accumulation of ß-catenin was toxic in striatal neurons. For this, we examined the effect of expressing ß-catenin in primary cultures of striatal neurons (Fig. 6E). We found that elevated levels of ß-catenin resulted in striatal cell death as compared to the control situation, suggesting that abnormal accumulation of ß-catenin in striatal neurons is toxic.

Example 10: Slimb rescues neurodegeneration in a *Drosophila* HD model.

We next evaluated the protective effect of ß-TrCP in a more physiological situation. For this purpose, we asked what could be the effect of Slimb, the *Drosophila* homologue of ß-TrCP in HD flies. We determined the phenotypes induced by mutant polyQ-huntingtin expression (htt-548-Q128) in the absence or in the presence of Slimb (Fig. 7*A*). Using a *gmr*-Gal4 line, driving expression in the eyes, we observed that the expression of wild-type htt-548-Q0 did not show any adult eye phenotype. In contrast, expressing mutant polyQ-huntingtin (htt-548-

Q128) in developing eyes led to adults with a loss of eye pigmentation (left panel) and a rough eye phenotype correlated with abnormal ommatidial array as detected by scanning electron microscopy (right panels) (Lee et al., 2004). This phenotype is related to a progressive neurodegeneration of eye retina with a loss of photoreceptors (Lee et al., 2004). Interestingly, expression of UAS-Slimb transgene in htt-548-Q128 flies rescued the eye depigmentation defect and improved the ommatidial morphology compared to htt-548-Q128 fly expressing neutral UAS transgene (UASLacZ). As a control, we ensured that Slimb transgene expression did not have a phenotype by itself by crossing UAS-Slimb with GMR-Gal4-UAS-GFP flies. We next quantified the rescue in loss of eye pigmentation. When the depigmentation was maximal in polyQ-huntingtin expressing flies (100% of flies), we calculated the percentage of flies expressing UAS-Slimb and htt-548-Q128 and showing a loss of eye pigmentation phenotype. We found that Slimb overexpression decreased over 60% the loss of pigmentation (Fig. 7*A*, graph). Together our results show that Slimb rescues the loss of eye pigmentation and the ommatidial morphology in *Drosophila*, two hallmarks of neuronal degeneration in this *in vivo* HD model.

Having shown that ß-catenin accumulated in HD, we next tested whether the restoration of polyQ-induced toxicity was concomitant with rescue of ß-catenin level. We analyzed the level of total armadillo by immunoblotting of extracts of *Drosophila* expressing wild-type or polyQ-huntingtin in the eye in the presence or absence of Slimb. When Slimb was overexpressed, armadillo failed to accumulate in htt-548-Q128 expressing fly (Fig. 7*B*). In our experimental condition, Slimb by itself did not modulate the level of armadillo expression (data not shown). In agreement, it has been previously shown that downregulation of armadillo by RNA interference suppress eye degenerescence in a drosophila model of HD (Kaltenbach et al., 2007). We also assessed the amount of polyQ-huntingtin in HD *Drosophila* expressing Slimb or not (Fig. 7*B*) and observed that those remained unmodified in both conditions. This shows that the neuroprotective effect of Slimb in HD is linked to its ability to degrade ß-catenin/armadillo *in vivo*.

Example 11: The anti-inflammatory drug indomethacin reduces htt-polyQ-induced toxicity in neurons and in *Drosophila.*

As indomethacin, an inhibitor of cyclooxygenase, was shown to downregulate ß-catenin mRNA and protein level in cells (Dihlmann et al., 2001; Hawcroft et al., 2002; Kapitanovic et al., 2006), we tested whether this drug could have neuroprotective properties in a neuronal model of HD. By immunoblotting analyses, we first observed that indomethacin treatment of neurons reduced the level of ß-catenin (Fig. 8*A*). Next, we transfected primary cultures of striatal neurons with wild-type or polyQ-htt (htt-480-17Q or htt-480-68Q) and treated these cells with indomethacin (600µM) or 1% DMSO for 24 hours. In agreement with a toxic role for the accumulation of ß-catenin in HD, indomethacin treatment resulted in a reduced polyQ-htt-induced neuronal toxicity (Fig. 8*B*).

We next asked whether indomethacin could promote such a neuroprotective effect in a more physiological situation. For this purpose, we analyzed the effect of indomethacin on wild-type htt-548-Q0 and polyQ htt-548-Q128 flies. As shown previously (Fig. 8*C*), expressing mutant polyQ-huntingtin (htt-548-Q128) in developing eyes led to adults with a loss of eye pigmentation (left panel). Interestingly, this defect was rescued by indomethacin treatment. We quantified this effect when the depigmentation was maximal in polyQ-huntingtin expressing flies (100% of flies) and found that indomethacin led significantly reduced the loss of pigmentation (Fig. 8*C*, graph). Immunoblotting experiments also revealed a lack of armadillo accumulation following the treatment of polyQ-huntingtin expressing-flies with indomethacin (Fig. 9A), demonstrating a correlation between the neuroprotective effect of indomethacin and the decrease in armadillo levels.

We analyzed the effect of indomethacin on the survival of adult flies with HD. The expression of UAS-htt^{548aa}-Q128 under the control of the pan neuronal *elav*-GAL4 driver resulted in fully viable adults with uncoordinated movement and abnormal grooming behavior (*elav*-Gal4/ UAS-htt^{548aa}-Q128 flies) (Lee *et al*, 2004). These behavioral defects worsened with age and the animals died prematurely. Indomethacin treatment increased the mean survival of UAS-htt^{548aa}-Q128 flies from 5.9 to 7.5 days, a 27% fold increase with respect to untreated UAS-htt^{548aa}-Q128 flies (Fig. 9B). Together our results show that indomethacin rescues

degeneration in cells and in an *in vivo* HD model strongly supporting the notion that this drug could be a suitable HD treatment.

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CLAIMS

1. An antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of ß-catenin.

2. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to claim 1, for interfering with cytoplasmic accumulation of ß-catenin in a neuronal cell.

3. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to claim 1 or 2, wherein said antagonist inhibits ß-catenin expression in a neuronal cell.

4. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to claim 1 or 2, wherein said antagonist increases ß-catenin degradation in a neuronal cell.

5. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to claim 1 or 2, wherein said antagonist induces ß-catenin relocalization at a neuronal cell membrane.

6. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to claim 1 or 2, wherein said antagonist interferes with ß-catenin binding to a transcription factor of the T-cell factor (TCF)/lymphocyte enhancer factor (LEF) family or to a ß-catenin co-activator.

7. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 6, wherein said antagonist is selected from the group consisting of a ß-catenin iRNA, a ß-catenin antisense oligonucleotide, a ß-catenin siRNA, a ß-catenin ribozyme, an antibody or aptamer or small molecule inhibitor directed against ß-catenin and which blocks interaction of ß-catenin with TCF/LEF, a ß-catenin co-activator, a vector containing a β-TrCP encoding sequence, and a F-box chimera.

8. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 7, wherein said antagonist is selected from the group consisting of, PKF115-854, CGP049090, ICG-001.

9. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 8, wherein said neurodegenerative disorder is a disorder in which neuronal toxicity is associated with cytoplasmic ß-catenin accumulation.

10. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 9, wherein said neurodegenerative disorder is selected from the group consisting of polyglutamine (polyQ) diseases, Alzheimer's disease, Parkinson's disease, prion diseases, multiple sclerosis, and amyotrophic lateral sclerosis.

11. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 10, wherein said neurodegenerative disorder is a polyglutamine (polyQ) disease selected from the group consisting of Huntington's disease, Kennedy disease, Haw River syndrome, Spinocerebellar ataxia type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3, Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 7, Spinocerebellar ataxia type 12 and Spinocerebellar ataxia type 17.

12. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 11, wherein said neurodegenerative disorder is Huntington's disease.

13. The antagonist of ß-catenin for use for preventing and/or treating a neurodegenerative disorder according to any one of claims 1 to 12, wherein said antagonist is administered simultaneously or sequentially with another active substance.

14. A method for screening molecules likely to prevent and/or treat Huntington's disease, which method comprises:

a) contacting a candidate molecule with a cell expressing polyglutamine-huntingtin (polyQ-Htt) and ß-catenin; and

b) detecting if :

i) polyQ-Htt interacts with ß-catenin in the presence of said candidate molecule wherein, detection of an interaction between polyQ-Htt and ß-catenin in the presence of said candidate molecule is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

ii) ß-catenin cellular level is decreased in the presence of said candidate molecule as compared with ß-catenin cellular level in the absence of said candidate molecule, wherein decreased ß-catenin cellular level is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

iii) ß-catenin localization at the cell membrane is increased in the presence of said candidate molecule as compared with ß-catenin localization at the cell membrane in the absence of said candidate molecule, wherein increased ß-catenin localization at the cell membrane is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease; and/or

iv) ß-catenin degradation is increased in the presence of said candidate molecule as compared with ß-catenin degradation in the absence of said candidate molecule, wherein increased ß-catenin degradation is indicative of a candidate molecule likely to prevent and/or treat Huntington's disease.

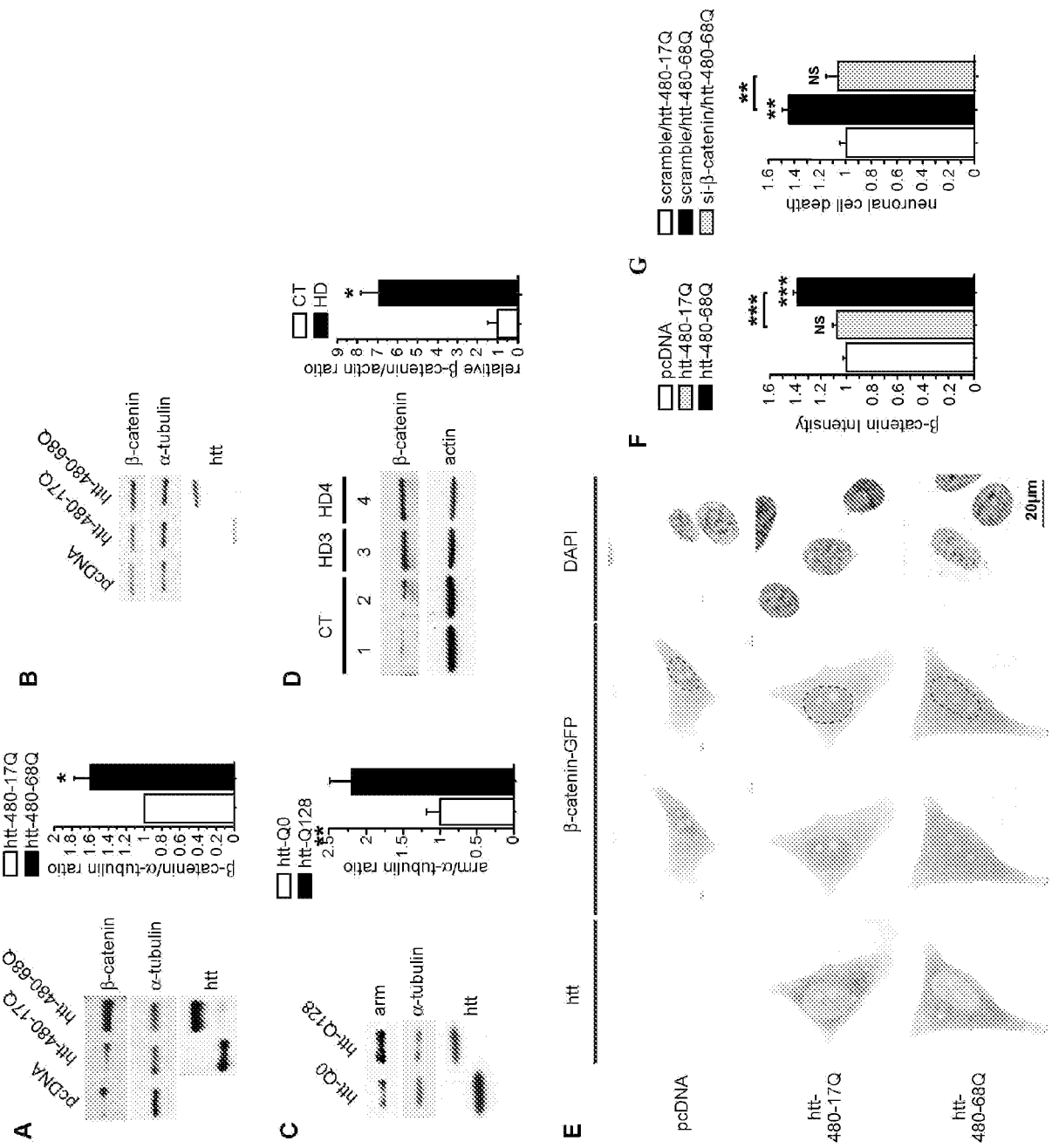
A
pcDNA
htt-480-17Q
htt-480-68Q
β-catenin
α-tubulin
htt
htt-480-17Q
htt-480-68Q
β-catenin/α-tubulin ratio
B
pcDNA
htt-480-17Q
htt-480-68Q
β-catenin
α-tubulin
htt
C
htt-Q0
htt-Q128
arm
α-tubulin
htt
htt-Q0
htt-Q128
arm/α-tubulin ratio
D
CT
HD
relative β-catenin/actin ratio
CT
HD3
HD4
1
2
3
4
β-catenin
actin
E
htt
β-catenin-GFP
DAPI
pcDNA
htt-480-17Q
htt-480-68Q
20µm
F
pcDNA
htt-480-17Q
htt-480-68Q
β-catenin Intensity
G
scramble/htt-480-17Q
scramble/htt-480-68Q
si-β-catenin/htt-480-68Q
neuronal cell death

FIG.1

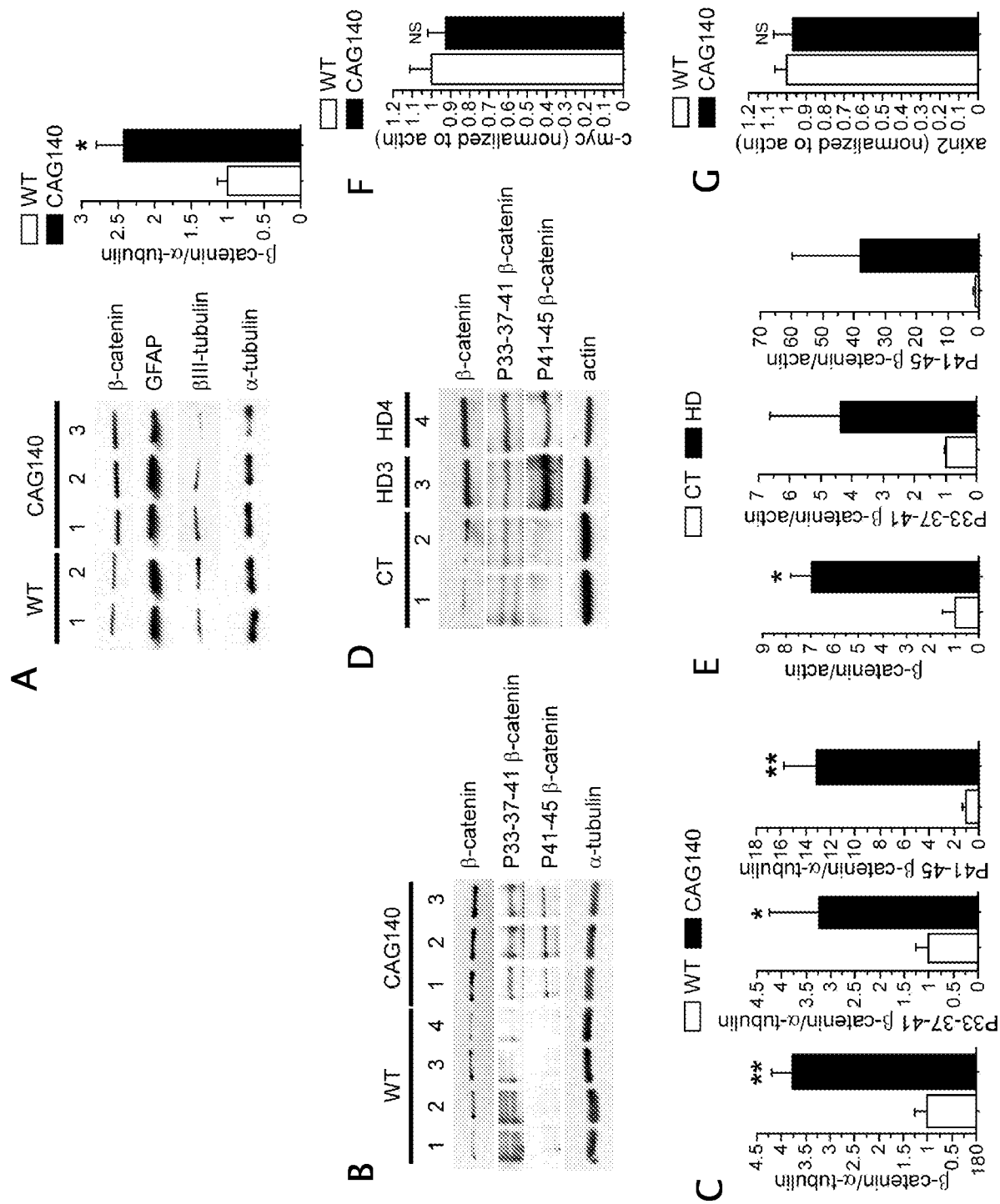
A
WT
CAG140
1 2 1 2 3
β-catenin
GFAP
βIII-tubulin
α-tubulin
β-catenin/α-tubulin
B
WT
CAG140
1 2 3 4 1 2 3
β-catenin
P33-37-41 β-catenin
P41-45 β-catenin
α-tubulin
C
WT
CAG140
β-catenin/α-tubulin
P33-37-41 β-catenin/α-tubulin
P41-45 β-catenin/α-tubulin
D
CT HD3 HD4
1 2 3 4
β-catenin
P33-37-41 β-catenin
P41-45 β-catenin
actin
E
CT
HD
β-catenin/actin
P33-37-41 β-catenin/actin
P41-45 β-catenin/actin
F
WT
CAG140
NS
c-myc (normalized to actin)
G
WT
CAG140
NS
axin2 (normalized to actin)

FIG.2

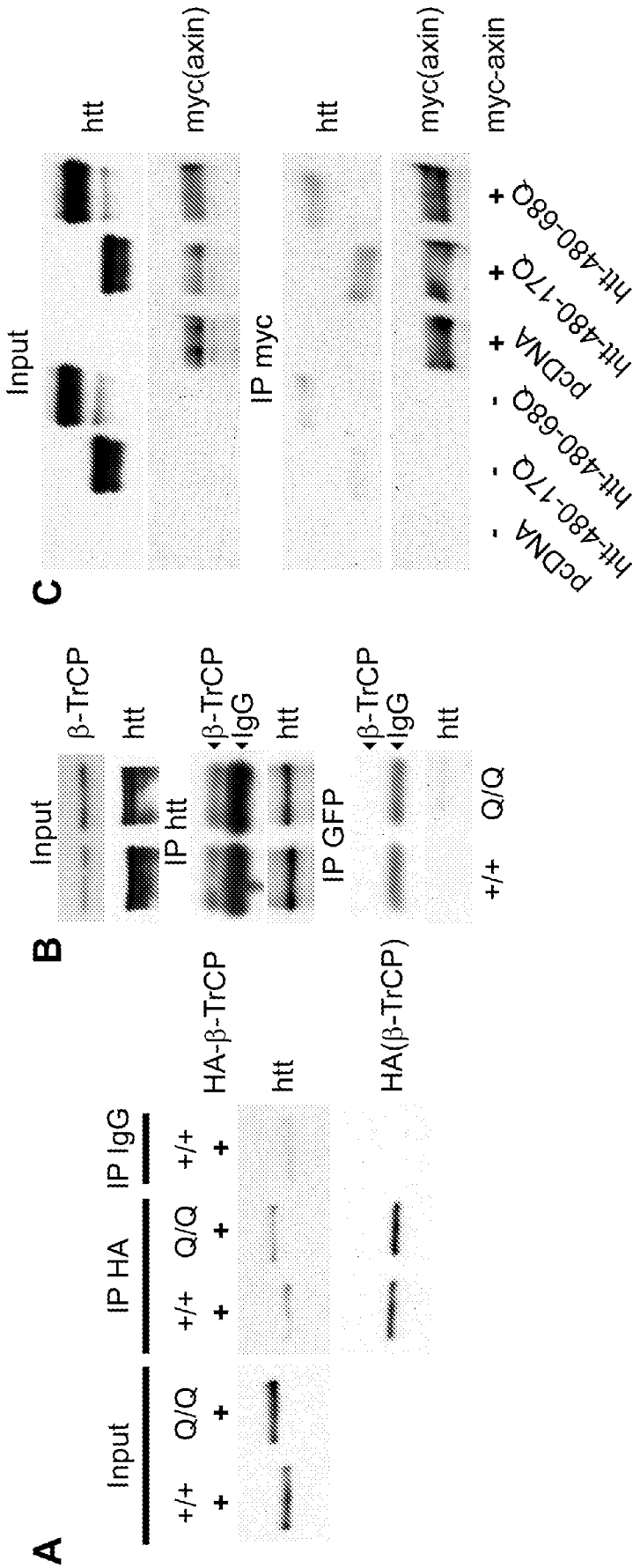
A
Input
IP HA
IP IgG
+/+
+
Q/Q
+
+/+
+
Q/Q
+
+/+
+
HA-β-TrCP
htt
HA(β-TrCP)
B
Input
β-TrCP
IP htt
htt
β-TrCP
IgG
htt
IP GFP
β-TrCP
IgG
htt
+/+ Q/Q
C
Input
htt
myc(axin)
IP myc
htt
myc(axin)
myc-axin
pcDNA -
htt-480-17Q -
htt-480-68Q -
pcDNA +
htt-480-17Q +
htt-480-68Q +

FIG.3

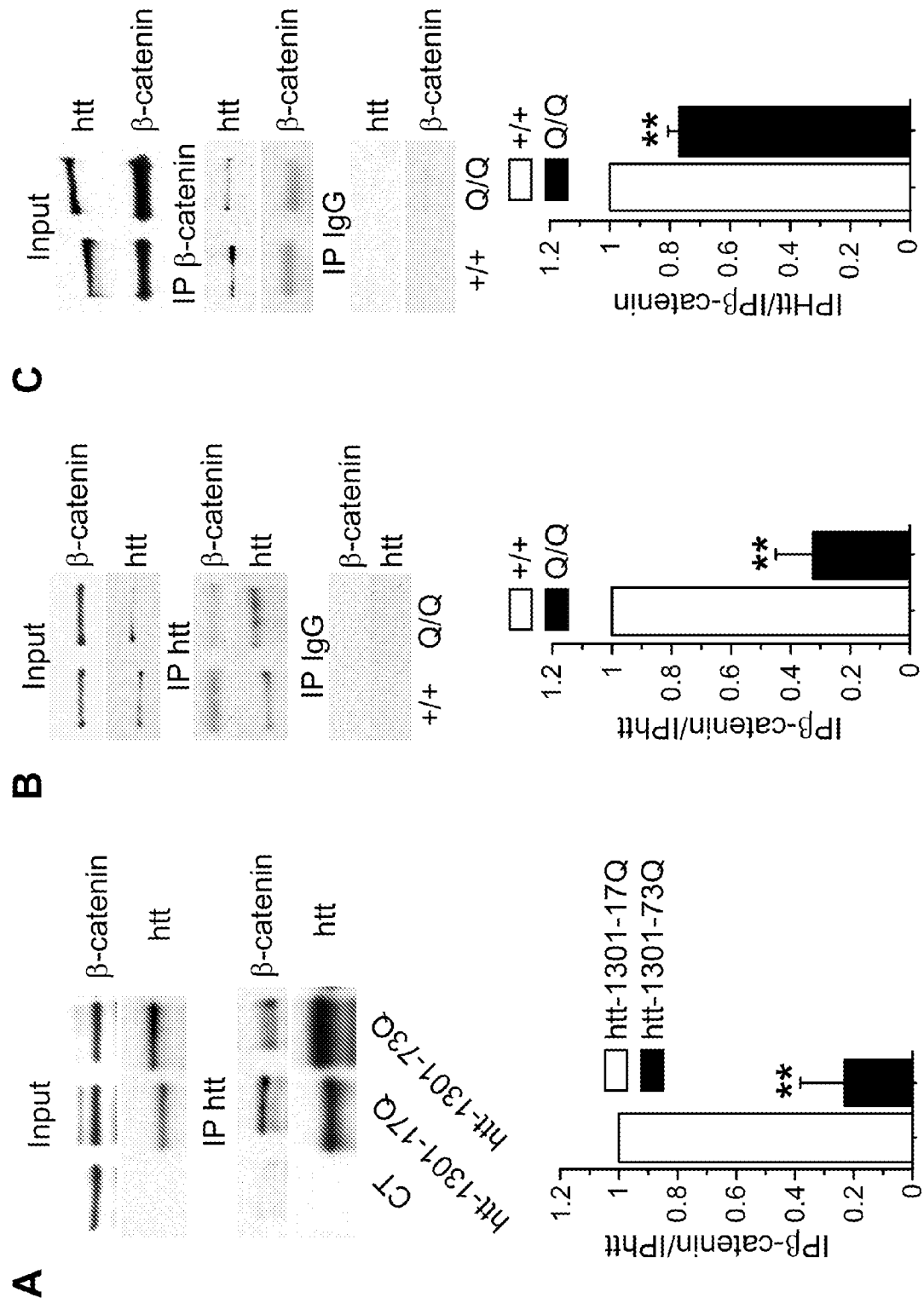
A
Input
β-catenin
htt
IP htt
β-catenin
htt
CT
htt-1301-17Q
htt-1301-73Q
IPβ-catenin/IPhtt
htt-1301-17Q
htt-1301-73Q
**
B
Input
β-catenin
htt
IP htt
β-catenin
htt
IP IgG
β-catenin
htt
+/+ Q/Q
IPβ-catenin/IPhtt
+/+
Q/Q
**
C
Input
htt
β-catenin
IP β-catenin
htt
β-catenin
IP IgG
htt
β-catenin
+/+ Q/Q
IPHtt/IPβ-catenin
+/+
Q/Q
**

FIG.4

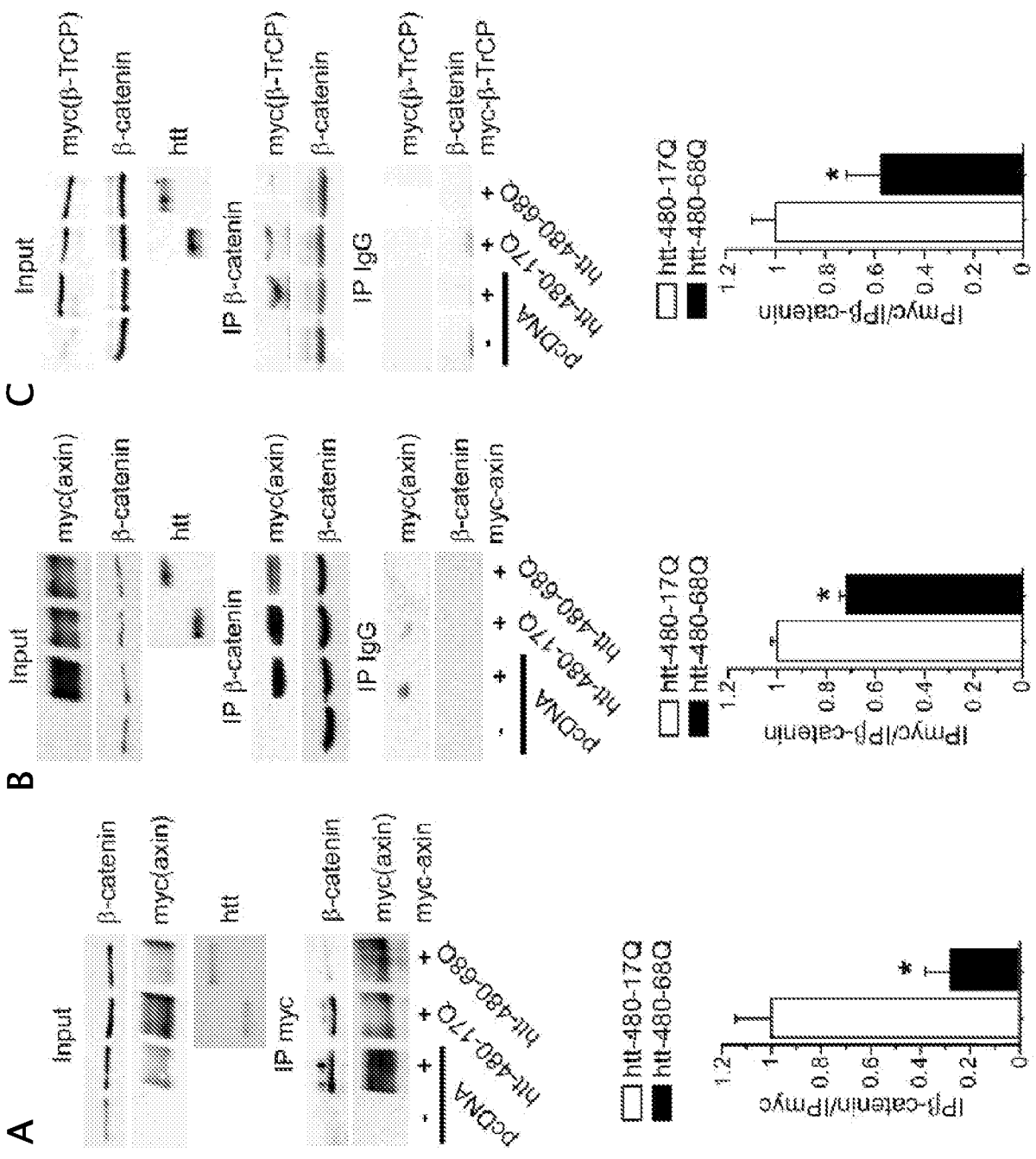
A
Input
β-catenin
myc(axin)
htt
IP myc
β-catenin
myc(axin)
myc-axin
pcDNA
htt-480-17Q
htt-480-68Q
htt-480-17Q
htt-480-68Q
IPβ-catenin/IPmyc
B
Input
myc(axin)
β-catenin
htt
IP β-catenin
myc(axin)
β-catenin
IP IgG
myc(axin)
β-catenin
myc-axin
pcDNA
htt-480-17Q
htt-480-68Q
htt-480-17Q
htt-480-68Q
IPmyc/IPβ-catenin
C
Input
myc(β-TrCP)
β-catenin
htt
IP β-catenin
myc(β-TrCP)
β-catenin
IP IgG
myc(β-TrCP)
β-catenin
myc-β-TrCP
pcDNA
htt-480-17Q
htt-480-68Q
htt-480-17Q
htt-480-68Q
IPmyc/IPβ-catenin

FIG.5

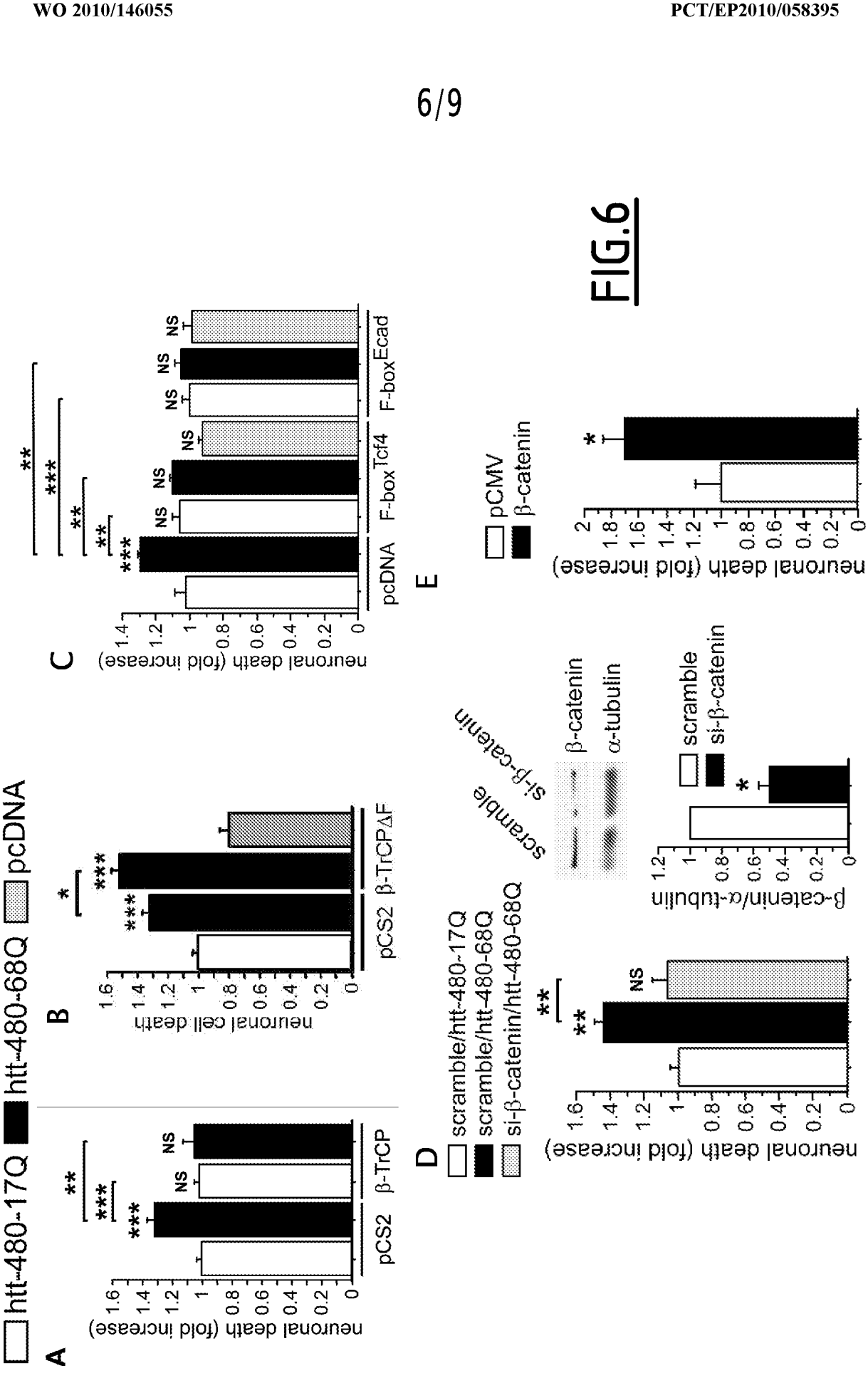
htt-480-17Q
htt-480-68Q
pcDNA
A
neuronal death (fold increase)
pCS2
β-TrCP
B
neuronal cell death
pCS2
β-TrCPΔF
C
neuronal death (fold increase)
pcDNA
F-box Tcf4
F-box Ecad
D
scramble/htt-480-17Q
scramble/htt-480-68Q
si-β-catenin/htt-480-68Q
neuronal death (fold increase)
scramble
si-β-catenin
β-catenin
α-tubulin
β-catenin/α-tubulin
scramble
si-β-catenin
E
pCMV
β-catenin
neuronal death (fold increase)

FIG.6

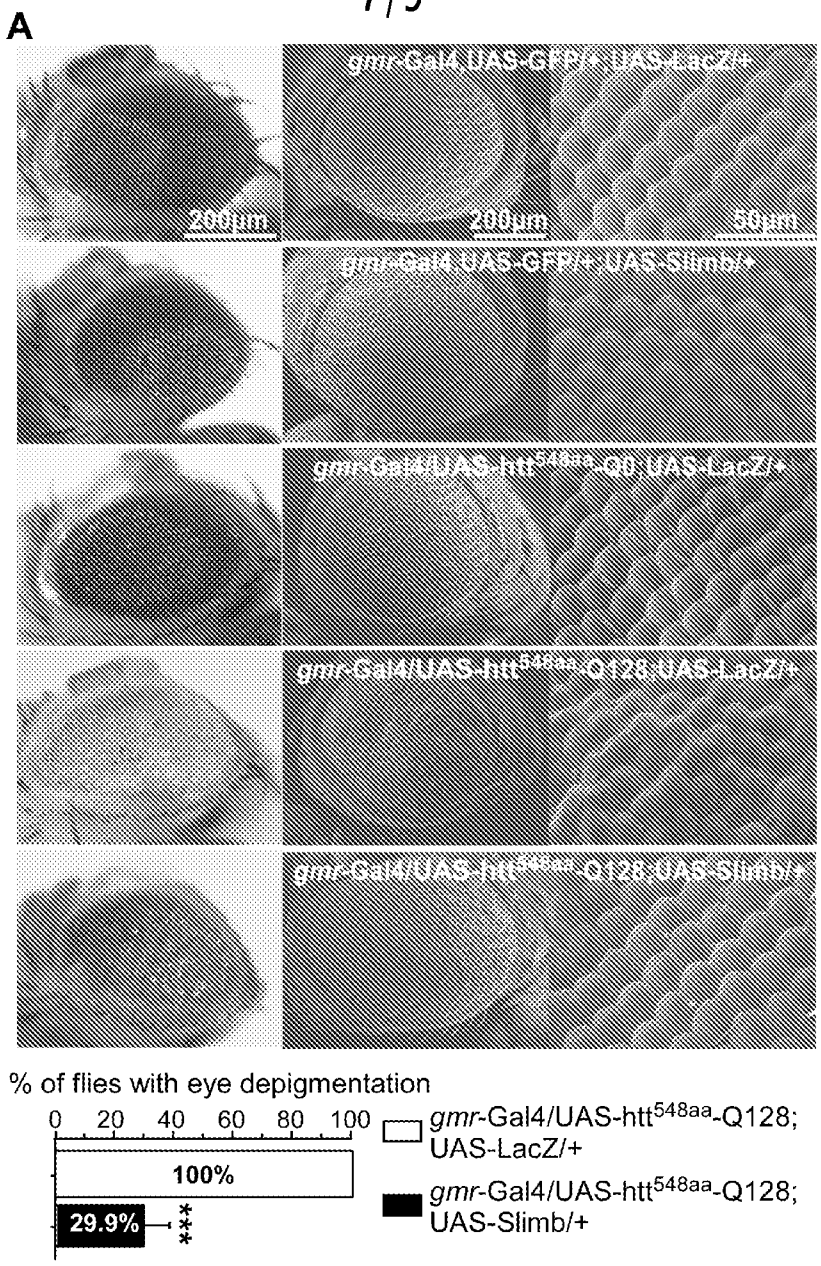
A
gmr-Gal4,UAS-GFP/+;UAS-LacZ/+
200µm
200µm
50µm
gmr-Gal4,UAS-GFP/+;UAS-Slimb/+
gmr-Gal4/UAS-htt548aa-Q0;UAS-LacZ/+
gmr-Gal4/UAS-htt548aa-Q128;UAS-LacZ/+
gmr-Gal4/UAS-htt548aa-Q128;UAS-Slimb/+
% of flies with eye depigmentation
0 20 40 60 80 100
100%
29.9%
***
gmr-Gal4/UAS-htt548aa-Q128; UAS-LacZ/+
gmr-Gal4/UAS-htt548aa-Q128; UAS-Slimb/+
B
gmr/htt548aa-Q0; LacZ/+
gmr/htt548aa-Q128; LacZ/+
gmr/htt548aa-Q128; Slimb/+
arm
α-tubulin
htt
gmr/htt548aa-Q0;LacZ/+
gmr/htt548aa-Q128;LacZ/+
gmr/htt548aa-Q128;Slimb/+
*
**
NS
arm/α-tubulin
2.5
2
1.5
1
0.5
0

FIG.7

A
IndoM
0.1 0.2 0.4 0.6 CT mM
β-catenin
α-tubulin
B
neuronal death (fold increase)
htt-480-17Q
htt-480-68Q
**
***
NS
**
DMSO IndoM
C
CT
IndoM
gmr-Gal4,UAS-GFP/ UAS-htt548aa-Q128
gmr-Gal4,UAS-GFP/ UAS-htt548aa-Q0
200µm
% of flies with eye depigmentation
120 100 80 60 40 20 0
CT
IndoM
100%
40.8%
***

FIG.8

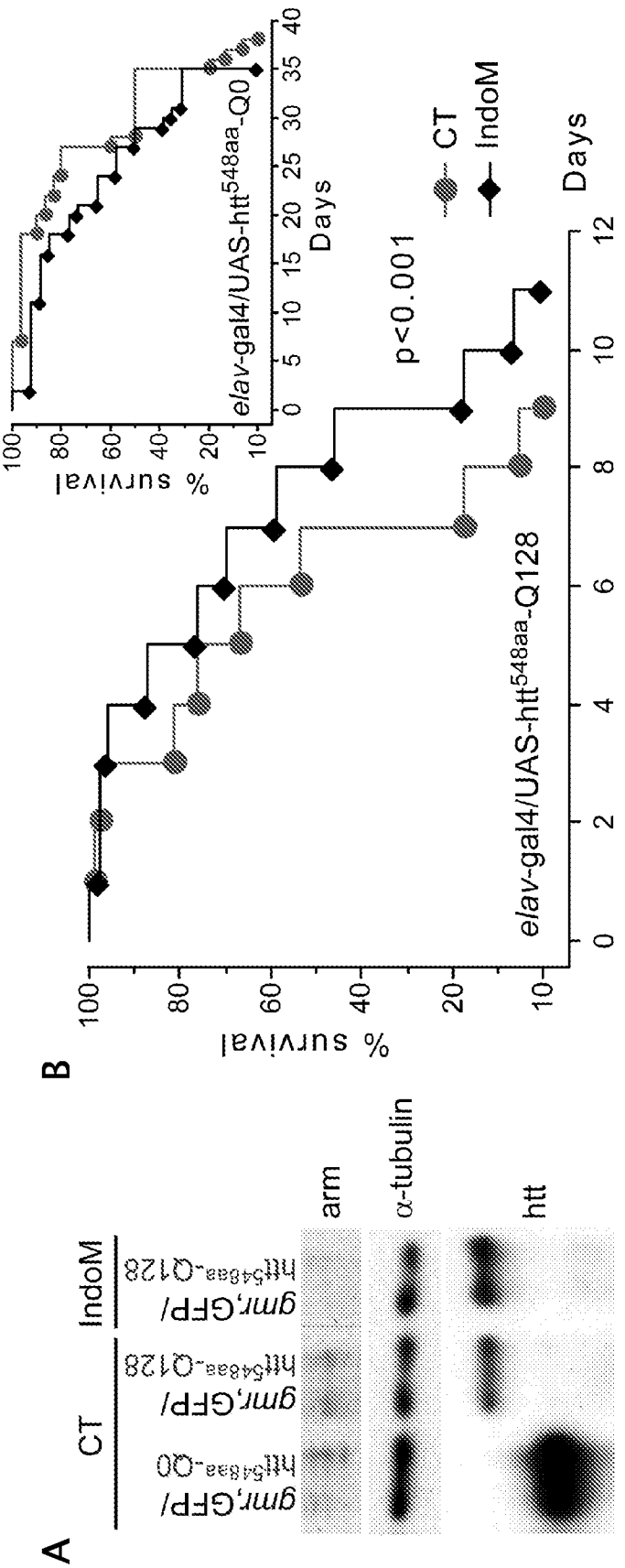
A
CT
IndoM
gmr,GFP/ htt548aa-Q0
gmr,GFP/ htt548aa-Q128
gmr,GFP/ htt548aa-Q128
arm
α-tubulin
htt
B
% survival
elav-gal4/UAS-htt548aa-Q128
p<0.001
CT
IndoM
Days
elav-gal4/UAS-htt548aa-Q0
% survival
Days

FIG.9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/058395

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/00 A61P25/14 A61P25/16 A61P25/28 A61K38/39
A61K31/7088 A61K33/00 A61K38/17 A61K39/395 A61K48/00
A61K45/06 G01N33/68 A61K31/713 C12N15/113

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 C12N

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 practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SALINS ET AL: "TGF-beta1 is increased in a transgenic mouse model of familial Alzheimer's disease and causes neuronal apoptosis" NEUROSCIENCE LETTERS, LIMERICK, IE LNKD-DOI:10.1016/J.NEULET.2007.10.025, vol. 430, no. 1, 30 October 2007 (2007-10-30), pages 81-86, XP022411299 ISSN: 0304-3940 whole article, particularly abstract; Fig. 4f; p. 86, left hand column, last paragraph ----- -/--	1-7,9, 10,13

[X] 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
22 September 2010	01/12/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheithe, Rupert

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CULLEN D A ET AL: "The effect of polyglutamine expansion in the human androgen receptor on its ability to suppress beta-catenin-Tcf/Lef dependent transcription." NEUROSCIENCE LETTERS, vol. 354, no. 1, 2 January 2004 (2004-01-02), pages 54-58, XP002601652 ISSN: 0304-3940 whole document, especially abstract; p. 57, left hand column, last sentence	11
A	BORRELL-PAGÈS M ET AL: "Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies" CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, BIRKHÄUSER-VERLAG, BA LNKD- DOI:10.1007/S00018-006-6242-0, vol. 63, no. 22, 16 October 2006 (2006-10-16), pages 2642-2660, XP019457697 ISSN: 1420-9071 whole document, especially p. 2644, Table 1; p. 2649, chapter "Towards therapies for HD", first paragraph; p. 2650, Table 2	1-7,9-13
A	KURKOWSKA-JASTRZEBSKA I ET AL: "Indomethacin protects against neurodegeneration caused by MPTP intoxication in mice" INTERNATIONAL IMMUNOPHARMACOLOGY, vol. 2, no. 8, July 2002 (2002-07), pages 1213-1218, XP002601653 ISSN: 1567-5769 whole article; particularly p. 1217, right hand column, last paragraph	1-7,9-13
A	KAPITANOVIC S ET AL: "Effect of indomethacin on E-cadherin and beta-catenin expression in HT-29 colon cancer cells" EXPERIMENTAL AND MOLECULAR PATHOLOGY, ACADEMIC PRESS, US, vol. 80, no. 1, 1 February 2006 (2006-02-01), pages 91-96, XP024944813 ISSN: 0014-4800 [retrieved on 2006-02-01] cited in the application abstract	1-7,9-13

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HAWCROFT GILLIAN ET AL: "Indomethacin induces differential expression of beta-catenin, gamma-catenin and T-cell factor target genes in human colorectal cancer cells" CARCINOGENESIS (OXFORD), vol. 23, no. 1, January 2002 (2002-01), pages 107-114, XP002601654 ISSN: 0143-3334 cited in the application abstract	1-7,9-13
T	GODIN JULIETTE D ET AL: "Mutant huntingtin-impaired degradation of beta-catenin causes neurotoxicity in Huntington's disease" EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 29, no. 14, July 2010 (2010-07), pages 2433-2445, XP002601655 ISSN: 0261-4189 the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2010/058395

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.:
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:

see additional sheet

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 an 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.:

1-7, 9-13(all partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a beta-catenin iRNA or beta-catenin siRNA.

---

2. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a beta-catenin antisense oligonucleotide.

---

3. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a beta-catenin ribozyme.

---

4. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is an antibody according to claim 7.

---

5. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is an aptamer according to claim 7.

---

6. claims: 8(completely); 1-7, 9-13(partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a small molecule inhibitor according to clam 7.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a beta-catenin co-activator.

---

8. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a vector according to claim 7.

---

9. claims: 1-7, 9-13(all partially)

An antagonist of beta-catenin for use for preventing and/or treating a neurodegenerative disorder, wherein said antagonist is a direct antagonist of beta-catenin and wherein said direct antagonist is a F-box-chimera.

---

10. claim: 14

A method of screening molecules according to claim 14.
