



(43) International Publication Date
28 August 2014 (28.08.2014)

(10) International Publication Number
WO 2014/128142 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/EP2014/053187

(22) International Filing Date:
19 February 2014 (19.02.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
13155742.3 19 February 2013 (19.02.2013) EP

(71) Applicants: **DEUTSCHES KREBSFORSCHUNG-SZENTRUM** [DE/DE]; Im Neuenheimer Feld 280, 69120 Heidelberg (DE). **RUPRECHT-KARLS-UNIVERSITÄT HEIDELBERG** [DE/DE]; Grabengasse 1, 69117 Heidelberg (DE).

(72) Inventors: **ERDMANN, Gerrit**; Poststrasse 36/1, 69115 Heidelberg (DE). **BOUTROS, Michael**; Marstallstrasse 11, 69117 Heidelberg (DE).

(74) Agent: **DICK, Alexander**; Herzog Fiesser & Partner, Patentanwälte, Dudenstrasse 46, 68167 Mannheim (DE).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: THE SIGNATURE OF WNT/ β -CATENIN SIGNALING IN CANCER

(57) Abstract: The present invention is concerned with compounds modulating Wnt/ β -catenin signaling in cancer cells. Furthermore, contemplated by the present invention are therapeutic and diagnostic methods and means for identifying such a compound, as well as the use of said compound for the manufacture of a drug against cancer. The present invention also encompasses therapeutic and diagnostic methods and means for diagnosing cancer in the subject and for determining whether a subject is in need of an anti-cancer therapy, as well as uses thereof. It is also referred to a device and kit for carrying out the methods of the present invention.



WO 2014/128142 A1

The signature of Wnt/ β -catenin signaling in Cancer

5 The present invention is concerned with compounds modulating Wnt/ β -catenin signaling in cancer cells. Furthermore, contemplated by the present invention are therapeutic and diagnostic methods and means for identifying such a compound, as well as the use of said compound for the manufacture of a drug against cancer. The present invention also encompasses therapeutic and
10 diagnostic methods and means for diagnosing cancer in the subject and for determining whether a subject is in need of an anti-cancer therapy, as well as uses thereof. It is also referred to a device and kit for carrying out the methods of the present invention.

15 The Wingless-type (Wnt)/ β -catenin signaling plays important roles in multiple biological processes, including embryonic development, tissue homeostasis and carcinogenesis, and is conserved across metazoans (see Logan and Nusse 2004, *Annu Rev Cell Dev Biol* 20: 781-810). In model organisms and man, secretion of Wnts, a class of 19 hydrophobic proteins, requires the
20 multi-pass transmembrane protein evenness-interrupted/Wntless (Evi/Wls; see Bänziger et al. 2006, *Cell* 125: 509-522; Bartscherer et al. 2006, *Cell* 125: 523-533; Adell et al. 2009, *Development* 136: 905-910; Augustin et al. 2012, *EMBO Mol Med* 4: 38-51). Upon binding to receptors of the Frizzled (FZD) family and their co-receptors lipoprotein receptor related proteins 5 and 6 (Lrp5/6), Wnt proteins activate canonical and non-canonical signaling cascades.
25 Canonical signaling prevents the constitutive degradation of cytosolic β -catenin, leading to its accumulation and translocation to the nucleus, where it acts as a cofactor for transcription factors of the T-cell specific (TCF) family. In the absence of signaling, degradation of β -catenin is initiated by adenomatous polyposis coli (APC), glycogen synthase kinase β (GSK3 β) and axis inhibition protein-1 (Axin1), which form the 'destruction complex'. APC recruits casein kinase 1
30 (CK1 α) and GSK3 β , which phosphorylate β -catenin and mark it for ubiquitination by the SKP1-Cullin 1-F-box (SCF β KP1-) E3 ubiquitin ligase complex, triggering its proteasomal degradation (see Klaus and Birchmeier 2008, *Nat Rev Cancer* 8: 387-398). Wnt signaling can be regulated by, in particular, secreted antagonists of Wnt signaling such as proteins of the Dickkopf family (DKK1-4) and secreted frizzled related proteins (sFRPs; see Buechling and Boutros 2011, *Curr.*
35 *Top. Dev. Biol.* 97: 21-53). Thus, it is to be understood that said secreted Wnt antagonists may also modulate Wnt signaling, by, e.g. inhibition and/or activation of Wnt signaling. Examples for such modulating secreted Wnt antagonists are sFRPs. In presence of canonical Wnt ligands,

phosphorylation of β -catenin by GSK3 β is reduced and dephosphorylation by serine/threonine phosphatases PP1 and PP2a relieves β -catenin from constant degradation, allowing it to accumulate in the cytoplasm. β -catenin can now enter the nucleus where it displaces the transcriptional repressor transducin-like enhancer of split (TLE) (Groucho in drosophila) from T-cell specific transcription factors (TCF) such as TCF/LEF1 or TCF4 (TCF7L2). This turns TCFs into transcriptional activators resulting in the expression of target genes, for instance cyclin D1 (CCND1), Axin2 and cMyc. This process is aided by co-activators, e.g. Bcl9, and leads to recruitment of histone acetylases (HATs) such as CBP/P300. Hence, APC and β -catenin constitute opposing factors in their regulation of canonical Wnt/ β -catenin target genes: APC blocks Wnt target gene expression, β -catenin expression of these genes. Accordingly depletion of APC or β -catenin by e.g. RNAi causes the opposite effects. Knockdown of APC strongly promotes expression of Wnt/ β -catenin target genes whereas knockdown of β -catenin strongly reduces their expression. In addition, numerous other signaling pathways can interact with the Wnt/ β -catenin signaling cascade at different levels. Unexpectedly, secreted Wnt antagonists were found to attenuate Wnt/ β -catenin signaling in colorectal cancer cell lines carrying APC mutations (see Suzuki et al. 2004, Nat. Genet. 36: 417-422; Baehs et al. 2009, Cancer Lett. 276: 152-159). The canonical Wnt signaling pathway is of particular importance for the development of colorectal cancer, which is one of the third most commonly diagnosed cancers worldwide with more than one million new cases each year. Colorectal cancer, also referred to as colon carcinoma or bowel cancer, is characterized by an uncontrolled cell growth in the colon, the rectum or in the appendix. Typically, colorectal cancer starts in the lining of the bowel, grows into the muscle layers underneath, and then through the bowel wall. Classic symptoms and signs of colorectal cancer are worsening constipation, blood in the stool, change in bowel habit, weight loss, fever, loss of appetite, and nausea or vomiting.

More than 90% of sporadic colorectal carcinomas are induced by loss-of-function mutations in the adenomatosis polyposis coli (APC) gene, which encodes a scaffolding protein mediating constitutive destruction of the β -catenin transcriptional coactivator in the absence of Wnt ligand (see Tang et al. 2009, Proc Natl Acad Sci U S A. 105(28): 9697-9702). Approximately 10% of sporadic colorectal carcinomas harbor mutations in β -catenin (see Morin et al. 1997, Science 275: 1787-1790; Sparks et al. 1998, Cancer Res 58: 1130-1134). Thus, loss-of-function mutations in APC or activating mutations in β -catenin constitute the earliest events of colon cancer development. These mutations lead to excessive Wnt/ β -catenin signaling, wherein said Wnt/ β -catenin signaling regulates proliferation and differentiation of intestinal stem and progenitor cells, and induces hyperplasia in intestinal crypts (see Humphries and Wright 2008, Nat Rev Cancer 8: 415-424; Reya and Clevers 2005, Nature 434: 843-850). Furthermore, Wnt pathway activity is not only required in the initiating phase of colon tumor development, but also for the maintenance of colon cancer cells during tumor progression and the formation of

metastases (see Scholer-Dahirel et al. 2011, Proc Natl Acad Sci U S A 108: 17135-17140). This makes the Wnt pathway an interesting target for therapeutic intervention in colon cancer patients (see Barker and Clevers 2006, Nat Rev Drug Discov 5, 997-1014).

- 5 As described above, the Wnt/ β -catenin signaling pathway has been causally linked to onset and progression of colon cancer, however, targeted therapeutics are still missing.

Thus, the technical problem underlying the present invention can be seen as the provision of means and methods for reliably and efficiently identifying a compound modulating Wnt/ β -catenin signaling to comply with the aforementioned needs. The technical problem is solved by
10 the embodiments characterized in the claims and herein below.

Accordingly, the present invention relates to a method for identifying a compound modulating
15 Wnt/ β -catenin signaling comprising the steps of:

- a) determining the amounts of at least the biomarkers NKD1 and C10orf54 in a sample comprising cells, wherein said cells have been brought into contact with a compound suspected to be a modulator of Wnt/ β -catenin signaling; and
- b) comparing the amount of the biomarker NKD1 determined in step a) to a
20 reference for NKD1 and comparing the amount of the biomarker C10orf54 determined in step a) to a reference for C10orf54, whereby a compound modulating Wnt/ β -catenin signaling is identified.

25 The method of the present invention, preferably, is an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to sample pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or assisted by automation. Preferably, step a) and/or b) may in total or in part be assisted by automation, e.g., by a suitable robotic and sensory equipment for the
30 determination in step a) or a computer-implemented comparison and/or diagnosis based on said comparison in step b).

The term "Wnt/ β -catenin signaling" as used herein refers to signaling through the cellular
35 signaling pathway well known to the skilled artisan (see, e.g., MacDonald 2009, Developmental Cell 17.1: 653-71). The term "Wnt/ β -catenin signaling gene" as used herein, refers to a polynucleotide, preferably DNA or RNA, that encodes a polypeptide or RNA involved in the Wnt/ β -catenin signaling pathway. Preferably, the term relates any gene affected, directly or indirectly, by a modulation of Wnt/ β -catenin signaling, including genes mediating Wnt/ β -catenin

signaling and genes affected by Wnt/ β -catenin signaling (Wnt/ β -catenin target genes). More preferably, the term relates to genes mediating Wnt/ β -catenin signaling. Preferably, the term "Wnt/ β -catenin signaling protein", as used herein, refers to a polypeptide encoded by the aforementioned Wnt/ β -catenin signaling gene. Preferably, said Wnt/ β -catenin signaling protein is selected from a Wnt protein encoded by any one gene of WNT1, WNT2, WNT2B, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, or WNT16, or from a WNT receptor being any one of FZD1 to FZD10, Axin, APC, β -catenin, GSK-3, and a TCF/LEF transcription factor such as LEF-2. Further known polypeptides and/or regulators of the Wnt/ β -catenin signaling pathway in humans are shown in Table 9. Preferred compounds modulating the Wnt/ β -catenin signaling pathway are described elsewhere herein.

The term "modulating" as used herein refers to blocking, de novo activating, enhancing or reducing Wnt/ β -catenin signaling. It is to be understood that, preferably, said modulation refers to statistically significant changes in signaling activity. Whether modulation is statistically significant, or not, can be determined by the skilled artisan without further ado, preferably, by applying standard statistics such as, e.g., the determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Modulation can, preferably, be conferred by alteration of protein-protein interactions, isomerization, proteolytic processing, intracellular translocation, ubiquitination, protein abundance or activity and/or RNA abundance.

The term "compound" as used herein encompasses any molecule, i.e. any organic or inorganic chemical substance. The organic molecule may belong to any known chemical class of molecules. Preferably, an organic molecule is a lipid, a fatty acid, a purine, a pyrimidine, an alkaloid, an amino acid, a peptide, an antibody, an aptamere, a protein, a biogenic amine, an isoprenoid, a steroid, or a single- or double- stranded nucleic acid. The compound may be a naturally occurring compound which can be obtained from a biological or environmental source or an artificially synthesized compound. In accordance with the present invention, a compound shall be used to modulate the Wnt/ β -catenin signaling. Modulation can be conferred by various effects elicited by a modulator compound as set forth above.

As used herein, a "compound suspected to be a modulator of Wnt/ β -catenin signaling" is a compound according to the present invention for which it cannot be excluded from the state of the art that it has activity in modulating Wnt/ β -catenin signaling. Preferably, the compound suspected to be a modulator of Wnt/ β -catenin signaling belongs to the same chemical family, gene class, gene family, protein class, or protein family as a compound known as a modulator of Wnt/ β -catenin signaling from the state of the art. More preferably, the compound suspected to be

a modulator of Wnt/ β -catenin signaling has a primary structure at least 80% identical, at least 85% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, at least 99.75% identical, at least 99.9% identical, or at least 99.95% identical to a compound known as a modulator of Wnt/ β -catenin signaling from the state of the art. Preferred examples of compounds known as a modulator of Wnt/ β -catenin signaling from the state of the art are IWR1, IWR2, IWP1, IWP2, C59-Wnt, XAV 939, ICG-001, BIO, CHIR and LiCL (see Hernández et al. 2009, Mini Rev Med Chem 9: 1024-1029; Huang et al. 2009, Nature 461: 614-620; Lu et al. 2009, Bioorg. Med. Chem. Lett 19: 3825-3827; Dodge et al. 2012, J. Biol. Chem. 287: 23246-23254). Preferably, the compound suspected to be a modulator of Wnt/ β -catenin signaling is a compound for which modulation of Wnt/ β -catenin signaling can be predicted. More preferably, the compound suspected to be a modulator of Wnt/ β -catenin signaling is selected from the list consisting of antisense RNA, siRNA, micro RNA, ribozymes, oligonucleotides, peptides, PNAs, proteins, antibodies, aptamers, and organic small molecule compounds.

Preferably, a modulating compound can elicit its modulating effect directly or indirectly. The directly modulating compound affects at least one Wnt/ β -catenin signaling molecule in the Wnt/ β -catenin signaling pathway. Direct modulation can, preferably, occur by modulating the biological activity of a Wnt/ β -catenin signaling protein, e.g. by binding to said protein. The indirectly modulating compounds affect regulators which govern the abundance of a Wnt/ β -catenin signaling protein. The abundance of a Wnt/ β -catenin signaling protein can, preferably, be modulated by altering the gene expression of the Wnt/ β -catenin signaling gene, e.g., preferably, the abundance of the RNA transcripts, the translation of said transcripts into protein, the stability of said Wnt/ β -catenin signaling protein, and the like. Suitable regulators governing the abundance of a Wnt/ β -catenin signaling protein which are preferably envisaged in accordance with the present invention are, e.g., transcription factors governing Wnt gene expression. The indirect modulation may also occur via modulating the biological activity or the abundance of a regulator of a Wnt/ β -catenin signaling protein and/or of a regulator of a gene encoding a Wnt/ β -catenin signaling protein.

Compounds according to the present invention which are preferably envisaged for the direct modulation of the biological activity of a Wnt/ β -catenin signaling protein are antibodies, aptamers, or small molecules that specifically bind to the Wnt/ β -catenin signaling protein and, preferably, thereby modulate the biological activity thereof.

Compounds according to the present invention which are preferably envisaged for the indirect modulation of the biological activity of a Wnt/ β -catenin signaling protein are single- or double-stranded nucleic acids and, in particular, antisense RNAs, inhibitory oligonucleotides, PNAs,

micro RNAs, siRNA, ribozymes or triple-helix forming agents or small molecules that specifically bind to the Wnt/ β -catenin signaling gene encoding said protein or RNA transcripts thereof and, preferably, thereby modulate the biological activity thereof. Similarly, compounds according to the present invention which are preferably envisaged for the indirect modulation of the biological activity of a regulator of the Wnt/ β -catenin signaling protein are single- or double-stranded nucleic acids and, in particular, antisense RNAs, oligonucleotides, micro RNAs, siRNA, ribozymes or triple-helix forming agents or small molecules that specifically bind to the gene encoding the regulator or RNA transcripts thereof and, preferably, thereby modulate the biological activity thereof.

An antisense RNA as referred to herein means a single-stranded RNA that is complementary to a messenger RNA (mRNA) strand transcribed within a cell. Antisense RNA can be introduced into a cell to inhibit translation of a complementary mRNA by base pairing to it and physically obstructing the translation machinery. Preferably, the Wnt/ β -catenin signaling pathway is inhibited, e.g. by degradation of RNA transcribed from a Wnt/ β -catenin signaling gene (target RNA) or by inhibition of translation of said target RNA. It is to be understood that inhibiting as used herein does not necessarily mean the complete inhibition of translation in all cases. Inhibition, preferably, reduces translation by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% as compared to the uninhibited translation level in a reference. Methods relating to the use of antisense RNA to inhibit translation in animals, including mammals, are known in the art (see, e.g. Weiss 1999, Cell. Mol. Life Sci. 55: 334-358).

RNA interference (RNAi) as used herein refers to sequence-specific, post-transcriptional gene silencing of a selected target gene. The RNAi in the context of the present invention, preferably, specifically silences the expression of a Wnt/ β -catenin signaling gene. It is to be understood that silencing as used herein does not necessarily mean the complete abolishment of gene expression in all cases. RNAi, preferably, silences gene expression by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% as compared to the expression level in a reference. RNAi requires in the cell the presence of a dsRNA, wherein said dsRNA is homologous in sequence to the target RNAs. The term double stranded RNA (dsRNA) as used herein refers to RNA having a duplex structure comprising two complementary and anti-parallel nucleic acid strands. Said strands have the same or a different number of nucleotides. It is, however, also contemplated by the present invention that the dsRNA is formed between two sequence stretches on the same RNA molecule. Also preferably, RNAi can be used for therapeutic approaches to treat several types of cancers as described elsewhere herein, wherein said cancer is accompanied with a modulated expression and/or activity of at least one of the Wnt/ β -catenin signaling genes of the present invention. For such therapeutic approaches, an expression construct for small interfering RNA (siRNA) is introduced into target cells of the

subject suffering from modulated expression of a Wnt/ β -catenin signaling gene. Accordingly, siRNA can be combined efficiently with other therapy approaches. Methods relating to the use of RNAi to silence genes in animals, including mammals, are known in the art (see, e.g., Hammond et al. 2001, Nature Rev. Genet. 2: 110-119; Bernstein et al. 2001, Nature 409: 363-366; WO 9932619; and Elbashir et al. 2001, Nature 411: 494-498). As used herein, RNAi of the present invention is of sufficient length and complementarity to stably interact with the target RNA, i.e. it comprises at least 15, at least 17, at least 19, at least 21, at least 22 nucleotides complementary to the target RNA. By stably interact is meant an interaction of the RNAi or its products produced by the cell with a target RNA, e.g., by forming hydrogen bonds with complementary nucleotides in the target RNA under physiological conditions. Not all nucleotides of said RNAi necessarily exhibit complete Watson-Crick base pairs in the interaction with the target RNA; the two RNA strands may be substantially complementary. Preferably, complementarity between the RNAi agent and the RNA target is 100%, but can be less if desired, preferably, is 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. For example, 19 bases out of 21 bases can be base-paired. In some instances, where distinction between various allelic variants is desired, 100% complementarity to the Wnt/ β -catenin signaling gene is required in order to effectively discern the target sequence from the other allelic sequence. When selecting between allelic targets, the choice of length is also an important factor because it is the other factor involved in the percent complementary and the ability to differentiate between allelic differences. It is known to the skilled artisan that two types of small RNA molecules, small interfering RNA (siRNA) and microRNA (miRNA) are suitable for RNA interference.

The term "siRNA", as used herein, refers to: a) a dsRNA consisting of at least 15, at least 17, at least 19, at least 21 consecutive nucleotides base-paired, i.e. forming hydrogen bonds with a complementary nucleotide, b) a siRNA molecule or a molecule comprising an siRNA molecule, or c) a polynucleotide encoding a) or b), wherein, preferably, said polynucleotide is operatively linked to an expression control sequence. The siRNA in the context of the present invention, preferably, specifically silences the expression of a Wnt/ β -catenin signaling gene. It is to be understood that silencing as used herein does not necessarily mean the complete abolishment of gene expression in all cases, as described elsewhere herein. The siRNA is a single-stranded RNA molecule with a length, preferably, greater than or equal to 15 nucleotides and, preferably, a length of 15 to 49 nucleotides, more preferably 17 to 30 nucleotides, and most preferably 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30 nucleotides. The function of the siRNA to inhibit expression of the Wnt/ β -catenin signaling gene is modulated by said expression control sequence. Preferred expression control sequences can be regulated by exogenous stimuli, e.g. the tet operator, whose activity can be regulated by tetracycline, or heat inducible promoters. Alternatively or in addition, one or more expression control sequences can be used which allow tissue-specific expression of the siRNA.

The term "micro RNA" or "miRNA" as used herein refers to a) a pre-microRNA, i.e. miRNA comprising at least 30, at least 40, at least 50, at least 60, at least 70 nucleotides base-paired to a complementary sequence on the same miRNA molecule (stem), i.e. as a dsRNA, separated by a stretch of non-base-paired nucleotides (loop), b) a pre-microRNA, i.e. a dsRNA molecule comprising a stretch of at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 base-paired nucleotides formed by nucleotides of the same RNA molecule (stem), separated by a loop, c) a microRNA (miRNA), i.e. a dsRNA comprising at least 15, at least 17, at least 18, at least 19, at least 21 nucleotides on each of the two separate RNA strands, or d) a polynucleic acid encoding a) or b), wherein, preferably, said polynucleic acid is operatively linked to an expression control sequence as specified above. The miRNA in the context of the present invention, preferably, specifically silences the expression of a Wnt/ β -catenin signaling gene. However, any type of RNA is encompassed by the present method. It is to be understood that silencing as used herein does not necessarily mean the complete abolishment of gene expression in all cases, as described elsewhere herein.

As used herein, the term "ribozyme" refers to an RNA molecule specifically hybridizing to a target RNA molecule (e.g. a RNA molecule transcribed from a Wnt/ β -catenin signaling gene) and catalysing the hydrolysis of one or more phosphodiester bonds in said target RNA molecule, causing the target RNA to be degraded by cellular enzymes. The ribozyme in the context of the present invention, preferably, specifically hybridizes to an RNA molecule transcribed from a Wnt/ β -catenin signaling gene and catalyses the hydrolysis of said RNA molecule. RNA sequences showing suitable catalytic properties, like hammerhead ribozyme, hairpin ribozyme, or RNase P are known in the art (see, e.g. Doherty and Doudna 2001, *Annu. Rev. Biophys. Biomol. Struct.* 30: 457-475). Sequence specificity and, thus, target RNA specificity is accomplished by specific binding of the ribozyme to the target RNA by means of Watson-Crick base pairing of complementary, anti-parallel RNA strands. Methods of generating ribozymes directed against RNA sequences of interest are known in the art (see, for example, Citti and Rainaldi 2005, *Curr. Gene Ther.* 5(1): 11-24).

The term "peptide nucleic acid" or "PNA", as used herein, refers to an artificially synthesized polymer similar to DNA or RNA (see, e.g. Nielsen 1991, *Science* 254(5037): 1497-1500). The PNA in the context of the present invention, preferably, modulates the Wnt/ β -catenin signaling pathway, e.g. by modulating gene expression of a Wnt/ β -catenin signaling gene, as antisense therapeutic agent, or anticancer agent. More preferably, said PNA is an inhibitor of the Wnt/ β -catenin signaling pathway. The PNA of the present invention is of sufficient length and binds complementary DNA or RNA, e.g., by forming hydrogen bonds with complementary nucleotides in the target DNA or RNA under physiological conditions. Not all PNAs necessarily exhibit complete Watson-Crick base pairs in the interaction with the target DNA or RNA. However, the preferred complementarity between the PNA and the DNA or RNA target is 100%, but can be

less if desired, preferably, is 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. The PNA can be prepared by well known methods in the art.

The term “antibody” as used herein refers to a molecule from the subgroup of gamma globulin proteins, also referred to as the immunoglobulins (Ig). The antibody is, preferably, of any subtype, i.e. IgA, IgD, IgE, IgM or, more preferably, IgG. The antibody in the context of the present invention is, preferably, an antibody against a gene product encoded by a Wnt/ β -catenin signaling gene. More preferably, said antibody is an inhibitor of the Wnt/ β -catenin signaling pathway. Such an antibody can be prepared by well-known methods using a purified polypeptide or a suitable fragment derived therefrom as an antigen. A fragment suitable as an antigen can be identified by antigenicity determining algorithms well known in the art. Such fragments can be obtained either by proteolytic digestion from a gene product encoded by a Wnt/ β -catenin signaling gene or can be synthetic peptides. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a human or humanized antibody, or primatized, chimerized or fragment thereof. Also comprised as antibody of the present invention is a bispecific or a trispecific antibody, a synthetic antibody, an antibody fragment, such as Fab, Fv, or scFv fragments etc., or a chemically modified derivative of any of these. An antibody of the present invention preferably binds specifically (i.e. does not cross react with other polypeptides or peptides) to one of the polypeptides of the invention. Specific binding can be tested by various well known techniques. An antibody or fragments thereof can be obtained by using methods well known in the art, e.g., described in Harlow and Lane “Antibodies, A Laboratory Manual”, CSH Press, Cold Spring Harbor, 1988. A monoclonal antibody can be prepared by the techniques originally described in Köhler and Milstein 1975, Nature 256: 495, and Galfré 1981, Meth. Enzymol. 73: 3, which comprise the fusion of mouse or other rodent myeloma cells to spleen cells derived from immunized mammals.

The term “aptamer” as used herein refers to an oligonucleotide or a peptide specifically binding to a gene product of one of the Wnt/ β -catenin signaling genes of the present invention. The aptamer in the context of the present invention, preferably, modifies the activity and/or stability of said gene product, more preferably, is an inhibitor of the Wnt/ β -catenin signaling pathway. Preferably, said aptamer is an RNA or a DNA aptamer comprising an RNA or DNA molecule that is able to specifically bind to the three-dimensional surface of a polypeptide and to inhibit the function of said polypeptide. Said RNA or DNA aptamer can be obtained, e.g. by in vitro selection, e.g. systematic evolution of ligands by exponential enrichment (SELEX). Methods relating to the development and use of RNA and DNA aptamers are known in the art (see, for example, Ulrich 2006, Handb. Exp. Pharmacol. 173: 305-326 and Ulrich 2005, Med. Chem. 1(2): 199-208). Preferably, said aptamer is a peptide aptamer comprising short variable peptide domains, wherein said domains are attached at both ends to a protein scaffold. Said peptide aptamer, preferably, comprises sequences of 8-80 amino acids, more preferably 10-50 amino

acids, and most preferably 15-30 amino acids. Said amino acid sequences can, e.g. be isolated from randomized peptide expression libraries in a suitable host system like baker's yeast (see, for example, Klevenz et al. 2002, Cell. Mol. Life Sci. 59: 1993-1998). Said peptide aptamer, preferably, is used as free peptide. The peptide aptamer of this invention, preferably, comprises chemically modified amino acids, e.g., amino acids which are biotinylated, or are coupled to fluorophores, such as fluorescein, or Cy 3, are conformationally restricted, e.g. by disulfide bridging or by stapling (Walensky 2004, Science 305(5689): 1466-1470), or are linked to cell penetration peptides or protein transduction domains (Snyder 2004, Pharm Res 21(3): 389-393). Such modifications can improve the biological properties of the peptide aptamers, e.g., cell penetration, binding, stability, or may be used as detection labels. The peptide aptamer of the present invention can be recombinantly manufactured or chemically synthesized. The peptide aptamer comprises further amino acids, wherein said amino acids may serve as a tag for purification or detection. Moreover, the peptide aptamer of the present invention can be comprised by a fusion polypeptide, wherein the fusion partner, e.g., serves as a scaffold, fixing the peptide aptamer in a defined conformation. The variant or modified peptide aptamers, preferably, retain the biological activity of the peptide aptamer, i.e. they are capable of specifically binding to a gene product of one of the Wnt/ β -catenin signaling genes of the present invention.

The term "biomarker" is well known to the skilled person and relates to a substance, preferably a cellular constituent, more preferably a cellular macromolecule, the concentration or chemical state of which is used or usable as an indicator of a biological state. Preferably, the biomarker is NKD1, C10orf54, PSCA, CPNE5, RAET1G, or UCP3. According to the method of the present invention, the amounts of at least the biomarkers NKD1 and C10orf54 are determined in a sample. More preferably, the amount of one biomarker selected from the group consisting of PSCA, CPNE5 and RAET1G is determined in a sample in addition to NKD1 and C10orf54. Even more preferably, the amounts of two biomarkers selected from the group consisting of PSCA, CPNE5 and RAET1G are determined in a sample in addition to NKD1 and C10orf54. Most preferably, the amounts of the biomarkers PSCA, CPNE5 and RAET1G are determined in a sample in addition to NKD1 and C10orf54. Preferably, the amount of the biomarker UCP3 is determined in a sample additionally to the biomarkers as described above.

The term "NKD1" as used herein refers to the Naked1 gene (see NCBI Gene ID: 85407, RefSeq ID: NM_033119, or NCBI Reference Sequence: NM_033119.4) and/or gene products encoded by said gene. The nucleic acid sequence, in particular the coding sequence (CDS), of NKD1 is shown in SEQ ID NO: 1, the encoded amino acid sequence is shown in SEQ ID NO: 2. NKD1 is known in the art to act as a negative regulator of the Wnt signaling pathway and interacts with

the cytoplasmic disheveled protein 1 (DVL1; see Van Raay et al. 2007, Dev. Biol. 309, 151-168; Van Raay et al. 2011, PLoS ONE 6, e18650).

The term "C10orf54" as used herein refers to the chromosome 10 open reading frame 54 gene (see NCBI Gene ID: 64115, RefSeq ID: NM_022153, or NCBI Reference Sequence: NM_022153.1) and/or gene products encoded by said gene. The nucleic acid sequence, in particular the CDS, of C10orf54 is shown in SEQ ID NO: 3, the encoded amino acid sequence is shown in SEQ ID NO: 4. C10orf54 has been linked to the regulation of matrix metalloproteinases and invasiveness of tumours (see Sakr et al. 2010, Cancer Sci. 101, 2368-2374).

The term "PSCA" as used herein refers to the prostate stem cell antigen (see NCBI Gene ID: 8000, RefSeq ID: NM_005672, or NCBI Reference Sequence: NM_005672.4) and/or gene products encoded by said gene. The nucleic acid sequence, in particular the CDS, of PSCA is shown in SEQ ID NO: 5, the encoded amino acid sequence is shown in SEQ ID NO: 6. It is known from the said RefSeq entry, that PSCA encodes a glycosylphosphatidylinositol-anchored cell membrane glycoprotein. PSCA is highly expressed in the prostate and is also expressed in the bladder, the placenta, the colon, the kidney, and the stomach. PSCA is up-regulated in a large proportion of prostate cancers and is also detected in cancers of the bladder and pancreas. It includes a polymorphism that results in translation starting from an upstream start codon in some individuals; this polymorphism is thought to be associated with a risk for certain gastric and bladder cancers. Alternative splicing results in multiple transcript variants.

The term "CPNE5" as used herein refers to the copine V gene (see NCBI Gene ID: 57699, RefSeq ID: NM_020939, or NCBI Reference Sequence: NM_020939.1) and/or gene products encoded by said gene. The nucleic acid sequence, in particular the CDS, of CPNE5 is shown in SEQ ID NO: 7, the encoded amino acid sequence is shown in SEQ ID NO: 8. It is known from the said RefSeq entry, that CPNE5 is one of several genes that encode a calcium-dependent protein containing two N-terminal type II C2 domains and an integrin A domain-like sequence in the C-terminus. It is known that calcium-dependent membrane-binding proteins may regulate molecular events at the interface of the cell membrane and cytoplasm. Sequence analysis identified multiple alternatively spliced transcript variants but their full-length natures could not be determined.

The term "RAET1G" as used herein refers to the retinoic acid early transcript 1G gene (see NCBI Gene ID: 353091, RefSeq ID: NM_001001788, or NCBI Reference Sequence: NM_001001788.2) and/or gene products encoded by said gene. The nucleic acid sequence, in particular the CDS, of RAET1G is shown in SEQ ID NO: 9, the encoded amino acid sequence is shown in SEQ ID NO: 10. It is known from the said RefSeq entry, that members of the RAET1

family, such as RAET1G, are major histocompatibility complex (MHC) class I-related genes located within a 180-kb cluster on chromosome 6q24.2-q25.3. RAET1 proteins contain MHC class I-like alpha-1 and alpha-2 domains. RAET1E (MIM 609243) and RAET1G differ from the other RAET1 proteins (e.g., RAET1I or ULBP1; MIM 605697) in that they have type I
5 membrane-spanning sequences at their C-termini rather than glycosylphosphatidylinositol anchor sequences (see Radosavljevic et al. 2002, Genomics 79(1): 114-23).

The term "UCP3" as used herein refers to the gene uncoupling protein 3 (mitochondrial, proton carrier; see NCBI Gene ID: 7352) and/or gene products encoded by said gene. In the context of
10 the present invention two different isoforms of the gene UCP3 are used: the isoform UCP3L which is encoded by transcript variant long (see RefSeq ID: NM_003356 or NCBI Reference Sequence: NM_003356.3) and the isoform UCP3S which is encoded by transcript variant short (see RefSeq ID: NM_022803 or NCBI Reference Sequence: NM_022803.2). The nucleic acid sequence, in particular the CDS, of UCP3L is shown in SEQ ID NO: 11, the encoded amino acid
15 sequence is shown in SEQ ID NO: 12. The nucleic acid sequence, in particular the CDS, of UCP3S is shown in SEQ ID NO: 13, the encoded translated amino acid sequence is shown in SEQ ID NO: 14. Mitochondrial uncoupling proteins (UCP) belong to the family of mitochondrial anion carrier proteins (MACP), acting by separating oxidative phosphorylation from ATP synthesis with energy dissipated as heat (mitochondrial proton leak). They facilitate
20 the transfer of ions between inner to the outer mitochondrial membrane and can also reduce the mitochondrial membrane potential in mammalian cells. The different UCPs have tissue-specific expression; this gene is primarily expressed in skeletal muscle. This gene's protein product is postulated to protect mitochondria against lipid-induced oxidative stress. Expression levels of UCPs increase when fatty acid supplies to mitochondria exceed their oxidation capacity and the
25 protein enables the export of fatty acids from mitochondria. UCPs contain the three solcar protein domains typically found in MACPs. Two splice variants have been found for this gene.

The term "determining" as used herein refers to measuring the amount or concentration of at
30 least one biomarker in a sample, preferably semi-quantitatively or quantitatively. Such a biomarker is an indicator of a biological state. Preferred biomarkers are defined elsewhere herein. Measuring can be done directly or indirectly. Direct measuring relates to measuring the amount or concentration of said biomarker based on a signal, wherein said signal is obtained from said biomarker itself and wherein the intensity of the signal directly correlates with the
35 number of molecules of said biomarker present in the sample. Such a signal can be obtained, e.g., by measuring an intensity value of a specific physical or chemical property of said biomarker. Indirect measuring includes measuring of a signal obtained from a secondary component, i.e., a component not being said biomarker itself, or a biological readout system, e.g., measurable cellular responses, ligands, labels, or enzymatic reaction products. Preferably,

measuring is performed on a processed sample, said processing comprising extraction of polynucleotides or polypeptides from the sample. It is, however, also envisaged by the present invention that the gene product is determined by qRT-PCR, hybridization techniques and massive parallel sequencing. As used herein, a qRT-PCR is well known to the skilled artisan and refers to a method where messenger RNA is repeatedly amplified and detected via fluorescent signals. Thus, a qRT-PCR allows to relatively and absolutely quantify a gene product. Hybridization based techniques as used herein are well known to the skilled artisan and, preferably, comprise NanoString and Padlock systems that allow direct detection and quantification of mRNA (see Geiss et al. 2008, Nat. Biotechnol. 26: 317-325; Stougaard et al. 2011, Integr Biol (Camb) 3: 982-992). Massive parallel sequencing techniques are also well known to the skilled artisan and can be applied to detect and quantify gene products by sequencing of RNA.

The term "amount" as used herein refers to the absolute amount of at least one biomarker, the relative amount or concentration of the said biomarker as well as any value or parameter correlating thereto. Such values or parameters comprise intensity signal values from all specific physical and/or chemical properties including biochemical properties of said biomarker. Such properties include, e.g., molecular weight, viscosity, density, electrical charge, spin, optical activity, colour, fluorescence, chemoluminescence, elementary composition, chemical structure, capability to react with other compounds, capability to elicit a response in a biological read out system (e.g., induction of a reporter gene) and the like. Values for said properties may serve as characteristic features and can be determined by techniques well known in the art. Moreover, encompassed are all values or parameters obtained by direct measurements, e.g. intensity values in mass spectra or NMR spectra, and/or indirect measurements specified elsewhere in this specification, e.g., biological readout systems in response to said biomarker or intensity signals obtained from specifically bound ligands. Furthermore, the characteristic feature is any feature derived from the values of the physical and/or chemical properties of a biomarker by standard operations, e.g., mathematical calculations such as multiplication, division or logarithmic calculus. Most preferably, the at least one characteristic feature allows the determination and/or chemical identification of the said at least one biomarker and its amount. Accordingly, the characteristic value, preferably, also comprises information relating to the abundance of the biomarker from which the characteristic value is derived. For example, a characteristic value of a biomarker may be a peak in a mass spectrum. Such a peak contains characteristic information of the biomarker, i.e. the mass-to-charge ratio (m/z) information, as well as an intensity value being related to the abundance of the said biomarker (i.e. its amount) in the sample.

Moreover, the analysis step may, preferably, be preceded by a compound separation and / or enrichment step. Preferably, said compound separation and / or enrichment step yields a time resolved separation and / or enrichment of the compound comprised by the sample. Suitable

techniques for separation and enrichment to be used preferably in accordance with the present invention, therefore, include all chromatographic separation techniques, i.e. liquid chromatography (LC), high performance liquid chromatography (HPLC), gas chromatography (GC), or thin layer chromatography. These techniques are well known in the art and can be applied by the person skilled in the art without further ado. The method of the present invention shall be, preferably, assisted by automation. For example, sample processing or pretreatment can be automated by robotics. Data processing and comparison is, preferably, assisted by suitable computer programs and databases. Automation as described herein before allows using the method of the present invention in high-throughput approaches.

Preferably, the biomarker is determined by a specific chemical or biological assay. Said assay shall comprise means allowing to specifically detect at least one biomarker in the sample. Preferably, said means are capable of specifically recognizing the chemical structure of the biomarker or are capable of specifically identifying the biomarker based on its capability to react with other compounds or its capability to elicit a response in a biological read out system (e.g., induction of a reporter gene). Means capable of specifically recognizing the chemical structure of a biomarker are, preferably, diagnostic antibodies or other proteins, wherein said proteins specifically interact with chemical structures, such as receptors or enzymes. Specific diagnostic antibodies, for instance, may be obtained using the biomarker as antigen by methods well known in the art. Diagnostic antibodies as referred to herein include both polyclonal and monoclonal antibodies, as well as fragments thereof, such as Fv, Fab and F(ab)₂ fragments that are capable of binding the antigen or hapten. The term diagnostic antibody according to the present invention also includes humanized hybrid antibodies wherein amino acid sequences of a non-human donor antibody exhibiting a desired antigen-specificity are combined with sequences of a human acceptor antibody. The sequences of the donor antibody used will usually include at least the antigen-binding amino acid residues of the donor but may comprise other structurally and/or functionally relevant amino acid residues of the donor antibody as well. Such hybrids can be prepared by several methods well known in the art. Moreover, encompassed are single chain antibodies, which are well known to the skilled artisan. Moreover, said antibodies may be used as a basis to generate oligopeptides specifically recognizing the biomarker. These oligopeptides shall, for example, comprise the enzyme's binding domains or pockets for the said biomarker. Suitable antibody and/or enzyme based assays may be RIA (radioimmunoassay), ELSA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electro-chemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFA) or solid phase immune tests. Moreover, the biomarker may also be identified based on its capability to react with other compounds, i.e. by a specific chemical reaction. Further, the biomarker may be determined in a sample due to its capability to elicit a response in a biological read out system. The biological response shall be detected as read out indicating the presence and/or the amount of the biomarker comprised by the sample. The

biological response may be, e.g., the induction of gene expression or a phenotypic response of a cell or a subject. Preferably, the biomarker is determined by qRT-PCR, hybridization techniques and massive parallel sequencing as described above herein.

- 5 Moreover, depending on the technique used for determining the amount of a biomarker, the analyte determined by the determination technique may differ with respect to its chemical nature from the biomarker found in the sample. Such analytes include derivatives of the biomarkers, wherein said derivatives of biomarkers are generated by the pretreatment processes used for the sample or by the determination technique as such. However, it is to be understood that the said
10 derivative of the biomarker represents the biomarker qualitatively or, preferably, quantitatively.

Preferably, the amount of a polynucleotide according to the present invention is determined by one of several methods well-known in the art. Quantification preferably is absolute, i.e. relating to a specific number of polynucleotides or, more preferably, relative, i.e. measured in arbitrary
15 normalized units. Methods allowing for absolute or relative quantification are well known in the art, e.g., quantitative PCR methods are methods for relative quantification; if a calibration curve is incorporated in such an assay, the relative quantification can be used to obtain an absolute quantification. Other methods known are, e.g. nucleic acid sequence-based amplification (NASBA) or the Branched DNA Signal Amplification Assay method, preferably in combination
20 with dot blot or luminex detection of amplified polynucleotides. Preferably, the polynucleotide amounts are normalized polynucleotide amounts, i.e. the polynucleotide amounts obtained are set into relation to at least one reference amplification product, thereby, preferably, setting the polynucleotide amounts into relation to the number of cells in the sample and/or the efficiency of polynucleotide amplification. Thus, preferably, the reference amplification product is a product
25 obtained from a polynucleotide known to have a constant abundancy in each cell, i.e. a polynucleotide comprised in most, preferably all, cells of a sample in approximately the same amount. More preferably, the reference amplification product is amplified from a chromosomal or mitochondrial gene or from the mRNA of a housekeeping gene. A preferred housekeeping gene is selected from the group consisting of: UBC, GAPDH, ACTB, B2M, and 18s. The skilled
30 artisan knows said housekeeping genes. It is to be understood that said housekeeping genes are used alone or in combination with each other.

In a preferred embodiment of the present invention the forward and reverse primer pairs specific for housekeeping genes are used as shown in Table 1. It is to be understood that also other
35 primer pairs for the said housekeeping genes can be used. The skilled artisan known how to generate suitable forward and primer pair sequences for housekeeping genes, e.g. by using different software tools. Table 2 shows the gene name and the NCBI gene ID of the said housekeeping genes.

Table 1: Forward and reverse primer pairs of the housekeeping genes used:

Gene	Forward Primer		Reverse Primer	
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
UBC	GGCAAAGATCCAAGATAAGGAA	15	GGACCAAGTGCAGAGTGGAC	16
GAPDH	AGCCACATCGCTCAGACAC	17	GCCCAATACGACCAAATCC	18
ACTB	ATTGGCAATGAGCGGTTTC	19	TGAAGGTAGTTTCGTGGATGC	20
B2M	TTCTGGCCTGGAGGCTATC	21	TCAGGAAATTTGACTTTCCATTC	22
18s	GGAGAGGGAGCCTGAGAAAC	23	TCGGGAGTGGGTAATTTGC	24

Table 2: Gene name and NCBI gene ID of the housekeeping genes used:

Gene Symbol	Gene Name	NCBI Gene ID
UBC	Ubiquitin C	7316
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	2597
ACTB	actin, beta	60
18s (RNA18S5)	RNA, 18S ribosomal 5	100008588

5

The term “subject” as used herein encompasses animals, preferably mammals, amphibians, and ray-finned fishes, and, more preferably, mouse, rat, cat, dog, cattle, rabbit, Guinean pig, pig, Chinese hamster, monkey, xenopus, zebrafish, and humans. Preferably, xenopus and/or zebrafish are used for initial in vivo testing of compounds. The subject, preferably, is apparently healthy, e.g. not suffering from cancer. Typically, such a subject is apparently healthy with respect to cancer prior to the onset of signs or symptoms of cancer or any cancer-related medical event. It is understood that in case the cells of the present invention are intentionally brought into contact with a compound suspected to be a modulator of Wnt/ β -catenin signaling, the subject, preferably, is not a human. It is, however, also envisaged that the method of the present invention is employed for identifying compounds present in the environment (e.g. pollutants) being modulators of Wnt/ β -catenin signaling using e.g. an epidemiologic approach. In such cases, the subject may be a human.

The term “sample”, as used herein, refers to a sample of cells obtained from cultured cells or from a subject. Preferably, the sample comprises isolated and/or separated cells or a cell lysate. Preferably, said cells are metazoan cells, more preferably mammalian cells; most preferably, the cells are selected from the group consisting of mouse, rat, feline, canine, bovine, rabbit, Guinea pig, pig, Chinese hamster, monkey, and human cells. Preferably, the sample is obtained from cultivated cells, more preferably from a cell line; even more preferably cells are cultivated cells of a cancer cell line. Most preferably, the cells are cells from a cell line selected from the group consisting of HCT116, DLD1, CACO-2, SW480, RKO, HT29, COLO320, HT55, SW48, and LoVo. Further preferred cells are cells from a cancer cell line found in Rowan et al. 2000, PNAS 97: 3352-3357.

It is, however, also envisaged by the present invention that the sample comprises cells obtained from a subject, e.g., from a body fluid, a tissue or an organ of said subject. Said cells obtained from a tissue or organ of a subject are, preferably, obtained from any tissue or organ by well known techniques, e.g., by biopsy or other surgical procedures. Said cells obtained from a body fluid of a subject are obtained by well-known techniques and include, preferably, blood. The sample is obtained by well-known techniques and includes, preferably, use of brushes, (cotton) swabs, spatula, rinse/wash fluids, punch biopsy devices, puncture of cavities with needles or surgical instrumentation. A tissue or organ sample is obtained from any tissue or organ by, e.g., biopsy or other surgical procedures. A sample of a body fluid is obtained by well-known techniques and includes, preferably, a sample of blood. Preferably, said sample of a subject is obtained from any tissue or organ of a subject.

The term "HCT116 cell line" as used herein refers to a continuous line of human colorectal carcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357 and Chan et al. 2002, Proc Natl Acad Sci U S A 99: 8265-8270).

The term "DLD1 cell line" as used herein refers to a continuous line of human epithelial colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

The term "CACO-2 cell line" as used herein refers to a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

The term "SW480 cell line" as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

The term "RKO cell line" as used herein refers to a continuous line of human epithelial colon carcinoma cells. Culturing said cell line is well known in the art (see, e.g., Zhang et al. 2010, Chem. Biol. Interact. 185: 174-181).

The term "HT29 cell line" as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

The term "COLO320 cell line" as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

The term “LoVo cell line” as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

5

The term “HT55 cell line” as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

10 The term “SW48 cell line” as used herein refers to a continuous line of human colorectal adenocarcinoma cells. Culturing said cell line is well known in the art (see, e.g., Rowan et al. 2000, PNAS 97: 3352-3357).

15 The term “comparing” as used herein refers to comparing the determined amount for at least one biomarker as referred to herein to a corresponding reference. It is to be understood that comparing as used herein refers to any kind of comparison made between the amounts of the biomarker with that of the reference. The comparison referred to in step b) of the method of the present invention may be carried out manually or computer assisted. The amounts of the
20 biomarker and the reference can be, e.g., compared to each other and the said comparison can be automatically carried out by a computer program executing an algorithm for the comparison. The computer program carrying out the said evaluation will provide the desired assessment in a suitable output format. For a computer assisted comparison, the determined amount of the biomarker is compared to the corresponding amount of a suitable reference stored in a database
25 by a computer program. The computer program may further evaluate the result of the comparison, i.e. automatically provide the desired assessment in a suitable output format. Based on the comparison of the amount determined in step a) and the reference, it is possible to assess whether a compound is capable of modulating Wnt/ β -catenin signaling, or not. Therefore, the reference is to be chosen so that either a difference or a similarity in the amounts of the
30 biomarker and the reference allows identifying such a compound. Preferably, comparing refers to assessing whether the results of the determination described hereinabove in detail, i.e. the results of the qualitative or quantitative determination of the amount of a biomarker, are identical or similar to a reference or differ therefrom.

35 The term “reference”, relates to an amount obtained, preferably, from a sample of cells, wherein said cells (i) have not been brought into contact with a compound suspected to modulate Wnt/ β -catenin signaling, or (ii) have been brought into contact with a compound known to modulate Wnt/ β -catenin signaling. Cells having not been brought into contact with a compound suspected to modulate Wnt/ β -catenin signaling, are, e.g., cells with an unchanged Wnt/ β -catenin signaling

and, more preferably, apparently healthy cells. Cells having been brought into contact with a compound known to modulate Wnt/ β -catenin signaling, are, e.g., cells with modulated Wnt/ β -catenin signaling. The reference amount is determined as described herein above for the amount of the at least one biomarker. It is understood that, according to the present invention, the value
5 determined for a given biomarker is compared to a corresponding reference. The term "corresponding reference" is understood by the skilled person and relates to an amount obtained for the same biomarker in a different, preferably control, sample, more preferably comprising cells of the same origin and/or kind as the cells in the sample. Preferably, the reference is calculated from the average or median for the relative or absolute amount for the at least one
10 biomarker (average reference). How to calculate a suitable reference value, preferably, the average or median, is well known in the art.

Preferably, the reference is obtained from a sample of cells corresponding to the cells of step a) and wherein said cells have not been brought into contact with a compound suspected to
15 modulate Wnt/ β -catenin signaling. In accordance with the present invention said cells corresponding to the cells of step a), preferably, are cells of the same origin, more preferably, the same kind of cells or cells of the same physiological state, or, most preferably, the same cells as the cells in the sample. Thus, a compound modulating Wnt/ β -catenin signaling can be identified based on the differences between the amounts of the at least one biomarker in a sample and the
20 aforementioned reference, i.e. differences in the qualitative or quantitative composition with respect to the aforementioned biomarkers. The same applies if an average reference as specified above is used. The difference can be an increase in the absolute or relative amount of the biomarker (sometimes referred to as up-regulation of the biomarker; see also Examples) or a decrease in either of said amounts or the absence of a detectable amount of the biomarker
25 (sometimes referred to as down-regulation of the biomarker; see also Examples). Preferably, the difference in the relative or absolute amount is significant, i.e. outside of the interval between 45th and 55th percentile, 40th and 60th percentile, 30th and 70th percentile, 20th and 80th percentile, 10th and 90th percentile, 5th and 95th percentile, 1st and 99th percentile of the reference amount. Preferably, an increased or decreased amount of the at least one biomarker in
30 comparison to the reference obtained from a sample of cells having not been brought into contact with a compound suspected to modulate Wnt/ β -catenin signaling or an average reference, is indicative for a compound modulating Wnt/ β -catenin signaling.

Also preferably, the reference is obtained from a sample of cells, wherein said cells are
35 corresponding to the cells of step a) and wherein said cells have been brought into contact with a compound known to modulate Wnt/ β -catenin signaling. In accordance with the present invention said cells corresponding to the cells of step a), preferably, are cells of the same origin, more preferably, the same kind of cells or cells of the same physiological state, or, most preferably, the same cells as the cells in the sample. Thus, a compound modulating Wnt/ β -catenin signaling can

be identified based on the degree of identity or similarity between the amounts of the at least one biomarker in a sample and the aforementioned reference, i.e. based on a similar, preferably essentially identical, or, more preferably identical qualitative or quantitative composition with respect to the aforementioned biomarkers. The amounts of the biomarker and the reference are essentially identical, if the values for the characteristic features and, in the case of quantitative determination, the intensity values are essentially identical. Said amounts are similar, if the values of the characteristic features are identical but the intensity values are different. Such a difference is, preferably, not significant and shall be characterized in that the values for the intensity are within at least the interval between 1st and 99th percentile, 5th and 95th percentile, 10th and 90th percentile, 20th and 80th percentile, 30th and 70th percentile, or 40th and 60th percentile of the reference amount. Preferably, an identical amount of the biomarker and the reference obtained from a sample of cells having been brought into contact with a compound known to modulate Wnt/ β -catenin signaling or an average reference, is indicative for a compound modulating Wnt/ β -catenin signaling.

Advantageously, it has been found in the context of the present invention that the biomarkers identified across a panel of cell lines ensuring greatest possible independence from genetic background offer reliable identification of differential gene expression then microarrays. Specifically, the markers allow for directly measuring the activity of a cancer signaling pathway, i.e. the wnt-pathway. Using the method of the present invention, candidate compounds can be screened for their specificity and for their impact on said cancer signaling pathway.

The definitions made above apply mutatis mutandis to the following.

Moreover, the present invention relates to a method for identifying a drug, comprising the steps of the method for identifying a compound modulating Wnt/ β -catenin signaling of the present invention and, further, the step of identifying a compound modulating Wnt/ β -catenin signaling as a drug.

The term “drug”, as used herein, relates to a chemical substance used in the treatment, cure, prevention, or diagnosis of disease in a subject. Preferably, the term relates to a chemical substance used in the treatment, cure, prevention, or diagnosis of cancer in a subject. Said drug, preferably, comprises the compound modulating Wnt/ β -catenin signaling of the present invention and, more preferably, one or more pharmaceutically acceptable carrier. Preferably, said compound is a drug suspected to be a drug against cancer, more preferably the drug is a drug against cancer. The compound modulating Wnt/ β -catenin signaling of the present invention can be formulated as a pharmaceutically acceptable salt. Acceptable salts comprise acetate,

methylester, HCl, sulfate, chloride and the like. The drug is, preferably, administered topically or systemically. Suitable routes of administration conventionally used for drug administration are intratumoral, peritumoral, oral, intravenous, or parenteral administration as well as application as suppository. Preferably, oral and intravenous drug administration is used. However, depending
5 on the nature and mode of action of the compound, the drug can be administered by other routes as well. For example, a polynucleotide compound is administered in a gene therapy approach by using viral vectors, viruses or liposomes. Moreover, the compound is administered in combination with other drugs either in a common drug or as separated drugs wherein said separated drugs can be provided in the form of a kit of parts. The compound is, preferably,
10 administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. The procedure comprises mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined,
15 the route of administration and other well-known variables.

The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed can be, for example, either a solid, a gel or a liquid. Exemplary of solid carriers are
20 lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. Said suitable
25 carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The diluent(s) is/are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the drug also include other carriers, adjuvants, or nontoxic, nontherapeutic,
30 non-immunogenic stabilizers and the like.

A therapeutically effective dose relates to an amount of the compound to be used as a drug of the present invention, wherein said compound prevents or treats the symptoms accompanying cancer. Therapeutic efficacy and toxicity of such a compound can be determined by standard
35 pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀. The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the

above described methods. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment. A typical dose can be, for example, in the range of 1 to 1000 μg ; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the drug should be in the range of 1 μg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 μg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range to provide from about 0.01 mg per kg body mass to about 10 mg per kg body mass. The drugs referred to herein is administered at least once in order to treat or ameliorate or prevent cancer. However, the said drug can be administered more than one time, for example from one to four times daily up to a non-limited number of days. A specific drug is prepared in a manner well known in the pharmaceutical art and comprises at least one active compound referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent. For making those specific drug, the active compound will usually be mixed with a carrier or the diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. The resulting formulations are to be adopted to the mode of administration, i.e. in the forms of tablets, capsules, suppositories, solutions, suspensions or the like. Dosage recommendation is indicated in the prescribers or users instructions in order to anticipate dose adjustments depending on the considered recipient.

The term "treatment", as used herein, refers to treatment of the diseases according to the present invention, preferably cancer, or of the symptoms accompanied therewith to a significant extent. The skilled artisan knows suitable therapies. Said therapy is, preferably, an anti-cancer therapy, more preferably, a therapy using a compound, wherein said compound is an inhibitor of the Wnt/ β -catenin signaling pathway. Said therapy as used herein also aims to entirely restore the health with respect to the cancer referred to herein. It is to be understood that the therapy as used in accordance with the present invention may not be effective in all subjects to be treated. However, the term shall require that a statistically significant portion of subjects suffering from cancer can be successfully treated. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc.. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the treatment shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

The term "prevention" refers to retainment of health with respect to the diseases according to the present invention, preferably cancer, or the symptoms referred to herein for a certain period of time in a subject. It will be understood that the said period of time is dependent on the amount of the drug compound administered and individual factors of the subject. It is to be understood that prevention may not be effective in all subjects treated with the compound according to the present invention. However, the term requires that a statistically significant portion of subjects of a cohort or population are effectively prevented from suffering from cancer or the symptoms referred to herein. Preferably, a cohort or population of subjects is envisaged in this context, wherein said cohort or population normally, i.e. without preventive measures according to the present invention, would develop cancer or symptoms as referred to herein. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools discussed above. Preferably, prevention shall be effective for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population. Preferably, prevention is prevention of tissue invasion and metastasis formation by cancer cells.

The term "cancer", as used herein, relates to a disease of a subject characterized by uncontrolled growth by a group of body cells (cancer cells). This uncontrolled growth may be accompanied by intrusion into and destruction of surrounding tissue and possibly spread of cancer cells to other locations in the body. Thus, the term, preferably, encompasses pre-malignant lesions, malignant lesions as well as solid tumors and metastatic cancer (both local metastasis and distant metastasis). The symptoms accompanying cancer are well known from standard text books of medicine. Preferably, cancer is selected from the group consisting of colorectal cancer, breast cancer, esophageal cancer, melanoma, hepatocellular carcinoma, desmoid tumor, pancreatic cancer, gastric cancer, ovarian cancer, and prostate cancer.

In a further embodiment, the present invention relates to the use of at least the biomarkers NKD1 and C10orf54 in a sample comprising cells for determining whether said cells have been brought into contact with a compound modulating Wnt/ β -catenin signaling. Preferably, said compound modulating Wnt/ β -catenin signaling is an inhibitor of Wnt/ β -catenin signaling. Preferably, said compound is a drug or suspected to be a drug against cancer. Preferably, said cancer is selected from the group consisting of: colorectal cancer, breast cancer, esophageal cancer, melanoma, hepatocellular carcinoma, desmoid tumor, pancreatic cancer, gastric cancer, ovarian cancer, and prostate cancer.

Moreover, the present invention relates to a method for diagnosing cancer in a subject suspected to suffer therefrom, comprising the steps of:

- a) determining the amounts of at least the biomarkers NKD1 and C10orf54 in a sample of the said subject; and
- b) comparing the amount of the biomarker NKD1 determined in step a) to a reference for NKD1 and comparing the amount of the biomarker C10orf54 determined in step a) to a reference for C10orf54, whereby cancer in the subject is to be diagnosed.

The term "diagnosing" as used herein refers to assessing whether a subject suffers from cancer, or not. As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be diagnosed. The term, however, requires that assessment of the presence or absence of cancer is correct for a statistically significant portion of the subjects (e.g. a cohort in a cohort study). Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools as described herein above.

In the context of the method for diagnosing cancer of the present invention, the term "reference" relates to an amount, preferably, determined in a sample from a subject, wherein said subject (i) is apparently healthy or (ii) suffers from cancer. Also preferably, such reference amount is determined in a sample obtained from cells, wherein said cells (i) have not been brought into contact with a compound known to modulate Wnt/ β -catenin signaling or (ii) have been brought into contact with a compound known to modulate Wnt/ β -catenin signaling. Cells having not been brought into contact with a compound known to modulate Wnt/ β -catenin signaling, are, e.g., cells with an unchanged Wnt/ β -catenin signaling and, more preferably, apparently healthy cells. Cells having been brought into contact with a compound known to modulate Wnt/ β -catenin signaling, are, e.g., cells with modulated Wnt/ β -catenin signaling. The reference amount is determined as described herein above for the amount of the biomarker. Moreover, the reference, also preferably, is an average reference as described above.

Preferably, a subject suffering from cancer can be diagnosed based on the differences between the amounts of the at least one biomarker in a sample and the aforementioned reference, i.e. differences in the qualitative or quantitative composition with respect to the aforementioned biomarkers. The same applies if an average reference as specified above is used. The difference can be an increase in the absolute or relative amount of the biomarker (sometimes referred to as up-regulation of the biomarker; see also Examples) or a decrease in either of said amounts or the absence of a detectable amount of the biomarker (sometimes referred to as down-regulation of the biomarker; see also Examples). Preferably, the difference in the relative or absolute amount is significant, i.e. outside of the interval between 45th and 55th percentile, 40th and 60th percentile, 30th and 70th percentile, 20th and 80th percentile, 10th and 90th percentile, 5th and

95th percentile, 1st and 99th percentile of the reference amount. Preferably, an increased or decreased amount of the at least one biomarker in comparison to the reference obtained in a sample from an apparently healthy subject, a sample obtained from cells having not brought into contact with a compound known to modulate Wnt/ β -catenin signaling, or an average reference is indicative for a subject suffering from cancer. Also preferably, such an increased or decreased amount of the at least one biomarker in comparison to the reference is indicative for a subject in need of an anti-cancer therapy.

Also preferably, a subject suffering from cancer can be identified based on the degree of identity or similarity between the amounts of the at least one biomarker in a sample and the aforementioned reference, i.e. based on an identical or similar qualitative or quantitative composition with respect to the aforementioned biomarkers. The amounts of the biomarker and the reference are identical, if the values for the characteristic features and, in the case of quantitative determination, the intensity values are identical. Said amounts are similar, if the values of the characteristic features are identical but the intensity values are different. Such a difference is, preferably, not significant and shall be characterized in that the values for the intensity are within at least the interval between 1st and 99th percentile, 5th and 95th percentile, 10th and 90th percentile, 20th and 80th percentile, 30th and 70th percentile, 40th and 60th percentile of the reference amount. Preferably, an identical amount of the biomarker and the reference obtained in a sample from a subject suffering from cancer, a sample obtained from cells having brought into contact with a compound known to modulate Wnt/ β -catenin signaling, or an average reference is indicative for a subject suffering from cancer. Also preferably, such an identical amount of the biomarker and the reference is indicative for a subject in need of an anti-cancer therapy.

Further, the present invention relates to a method for determining whether a subject is in need of an anti-cancer therapy comprising the steps of:

- a) determining the amounts of at least the biomarkers NKD1 and C10orf54 in a sample of the said subject; and
- b) comparing the amount of the biomarker NKD1 determined in step a) to a reference for NKD1 and comparing the amount of the biomarker C10orf54 determined in step a) to a reference for C10orf54, whereby it is determined whether the subject is in need of the anti-cancer therapy.

Furthermore, the term "subject in need of an anti-cancer therapy" as used herein relates to a subject as described above suffering from cancer. In order to treat said cancer or the symptoms accompanied therewith to a significant extent, said subject is in need of an anti-cancer therapy. Preferably, said therapy uses an inhibitor of the Wnt/ β -catenin signaling pathway.

The present invention also relates to a device adapted for carrying out a method of the invention comprising:

- a) an analyzing unit comprising a detection agent, wherein said detection agent specifically determines the amounts of at least the biomarkers NKD1 and C10orf54 in a sample obtained from cells; and
- b) an evaluation unit for comparing the determined amounts with reference amounts, said unit comprising a database with reference amount values and a computer-implemented algorithm for carrying out the comparison.

The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow the identification. Preferred means for determining the amount of the said biomarkers and means for carrying out the comparison are disclosed above in connection with the methods of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of the biomarkers are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to establish an identification (i.e. of a compound modulating Wnt/ β -catenin signaling), a diagnosis (i.e. a subject suffering from cancer), and/or a determination (i.e. whether a subject is in need of an anti-therapy). Preferably, said device allows the identification of a compound modulating Wnt/ β -catenin signaling, the diagnosis a subject suffering from cancer, and/or the determination whether a subject is in need of an anti-cancer therapy. More preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the biomarker in a sample and an evaluation unit for processing the resulting data for the diagnosis. Alternatively, where means such as test stripes are used for determining the amount of the gene products, the means for diagnosing may comprise control stripes or tables allocating the determined amount to an amount known to modulate Wnt/ β -catenin signaling. Preferred means for detection are disclosed in connection with embodiments relating to the methods of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the identification and/or diagnostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further inventive skills. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of parametric diagnostic raw data, preferably, as absolute or relative amounts. It is to be understood that these data will need interpretation by the clinician. However, also envisaged are

expert system devices wherein the output comprises processed diagnostic raw data the interpretation of which does not require a specialized clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the polypeptides, Plasmon surface resonance devices, NMR spectro-
5 meters, mass- spectrometers etc.) or evaluation units/devices referred to above in accordance with the methods of the invention.

As used herein, the term “detection agent” relates to an agent specifically interacting with, and thus recognizing, the biomarkers defined above. Preferably, said detection agent is labeled in a
10 way allowing detection of said detection agent inside the human body. Preferably, said detection agent is a polypeptide, e.g. an anticalin, a designed ankyrin repeat protein (DARPin), a single-chain T-cell receptor, or an antibody. Preferably, the detection agent is water soluble and can be transported to the cell via the bloodstream. Also preferably, the detection agent recognizes the biomarkers defined above by the presence of said biomarkers or a fragment thereof on the
15 surface of a cell, like e.g. a single-chain T-cell receptor or an specifically recognizing antibody. Most preferably, the detection agent enters the cell, e.g. by endocytosis, by receptor mediated endocytosis or mediated by a protein transduction domain or the like. Preferably, the label allowing detection of the detection reagent inside the human body is a label as described herein above. More preferably, said label is detectable by computer tomography (CT, e.g. Iodine), by
20 magnet resonance tomography (MRT, e.g. gadolinium), or by positron emission tomography (PET, e.g. ^{18}F , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{131}I , or ^{186}Re ; see van Dongen et al. 2007, The Oncologist 12(12): 1379-1389) and non-invasive tomography methods well known to the skilled artisan.

25 The present invention further relates to a kit comprising instructions to carry out one of the methods of the present invention, and means for determining the amounts of at least the biomarkers NKD1 and C10orf54 in a sample comprising cells.

30 The term “kit” as used herein refers to a collection of the aforementioned components, preferably, provided separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. The kit, preferably, contains the aforementioned components in a ready-to-use formulation. Preferably, the kit may additionally comprise instructions, e.g., a user’s manual for interpreting the results of any
35 identification, diagnosis, and/or determination with respect to the methods of the present invention. Particularly, such manual may include information for allocating the amounts of the determined biomarkers to the kind of identification, diagnosis, and/or determination. Details are to be found elsewhere in this specification. Additionally, such user’s manual may provide instructions about correctly using the components of the kit for determining the amount(s) of the

respective biomarker. A user's manual may be provided in paper or electronic form, e.g., stored on CD or CD ROM, or downloadable via a web-interface from an online repository. The present invention also relates to the use of said kit in any of the methods according to the present invention.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

FIGURES

Figure 1: Relative gene expression after knockdown with siCTNNB1 are shown for CTNNB1 and NKD. Gene expression was measured using quantitative Real-Time PCR.

EXAMPLES

Example 1: Identification of biomarkers modulating Wnt/ β -catenin signaling

RNAi Experiments

siRNAs against CTNNB1/ β -catenin, APC and a control were obtained from Ambion (Applied Biosystems, Foster City, CA). Cells were transfected in 6-well plates with 10 nM siRNA using a total of 0.1% DharmaFECT 1 (Thermo Fischer Scientific, Waltham, MA) or RNAImax (Invitrogen, Carlsbad, CA). Cells were incubated for 72 hrs at 37°C and 5% CO₂ and two wells per knockdown were pooled for total RNA extraction using the RNeasy kit (Qiagen, Hilden).

Expression profiling by RNA sequencing (RNAseq)

Total RNA was extracted using the RNeasy Mini kit (Qiagen, Hilden). RNA integrity was verified on an Agilent 2100 Bioanalyzer. mRNA was isolated with the Dynabeads mRNA Purification Kit (Invitrogen). For each sample, a library for SOLiD sequencing was prepared using the "Whole transcriptome analysis kit" (4409491 Rev. D, Applied Biosystems). In brief, polyA RNA was fragmented using RNaseIII, purified, hybridized and ligated to RNA adapters.

Ligation products were reverse transcribed, purified and approx. 200 nt cDNA fragments were isolated after electrophoresis on a 6% TBE-Urea gel. cDNA was PCR amplified with SOLiD sequencing primers and purified to produce a library suitable as a template for emulsion PCR. DNA concentrations were determined using qPCR. A barcoded pooled library was generated by combining equimolar amounts from all samples. Emulsion PCR and sequencing with the SOLiD system was performed according to the manufacturers' protocol (Applied Biosystems).

Bioinformatic analysis

Mapping of SOLiD reads was performed using the mapReads algorithm, supplied by Applied Biosystems as part of the whole transcriptome analysis pipeline (v1.2). Two mismatches were allowed in both seed regions (bases 1-25, and bases 20-45) of the aligned read. Reads that mapped uniquely to the Human Genome (Hg18) were retained for further analysis. Reads were also mapped to a filter reference (Applied Biosystems). The filter library removed from further analysis reads that aligned to sequences from ribosomal RNA, tRNAs, single base repeats, adapter sequences. The python package HTSeq (v0.4.7), was used to count the number of reads mapping to each gene. Expression values (read counts) were obtained by summing the number of reads that mapped uniquely to annotated regions of the Human Genome (Hg18). Reads that mapped to a locus that had more than one annotated gene were considered ambiguous and not used. Bioconductor package, DESeq (v1.3) (Anders and Huber, 2010, Genome Biol. 11(10): R106), was used to identify differentially expressed genes between siRNA knockdown samples and control siRNA. Differential expression was determined from read count data scaled for different sequencing depth. Reads per kilo base per million reads mapped (RPKM) values were calculated to estimate and compare mRNA expression levels across samples. Gene length was defined as the region that encompasses the union of all transcript variants of a gene that do not overlap other genes.

Using the above mentioned bioinformatics analysis, biomarkers, which are modulated in 3 or more cell lines could be identified (see Tables 3 and 4).

Table 3: Shown are biomarkers being modulated in 3 or more cell lines according to the following conditions: opposite regulation in APC vs. CTNNB1 RNAi, AND (abs. $\log_2$ FC APC > 1 AND abs. $\log_2$ FC CTNNB1 > 1.5). Due to the aforementioned opposing roles of APC and β -catenin (CTNNB1) in regulating the expression of Wnt/ β -catenin target genes, opposite regulation after knockdown of either genes is central to ensure the selection of genes that are targets of the Wnt/ β -catenin signaling pathway. Logarithmic conversion is a frequently used method to present fold changes (FC). Thresholds for FC changes were used to remove genes which do not respond strongly to the Wnt/ β -catenin signaling pathway and, thus, would not be reliable to detect changes in the said Wnt/ β -catenin signaling pathway. As β -catenin is the most central component in Wnt/ β -catenin signaling pathway, a more stringent cutoff was placed on

this parameter as opposed to APC, which is one of several factors blocking Wnt/ β -catenin signaling pathway. Hence, APC knockdown phenotypes may be less pronounced than β -catenin knockdown phenotypes, especially, when comparing several cell lines. The efficiency of knockdown may vary between cell lines: while most CTNNB1 siRNAs are good knockdowns, APC knockdown efficiency shows more variation. The term “n.d.” (not detectable) applies to down-regulated genes only, where no reads were found after knockdown.

Gene Symbol	ENSBG_ID; ENTREZ_ID	Caco2; siCTNN B1 knock- down	Colo320; siCTNN B1 knock- down	DLD1; siCTNN B1 knock- down	HCT116; siCTNN B1 knock- down	HT29; siCTNN B1 knock- down	RKO; siCTNN B1 knock- down	SW480; siCTNN B1 knock- down
NKD1	ENSBG00000140807; 85407	-2.104	n.d.	-2.159	-2.093	n.d.	n.d.	-1.528
C10orf54	ENSBG00000107738; 64115	1.367	2.027	2.496	n.d.	1.592	1.55	n.d.
PSCA	ENSBG00000167653; 8000	n.d.	2.101	4.110	n.d.	1.687	n.d.	2.157
CPNE5	ENSBG00000124772; 57699	2.053	2.632	2.497	n.d.	n.d.	n.d.	n.d.

Table 4: Shown are biomarkers being modulated in 4 or more cell lines according to the following conditions opposite regulation in APC vs. CTNNB1 RNAi, AND (abs. log₂ FC APC > 1 AND abs. log₂ FC CTNNB1 > 1.5). Due to the aforementioned opposing roles of APC and β -catenin (CTNNB1) in regulating the expression of Wnt/ β -catenin target genes, opposite regulation after knockdown of either genes is central to ensure the selection of genes that are targets of the Wnt/ β -catenin signaling pathway. Logarithmic conversion is a frequently used method to present fold changes (FC). Thresholds for FC changes were used to remove genes which do not respond strongly to the Wnt/ β -catenin signaling pathway and, thus, would not be reliable to detect changes in the said Wnt/ β -catenin signaling pathway. As β -catenin is the most central component in Wnt/ β -catenin signaling pathway, a more stringent cutoff was placed on this parameter as opposed to APC which is one of several factors blocking Wnt/ β -catenin signaling pathway. Hence, APC knockdown phenotypes may be less pronounced than β -catenin knockdown phenotypes, especially when comparing several cell lines. The efficiency of knockdown may vary between cell lines: while most CTNNB1 siRNAs are good knockdowns, APC knockdown efficiency shows more variation. The term “n.d.” (not detectable) applies to down-regulated genes only, where no reads were found after knockdown.

Gene Symbol	ENSBG_ID; ENTREZ_ID	Caco2; siCTNN B1 knock- down	Colo320; siCTNN B1 knock- down	DLD1; siCTNN B1 knock- down	HCT116; siCTNN B1 knock- down	HT29; siCTNN B1 knock- down	RKO; siCTNN B1 knock- down	SW480; siCTNN B1 knock- down
NKD1	ENSBG00000140807; 85407	-2.104	n.d.	-2.159	-2.093	n.d.	n.d.	-1.528
C10orf54	ENSBG00000107738; 64115	1.367	2.027	2.496	n.d.	1.592	1.55	n.d.

PSCA	ENSG00000167653; 8000	n.d.	2.101	4.110	n.d.	1.687	n.d.	2.157
CPNE5	ENSG00000124772; 57699	2.053	2.632	2.497	n.d.	n.d.	n.d.	n.d.
RAET1 G	ENSG00000203722; 353091	n.d.	1.516	1.513	n.d.	2.691	n.d.	1.741

The p-values of the biomarkers displayed in Tables 3 and 4 reflect significance of the differential expression for each individual biomarker (see Tables 5 and 6 below). However, said p-values do not reflect significance over the whole set of cell lines and they do not reflect any additional parameter, e.g. that APC and CTNNB1 knockdown would cause opposite phenotypes. Due to differences in sequencing depth of the samples (i.e. lower sequencing depth), some of the p-values displayed in Tables 5 and/or 6 seem to be not significant.

Table 5: P-values of the biomarkers displayed in Tables 3 and 4.

Gene Symbol	ENSBL_ID; ENTREZ_ID	Caco2; siCTNN B1 knock- down	Colo320; siCTNN B1 knock- down	DLD1; siCTNN B1 knock- down	HCT116; siCTNN B1 knock- down	HT29; siCTNN B1 knock- down	RKO; siCTNN B1 knock- down	SW480; siCTNN B1 knock- down
NKD1	ENSG00000140807; 85407	0.085		0.011	0.005		0.540	1.950E-05
C10orf 54	ENSG00000107738; 64115	0.047	0.097	4.430E-06		0.000		
PSCA	ENSG00000167653; 8000		0.844	0.844		0.035		0.608
CPNE5	ENSG00000124772; 57699	0.791	0.344	0.344				
RAET1 G	ENSG00000203722; 353091		0.805	0.560		0.918		0.765
UCP3	ENSG00000175564; 7352	1.000	0.705		0.968		0.599	0.637

Table 6: Benjamini corrected p-values of the biomarkers displayed in Tables 3 and 4.

Gene Symbol	ENSBL_ID; ENTREZ_ID	Caco2; siCTNN B1 knock- down	Colo320; siCTNN B1 knock- down	DLD1; siCTNN B1 knock- down	HCT116; siCTNN B1 knock- down	HT29; siCTNN B1 knock- down	RKO; siCTNN B1 knock- down	SW480; siCTNN B1 knock- down
NKD1	ENSG00000140807; 85407	1.000		0.265	0.589		1.000	0.009
C10orf 54	ENSG00000107738; 64115	1.000	1.000	0.001		0.062		
PSCA	ENSG00000167653; 8000		1.000	1.000		1.000		1.000
CPNE5	ENSG00000124772; 57699	1.000	1.000	1.000				
RAET1 G	ENSG00000203722; 353091		1.000	0.989		1.000		1.000

UCP3	ENSG00000175564; 7352	1.000	1.000	1.000	1.000	1.000
------	-----------------------	-------	-------	-------	-------	-------

Example 2: Identification of individual cell line response genes

5 siRNA Transfection

siRNAs against CTNNB1/ β -catenin, Evi, APC and the control were obtained from Ambion (Applied Biosystems, Foster City, CA). Cells were transfected in 12- or 6-well plates with 10 nM siRNA using a total of 0.1% DharmaFECT 1 (Thermo Fischer Scientific, Waltham, MA) or RNAImax (Invitrogen, Carlsbad, CA).

10

Expression profiling by RNA sequencing (RNAseq)

Total RNA was extracted as described below and RNA integrity was verified on an Agilent 2100 Bioanalyzer. mRNA was isolated with the Dynabeads mRNA Purification Kit (Invitrogen). For each sample, a library for SOLiD sequencing was prepared using the 'Whole transcriptome analysis kit' (4409491 Rev. D, Applied Biosystems). In brief, polyA RNA was fragmented using
15 RNAseIII, purified, hybridized and ligated to RNA adapters. Ligation products were reverse transcribed, purified and approx. 200 nt cDNA fragments were isolated after electrophoresis on a 6% TBE-Urea gel. cDNA was PCR amplified with SOLiD sequencing primers and purified to produce a library suitable as a template for emulsion PCR. DNA concentrations were determined
20 using qPCR. A barcoded pooled library was generated by combining equimolar amounts from all samples. Emulsion PCR and sequencing with the SOLiD system was performed according to the manufacturers' protocol (Applied Biosystems).

Bioinformatic analysis

25 Mapping of SOLiD reads was performed using the mapReads algorithm, supplied by Applied Biosystems as part of the whole transcriptome analysis pipeline (v1.2). Two mismatches were allowed in both seed regions (bases 1-25, and bases 20-45) of the aligned read. Reads that mapped uniquely to the Human Genome (Hg18) were retained for further analysis. Reads were also mapped to a filter reference (Applied Biosystems). The filter library removed from further
30 analysis reads that aligned to sequences from ribosomal RNA, tRNAs, single base repeats, adapter sequences. The python package HTSeq (v0.4.7), was used to count the number of reads mapping to each gene (<http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html>). Expression values (read counts) were obtained by summing the number of reads that mapped uniquely to annotated regions of the Human Genome (Hg18). Reads that mapped to a locus that
35 had more than one annotated gene were considered ambiguous and not used. Bioconductor package, DESeq (v1.3; Anders and Huber, 2010, Genome Biol. 11(10): R106), was used to identify differentially expressed genes between siRNA knockdown samples and control siRNA.

Differential expression was determined from read count data scaled for different sequencing depth. Reads per kilo base per million reads mapped (RPKM) values were calculated to estimate and compare mRNA expression levels across samples. Gene length was defined as the region that encompasses the union of all transcript variants of a gene that do not overlap other genes.

5

Table 7 shows the five most significant up- and down-regulated biomarkers determined in 7 individual cell lines according to the following conditions: opposite regulation in APC vs. CTNNB1 RNAi, AND (abs. log₂ FC APC > 1 AND abs. log₂ FC CTNNB1 > 1.5).

- 10 **Table 7:** The five most significant up- and down-regulated biomarkers were determined in several individual cell lines according to the following conditions: opposite regulation in APC vs. CTNNB1 RNAi, AND (abs. log₂ FC APC > 1 AND abs. log₂ FC CTNNB1 > 1.5). Due to the
- 15 aforementioned opposing roles of APC and β -catenin (CTNNB1) in regulating the expression of Wnt/ β -catenin target genes, opposite regulation after knockdown of either genes is central to ensure the selection of genes that are targets of the Wnt/ β -catenin signaling pathway. Logarithmic conversion is a frequently used method to present fold changes (FC). Thresholds for FC changes were used to remove genes which do not respond strongly to the Wnt/ β -catenin signaling pathway and, thus, would not be reliable to detect changes in the said Wnt/ β -catenin signaling pathway. As β -catenin is the most central component in Wnt/ β -catenin signaling
- 20 pathway, a more stringent cutoff was placed on this parameter as opposed to APC which is one of several factors blocking Wnt/ β -catenin signaling pathway. Hence, APC knockdown phenotypes may be less pronounced than β -catenin knockdown phenotypes, especially, when comparing several cell lines. The efficiency of knockdown may vary between cell lines: while most CTNNB1 siRNAs are good knockdowns, APC knockdown efficiency shows more
- 25 variation.

Caco2				
Gene Symbol	ENSBLE_ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
LGR5	ENSG00000139292; 8549	down	-3,328	1,392
FAM46C	ENSG00000183508; 54855	down	-1,791	1,032
NKD1	ENSG00000140807; 85407	down	-2,104	1,217
ITGA9	ENSG00000144668; 3680	down	-2,502	1,04
WNT5B	ENSG00000111186; 81029	down	-2,629	1,394
SYTL5	ENSG00000147041; 94122	up	1,877	-1,647
PTPRR	ENSG00000153233; 5801	up	1,875	-1,135
PHEX	ENSG00000102174; 5251	up	1,574	-1,323

PIK3CD	ENSG00000171608; 5293	up	2,316	-1,945
PIK3IP1	ENSG00000100100; 113791	up	2,178	-1,024

Colo320				
Gene Symbol	ENSBL_ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
MANF	ENSG00000145050; 7873	down	-1,584	1,509
TMEM200A	ENSG00000164484; 114801	down	-1,83	1,409
FABP2	ENSG00000145384; 2169	down	-2,291	2,388
ST8SIA4	ENSG00000113532; 7903	down	-1,806	1,241
LACE1	ENSG00000135537; 246269	down	-2,654	1,239
IGFBP5	ENSG00000115461; 3488	up	5,866	-1,297
EPAS1	ENSG00000116016; 2034	up	3,708	-1,469
CLUL1	ENSG00000079101; 27098	up	5,612	-1,771
PLCE1	ENSG00000138193; 51196	up	5,217	-1,449
SHISA3	ENSG00000178343; 152573	up	5,47	n.d.

DLD1				
Gene Symbol	ENSBL_ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
PRR9	ENSG00000203783; 574414	down	-2,403	2,08
CADPS	ENSG00000163618; 8618	down	-1,773	2,082
NKD1	ENSG00000140807; 85407	down	-2,159	2,396
BCL11A	ENSG00000119866; 53335	down	-4,501	2,007
PTGS2	ENSG00000073756; 5743	down	-2,627	2,278
REG4	ENSG00000134193; 83998	up	5,173	-1,058
PSCA	ENSG00000167653; 8000	up	4,11	-1,313
KRT4	ENSG00000170477; 3851	up	5,577	-1,682
CD177	ENSG00000204936; 57126	up	4,038	-1,421
RBP4	ENSG00000138207; 5950	up	3,754	-2,042

HCT116				
Gene Symbol	ENSL ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
ADAM19	ENSG00000135074; 8728	down	-1,922	1,156
ADAMTS14	ENSG00000138316; 140766	down	-2,699	1,157
AXIN2	ENSG00000168646; 8313	down	-2,277	3,272
DUSP6	ENSG00000139318; 1848	down	-1,525	1,175
KIAA1199	ENSG00000103888; 57214	down	-1,825	1,865
RGS2	ENSG00000116741; 5997	up	1,672	-2,435
GPR87	ENSG00000138271; 53836	up	2,532	-1,009
DPP4	ENSG00000197635; 1803	up	1,521	-1,822
THSD7A	ENSG00000005108; 221981	up	2,884	n.d.
LAMA4	ENSG00000112769; 3910	up	1,545	-2,556
RUNX2	ENSG00000124813; 860	down	-2,486	1,953
NKD1	ENSG00000140807; 85407	down	-2,093	2,396

HT29				
Gene Symbol	ENSL ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
WNT11	ENSG00000085741; 7481	down	-2,023	2,582
PTPRO	ENSG00000151490; 5800	down	-1,674	1,168
CD83	ENSG00000112149; 9308	down	-2,279	1,035
LRRC2	ENSG00000163827; 79442	down	-4,253	1,115
ATOH8	ENSG00000168874; 84913	down	-3,62	1,403
CEACAM5	ENSG00000105388; 1048	up	4,842	-3,45
SDCBP2	ENSG00000125775; 27111	up	2,365	-1,338
VSIG1	ENSG00000101842; 340547	up	5,042	-1,865
TFF3	ENSG00000160180; 7033	up	2,023	-1,523
C10orf54	ENSG00000107738; 64115	up	1,592	-1,114

RKO				
Gene Symbol	ENSL_ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
SNAR-F	Not available; 100126781	down	n.d.	1,55
SNORD31	ENSG00000201669; ENSG00000201847; 9298	down	n.d.	1,55
GC	ENSG00000145321; 2638	down	n.d.	1,064
IMMP1L	ENSG00000148950; 196294	down	n.d.	1,479
UCP3	ENSG00000175564; 7352	down	n.d.	1,479
CREB3L1	ENSG00000157613; 90993	up	3,496	n.d.
MACC1	ENSG00000183742; 346389	up	3,904	n.d.
KRT20	ENSG00000171431; 54474	up	1,965	-2,164
ARL9	ENSG00000196503; 132946	up	1,782	-1,48
DISP2	ENSG00000140323; 85455	up	2,319	n.d.

SW480				
Gene Symbol	ENSL_ID; ENTREZ_ID	Regulation by CTNNB1 knockdown	CTNNB1 knockdown; log 2 fold change	APC; log 2 fold change
CPXM1	ENSG00000088882; 56265	up	n.d.	1,236
MSI1	ENSG00000135097; 4440	up	-2,428	1,099
CHRNA4	ENSG00000117971; 1143	up	-2,165	2,262
SLC19A3	ENSG00000135917; 80704	up	n.d.	1,999
DKK4	ENSG00000104371; 27121	up	n.d.	1,777
GPR39	ENSG00000183840; 2863	down	2,819	-1,808
CCDC159	ENSG00000183401; 126075	down	2,741	n.d.
TIMP4	ENSG00000157150; 7079	down	2,741	n.d.
APOL3	ENSG00000128284; 80833	down	1,519	-2,03
MEGF10	ENSG00000145794; 84466	down	2,031	n.d.

Example 3: Gene expression after knockdown with siCTNNB1

5 Relative gene expression after knockdown with siCTNNB1 is shown in Figure 1. Gene expression was measured using quantitative Real-Time PCR. siRNAs against CTNNB1/ β -catenin, Evi, APC and the control were obtained from Ambion (Applied Biosystems, Foster City, CA). Cells were transfected in 12- or 6-well plates with 10 nM siRNA using a total of 0.1% DharmaFECT 1 (Thermo Fischer Scientific, Waltham, MA) or RNAiMax (Invitrogen, Carlsbad, CA). cDNA was prepared from 0.5 or 2 μ g total RNA using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fischer Scientific). Oligo-dT primers were used to prime the reaction. Subsequently the cDNA was diluted to 5ng/ μ l and used for qPCR on the Lightcycler480 (Roche) using the universal probe library system (Roche) in a 384-well format. Primer probe combinations for each quarry gene were constructed using the Roche ProbeFinder V2.45, automatically selecting intron-spanning primers. All samples were amplified as triplicates and differential expression was analyzed using the LightCycler®480 Software. The reference refers to UBC and GAPDH combined to serve as a housekeeping gene. Corresponding relative gene expression amounts are shown in Table 8.

Table 8: Relative gene expression amounts of CTNNB1 and NKD.

	Replicate 1	Replicate 2	Replicate Average	Replicate Average [log ₂]
Reference	1.000	1.000	1.000	0.000
CTNNB1	0.250	0.325	0.288	-1.797
NKD	0.225	0.350	0.287	-1.801

	Error rate Replicate 1	Error rate Replicate 2	Standarddeviation
Reference	0.000	0.000	0.000
CTNNB1	0.047	0.254	0.053
NKD	0.069	0.067	0.088

Example 4: Known polypeptides and/or regulators of the Wnt/ β -catenin signaling pathway in humans

25

About 150 known polypeptides and/or regulators of the Wnt/ β -catenin signaling pathway in humans are shown in Table 9.

Table 9: Known polypeptides and/or regulators of the Wnt/ β -catenin signaling pathway in humans.

ENSEMBL GENE ID	ENTREZ GENE ID	Description
ENSG00000159692	1487	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa05200:Pathways in cancer, hsa05220:Chronic myeloid leukemia,
ENSG00000175029	1488	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa05200:Pathways in cancer, hsa05220:Chronic myeloid leukemia,
ENSG00000005339	1387	hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04520:Adherens junction, hsa04630:Jak-STAT signaling pathway, hsa04720:Long-term potentiation, hsa04916:Melanogenesis, hsa05016:Huntington's disease, hsa05200:Pathways in cancer, hsa05211:Renal cell carcinoma, hsa05215:Prostate cancer,
ENSG00000168772	80319	hsa04310:Wnt signaling pathway,
ENSG00000100393	2033	hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04520:Adherens junction, hsa04630:Jak-STAT signaling pathway, hsa04720:Long-term potentiation, hsa04916:Melanogenesis, hsa05016:Huntington's disease, hsa05200:Pathways in cancer, hsa05211:Renal cell carcinoma, hsa05215:Prostate cancer,
ENSG00000072803	23291	hsa04114:Oocyte meiosis, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway,
ENSG00000175592	8061	hsa04310:Wnt signaling pathway,
ENSG00000134318	9475	hsa04062:Chemokine signaling pathway, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04360:Axon guidance, hsa04510:Focal adhesion, hsa04670:Leukocyte transendothelial migration, hsa04810:Regulation of actin cytoskeleton, hsa05130:Pathogenic Escherichia coli infection,
ENSG00000175792	8607	hsa04310:Wnt signaling pathway,
ENSG00000113558	6500	hsa04110:Cell cycle, hsa04114:Oocyte meiosis, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway,
ENSG00000175387	4087	hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04520:Adherens junction, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer,
ENSG00000166949	4088	hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04520:Adherens junction, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05220:Chronic myeloid leukemia,
ENSG00000141646	4089	hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04520:Adherens junction, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05220:Chronic myeloid

		leukemia,
ENSG00000164736	64321	hsa04310:Wnt signaling pathway,
ENSG00000163904	59343	hsa04310:Wnt signaling pathway,
ENSG00000156076	11197	hsa04310:Wnt signaling pathway,
ENSG00000115266	10297	hsa04310:Wnt signaling pathway, hsa04810:Regulation of actin cytoskeleton, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05217:Basal cell carcinoma,
ENSG00000134982	324	hsa04310:Wnt signaling pathway, hsa04810:Regulation of actin cytoskeleton, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05217:Basal cell carcinoma,
ENSG00000103126	8312	hsa04310:Wnt signaling pathway, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05217:Basal cell carcinoma,
ENSG00000168646	8313	hsa04310:Wnt signaling pathway, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05217:Basal cell carcinoma,
ENSG00000166167	8945	hsa04114:Oocyte meiosis, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway,
ENSG00000166869	63928	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000187446	11261	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000070808	815	hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04720:Long-term potentiation, hsa04722:Neurotrophin signaling pathway, hsa04740:Olfactory transduction, hsa04912:GnRH signaling pathway,
ENSG00000058404	816	hsa04916:Melanogenesis, hsa05214:Glioma, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04720:Long-term potentiation, hsa04722:Neurotrophin signaling pathway, hsa04740:Olfactory transduction, hsa04912:GnRH signaling pathway,
ENSG00000145349	817	hsa04916:Melanogenesis, hsa05214:Glioma, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04720:Long-term potentiation,

ENSG00000148660	818	hsa04722:Neurotrophin signaling pathway, hsa04740:Olfactory transduction, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa05214:Glioma, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04720:Long-term potentiation, hsa04722:Neurotrophin signaling pathway, hsa04740:Olfactory transduction, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa05214:Glioma, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway,
ENSG00000113712	1452	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway,
ENSG00000180138	122011	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway,
ENSG00000213923	1454	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04710:Circadian rhythm,
ENSG00000101266	1457, 283106	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04530:Tight junction,
ENSG00000070770	1459	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04530:Tight junction,
ENSG00000168036	1499	hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04520:Adherens junction, hsa04530:Tight junction, hsa04670:Leukocyte transendothelial migration, hsa04916:Melanogenesis, hsa05130:Pathogenic Escherichia coli infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05217:Basal cell carcinoma, hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC),
ENSG00000178585	56998	hsa04310:Wnt signaling pathway,
ENSG00000147869	9350	hsa04310:Wnt signaling pathway,
ENSG00000100888	57680	hsa04310:Wnt signaling pathway,
ENSG00000055130	8454	hsa04110:Cell cycle, hsa04114:Oocyte meiosis, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04110:Cell cycle, hsa04115:p53 signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04630:Jak-STAT signaling pathway, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05213:Endometrial cancer, hsa05214:Glioma, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05218:Melanoma, hsa05219:Bladder cancer, hsa05220:Chronic myeloid leukemia, hsa05221:Acute myeloid leukemia, hsa05222:Small cell lung cancer, hsa05223:Non-small cell lung cancer, hsa05416:Viral myocarditis,
ENSG00000110092	595	hsa04110:Cell cycle, hsa04115:p53 signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04630:Jak-STAT signaling pathway,
ENSG00000118971	894	hsa04110:Cell cycle, hsa04115:p53 signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04630:Jak-STAT signaling pathway,
ENSG00000112576	896	hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04630:Jak-STAT signaling pathway,
ENSG00000107984	22943	hsa04310:Wnt signaling pathway,

ENSG00000155011	27123	hsa04310:Wnt signaling pathway,
ENSG00000104371	27121	hsa04310:Wnt signaling pathway,
ENSG00000100592	23002	hsa04310:Wnt signaling pathway,
ENSG00000146122	23500	hsa04310:Wnt signaling pathway,
ENSG00000107404	1855, 8215	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000004975	1856	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000161202	1857	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000165879	10023	hsa04310:Wnt signaling pathway,
ENSG00000181274	23401	hsa04310:Wnt signaling pathway,
ENSG00000157240	8321	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000111432	11211	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000180340	2535	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000104290	7976	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000174804	8322	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000163251	7855	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000164930	8323	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000155760	8324	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000177283	8325	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000188763	8326	hsa04310:Wnt signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05217:Basal cell carcinoma,
ENSG00000082701	2932	hsa04012:ErbB signaling pathway, hsa04062:Chemokine signaling pathway, hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04360:Axon guidance, hsa04510:Focal adhesion, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,

ENSG00000177606	3725	hsa04722:Neurotrophin signaling pathway, hsa04910:Insulin signaling pathway, hsa04916:Melanogenesis, hsa05010:Alzheimer's disease, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05215:Prostate cancer, hsa05217:Basal cell carcinoma, hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04620:Toll-like receptor signaling pathway, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04722:Neurotrophin signaling pathway, hsa04912:GnRH signaling pathway, hsa05120:Epithelial cell signaling in Helicobacter pylori infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05211:Renal cell carcinoma,
ENSG00000162337	4041	hsa04310:Wnt signaling pathway,
ENSG00000070018	4040	hsa04310:Wnt signaling pathway,
ENSG00000204435, ENSG00000206300, ENSG00000206406,	1460, 58496	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04530:Tight junction,
ENSG00000138795	51176	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05217:Basal cell carcinoma, hsa05221:Acute myeloid leukemia, hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC),
ENSG00000137673	4316	hsa04310:Wnt signaling pathway,
ENSG00000109339	5602	hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04620:Toll-like receptor signaling pathway, hsa04621:NOD-like receptor signaling pathway, hsa04622:RIG-I-like receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04722:Neurotrophin signaling pathway, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04920:Adipocytokine signaling pathway, hsa04930:Type II diabetes mellitus, hsa05120:Epithelial cell signaling in Helicobacter pylori infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04620:Toll-like receptor signaling pathway, hsa04621:NOD-like receptor signaling pathway, hsa04622:RIG-I-like receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04722:Neurotrophin signaling pathway,
ENSG00000107643	5599	hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04920:Adipocytokine signaling pathway, hsa04930:Type II diabetes mellitus, hsa05120:Epithelial cell signaling in Helicobacter pylori infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer,

ENSG00000050748	5601	hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04310:Wnt signaling pathway, hsa04510:Focal adhesion, hsa04620:Toll-like receptor signaling pathway, hsa04621:NOD-like receptor signaling pathway, hsa04622:RIG-I-like receptor signaling pathway, hsa04660:T cell receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04722:Neurotrophin signaling pathway, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04920:Adipocytokine signaling pathway, hsa04930:Type II diabetes mellitus, hsa05120:Epithelial cell signaling in Helicobacter pylori infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa04010:MAPK signaling pathway, hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04620:Toll-like receptor signaling pathway, hsa04621:NOD-like receptor signaling pathway, hsa04622:RIG-I-like receptor signaling pathway, hsa04660:T cell receptor signaling pathway,
ENSG00000135341	6885	hsa04660:T cell receptor signaling pathway,
ENSG00000140807	85407	hsa04310:Wnt signaling pathway,
ENSG00000145506	85409	hsa04310:Wnt signaling pathway,
ENSG00000087095	51701	hsa04010:MAPK signaling pathway, hsa04310:Wnt signaling pathway, hsa04520:Adherens junction,
ENSG00000102908	10725	hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,
ENSG00000131196	4772	hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,
ENSG00000101096	4773	hsa04010:MAPK signaling pathway, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,
ENSG00000072736	4775	hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,
ENSG00000100968	4776	hsa04010:MAPK signaling pathway, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway,
ENSG00000112033	5467	hsa03320:PPAR signaling pathway, hsa04310:Wnt signaling pathway, hsa05200:Pathways in cancer, hsa05221:Acute myeloid leukemia,
ENSG00000182621	23236	hsa00562:Inositol phosphate metabolism, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis,

		hsa05010:Alzheimer's disease, hsa05016:Huntington's disease,
ENSG00000137841	5330	hsa00562:Inositol phosphate metabolism, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04742:Taste transduction, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa05010:Alzheimer's disease, hsa05016:Huntington's disease,
ENSG00000149782	5331	hsa00562:Inositol phosphate metabolism, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa05010:Alzheimer's disease, hsa05016:Huntington's disease,
ENSG00000101333	5332	hsa00562:Inositol phosphate metabolism, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa05010:Alzheimer's disease, hsa05016:Huntington's disease,
ENSG00000102312	64840	hsa04310:Wnt signaling pathway,
ENSG00000080815	5663	hsa04310:Wnt signaling pathway, hsa04330:Notch signaling pathway, hsa04722:Neurotrophin signaling pathway, hsa05010:Alzheimer's disease,
ENSG00000139174	144165	hsa04310:Wnt signaling pathway,
ENSG00000163637	166336	hsa04310:Wnt signaling pathway,
ENSG00000154229	5578	hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04530:Tight junction, hsa04540:Gap junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04664:Fc epsilon RI signaling pathway, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa04960:Aldosterone-regulated sodium reabsorption, hsa05110:Vibrio cholerae infection, hsa05130:Pathogenic Escherichia coli infection, hsa05200:Pathways in cancer, hsa05214:Glioma, hsa05223:Non-small cell lung cancer,

		hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04530:Tight junction, hsa04540:Gap junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04662:B cell receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04912:GnRH signaling pathway, hsa04916:Melanogenesis, hsa04960:Aldosterone-regulated sodium reabsorption, hsa05110:Vibrio cholerae infection, hsa05200:Pathways in cancer, hsa05214:Glioma, hsa05223:Non-small cell lung cancer,
ENSG00000166501	5579	hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04020:Calcium signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04530:Tight junction, hsa04540:Gap junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04916:Melanogenesis, hsa04960:Aldosterone-regulated sodium reabsorption, hsa05110:Vibrio cholerae infection, hsa05200:Pathways in cancer, hsa05214:Glioma, hsa05223:Non-small cell lung cancer,
ENSG00000126583	5582	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04070:Phosphatidylinositol signaling system, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04530:Tight junction, hsa04540:Gap junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04720:Long-term potentiation, hsa04730:Long-term depression, hsa04916:Melanogenesis, hsa04960:Aldosterone-regulated sodium reabsorption, hsa05110:Vibrio cholerae infection, hsa05200:Pathways in cancer, hsa05214:Glioma, hsa05223:Non-small cell lung cancer,
ENSG00000183943	5613	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04740:Olfactory transduction, hsa04742:Taste transduction, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04916:Melanogenesis, hsa05020:Prion diseases, hsa05110:Vibrio cholerae infection, hsa05414:Dilated cardiomyopathy,
ENSG00000072062	5566	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04740:Olfactory transduction, hsa04742:Taste transduction, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04916:Melanogenesis, hsa05020:Prion diseases, hsa05110:Vibrio cholerae infection, hsa05414:Dilated cardiomyopathy,

ENSG00000142875	5567	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04740:Olfactory transduction, hsa04742:Taste transduction, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04916:Melanogenesis, hsa05020:Prion diseases, hsa05110:Vibrio cholerae infection, hsa05414:Dilated cardiomyopathy,
ENSG00000165059	5568	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04062:Chemokine signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04540:Gap junction, hsa04720:Long-term potentiation, hsa04740:Olfactory transduction, hsa04742:Taste transduction, hsa04910:Insulin signaling pathway, hsa04912:GnRH signaling pathway, hsa04914:Progesterone-mediated oocyte maturation, hsa04916:Melanogenesis, hsa05020:Prion diseases, hsa05110:Vibrio cholerae infection, hsa05414:Dilated cardiomyopathy,
ENSG00000113575	5515	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04530:Tight junction, hsa04730:Long-term depression,
ENSG00000104695	5516	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04530:Tight junction, hsa04730:Long-term depression,
ENSG00000105568	5518	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04530:Tight junction, hsa04730:Long-term depression,
ENSG00000137713	5519	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04530:Tight junction, hsa04730:Long-term depression,
ENSG00000066027	5525	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway,
ENSG00000068971	5526	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway,
ENSG00000112640	5528	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway,
ENSG00000154001	5529	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway,
ENSG00000078304	5527	hsa04114:Oocyte meiosis, hsa04310:Wnt signaling pathway,
ENSG00000138814	5530	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),

ENSG00000107758	5532	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000120910	5533	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000115953	5534	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000188386	5535	hsa04010:MAPK signaling pathway, hsa04020:Calcium signaling pathway, hsa04114:Oocyte meiosis, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04660:T cell receptor signaling pathway, hsa04662:B cell receptor signaling pathway, hsa04720:Long-term potentiation, hsa05010:Alzheimer's disease, hsa05014:Amyotrophic lateral sclerosis (ALS),
ENSG00000067560	387	hsa04062:Chemokine signaling pathway, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04360:Axon guidance, hsa04510:Focal adhesion, hsa04520:Adherens junction, hsa04530:Tight junction, hsa04660:T cell receptor signaling pathway, hsa04670:Leukocyte transendothelial migration, hsa04722:Neurotrophin signaling pathway, hsa04810:Regulation of actin cytoskeleton, hsa05130:Pathogenic Escherichia coli infection, hsa05200:Pathways in cancer,
ENSG00000136238	5879	hsa04010:MAPK signaling pathway, hsa04062:Chemokine signaling pathway, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04520:Adherens junction, hsa04620:Toll-like receptor signaling pathway, hsa04650:Natural killer cell mediated cytotoxicity, hsa04662:B cell receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04722:Neurotrophin signaling pathway, hsa04810:Regulation of actin cytoskeleton, hsa05014:Amyotrophic lateral sclerosis (ALS), hsa05120:Epithelial cell signaling in Helicobacter pylori infection, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05211:Renal cell carcinoma, hsa05212:Pancreatic cancer, hsa05416:Viral

		myocarditis,
ENSG00000128340	5880	hsa04010:MAPK signaling pathway, hsa04062:Chemokine signaling pathway, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04520:Adherens junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04662:B cell receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04666:Fc gamma R-mediated phagocytosis, hsa04670:Leukocyte transendothelial migration, hsa04810:Regulation of actin cytoskeleton, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05416:Viral myocarditis,
ENSG00000169750	5881	hsa04010:MAPK signaling pathway, hsa04310:Wnt signaling pathway, hsa04360:Axon guidance, hsa04370:VEGF signaling pathway, hsa04510:Focal adhesion, hsa04520:Adherens junction, hsa04650:Natural killer cell mediated cytotoxicity, hsa04662:B cell receptor signaling pathway, hsa04664:Fc epsilon RI signaling pathway, hsa04810:Regulation of actin cytoskeleton, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05416:Viral myocarditis,
ENSG00000100387	9978	hsa03420:Nucleotide excision repair, hsa04110:Cell cycle, hsa04114:Oocyte meiosis, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa05200:Pathways in cancer, hsa05211:Renal cell carcinoma,
ENSG00000104332	6422	hsa04310:Wnt signaling pathway,
ENSG00000145423	6423	hsa04310:Wnt signaling pathway,
ENSG00000106483	6424	hsa04310:Wnt signaling pathway,
ENSG00000120057	6425	hsa04310:Wnt signaling pathway,
ENSG00000196470	6477	hsa04115:p53 signaling pathway, hsa04120:Ubiquitin mediated proteolysis, hsa04310:Wnt signaling pathway, hsa04062:Chemokine signaling pathway, hsa04270:Vascular smooth muscle contraction, hsa04310:Wnt signaling pathway,
ENSG00000067900	6093, 727758	hsa04350:TGF-beta signaling pathway, hsa04360:Axon guidance, hsa04510:Focal adhesion, hsa04670:Leukocyte transendothelial migration, hsa04810:Regulation of actin cytoskeleton, hsa05130:Pathogenic Escherichia coli infection,
ENSG00000116161	27101, 644877	hsa04310:Wnt signaling pathway,
ENSG00000081059	6932	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05217:Basal cell carcinoma, hsa05221:Acute myeloid leukemia, hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC),

ENSG00000148737	6934	hsa04310:Wnt signaling pathway, hsa04520:Adherens junction, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05217:Basal cell carcinoma, hsa05221:Acute myeloid leukemia, hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC),
ENSG00000177565	79718	hsa04310:Wnt signaling pathway,
ENSG00000101849	6907	hsa04310:Wnt signaling pathway,
ENSG00000092377	90665	hsa04310:Wnt signaling pathway,
ENSG00000141510	7157	hsa04010:MAPK signaling pathway, hsa04110:Cell cycle, hsa04115:p53 signaling pathway, hsa04210:Apoptosis, hsa04310:Wnt signaling pathway, hsa04722:Neurotrophin signaling pathway, hsa05014:Amyotrophic lateral sclerosis (ALS), hsa05016:Huntington's disease, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05212:Pancreatic cancer, hsa05213:Endometrial cancer, hsa05214:Glioma, hsa05215:Prostate cancer, hsa05216:Thyroid cancer, hsa05217:Basal cell carcinoma, hsa05218:Melanoma, hsa05219:Bladder cancer, hsa05220:Chronic myeloid leukemia, hsa05222:Small cell lung cancer, hsa05223:Non-small cell lung cancer,
ENSG00000136997	4609	hsa04010:MAPK signaling pathway, hsa04012:ErbB signaling pathway, hsa04110:Cell cycle, hsa04310:Wnt signaling pathway, hsa04350:TGF-beta signaling pathway, hsa04630:Jak-STAT signaling pathway, hsa05200:Pathways in cancer, hsa05210:Colorectal cancer, hsa05213:Endometrial cancer, hsa05216:Thyroid cancer, hsa05219:Bladder cancer, hsa05220:Chronic myeloid leukemia, hsa05221:Acute myeloid leukemia, hsa05222:Small cell lung cancer,
ENSG00000173218	81839	hsa04310:Wnt signaling pathway,
ENSG00000162738	57216	hsa04310:Wnt signaling pathway,
ENSG00000105989	7472	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000125084	7471	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000135925	80326	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000169884	7480	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000085741	7481	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000002745	51384	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,

ENSG00000134245	7482	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000108379	7473	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000154342	89780	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000162552	54361	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000114251	7474	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000111186	81029	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000115596	7475	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000154764	7476	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000188064	7477	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000061492	7478	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000075290	7479	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000143816	7483	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,
ENSG00000158955	7484	hsa04310:Wnt signaling pathway, hsa04340:Hedgehog signaling pathway, hsa04916:Melanogenesis, hsa05200:Pathways in cancer, hsa05217:Basal cell carcinoma,

Claims

1. A method for identifying a compound modulating Wnt/ β -catenin signaling comprising the steps of:
 - 5 a) determining the amounts of at least the biomarkers NKD1 and C10orf54 in a sample comprising cells, wherein said cells have been brought into contact with a compound suspected to be a modulator of Wnt/ β -catenin signaling; and
 - 10 b) comparing the amount of the biomarker NKD1 determined in step a) to a reference for NKD1 and comparing the amount of the biomarker C10orf54 determined in step a) to a reference for C10orf54, whereby a compound modulating Wnt/ β -catenin signaling is identified.
2. The method of claim 1, wherein said reference is obtained from a sample
15 comprising cells corresponding to the cells of step a) and wherein said cells have not been brought into contact with a compound suspected to modulate Wnt/ β -catenin signaling.
3. The method of claim 2, wherein amounts of said biomarkers determined in step a)
20 differing from the corresponding references are indicative for a compound modulating Wnt/ β -catenin signaling.
4. The method of claim 1, wherein said reference is obtained from a sample of cells
25 corresponding to the cells of step a) and wherein said cells have been brought into contact with a compound known to modulate Wnt/ β -catenin signaling.
5. The method of claim 4, wherein amounts of said biomarkers determined in step a)
which are essentially identical to the corresponding reference are indicative for a
30 compound modulating Wnt/ β -catenin signaling.
6. The method of any one of claims 1 to 5, wherein said compound modulating
Wnt/ β -catenin signaling is selected from the group consisting of: antisense RNA,
siRNA, micro RNA, ribozymes, oligonucleotides, peptides, PNAs, proteins,
antibodies, aptamers, and organic small molecule compounds.
35
7. The method of any one of claims 1 to 6, wherein said cells are human or animal
cells.

8. The method of any one of claims 1 to 7, wherein said compound modulating Wnt/ β -catenin signaling is an inhibitor of said Wnt/ β -catenin signaling.
9. A method for identifying a drug comprising the steps of the method for identifying a compound modulating Wnt/ β -catenin signaling of any one of claims 1 to 8 and, further, the step of identifying a compound modulating Wnt/ β -catenin signaling as a drug.
10. The method of claim 9, wherein said drug is a drug against cancer.
11. The method of claim 10, wherein said cancer is selected from the group consisting of: colorectal cancer, breast cancer, esophageal cancer, melanoma, hepatocellular carcinoma, desmoid tumor, pancreatic cancer, gastric cancer, ovarian cancer, and prostate cancer.
12. Use of at least the biomarkers NKD1 and C10orf54 in a sample comprising cells for determining whether said cells have been brought into contact with a compound modulating Wnt/ β -catenin signaling.
13. The use of claim 12, wherein said compound modulating Wnt/ β -catenin signaling is an inhibitor of Wnt/ β -catenin signaling.
14. The use of claim 12 or 13, wherein said compound modulating Wnt/ β -catenin signaling is a drug or suspected to be a drug against cancer.
15. The use of claim 14, wherein said cancer is selected from the group consisting of: colorectal cancer, breast cancer, esophageal cancer, melanoma, hepatocellular carcinoma, desmoid tumor, pancreatic cancer, gastric cancer, ovarian cancer, and prostate cancer.

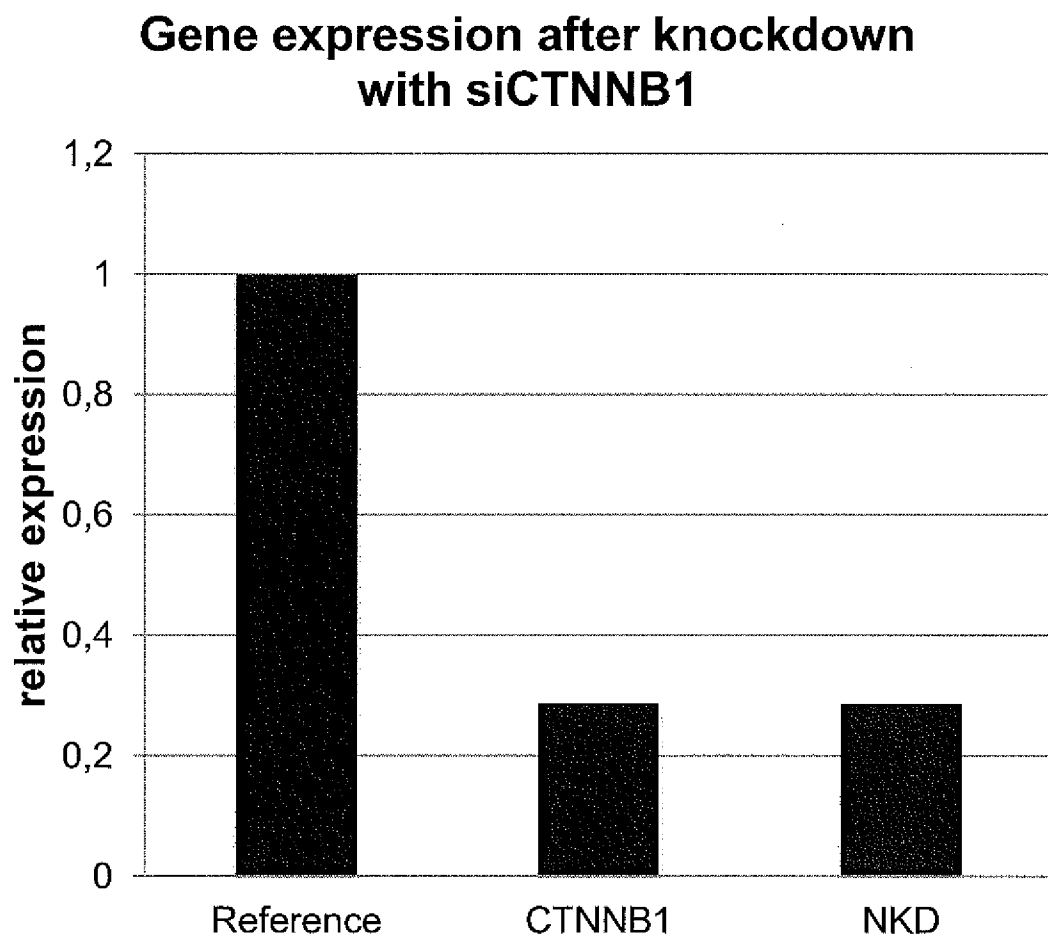


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/053187

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

ADD.

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)

C12Q

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/252689 A1 (WU ZHONG [US] ET AL) 4 October 2012 (2012-10-04) paragraph [0050]; claims 14,24 -----	1-15
A	WHARTON KEITH A JR ET AL: "Vertebrate proteins related to Drosophila naked cuticle bind dishevelled and antagonize Wnt signaling", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 234, no. 1, 1 June 2001 (2001-06-01), pages 93-106, XP055072321, ISSN: 0021-9258 ----- -/--	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 March 2014

Date of mailing of the international search report

18/03/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Cornelis, Karen

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2014/053187

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. P. CREYGHTON ET AL: "PR130 is a modulator of the Wnt-signaling cascade that counters repression of the antagonist Naked cuticle", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 103, no. 14, 4 April 2006 (2006-04-04), pages 5397-5402, XP055065674, ISSN: 0027-8424, DOI: 10.1073/pnas.0507237103 abstract -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2014/053187

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012252689 A1	04-10-2012	EP 2694963 A1	12-02-2014
		US 2012252689 A1	04-10-2012
		WO 2012135845 A1	04-10-2012
