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(54) Title: A NEW ELEMENT OF THE WG/WNT SIGNALING PATHWAY

(57) Abstract: The present invention relates to a new element of the Wg/Wnt signaling pathway. In particular, the invention relates to the fact that Parafibromin is a β -catenin interacting protein. The invention relates to methods for screening for a substance that inhibits or enhances the binding between Parafibromin and β -catenin.



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BACKGROUND OF THE INVENTION

Wnt genes of vertebrates and invertebrates encode a large family of secreted, cystein rich proteins that play key roles as intercellular signaling molecules in a wide variety of biological processes (for an extensive review see Wodarz and Nusse 1998). The first Wnt gene, mouse *wnt-1*, was discovered as a proto-oncogene activated by integration of mouse mammary tumor virus in mammary tumors (Nusse and Varmus 1982). Consequently, the involvement of the Wnt/Wg pathway in cancer has been an area of intensive study. With the identification of the *Drosophila* polarity gene *wingless* as a *wnt-1* homologue (Cabrera, Alonso et al. 1987; Perrimon and Mahowald 1987; Rijsewijk, Schuermann et al. 1987), it became clear that Wnt genes are important developmental regulators. Thus, although at first glance dissimilar, biological processes, like embryogenesis and carcinogenesis, both rely on cell communication via identical signaling pathways.

In a current model of the pathway, the secreted Wnt protein binds to Frizzled (Fz) cell surface receptors and thereby activates the cytoplasmic protein Dishevelled (Dsh). Dsh then transmits the signal to a complex of several proteins, including the protein kinase GSK3- β /Shaggy (Sgg), the scaffold proteins Axin and Adenomatous Polyposis Coli (APC), as well as β -catenin, the vertebrate homologue of Armadillo (Arm). In this complex, β -catenin is normally targeted for degradation after being phosphorylated by GSK3- β . In response to Wnt signaling and the resulting down-regulation of GSK3- β activity, β -catenin escapes from degradation and accumulates in the cytoplasm. Free cytoplasmic β -catenin can translocate to the nucleus, where it modulates gene transcription by binding the TCF/LEF family of transcription factors (Grosschedl 1999).

This setup, in which the key transducer is continuously held in check, is highly susceptible to mutations in its inhibitory components. The loss of any of the three elements of the β -catenin destruction complex, or mutations in β -catenin which render it insusceptible to degradation, lead to an increase in β -catenin levels and hence to the constitutive activation of the pathway.

This can lead to cell fate changes, uncontrolled proliferation and tumorigenic behaviour, as occurring with loss of APC or Axin function (Barker 1999; Morin 1999; Potter 1999; Roose and Clevers 1999; Waltzer and Bienz 1999). There are several possibilities to counter the negative effects of uncontrolled Wnt signaling for therapeutic intervention, however, most potent would be interference with the transcriptional activator function of β -catenin.

Currently, there are no known therapeutic agents effectively inhibiting β -catenin transcriptional activation. Consequently, there is a dire need to improve our understanding of the Wnt pathway in order to better develop drugs against diseases where Wnt signaling is implicated.

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In a study of Wnt/Wg signaling, the inventors identified the novel *Drosophila* gene *hyrax* (*hyx*) as an auxilliary nuclear Wg component. Overexpression and loss of function *in vitro* and *in vivo* influenced various Wnt/Wg signaling readouts downstream of the cytoplasmic β -catenin/Arm degradation complex. *hyx* encodes a highly conserved nuclear protein which is related to yeast Cdc73p, a factor of the Paf1 complex that is involved in RNA Polymerase II (RNAPII)-associated histone modifications during transcription. Additionally, the product of the human tumor suppressor *HRPT2*, Parafibromin, is the direct functional ortholog of *Drosophila* Hyx. The N-terminus of Parafibromin/Hyx interacts directly with the C-terminal Armadillo (Arm) repeats and the proximal C-terminus of β -catenin/Arm *in vitro* and *in vivo*. Together, this data suggests the involvement of Parafibromin/Hyx in Wnt/Wg signaling via a nuclear interaction with β -catenin/Arm to transmit the Wnt/Wg signal.

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SUMMARY OF THE INVENTION

The inventors have presented evidence that human Parafibromin and *Drosophila* Hyx are a novel nuclear element of the Wnt/Wg signaling cascade. In particular, they have demonstrated that Parafibromin is a novel β -catenin interacting protein, an interaction that is conserved between their respective orthologs Hyx and Arm in *Drosophila*. In addition, they have also identified minimal regions within β -catenin and Parafibromin that are required for their binding.

The invention relates to a method for screening for a substance that inhibits or enhances the binding between Parafibromin and β -catenin comprising the steps of:

a) bringing a candidate substance into contact with a (poly)peptide with the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14 under conditions that permit binding of said substance to said (poly)peptide; or

b) bringing a candidate substance into contact with a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 12 extending from amino acid residue 637 to 781 or from 637 to 722, or with the homologue (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 14 under conditions that permit binding of said substance to said (poly)peptide fragment; or

c) bringing a candidate substance into contact with a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending from amino acid residue 1 to 343, from 200 to 343, from 200 to 263, from 200 to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or with the homologue (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. Nos 8, 9, 10 or 11 under conditions that permit binding of said substance to said (poly)peptide fragment; or

d) bringing a candidate substance into contact with a derivate of the (poly)peptide with the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or of the (poly)peptide fragment according to b) or c) under conditions that permit binding of said substance to said derivate; or

e) bringing a candidate substance into contact with a (poly)peptide which is at least 50 % homologous to the (poly)peptide with the amino acid sequence of

SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or with a (poly)peptide fragment which is at least 50 % homologous to the (poly)peptide fragment as stated under b) or c) under conditions that permit binding of said substance to said (poly)peptide or (poly)peptide fragment; and

- 5 f) detecting if the candidate substance is having inhibitory activity or enhancing activity on the binding between Parafibromin and β -catenin or their respective orthologs.

10 This means that if the candidate substance binds to a (poly)peptide with the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to a (poly)peptide fragment as stated under b) or c), or to a derivate, (poly)peptide or (poly)peptide fragment as stated under d) or e), respectively, it is detected if the candidate substance is having inhibitory activity or enhancing activity on the binding between Parafibromin and β -catenin or their respective orthologs.

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A derivate as stated under d) is a (poly)peptide with the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or a (poly)peptide fragment according to b) or c) with an arbitrary molecule attached to the N- or C-terminal part or to a side chain of an amino acid, and a candidate substance showing an affinity to said derivate which is at the most 50% increased or decreased compared to the affinity between the candidate substance and the corresponding unmodified (poly)peptide or (poly)peptide fragment, respectively.

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"Percent (%) homologous" with respect to the mentioned (poly)peptide sequences is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the sequences with SEQ ID. Nos 7, 8, 9, 10, 11, 12 und 14, after aligning the sequence and introducing gaps, if necessary, to achieve the maximum percentage sequence identity, and not considering any conservative amino acid substitution as part of the sequence identity. The % identity values used herein can be generated by WU-BLAST-2, which was obtained from (Tatusova TA 1999). WU-BLAST-2 uses several search parameters, most of which are set to the default values.

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In a similar manner, "percent (%) homologous" with respect to nucleic acid sequences with the SEQ ID. Nos 1, 2, 3, 4, 5, 6 and 13 is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the nucleic acid

sequences of Parafibromin, β -catenin or their respective orthologs. The identity values used herein can be generated using BLAST module of WU-BLAST-2 set to the default parameters.

In a preferred embodiment of the invention the (poly)peptide or the (poly)peptide fragment
5 stated under e) is at least 52 % homologous to the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c), preferably at least 55 % homologous, more preferably at least 60 % homologous, even more preferably at least 65 % homologous, yet even more preferably at least 70 % homologous.

10 In another preferred embodiment of the invention the (poly)peptide or the (poly)peptide fragment stated under e) is at least 75 % homologous to the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c), preferably at least 80 % homologous, more preferably at least 85 % homologous, even more preferably at least 86 % homologous, yet even more preferably at least 87 % homologous.

15 In another preferred embodiment of the invention the (poly)peptide or the (poly)peptide fragment stated under e) is at least 88 % homologous to the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c), preferably at least 89 % homologous, more preferably at least 90 % homologous, even more
20 preferably at least 91 % homologous, yet even more preferably at least 92 % homologous.

In another preferred embodiment of the invention the (poly)peptide or the (poly)peptide fragment stated under e) is at least 93 % homologous to the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c),
25 preferably at least 94 % homologous, more preferably at least 95 % homologous, even more preferably at least 96 % homologous, yet even more preferably at least 97 % homologous.

In further preferred embodiment of the invention the (poly)peptide or the (poly)peptide fragment stated under e) is at least 98 % homologous to the amino acid sequence of SEQ ID.
30 Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c), preferably at least 99 % homologous.

Further, the invention relates to an antibody which specifically binds, in part at least, to a (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 12

extending from amino acid residue 637 to 781 or from 637 to 722, or to the homologue (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 14.

Additionally, another aspect of the invention provides for an antibody which specifically
5 binds, in part at least or fully, to a (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending from amino acid residue 200 to 343, from 200 to 263, from 200 to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or to the homologue (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. Nos 8, 9, 10 or 11.

10 Further, the invention relates to a method for screening for a substance that inhibits or enhances the binding between Parafibromin and β -catenin comprising the steps of:

- 15 a) bringing a candidate substance into contact with a nucleic acid molecule with the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13 under conditions that permit binding of said substance to said nucleic acid molecule; or
- b) bringing a candidate substance into contact with a third molecule containing the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13 under
20 conditions that permit binding of said substance to said nucleic acid molecule; or
- c) bringing a candidate substance into contact with a fourth molecule being a derivate of the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13 under conditions that permit binding of said substance to said nucleic acid
25 molecule; or
- d) bringing a candidate substance into contact with a nucleic acid molecule which is at least 50 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13 under conditions that permit binding of said substance to said nucleic acid molecule; or
- 30 e) bringing a candidate substance into contact with a nucleic acid molecule which contains a fragment of the nucleic acid sequence of SEQ ID. No 1, 2, 3, 4, 5, 6 or 13 under conditions that permit binding of said substance to said nucleic acid molecule, wherein the fragment comprises at least 240 nucleotides; and

- f) detecting if the candidate substance is having inhibitory activity or enhancing activity on the binding between Parafibromin and β -catenin or their respective orthologs.

5 A derivate as stated under c) is a nucleic acid molecule with a nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13 with an arbitrary molecule attached to the said nucleic acid molecule, said derivate having the characteristic that a candidate substance showing an affinity to said derivate which is at the most 50% increased or decreased compared to the affinity between the candidate substance and the corresponding unmodified nucleic acid
10 molecule.

In one embodiment of the invention, the nucleic acid molecule stated under d) is at least 52 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13, preferably at least 55 % homologous, more preferably at least 60 % homologous, even more preferably at
15 least 65 % homologous, yet even more preferably at least 70 % homologous.

In another preferred embodiment, the nucleic acid molecule stated under d) is at least 75 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13, preferably at least 80 % homologous, more preferably at least 85 % homologous, even more preferably at
20 least 86 % homologous, yet even more preferably at least 87 % homologous.

In a further preferred embodiment, the nucleic acid molecule stated under d) is at least 88 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5 6 or 13, preferably at least 89 % homologous, more preferably at least 90 % homologous, even more preferably at
25 least 91 % homologous, yet even more preferably at least 92 % homologous.

In a further preferred embodiment, the nucleic acid molecule stated under d) is at least 93 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13, preferably at least 94 % homologous, more preferably at least 95 % homologous, even more preferably at
30 least 96 % homologous, yet even more preferably at least 97 % homologous.

In a further preferred embodiment, the nucleic acid molecule stated under d) is at least 98 % homologue to the nucleic acid sequence of SEQ ID. Nos 1, 2, 3, 4, 5, 6 or 13, preferably at least 99 % homologous.

In another aspect, the invention relates to a pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 12 extending from amino acid residue 637 to 781 or from 637 to 722 or the homologue (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 14, or a functionally equivalent derivate thereof.

In a further aspect, the invention relates to a pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending from amino acid residue 1 to 343, from 200 to 343, from 200 to 263, from 200 to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or the homologue (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. Nos 8, 9, 10 or 11, or a functionally equivalent derivate thereof.

A functionally equivalent derivate is a pharmaceutically acceptable salt of said (poly)peptide fragments or a said (poly)peptide fragment with an arbitrary molecule attached to the N- or C-terminal part or to a side chain of an amino acid, said derivate showing an affinity to Parafibromin or β -catenin, respectively, which is at the most 50% increased or decreased compared to the affinity between the unmodified (poly)peptide fragment and Parafibromin or β -catenin, respectively.

Additionally, the invention relates to a pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising one of the mentioned antibodies.

Preferred cancer types are Wnt-dependent cancer types, preferably colorectal cancer, lung cancer, nasopharyngeal carcinoma, preferably Wnt-2 dependent nasopharyngeal carcinoma, small intestinal adenocarcinoma, fundic gland polyps (gastric), gastric carcinoma, gastric (intestinal-like), gastric adenoma (without associated adenocarcinoma), gastrointestinal carcinoid tumor, esophageal adenocarcinoma, juvenile nasopharyngeal angiofibromas, melanoma, pilamatricomas, lung adenocarcinomas, ovarian carcinoma, uterine cervix, uterine endometrial, breast fibromatoses, prostate, thyroid carcinoma, hepatoblastoma, hepatocellular carcinoma, hepatocellular carcinoma associated with hepatitis C, medulloblastoma, desmoid

tumor, Wilm's tumor (kidney), pancreatic (non-ductal acinar cell carcinomas), pancreatoblastoma and synovial sarcoma.

5 Preferred types of bone or joint disorders are osteoarthritis or rheumatoid arthritis, respectively.

10 The invention relates further to an assay for studying diseases induced by a enhanced or reduced binding between Parafibromin and β -catenin or their orthologs or for drug screening comprising the use of a cell line or organism selected from the group comprising Drosophila, mice, rats, rabbits, chicken, frogs, pigs, fishes, worms or sheep, said organism showing enhanced or reduced binding between Parafibromin and β -catenin or their respective orthologs in at least one tissue or organ and said cell line showing enhanced or reduced binding between Parafibromin and β -catenin or their respective orthologs.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 13A: Hyx is an evolutionary conserved Cdc73 homology protein with homology and functional relationship to the human tumour suppressor protein Parafibromin. This figure depicts a dendrogram showing the evolutionary relationship of the Parafibromin/Hyx homologs.

Fig. 13B: Hyx is an evolutionary conserved Cdc73 homology protein with homology and functional relationship to the human tumour suppressor protein Parafibromin. This figure depicts an alignment of Parafibromin, Hyx, and their homologues from *D. rerio* (NP_956642), *C. elegans* (F35F11.1) and *S. cerevisiae* (Cdc73p).

Fig. 14: *hyx* RNAi decreases Wg signaling downstream of axin/dAPC2 in S2 cells. This figure depicts the effects of RNAi against *hyx* on Wg reporter activation in *Drosophila* S2 cells.

Fig. 15: Somatic *hyx* loss of function clones show cell-autonomous decrease in *Distal-less* (*Dll*) expression and other developmental defects, but not general transcription impairment. This figure depicts the effect of somatic loss of function of *hyx* on the expression of Wg target genes and other unrelated genes.

Fig. 16: Identification Of *hyx* as a novel essential gene which by overexpression counter-acts the dominant-negative effect of overexpressed *lgs*^{17E}. This figure depicts the reversion of the overexpression phenotype of a dominant negative inhibitor of Wg signaling, *lgs*^{17E}, by co-overexpression of *hyx*. This overexpression has no general effect on transgene expression. Additionally, hypomorphic *hyx* loss of function rescue leads to leg development impairment.

Fig. 17: Parafibromin is a nuclear protein that can substitute Hyx *in vivo* and influences the TOP-Flash Wnt signaling reporter in 293T cells. This figure depicts experiments demonstrating that Hyx and Parafibromin can functionally replace each other in flies and in mammalian tissue culture, as well as the effect of

Parafibromin overexpression and reduction on Wnt signaling in mammalian cells.

Fig. 18: Parafibromin/Hyx directly interacts independently of its Cdc73 motif in the region between Arm repeat 12 and the proximal C-terminus of beta-catenin/Arm. This figure depicts the results of interaction studies demonstrating that Parafibromin and β -catenin can interact and the experiments beginning to delineate the minimal interaction domains.

Fig. 19: The Parafibromin N-terminus, and the β -catenin interaction domain (CID) region in particular, are sufficient for β -catenin binding. Numbers indicate amino acid residues of Parafibromin used to generate GST fusion proteins. GST alone, as well as the unrelated GST-GFP and GST-dTAB2 fusion proteins served as negative controls and GST-hTCF¹⁻¹³⁰ as positive control.

DETAILED DESCRIPTION OF THE INVENTION

The Wnt signaling cascade is essential for the development of both vertebrates and invertebrates, and has been implicated in tumorigenesis. The *Drosophila* Wnt genes are one of the best characterized within the Wnt-protein family, which includes more than hundred genes across all species. In the *Drosophila* embryo, Wg is required for formation of parasegment boundaries and for maintenance of *engrailed* (*en*) expression in adjacent cells. The epidermis of embryos defective in Wg function shows only a rudimentary segmentation, which is reflected in an abnormal cuticle pattern. While the ventral cuticle of wild type larvae displays denticle belts alternating with naked regions, the cuticle of Wg mutant larvae is completely covered with denticles. During imaginal disc development, Wg controls dorso-ventral positional information. In the leg disc, Wg patterns the future leg by the induction of ventral fate (Struhl and Basler 1993). In animals with reduced Wg activity, the ventral half of the leg develops into a mirror image of the dorsal side (Baker 1988). Accordingly, reduced Wg activity leads to the transformation of wing to notal tissue, hence the name of the gene (Sharma and Chopra 1976). In the eye disc, Wg suppresses ommatidial differentiation in favour of head cuticle development, and is involved in establishing the dorso-ventral axis across the eye field (Heberlein, Borod et al. 1998).

Additional genes have been implicated in the secretion, reception or interpretation of the Wg signal. For instance, genetic studies in *Drosophila* revealed the involvement of *frizzled* (*Dfz*), *dishevelled* (*dsh*), *shaggy/zeste-white-3* (*sgg/zw-3*), *armadillo* (*arm*), *adenomatous polyposis coli* (*E-apc*), *axin*, and *pangolin* (*pan*) in Wg signaling. The genetic order of these transducers has been established in which Wg acts through Dsh to inhibit Sgg, thus relieving the repression of Arm by Sgg, resulting in the cytoplasmic accumulation of Arm and its translocation to the nucleus. In the nucleus Arm interacts with Pan to activate transcription of target genes. Vertebrate homologues have been identified for all these components (for an updated review see Peifer and Polakis 2000), suggesting that novel identified members of the *Drosophila* signaling pathway most likely have vertebrate counterparts.

Mutations leading to nuclear accumulation of the mammalian homologue of Arm, β -catenin, and consequently to constitutive activation of the Wg/Wnt pathway have been observed in many types of cancer, including colon cancer, breast cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastomas, pilomatricomas, and

prostate cancer (Morin 1999; Polakis, Hart et al. 1999). It is now apparent that deregulation of β -catenin signaling is an important event in the genesis of these malignancies. However, there are still no known therapeutic agents effectively inhibiting β -catenin transcriptional activation. This is partly due to the fact that many of the essential components required for its full activation and nuclear translocation are still unknown.

With different tools the inventors showed that the *Drosophila* gene *CG11990* (*hyx*) and its human homolog *HRPT2*, encoding Parafibromin, are involved in Wg/Wnt signaling. More specifically, it was shown that Hyx and Parafibromin are novel components of the Wg/Wnt pathway and interact with a central Wg/Wnt pathway component, Armadillo/ β -catenin. Further, minimal regions were identified required for this interaction, which provide the basis to screen for and develop novel therapeutics which modulate this interaction.

RNA interference (RNAi) in *Drosophila* Schneider 2 (S2) cells, combined with a Dual-Luciferase reporter readout, is an established system to analyse the impact of gene knock-down on signal transduction pathways (Boutros et al., 2004; Caplen et al., 2000; Clemens et al., 2000; Kiger et al., 2003; Lum et al., 2003). The inventors developed an S2-cell based, Wg-sensitive Luciferase reporter system based on the LEF-luc reporter system described previously (Schweizer and Varmus, 2003) to monitor Wg pathway activation. Targeting known positive Wg pathway components *dsh*, *arm*, *lgs* and *pygo* by RNAi strongly decreased the LEF-luc readout (Fig. 14 A and data not shown). Reciprocally, RNAi against the negative regulators *daxin* and *APC2*, but not the absent *APC*, increased the pathway readout, even without Wg-mediated induction (Fig. 14 A). RNAi against Hyx in this system, using dsRNA against different regions of the Hyx mRNA, severely decreased the LEF-luc readout, without significant impairment of the constitutive Renilla Luciferase levels (Fig. 14 A and data not shown). Moreover, independent Dual Luciferase reporter systems for heavy metal stress induction and *brinker* (*brk*) enhancer control (Müller et al., 2003) were not significantly affected by *hyx* RNAi (Fig. 14 B). Thus, the *hyx* RNAi results indicate that Wg reporter gene activity in S2 cells is crucially dependent on sufficient *hyx* transcript, providing evidence that *hyx* can be a positive component in the Wg/Wnt signaling cascade.

To epistatically map *hyx* in the Wg/Wnt pathway, combinatorial RNAi (Kuznicki et al., 2000; Lum et al., 2003; Schmid et al., 2002) was employed in the S2-cell reporter systems mentioned above, by targeting two genes simultaneously. When the positive downstream

component *lgs* was targeted together with *APC2* or *daxin* in the S2-cell Wg reporter system, a reduction of the Luciferase readout levels was observed comparable to *lgs* RNAi alone, but not when using GFP dsRNA instead (Fig. 14 C). Co-administration of *hyx* RNAi decreased the elevated Luciferase levels caused by *APC2* or *daxin* dsRNA as efficiently as *lgs* RNAi (Fig. 14 C). This suggests that *hyx* is, like *lgs*, epistatic to the cytoplasmic Arm degradation complex, indicating that Hyx acts as a positive, presumably nuclear Wg signaling component. The results of *in vivo* loss of function and overexpression experiments (presented below) in *Drosophila* are consistent with this model.

To gain more insight into *hyx* function, the inventors generated mutant alleles using a standard EMS mutagenesis screen approach, using reversion of the *salE>lgs^{17E}* phenotype by *EP-hyx* (*P9*) as the basis for an EMS mutagenesis screen (Fig. 16 A-F, see below). Two potential alleles were identified which upon sequencing of the *hyx* coding region revealed premature stop codons (R505X in *hyx¹*, and Q461X in *hyx²*, Fig. 16 G). Subsequently the inventors identified the EP-insertion *EY6898* (Bellen et al., 2004), which maps to the *hyx* 5' UTR as a third, recessive lethal allele which was designated *hyx^{EY}* (Fig. 16 G).

One copy of *act5c-Gal4* rescued the homozygous lethality of *hyx^{EY}* to pharate adult stage with high efficiency (data not shown). However, these pharates are rarely eclosed from their pupal cases. Examination of fully eclosed animals and pharate pupae revealed severe leg defects, mostly in the second and third leg pairs. Although all segments were present, segments showed shortening, twists and potential polarization (Fig. 16 K and L). This phenotype is reminiscent of hypomorphic phenotypes of the established Wg components *pan* and *pygo* (Brunner et al., 1997; Parker et al., 2002; Thompson et al., 2002). This data suggests an important function for *hyx* during development.

To further analyze the effect of homozygous *hyx* loss of function on Wg signaling *in vivo*, the FRT/FLP system (Xu and Rubin, 1993) was used to generate somatic clones with the *hyx* alleles in *Drosophila* imaginal discs to monitor target gene expression by immunofluorescence. By monitoring clones mutant for *hyx*, it was found that the expression levels of the long-range Wg target *Distal-less* (*Dll*) (Neumann and Cohen, 1997; Zecca et al., 1996), were severely reduced or lost in a cell-autonomous fashion (Fig. 15 A-C). This downregulation of *Dll* was caused by an apparently direct effect on Wg signaling rather than impairing general transcription and viability, since expression of the Hedgehog (Hh) target

genes *decapentaplegic* (*dpp*) and *patched* (*ptc*), the Dpp target *optomotor-blind* (*omb*), and the selector gene *engrailed* (*en*) was not impaired in *hyx* mutant clones, as monitored by corresponding *lacZ* reporter expression (Fig. 15 D-I and data not shown). Taken together, these results show specific involvement of *hyx* in controlling the Wg target gene *Dll*.

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To further analyze *hyx* loss of function effects, the inventors wanted to monitor *hyx* mutant clones in adult structures. However, since such clones disappear from imaginal discs before reaching the adult stage, the inventors utilized the FRT/FLP system together with the Minute (Min) technique to generate mutant clones observable in adult structures. In this setup, adults were recovered showing a wide variety of phenotypes. Common abnormalities the inventors observed phenotypes associated with impaired Wg signaling: leg defects of the same type as found in the *act5c-Gal4*, *hyx^{EY}* rescue, as well as notches around the wing blade margin (Fig. 15 J and K). Additionally, large *hyx* mutant clones in the notum region showed loss or severe impairment of thoracic dorsocentral macrochaetae with high penetrance (data not shown), a phenotype described for Wg signaling impairment (Gerlitz and Basler, 2002; Phillips and Whittle, 1993). However, presumably Wg-independent wing blade patterning defects in *Hyx* mutant clones were also noted, namely the formation of ectopic vein material (Fig. 15 J). Also noted were severe impairment of eye development and outgrowths when clones were induced selectively in the head region by *eyeless* (*ey*)-*flp* (data not shown), yet only minor defects in antenna development when compared to mutant clones of the Wg component *pygo* (data not shown). Thus, it was concluded from the *in vivo* loss of function analysis that a) *hyx* influences certain, but not all, Wg signaling readouts, and that b) its loss clearly also impacts other, Wg-independent developmental processes. Therefore, *hyx* does not fall into the category of core nuclear Wg signaling components such as *arm*, *lgs* or *pygo*, but rather represents an auxiliary, more pleiotropic component, similar to CBP/p300, BRG-1/brm, TIP48/49 or tsh (Barker et al., 2001; Bauer et al., 2000; Collins and Treisman, 2000; Gallet et al., 1999; Gallet et al., 1998; Hecht et al., 2000; Takemaru and Moon, 2000; Waltzer et al., 2001).

30 *lgs^{17E}* is a mutant allele of the Wg component *lgs* which encodes a protein product with severely impaired Armadillo interaction capability, but normal binding potential to the nuclear factor Pygo (Kramps et al., 2002). Using the Gal4/UAS-system (Brand and Perrimon, 1993), the inventors overexpressed a *UAS-lgs^{17E}* transgene using *spalt major-enhancer Gal4* (*sale-Gal4*), a strong Gal4 driver derived from regulatory elements of *sal* (Barrio, 2004)

with activity in the area between the presumptive wing veins L2 and L5 during larval development (D. Nellen and K. B., data not shown). *salE>lgs^{17E}* lead to margin notches in the adult wing, typical for late loss of Wg signal transduction (Fig. 16 A-F). This effect is probably caused by a dominant-negative action of *lgs^{17E}* when overexpressed, presumably by competition with endogenous *lgs* and thus interfering with Armadillo-mediated target gene activation.

Afterwards, various double-headed EP-element insertions recovered in a screen for genes involved in wing patterning and growth (D. Nellen and K. B., data not shown) were tested to analyze their effect on the *salE>lgs^{17E}* phenotype. EP-insertions (P9 and P10) which map to the *hyx* locus as well as direct UAS constructs driving expression of *hyx* showed a complete phenotype reversion (Fig. 16 D and E). This reversion was specific, since overexpression of *hyx* did not lead to reversions of unrelated phenotypes in other pathway-specific screening systems and did also not impair general transgene expression (Fig. 16 H-J and data not shown).

In summary, the evidence suggests that *hyx* encodes a normally non-limiting product which is specifically able, when overexpressed, to overcome the dominant-negative effect of excess *lgs^{17E}* on Wg signaling.

Analysis by motif and BLAST searches of the primary amino acid sequence of the *hyx* protein product revealed that it is part of a conserved group of proteins represented in every annotated metazoan and plant genome, as well as genomes of lower eukaryotes (Fig. 13A). Usually, only a single putative Hyx homolog is present in each species except in *Drosophila*, which has a second Cdc73 homology-encoding gene, *CG6220* (Fig. 13A). It is unlikely that *CG6220* interferes with redundancy as it did not have a detectable effect on the systems described below (data not shown). Moreover, its expression pattern is probably biased to the testis and thus unlikely to be of relevance in somatic tissue (Parisi et al., 2004).

With 59% protein sequence identity, the human Hyx homolog is Parafibromin, encoded by the *HRPT2 (c1orf28)* tumor suppressor gene involved in the Hyperparathyroidism-Jaw Tumor Syndrome (HPT-JT) (Carpten et al., 2002; Howell et al., 2003; Shattuck et al., 2003) (Fig. 13B). HPT-JT features predominantly parathyroid tumors, ossifying fibromas of the jaw, and

renal cysts, caused by loss of heterozygosity of *HRPT2* in both familial and sporadic cases (Chen et al., 2003).

Although the exact molecular nature of Parafibromin function is unknown at present, a clue as to the potential function of Hyx/Parafibromin-related proteins was initially provided by the *Saccharomyces cerevisiae* Hyx homolog, Cdc73p (Fig. 13B), which is associated with the nuclear Polymerase-Associated Factor 1 (Paf1) complex (Chang et al., 1999; Krogan et al., 2003; Krogan et al., 2002; Mueller and Jaehning, 2002; Porter et al., 2002; Shi et al., 1997; Squazzo et al., 2002). The Paf1 complex binds to initiating and elongating RNA polymerase II (RNAPII) directly via Cdc73p and coordinates histone modifications associated with transcriptional initiation and elongation (Krogan et al., 2003; Mueller et al., 2004; Rondon et al., 2004; Wood et al., 2003). There are potential homologs for most Paf1 complex components in all species, suggesting an evolutionary conservation of this complex. Recently, a mammalian Paf1 complex was isolated and shown to contain Parafibromin, further suggesting that a role in transcriptional initiation and elongation is probably conserved (Rozenblatt-Rosen et al., 2005). Parafibromin and Hyx contain a metazoan-specific, long N-terminal region which is absent in Cdc73p. However, the C-terminal portion of all these proteins is highly homologous and presumably contains an evolutionarily conserved function.

Given the strong evidence implicating *hyx* as component of Wg signalling, the inventors examined if this role was evolutionarily conserved in mammals. More specifically, it was examined if the human homolog *HRPT2*, encoding Parafibromin, also positively influences Wnt signaling in human cells. Therefore, the inventors used the Wnt-responsive TOP-Flash Luciferase assay (van de Wetering et al., 1997) in HEK 293T cells and modified *HRPT2* levels by overexpression and RNAi. In the TOP/FOP-Flash system, the TOP-Flash construct harbouring tandem TCF consensus binding sites in front of a Firefly Luciferase was transfected together with a constitutively transcribed Renilla Luciferase (CMV-RL) to monitor β -catenin-mediated Wnt pathway activity. To activate the Wnt pathway, the inventors treated the transfected cells 24h before Luciferase measurement with 25mM Lithium Chloride (LiCl), which potently inhibits GSK-3 β and thus blocks β -catenin degradation, consequently inducing Wnt target genes (Jho et al., 2002).

When a CMV promoter-driven *HA-HRPT2* or *HA-hyx* construct together with the TOP-Flash reporter was transfected, a two- to threefold increase in TOP-Flash activity was observed

when compared to transfection of an EGFP control after LiCl stimulation (Fig. 17 K). Parafibromin levels in this system were quickly saturated, since introducing half the amount of *HA-HRPT2* construct resulted in a similar degree of activation, while four times less increased the induction by 50% (Fig. 17 K and data not shown). *HA-hyx* overexpression had a similar effect on the reporter readout (Fig. 17 K). This overexpression data suggests that Parafibromin positively synergizes with Wnt signaling components downstream of GSK-3 β in activation of the TOP-Flash Luciferase.

To decrease *HRPT2* levels, two independent small interfering RNAs (siRNAs) against *HRPT2* were used and their RNAi effect after transfection on *HRPT2* transcript levels in HEK 293T cells were monitored. Both siRNAs knocked the *HRPT2* mRNA levels down to approximately 20% within 48h after transfection, as determined by RT-PCR, without any obvious deleterious effects on cell viability and control gene expression (P. Brugger, P. Zipperlen, C. M. and K. B., data not shown). Next, the effect of siRNA-mediated *HRPT2* knock-down on Wnt pathway activity was analysed. When monitored by the TOP-FLASH assay, the *HRPT2* siRNAs significantly reduced the TOP-Flash readout 48h after transfection (data not shown). The decrease was more pronounced when the cells were harvested 72h after transfection (Fig. 17 L). A similar decrease was observed when a siRNA against the known positive Wnt component *hPYGO-2* was used, but not when using a *GFP* control siRNA (Fig. 17 L). This suggests that Parafibromin is a rather stable protein or present at high levels, and that its amount is crucial for Wnt signaling transmission in HEK 293T cells downstream of GSK-3 β .

It was reasoned that if the *HRPT2* siRNA effect on the TOP-Flash readout was specific, it could be restored by supplying the *Drosophila* ortholog *hyx* into the system, analogous to the *in vivo* experiments (see below). The *hyx* mRNA sequence does not align to any of the siRNAs which were used to target *HRPT2* and thus should not be targeted in these assays. When co-transfected with a *HRPT2* siRNA, *HA-hyx* restored the relative Luciferase levels in the TOP-Flash assay to a level comparable to the expression *HA-hyx* alone (Fig. 17 L).

The converse question of whether *HRPT2* could functionally replace *hyx* in *Drosophila*, and if yes, what is the functional sub-cellular localization of Parafibromin, was also investigated. In order to address these questions, the inventors assumed with respect to their previous findings that 1) overexpression of *HRPT2* should reverse the dominant-negative effect of *salE>lgs^{17E}*,

analogous to *hyx* overexpression; 2) more directly, ubiquitously expressed *HRPT2* should be able to rescue *hyx* loss of function; finally, 3) functional Parafibromin should localize to the nucleus, at least in Wg signal-receiving cells.

5 To address the first point, transgenic lines were generated carrying a *UAS-HRPT2* construct containing the full Parafibromin-encoding open reading frame. Several independent insertion lines reversed the effects of *salE-Gal4*-mediated overexpression of *UAS-Igs^{17E}* (Fig 17 A-C and data not shown). Similar to overexpression of *hyx*, overexpression of *HRPT2* with various Gal4 drivers did not generate any obvious phenotypes, besides a slightly distorted ommatidial
10 pattern with *GMR-Gal4* (data not shown). Therefore, these results fully recapitulated the *hyx* overexpression findings.

In order to address points 2 and 3, transgenic lines were established harbouring a construct encoding N-terminally HA-tagged Parafibromin controlled by the weak and ubiquitous
15 *tubulin-1a* promoter (*tub1a-HA-HRPT2*). Using three different, independent transgenic insertions, the inventors were able to fully rescue all mutant *hyx* allele combinations as well as *hyx^{EY}* homozygosity to adulthood without obvious phenotypic defects (Fig. 17 D). Since this showed that Parafibromin can indeed functionally substitute Hyx, larval tissues for the HA epitope of the transgene was monitored by immunofluorescence to analyze its sub-cellular
20 localization. As expected, HA-Parafibromin was found to be exclusively nuclear in all cells of all tissues examined, independent of cellular Wg pathway activity (Fig. 17 E-J). These results strongly suggest that nuclear Parafibromin can fully replace Hyx in *Drosophila*, and that Hyx is presumably also a nuclear protein. Supporting this, the inventors observed HA- and EGFP-tagged Hyx, like Parafibromin fusion proteins, to localize to the nuclei of HEK 293T
25 embryonic kidney cells (data not show), in agreement with the localization prediction based on Cdc73p homology, protein sequence analysis, RNAi epistasis and data from other reports (Cronshaw et al., 2002; Rozenblatt-Rosen et al., 2005; Tan et al., 2004; Woodard et al., 2005). These findings further support the idea that Parafibromin/Hyx is a nuclear component involved in Wnt/Wg target gene activation.

30 In summary, these results indicate that Hyx can substitute for Parafibromin, and further suggests that these proteins are true orthologs. Moreover, the results indicate a conserved role of metazoan Cdc73 homology proteins in nuclear Wnt/Wg signaling.

The Cdc73p similarity and the results so far confirm that Parafibromin/Hyx is a pleiotropic factor which assists in Wnt/Wg target gene activation, potentially by acting in a nuclear, yeast Paf1 complex-like entity. It was therefore reasoned that Parafibromin/Hyx could represent a linker between a Paf1 complex and known nuclear Wnt/Wg signaling components. To test this assumption, the inventors used the yeast-2-hybrid technique to look for interactions between a *HRPT2* bait and prey constructs containing different known nuclear Wnt signalling effectors and the unrelated Sp1/egr-like zinc finger transcription factor Hucklebein (Hkb). Intriguingly, a specific interaction between both Parafibromin and Hyx to β -catenin was found (Fig. 18 A). The inventors confirmed the interaction using GST-Parafibromin and GST-Hyx coupled sepharose beads, which were able to efficiently pull-down *in vitro* translated full-length β -catenin and N-terminally truncated Arm (Δ N-Arm), respectively, as well as β -catenin from lysates of LiCl-treated HEK 293T cells (Fig. 18 D and data not shown). In an analogous set of pull-downs, GST- Δ N- β -catenin and GST- Δ N-Arm showed interactions with *in vitro* translated Parafibromin and Hyx as well as HA-Parafibromin from transiently transfected HEK 293T cells (Fig. 18 E and data not shown).

To verify the interaction *in vivo*, co-immunoprecipitation experiments were performed. For this task, the inventors transiently expressed HA-Parafibromin and FLAG-tagged β -catenin which contained the stabilizing S33Y mutation (β -catenin^{S33Y}-FLAG) in HEK 293T cells, and immunoprecipitated lysates with α -FLAG or a control antibody. α -FLAG specifically precipitated HA-Parafibromin when β -catenin^{S33Y}-FLAG was present in the lysates (Fig. 18 B and data not shown). More directly, the inventors succeeded in co-precipitating endogenous β -catenin together with Parafibromin from lysates of LiCl-treated 293T cells by using the α -Parafibromin antibody BL-649 (Fig. 18 C). Additionally, α -HA pulldowns from LiCl-treated, with HA-Hyx transiently transfected S2 cells specifically co-precipitated endogenous Arm (data not shown). This data strongly suggests that Parafibromin/Hyx and β -catenin/Arm co-exist in a complex *in vivo*.

β -catenin, and by homology Armadillo, is a modular protein featuring an N-terminal domain (NTD), followed by 12 Armadillo repeats and a C-terminal domain (CTD) (Fig. 18 D) (Cox et al., 1999; Daniels and Weis, 2002; Hecht et al., 1999; Huber et al., 1997; Orsulic and Peifer, 1996). While the central repeats 4-8 are necessary for coupling β -catenin to TCF/LEF factors in the nucleus, the surrounding structure has been shown to interact with various transcriptional modulators. To determine the β -catenin region necessary for Parafibromin

recruitment, the inventors performed a series of GST pull-downs using *in vitro* translated β -catenin fragments and analyzed their binding to GST-Parafibromin. From these assays, and taking into account the possible instability of terminal Arm repeats in peptides, the inventors conclude that the β -catenin region of repeat 12 to the proximal C-terminus (amino acids 637-781) harbours a minimal Parafibromin binding motif with full binding capacity (Fig. 18 D). This conclusion is further supported by the observation that separating the CTD from the Arm repeats of β -catenin abolishes binding of either fragment to Parafibromin (Fig. 18 D). The inventors also found that Hyx interacts with the corresponding part of Armadillo in a similar set of experiments (data not shown), indicating that the interaction region is conserved. Intriguingly, the Parafibromin/Hyx binding area at the repeat 12 to CTD joint is embedded in the β -catenin interaction regions for the chromatin remodelling factors CBP/p300 (repeat 10-C) (Hecht et al., 2000; Takemaru and Moon, 2000) and Brg-1 (repeats 7-12) (Barker et al., 2001).

The inventors next determined the β -catenin/Arm binding domain of Parafibromin/Hyx. Results to date indicate that the region of Parafibromin spanning amino acids 1-343 is required for the interaction (Fig 18 E and data not shown). Importantly this region lies outside the Cdc73 domain and within the extended N-terminal portion of Hyx/Parafibromin that is conserved in metazoans (Fig. 13A). The inventors postulate that the N-terminal region of Hyx/Parafibromin may contain motifs responsible for the binding to β -catenin and potentially other pathway-specific transcription units.

Subsequently, the inventors analyzed if hTCF-4 and Parafibromin can simultaneously interact with β -catenin. For these experiments, a GST-coupled hTCF-4 fragment was used comprising amino acids 1-130 (GST-hTCF-4¹⁻¹³⁰), which includes the N-terminal β -catenin binding motif. Although GST-hTCF-4¹⁻¹³⁰ strongly interacts with β -catenin, it does not bind *in vitro* translated Parafibromin, even if provided in great excess (data not shown). In preliminary experiments, the inventors attempted to precipitate Parafibromin indirectly via β -catenin from lysates of HEK 293T cells transfected with HA-Parafibromin and β -catenin^{S33Y}-FLAG, using GST-hTCF-4¹⁻¹³⁰ or GST alone. They found that HA-Parafibromin is significantly enriched by GST-hTCF-4¹⁻¹³⁰ in the presence of β -catenin^{S33Y}-FLAG compared to the control (data not shown). This suggests that TCF/LEF factors and Parafibromin can interact with β -catenin simultaneously.

In summary, the inventors have presented several complementary lines of evidence that Parafibromin/Hyx is a novel nuclear component of the Wnt/Wg signaling cascade both in mammals and in *Drosophila*. In particular, they have demonstrated that Parafibromin is a novel β -catenin interacting protein, an interaction that is conserved in *Drosophila*. In addition, they have also identified minimal regions within β -catenin and Hyx/Parafibromin that are required for their interaction. The delineation of this minimal interaction region provides an avenue to modulate Wnt/Wg signaling for therapeutic purposes.

In addition, the observation of a minimal interaction domain within the extended N-terminal region of metazoan Hyx/Parafibromin homologs lends support to the hypothesis that additional transcriptional units may bind to the other conserved motifs within the N-terminal region. It is important to note that the evidence presented here and the data available to date do not imply that Wnt/Wg signaling is involved directly in the etiology of Hyperparathyroidism, where Parafibromin has a tumor suppressor role. The novel implication from the results is that Hyx/Parafibromin can act as a direct link to pathway-specific transcriptional units and thereby serves as a therapeutic target to control those pathways.

EXPERIMENTAL PART

Example 15 EP-lines, *hyx* mutants and flystocks:

The *UAS-lgs^{17E}* construct was generated using the site-directed mutagenesis kit (Stratagene). The EP-insertions *EP-hyx* (*P9*) and *P10* have been mapped by plasmid rescue and PCR and balanced with *TM6b*. *UAS-hyx* was created by cloning the full-length EST *LD47989* into *pUAS-T*. The recessive lethal alleles *hyx¹*, *hyx²* and *hyx^{EY}* (*EY6898*, Drosophila Gene Disruption Project) were analyzed by PCR and balanced over *TM6b*. *EP-hyx*, *P10* and all *hyx* alleles reported in this study fully complement *neur^{A101}*, *neur^{j6C12}* and *neur¹* (Bloomington Stock Center). Transgenics carrying *UAS-HRPT2* and *tub1α-HA-HRPT2* were created by standard transformation. *UAS-CG6220* was created using the full-length EST *AT09112*; *EY3881* was obtained from the *Drosophila* Gene Disruption Project. The following additional flystocks were used: *salE-Gal4/TM6b* (also combined with *UAS-lgs^{17E}* to generate *yw*; *UAS-lgs^{17E}*; *salE-Gal4*) and *salE-lacZ* (D. Nellen and K. B.); *act5c-FRT-CD2-FRT-Gal4*, with and without recombined *UAS-GFP^{nl}*; *C765-Gal4* (also combined with *UAS-lgs^{17E}* to generate *yw*; *UAS-lgs^{17E}*; *C765-Gal4*); *GMR-Gal4*; *act5c-Gal4*; *dpp-lacZ*; *ptc-lacZ*; *salm-lacZ*; *yw*, *hs-flp*; *FRT82*, *ubi-GFP/TM6b*; *yw*, *hs-flp*, *f*; *FRT82*, *Min*, *f⁺/TM2*; *TM6b*, *ubi-GFP*; *UAS-GFP^{nl}* (Bloomington Stock Center) and *UAS-lacZ*. All flystocks and crosses were kept at 25°C if not otherwise indicated.

Example 2

25 EMS mutagenesis screen:

EP-element *P9* reverses the wing phenotype of *salE>lgs^{17E}* by *hyx* overexpression (see text). To generate *hyx* mutant alleles, adult *P9* males of an age between 1-2 days were collected and starved for 8h in empty bottles. They were subsequently transferred into bottles containing

filter papers moistened with a 1% saccharose-water solution containing 0.4ml/100ml Ethyl methanesulfonate (EMS purum, Fluka). After 24h, the males were put into bottles containing regular food to recover for 24h, before they were mated in bulk to *salE> lgs^{17E}* virginal females. F1 males carrying the mutagenized P9* chromosome marked with *y⁺* were then
5 selected for re-occurrence of the *salE>lgs^{17E}* wing phenotype. Positive individuals were crossed to a *salE-Gal4* stock to re-screen, and positive lines out of this selection were selected for the *y⁺* marker and balanced.

After genetic selection for candidates, 25-50 males with the 3rd chromosome genotype
10 *P9*/salE-Gal4* were collected and frozen for 20min. DNA preparations were then performed following standard protocols. A 2119bp fragment, containing the start and stop codon of the CG11990 ORF was amplified by PCR (Expand High Fidelity PCR System, Roche) using primers with SEQ ID. Nos 15 and 20 out of the genomic DNA preparation, purified using the QIAquick® PCR purification kit (Qiagen) after quality control on a 1-1.5% agarose gel and
15 sequenced using primers with SEQ ID. Nos 15 to 20.

Example 3

Clonal analysis and immunohistochemistry:

20 *yw, hs-flp; FRT82, hyx^{EY}/TM6b* males were crossed to *yw, hs-flp; FRT82, ubi-GFP* virgins that carried *lac-Z* insertions of interest on the second chromosome. Larvae of these crosses were heat-shocked 48-72h after egg laying at 37.5°C for 45 min. 3rd instar larval tissue was stained with mouse monoclonal anti-β-Gal (1:2000, Promega), mouse monoclonal anti-Dll (1:2000), mouse polyclonal anti-CD2 (1:2000), rabbit monoclonal anti-Haemagglutinin (HA)
25 (1:500, Promega) or mouse monoclonal anti-HA (HA11, 1:1000) (BAbCO). Goat secondary antibodies used were Alexa488 and Alexa594 (1:400) (Molecular Probes). Minute

background clones were created as above but by crossing the males to *yw, hs-flp; FRT82, Min, ubi-GFP/TM6b* virgins.

Vectors and Constructs:

5 *hyx* constructs were PCR generated using the EST *LD47989*. *HRPT2* constructs used the full-length EST *V-1314* (Genecopoeia) as basis for PCR. *CTNNB1* and *armadillo* fragments for IVT production were PCR amplified and cloned into *pcDNA3*. β -catenin protein regions were taken as defined by GeneBank entry PRO2286). GST fusion constructs were cloned using *pGEX-KG* as backbone (Guan and Dixon, 1991). *pTOPFLASH* has been described previously
10 (van de Wetering et al., 1997). The β -cateninS33Y-FLAG expression construct is as described previously (Kolligs et al., 1999). *CMV-RL* was obtained from Promega, and *CMV-EGFP-C1* was obtained from Clontech.

Example 4

15

Mammalian cell culture experiments:

HEK 293T cells were seeded at a density of $2-2.5 \times 10^5$ cells/ml in DMEM with 10% FCS and transfected 24h later with a total of 1.25 μ g plasmid DNA per ml of medium (0.5 μ g TOP/FOP-Flash, 0.25 μ g CMV-RL and 0.5 μ g cargo or empty vector, plus 1% of *CMV-EGFP-C1* (Clontech) as transfection control) using CaCl₂. In siRNA experiments, 3 μ g siRNA per ml of medium was additionally co-transfected. After 16h, the medium was changed and transfection efficiency verified by fluorescence microscopy for EGFP. If necessary, cells were treated by addition of 25mM LiCl (Sigma) 24h before harvesting. Cells were harvested 48h or 72h after transfection. Dual-Luciferase assays were performed as described below.
25 siRNAs with SEQ ID. Nos 21 and 22 were used for *HRPT2*, siRNA with SEQ ID. No 23 were used for *hPYGO2* and standard GFP-22 control (Quiagen).

Example 5

Protein interaction studies:

The yeast-2-hybrid system was used as described in Bartel and Fields, 1997. Radioactively
5 labelled proteins for GST pulldowns were generated with the T7 TNT kit (Promega), GST
pulldowns were done according to standard methods (Sambrook and Russell, 2001). For
immunoprecipitation (IP) experiments, petri dishes seeded with HEK 293T cells (2×10^6) were
transfected as described above. Cells were lysed in NP-40 lysis buffer (Rozenblatt-Rosen et
al., 2005), the lysates cleared by centrifugation and subsequently used for precipitation
10 experiments (275 μ l lysate, 30 μ l Protein-A Sepharose (Amersham Biosciences), 2 μ l
antibody or the equivalent of 2 μ g GST-fusion protein-coupled Sepharose plus Glutathione
Sepharose (Amersham Biosciences) instead of Protein-A Sepharose). After 2h incubation at
4°C, pulldowns were analyzed on NuPAGE gels (Invitrogen). Antibodies used for IP were
mouse α -FLAG (M2, Sigma), mouse α -Myc (9E10, *Drosophila* Hybridoma Bank), mouse
15 HA-11 (BAbCO), rabbit α -HA (ICL) and rabbit α -Parafibromin (BL649, Bethyl Labs).
Antibodies for western blotting also included rabbit α -Parafibromin (BL648, Bethyl Labs,
1:10.000) and mouse α - β -catenin (BD Biosciences, 1:2.000).

Example 6

20

Insect cell culture:

S2 cells were propagated in $1 \times$ Schneider's *Drosophila* media (Invitrogen) supplemented with
10% FBS, 50 units/ml penicillin, and 50 μ g/ml streptomycin in 75-cm² T-flasks (Sarstedt) at
room temperature. S2 cells stable lines were propagated in the same media supplemented with
25 200 μ g/ml hygromycin (Roche Molecular Biochemicals) and/or 100 μ g/ml blasticidin
(Invitrogen).

Example 7

Transient transfection and generation of stable lines:

Standard protocols for transfection and the generation of stable cell lines were utilised, as detailed in the Drosophila Expression System manual (Invitrogen). Briefly, S2 cells were routinely transfected with Cellfectin (Invitrogen Life Technologies) using the following amounts for one well of a 6 well plate: 4 µg DNA, 16 µl Cellfectin per 3x10⁶ cells in 1 ml of Drosophila expression system (DES) serum-free medium (Invitrogen). Three hours after addition of the DNA/Cellfectin mix 1 ml of 1× Schneider's media containing 20% FBS. For transient transfections the cells were harvested 48 hr post transfection. For the generation of the stable line the cells were allowed to grow for 24 hr post transfection before the selection medium was added. 0.2 µg of resistance plasmid were used (*pCoHygro* and or *pCoBlast*) per 4.0 µg total DNA. Polyclonal lines stably expressing the transfected constructs were allowed to grow out subsequently expanded.

For the generation of the Wg stable line (referred to as 197.10 2/3) the following plasmids were used: 0.1 µg *LEF-luciferase* reporter, 0.1 µg mouse *LEF-1*, 0.05 µg *Renilla luciferase* and 0.1 µg *pTub_Dfz2*.

For the Wg reporter system (transient) the following plasmids were used: 1.0 µg *LEF-luciferase* reporter, 1.0 µg mouse *LEF-1*, 0.2 µg *Renilla luciferase* and 0.5 µg *pTub_Dfz2*.

For the brinker reporter system the following plasmids were transfected (no stable lines used for this reporter): 1.0 µg *brinker-luciferase* reporter and 0.2 µg *Renilla luciferase*.

For the metallothionein reporter system the following plasmids were transfected (no stable lines used for this reporter): 1.0 µg *pMT-luciferase reporter* and 0.2 µg *Renilla luciferase*.

Renilla luciferase was included in each transfection as a control for the efficiency of transfection. The final amount of DNA was adjusted to 4.0 µg using *pBluescript* vector DNA. The published *Drosophila* expression constructs were cloned in the vectors as described for the *LEF-luc* reporter (Schweizer et al., 2003), the brinker reporter (Mueller et al., 2003). The *tubulin* driven constructs were generated by inserting the relevant coding sequences, with Kozak sequence, downstream of the *tubulin* promoter (*pTub_DFz2*). The metallothionein inducible constructs were generated by inserting the relevant coding sequences with Kozak consensus downstream of the metallothionein promoter (*pMT-Luc*) in the *pMT/V5-His B* vector (Invitrogen).

Example 8

dsRNA production:

The method was adapted from the procedure published by the Dixon laboratory (Worby et al., 2001). Individual DNA fragments approximately 700 bp in length, containing coding sequences for the proteins to be "knocked out" were amplified by using PCR. Each primer used in the PCR contained a 5' T7 RNA polymerase binding site (SEQ ID. No 24) followed by sequences specific for the targeted genes. The PCR products were purified by using the High Pure PCR Purification Kit (Roche Molecular Biochemicals). The purified PCR products were used as templates by using a MEGASCRIP T7 transcription kit (Ambion, Austin, TX) to produce dsRNA. The dsRNA products were ethanol-precipitated and resuspended in water. The dsRNAs were annealed by incubation at 65°C for 30 min followed by slow cooling to

room temperature. The dsRNA were analyzed by 1% agarose gel electrophoresis to ensure that the majority of the dsRNA existed as a single band of approximately 700 bp. The dsRNA were stored at -20°C.

- 5 Primer sequences used to generate specific dsRNAs were obtained as follows:

<i>Hyx</i>	<i>SEQ ID. Nos 25 and 26</i>
<i>hyx b</i>	<i>SEQ ID. Nos 27 and 28</i>
<i>APC</i>	<i>SEQ ID. Nos 29 and 30</i>
<i>dAPC-2</i>	<i>SEQ ID. Nos 31 and 32</i>
<i>Arm</i>	<i>SEQ ID. Nos 33 and 34</i>
<i>dAxin</i>	<i>SEQ ID. Nos 35 and 36</i>
<i>lgs</i>	<i>SEQ ID. Nos 37 and 38</i>
<i>CG6220</i>	<i>SEQ ID. Nos 39 and 40</i>
<i>GFP</i>	<i>SEQ ID. Nos 41 and 42</i>

Example 9

Conditions for RNAi in *Drosophila* cell culture:

- 10 *Drosophila* cell culture cells were diluted to a final concentration of 1×10^6 cells/ml in *Drosophila* expression system (DES) serum-free medium (Invitrogen). One milliliter of cells was plated per well of a six-well cell culture dish (Corning). dsRNA was added directly to the media to a final concentration of approximately 15 µg of dsRNA per ml. This was followed immediately by vigorous agitation. The cells were incubated for 30 min at room temperature
- 15 followed by addition of 1 ml of $1 \times$ Schneider's media containing 20% FBS. The cells were incubated for 24 hr to allow for turnover of the target protein and then the pathway was induced. The Wg pathway was activated by co-culture with Wg-producing cells generated by the Nusse laboratory (van Leeuwen et al., 1994). The Hh pathway was activated by inducing the expression of HhN by the addition of CuSO₄ to a final concentration of 200 µM to the
- 20 culture media.

Example 10

Luciferase assays:

Approximately 48 hours after induction of the pathway, the cells were lysed in a passive lysis
5 buffer (Promega) by shaking at room temperature for 30 minutes. The luciferase activities of
the lysates were then analyzed by a luminometer (Wallac 1420 Victor 2 multilabel, multitask
plate reader, Perkin Elmer) to measure the dual luciferase activities of LEF-luciferase reporter
and Renilla luciferase using the Promega Dual-Luciferase reporter assay system. All the
experiments were performed at least three times, and each time in triplicates.

10

Example 11

Mapping of the β -catenin binding domain in Parafibromin:

15 A GST-fusion comprising the N-terminus (amino acids 1-343) of Parafibromin interacted
with β -catenin *in vitro*, while the C-terminal half of Parafibromin (amino acids 344-531)
failed to do so (Figure 19). Further truncations allowed the inventors to define a stretch of
amino acids (200-250) essential for β -catenin binding (Figure 19). This stretch corresponds to
an evolutionarily conserved block in Parafibromin and its homologs centered on amino acids
20 218-263, which itself turned out to be sufficient for potent β -catenin binding (Figure 19).
Therefore evidence exists that amino acids 218-263 of Parafibromin constitute a discrete β -
catenin interaction domain (CID).

However, this minimal binding site is presumably only the core of the real interaction
25 interface. Thus, referring to the experimental data, the Parafibromin fragment spanning amino
acids 200-343 seems the smallest protein fragment with full binding capacity. Interestingly,
amino acids 200-343 of Parafibromin are 60% identical with the corresponding fragment in

Drosophila Hyrax, which is functionally equivalent to Parafibromin and hence also bind β -catenin.

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Claims

1. A method for screening for a substance that inhibits or enhances the binding between
5 Parafibromin and β -catenin comprising the steps of:

a) bringing a candidate substance into contact with a (poly)peptide with the
amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14 under conditions
that permit binding of said substance to said (poly)peptide; or

10 b) bringing a candidate substance into contact with a (poly)peptide fragment of
the (poly)peptide with the amino acid sequence of SEQ ID. No 12 extending
from amino acid residue 637 to 781 or from 637 to 722, or with the homologue
(poly)peptide fragment of the (poly)peptide with the amino acid sequence of
SEQ ID. No 14 under conditions that permit binding of said substance to said
15 (poly)peptide fragment; or

c) bringing a candidate substance into contact with a (poly)peptide fragment of
the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending
from amino acid residue 1 to 343, from 200 to 343, from 200 to 263, from 200
to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or with the
20 homologue (poly)peptide fragment of the (poly)peptide with the amino acid
sequence of SEQ ID. Nos 8, 9, 10 or 11 under conditions that permit binding
of said substance to said (poly)peptide fragment; or

d) bringing a candidate substance into contact with a derivate of the (poly)peptide
with the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or of
25 the (poly)peptide fragment according to b) or c) under conditions that permit
binding of said substance to said derivate; or

e) bringing a candidate substance into contact with a (poly)peptide which is at
least 50 % homologous to the (poly)peptide with the amino acid sequence of
SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or with a (poly)peptide fragment which
30 is at least 50 % homologous to the (poly)peptide fragment as stated under b) or
c) under conditions that permit binding of said substance to said (poly)peptide
or (poly)peptide fragment; and

- f) detecting if the candidate substance is having inhibitory activity or enhancing activity on the binding between Parafibromin and β -catenin or their respective orthologs.

- 5 2. A method according to claim 1, characterized in that the (poly)peptide or the (poly)peptide fragment stated under e) is at least 88 % homologous to the amino acid sequence of SEQ ID. Nos 7, 8, 9, 10, 11, 12 or 14, or to the (poly)peptide fragment as stated under b) or c), preferably at least 89 % homologous, more preferably at least 90 % homologous, even more preferably at least 91 % homologous, yet even more preferably at least 92 % homologous.
- 10
3. An antibody which binds to a (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 12 extending from amino acid residue 637 to 781 or from 637 to 722 or to the homologue (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 14.
- 15
4. An antibody which binds to a (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending from amino acid residue 200 to 343, from 200 to 263, from 200 to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or to the homologue (poly)peptide domain of the (poly)peptide with the amino acid sequence of SEQ ID. Nos 8, 9, 10 or 11.
- 20
5. A pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 12 extending from amino acid residue 637 to 781 or from 637 to 722, or the homologue (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 14, or a functionally equivalent derivate thereof.
- 25
6. A pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising a (poly)peptide fragment of the (poly)peptide with the amino acid sequence of SEQ ID. No 7 extending from amino acid residue 1 to 343, from 200 to 343, from 200 to 263, from 200 to 250, from 218 to 343, from 218 to 263 or from 218 to 250, or the homologue (poly)peptide fragment of the
- 30

(poly)peptide with the amino acid sequence of SEQ ID. Nos 8, 9, 10 or 11, or a functionally equivalent derivate thereof.

- 5 7. A pharmaceutical composition for the treatment of cancer, bone or joint disorders or developmental disorders comprising an antibody according to claim 3 or 4.
- 10 8. An assay for studying diseases induced by a enhanced or reduced binding between Parafibromin and β -catenin or their orthologs or for drug screening comprising the use of a cell line or organism selected from the group comprising *Drosophila*, mice, rats, rabbits, chicken, frogs, pigs, fishes, worms or sheep, said organism showing enhanced or reduced binding between Parafibromin and β -catenin or their respective orthologs in at least one tissue or organ and said cell line showing enhanced or reduced binding between Parafibromin and β -catenin or their respective orthologs.

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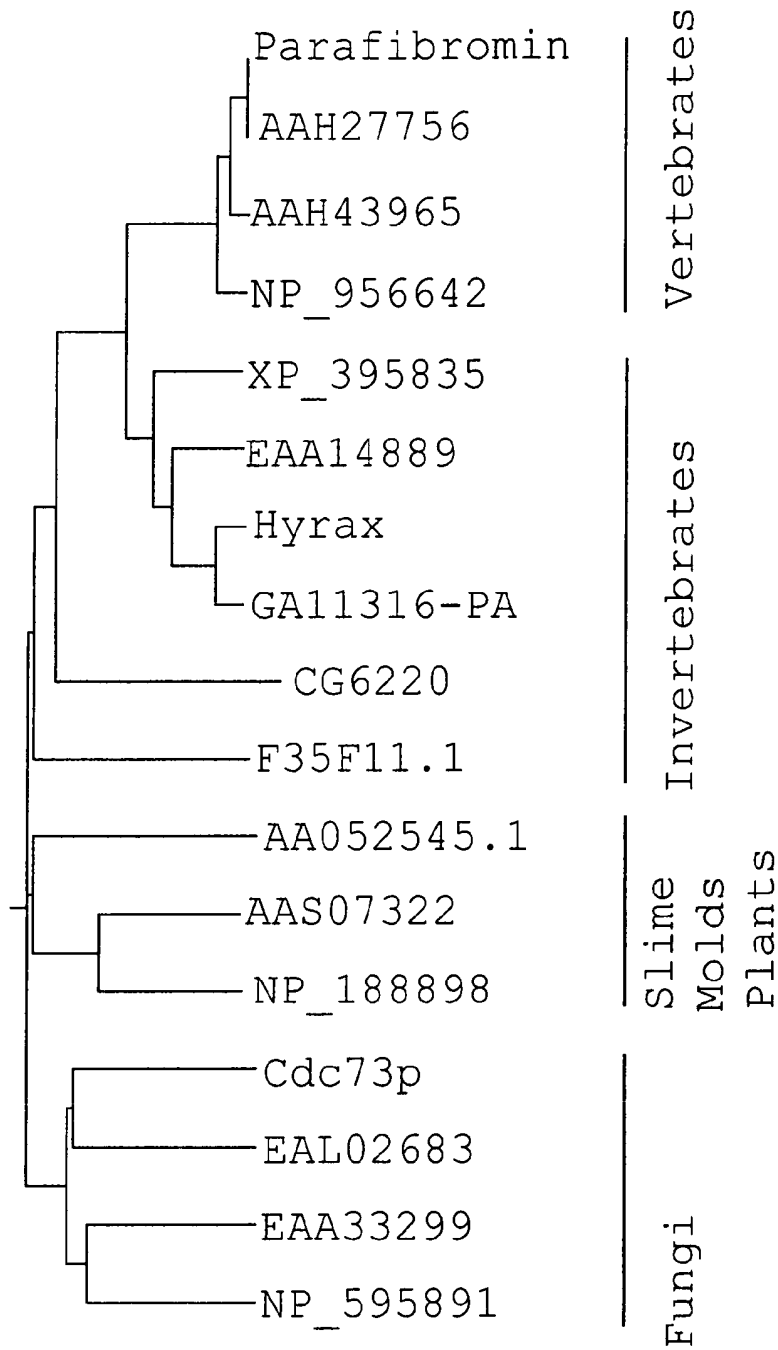


Figure 1

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PARAFIBROMIN MADVLSVLRQYNIQKK-----EIVVKGD-EVIFGEFSWPKNVKTNY
 NP_956642 MADVLSVLRQYNIQKK-----EIVAKGD-EVIFGEFSWPKNVKTNY
 HYRAX MADPLSLLRQYNINKK-----EIVERDS-QIIFGEFSWPKSVKTNY
 F35F11.1 -MDPLEALQKHVQRPEEFPLREVTVSGISYVAFGDYAYKKDTETSL
 Cdc73p MANSLDRLLREHLKNGD-----KLVLKNN---EG-QSTDDITKATMV

 VVWGTGKEGQPREYTTLSILFLLNNVHL SHPVYVRRRAATENIPVRRRPDRKDLLGYLNG
 VIWGTGKEGQKEYTTLSILFLLNNVHLPHPSYVRRRAATENIPVRRRPDRKDLLSYLNG
 LKYGSGKKGAPREYTTLECLLYLLKNVMLQHSVYVRQCAAEDIPAVNRPPDRKELLAYLNG
 QIYG---KSD--EFYSLVFLKYSHENHG VYVKEAAAAGVRAVTRIDRKNVTEYLVQ
 ETLSS---DGS---TQDSFPLNEETEIEIDGSLVQ-----LRIIVHCWMNK

 E-ASTSASIDRSAPLEIGLQIRSTQVKRAADEVLAEAKKPRIEDEEC-VRLDKERLAARLEGH
 E-SSSSTSIDRSAPLEIGLQRPSTQVKRAADEVSSEAKKPRVEDEER-VRLDKERLAARLEGH
 E-TPTCASIDKSAPLEIPTQVKRAAEGEPSSVEAAKKARFEETQV-QKVREQLAARWDVNQ
 DRTDFPALMNQVNPLSLRQLLSSEP-----EAKKPRLDGEAAGEPMDTSTSDPEQESA
 D-----SSAADYLAADCQN-----KQLTN

Figure 2A

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KEGIVQTEQIRSLSEAMSVEKIAAIKAKIMAKKR---STIKTDL---DDDITALKQRSFVDAEVDV
 KESIVQTDQIRSLSEAMSVEKIAAIKAKIMAKKR---STIKTDL---DDDITQK-QRSFVDAEVDV
 KETAVNMDNIKSLSETMSVEKIAAIKAKRLANKR---TTIKRTD---NDEAMGTDLRAILDYDVIDS
 VSAAKKEVEIRALNDNLTKDRIAEMRRKRQSHREKGIVTIETFPQKPDFLIKFFQFFSESLSLTL
 VSFLQRTDLINWLSGNTESQYLKAP---GQKG-----ETSD---KVDIENKTLAGELSTVKST

TRDIVSRERVWRTRTTILQST--GKNFSKNIFAILQSVKAREEGRAPEQRPAPNAAPVDPTLRTK
 TRDIVSRERVWRTRTTILQSS--GKNFSKNIFAILQSVKAREEGRAPEQRQTQNTQVDPAIRNK
 TKDII SRERQWRTRTSILQST--GKIFAKNIFAMLQGIKAREEGRNRPQVPNPIKMPEPARIAKP
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Figure 2B

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Figure 2C

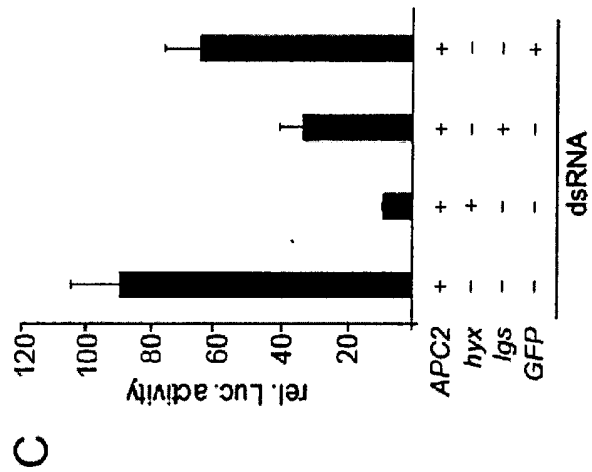
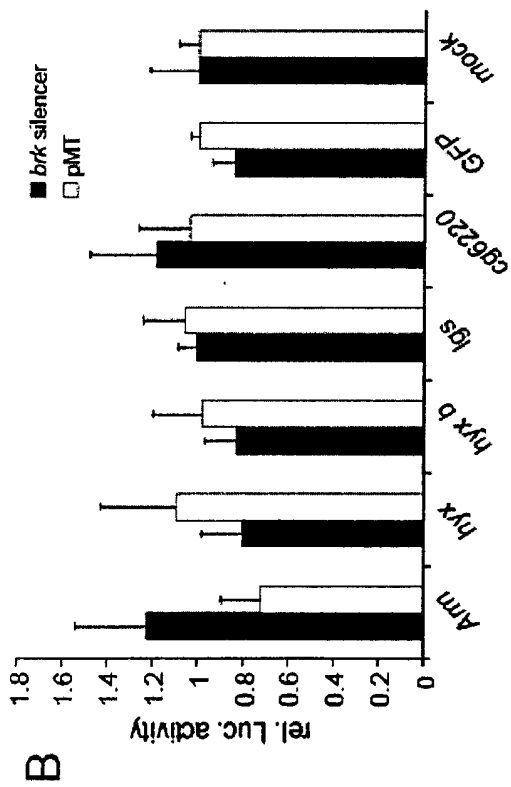
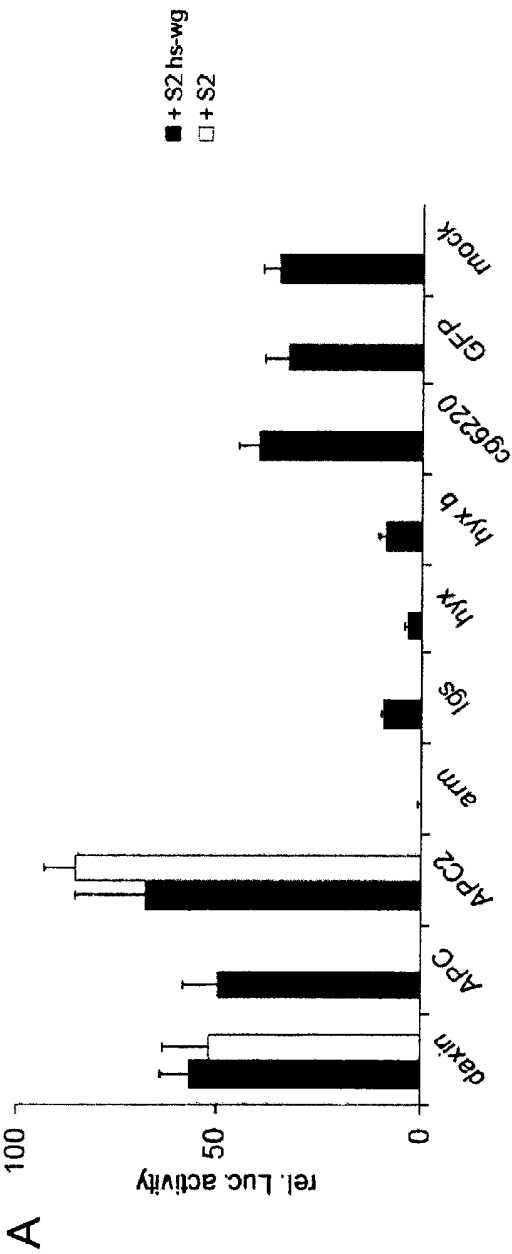
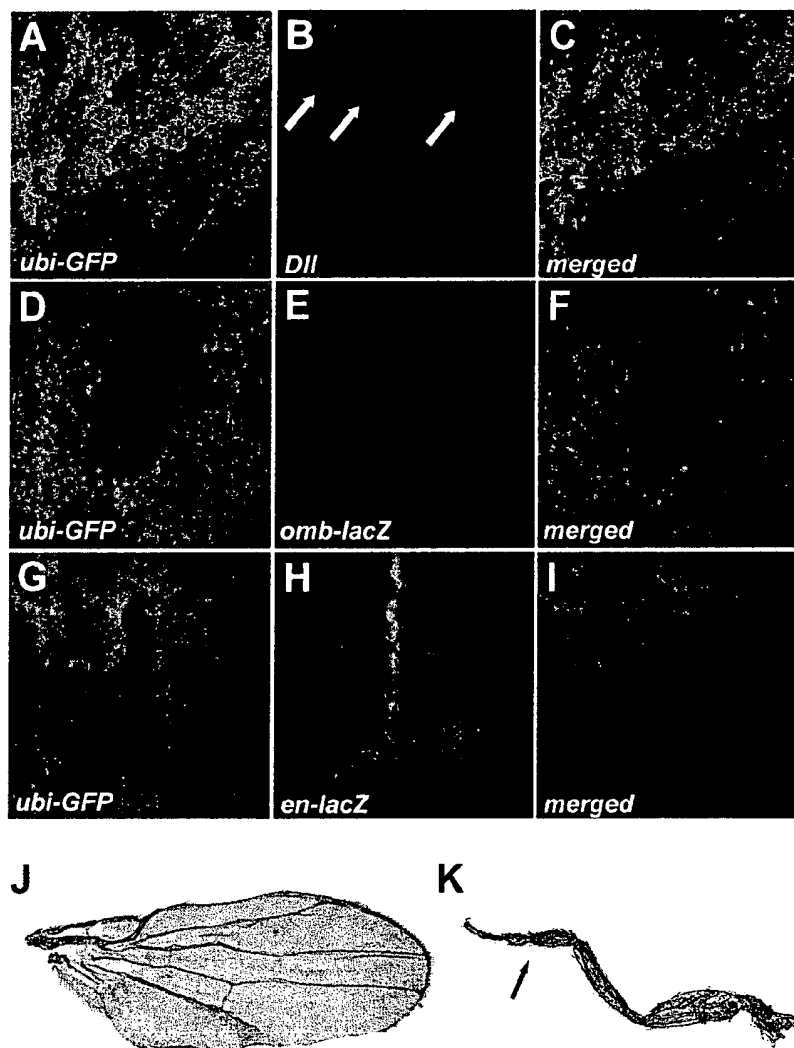


Figure 3

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**Figure 4**

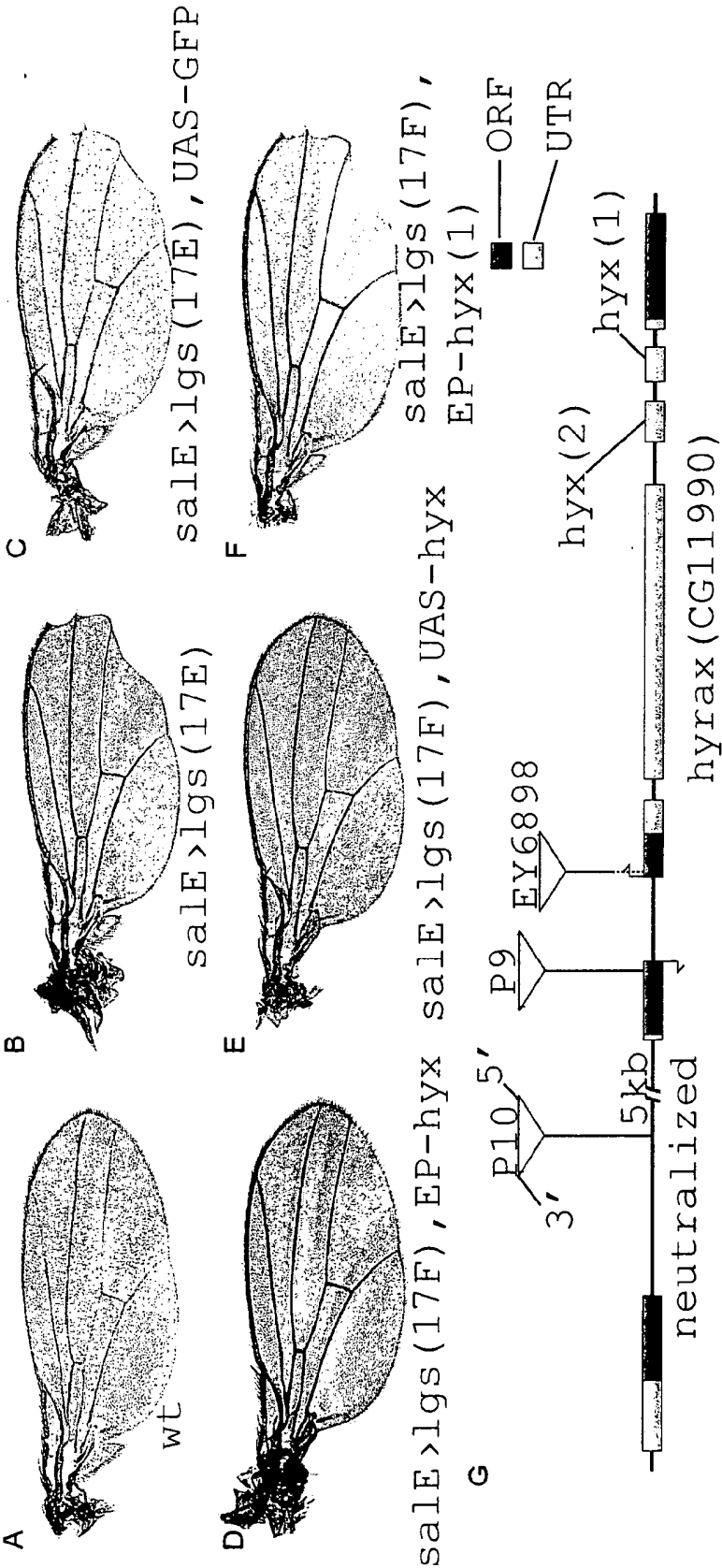
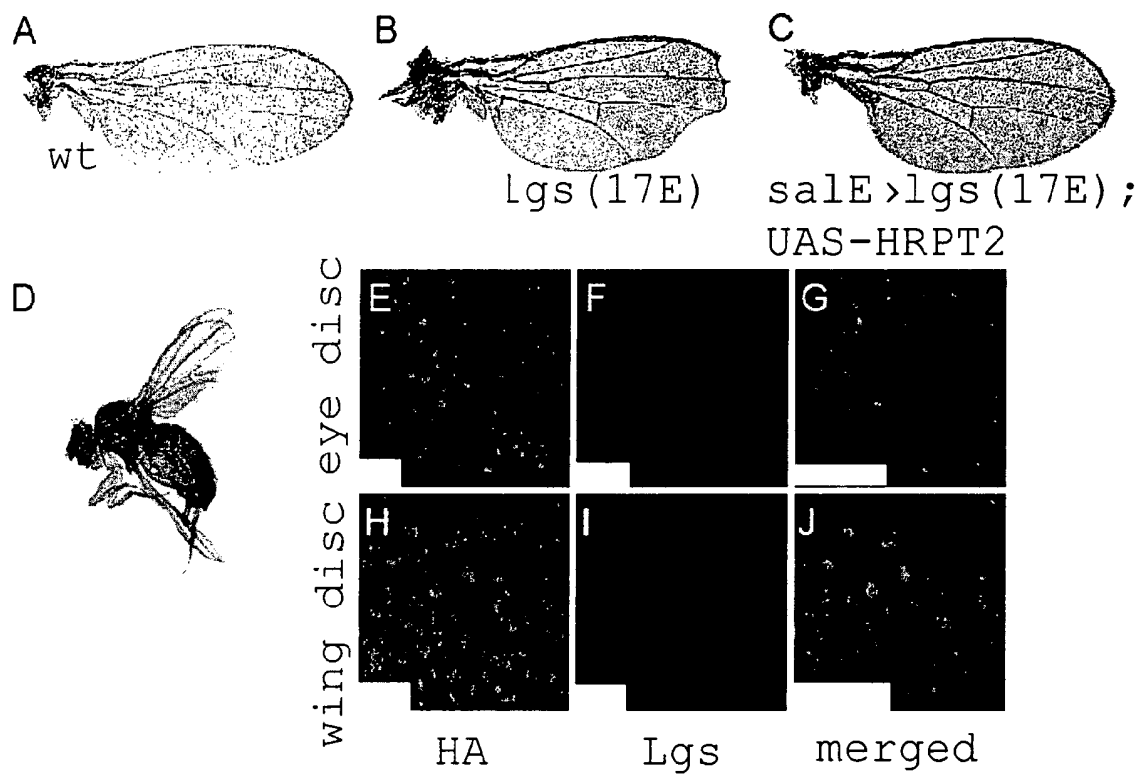


Figure 5

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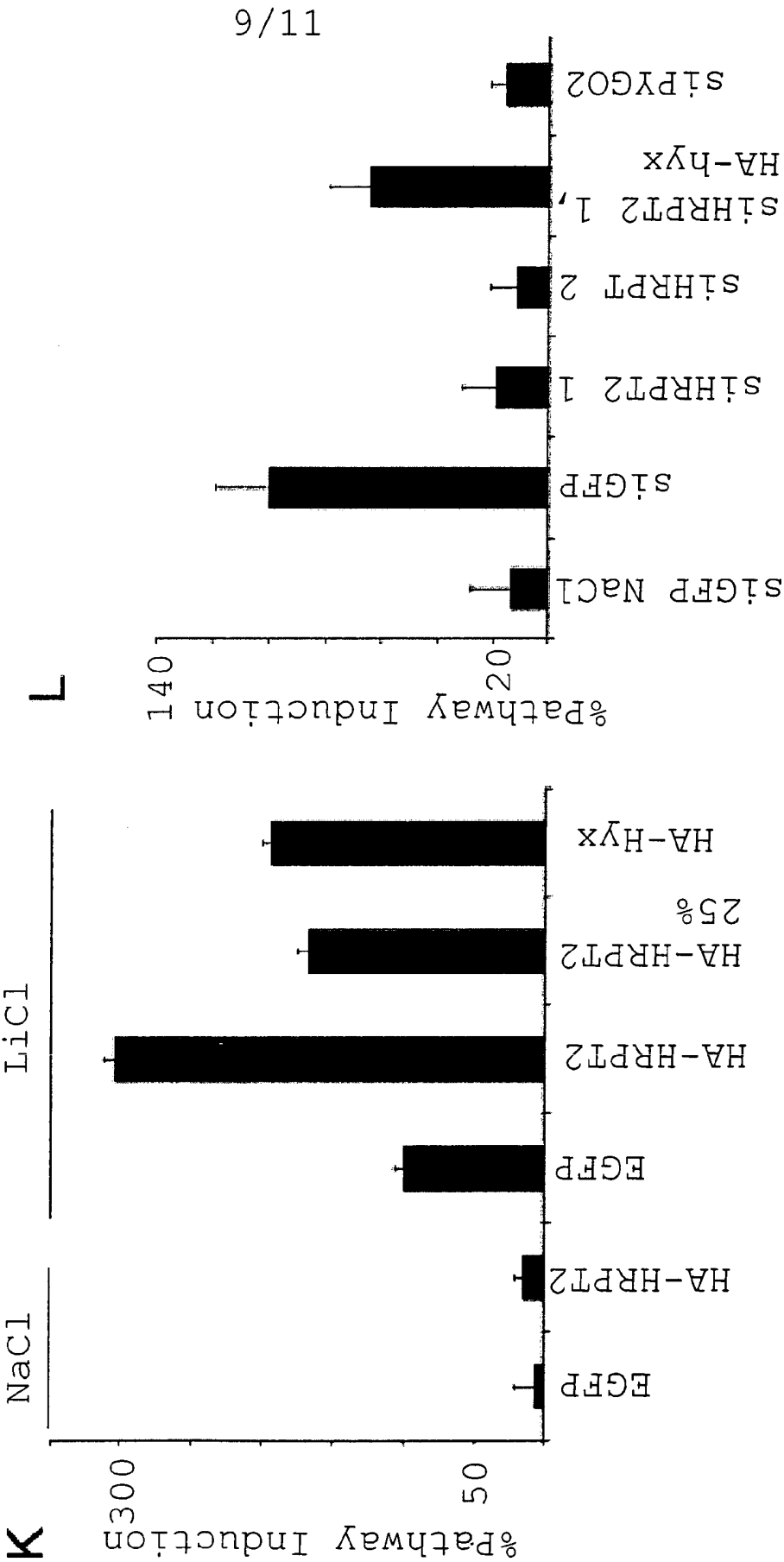


Figure 6K-L

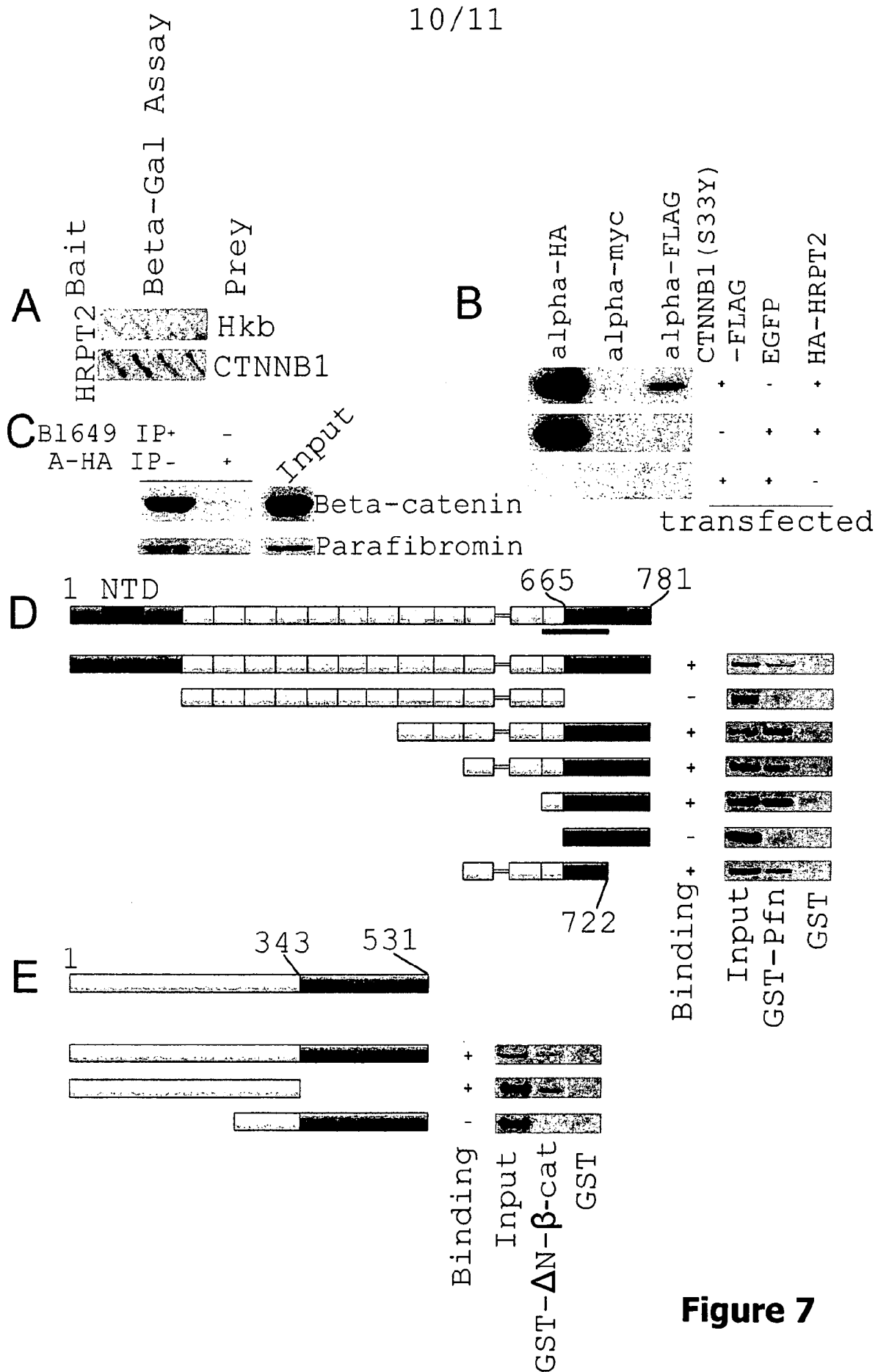


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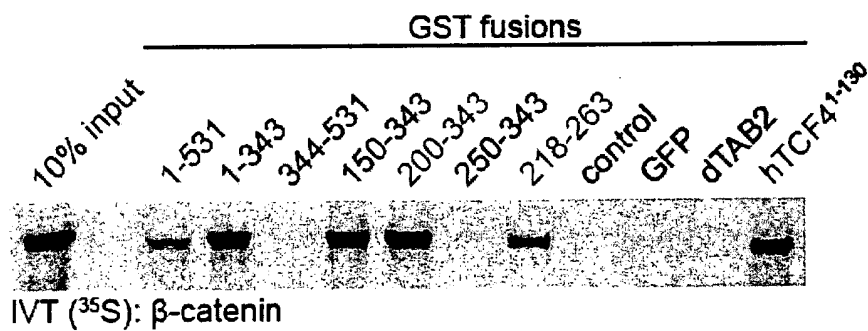


Figure 8

Sequence listing 20503-TGC-P-WO
SEQUENCE LISTING

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Sequence Listing 20503-TGC-P-WO

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Sequence listing 20503-TGC-P-WO

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Sequence listing 20503-TGC-P-WO

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<210> 7
 <211> 531
 <212> PRT
 <213> Homo sapiens

Sequence listing 20503-TGC-P-WO

<220>
 <221> misc_feature
 <223> Description of sequence: Protein Parafinbromin - NP_078805
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 35 40 45
 Gly Gln Pro Arg Glu Tyr Tyr Thr Leu Asp Ser Ile Leu Phe Leu Leu
 50 55 60
 Asn Asn Val His Leu Ser His Pro Val Tyr Val Arg Arg Ala Ala Thr
 65 70 75 80
 Glu Asn Ile Pro Val Val Arg Arg Pro Asp Arg Lys Asp Leu Leu Gly
 85 90 95
 Tyr Leu Asn Gly Glu Ala Ser Thr Ser Ala Ser Ile Asp Arg Ser Ala
 100 105 110
 Pro Leu Glu Ile Gly Leu Gln Arg Ser Thr Gln Val Lys Arg Ala Ala
 115 120 125
 Asp Glu Val Leu Ala Glu Ala Lys Lys Pro Arg Ile Glu Asp Glu Glu
 130 135 140
 Cys Val Arg Leu Asp Lys Glu Arg Leu Ala Ala Arg Leu Glu Gly His
 145 150 155 160
 Lys Glu Gly Ile Val Gln Thr Glu Gln Ile Arg Ser Leu Ser Glu Ala
 165 170 175
 Met Ser Val Glu Lys Ile Ala Ala Ile Lys Ala Lys Ile Met Ala Lys
 180 185 190
 Lys Arg Ser Thr Ile Lys Thr Asp Leu Asp Asp Asp Ile Thr Ala Leu
 195 200 205
 Lys Gln Arg Ser Phe Val Asp Ala Glu Val Asp Val Thr Arg Asp Ile
 210 215 220
 Val Ser Arg Glu Arg Val Trp Arg Thr Arg Thr Thr Ile Leu Gln Ser
 225 230 235 240

Sequence listing 20503-TGC-P-WO

Thr Gly Lys Asn Phe Ser Lys Asn Ile Phe Ala Ile Leu Gln Ser Val
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 Lys Ala Arg Glu Glu Gly Arg Ala Pro Glu Gln Arg Pro Ala Pro Asn
 260 265 270
 Ala Ala Pro Val Asp Pro Thr Leu Arg Thr Lys Gln Pro Ile Pro Ala
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 Ala Tyr Asn Arg Tyr Asp Gln Glu Arg Phe Lys Gly Lys Glu Glu Thr
 290 295 300
 Glu Gly Phe Lys Ile Asp Thr Met Gly Thr Tyr His Gly Met Thr Leu
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 Lys Ser Val Thr Glu Gly Ala Ser Ala Arg Lys Thr Gln Thr Pro Ala
 325 330 335
 Ala Gln Pro Val Pro Arg Pro Val Ser Gln Ala Arg Pro Pro Pro Asn
 340 345 350
 Gln Lys Lys Gly Ser Arg Thr Pro Ile Ile Ile Ile Pro Ala Ala Thr
 355 360 365
 Thr Ser Leu Ile Thr Met Leu Asn Ala Lys Asp Leu Leu Gln Asp Leu
 370 375 380
 Lys Phe Val Pro Ser Asp Glu Lys Lys Lys Gln Gly Cys Gln Arg Glu
 385 390 395 400
 Asn Glu Thr Leu Ile Gln Arg Arg Lys Asp Gln Met Gln Pro Gly Gly
 405 410 415
 Thr Ala Ile Ser Val Thr Val Pro Tyr Arg Val Val Asp Gln Pro Leu
 420 425 430
 Lys Leu Met Pro Gln Asp Trp Asp Arg Val Val Ala Val Phe Val Gln
 435 440 445
 Gly Pro Ala Trp Gln Phe Lys Gly Trp Pro Trp Leu Leu Pro Asp Gly
 450 455 460
 Ser Pro Val Asp Ile Phe Ala Lys Ile Lys Ala Phe His Leu Lys Tyr
 465 470 475 480
 Asp Glu Val Arg Leu Asp Pro Asn Val Gln Lys Trp Asp Val Thr Val
 485 490 495
 Leu Glu Leu Ser Tyr His Lys Arg His Leu Asp Arg Pro Val Phe Leu
 500 505 510

Sequence listing 20503-TGC-P-WO

Arg Phe Trp Glu Thr Leu Asp Arg Tyr Met Val Lys His Lys Ser His
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Leu Arg Phe
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<210> 8
 <211> 521
 <212> PRT
 <213> Danio rerio

<220>
 <221> misc_feature
 <223> Description of sequence: Protein D. rerio - NP_956642

<400> 8

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Glu Ile Val Ala Lys Gly Asp Glu Val Ile Phe Gly Glu Phe Ser Trp
 20 25 30

Pro Lys Asn Val Lys Thr Asn Tyr Val Ile Trp Gly Thr Gly Lys Glu
 35 40 45

Gly Gln Pro Lys Glu Tyr Tyr Thr Leu Asp Ser Ile Leu Phe Leu Leu
 50 55 60

Asn Asn Val His Leu Pro His Pro Ser Tyr Val Arg Arg Ala Ala Thr
 65 70 75 80

Glu Asn Ile Pro Val Val Arg Arg Pro Asp Arg Lys Asp Leu Leu Ser
 85 90 95

Tyr Leu Asn Gly Glu Ser Ser Ser Ser Thr Ser Ile Asp Arg Ser Ala
 100 105 110

Pro Ile Glu Ile Gly Leu Gln Arg Pro Thr Gln Val Lys Arg Ala Ala
 115 120 125

Asp Glu Val Ser Ser Glu Ala Lys Lys Pro Arg Val Glu Asp Glu Glu
 130 135 140

Arg Val Arg Leu Asp Lys Glu Arg Leu Ala Ala Arg Leu Glu Gly His
 145 150 155 160

Lys Glu Ser Ile Val Gln Thr Asp Gln Ile Arg Ser Leu Ser Glu Ala
 165 170 175

Met Ser Val Glu Lys Ile Ala Ala Ile Lys Ala Lys Ile Met Ala Lys
 180 185 190

Sequence listing 20503-TGC-P-WO

Lys Arg Ser Thr Ile Lys Thr Asp Leu Asp Asp Asp Ile Thr Gln Lys
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 Gln Arg Ser Phe Val Asp Ala Glu Val Asp Val Thr Arg Asp Ile Val
 210 215 220
 Ser Arg Glu Arg Val Trp Arg Thr Arg Thr Thr Ile Leu Gln Ser Ser
 225 230 235 240
 Gly Lys Asn Phe Ser Lys Asn Ile Phe Ala Ile Leu Gln Ser Val Lys
 245 250 255
 Ala Arg Glu Glu Gly Arg Ala Pro Glu Gln Arg Gln Thr Gln Asn Gln
 260 265 270
 Thr Gln Val Asp Pro Ala Ile Arg Asn Lys Gln Pro Val Pro Ala Ala
 275 280 285
 Tyr Asn Arg Tyr Asp Gln Glu Arg Phe Lys Gly Lys Glu Glu Thr Glu
 290 295 300
 Gly Phe Lys Ile Asp Thr Met Gly Thr Tyr His Gly Met Thr Leu Lys
 305 310 315 320
 Ser Val Thr Glu Gly Ala Ser Ala Arg Lys Ala Gln Thr Pro Ala Met
 325 330 335
 Gln Pro Val Pro Arg Pro Val Ser Gln Ala Arg Pro Pro Pro Asn Gln
 340 345 350
 Lys Lys Gly Ser Arg Thr Pro Ile Ile Ile Ile Pro Ala Ala Thr Thr
 355 360 365
 Ser Leu Ile Thr Met Leu Asn Ala Lys Asp Leu Leu Gln Asp Leu Lys
 370 375 380
 Phe Val Thr Ser Glu Glu Lys Lys Lys Gln Gly Ile Gln Arg Asp Asn
 385 390 395 400
 Glu Val Leu Leu Gln Arg Arg Lys Asp Gln Val Gln Pro Gly Gly Ala
 405 410 415
 Thr Leu Ser Val Thr Val Pro Tyr Arg Val Ile Asp Gln Pro Leu Lys
 420 425 430
 Leu Ala Pro Gln Asp Trp Asp Arg Val Val Ala Val Phe Val Gln Gly
 435 440 445
 Pro Ala Trp Gln Phe Lys Gly Trp Pro Trp Leu Leu Pro Asp Gly Ser
 450 455 460

Sequence listing 20503-TGC-P-WO

Pro Val Asp Ile Phe Ala Lys Ile Arg Ala Phe His Leu Lys Tyr Asp
 465 470 475 480

Glu Ala Arg Met Asp Pro Asn Val Gln Lys Trp Asp Val Thr Val Leu
 485 490 495

Glu Leu Ser His His Lys Arg His Leu Asp Arg Pro Val Phe Leu Arg
 500 505 510

Phe Trp Glu Thr Leu Asp Arg Ser Gly
 515 520

<210> 9

<211> 538

<212> PRT

<213> *Drosophila melanogaster*

<220>

<221> misc_feature

<223> Description of sequence: Protein Hyrax - NP649863

<400> 9

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Pro Lys Ser Val Lys Thr Asn Tyr Leu Lys Tyr Gly Ser Gly Lys Lys
 35 40 45

Gly Ala Pro Arg Glu Tyr Tyr Thr Leu Glu Cys Leu Leu Tyr Leu Leu
 50 55 60

Lys Asn Val Met Leu Gln His Ser Val Tyr Val Arg Gln Cys Ala Ala
 65 70 75 80

Glu Asp Ile Pro Ala Val Asn Arg Pro Asp Arg Lys Glu Leu Leu Ala
 85 90 95

Tyr Leu Asn Gly Glu Thr Pro Thr Cys Ala Ser Ile Asp Lys Ser Ala
 100 105 110

Pro Leu Glu Ile Pro Thr Gln Val Lys Arg Ala Ala Glu Gly Glu Pro
 115 120 125

Ser Ser Val Glu Val Ala Ala Lys Lys Ala Arg Phe Glu Glu Thr Gln
 130 135 140

Val Gln Lys Val Arg Glu Gln Leu Ala Ala Arg Trp Asp Val Asn Gln
 145 150 155 160

Sequence listing 20503-TGC-P-WO

Lys Glu Thr Ala Val Asn Met Asp Asn Ile Lys Ser Leu Ser Glu Thr
 165 170 175
 Met Ser Val Glu Lys Ile Ala Ala Ile Lys Ala Lys Arg Leu Ala Asn
 180 185 190
 Lys Arg Thr Thr Ile Lys Arg Thr Asp Asn Asp Glu Ala Met Gly Thr
 195 200 205
 Asp Leu Arg Ala Ile Leu Asp Tyr Asp Val Asp Ser Thr Lys Asp Ile
 210 215 220
 Ile Ser Arg Glu Arg Gln Trp Arg Thr Arg Thr Ser Ile Leu Gln Ser
 225 230 235 240
 Thr Gly Lys Ile Phe Ala Lys Asn Ile Phe Ala Met Leu Gln Gly Ile
 245 250 255
 Lys Ala Arg Glu Glu Gly Arg Asn Arg Pro Gln Val Pro Asn Pro Ile
 260 265 270
 Lys Met Pro Glu Pro Ala Arg Ile Ala Lys Pro Gln Pro Gln Leu Ser
 275 280 285
 Gln Tyr Asn Arg Tyr Asp Gln Glu Arg Phe Asn Arg Gln Lys Glu Glu
 290 295 300
 Thr Glu Gly Phe Lys Ile Asp Thr Leu Gly Thr Tyr His Gly Met Ser
 305 310 315 320
 Leu Lys Ser Val Thr Glu Gly Ser Leu Ala Gln Arg Lys Ala Gln Ala
 325 330 335
 Asn Asn Leu Pro Gly Ala Pro Gly Val Ile Ala Gly Ala Ala Ala Gly
 340 345 350
 Arg Pro Lys Glu Leu Leu Pro Ala Ala Gln Ala Arg Gln Leu Pro Ala
 355 360 365
 Asn Gly Pro Ser Lys Arg Thr Ser Arg Thr Pro Ile Ile Ile Ile Pro
 370 375 380
 Ser Ala Asn Thr Ser Leu Ile Thr Met Leu Asn Ala Lys Asp Ile Leu
 385 390 395 400
 Gln Glu Leu Arg Phe Met Ser Thr Ser Asp Lys Lys Leu Gln Gly Cys
 405 410 415
 Gln Arg Glu Cys Glu Val Leu Leu Gln Arg Lys Arg Asn Asn Gln Thr
 420 425 430

Sequence listing 20503-TGC-P-WO

Val Ser Tyr Arg Val Ile Asp Asn Pro Thr Lys Leu Ser Gln Gln Glu
 435 440 445

Trp Gln Arg Val Val Ala Val Phe Val Met Gly Pro Gln Trp Gln Phe
 450 455 460

Lys Gly Trp Pro Trp Glu Gly Asn Pro Val Asp Ile Phe Ser Lys Ile
 465 470 475 480

Cys Ala Phe His Leu Cys Phe Ser Glu Met Lys Leu Asp Ser Asn Val
 485 490 495

Glu Arg Trp Ser Val Thr Leu Leu Arg Leu Ser Gln Asn Lys Arg His
 500 505 510

Met Asp Arg Ala Val Leu Ser Lys Phe Trp Glu Thr Leu Asp Lys Tyr
 515 520 525

Ile Ala Lys Tyr Lys Pro Asp Leu Arg Tyr
 530 535

<210> 10
 <211> 554
 <212> PRT
 <213> C. elegans

<220>
 <221> misc_feature
 <223> Description of sequence: Protein C. elegans - NP_500465
 <400> 10

Met Asp Pro Leu Glu Ala Leu Gln Lys His Val Gln Arg Pro Glu Glu
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Phe Pro Leu Arg Glu Val Thr Val Ser Gly Ile Ser Tyr Val Ala Phe
 20 25 30

Gly Asp Tyr Ala Tyr Lys Lys Asp Thr Glu Thr Ser Leu Gln Ile Tyr
 35 40 45

Gly Lys Ser Asp Glu Phe Tyr Ser Leu Glu Ser Leu Val Val Phe Leu
 50 55 60

Lys Tyr Ser His Glu Asn His Gly Val Tyr Val Lys Glu Ala Ala Ala
 65 70 75 80

Ala Gly Val Arg Ala Val Thr Arg Ile Asp Arg Lys Asn Val Thr Glu
 85 90 95

Tyr Leu Gln Gly Asp Arg Thr Asp Phe Pro Ala Leu Met Asn Gln Val
 100 105 110

Sequence listing 20503-TGC-P-WO

Asn Pro Leu Ser Leu Arg Gln Leu Leu His Ser Ser Glu Pro Glu Ala
 115 120 125
 Lys Lys Pro Arg Leu Asp Gly Glu Ala Ala Gly Glu Pro Met Asp Thr
 130 135 140
 Ser Thr Ser Ala Glu Ile Cys Ser Lys Ile Pro Ile Phe Leu Lys Ile
 145 150 155 160
 Asn Ser Ile Phe Phe Pro Asp Glu Pro Gln Glu Ser Ala Val Ser Ala
 165 170 175
 Ala Lys Lys Glu Val Glu Ile Arg Ala Leu Asn Asp Asn Leu Thr Lys
 180 185 190
 Asp Arg Ile Ala Glu Met Arg Arg Lys Arg Gln Ser His Arg Glu Lys
 195 200 205
 Gly Ile Val Thr Ile Glu Thr Thr Phe Pro Gln Lys Pro Asp Phe Leu
 210 215 220
 Ile Lys Lys Phe Gln Phe Phe Ser Glu Ser Leu Ser Thr Leu Thr Ser
 225 230 235 240
 Ala Ser Leu Pro Lys Thr Arg Ile His Lys Thr Arg Glu Asn Val Met
 245 250 255
 Leu Gly Ala Arg Asp Leu Ser Asn Val Leu Asp Ile Ile Thr Ser Ala
 260 265 270
 Gln Arg Gln Trp Asp Leu Asn Glu Lys Lys Glu Lys Val Ala Ala Val
 275 280 285
 His Ala Thr Asn Leu Ser Lys Asp Gln Ser Gly Ala Ala Gly Gly Gln
 290 295 300
 Gln Gln Arg Ser Gly Tyr Ser Arg Tyr Ala Gln Glu Ala Phe Ala His
 305 310 315 320
 Glu Lys Thr Lys Glu Ile Gln Thr Glu Gly Ser Phe Ile Gly Ser Asn
 325 330 335
 Phe Ser Ser Ile Lys Gln Gly His His Ala Val Gln Lys Ala Pro Asp
 340 345 350
 Ala Pro Pro Gly Arg Pro Pro Leu Ala Lys Pro Ile Gln Leu Leu Thr
 355 360 365
 Ser Ser Thr Ala Thr Ser Ser Gly Ser Ser Ala Ala Gln Asn Gly Ser
 370 375 380

Sequence listing 20503-TGC-P-WO

Lys Arg Thr Ser Arg Ser Pro Ile Ile Ile Val Pro Ser Ala Met Asn
385 390 395 400

Thr Met Ile Asn Leu Tyr Asn Val Arg Asp Ile Leu Gln Asn Phe Ser
405 410 415

Tyr Val Pro Val Asp Gln Arg Arg Lys Glu Thr Asn Lys Lys Pro Val
420 425 430

Asp Leu Ala Ile Gln Arg Gln Lys Asn Gly Val Thr Tyr Asn Ile Arg
435 440 445

Val Ile Asp Asn Ala Glu Lys Leu Ala Asn Asp Asp Trp Asp Arg Val
450 455 460

Ile Ala Val Phe Val Met Gly Val Ala Trp Gln Phe Lys Gly Trp Lys
465 470 475 480

Trp Asn Gly Asn Pro Thr Asp Ile Phe Thr His Ile Pro Ala Phe His
485 490 495

Phe His Val Asp Gln Asp Lys Pro Val Ala Gln Val Met Gln Trp Asn
500 505 510

Val His Lys Ile Pro Val Ser Ala Thr Lys Arg His Met Asp Lys Ala
515 520 525

Arg Phe Ser Gln Val Trp Glu Thr Ile Glu Asn Phe Val Arg Lys Asn
530 535 540

Lys Pro His Leu Thr Ala Arg Leu Gly Leu
545 550

<210> 11
<211> 393
<212> PRT
<213> *Saccharomyces cerevisiae*

<220>
<221> misc_feature
<223> Description of sequence: Protein Cdc73p - YLR418C

<400> 11

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Lys Leu Val Leu Lys Asn Asn Glu Gly Gln Ser Thr Asp Asp Ile Thr
20 25 30

Lys Ala Thr Met Val Glu Thr Leu Ser Ser Asp Gly Ser Thr Gln Asp
35 40 45

Sequence listing 20503-TGC-P-WO

Ser Phe Pro Leu Asn Glu Glu Thr Glu Ile Glu Ile Asp Gly Ser Leu
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 Val Gln Leu Arg Ile Ile Val His Cys Trp Met Asn Lys Asp Ser Ser
 65 70 75 80
 Ala Ala Asp Tyr Leu Ala Asp Cys Gln Asn Lys Gln Leu Thr Asn Val
 85 90 95
 Ser Phe Leu Gln Arg Thr Asp Leu Ile Asn Trp Leu Ser Gly Asn Thr
 100 105 110
 Glu Ser Ser Gln Tyr Leu Lys Ala Pro Gly Gln Lys Gly Glu Thr Ser
 115 120 125
 Asp Lys Val Asp Ile Glu Asn Lys Thr Leu Ala Gly Glu Leu Ser Thr
 130 135 140
 Val Lys Ser Thr Thr Ser Ala Ser Leu Glu Asn Asp Ser Glu Val Ser
 145 150 155 160
 Asp Pro Val Val Val Glu Thr Met Lys His Glu Arg Ile Leu Val Asp
 165 170 175
 His Asn Ser Ala Leu Arg Gly Ala Lys Pro Ile Asn Phe Gly Tyr Leu
 180 185 190
 Ile Lys Asp Ala Glu Leu Lys Leu Val Gln Ser Ile Lys Gly Ser Leu
 195 200 205
 Arg Gly Ser Lys Leu Pro Pro Gly His Lys Gly Ala His Gly Arg Ile
 210 215 220
 Ser Lys Thr Asn Gly Ser Ser Gly Gly Pro Arg Lys Asp Pro Ile Ile
 225 230 235 240
 Leu Ile Pro Ser Ala Ala Ser Ser Ile Leu Thr Val Ala Asn Ile Lys
 245 250 255
 Gln Phe Leu Leu Glu Ser Lys Tyr Val Asn Pro Arg Asn Leu Pro Ser
 260 265 270
 Val Pro Asn Gly Leu Val Asn Ile Glu Lys Asn Phe Glu Arg Ile Ser
 275 280 285
 Arg Pro Ile Arg Phe Ile Ile Val Asp Asn Thr Arg Met Phe Thr Lys
 290 295 300
 Pro Glu Tyr Trp Asp Arg Val Val Ala Ile Phe Thr Thr Gly His Thr
 305 310 315 320

Sequence listing 20503-TGC-P-WO

Trp Gln Phe Asn Asn Tyr Gln Trp Asn Ser Pro Gln Glu Leu Phe Gln
 325 330 335

Arg Cys Lys Gly Tyr Tyr Phe His Phe Ala Gly Asp Ser Val Pro Gln
 340 345 350

His Val Gln Gln Trp Asn Val Glu Lys Val Glu Leu Asp Lys Asn Lys
 355 360 365

Arg Phe Lys Asp Val Glu Val Val Arg Tyr Phe Trp His Ser Leu Glu
 370 375 380

Lys Glu Leu Ile Ser Arg Gly Tyr Arg
 385 390

<210> 12
 <211> 781
 <212> PRT
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Description of sequence: Protein beta-catenin - P35222

<400> 12

Met Ala Thr Gln Ala Asp Leu Met Glu Leu Asp Met Ala Met Glu Pro
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Asp Arg Lys Ala Ala Val Ser His Trp Gln Gln Gln Ser Tyr Leu Asp
 20 25 30

Ser Gly Ile His Ser Gly Ala Thr Thr Thr Ala Pro Ser Leu Ser Gly
 35 40 45

Lys Gly Asn Pro Glu Glu Glu Asp Val Asp Thr Ser Gln Val Leu Tyr
 50 55 60

Glu Trp Glu Gln Gly Phe Ser Gln Ser Phe Thr Gln Glu Gln Val Ala
 65 70 75 80

Asp Ile Asp Gly Gln Tyr Ala Met Thr Arg Ala Gln Arg Val Arg Ala
 85 90 95

Ala Met Phe Pro Glu Thr Leu Asp Glu Gly Met Gln Ile Pro Ser Thr
 100 105 110

Gln Phe Asp Ala Ala His Pro Thr Asn Val Gln Arg Leu Ala Glu Pro
 115 120 125

Ser Gln Met Leu Lys His Ala Val Val Asn Leu Ile Asn Tyr Gln Asp
 130 135 140

Sequence listing 20503-TGC-P-WO

Asp Ala Glu Leu Ala Thr Arg Ala Ile Pro Glu Leu Thr Lys Leu Leu
 145 150 155 160

Asn Asp Glu Asp Gln Val Val Val Asn Lys Ala Ala Val Met Val His
 165 170 175

Gln Leu Ser Lys Lys Glu Ala Ser Arg His Ala Ile Met Arg Ser Pro
 180 185 190

Gln Met Val Ser Ala Ile Val Arg Thr Met Gln Asn Thr Asn Asp Val
 195 200 205

Glu Thr Ala Arg Cys Thr Ala Gly Thr Leu His Asn Leu Ser His His
 210 215 220

Arg Glu Gly Leu Leu Ala Ile Phe Lys Ser Gly Gly Ile Pro Ala Leu
 225 230 235 240

Val Lys Met Leu Gly Ser Pro Val Asp Ser Val Leu Phe Tyr Ala Ile
 245 250 255

Thr Thr Leu His Asn Leu Leu Leu His Gln Glu Gly Ala Lys Met Ala
 260 265 270

Val Arg Leu Ala Gly Gly Leu Gln Lys Met Val Ala Leu Leu Asn Lys
 275 280 285

Thr Asn Val Lys Phe Leu Ala Ile Thr Thr Asp Cys Leu Gln Ile Leu
 290 295 300

Ala Tyr Gly Asn Gln Glu Ser Lys Leu Ile Ile Leu Ala Ser Gly Gly
 305 310 315 320

Pro Gln Ala Leu Val Asn Ile Met Arg Thr Tyr Thr Tyr Glu Lys Leu
 325 330 335

Leu Trp Thr Thr Ser Arg Val Leu Lys Val Leu Ser Val Cys Ser Ser
 340 345 350

Asn Lys Pro Ala Ile Val Glu Ala Gly Gly Met Gln Ala Leu Gly Leu
 355 360 365

His Leu Thr Asp Pro Ser Gln Arg Leu Val Gln Asn Cys Leu Trp Thr
 370 375 380

Leu Arg Asn Leu Ser Asp Ala Ala Thr Lys Gln Glu Gly Met Glu Gly
 385 390 395 400

Leu Leu Gly Thr Leu Val Gln Leu Leu Gly Ser Asp Asp Ile Asn Val
 405 410 415

Sequence listing 20503-TGC-P-WO

Val Thr Cys Ala Ala Gly Ile Leu Ser Asn Leu Thr Cys Asn Asn Tyr
420 425 430

Lys Asn Lys Met Met Val Cys Gln Val Gly Gly Ile Glu Ala Leu Val
435 440 445

Arg Thr Val Leu Arg Ala Gly Asp Arg Glu Asp Ile Thr Glu Pro Ala
450 455 460

Ile Cys Ala Leu Arg His Leu Thr Ser Arg His Gln Glu Ala Glu Met
465 470 475 480

Ala Gln Asn Ala Val Arg Leu His Tyr Gly Leu Pro Val Val Val Lys
485 490 495

Leu Leu His Pro Pro Ser His Trp Pro Leu Ile Lys Ala Thr Val Gly
500 505 510

Leu Ile Arg Asn Leu Ala Leu Cys Pro Ala Asn His Ala Pro Leu Arg
515 520 525

Glu Gln Gly Ala Ile Pro Arg Leu Val Gln Leu Leu Val Arg Ala His
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Gln Asp Thr Gln Arg Arg Thr Ser Met Gly Gly Thr Gln Gln Gln Phe
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Val Glu Gly Val Arg Met Glu Glu Ile Val Glu Gly Cys Thr Gly Ala
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Leu His Ile Leu Ala Arg Asp Val His Asn Arg Ile Val Ile Arg Gly
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Leu Asn Thr Ile Pro Leu Phe Val Gln Leu Leu Tyr Ser Pro Ile Glu
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Asn Ile Gln Arg Val Ala Ala Gly Val Leu Cys Glu Leu Ala Gln Asp
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Lys Glu Ala Ala Glu Ala Ile Glu Ala Glu Gly Ala Thr Ala Pro Leu
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Thr Glu Leu Leu His Ser Arg Asn Glu Gly Val Ala Thr Tyr Ala Ala
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Ala Val Leu Phe Arg Met Ser Glu Asp Lys Pro Gln Asp Tyr Lys Lys
660 665 670

Arg Leu Ser Val Glu Leu Thr Ser Ser Leu Phe Arg Thr Glu Pro Met
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Sequence listing 20503-TGC-P-WO

Ala Trp Asn Glu Thr Ala Asp Leu Gly Leu Asp Ile Gly Ala Gln Gly
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Glu Pro Leu Gly Tyr Arg Gln Asp Asp Pro Ser Tyr Arg Ser Phe His
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Ser Gly Gly Tyr Gly Gln Asp Ala Leu Gly Met Asp Pro Met Met Glu
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His Glu Met Gly Gly His His Pro Gly Ala Asp Tyr Pro Val Asp Gly
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Sequence listing 20503-TGC-P-WO

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Sequence listing 20503-TGC-P-WO

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35 40 45

Gly Ala Val Thr Gln Val Pro Ser Leu Ser Gly Lys Glu Asp Glu Glu
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Thr Arg Ser Gln Arg Val Arg Ala Ala Met Phe Pro Glu Thr Leu Glu
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Ala Val Gln Arg Leu Ser Glu Pro Ser Gln Met Leu Lys His Ala Val
130 135 140

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Ile Pro Glu Leu Ile Lys Leu Leu Asn Asp Glu Asp Gln Val Val Val
165 170 175

Ser Gln Ala Ala Met Met Val His Gln Leu Ser Lys Lys Glu Ala Ser
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Arg His Ala Ile Met Asn Ser Pro Gln Met Val Ala Ala Leu Val Arg
195 200 205

Sequence listing 20503-TGC-P-WO

Ala Ile Ser Asn Ser Asn Asp Leu Glu Ser Thr Lys Ala Ala Val Gly
 210 215 220

Thr Leu His Asn Leu Ser His His Arg Gln Gly Leu Leu Ala Ile Phe
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Lys Ser Gly Gly Ile Pro Ala Leu Val Lys Leu Leu Ser Ser Pro Val
 245 250 255

Glu Ser Val Leu Phe Tyr Ala Ile Thr Thr Leu His Asn Leu Leu Leu
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Gly Gly Met Gln Ala Leu Ala Met His Leu Gly Asn Met Ser Pro Arg
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Leu Gly Ser Thr Asp Val Asn Val Val Thr Cys Ala Ala Gly Ile Leu
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Ser Asn Leu Thr Cys Asn Asn Gln Arg Asn Lys Ala Thr Val Cys Gln
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Val Gly Gly Val Asp Ala Leu Val Arg Thr Ile Ile Asn Ala Gly Asp
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Arg Glu Glu Ile Thr Glu Pro Ala Val Cys Ala Leu Arg His Leu Thr
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Sequence listing 20503-TGC-P-WO

Ser Arg His Val Asp Ser Glu Leu Ala Gln Asn Ala Val Arg Leu Asn
 485 490 495
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 515 520 525
 Pro Ala Asn His Ala Pro Leu Arg Glu His Gly Ala Ile His His Leu
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 Val Arg Leu Leu Met Arg Ala Phe Gln Asp Thr Glu Arg Gln Arg Ser
 545 550 555 560
 Ser Ile Ala Thr Thr Gly Ser Gln Gln Pro Ser Ala Tyr Ala Asp Gly
 565 570 575
 Val Arg Met Glu Glu Ile Val Glu Gly Thr Val Gly Ala Leu His Ile
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 595 600 605
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 Arg Val Ala Ala Gly Val Leu Cys Glu Leu Ala Ala Asp Lys Glu Gly
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 Glu Glu Ala Tyr Glu Gly Leu Tyr Gly Gln Gly Pro Pro Ser Val His
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 Ser Ser His Gly Gly Arg Ala Phe His Gln Gln Gly Tyr Asp Thr Leu
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Sequence listing 20503-TGC-P-WO

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Gly Gly Ala Gly Gly Ala Pro Gly Asn Gly Gly Ala Val Gly Gly Ala
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Ser Gly Gly Gly Gly Asn Ile Gly Ala Ile Pro Pro Ser Gly Ala Pro
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Thr Ser Pro Tyr Ser Met Asp Met Asp Val Gly Glu Ile Asp Ala Gly
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Asn Asn Leu Ala Ala Trp Tyr Asp Thr Asp Cys
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 <223> Description of sequence: Primer

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<210> 17
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 <212> DNA
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<220>
 <223> Description of sequence: Primer

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<210> 18
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Sequence listing 20503-TGC-P-WO

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Sequence listing 20503-TGC-P-WO

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 <210> 32
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 <220>
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 <210> 34
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Sequence listing 20503-TGC-P-WO

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<210> 35
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<400> 35
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<210> 36
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<212> DNA
<213> Artificial

<220>
<223> Description of sequence: primer sequence dAxi used to generate specific dsRNA

<400> 36
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<210> 37
<211> 39
<212> DNA
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<220>
<223> Description of sequence: primer sequence lgs used to generate specific dsRNA

<400> 37
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<210> 38
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<220>
<223> Description of sequence: primer sequence lgs used to generate specific dsRNA

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<210> 39
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<220>
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Sequence listing 20503-TGC-P-WO

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<210> 41
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<210> 42
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<212> DNA
<213> Artificial

<220>
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<400> 42
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/002533

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/32 C07K14/47 A61K38/17 G01N33/68 G01N33/574
G01N33/566

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)

C07K A61K G01N

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

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

EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MOSIMANN CHRISTIAN ET AL: "Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo." CELL. 21 APR 2006, vol. 125, no. 2, 21 April 2006 (2006-04-21), pages 327-341, XP002389728 ISSN: 0092-8674 the whole document ----- -/--	1-8

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

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

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

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

Z document member of the same patent family

Date of the actual completion of the international search

19 July 2006

Date of mailing of the international search report

09/08/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Pilch, B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/002533

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUELSKEN J ET AL: "Requirement for beta-catenin in anterior-posterior axis formation in mice." THE JOURNAL OF CELL BIOLOGY. 7 FEB 2000, vol. 148, no. 3, 7 February 2000 (2000-02-07), pages 567-578, XP002389730 ISSN: 0021-9525 page 568, right-hand column, paragraph 4; figure 2	3
X	----- ABERLE H ET AL: "Assembly of the cadherin-catenin complex in vitro with recombinant proteins." JOURNAL OF CELL SCIENCE. DEC 1994, vol. 107 (Pt 12), December 1994 (1994-12), pages 3655-3663, XP002389731 ISSN: 0021-9533 page 3656, left-hand column, paragraph 3; figure 4; table 1	3
X	----- ROZENBLATT-ROSEN ORIT ET AL: "The parafibromin tumor suppressor protein is part of a human Paf1 complex." MOLECULAR AND CELLULAR BIOLOGY. JAN 2005, vol. 25, no. 2, January 2005 (2005-01), pages 612-620, XP002389729 ISSN: 0270-7306 page 613, left-hand column, paragraph 2; figures 1,3	4
X	----- WO 01/66689 A (HYSEQ, INC; TANG, Y., TOM; LIU, CHENGHUA; ASUNDI, VINOD; XU, CHONGJUN;) 13 September 2001 (2001-09-13) SEQ ID No: 012 has 100.00 % identity (100.00 % ungapped) over 781 (q:s=1-781:1-781) with subject GSP:AAU28118 disclosed in patent W00166689 published on 20010913. claims 27,28	7
X	----- WO 02/078524 A (ZYCOS INC; CHICZ, ROMAN, M; TOMLINSON, ANDREW, J; URBAN, ROBERT, G) 10 October 2002 (2002-10-10) SEQ ID No: 012 has 100.00 % identity (100.00 % ungapped) over 781 (q:s=1-781:1-781) with subject GSP:ABU05111 disclosed in patent W002078524 published on 20021010. claims 58,59 ----- -/--	7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/002533

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with Indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/024097 A (GENENTECH, INC; CHIU, HENRY; CLARK, HILARY; DENNIS, KATHRYN; FONG, SHE) 25 March 2004 (2004-03-25) SEQ ID No: 007 has 99.81 % identity (99.81 % ungapped) over 531 (q:s=1-531:1-531) with subject GSP:ADL83181 disclosed in patent WO2004024097 published on 20040325. claims 20,21	7
A	----- CARPTEN J D ET AL: "HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 32, December 2002 (2002-12), pages 676-680, XP002984588 ISSN: 1061-4036 the whole document -----	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2006/002533

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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