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(71) Applicant: **THE CHAIM SHEBA MEDICAL CENTER** [IL/IL]; Clinical Research Center & Advanced Technologies, Hanhala Bldg, 5262100 Tel Hashomer (IL).

(72) Inventors: **DEKEL, Benjamin**; 4 Zangvil Street, 6259902 Tel Aviv (IL). **MARK-DANIELI, Michal**; 4 Simtat Haanafa, 4281000 Zur-Moshe (IL). **VAX, Einav**; 7 Haitshar St., Apt. 11, 7549330 Rishon Lezion (IL). **SHAKKED, Naomi Pode**; 4/9 Yitzhak Rabin St., 5551009 Kiryat Ono (IL).

(74) Agents: **PEARL COHEN ZEDEK LATZER BARATZ** et al.; P.O.Box 12704, 4673339 Herzliya (IL).

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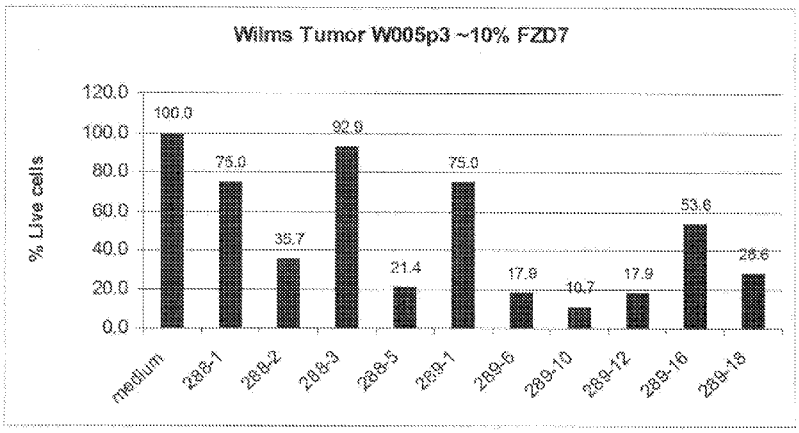


Figure 4A

(57) Abstract: The invention provides antibodies or an antigen-binding portion thereof, which recognize the human Frizzled 7 receptor. Further, the invention provides methods of using these antibodies for the treatment cancer in a subject.

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FRIZZLED RECEPTOR ANTIBODIES FOR TREATMENT OF CANCER

FIELD OF THE INVENTION

[001] The present invention relates to compositions for treatment of diseases such as cancer. Specifically, the invention relates to antibodies that bind to human frizzled receptors, as well as to methods of using the antibodies or for the treatment cancer.

BACKGROUND OF THE INVENTION

[002] It is becoming clear that many, if not most, malignancies arise from a population of cells that exclusively maintain the ability to self-renew and sustain the tumor via the expression of tumor progenitor genes. These ‘‘cancer stem cells’’ are often biologically distinct from the differentiated cancer cells that comprise most of the tumor bulk. Because cancer stem cells are believed to be primarily responsible for tumor initiation as well as resistance to chemo- and radiotherapy, their persistence may account for relapsing disease in cancer patients.

[003] Wilms’ tumor, a type of kidney cancer or nephroblastoma is one of the most common solid tumors of childhood, occurring in 1 in 10,000 children and accounting for 8% of childhood cancers. With improved multimodality therapy, WT survival rates have risen over the last 40 years to 85%-90%; however, for those whose disease relapses or metastasizes, even intensive salvage regimens result in subsequent survival closer to 50%. Moreover, survivors are at increased risk for a broad spectrum of adverse outcomes caused by chemotherapy and radiation therapy, such as late mortality and secondary cancers.

[004] Wilms tumor is believed to result from malignant transformation of abnormally persistent renal stem cells which retain embryonic differentiation potential. Indeed, recent molecular data obtained by microarrays demonstrate that Wilms’ tumor and Wilms’ tumor-stem like xenografts systematically overexpress nephric-progenitor genes corresponding to the earliest stages of normal metanephric kidney development connecting tumorigenesis and organogenesis in the kidney.

[005] In addition to genes that specify the renal lineage, Wnt pathway-related molecules including β-catenin (CTNNB1), frizzled7 (FZD7) and frizzled2 (FZD2), are concomitantly induced in Wilms’ tumor. In general, the binding of Wnt ligand to frizzled cell surface receptors normally leads to inhibition of a “destruction complex” consisting of APC/Axin/GSK-3β/Ck1/Dvl and other factors, with subsequent accumulation of

dephosphorylated stabilized β-catenin, and transcription of its target genes. Interestingly, recent data have demonstrated an essential role for the Wnt–β-catenin signaling pathway in nephrogenesis. Also, a genomic screen of Wilms' tumor identified FZD7, a Wnt receptor, to be a biomarker of cancer stem cells in Wilms' tumor. Additionally, a striking link between β-catenin signaling and the development of Wilms tumor has been demonstrated.

[006] In recent years the involvement of the Wnt pathway has been shown in various other tumors (e.g. colon, breast) and in the self-renewal mechanism of stem cells. Unregulated activation of the Wnt can alter the developmental fate of tumor cells to maintain them in an undifferentiated and proliferative state. Thus carcinogenesis can proceed in the context of altered homeostatic mechanisms controlling normal development and tissue repair by stem cells.

[007] Secreted Wnt ligands activate a receptor complex consisting of a Fzd receptor family member and low-density lipoprotein (LDL) receptor-related protein 5 or 6 (LPR5/6). The Fzd receptors are seven transmembrane domain receptors of the G-protein coupled receptor (GPCR) superfamily and contain a large extracellular N-terminal ligand binding domain with 10 conserved cysteines, known as the cysteine rich domain. There are ten known human FZD receptors: FZD1-10. Different Fzd cysteine rich domains have different binding affinities for specific Wnts. Fzd receptors have been grouped into those that activate the canonical β-catenin pathway and those that activate noncanonical pathways.

[008] To form the receptor complex that binds the FZD ligands, FZD receptors interact with LRP5/6, single pass transmembrane proteins. The canonical Wnt signaling pathway activated upon receptor binding is mediated by the cytoplasmic protein Dishevelled (Dsh) interacting directly with the Fzd receptor and results in the cytoplasmic stabilization and accumulation of β-catenin. In the absence of a Wnt signal, β-catenin is localized to a cytoplasmic destruction complex that includes the tumor suppressor proteins adenomatous polyposis coli (APC) and Axin. Activation of Dsh results in the dissociation of the destruction complex.

[009] In addition to the canonical signaling pathway, Wnt ligands also activate β-catenin - independent pathways (i.e. non-canonical Wnt signaling). Non-canonical Wnt signaling has been implicated in the process of gastrulation. Antagonism is often reported between the canonical and non-canonical pathways, and some evidence indicates that non-canonical signaling can suppress cancer formation. Thus, in certain contexts, Fzd receptors act as negative regulators of the canonical Wnt signaling pathway. For example, FZD6 represses Wnt canonical signaling when co-expressed with FZD1.

[0010] The canonical Wnt signaling pathway also plays a central role in the maintenance of stem cell populations in the small intestine and colon, and the inappropriate activation of this pathway plays a prominent role in colorectal cancers; colorectal cancer is most commonly initiated by activating mutations in the Wnt signaling cascade. Approximately 5-10% of all colorectal cancers are hereditary with one of the main forms being familial adenomatous polyposis (FAP), an autosomal dominant disease in which about 80% of affected individuals contain a germline mutation in the adenomatous polyposis coli (APC) gene. Mutations have also been identified in other Wnt pathway components including Axin and β-catenin.

[0011] In human breast cancer, β-catenin accumulation implicates activated Wnt signaling in over 50% of carcinomas, and though specific mutations have not been identified, upregulation of Frizzled receptor expression has been observed.

[0012] Thus, there remains a need to identify molecules that may halt the inappropriate activation of the Wnt signaling pathway in cancers, including but not limited to colorectal cancer, melanomas and breast cancer. The present invention addresses this need by providing an antibody specific to the FZD7 receptor.

SUMMARY OF THE INVENTION

[0013] In one embodiment, the invention provides an isolated antibody or antigen-binding portion thereof that binds specifically to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of said receptor. In another embodiment the antibody may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0014] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid (AA) portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor. In another embodiment the antibody may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0015] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on a Frizzled receptor.

[0016] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on a Frizzled receptor.

[0017] In another embodiment, the invention provides an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:4 is present on a Frizzled receptor. In another embodiment the antibody may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 289-6;

b) the antibody produced by the hybridoma cell line 289-12; and

c) the antibody produced by the hybridoma cell line 289-18.

[0018] In another embodiment, the invention provides a method for treating a tumor in a subject. The method comprises the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of the receptor. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0019] In another embodiment, the invention provides a method for treating a tumor in a subject. The method comprises the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 AA portion of SEQ ID NO: 1, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0020] In another embodiment, the invention provides a method for treating a tumor in a subject. The method comprises the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 AA portion of SEQ ID NO: 2, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

[0021] In another embodiment, the invention provides a method for treating a tumor in a subject. The method comprises the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 AA portion of SEQ ID NO: 3, wherein said portion of SEQ ID NO:3 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

[0022] In another embodiment, the invention provides a method for treating a tumor in a subject. The method comprises the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 AA portion of SEQ ID NO: 4, wherein said portion of SEQ ID NO:4 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 289-6;

b) the antibody produced by the hybridoma cell line 289-12; and

c) the antibody produced by the hybridoma cell line 289-18.

[0023] In another embodiment, the invention provides a method of detecting a tumor in a subject. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for Frizzled7 by an isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of said receptor. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0024] In another embodiment, the invention provides a method of detecting a tumor in a subject. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on the Frizzled receptor. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 288-1;

b) the antibody produced by the hybridoma cell line 288-2;

c) the antibody produced by the hybridoma cell line 288-3; and

d) the antibody produced by the hybridoma cell line 288-5.

[0025] In another embodiment, the invention provides a method of detecting a tumor in a subject. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on the Frizzled receptor.

[0026] In another embodiment, the invention provides a method of detecting a tumor in a subject. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on the Frizzled receptor.

[0027] In another embodiment, the invention provides a method of detecting a tumor in a subject. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 AA portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:4 is present on the Frizzled receptor. In another embodiment the antibody used in this method may be selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 289-6;

b) the antibody produced by the hybridoma cell line 289-12; and

c) the antibody produced by the hybridoma cell line 289-18.

[0028] In some embodiments, methods of this invention are directed to a Wilm's tumor, a colorectal tumor, a colon cancer, a tumor comprising melanoma cells, or a breast tumor, or any combination thereof.

[0029] Other features and advantages of the present invention will become apparent from the

following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0031] **Figure 1A** shows a tissue specificity comparison between FZD7 (left) and FZD2 (right).

[0032] **Figure 1B** shows a comparison of the 10 cysteine residues typical of the cysteine-rich extracellular domain of Fz family members between the human FZD2 and human FZD7. 95% (142/150) homology is shown. Black rectangles indicate selected epitope locations. Adjacent squares indicate the epitope's corresponding SEQ ID NO: (for example, the square with the number "1" in it labels the rectangle containing the sequence which is SEQ ID NO: 1).

[0033] **Figure 2** shows inhibition of malignant cell proliferation by FZD7-specific monoclonal antibodies produced by hybridoma clones generated with selected epitopes. Numbers indicate the hybridoma clone from which an antibody was taken (for example, 288-1 indicates a monoclonal antibody generated by hybridoma clone 288-1). Wilms' Tumor cells (W005) were seeded in 96-well plates and were incubated with antibodies for 48 hours and then were assayed by MTS (cell proliferation assay). Same was done in melanoma cell line cells (SK-MEL28), Wilms Tumor cells (WT): and in HeLa cervical cancer cells. FZD7 expression levels for the Wilms' Tumor cells (WT), SK-MEL-28 cells and HeLA cells are 10%, 11% and 3% respectively.

[0034] **Figure 3** shows inhibition of malignant cell proliferation by FZD7-specific antibodies from selected hybridoma clones. SK-MEL-28 melanoma cells were seeded in 96 well plates. Cells were incubated with antibodies from the indicated clones for 48 hours and then were assayed by MTS (cell proliferation assay). Same was done in HeLa and in HT-29 colorectal adenocarcinoma cells. FZD7 expression levels are indicated for SK-MEL-28 (11-22%), HeLA

(3%) and HT-29 (2.6%). The circled stars indicate the clones shown to be effective. (n=5)

[0035] **Figure 4A** shows killing of primary tumor malignant cells by FZD7-specific monoclonal antibodies. Primary tumor was Wilms' tumor and antibodies were FZD7-specific monoclonal antibodies produced by hybridoma clones generated with selected epitopes. Numbers indicate the hybridoma clone from which an antibody was taken (for example, 288-1 indicates a monoclonal antibody generated by hybridoma clone 288-1). Wilms' Tumor cells (W005) were seeded in 96-well plates and were incubated with antibodies for 48 hours and then were assayed for viability by trypan blue. Percent of live cells from total cells is indicated.

[0036] **Figure 4B** shows killing of SK-MEL-28 and SK-MEL-28 melanoma cells by FZD7-specific monoclonal antibodies produced by hybridoma clones generated with selected epitopes. Numbers indicate the hybridoma clone from which an antibody was taken (for example, 288-1 indicates a monoclonal antibody generated by hybridoma clone 288-1). SK-MEL-28 and SK-MEL-28 melanoma cells were seeded in 96-well plates and were incubated with antibodies for 48 hours and then were assayed for viability by trypan blue. Absolute cell counts of live cells are shown.

[0037] **Figure 4C** shows representative micro-images of SK-MEL-28 cells after treatment with one of the monoclonal antibodies (clone 288-2, right image) or without antibody treatment (left). Cell death and is visible in treated cells (right).

[0038] **Figure 5A** shows the dose response in terms of inhibition of cell proliferation upon exposure to different concentrations of anti FZD7 monoclonal antibody produced in clones 288-1 (top) and 288-5 (bottom). The response of a high FZD7- expressing malignant tissue (SK-MEL28, melanoma cells) is compared with that of a low FZD7-expressing malignant tissue (HeLa, cervical cancer cells). Cells were exposed to antibody for 48h and proliferation was assessed by the MTS assay and compared to cells not treated with antibody. Proliferation was inhibited in the presence of either FZD7 antibody in high FZD7 expressing cells but not in low expressing cells (HeLA). Optimal inhibition of proliferation was observed at an antibody concentration of about 5ug/ml. Data shown is normalized to control untreated cells for each cell line.

[0039] **Figure 5B** shows the data from Figure 5A in a way which highlights the effect the culturing density of SK-MEL28 melanoma cells has on the level of inhibition of proliferation by 5ug/ml of the FZD7 monoclonal Ab produced by hybridoma clones 288-1 and 288-5. It is observed that at this Ab concentration, inhibition is greater when a 4000 cell/well density is employed in the case of 288-5 and similar in the case of 288-1.

[0040] **Figure 6** shows Western blot analysis for active β-Catenin (Millipore 05665) in HeLa and SK-MEL28 cells after 48h treatment with 288-1 Ab at the indicated concentration. β-Catenin inhibition by the 288-1 FZD7 mAb is demonstrated.

[0041] **Figure 7A** shows apoptosis of malignant cells treated with antibodies of the invention. Specifically, Flow cytometry of APC conjugated Annexin V to Melanoma and HeLa cells is shown. Levels of Annexin staining are compared in the presence of the monoclonal antibody produced by hybridoma 288-1 at different concentrations or without antibody.

[0042] **Figure 7B** shows Expression of WNT targeted genes using RQ PCR. Untreated and treated Wilms' tumor and HeLa cells.

[0043] **Figure 8** illustrates the arrangement of the FZD7 polypeptide within the plasma membrane and identifies the locations of peptides 1-4.

[0044] **Figure 9** presents data showing the inhibition of cell proliferation by FZD7 specific monoclonal antibodies secreted by hybridomas in relationship to percent proliferation of control.

[0045] **Figure 10** presents a micrograph showing Anti FZD7 Ab288-1 double labeling with a commercial anti FZD7 antibody of SK-MEL28 cells.

[0046] **Figure 11A** presents the results of an immune-precipitation assay confirming the specificity of monoclonal antibody 228-1 binding to the FZD7 receptor in an SK-MEL28 cell lysate.

[0047] **Figure 11B** presents the results of a peptide blocking assay demonstrating the specificity of monoclonal antibody 288-1 to antigen C of the FZD7 receptor.

[0048] **Figure 11C** presents Western Blot data showing serial dilutions of the monoclonal antibody 288-1 (4ug/ml, 2ug/ml and 1ug/ml), confirming specificity of this antibody for FZD7.

[0049] **Figure 12** presents a bar graph showing that stabilized anti FZD7 Ab 288-1 reduces proliferation in melanoma cells. Control untreated cells were set as 100%, average of % proliferation as indicated by the range markers in the bar graph.

[0050] **Figure 13A** presents results (bar graphs) demonstrating MAb 288-1 inhibits expression of Wnt Signaling pathway target genes AXIN, CCND1, C-MYC, FZD7, and CTNNB (β-catenin), and Wnt pathway inhibitors DKK1 and sFRP1 in SK-MEL28 cells.

[0051] **Figure 13B** presents results (bar graphs) demonstrating MAb 288-1 inhibits expression of Wnt Signaling pathway target genes: AXIN, CCND1, C-MYC; FZD7; CTNNB (β-catenin), and Wnt pathway inhibitors: DKK1 and sFRP1; in Wilms' tumor cells.

[0052] **Figure 13C** presents Western blot data showing that protein levels of active β-Catenin were reduced by 5 ug/ml anti FZD7 Ab 288-1 in Wilms' tumor cells

[0053] **Figure 14A** shows the results of flow cytometry analysis of MAb 288-1 treated and untreated cells using annexin V and 7-Amino actinmyosin D (7AAD) staining, wherein the is an increase in the percent of pre-apoptotic cells in SK-MEL28 (71%) and WT (47%) treated cells.

[0054] **Figure 14B** presents data (bar graph) showing increased % of dead SK-MEL28 cells following MAb-288-1 treatment.

[0055] **Figure 14C** presents data (bar graphs) showing increased % of dead WT cells following MAb-288-1 treatment. W002, W026, W0027 and W011Xn represent Wilms' tumor derived cells from different donors.

[0056] **Figure 15A** presents Colony Forming Units (CFU) data (bar graph) demonstrating that the number of CFU decreased in SK-MEL28 cells treated with MAb 288.1.

[0057] **Figure 15B** shows micrographs of untreated and MAb 288.1 treated SK-MEL28 cells, demonstrating smaller colony size in SK-MEL28 cells treated with MAb 288.1.

[0058] **Figure 15C** presents the results of a sphere formation assay (bar graph) demonstrating that the number of spheres formed decreased in SK-MEL28 cells treated with MAb 288.1.

[0059] **Figure 15D** presents the results of a sphere formation assay (bar graph) demonstrating that the number of spheres formed decreased in WT cells treated with MAb 288.1.

[0060] **Figure 15E** shows representative micrographs of spheres formed from SK-MEL28 cells in the presence or absence (untreated) of MAb 288.1. Less condensed and smaller spheres were formed following MAb 288.1 treatment.

[0061] **Figure 15F** shows representative micrographs of spheres formed from WT cells in the presence or absence (untreated) of MAb 288.1. Less condensed and smaller spheres were formed following MAb 288.1 treatment.

[0062] **Figure 16A** presents micrographs showing morphological changes in SK-MEL28 cells treated with MAb 288.1 as compared with untreated cells.

[0063] **Figure 16B** presents micrographs of a wound healing migration assay showing that migration of SK-MEL28 cells treated with MAb 288.1 was significantly inhibited, as compared with untreated cells.

[0064] It will be appreciated that for simplicity and clarity of illustration, elements shown in the figures have not necessarily been drawn to scale. For example, the dimensions of some of the elements may be exaggerated relative to other elements for clarity. Further, where

considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0065] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that the present invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the present invention.

[0066] Although examples are not limited in this regard, the terms "plurality" and "a plurality" as used herein may include, for example, "multiple" or "two or more". The terms "plurality" or "a plurality" may be used throughout the specification to describe two or more components, elements, units, parameters, or the like. Unless explicitly stated, the method examples described herein are not constrained to a particular order or sequence. Additionally, some of the described method examples or elements thereof can occur or be performed at the same point in time.

DEFINITIONS

[0067] The term "antibody," as used herein, includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion" or "antigen-binding domain") or single chain thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH). Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region (abbreviated herein as CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component of the classical complement system.

[0068] The term "epitope," as used herein, means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0069] The term "discontinuous epitope," as used herein, means a conformational epitope on a protein antigen which is formed from at least two separate regions in the primary sequence of the protein.

[0070] The term "isolated antibody," as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that binds specifically to amino acid residues in the cytoplasmic domain of FZD7 is substantially free of antibodies that specifically bind other antigens). An isolated antibody that specifically binds to an epitope, isoform or variant of a human Frizzled receptor may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., Frizzled species homologs). An isolated antibody may have cross-reactivity with other antigens yet still remain specific, namely bind only to a particular epitope. In the case of cross-reactivity, this epitope may be present on more than one protein or antigen. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0071] The term "monoclonal antibody," as used herein, refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0072] The term "subject," as used herein, includes any human or non-human animal. The term "non-human animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

[0073] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention

[0074] The present invention provides isolated antibodies or antigen binding domains thereof that specifically bind one or more human frizzled receptors (FZDs). In certain embodiments, the antibodies or antigen binding domains thereof specifically bind two, three, four, five, six, seven, eight, nine, or ten frizzled receptors. The human frizzled receptor or receptors bound by the antibodies may be selected from the group consisting of FZD 1, FZD2, FZD3, FZD4,

FZD5, FZD6, FZD7, FZD8, FZD9, and FZD10. In certain embodiments, the one or more human frizzled receptors comprise FZD1, FZD2, FZD5, FZD7, and/ or FZD8. In certain embodiments, the one or more human frizzled receptors comprise FZD7. In certain embodiments, the one or more human frizzled receptors comprise FZD7, FZD5 and/or FZD8. In certain embodiments, the antibody specifically binds FZD7. In other embodiments, the antibody specifically binds to both FZD7 and FZD1. In certain embodiments, the antibody specifically binds to both FZD7 and FZD2. In certain embodiments, the antibody specifically binds to both FZD7 and FZD3. In certain embodiments, the antibody specifically binds to both FZD7 and FZD4. In certain embodiments, the antibody specifically binds to both FZD7 and FZD5. In certain embodiments, the antibody specifically binds to both FZD7 and FZD6. In certain embodiments, the antibody specifically binds to both FZD7 and FZD8. In certain embodiments, the antibody specifically binds to both FZD7 and FZD9. In certain embodiments, the antibody specifically binds to both FZD7 and FZD10. In certain embodiments, the antibody specifically binds to FZD7, FZD1, FZD2, FZD5, FZD7, and FZD8. The full-length amino acid (AA) sequences for human FZD1-10 are known in the art and also provided herein as SEQ ID NO:5 (FZD1 AA), SEQ ID NO:6 (FZD2 AA), SEQ ID NO:7 (FZD3 AA), SEQ ID NO:8 (FZD4 AA), SEQ ID NO:9 (FZD5 AA), SEQ ID NO:10 (FZD6 AA), SEQ ID NO:11 (FZD7 AA), SEQ ID NO:12 (FZD8 AA), SEQ ID NO:13 (FZD9 AA) and SEQ ID NO:14 (FZD10 AA).

[0075] In certain embodiments, the antibody or antigen binding portion thereof described herein specifically binds to human FZD7. In other embodiments, that antibody, may further specifically bind or cross-react with one or more additional human frizzled receptors. In certain embodiments, the one or more additional human frizzled receptors are selected from the group consisting of FZD2, FZD5, and FZD8. In certain embodiments, the one or more additional human frizzled receptors are selected from the group consisting of FZD1, FZD2, FZD5, and FZD8. In certain embodiments, the one or more additional human frizzled receptors are selected from the group consisting of FZD1, FZD2, FZD5, and FZD8. In certain embodiments, the one or more additional human frizzled receptors are selected from the group consisting of FZD1, FZD2, FZD5, and FZD8. In certain embodiments, the one or more additional human frizzled receptors comprise both FZD5 and FZD8. In certain embodiments, the one or more additional human frizzled receptors comprise are selected from the group consisting of FZD1, FZD2, FZD5, and FZD8.

[0076] In certain embodiments, the antibody specifically binds to three or more human

frizzled receptors. In certain embodiments, the three or more human frizzled receptors comprise three or more frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the antibody further specifically binds to one or more additional human frizzled receptors.

[0077] In certain embodiments, the antibody specifically binds to the extracellular domain within the one or more human frizzled receptors to which it binds. Sequences of the extracellular domain of each of the human frizzled receptors are known in the art.

[0078] In certain embodiments, the antibody specifically binds to the Fri domain (FRI) (also known as the cysteine-rich domain (CRD)) within the human frizzled receptor(s) to which it binds. Sequences of the Fri domain of each of the human frizzled receptors are known in the art.

[0079] In certain embodiments, the antibody specifically binds to the Fri domain (FRI) (cysteine-rich domain (CRD)) within the human frizzled 7 receptor. In certain embodiments the antibody binds specifically to SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to all of the amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to a fraction of the amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least five contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least four contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least five contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least six contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least seven contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least eight contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least nine contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least ten contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In

certain embodiments the antibody binds specifically to at least eleven contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least twelve contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to at least thirteen contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 7.

[0080] In certain embodiments, the antibody specifically binds to the Fri domain (FRI) (cysteine-rich domain (CRD)) within the human frizzled 2 receptor. In certain embodiments the antibody binds specifically to SEQ ID NO:2 within the FRI domain of Frizzled 7. In certain embodiments the antibody binds specifically to all of the amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to a fraction of the amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least five contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least four contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least five contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least six contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least seven contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least eight contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least nine contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least ten contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least eleven contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least twelve contiguous or non-contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2. In certain embodiments the antibody binds specifically to at least thirteen contiguous or non-

contiguous amino acids (AAs) of SEQ ID NO:2 within the FRI domain of Frizzled 2.

[0081] In certain embodiments, the antibody specifically binds to a transmembrane portion of the human frizzled receptor(s). Sequences of the transmembrane portion of each of the human frizzled receptors are known in the art.

[0082] In certain embodiments, the antibody specifically binds to a cytoplasmic portion of the human frizzled receptor(s). Sequences of the cytoplasmic portion of each of the human frizzled receptors are known in the art.

[0083] In certain embodiments, the antibody specifically binds to a portion of the human frizzled receptor(s), wherein this portion consists of both transmembrane and cytoplasmic sections.

[0084] In certain embodiments, the antibody specifically binds to a transmembrane portion of the human frizzled 7 receptor. In certain embodiments, the antibody binds to all or part of SEQ ID NO: 1 within this transmembrane portion.

[0085] In certain embodiments, the antibody specifically binds to a cytoplasmic portion of the human frizzled 7 receptor. In certain embodiments, the antibody binds to all or part of SEQ ID NO: 1 within this cytoplasmic portion.

[0086] In certain embodiments, the antibody specifically binds to a portion of the human frizzled 7 receptor, wherein this portion consists of both transmembrane and cytoplasmic sections. In certain embodiments, the antibody binds to all or part of SEQ ID NO: 1 within this portion

[0087] In certain embodiments, an individual antigen-binding site of a FZD-binding antibody described herein is capable of binding the one, two, three, four, or five or more human frizzled receptors. In certain embodiments, an individual antigen-binding site of the FZD-binding antibody is capable of specifically binding one, two, three, four, or five human frizzled receptors selected from the group consisting of FZD1, FZD2, FZD5, FZD7, and FZD8.

[0088] In certain embodiments, the FZD-binding antibody binds to one or more (for example, two or more, three or more, or four or more) human frizzled receptors with a dissociation constant (KQ) of about 1000 nM or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, or about 10 nM or less. For example, in certain embodiments, a FZD-binding antibody described herein that binds to more than one FZD receptor, binds to those FZDs receptors with a KD of about 100 nM or less, about 20 nM or less, or about 10 nM or less. In certain embodiments, the FZD-binding antibody binds to each of one or more (e.g., 1, 2, 3, 4, or 5) of the following FZDs with a dissociation constant of about 40 nM or less: FZD1, FZD2,

FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding antibody binds to each of one or more of the following FZDs with a dissociation constant of about l0 nM or less: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding antibody binds to each of the following FZDs with a dissociation constant of about 10 nM or less: FZD1, FZD2, FZD5, FZD7, and FZD8.

[0089] In certain embodiments, the FZD-binding antibody binds to one or more human frizzled receptors with an EC50 of about 1000nM or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, or about 1 nM or less. For example, in certain embodiments, a FZD-binding antibody described herein that binds to more than one FZD has an EC50 of about 40 nM or less, about 20 nM or less, or about 10 nM or less, with respect to those FZDs. In certain embodiments, the FZD-binding antibody has an EC50 of about 20 nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of the following FZDs: FZD1, FZD2, FZD5, FZD7, and FZD8. In certain embodiments, the FZD-binding antibody has an EC50 of about 10 nM or less with respect to one or more (e.g., 1, 2, 3, 4, or 5) of the following FZDs: FZDl, FZD2, FZD5, FZD7, and FZD8.

[0090] In certain embodiments, the FZD-binding antibody specifically binds to the sequence RFYHRLSHSSKGETAV (SEQ ID NO: 1) within the human frizzled 7 receptor. In certain embodiments, the FZD-binding antibody specifically binds to a sequence comprising at least 5 amino acids of the sequence RFYHRLSHSSKGETAV (SEQ ID NO: 1) within a human frizzled receptor. In certain embodiments, these five amino acids are contiguous. In certain embodiments, these five amino acids are not contiguous. In certain embodiments, these five amino acids are contiguous amino acids RFYHR. In certain embodiments, these five amino acids are contiguous amino acids FYHRL. In certain embodiments, these five amino acids are contiguous amino acids YHRLS. In certain embodiments, these five amino acids are contiguous amino acids HRLSH. In certain embodiments, these five amino acids are contiguous amino acids RLSHS. In certain embodiments, these five amino acids are contiguous amino acids LSHSS. In certain embodiments, these five amino acids are contiguous amino acids SHSSK. In certain embodiments, these five amino acids are contiguous amino acids HSSKG. In certain embodiments, these five amino acids are contiguous amino acids SSKGE. In certain embodiments, these five amino acids are contiguous amino acids SKGET. In certain embodiments, these five amino acids are contiguous amino acids KGETA. In certain embodiments, these five amino acids are contiguous amino acids GETAV. In one embodiment, the FZD-binding antibody may be the

antibody produced by the hybridoma cell line 288-1. In another embodiment, the FZD-binding antibody may be the antibody produced by the hybridoma cell line 288-2. In another embodiment, the FZD-binding antibody may be the antibody produced by the hybridoma cell line 288-5.

[0091] In certain embodiments, the FZD-binding antibody binds to at least part of a sequence in FZD1, FZD2, FZD3, FZD4,FZD5, FZD6, FZD8, FZD9 and/or FZD 10 that corresponds to the sequence RFYHRLSHSSKGETAV (SEQ ID NO: 1) in FZD7.

[0092] In certain embodiments, the FZD-binding antibody specifically binds to the sequence QEDAGLEVHQFYPL (SEQ ID NO: 2) within the human frizzled receptors 2 or 7. In certain embodiments, the FZD-binding antibody specifically binds to a sequence comprising at least 5 amino acids of the sequence QEDAGLEVHQFYPL (SEQ ID NO: 2) within a human frizzled receptor. In certain embodiments, these five amino acids are contiguous. In certain embodiments, these five amino acids are not contiguous. In certain embodiments, these five amino acids are contiguous amino acids QEDAG. In certain embodiments, these five amino acids are contiguous amino acids EDAGL. In certain embodiments, these five amino acids are contiguous amino acids DAGLE. In certain embodiments, these five amino acids are contiguous amino acids AGLEV. In certain embodiments, these five amino acids are contiguous amino acids GLEVH. In certain embodiments, these five amino acids are contiguous amino acids LEVHQ. In certain embodiments, these five amino acids are contiguous amino acids EVHQF. In certain embodiments, these five amino acids are contiguous amino acids VHQFY. In certain embodiments, these five amino acids are contiguous amino acids HQFYP. In certain embodiments, these five amino acids are contiguous amino acids QFYPL. In one embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 288-4. In another embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 289-5. In another embodiment the FZD-antibody may be the antibody produced by the hybridoma cell line number 4.

[0093] In certain embodiments, the antibody binds to at least part of a sequence in FZD1, FZD3, FZD4,FZD5, FZD6, FZD8, FZD9 and/or FZD 10 that corresponds to the sequence QEDAGLEVHQFYPL (SEQ ID NO: 2) in FZD2 or FZD7.

[0094] In certain embodiments, the FZD-binding antibody specifically binds to the sequence PGASDGRGRPAFPFS (SEQ ID NO: 3) within human frizzled receptor 7. In certain embodiments, the FZD-binding antibody specifically binds to a sequence comprising at least 5

amino acids of the sequence PGASDGRGRPAFPFS (SEQ ID NO: 3) within a human frizzled receptor. In certain embodiments, these five amino acids are contiguous. In certain embodiments, these five amino acids are not contiguous. In certain embodiments, these five amino acids are contiguous amino acids PGASD. In certain embodiments, these five amino acids are contiguous amino acids GASDG. In certain embodiments, these five amino acids are contiguous amino acids ASDGR. In certain embodiments, these five amino acids are contiguous amino acids SDGRG. In certain embodiments, these five amino acids are contiguous amino acids DGRGR. In certain embodiments, these five amino acids are contiguous amino acids GRGRP. In certain embodiments, these five amino acids are contiguous amino acids RGRPA. In certain embodiments, these five amino acids are contiguous amino acids GRPAF. In certain embodiments, these five amino acids are contiguous amino acids RPAFP. In certain embodiments, these five amino acids are contiguous amino acids PAFPF. In certain embodiments, these five amino acids are contiguous amino acids AFPFS.

[0095] In certain embodiments, the antibody binds to at least part of a sequence in FZD1, FZD3, FZD4,FZD5, FZD6, FZD8, FZD9 and/or FZD 10 that corresponds to the sequence PGASDGRGRPAFPFS (SEQ ID NO: 3) in FZD7.

[0096] In certain embodiments, the FZD-binding antibody specifically binds to the sequence DGSGGPGGGPTAYPTA (SEQ ID NO: 4) within human frizzled receptor 7. In certain embodiments, the FZD-binding antibody specifically binds to a sequence comprising at least 5 amino acids of the sequence DGSGGPGGGPTAYPTA (SEQ ID NO: 4) within a human frizzled receptor. In certain embodiments, these five amino acids are contiguous. In certain embodiments, these five amino acids are not contiguous. In certain embodiments, these five amino acids are contiguous amino acids DGSGG. In certain embodiments, these five amino acids are contiguous amino acids GSGGP. In certain embodiments, these five amino acids are contiguous amino acids SGGPG. In certain embodiments, these five amino acids are contiguous amino acids GGPGG. In certain embodiments, these five amino acids are contiguous amino acids GPGGG. In certain embodiments, these five amino acids are contiguous amino acids PGGGP. In certain embodiments, these five amino acids are contiguous amino acids GGGPT. In certain embodiments, these five amino acids are contiguous amino acids GGPTA. In certain embodiments, these five amino acids are contiguous amino acids GPTAY. In certain embodiments, these five amino acids are contiguous amino acids PTAYP. In certain embodiments, these five amino acids are

contiguous amino acids TAYPT. In certain embodiments, these five amino acids are contiguous amino acids AYPTA. In one embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 289-6. In another embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 289-12. In another embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 289-18. In another embodiment the FZD-binding antibody may be the antibody produced by the hybridoma cell line 289-3.I n another embodiment the FZD-antibody may be the antibody produced by the hybridoma cell line number 13.

[0097] In certain embodiments, the FZD-binding antibody binds to at least part of a sequence in FZD1, FZD3, FZD4,FZD5, FZD6, FZD8, FZD9 and/or FZD 10 that corresponds to the sequence DGSGGPGGGPTAYPTA (SEQ ID NO: 4) in FZD7.

[0098] In certain embodiments, the FZD-binding antibody inhibits binding of a ligand (e.g, a Wnt) to a portion of the FZD receptor (e.g, the BBS of the FZD). In certain embodiments, the FZD-binding antibody inhibits binding of a signaling molecule (e.g, dishevelled (Dvl), an intracellular protein) to a portion of the FZD receptor (e.g, the cytosolic portion).

[0099] In certain embodiments, the FZD-binding antibody is an IgG1 antibody. In certain embodiments, the FZD-binding antibody is an IgG2 antibody. In certain embodiments, the FZD-binding antibody is an IgG3 antibody. In certain embodiments, the FZD-binding antibody is an IgG4 antibody. In certain embodiments, the FZD-binding antibody is an IgA antibody. In certain embodiments, the FZD-binding antibody is an IgD antibody. In certain embodiments, the FZD-binding antibody is an IgE antibody. In certain embodiments, the FZD-binding antibody is an IgM antibody. In certain embodiments, the antibody is a monoclonal antibody. In certain embodiments, the antibody is a human antibody or a humanized antibody. In certain embodiments, the antibody is a chimeric antibody or a chimeric/humanized antibody. In certain embodiments, the antibody is an antibody fragment.

[00100] The antibodies of the present invention can be assayed for specific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as BIAcore analysis, FACS analysis, immunofluorescence, immunocytochemistry, West-ern blots, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitation reac-tions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well known in the

art.

[00101] For example, the specific binding of an antibody to a human frizzled receptor may be determined using ELISA.

[00102] An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding the FZD-binding antibody conjugated to a detectable compound such as an enzymatic substrate (e.g. horseradish peroxidase or alkaline phosphatase) to the well, incubating for a period of time and detecting the presence of the antigen. In some embodiments, the FZD-binding antibody is not conjugated to a detectable compound, but instead a second conjugated antibody that recognizes the FZD-binding antibody is added to the well. In some embodiments, instead of coating the well with the antigen, the FZD-binding antibody can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of the antigen to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art.

[00103] The binding affinity of an antibody to a human frizzled receptor and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled anti-gen (e.g. ^{3}H or ^{125}I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen followed by the detection of the antibody bound to the labeled antigen. The affinity of the antibody against a frizzled receptor and the binding off-rates can be determined from the data by scatchard plot analysis. In some embodiments, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies that bind one or more human frizzled receptors. BIAcore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized FZD antigens on their surface.

[00104] In certain embodiments, the antibody is an antagonist of at least one human frizzled receptor (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 FZDs) bound by the antibody. In certain embodiments, the antibody inhibits at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100% of one or more activity of the bound human frizzled receptor.

[00105] In certain embodiments, the FZD-binding antibody inhibits binding of a ligand to at least one human frizzled receptor. In certain embodiments, the FZD-binding antibody inhibits binding of a ligand to the Biological Binding site (BBS) of the human frizzled receptor. In

certain embodiments, the ligand is a human Wnt protein. In certain embodiments, the inhibition of binding of a particular ligand to a particular human frizzled protein provided by the FZD-binding antibody is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, an antibody that inhibits binding of a ligand such as a Wnt to a FZD, further inhibits Wnt signaling (e.g, inhibits canonical Wnt signaling).

[00106] In certain embodiments, the FZD-binding antibody inhibits Wnt signaling. It is understood that a FZD-binding antibody that inhibits Wnt signaling may, in certain embodiments, inhibit signaling by one or more Wnts, but not necessarily by all Wnts. In certain alternative embodiments, signaling by all human Wnts may be inhibited. In certain embodiments, signaling by one or more Wnts selected from the group consisting of WNT 1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A (previously WNT 14), WNT9B (previously WNT15), WNT10A, WNT10B, WNT11, and WNT16 is inhibited. In certain embodiments, the Wnt signaling that is inhibited is signaling by WNT1, WNT2, WNT3, WNT3A, WNT7a, WNT7b, and/or WNT10B. In certain embodiments, the antibody inhibits signaling by (at least) WNT1, WNT3A, WNT7b, and WNT10B. In particular embodiments, the antibody inhibits signaling by (at least) WNT3A. In certain embodiments, the inhibition of signaling by a Wnt provided by the FZD-binding antibody is a reduction in the level of signaling by the Wnt of least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain embodiments, the Wnt signaling that is inhibited is canonical Wnt signaling. In certain embodiments, the Wnt signaling that is inhibited is non-canonical Wnt signaling.

[00107] *In vivo* and *in vitro* assays for determining whether a FZD-binding antibody inhibits Wnt signaling are known in the art. For example, cell-based, luciferase reporter assays utilizing a TCF/Luc reporter vector containing multiple copies of the TCF-binding domain upstream of a firefly luciferase reporter gene may be used to measure canonical Wnt signaling levels in vitro. The level of Wnt signaling in the presence of one or more Wnts with the FZD-binding antibody present is compared to the level of signaling without the FZD-binding antibody present. In addition to the TCF/luc reporter assay, the effect of a FZD-binding antibody on canonical Wnt signaling may be measured in vitro or in vivo by measuring the effect of the antibody on the level of expression of beta-catenin (as demonstrated in Figure 6) or beta-catenin-regulated genes, such as c-myc.

[00108] In certain embodiments, the effect of an antibody on Wnt signaling may also be assessed by measuring the effect of the antibody on the phosphorylation state of Dishevelled-1, Dishevelled-2, Dishevelled-3, LRP5, LRP6, and/or beta-catenin. In still further embodiments, the effect of a FZD-binding antibody on Wnt signaling is determined by assessing the impact of the FZD-binding antibody on the expression level of one or more genes in a Wnt signature.

[00109] In certain embodiments, the FZD-binding antibodies have one or more of the following effects: inhibit proliferation of tumor cells, reduce the tumorigenecity of a tumor by reducing the frequency of cancer stem cells in the tumor, inhibit tumor growth, increase survival, trigger cell death of tumor cells, differentiate tumorigenic cells to a non-tumorigenic state, or prevent metastasis of tumor cells.

[00110] In certain embodiments, antibodies that specifically bind one or more human frizzled receptors trigger cell death via a conjugated toxin, chemotherapeutic antibody, radioisotope, or other such antibody. For example, in certain embodiments, an antibody to a human frizzled antibody is conjugated to a toxin that is activated in tumor cells expressing the FZD by protein internalization. In certain alternative embodiments, the antibody is not conjugated to a toxin, chemotherapeutic antibody, or radioisotope.

[00111] In another embodiment, the antibodies of the invention are antibody "fusion proteins," sometimes referred to herein as "antibody conjugates." The fusion partner or conjugate partner can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the antibody and on the conjugate partner. Conjugate and fusion partners may be any molecule, including small molecule chemical compounds and polypeptides. Possible conjugate partners include but are not limited to cytokines, cytotoxic antibodies, toxins, radioisotopes, chemotherapeutic antibody, anti-angiogenic antibodies, tyrosine kinase inhibitors, and other therapeutically active antibodies. The designation of an antibody as a fusion or conjugate is not meant to constrain it to any particular embodiment of the present invention. Rather, these terms are used to convey the broad concept that any antibody of the present invention may be linked genetically, chemically, or otherwise, to one or more polypeptides or molecules to provide some desirable property.

[00112] Suitable conjugates include, but are not limited to, drugs and cytotoxic antibodies including, for example, cytotoxic drugs (e.g., chemotherapeutic antibodies) or toxins or active fragments of such toxins. Cytotoxic antibodies also include radiochemicals made by conjugating radioisotopes to antibodies, or binding of a radionuclide to a chelating antibody that has been covalently attached to the antibody.

[00113] In certain embodiments, the FZD-binding antibodies are capable of inhibiting tumor growth. In certain embodiments, the FZD-binding antibodies are capable of inhibiting tumor growth in vivo (e.g, in a xenograft mouse model and/or in a human having cancer).

[00114] In certain embodiments, the FZD-binding antibodies are capable of reducing the tumorigenicity of a tumor. In certain embodiments, the antibody or antibody is capable of reducing the tumorigenicity of a tumor comprising cancer stem cells in an animal model, such as a mouse xenograft model. In certain embodiments, the number or frequency of cancer stem cells in a tumor is reduced by at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold. In certain embodiments, the reduction in the number or frequency of proliferating cancer cells is determined by a tumor cell proliferation assay. An example of a proliferation inhibition assay (MTS) used to test the efficacy of an anti-FZD antibody is provided in Example 2, below. In certain embodiments, the reduction in the number or frequency of proliferating cancer cells is determined by a tumor cell viability assay. An example of a viability assay (trypan blue) used to test the efficacy of an anti-FZD antibody is provided in Example 3, below. In certain embodiments, the reduction in the number or frequency of proliferating cancer cells is determined by a tumor cell apoptosis assay. An example of an apoptosis assay (annexin V staining) used to test the efficacy of an anti-FZD antibody is provided in Example 4, below.

[00115] In certain embodiments, antibodies to human frizzled receptors mediate cell death of a cell expressing the FZD protein via antibody-dependent cellular cytotoxicity (ADCC). ADCC involves cell lysis by effector cells that recognize the Fc portion of an antibody. Many lymphocytes, monocytes, tissue macrophages, granulocytes and eosinophils.

[00116] In certain embodiments, antibodies to one or more FZDs trigger cell death of a cell expressing the FZD protein (s) by activating complement-dependent cytotoxicity (CDC).

[00117] The ability of any particular antibody against one or more FZDs to mediate lysis of the target cell by complement activation and/or ADCC can be assayed. The cells of interest are grown and labeled in vitro; the antibody is added to the cell culture in combination with either serum complement or immune cells which can be activated by the antigen antibody complexes. Cytolysis of the target cells is detected, for example, by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the in vitro test can then be used therapeutically in that particular patient.

[00118] In certain embodiments, the FZD-binding antibody has a circulating half-life in

mice, cynomologous monkeys, or humans of at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain embodiments, the FZD-binding antibody is an IgG antibody that has a circulating half-life in mice, cynomologous monkeys, or humans of at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. Methods of increasing the half-life of antibodies such as polypeptides and antibodies are known in the art. For example, known methods of increasing the circulating half-life of IgG antibodies include the introduction of mutations in the Fc region which increase the pH-dependent binding of the antibody to the neonatal Fc receptor (FcRn) at pH 6.0. Known methods of increasing the circulating half-life of antibody fragments lacking the Fc region include such techniques as PEGylation.

[00119] Polyclonal antibodies can be prepared by any known method. Polyclonal antibodies are raised by immunizing an animal (e.g. a rabbit, rat, mouse, donkey, etc) by multiple subcutaneous or intraperitoneal injections of the relevant antigen (a purified peptide fragment, full-length recombinant protein, fusion protein, etc) optionally conjugated to keyhole limpet hemocyanin (KLH), serum albumin, etc. diluted in sterile saline and combined with an adjuvant (e.g. Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. The polyclonal antibody is then recovered from blood, ascites and the like, of an animal so immunized. Collected blood is clotted, and the serum decanted, clarified by centrifugation, and assayed for antibody titer. The poly-clonal antibodies can be purified from serum or ascites according to standard methods in the art including affinity chromatography, ion-exchange chromatography, gel electrophoresis, dialysis, etc.

[00120] Monoclonal antibodies can be prepared using hybridoma methods. Using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit the production by lymphocytes of antibodies that will specifically bind to an immunizing antigen. Lymphocytes can also be immunized in vitro. Following immunization, the lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol, to form hybridoma cells that can then be selected away from unfused lymphocytes and myeloma cells. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen as determined by immunoprecipitation, immunoblotting, or by an in vitro binding assay (e.g. radioimmunoassay (RIA); enzyme-linked immunosorbent assay (ELISA)) can then be propagated either in vitro culture using standard methods or in vivo as ascites tumors in an animal. The monoclonal antibodies can then be

purified from the culture medium or ascites fluid as described for polyclonal antibodies above.

[00121] Alternatively monoclonal antibodies can also be made using recombinant DNA methods. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using conventional procedures. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors, which when transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, monoclonal antibodies are generated by the host cells. Also, recombinant monoclonal antibodies or fragments thereof of the desired species can be isolated from phage display libraries expressing CDRs of the desired species as described.

[00122] The polynucleotide(s) encoding a monoclonal antibody can further be modified in a number of different manners using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

[00123] In some embodiments, the monoclonal antibody against the human frizzled receptor(s) is a humanized antibody. In certain embodiments, such antibodies are used therapeutically to reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. Humanized antibodies can be produced using various techniques known in the art. A common method for producing humanized antibodies is to graft CDR sequences from a MAb (produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells which in turn produce a functional Ab that is secreted by the CHO cells. The methods described within this application are also useful for generating genetic alterations within Ig genes or chimeric Igs transfected within host cells. Humanized antibodies can also be made in transgenic mice containing human immunoglobulin loci that are capable upon immunization of producing the full repertoire of human antibodies in the absence of endogenous

immunoglobulin production.

[00124] In certain alternative embodiments, the antibody to the human frizzled receptor(s) is a human antibody.

[00125] Human antibodies can be directly prepared using various techniques known in the art. Immortalized human B lymphocytes immunized in vitro or isolated from an immunized individual that produce an antibody directed against a target antigen can be generated Also, the human antibody can be selected from a phage library, where that phage library expresses human antibodies.

[00126] This invention also encompasses bispecific antibodies that specifically recognize a human frizzled receptor. Bispecific antibodies are antibodies that are capable of specifically recognizing and binding at least two different epitopes. The different epitopes can either be within the same molecule (e.g. the same human frizzled receptor) or on different molecules such that bispecific the antibodies can specifically recognize and bind a human frizzled receptor as well as, for example, 1) an effector molecule on a leukocyte such as a T-cell receptor (e.g. CD3) or Fc receptor (e.g. CD64, CD32, or CD16) or 2) a cytotoxic antibody as described in detail below. In certain embodiments, the bispecific antibody specifically binds at least one human frizzled receptor, as well as either VEGF, a Notch ligand, such as a delta-like ligand (for example, DLL4) or jagged, or at least one Notch receptor selected from the group consisting of Notch 1, Notch 2, Notch 3, and Notch 4. Bispecific antibodies can be intact antibodies or antibody fragments.

[00127] Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in a polypeptide of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic antibodies to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic antibody or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Techniques for making bispecific antibod-ies are common in the art. Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Thus, in certain embodiments the antibodies to human frizzled receptor(s) are multispecific.

[00128] In certain embodiments, the antibodies described herein may be monospecific. For

example, in certain embodiments, each of the one or more antigen-binding sites that an antibody contains is capable of binding the same one or more human FZD receptors (e.g, FZDl, FZD2, FZD5, FZD7, or FZD8, or a homologous epitope on some combination of the FZDs). In certain embodiments, an antigen-binding site of a monospecific antibody described herein is capable of binding one, two, three, four, or five (or more) human frizzled receptors.

[00129] According to the present invention, techniques can be adapted for the production of single-chain antibodies specific to one or more human frizzled receptors. In addition, methods can be adapted for the construction of Fab expression libraries to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a FZD receptor, or derivatives, fragments, analogs or homologs thereof. Antibody fragments may be produced by techniques in the art including, but not limited to: (a) a F(ab')2 fragment produced by pepsin digestion of an antibody molecule; (b) a Fab fragment generated by reducing the disulfide bridges of an F(ab')2 fragment, (c) a Fab fragment generated by the treatment of the antibody molecule with papain and a reducing antibody, and (d) Fv fragments.

[00130] In certain embodiments, It can further be desirable, especially in the case of antibody fragments, to modify an antibody in order to increase its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis).

[00131] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells. It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking antibodies. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond.

[00132] For the purposes of the present invention, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the polypeptides of a human FZD receptor. In this regard, the variable region may comprise or be derived from any type of mammal that can be induced to mount a humoral response and generate immunoglobulins against the desired tumor associated antigen. As such, the variable region of the modified antibodies can be, for example, of human, murine, non-human primate (e.g. cynomolgus monkeys, macaques, etc.) or lupine origin. In some

embodiments both the variable and constant regions of the modified immunoglobulins are human. In other embodiments the variable regions of compatible antibodies (usually derived from a non-human source) can be engineered or specifically tailored to improve the binding properties or reduce the immunogenicity of the molecule. In this respect, variable regions useful in the present invention can be humanized or otherwise altered through the inclusion of imported amino acid sequences.

[00133] In certain embodiments, the variable domains in both the heavy and light chains are altered by at least partial replacement of one or more CDRs and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the antigen binding site.

[00134] Alterations to the variable region notwithstanding, those skilled in the art will appreciate that the modified antibodies of this invention will comprise antibodies (e.g., full-length antibodies or immunoreactive fragments thereof) in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as increased tumor localization or reduced serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some embodiments, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region compatible with this invention comprise additions, deletions or substitutions of one or more amino acids in one or more domains. That is, the modified antibodies dis-closed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some embodiments, modified constant regions wherein one or more domains are partially or entirely deleted are contemplated. In some embodiments, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (ΔCH2 constructs). In some embodiments, the omitted constant region domain will be replaced by a short amino acid spacer (e.g. 10 residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

[00135] In certain embodiments, the FZD-binding antibodies provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it may be that constant region modifications, consistent with this invention, moderate complement binding and thus reduce the serum half-life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region may be used to eliminate disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. Similarly, modifications to the constant region in accordance with this invention may easily be made using well known biochemical or molecular engineering techniques well within the purview of the skilled artisan.

[00136] In certain embodiments, a FZD-binding antibody that is an antibody does not have one or more effector functions. For instance, in some embodiments, the antibody has no antibody-dependent cellular cytoxicity (ADCC) activity and/or no complement-dependent cytoxicity (CDC) activity. In certain embodiments, the antibody does not bind to an Fc receptor and/or complement factors. In certain embodiments, the antibody has no effector function.

[00137] It will be noted that in certain embodiments, the modified antibodies may be engineered to fuse the CH3 domain directly to the hinge region of the respective modified antibodies. In other constructs it may be desirable to provide a peptide spacer between the hinge region and the modified CH2 and/or CH3 domains. For example, compatible constructs could be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added, for instance, to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible. However, it should be noted that amino acid spacers can, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct. Accordingly, in certain embodiments, any spacer added to the construct will be relatively non-immunogenic, or even omitted altogether, so as to maintain the desired biochemical qualities of the modified antibodies.

[00138] Besides the deletion of whole constant region domains, it will be appreciated that the antibodies of the present invention may be provided by the partial deletion or substitution

of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase tumor localization. Similarly, it may be desirable to simply delete that part of one or more constant region domains that control the effector function (e.g. complement CLQ binding) to be modulated. Such partial deletions of the constant regions may improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g. Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. Certain embodiments can comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as decreasing or increasing effector function or provide for more cytotoxin or carbohydrate attachment. In such embodiments it can be desirable to insert or replicate specific sequences derived from selected constant region domains.

[00139] The present invention further embraces variants and equivalents which are substantially homologous to the chimeric, humanized and human antibodies, or antibody fragments thereof, set forth herein. These can contain, for example, conservative substitution mutations, i.e. the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art.

[00140] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic antibody. Cytotoxic antibodies include chemotherapeutic antibodies, growth inhibitory antibodies, toxins (e.g, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), radioactive isotopes (i.e., a radioconjugate), etc. Chemotherapeutic antibodies useful in the generation of such immunoconjugates include, for example, methotrexate, adriamicin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating antibodies. Enzymati-cally active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria

toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii pro-teins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, andthetricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies including 212Bi, 1311, 131In, 90Y, and 186Re. Conjugates of the antibody and cytotoxic antibody are made using a variety of bifunctional protein-coupling antibodies such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional deriva-tives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diiso-cyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as l,5-difluoro-2,4-dinitroben-zene). Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, can also be used.

[00141] Conjugate antibodies are composed of two covalently joined antibodies. It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking antibodies. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioetherbond. Examples for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[00142] Regardless of how useful quantities are obtained, the antibodies of the present invention can be used in any one of a number of conjugated (i.e. an immunoconjugate) or unconjugated forms. Alternatively, the antibodies of this invention can be used in a nonconjugated or "naked" form. In certain embodiments, the antibodies are used in nonconjugated form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity (CDC) and antibody dependent cellular toxicity (ADCC) to eliminate the malignant cells. In some embodiments, the antibodies can be conjugated to radioisotopes, such as 90Y, 1251,131I, 123I, 111In, 105Rh, 153Sm, 67Cu, 67Ga, 166Ga, 166Ho, 177Lu, 186Re and 188Re using anyone of a number of well-known chelators or direct labeling. In other embodiments, the dis-closed compositions can comprise antibodies coupled to drugs, prodrugs or biological response modifiers such as methotrexate, adriamycin, and lymphokines such as interferon. Still other embodiments of the present invention comprise the

use of antibodies conjugated to specific biotoxins such as ricin or diphtheria toxin. In yet other embodiments the modified antibodies can be complexed with other immunologically active ligands (e.g. antibodies or fragments thereof) wherein the resulting molecule binds to both the neoplastic cell and an effector cell such as a T cell. The selection of which conjugated or unconjugated modified antibody to use will depend of the type and stage of cancer, use of adjunct treatment (e.g., chemotherapy or external radiation) and patient condition. It will be appreciated that one skilled in the art could readily make such a selection in view of the teachings herein.

[00143] The antibodies of the present invention can be recombinant antibodies, natural antibodies, or synthetic antibodies or fragment thereof, against a human FZD receptor. It will be recognized in the art that some amino acid sequences of the invention can be varied without significant effect of the structure or function of the protein. Thus, the invention further includes variations of the polypeptides which show substantial activity or which include regions of an antibody, or fragment thereof, against a human FZD receptor protein. Such mutants include deletions, insertions, inversions, repeats, and type substitutions.

[00144] The polypeptides and analogs can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half-life or absorption of the protein. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like.

[00145] The isolated antibodies described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable trans-formed host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof.

[00146] In some embodiments a DNA sequence encoding an antibody of interest would be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligo-nucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer

containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly. Once assembled (by synthesis, site-directed mutagenesis or another method), the polynucleotide sequences encoding a particular isolated polypeptide of interest will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction map-ping, and expression of a biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00147] In certain embodiments, recombinant expression vectors are used to amplify and express DNA encoding antibodies, or fragments thereof, against human frizzled receptors. Recombinant expression vectors are replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an anti-FZD antibody, or fragment thereof, operatively linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences, as described in detail below. Such regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are operatively linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. Structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without

a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00148] The choice of expression control sequence and expression vector will depend upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *Esherichia coli*, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as Ml 3 and filamentous single-stranded DNA phages.

[00149] Suitable host cells for expression of a FZD-binding polypeptide or antibody (or a FZD protein to use as an antigen) include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *bacilli*. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are known in the art.

[00150] Various mammalian or insect cell culture systems are also advantageously employed to express recombinant protein. Expression of recombinant proteins in mammalian cells can be performed because such proteins are generally correctly folded, appropriately modified and completely functional. Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells and other cell lines capable of expressing an appropriate vector including, for example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines.

[00151] In another embodiment, the invention provides a method of producing an antibody comprising culturing the host cell comprising the vector comprising the nucleic acid sequence encoding for the antibodies of the invention under conditions permitting expression of the antibody. Following expression in a host cell maintained in a suitable medium, the polypeptide or peptide to be expressed, such as that encoding the antibodies of the invention, antibody equivalents thereof, maybe isolated from the medium, and purified by methods known in the art. If the polypeptide or peptide is not secreted into the culture medium, the host cells are lysed prior to isolation and purification.

[00152] The present invention further provides methods of screening antibodies for efficacy in inhibiting Wnt signaling, for anti-tumor efficacy, and/or efficacy against cancer stem cells. These methods include, but are not limited to, methods comprising comparing the levels of one or more differentiation markers in a first solid tumor that has been exposed to the antibody relative to the levels of the one or more differentiation markers in a second solid tumor that has not been exposed to the antibody. In certain embodiments, these methods include (a) exposing a first solid tumor, but not a second solid tumor, to the antibody; (b) assessing the levels of one or more differentiation markers in the first and second solid tumors; and (c) comparing the levels of the one or more differentiation markers in the first and second solid tumors. In certain embodiments, the antibody is an inhibitor of the canonical Wnt signaling pathway, and/or inhibits binding of one or more human Wnt proteins to one or more human frizzled receptors. In certain embodiments, the antibody is an antibody that specifically binds to one or more human frizzled receptor. In certain embodiments, increased levels of one or more differentiation markers in the first solid tumor relative to the second solid tumor indicates efficacy against solid tumor stem cells. In certain alternative embodiments, decreased levels of one or more differentiation markers (i.e., negative markers for differentiation) in the first solid tumor relative to the second solid tumor indicates efficacy against solid tumor stem cells. In certain embodiments, the solid tumor is a pancreatic tumor. In certain embodiments, the solid tumor is a pancreatic tumor and the one or more differentiation markers may comprise one or more mucins (e.g, Mucl 6) and/or chromogranin A (CHGA). In certain alternative embodiments, the solid tumor is a colon tumor. In some embodiments, the solid tumor is a colon tumor and the one or more differentiation markers comprise cytokeratin 7. Other potential differentiation markers for pancreas and colon as well as other tumor types are known to those skilled in the art. The usefulness of potential differentiation markers in a screening method can be readily assessed by one skilled in the art by treating the desired tumor type with one or more of the anti-FZD antibodies disclosed herein such as 18R5 and/or 44R24 and then assessing for changes in expression of the marker by the treated tumor relative to control. Non-limiting examples of such methods, can for instance, be found in the specific Examples below.

[00153] In certain embodiments, the invention encompasses polynucleotides comprising polynucleotides that encode the antibodies of the invention or antigen-binding portions thereof. The polynucleotides of the invention can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-

stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

[00154] In certain embodiments, the polynucleotides are isolated. In certain embodiments, the polynucleotides are substantially pure.

[00155] In certain embodiments, variants of the polynucleotides of the invention can contain alterations in the coding regions, non-coding regions, or both. In some embodiments the polynucleotide variants contain alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. In some embodiments, nucleotide variants are produced by silent substitutions due to the degeneracy of the genetic code. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli). Vectors and cells comprising the polynucleotides described herein are also provided.

[00156] The FZD-binding antibodies of the invention are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In certain embodiments, the antibodies are useful for inhibiting Wnt signaling (e.g., canonical Wnt signaling), inhibiting tumor growth, inducing differentiation, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use may be in vitro, ex vivo, or in vivo methods. In certain embodiments, the FZD-binding antibody or polypeptide or antibody is an antagonist of the one or more human frizzled receptors to which it binds.

[00157] In certain embodiments, the FZD-binding antibodies or antagonists are used in the treatment of a disease associated with Wnt signaling activation. In particular embodiments, the disease is a disease dependent upon Wnt signaling. In particular embodiments, the Wnt signaling is canonical Wnt signaling. In certain embodiments, the FZD-binding antibodies or antagonists are used in the treatment of disorders characterized by increased levels of stem cells and/or progenitor cells.

[00158] In certain embodiments, the disease treated with the FZD-binding antibody is a cancer. In certain embodiments, the cancer is characterized by Wnt-dependent tumors. In certain embodiments, the cancer is characterized by tumors expressing one or more frizzled receptors to which the FZD-binding antibody binds. In certain embodiments, the cancer is characterized by tumors expressing one or more genes in a Wnt gene signature.

[00159] In certain embodiments, the disease treated with the FZD-binding antibody or antagonist is not a cancer. For example, the disease may be a metabolic disorder such as obesity or diabetes. Alternatively, the disease may be a bone disorder such as osteoporosis,

osteoarthritis, or rheumatoid arthritis. The disease may also be a kidney disorder, such as a polycystic kidney disease. Alternatively, eye disorders including, but not limited to, macular degeneration and familial exudative vitreoretinopathy may be treated. Cardiovascular disorders, including myocardial infarction, atherosclerosis, and valve disorders, may also be treated. In some embodiments, the disease is a pulmonary disorder such as idiopathic pulmonary arterial hypertension or pulmonary fibrosis. In some embodiments, the disease treated with the FZD-binding antibody is a liver disease, such as cirrhosis or liver fibrosis.

[00160] The present invention provides for methods of treating cancer comprising administering a therapeutically effective amount of a FZD-binding antibody to a subject (e.g., a subject in need of treatment). In certain embodiments, the cancer is a cancer selected from the group consisting of colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, liver cancer, breast cancer, kidney cancer (e.g. Wilms' tumor), prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, and head and neck cancer. In certain embodiments, the cancer is pancreatic cancer. In another embodiment, the condition is an ovarian cancer. In another embodiment, the condition is a lung cancer. In another embodiment, the condition is "lung" + "ovary" tumor. In another embodiment, the condition is a tumor metastasized from lung cancer. In another embodiment, the condition is a non-small cell lung cancer (NSCLC). In another embodiment, the condition is an NSCLC-Bev eligible (Avastin). In another embodiment, the condition is an NSCLC-Bev ineligible (Avastin). In another embodiment, the condition is a prostate cancer. In another embodiment, the condition is a tumor metastasized from prostate cancer. In another embodiment, the condition is a benign prostatic hyperplasia. In another embodiment, the condition is a hormone refractory prostate cancer (HRPC In another embodiment, the condition is a bladder cancer. In another embodiment, the condition is a gall bladder cancer. In another embodiment, the condition is a bone cancer. In another embodiment, the condition is a cervical cancer. In another embodiment, the condition is an adrenal cortical cancer. In another embodiment, the condition is an adrenal cancer. In another embodiment, the condition is a retinal cancer. In another embodiment, the condition is a retinoblastoma. In another embodiment, the condition is a gastric cancer. In another embodiment, the condition is aneuroendocrine cancer. In another embodiment, the condition is a bile duct cancer, for example, cholangiocarcinoma. In another embodiment, the condition is a myeloma. In another embodiment, the condition is an androgen-dependent tumor. In another embodiment, the condition is an androgen-independent tumor. In another embodiment, the condition is an acromegaly. In another embodiment, the

condition is a synovial sarcoma. In another embodiment, the condition is a diarrhea associated with metastatic carcinoid. In another embodiment, the condition is a vasoactive intestinal peptide secreting tumor. In another embodiment, the condition is a gigantism. In another embodiment, the condition is a psoriasis. In another embodiment, the condition is an atherosclerosis. In another embodiment, the condition is a smooth muscle restenosis of blood vessels. In another embodiment, the condition is an inappropriate microvascular proliferation. In another embodiment, the condition is a neuroblastoma. In another embodiment, the condition is a glioblastoma. In another embodiment, the condition is embryonal (EMB) carcinoma. In another embodiment, the condition is ALV. In another embodiment, the condition is a medulloblastoma. In another embodiment, the condition is ependymoma. In another embodiment, the condition is aHCC / hepatocellular cancer. In another embodiment, the condition is a hepatoblastoma. In another embodiment, the condition is Wilms' cancer. In another embodiment, the condition is Ewing cancer. In another embodiment, the condition is a Rhabdoid. In another embodiment, the condition is leukemia. In another embodiment, the condition is an esophageal. In another embodiment, the condition is a pediatric solid tumor, specifically including Ewing's/PNET. In another embodiment, the condition is an adult solid tumor. In another embodiment, the condition is an osteosarcoma. In another embodiment, the condition is a rhabdomyosarcoma (RMS). In another embodiment, the condition is a soft tissue sarcoma. In another embodiment, the condition is a soft tissue sarcoma including embryonal and alveolar rhabdomyosarcoma, GIST, alveolar soft part sarcoma, and clear cell sarcoma. In another embodiment, the condition is a thymoma. In another embodiment, the condition is a thymic carcinoma. In certain embodiments, the cancer is colorectal cancer. In certain embodiments, the subject is a human.

[00161] In some embodiments, the invention provides a method for treating a tumor in a subject comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor. The antibody of this method may bind to the cytoplasmic portion of the receptor and, optionally, to the transmembrane portion of the receptor. In one embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-1. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-2. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-5.

[00162] A method for treating a tumor in a subject, said method comprising the step of

administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

[00163] In some embodiments, the invention provides a method for treating a tumor in a subject comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on a frizzled receptor. In one embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-1. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-2. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-5. In one embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor expression. In another embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor activity.

[00164] In some embodiments, the invention provides a method for treating a tumor in a subject comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on a frizzled receptor. In one embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 288-4. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 289-5. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line number 4. In one embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor expression. In another embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor activity.

[00165] In some embodiments, the invention provides a method for treating a tumor in a subject comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on a

frizzled receptor. In one embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor expression. In another embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor activity.

[00166] In some embodiments, the invention provides a method for treating a tumor in a subject comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:4 is present on a frizzled receptor. In one embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 289-6. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 289-18. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 289-12. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line 289-3. In another embodiment, the antibody used in this method may be the antibody produced by the hybridoma cell line number 13. In one embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor expression. In another embodiment, treatment with antibody may be upon detection of an abnormal (e.g. high) level of frizzled receptor activity.

[00167] Treatment means any treatment of a disease in a mammal and includes: (1) preventing the disease from occurring in a mammal which may be predisposed to the disease but does not yet experience or display symptoms of the disease; e.g. prevention of the outbreak of the clinical symptoms; (2) inhibiting the disease, e.g., arresting its development; or (3) relieving the disease, e.g., causing regression of the symptoms of the disease.

[00168] Effective dosage for the treatment of a disease means that amount which, when administered to a mammal in need thereof, is sufficient to effect treatment, as defined above, for that disease. The method of treatment described herein can be used to treat any suitable mammal, preferably the mammal is a human.

[00169] The present invention further provides methods for inhibiting tumor growth using the antibodies or other antibodies described herein. In certain embodiments, the method of inhibiting the tumor growth comprises contacting the cell with a FZD-binding antibody in vitro. For example, an immortalized cell line or a cancer cell line that expresses the targeted FZD(s) is cultured in medium to which is added the antibody or other antibody to inhibit tumor growth. In some embodiments, tumor cells are isolated from a patient sample such as, for

example, a tissue biopsy, pleural effusion, or blood sample and cultured in medium to which is added an FZD-binding antibody to inhibit tumor growth.

[00170] In some embodiments, the method of inhibiting tumor growth comprises contacting the tumor or tumor cells with the FZD-binding antibody in vivo. In certain embodiments, contacting a tumor or tumor cell with a FZD-binding antibody is undertaken in an animal model. For example,

[00171] FZD-binding antibodies may be administered to xenografts expressing one or more FZDs that have been grown in immunocompromised mice (e.g. NOD/SCID mice) to inhibit tumor growth. In some embodiments, cancer stem cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and injected into immunocompromised mice that are then administered a FZD-binding antibody to inhibit tumor cell growth. In some embodiments, the FZD-binding antibody is administered at the same time or shortly after introduction of tumorigenic cells into the animal to prevent tumor growth. In some embodiments, the FZD-binding antibody is administered as a therapeutic after the tumorigenic cells have grown to a specified size.

[00172] In certain embodiments, the method of inhibiting tumor growth comprises administering to a subject a therapeutically effective amount of a FZD-binding antibody. In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or has had a tumor removed. In certain embodiments, the tumor is a tumor in which Wnt signaling is active. In certain embodiment, the Wnt signaling that is active is canonical Wnt signaling. In certain embodiments, the tumor is a Wnt-dependent tumor. For example, in some embodiments, the tumor is sensitive to axin over-expression. In certain embodiments, the tumor does not comprise an inactivating mutation (e.g, a truncating mutation) in the adenomatous polyposis coli (APC) tumor suppressor gene or an activating mutation in the beta-catenin gene. In certain embodiments, the tumor expresses one or more genes in a Wnt gene signature. In certain embodiments, the cancer for which a subject is being treated involves such a tumor.

[00173] In certain embodiments, the tumor expresses the one or more human frizzled receptors to which the FZD-binding antibody or antibody binds. In certain embodiments, the tumor overexpresses the human frizzled receptor(s). In certain embodiments, the tumor is a tumor selected from the group consisting of colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor (e.g Wilms' tumor), prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and

neck tumor. In certain embodiments, the tumor is a colorectal tumor. In certain embodiments, the tumor is a pancreatic tumor.

[00174] The invention also provides a method of inhibiting Wnt signaling in a cell comprising contacting the cell with an effective amount of a FZD-binding antibody. In certain embodiments, the cell is a tumor cell. In certain embodiments, the method is an in vivo method wherein the step of contacting the cell with the antibody comprises administering a therapeutically effective amount of the antibody to the subject. In some alternative embodiments, the method is an in vitro or ex vivo method. In certain embodiments, the Wnt signaling that is inhibited is canonical Wnt signaling. In certain embodiments, the Wnt signaling is signaling by WNT1, WNT2, WNT3, WNT3A, WNT7a, WNT7b, and/or WNT10B. In certain embodiments, the Wnt signaling is signaling by WNT1, WNT3A, WNT7b, and/or WNT 1 OB.

[00175] In addition, the invention provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering a therapeutically effective amount of a FZD-binding antibody to the subject. In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the antibody. Thus, the invention also provides a method of reducing the frequency of cancer stem cells in a tumor, comprising contacting the tumor with an effective amount of a FZD-binding antibody. The invention further provides methods of differentiating tumorigenic cells into non-tumorigenic cells comprising contacting the tumorigenic cells with a FZD-binding antibody (for example, by administering the FZD-binding antibody to a subject that has a tumor comprising the tumorigenic cells or that has had such a tumor removed). In certain embodiments, the tumor is a pancreatic tumor. In certain other embodiments, the tumor is a colon tumor. In certain other embodiments, the tumor is a Wilms' tumor.

[00176] The use of the FZD-binding antibodies, or antigen-binding portions thereof, described herein to induce the differentiation of cells, including, but not limited to tumor cells, is also provided. For example, methods of inducing cells to differentiate comprising contacting the cells with an effective amount of a FZD-binding antibody (i.e, an anti-FZD antibody) described herein are envisioned. Methods of inducing cells in a tumor in a subject to differentiate comprising administering a therapeutically effective amount of a FZD-binding antibody, to the subject are also provided. In certain embodiments, the tumor is a pancreatic tumor. In certain other embodiments, the tumor is a colon tumor. In certain other embodiments, the tumor is a Wilms' tumor.

[00177] Methods of treating a disease or disorder in a subject, wherein the disease or disorder is associated with Wnt signaling activation and/or is characterized by an increased level of stem cells and/or progenitor cells are further provided. In some embodiments, the treatment methods comprise administering a therapeutically effective amount of the FZD-binding antibody, polypeptide, or antibody to the subject. In certain embodiments, the Wnt signaling is canonical Wnt signaling.

[00178] In another embodiment, the invention provides a method for treating a medical condition mediated by elevated expression or activity of Frizzled receptors. In one embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to SEQ ID NO:1 within FZD7. In one embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to SEQ ID NO:2 within FZD7 or FZD2.. In one embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to SEQ ID NO:3 within FZD7. In one embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to SEQ ID NO:4 within FZD7. In another embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to a transmembrane domain in the FZD7 receptor. In another embodiment, the method comprises the step of administering, to a subject, a therapeutically effective dose of the antibody that binds specifically to a cytoplasmic domain with the FZD7 receptor.

[00179] The present invention further provides pharmaceutical compositions comprising one or more of the FZD-binding antibodies described herein. In certain embodiments, the pharmaceutical compositions further comprise a pharmaceutically acceptable vehicle. These pharmaceutical compositions find use in inhibiting tumor growth and treating cancer in human patients.

[00180] In certain embodiments, formulations are prepared for storage and use by combining a purified antibody of the present invention with a pharmaceutically acceptable vehicle (e.g. carrier, excipient). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; anti-oxidants including ascorbic acid and methionine; preservatives (e.g. octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as

methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight polypeptides (e.g. less than about 10 amino acid resi-dues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating antibodies such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG).

[00181] The pharmaceutical compositions of the present invention can be administered in any number of ways for either local or systemic treatment. Administration can be topical (such as to mucous membranes including vaginal and rectal delivery) such as transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary (e.g, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal); oral; or parenteral including intravenous, intraarterial, subcutaneous, intraperi-toneal or intramuscular injection or infusion; or intracranial (e.g, intrathecal or intraventricular) administration.

[00182] The therapeutic formulation of the antibodies of the invention may be in, certain embodiments, in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories for oral, parenteral, or rectal administration or for administration by inhalation. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other diluents (e.g. water) to form a solid pre-formulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of the type described above. The tablets, pills, etc of the novel composition can be coated or otherwise compounded to pro-vide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac,

cetyl alcohol and cellulose acetate.

[00183] In certain embodiments, the antibodies may also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial poly-merization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions as described in the art.

[00184] In certain embodiments, pharmaceutical formulations include antibodies of the present invention complexed with liposomes. Liposomes with enhanced circulation time are known in the art. In addition sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles (e.g. films, or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[00185] In certain embodiments, in addition to administering the FZD-binding antibody, the method or treatment further comprises administering a second anti-cancer antibody (prior to, concurrently with, and/or subsequently to administration of the FZD-binding antibody). Pharmaceutical compositions comprising the FZD-binding antibody and the second anti-cancer antibody are also provided. In selected embodiments, the FZD-binding antibodies will be administered to patients that have previously undergone treatment with the second anti-cancer antibody. In certain other embodiments, the FZD-binding antibody and the second anti-cancer antibody will be administered substantially simultaneously or concurrently. For example, a subject may be given the FZD-binding antibody while undergoing a course of treatment with the second anti-cancer antibody (e.g, chemotherapy). In certain embodiments, the FZD-binding antibody will be administered within 1 year of the treatment with the second anti-cancer antibody. In certain alternative embodiments, the FZD-binding antibody will be administered within 10, 8, 6, 4, or 2 months of any treatment with the second anti-cancer antibody. In certain other embodiments, the FZD-binding antibody will be administered within 4, 3, 2, or 1 week of any treatment with the second anti-cancer antibody. In some embodiments, the FZD-binding antibody will be administered within 5, 4, 3, 2, or 1 days of

any treatment with the second anti-cancer antibody. It will further be appreciated that the two antibodies or treatment may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[00186] Useful classes of anti-cancer antibodies that may be administered in combination with the antibodies of the invention include, for example, antitubulin antibodies, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating antibodies (e.g, platinum complexes such as cis-platin, mono(platinum), bis (platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluori-nated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, performing compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second anti-cancer antibody is an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[00187] Anticancer antibodies that may be administered in combination with the FZD-binding antibodies include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the combined administration of an antibody of the present invention and a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. Treatment with an antibody can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Chemotherapies contemplated by the invention include chemical substances or drugs which are known in the art and are commercially available, such as Gemcitabine, Irinotecan, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Busulfan, Cytoxin, TAXOL, Methotrexate, Cisplatin, Melphalan, Vinblastine and Carboplatin. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active antibodies can exert their biological activities simultaneously.

[00188] Chemotherapeutic agents useful in the instant invention also include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CY-TOXAN); alkyl sulfonates such as busulfan, improsulfanand piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methy-lamelamines including altretamine, triethylenemelamine, tri-etylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime nitrogen mustards such as chloram-bucil, chlomaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide

hydrochloride, melphalan, novembichin, phenesterine, pred-nimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimus-tine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esoru-bicin, idarubicin, marcellomycin, mitomycins, mycophe-nolic acid, nogalamycin, olivomycins, peplomycin, potfiro-mycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrex-ate, pteropterin, trimetrexate; purine analogs such as fludara-bine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimi-dine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocit-abine, floxuridine, 5-FU; androgens such as calusterone, dro-mostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; acegla-tone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitra-crine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizo-furan; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacy-tosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gem-citabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblas-tine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; dif-luoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal antibodies that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxy tamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide,

bicalutamide, leu-prolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. [0282] In certain embodiments, the chemotherapeutic agentis a topoisomerase inhibitor. Topoi-somerase inhibitors include, but are not limited to, doxorubicin HCL, daunorubicin citrate, mitoxantrone HCL, actino-mycin D, etoposide, Topotecan HCL, teniposide (VM-26), and irinotecan. In certain embodiments, the second anticancer agent is irinotecan. In certain embodiments, the tumor to be treated is a colorectal tumor and the second anticancer agent is a topoisomerase inhibitor, such as irinotecan. In certain embodiments, the chemotherapeutic agent is an anti-metabolite. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, Pemetrexed, tegafur, cytosine arabinoside, THIOGUANINE (GlaxoSmithKline), 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, the second anticancer agent is gemcitabine. In certain embodiments, the tumor to be treated is a pancreatic tumor and the second anticancer agent is an anti-metabolite (e.g., gemcitabine). In certain embodiments, the chemotherapeutic agent is an antimitotic antibody, including, but not limited to, antibodies that bind tubulin. By way of non-limiting example, the agent comprises a taxane. In certain embodiments, the agent comprises paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain embodiments, the antibody is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (e.g., ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinka alkaloid, such as vincristine, binblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of Eg5 kinesin or an inhibitor of a mitotic kinase such as Aurora A or Plkl. In certain embodiments where the chemotherapeutic agent administered in combination with the FZD-binding antibody or polypeptide or antibody comprises an antimitotic agent.

[00189] In certain embodiments, the treatment of cancer or a tumor in a subject involves the combined administration of an antibody of the present invention and radiation therapy. Treatment with the antibody can occur prior to, concurrently with, or subsequent to administration of radiation therapy.

[00190] In some embodiments, the treatment can involve the combined administration of antibodies of the present invention with other antibodies against additional tumor-associated antigens including, but not limited to, antibodies that bind to EGFR, ErbB2, HER2, DLL4,

Notch and/ or VEGF. In certain embodiments, the second anti-cancer antibody is an antibody that is an angiogenesis inhibitor (e.g, an anti-VEGF antibody). In certain embodiments, the second anti-cancer antibody is an inhibitor of Notch signaling. In certain embodiments, the second anti-cancer antibody is AVASTIN (Bevaci-zumab), Herceptin (Trastuzumab), VECTIBIX (Panitu-mumab), or Erbitux (Cetuximab). Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active antibodies can exert their biological activities simultaneously.

[00191] Furthermore, treatment of cancer or a tumor in a subject can include administration of one or more cytokines (e.g, lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of cancer cells or any other therapy deemed necessary by a treating physician.

[00192] For the treatment of the disease, the appropriate dosage of an of the present invention depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the antibody or antibody is administered for therapeutic or preventative purposes, previous therapy, or patient's clinical history. The antibody or antibody can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g. reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody or antibody. In certain embodiments, dosage is from 0.01 mg to l00mg/kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain embodiments, the FZD-binding antibody is given once every two weeks or once every three weeks. In certain embodiments, the dosage of the antibody or other FZD-binding antibody is from about 0.1 mg to about 20 mg/ kg of body weight.

[00193] The present invention provides kits that comprise the antibodies described herein and that can be used to perform the methods described herein. In certain embodiments, a kit comprises at least one purified antibody against one or more human frizzled receptors in one or more containers. In some embodiments, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results. One skilled in the art will readily recognize that the disclosed antibodies or antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the

art.

[00194] Further provided are kits comprising a FZD-binding antibody as well as a second anti-cancer agent. In certain embodiments, the second anti-cancer agent is a chemotherapeutic agent. In certain embodiments, the second anti-cancer agent is an angiogenesis inhibitor. In certain embodiments, the second anti-cancer agent is an inhibitor of Notch signaling (e.g, an anti-DLL4 or anti-Notch antibody).

[00195] In another embodiment, the invention provides a method of diagnosing a medical condition mediated by elevated expression or activity of frizzled receptors. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a frizzled receptor with an antibody of the invention. Methods for testing a reactivity of a biological sample with antibodies are well known in the art. Examples of testing methods include, but are not limited to, enzyme-linked immunosorbant assay (ELISA), immunocytochemistry, and immunoprecipitation.

[00196] In another embodiment, the invention provides a method of diagnosing a medical condition mediated by elevated expression or activity of the frizzled 7 receptor. The method comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a frizzled 7 receptor with an antibody of the invention.

[00197] In certain embodiment, the invention provides a method of detecting a tumor in a subject. The method comprised the steps of obtaining a biological sample from the subject and testing the biological sample for frizzled 7 by an isolated antibody of the invention or antigen-binding portion thereof that binds to a frizzled 7 receptor. The antibody may bind to the cytoplasmic portion and optionally, the transmembrane portion of the receptor. In one embodiment, the presence of a tumor is determined by assessing the level of frizzled 7 expression in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In another embodiment, the presence of a tumor is determined by assessing the level of activity of frizzled 7 in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy.

[00198] In another embodiment, a method of detecting a tumor in a subject. Comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on the Frizzled receptor. In one embodiment, the presence of a tumor is determined by assessing the level of a frizzled receptor expression in the obtained biological

sample. If it is abnormal (e.g., high), this may indicate a malignancy. In another embodiment, the presence of a tumor is determined by assessing the level of activity of a frizzled receptor in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In one embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 288-1. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 288-2. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 288-3. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 288-5.

[00199] In another embodiment, a method of detecting a tumor in a subject. Comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on the Frizzled receptor. In one embodiment, the presence of a tumor is determined by assessing the level of a frizzled receptor expression in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In another embodiment, the presence of a tumor is determined by assessing the level of activity of a frizzled receptor in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In one embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 288-4. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 289-5. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line number 4.

[00200] In another embodiment, a method of detecting a tumor in a subject. Comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on the Frizzled receptor. In one embodiment, the presence of a tumor is determined by assessing the level of a frizzled receptor expression in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In another embodiment, the presence of a tumor is determined by assessing the level of activity of a frizzled receptor in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy.

[00201] In another embodiment, a method of detecting a tumor in a subject. Comprises the steps of obtaining a biological sample from the subject; and testing the biological sample for a

Frizzled receptor by an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:4 is present on the Frizzled receptor. In one embodiment, the presence of a tumor is determined by assessing the level of a frizzled receptor expression in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In another embodiment, the presence of a tumor is determined by assessing the level of activity of a frizzled receptor in the obtained biological sample. If it is abnormal (e.g., high), this may indicate a malignancy. In one embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 289-6. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 289-18. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 289-12. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line 289-3. In another embodiment the antibody used in this method may be the antibody produced by the hybridoma cell line number 13.

[00202] The biological sample used in the methods described herein is a body fluid that is tested by methods of the present invention is, in another embodiment, a cerebrospinal fluid (CSF). In another embodiment, the body fluid is plasma. In another embodiment, the body fluid is any other type of fluid known in the art. Each possibility represents a separate embodiment of the present invention. In another embodiment, the biological sample is amniotic fluids, blood, sera, saliva, or their combination. In another embodiment the biological sample is a tissue biopsy. In another embodiment the biological sample is obtained from stool or urine or any other excretion (e.g. perspiration).

EXAMPLES

Example 1: Selection of Epitopes for FZD7 Monoclonal Antibody Generation

[00203] Frizzled family receptors share a high level of homology. To generate monoclonal antibodies which are specific for FZD7, non-overlapping sequences between FZD7 and a different Frizzled receptor- FZD2 were identified.

[00204] The expression of the FZD2 and FZD7 genes appears to be developmentally regulated, with high levels of expression in fetal kidney and lung and in adult colon and ovary (**Figure 1A**). FZD7 and FZD2 are highly homologous transmembrane proteins, especially in the ligand binding domain (**Figure 1B**).

[00205] **Materials and Methods**

[00206] *Epitope Mapping*: A peptide scanning method was used for epitope mapping. This

technique uses a library of many short peptide sequences of equal length of overlapping segments from a target protein and tests for their ability to bind the antibody of interest. The short overlapping peptides were designed for the desired sequenced with over hanged edges and additional external areas.

[00207] *Vaccination:* Peptide(s) corresponding to sequences specific to FZD7 from 4 different areas were used for immunization. For immunization, the peptides were conjugated to KLH using Maleimide Activated BSA/KLH conjugation kit (Sigma, MBK-1). BALB/c mice were immunized and boosted.

[00208] *Hybridoma Generation:* Spleens were harvested from immunized mice and cells isolated. Cells were fused with mouse myeloma cells (NS-1). Hybridoma supernatant was screened for peptide-specific antibodies by ELISA.

[00209] *Antibody Purification and Screening*: The hybridomas were grown in bioreactors and the antibodies were purified from the culture supernatant using Protein A agarose columns. Screening for FZD7-specific antibodies was done by ELISA on peptide conjugated BSA and is done by the following methods:

a. ELISA on CHO cells stably expressing full length FZD7 and fixed on ELIS plates.

b. Western blotting using cell extracts of CHO cells stably expressing full length FZD7.

[00210] Clones that were positive by the 3 methods above were further assayed and validated for selectivity and efficacy.

[00211] *ELISA:* BSA conjugates of the screened peptides were used, and peptide positive clones were further screened by Western blotting on cell extracts. The isotype was determined by ELISA using Mouse Monoclonal Antibody Isotyping Reantibodies (Sigma ISO-2).

[00212] **Results**

[00213] Towards the goal of generating FZD7 specific monoclonal antibodies, epitope mapping of the FZD7 protein was performed and generated four epitopes including those of the C- and N-terminus as well as non-overlapping sequences with FZD2 (**Figure 1B**). The amino acid sequences of these four epitopes are embodied in SEQ ID NOs: 1-4. These FZD7 sequences share a 56%, 100%, 40% and 25% homology with FZD2, respectively. Epitopes were injected into mice to generate hybridomas. In addition, a FZD7 construct was cloned, which was overexpressed in CHO cells for ELISA- based screening of hybridoma clones. Moreover, a secreted FZD7 vector was cloned that included FLAG and MYC-tag. This

approach enabled further purification of the MAb's generated via specific binding to secreted FZD7 on a FLAG/MYC column. Therefore, the sequenced peptides that have been selected can be tested for MAb specificity and to the degree for which they are homologous, with FZD2.

[00214] Overall, from the hybridomas generated, twenty clones were chosen for in-vitro studies. The selected clones, were those that were positive in ELISA testing for their respective specific antigen (peptide). **Table 1** shows the selected hybridomas and the specificity of the monoclonal antibody they produce. Ten (10) additional hybridomas tested did not show antigen specificity in ELISA assays.

[00215] **Table 1**

Clone Number	Antigen Specificity
288-1[1]	RFYHRLSHSSKGETAV (SEQ ID NO: 1)
288-2	RFYHRLSHSSKGETAV (SEQ ID NO: 1)
288-3	RFYHRLSHSSKGETAV (SEQ ID NO: 1)
288-5	RFYHRLSHSSKGETAV (SEQ ID NO: 1)
289-6	DGSGGPGGGPTAYPTA (SEQ ID NO: 4)
289-12	DGSGGPGGGPTAYPTA (SEQ ID NO: 4)
289-18	DGSGGPGGGPTAYPTA (SEQ ID NO: 4)

[00216] **Conclusions**

[00217] Hybridomas producing monoclonal antibodies which are specific for four different epitopes on the Frizzled 7 receptor have been produced. The likelihood of specificity of these antibodies to FZD7 is inversely related to the homology of the epitope sequences between FZD7 and FZD2.

Example 2: Effect of FZD7 Monoclonal Antibodies on Malignant Cell Proliferation

[00218] FZD7 monoclonal antibodies generated as described in Example 1 were evaluated for their ability to inhibit proliferation of both primary malignant tissue and malignant cell lines.

[00219] **Materials and Methods**

[00220] *MTS Assay:* Cells were seeded on a 96 well plates in triplicates. After 24 hours cells

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were treated with the desired antibody at increasing concentrations for 48 hours and evaluated by the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS) kit (Promega).

[00221] **Results**

[00222] FZD7 monoclonal antibodies were assayed for their ability to inhibit proliferation of malignant cells of different FZD7 expression levels. The analysis was performed on the following cell types: primary Wilms' tumor cells, melanoma (SK-MEL-28), colon (HT29), glioblastoma (U87) and a negative control HeLa cells (express low levels of FZD7). FACS analysis was used to determine FZD7 levels in each of the tumor types: approximately 10% for Wilms' tumor, 11% for melanoma, 2.6% for colon cancer and 3% for HeLa cells. The MTS assay was used to assess inhibition of malignant cell proliferation. Data are shown in **Figure 2** on Wilms' tumor (WT), melanoma (SK-MEL-28) and cervical cancer (HeLA) cells and in **Figure 3** on melanoma (SK-MEL-28), cervical cancer (HeLA) and colon cancer (HT-29). Note how inhibition is associated with expression level of frizzled 7.

[00223] **Conclusions**

Hybridoma clones were isolated that produce monoclonal antibodies that show beneficial effects in vitro on the inhibition of proliferation of tumor cells. Frizzled7-expressing tumor cell are especially vulnerable to the activity of the antibodies.

Example 3: Effect of FZD7 Monoclonal Antibodies on Malignant Cell Viability

[00224] FZD7 monoclonal antibodies generated as described in Example 1 were evaluated for their ability to induce cell death of both primary malignant tissue and malignant cell lines.

[00225] **Materials and Methods**

[00226] *MTS Assay*: Cells were seeded on a 96 well plates in triplicates. After 24 hours cells were treated with the desired antibody at increasing concentrations for 48 hours and evaluated by the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS) kit (Promega).

[00227] **Results**

[00228] FZD7 monoclonal antibodies were assayed for their ability to kill malignant cells of different FZD7 expression levels. First, killing of primary tumor malignant cells by the different FZD7-specific monoclonal antibodies was evaluated. As a source of primary tumor, Wilms' tumor, which has an approximately 10% expression level of FZD7, was used. Antibodies were the FZD7-specific monoclonal antibodies produced by hybridoma clones generated with selected epitopes as described in Example 1. After 48 hours of incubation with

antibodies cells were assayed for viability by trypan blue. Percent of live cells from total cells for each monoclonal Ab used is shown in **Figure 4A**. Next, killing of SK-MEL-28 and SK-MEL-29 melanoma cells by the FZD7-specific monoclonal antibodies was evaluated. SK-MEL-28 and SK-MEL-cells were seeded in 96-well plates and were incubated with antibodies for 48 hours and then were assayed for viability by trypan blue. Absolute cell counts of live cells are shown in **Figure 4B**. Finally, microimages of SK-MEL-28 cells after treatment with one of the monoclonal antibodies (clone 288-2) or without antibody treatment were obtained. Cell death and was readily visible in treated cells (**Figure 4C**).

Example 4: Determination of Optimal Concentration of FZD7 Monoclonal Antibodies for Induction of Tumor Inhibition.

[00229] FZD7 monoclonal antibodies generated as described in Example 1 were evaluated at different concentrations for their ability to inhibit of malignant cell proliferation.

[00230] **Materials and Methods**

[00231] *MTS Assay*: Cells were seeded on a 96 well plates in triplicates. After 24 hours cells were treated with the desired antibody at increasing concentrations for 48 hours and evaluated by the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS) kit (Promega).

[00232] **Results**

[00233] The dose response in terms of inhibition of cell proliferation upon exposure to different concentrations of anti FZD7 monoclonal antibody produced in clones 288-1 and 288-5 was determined. The response of a high FZD7- expressing malignant tissue (SK-MEL28, melanoma cells) was compared with that of a low FZD7-expressing malignant tissue (HeLa, cervical cancer cells). Cells were exposed to antibody for 48h and proliferation was assessed by the MTS assay and compared to cells not treated with antibody. Proliferation was inhibited in the presence of either FZD7 antibody in high FZD7 expressing cells but not in low expressing cells such as HeLa (**Figure 5A**). Optimal inhibition of proliferation was observed at an antibody concentration of about 5ug/ml. Data shown is normalized to control untreated cells for each cell line. **Figure 5B** shows the data from **Figure 5A** in a way which highlights the effect the culturing density of SK-MEL28 melanoma cells has on the level of inhibition of proliferation by 5ug/ml of the FZD7 monoclonal Ab produced by hybridoma clones 288-1 and 288-5. It is observed that at this Ab concentration, inhibition is greater when a 4000 cell/well density is employed in the case of 288-5 and similar in the case of 288-1.

[00234] **Conclusions**

[00235] Optimal concentration of monoclonal antibodies isolated from hybridoma clones for inducing cell death in tumor cells was determined. This is shown both in cell lines and in primary tumors.

Example 5: Effect of FZD7 Monoclonal Antibodies on Malignant Cell Wnt Signaling

[00236] FZD7 monoclonal antibodies generated as described in Example 1 were evaluated for their ability to inhibit Wnt signaling of malignant cells.

[00237] **Results:**

[00238] Western blot analysis was performed for active β-Catenin (millipore 05665) in HeLa and SK-MEL28 cells after 48h treatment with 288-1 Ab at the indicated concentration (**Figure 6**). β-catenin inhibition by the 288-1 FZD7 mAb is demonstrated. Also, in **Figure 7B** the expression of WNT targeted genes was evaluated using RQ PCR in Untreated & antibody treated Wilms tumor & HeLa cells (**Figure 7B**).

[00239] **Conclusion:** FZD7 monoclonal antibodies generated as described in Example 1 were able to inhibit Wnt signaling upon binding the receptor. This inhibition is associated with malignant cell death as demonstrated in Example 3 and apotosis as demonstrated in Example 6 below.

Example 6: Induction of Apoptosis in Malignant Cells by FZD7 Monoclonal Antibodies.

[00240] The ability of antibodies of the invention to induce apoptosis in malignant cells was determined.

[00241] **Results**

[00242] Apoptosis of malignant cells treated with antibodies of the invention was observed (**Figure 7A**). Specifically, Flow cytometry of APC conjugated Annexin V binding to Melanoma and HeLa cells is shown. Levels of Annexin staining are compared in the presence of the monoclonal antibody produced by hybridoma 288-1 at different concentrations or without antibody (**Figure 7A**).

[00243] **Conclusion:** The ability of antibodies of the invention to induce apoptosis in malignant cells was observed.

Example 7: Inhibition of cell proliferation by FZD7 Specific Monoclonal Antibodies Secreted by Hybridoma Clones

[00244] The ability of specific monoclonal antibodies of the invention to inhibit proliferation

in cancer cells was determined. Cells were seeded in 96 well plates. Melanoma cell line (SK-MEL28), Colon cancer cell line (HT-29), and primary Wilms' tumor (WT) cells were incubated with antibodies from the indicated clones for 48 hours and then were assayed by the cell proliferation assay (PROMEGA).

[00245] **Results: Figure 8** depicts the arrangement of the FZD7 polypeptide within the plasma membrane and identifies the locations of peptides 1-4. The homology between these peptides and other members of the Frizzled family of receptors is shown in the table below, wherein "Homology" indicates the number of amino acid residues that are similar between the peptide and the antigen area of the indicated FZD receptor:

Frizzled receptor	Peptide 1		Peptide 2		Peptide 3		Peptide 4	
	Homology	%	Homology	%	Homology	%	Homology	%
FZD1	14	100	6	38	4	25	9	56
FZD2	14	100	8	50	4	25	9	56
FZD3	5	36	1	6	1	6	5	31
FZD4	7	50	2	13	4	25	8	50
FZD5	11	79	4	25	3	19	6	38
FZD6	6	43	3	19	1	6	8	50
FZD7	14	100	16	100	16	100	16	100
FZD8	11	79	6	38	3	19	5	31
FZD9	6	43	6	38	4	25	3	19
FZD10	6	43	2	13	5	31	3	19
FZD11 (SMO) (SEQ ID NO 15)	2	14	1	6	2	13	4	25

[00246] **Figure 9** shows the inhibition of cell proliferation by FZD7 specific monoclonal antibodies secreted by hybridomas in relationship to percent proliferation of control. The numbers indicate the hybridoma clone from which the antibody was taken and "*" indicates the effective clones to be 288-1, 288-2, 288-5, 289-10, 289-16 and 289-13. The classification of the effective clones by peptide matching and isotype is presented in the table below:

Anti FZD7-Ab	Clone Isotype	Peptide
288-5	IgG1	4
288-1	IgG2b	4
289-13	IgG1	3
288-2	UD	1
289-10	UD	UD
289-16	UD	UD

Example 8: Anti FZD7 Antibody 288-1 Specificity - Specificity of Anti-FZD7 Monoclonal Antibody 288-1 for C-terminus Antigen of FZD7 Receptor Protein

[00247] A protein immunoprecipitation assay was used to validate specificity of monoclonal antibody (MAb) MAb-288.1, MAb-288.5, and MAb-289.13 binding to the FZD7 receptor in SK-MEL28 cell lysate.

[00248] **Methods**: For immunization mice were injected with both peptide 1 and peptide 4. In order to determine antibody specificity a blocking assay was performed. Briefly, cells' lysate was incubated with the peptide to block the binding antigen, then a Western Blot analysis was performed using the indicated anti-FZD7 Ab. FZD7 Input (total cell lysate), and FZD7 bound fractions were separated by 10% SDS-PAGE followed by Western Blotting and detected using an anti-FZD7 antibodies. Following an overnight incubation with the different MAbs, proteins were immune-precipitated using protein A agarose beads. Mouse IgG served as negative control. A peptide block assay was performed to verify the specificity of MAb-288.1 to antigen C of the FZD7 receptor. MAb-288.1 was incubated with the immunizing peptide for 4h. Lysate of HEK 293 cells was separated by 10% SDS-PAGE followed by Western Blotting. The detection of the FZD7 protein was done by either using MAb-288.1, MAb-288.1 + peptide C or a negative control Ab. MAb-288.1 binding to the FZD7 receptor was examined using HEK 293 cells over expressing FZD7. Cell extract was separated on SDS-PAGE and probed with different concentrations of MAb-288.1.

[00249] In order to further confirm specificity, immuno-precipitation (IP) was performed. Protein IP was performed on the melanoma cell-line cells using the indicated Abs.

[00250] **Results: Figure 11A** shows the results for IP by WB analysis. **Figure 11B** shows that anti-FZD7 288-1 antibody binding site was blocked by peptide 4 and not by peptide 1. IP was successful when using 288-1 Ab. (Input – untreated fraction taken from cell lysate before IP was preformed; IP – immuno-precipitated fraction; control – nonspecific Ab control; mock – mouse anti human IgG control). **Figure 11A** demonstrates that Anti FZD7 Ab 288-1 specifically immuno-precipitates the FZD7 protein. Antibody labeling of melanoma cells shows that an anti FZD7 Ab 288-1 conjugated to Cy3 labels melanoma cells (**Figure 10**).

[00251] **Conclusion:** Specific binding of the Ab to FZD7 expressing cells was observed. **Figure 11A** validates specific MAb-288-1 binding to FZD7 receptor. Further, the results shown in **Figure 11B** demonstrate that MAb 288-1 binding FZD7 is blocked by peptide-C. **Figure 11C** presents additional Western Blot results supporting specificity of MAb 288-1, wherein serial dilution of antibody corresponds with reduced signal.

Example 9: Functional Effect of Anti FZD7 Antibody 288-1 *in vitro*

[00252] Percent (%) proliferation inhibition was measured in melanoma cells. Average % proliferation (n>5) in melanoma cell-line cells was measured by incubating cells for 48 hours in the absence (untreated) or in the presence of anti FZD7-Ab 288-1 at 5μg/ml. Control untreated cells were set as 100%, average of % proliferation as indicated by the range markers in the bar graphs **Figure 12**

Results:

[00253] **Figure 12** shows that anti FZD7 Ab 288-1 reduces proliferation in melanoma cells.

Example 10: Monoclonal Antibody 288-1 Inhibits Malignant cells' Wnt Signaling *in vitro*

[00254] Expression of Wnt pathway target genes (AXIN2, CCND1, MYC-C), FZD7, β-catenin, and Wnt pathway inhibitors (DKK1, sFRP1) was measured in SK-MEL28 (**Figure 13A**) and WT cells (**Figure 13B).**

[00255] Western Blot analysis was performed to identify active β-Catenin in melanoma cell-line cells and in Wilms' tumor cells after incubation for 48 hours in the absence (untreated) or in the presence of the anti FZD7 Ab 288-1 at the indicated concentrations.(**Figures 6** and **13C**)

[00256] **Results:** Results presented in **Figure 13A** and **13B** show MAb-288.1 inhibits expression of Wnt pathway related genes. For each cell type data were calculated as mean ±

S.E.M of five separate experiments, $p<0.05$. This correlates well with the reduction of active β-catenin expression shown in **Figure 6** and **Figures 13C**.

Example 11: Monoclonal Antibody 288-1 Induces Apoptosis in Malignant Cells

[00257] Targeting FZD7 with MAb-288.1 induced cell death of SK-MEL28 and WT cells.

[00258] **Methods**: Cell were treated with 5μg/ml MAb-288.1 for 48h and then stained for annexinV and 7AAD. Flow cytometry analysis of melanoma cell-line cells and Wilms' tumor cells was used. Cells stained positive for annexinV antibodies are either at pre-apoptotic, apoptotic, or necrotic stages. Gates were placed according to isotype control staining (IC<1%)

[00259] **Results**: **Figure 14A** presents the flow cytometry analysis of annexinV staining of cells following treatment with MAb-288.1. Results show a marked increase in % of pre-apoptotic cells in SK-MEL28 cells **(left panels)** as well as in WT cells **(right panels)**. Further, Cells were treated with MAb-288.1, stained with 0.4% trypan blue and counted. % of dead cells was calculated. Results show % of dead cells was significantly lower in both SK-MEL28 **(Figure 14B)** and WT **(Figure 14C)** cells ($p<0.05$; $n=4$).

Example 12: Monoclonal Antibody 288-1 Prompts Depletion of Stemness Traits *in vitro*

[00260] *In vitro* treatment with MAb-288.1 diminished stemness traits. Colony forming ability (CFU) was compared between untreated and treated SK-MEL28 cells. The results presented in **Figure 15A** demonstrate that the number of colonies formed by the treated cells was significantly lower than the untreated cells. Representative phase-contrast images of colonies formed from treated and untreated SK-MEL28 cells showing smaller colonies in the treated cells are presented in **Figure 15B**. (scale bars=100μm, magnification X20). Sphere formation assays were performed on SK-MEL28 and WT cells. Number of spheres formed from the treated cells was significantly reduced in both SK-MEL28 **(Figure 15C)** and WT **(Figure 15D)** cells. Data is presented as mean±SEM of at least three experiments performed in triplicates ($p=0.00098$, $p=0.00065$, respectively). Representative phase-contrast images of spheres formed from treated and untreated SK-MEL28 cells **(Figure 15E)** and WT cells **(Figure 15F)** show much smaller, less condensed spheres in the treated cells (scale bars=100μm, magnification X10).

Example 13: Monoclonal Antibody 288-1 Treatment of SK-MEL28 Cells Demonstrates a Functional Effect-Inhibition of Cell Migration

[00261] MAb-288.1 treatment of SK-MEL28 growth in tissue culture induced morphological changes on SK-MEL28 cells, as seen in **Figure 16A**, wherein treated cells show a reduced spreading phenotype. Scale bar, 100μm. **Figure 16B** presents the results of a migration assay, demonstrating that 5μg/ml MAb-288.1 treatment of SK-MEL28 for 48h significantly inhibited SK-MEL28 cells' migration capacity, as compared to untreated cells. Scale bar, 1000μm.

[00262] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

What is claimed is:

1. An isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of said receptor.

2. An isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor.

3. The isolated antibody of any one of claims 1-2, wherein the antibody is selected from the group consisting of:
a) the antibody produced by the hybridoma cell line 288-1;
b) the antibody produced by the hybridoma cell line 288-2;
c) the antibody produced by the hybridoma cell line 288-3; and
d) the antibody produced by the hybridoma cell line 288-5.

4. An isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on a Frizzled receptor.

5. An isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on a Frizzled receptor.

6. An isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:2 is present on a Frizzled receptor.

7. The isolated antibody of claim 6, wherein the antibody is selected from the group consisting of:

a) the antibody produced by the hybridoma cell line 289-6;
b) the antibody produced by the hybridoma cell line 289-12; and
c) the antibody produced by the hybridoma cell line 289-18.

8. The isolated antibody of any one of claims 1-7 or an antigen-binding portion thereof, wherein the antibody binds to the Frizzled7 receptor but not to other Frizzled receptors.

9. The isolated antibody of any one of claims 1-8 or an antigen-binding portion thereof, wherein the antibody inhibits Wnt signaling in a cell expressing a Frizzled receptor.

10. The isolated antibody of any one of claims 1-9 wherein the antibody or antigen-binding portion thereof is coupled to a cytotoxic moiety.

11. The isolated antibody of any one of claims 1-10 or antigen-binding portion thereof, wherein the antibody is a monoclonal antibody.

12. The isolated antibody of any one of claims 1-10 or antigen-binding portion thereof, wherein the antibody is a polyclonal antibody.

13. The isolated antibody of any one of claims 1-12 or antigen-binding portion thereof, wherein the antibody is a recombinant antibody.

14. The isolated antibody of claim 13, wherein said antibody or antigen-binding portion is a single chain antibody, Fab, Fv, diabody or triabody.

15. The isolated antibody of any one of claims 1-14, wherein said antibody is humanized, chimeric or chimeric/humanized.

16. A pharmaceutical composition comprising the isolated antibody or antigen-binding portion thereof of anyone of claims 1-15 and a pharmaceutically acceptable carrier.

17. A method of reducing the tumorigenicity of a tumor that comprises cancer stem cells

in a subject, said method comprising the step of administering a therapeutically effective dose of the isolated antibody of any one of claims 1-16 to the subject, wherein the frequency of cancer stem cells in the tumor is reduced by administration of the isolated antibody or antigen-binding portion thereof.

18. A method for treating a tumor in a subject, said method comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of said receptor.

19. A method for treating a tumor in a subject, said method comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1, wherein said portion of SEQ ID NO:1 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

20. The method of any one of claims 18-19, wherein the isolated antibody is selected from the group consisting of:
a) the antibody produced by the hybridoma cell line 288-1;
b) the antibody produced by the hybridoma cell line 288-2;
c) the antibody produced by the hybridoma cell line 288-3; and
d) the antibody produced by the hybridoma cell line 288-5.

21. A method for treating a tumor in a subject, said method comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO: 2, wherein said portion of SEQ ID NO:2 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

22. A method for treating a tumor in a subject, said method comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-

binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO: 3, wherein said portion of SEQ ID NO:3 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

23. A method for treating a tumor in a subject, said method comprising the step of administering a therapeutically effective dose of an isolated antibody or antigen-binding portion thereof to a subject, wherein said isolated antibody binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO: 4, wherein said portion of SEQ ID NO:4 is present on a Frizzled receptor, and wherein said tumor has elevated expression or activity of Frizzled receptors.

24. The method of claim 23, wherein the isolated antibody is selected from the group consisting of:
a) the antibody produced by the hybridoma cell line 289-6;
b) the antibody produced by the hybridoma cell line 289-12; and
c) the antibody produced by the hybridoma cell line 289-18.

25. The method of any one of claims 19-24, wherein the isolated antibody binds to the Frizzeled7 receptor but does not bind to other Frizzled receptors.

26. The method of any one of claims 19-25, wherein the isolated antibody inhibits Wnt signaling in said tumor.

27. The method of any one of claims 19-26, wherein the isolated antibody or antigen-binding portion thereof is coupled to a cytotoxic moiety.

28. The method of any one of claims 19-27, wherein the isolated antibody is a monoclonal antibody.

29. The method of any one of claims 19-27, wherein the isolated antibody is polyclonal antibody.

30. The method of any one of claims 19-29, wherein the isolated antibody is recombinant antibody.

31. The method of claim 30, wherein the isolated antibody is a single chain antibody, Fab, Fv, diabody or triabody.

32. The isolated antibody of any one of claims 19-31, wherein said antibody is humanized, chimeric or chimeric/humanized.

33. The method of any one of claims 19-32, wherein said tumor is Wilm's tumor.

34. The method of any one of claims 19-32, wherein said tumor is comprised of melanoma cells.

35. The method of any one of claims 19-32, wherein said tumor is comprised of colon cancer cells.

36. The method of any one of claims 19-35, wherein the method further comprises the step of administering a chemotherapy antibody.

37. The method of any one of claims 19-36, wherein the method comprises the step of administering a second antibody or antigen-binding portion thereof.

38. A method of detecting a tumor in a subject, said method comprising the steps of obtaining a biological sample from the subject; and testing the biological sample for Frizzled7 by an isolated antibody or antigen-binding portion thereof that binds to a Frizzled7 receptor, wherein the antibody binds to the cytoplasmic portion and optionally, the transmembrane portion of said receptor.

39. A method of detecting a tumor in a subject, said method comprising the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:1,

wherein said portion of SEQ ID NO:1 is present on the Frizzled receptor.

40. The method of any one of claims 38-39, wherein the isolated antibody is selected from the group consisting of:
a) the antibody produced by the hybridoma cell line 288-1;
b) the antibody produced by the hybridoma cell line 288-2;
c) the antibody produced by the hybridoma cell line 288-3; and
d) the antibody produced by the hybridoma cell line 288-5.

41. A method of detecting a tumor in a subject, said method comprising the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:2, wherein said portion of SEQ ID NO:2 is present on the Frizzled receptor.

42. A method of detecting a tumor in a subject, said method comprising the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:3, wherein said portion of SEQ ID NO:3 is present on the Frizzled receptor.

43. A method of detecting a tumor in a subject, said method comprising the steps of obtaining a biological sample from the subject; and testing the biological sample for a Frizzled receptor by. an isolated antibody or antigen-binding portion thereof that binds to a sequence comprising at least a 5 amino acid portion of SEQ ID NO:4, wherein said portion of SEQ ID NO:4 is present on a Frizzled receptor.

44. The method of claim 43, wherein the isolated antibody is selected from the group consisting of:
a) the antibody produced by the hybridoma cell line 289-6;
b) the antibody produced by the hybridoma cell line 289-12; and
c) the antibody produced by the hybridoma cell line 289-18.

45. The method of any one of claims 39-44, wherein the isolated antibody or antigen-binding portion thereof binds the Frizzled7 receptor and does not bind to other Frizzled receptors.

46. The method of any one of claims 38-45, wherein the isolated antibody is a monoclonal antibody.

47. The method of any one of claims 38-45, wherein the isolated antibody is a polyclonal antibody.

48. The method of any one of claims 38-47, wherein the isolated antibody is recombinant antibody.

49. The method of any one of claims 38-48 wherein the isolated antibody is coupled to a chromogenic recombinant antibody or a radioactive antibody.

50. The method of any one of claims 38-49, wherein said tumor is Wilms' tumor.

51. The method of any one of claims 38-49, wherein said tumor is comprised of melanoma cells.

52. The method of any one of claims 38-49, wherein said tumor is comprised of colon cancer cells.

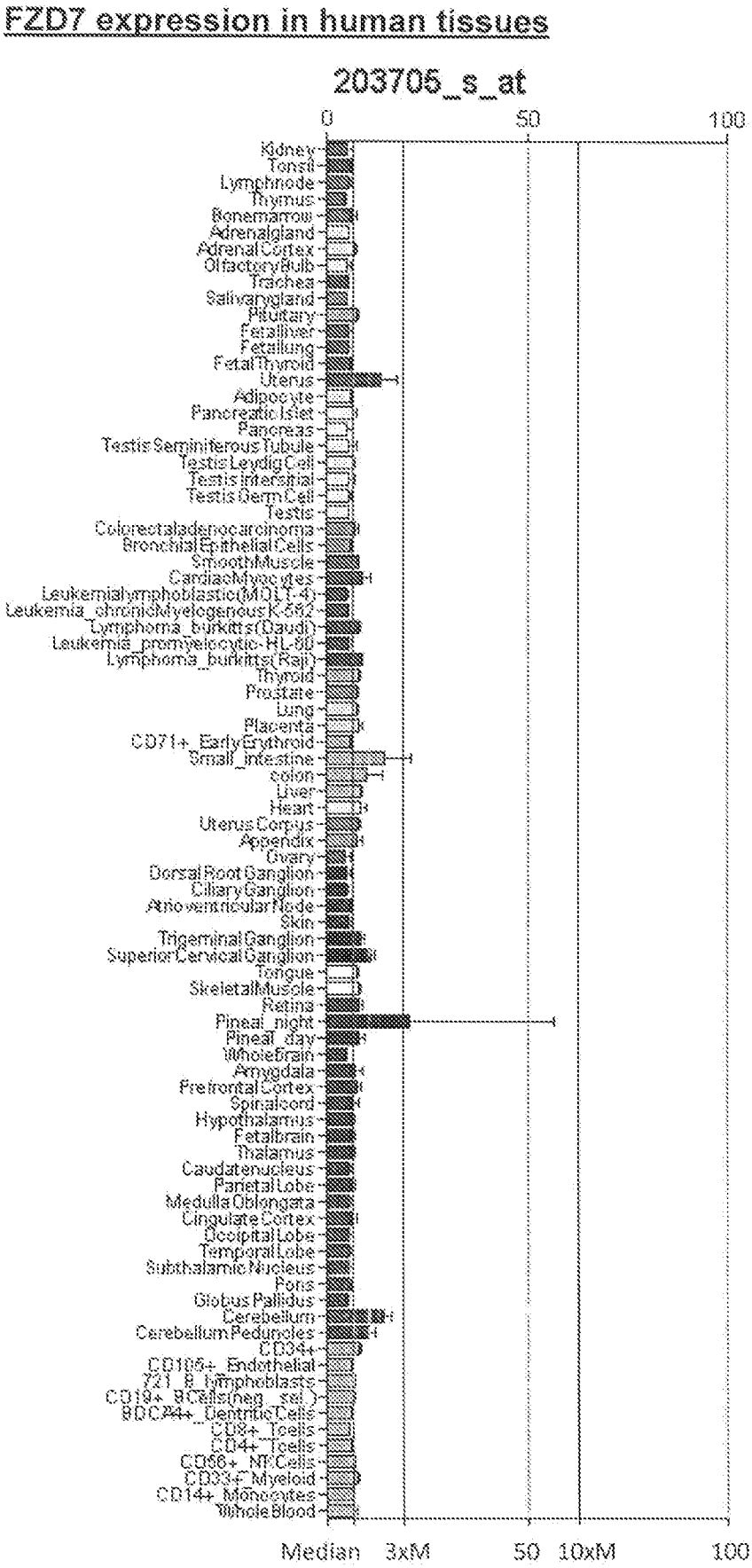

FZD7 expression in human tissues
203705_s_at
0
50
100
Kidney
Tonsil
Lymphnode
Thymus
Bonemarrow
Adrenalgland
Adrenal Cortex
Olfactory Bulb
Trachea
Salivarygland
Pituitary
Fetalliver
Fetallung
Fetal Thyroid
Uterus
Adipocyte
Pancreatic Islet
Pancreas
Testis Seminiferous Tubule
Testis Leydig Cell
Testis Interstitial
Testis Germ Cell
Testis
Colorectaladenocarcinoma
Bronchial Epithelial Cells
SmoothMuscle
CardiacMyocytes
Leukemia lymphoblastic(MOLT-4)
Leukemia_chronicMyelogenous K-562
Lymphoma_burkitts(Daudi)
Leukemia_promyelocytic-HL-60
Lymphoma_burkitts(Raji)
Thyroid
Prostate
Lung
Placenta
CD71+_EarlyErythroid
Small_Intestine
colon
Liver
Heart
Uterus Corpus
Appendix
Ovary
Dorsal Root Ganglion
Ciliary Ganglion
Atrioventricular Node
Skin
Trigeminal Ganglion
Superior Cervical Ganglion
Tongue
SkeletalMuscle
Retina
Pineal_night
Pineal_day
WholeBrain
Amygdala
Prefrontal Cortex
Spinalcord
Hypothalamus
Fetalbrain
Thalamus
Caudatenucleus
Parietal Lobe
Medulla Oblongata
Cingulate Cortex
Occipital Lobe
Temporal Lobe
Subthalamic Nucleus
Pons
Globus Pallidus
Cerebellum
Cerebellum Peduncles
CD34+
CD105+_Endothelial
721_B_lymphoblasts
CD19+_BCells(neg._sel.)
BDCA4+_DentriticCells
CD8+_Tcells
CD4+_Tcells
CD56+_NKCells
CD33+_Myeloid
CD14+_Monocytes
Whole Blood
Median
3xM
50
10xM
100

Figure 1A

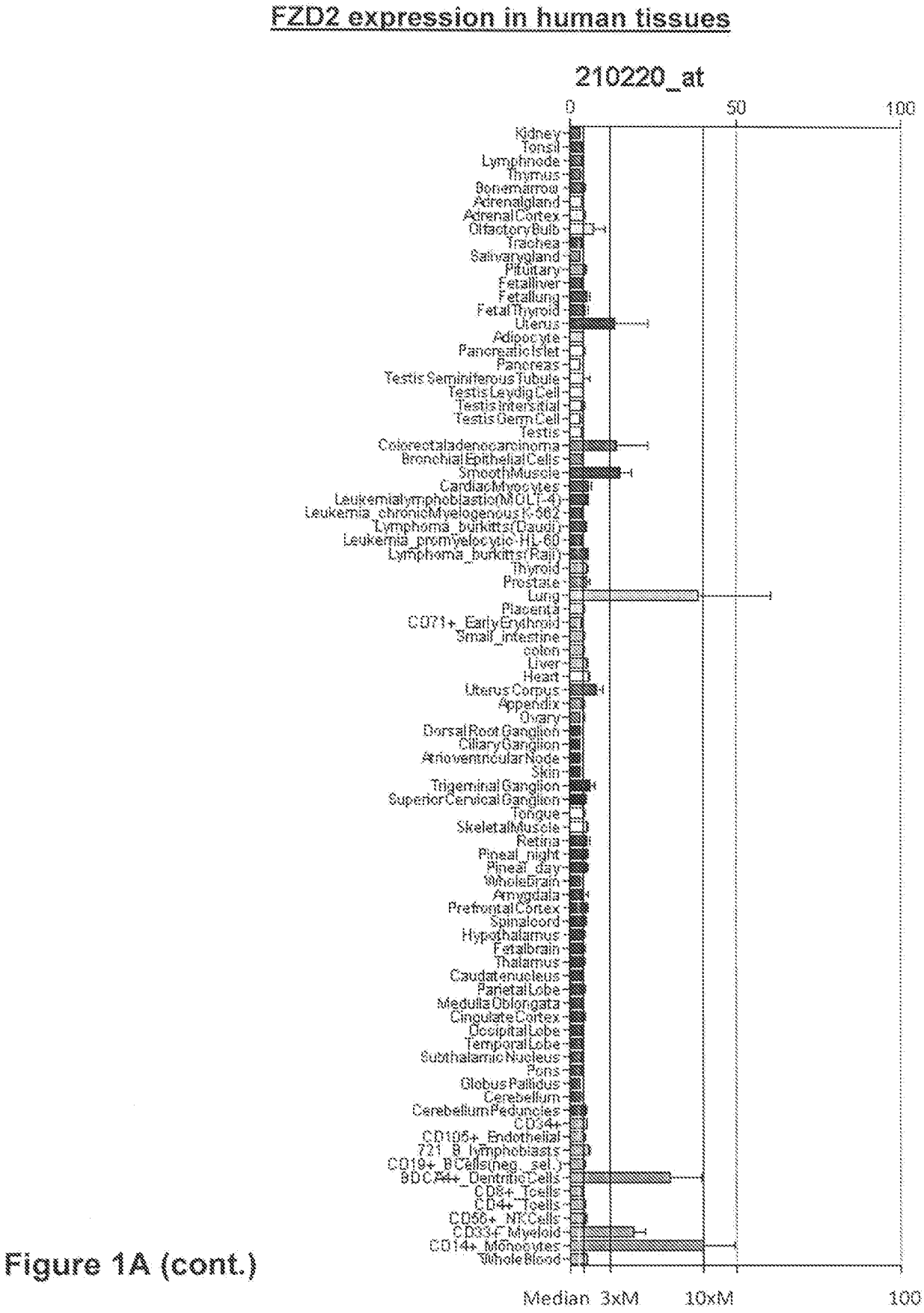


Figure 1A (cont.)

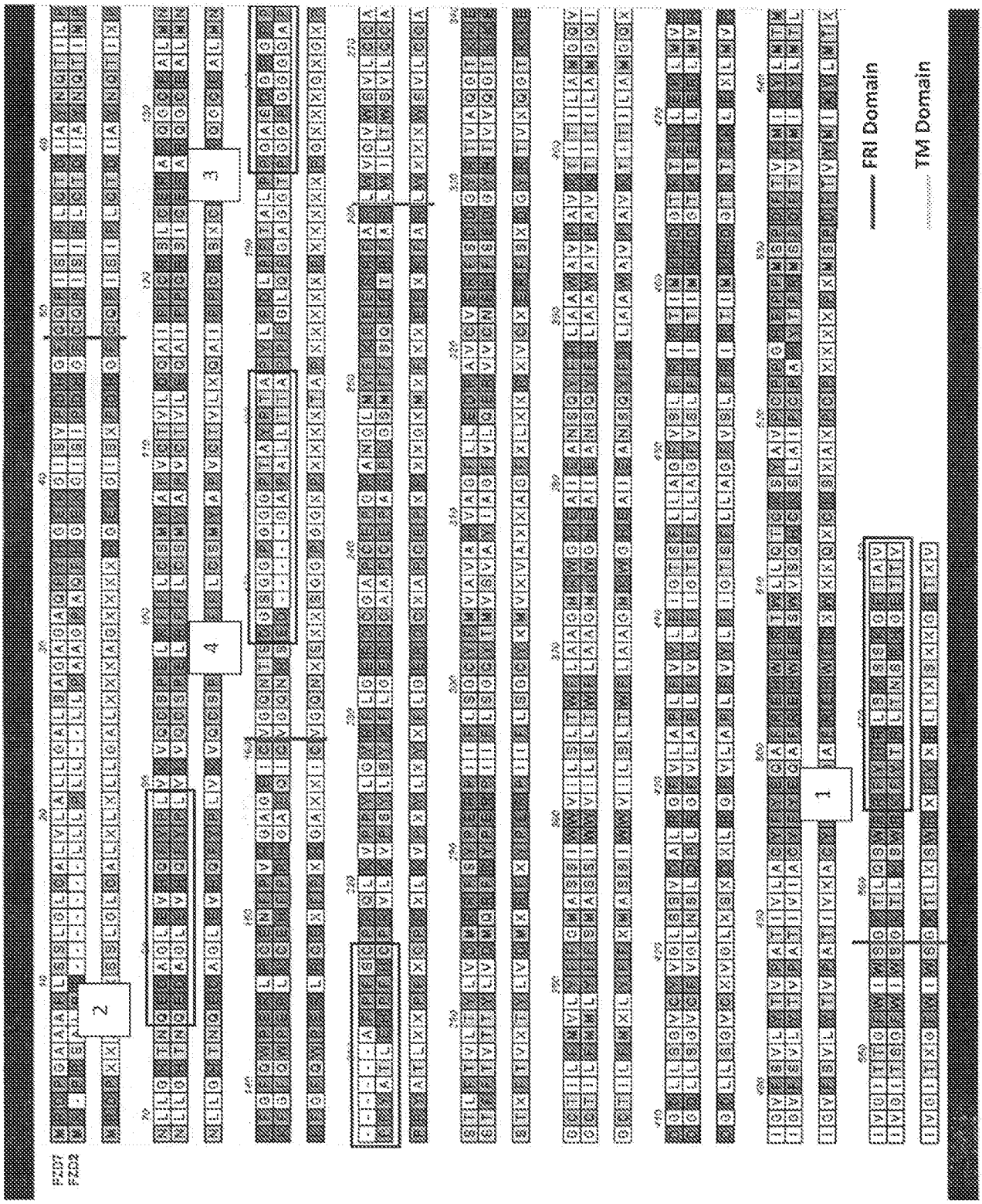


Figure 1B

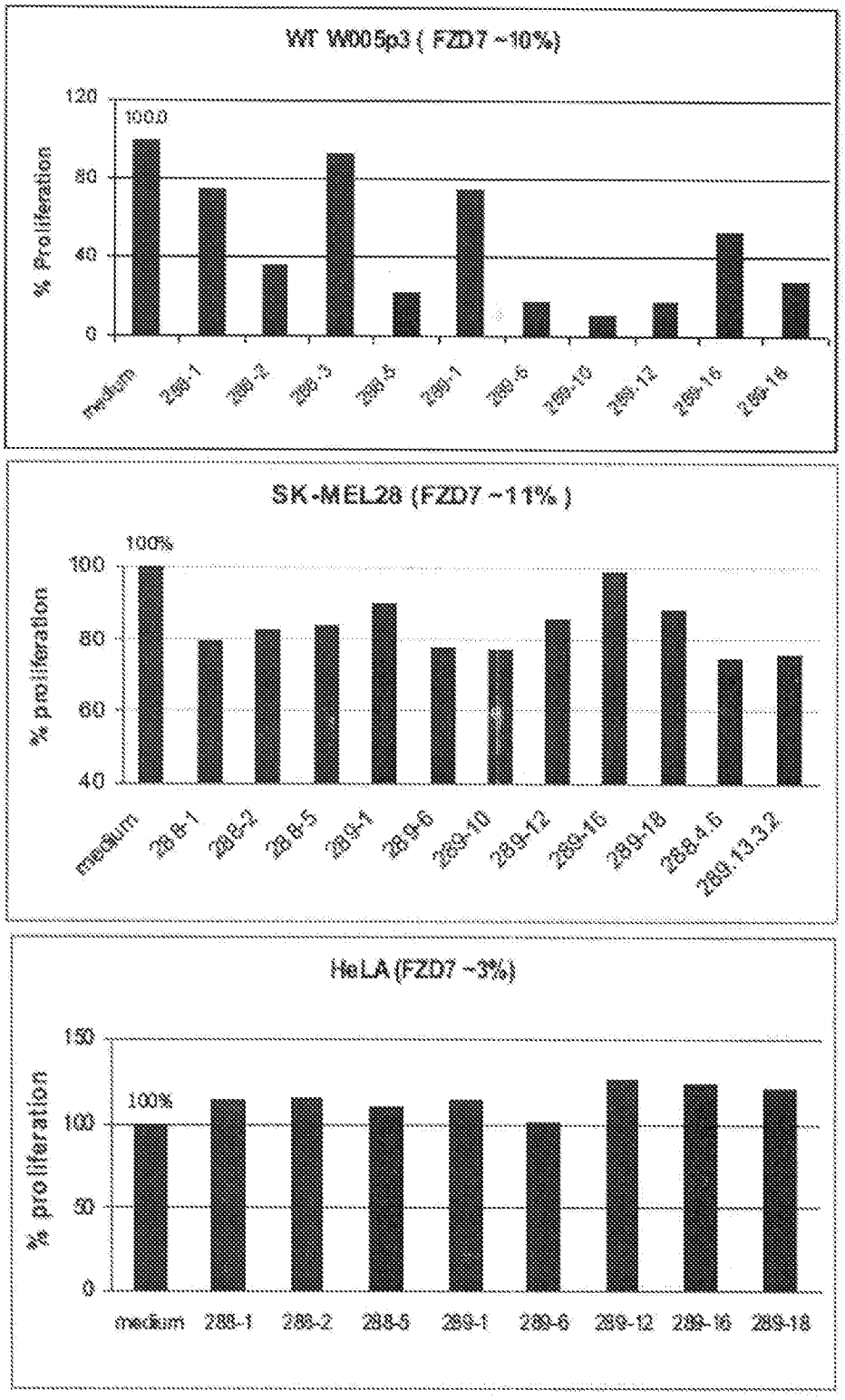

WT W0Sp3 (FZD7 ~10%)
% Proliferation
120
100
80
60
40
0
100.0
medium
SK-MEL28 (FZD7 ~11%)
% proliferation
100%
100
80
60
40
medium
HeLA (FZD7 ~3%)
% proliferation
150
100
50
0
100%
medium

Figure 2

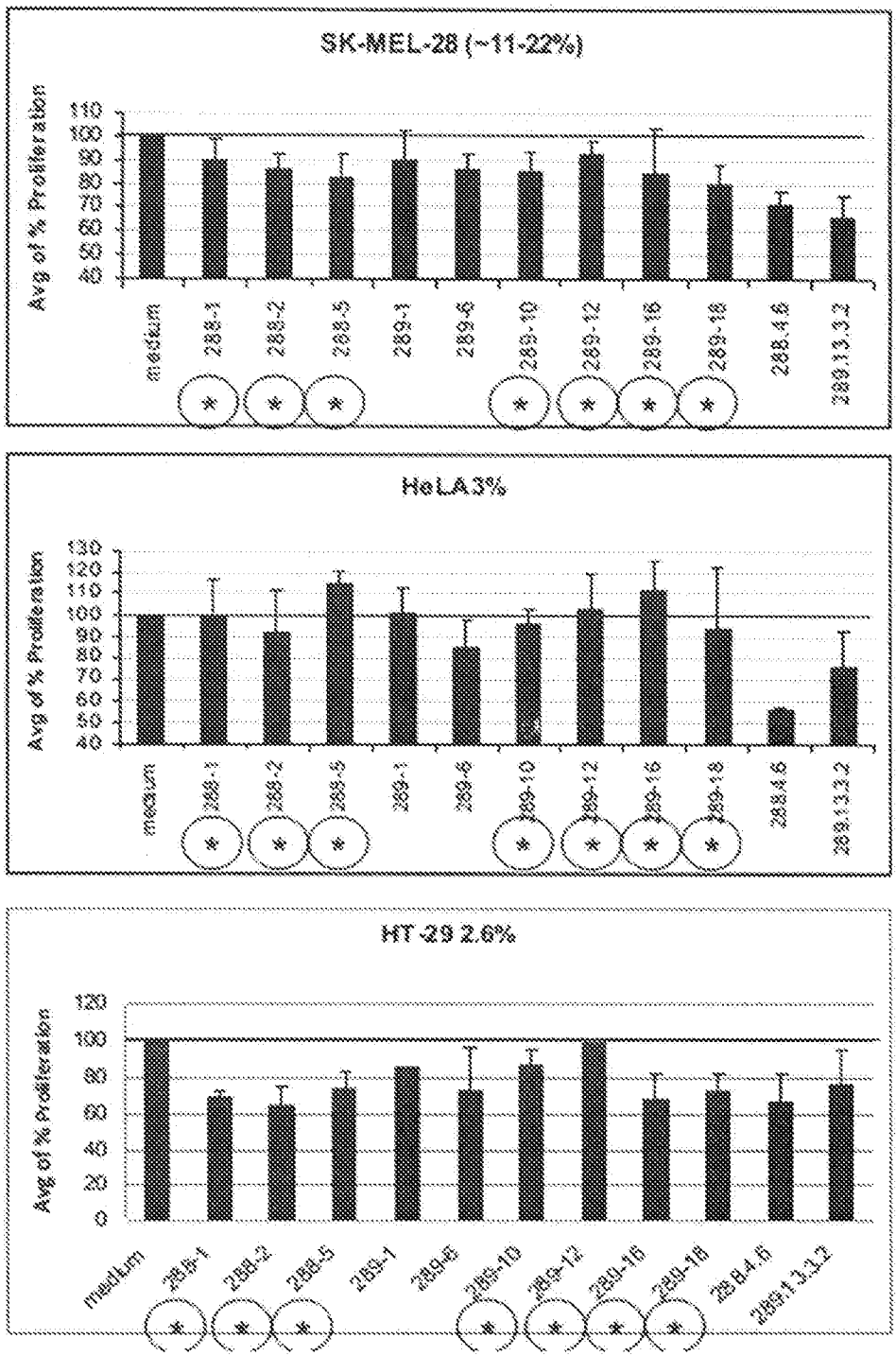

SK-MEL-28 (~11-22%)
Avg of % Proliferation
medium
HeLA 3%
Avg of % Proliferation
medium
HT-29 2.6%
Avg of % Proliferation
medium

Figure 3

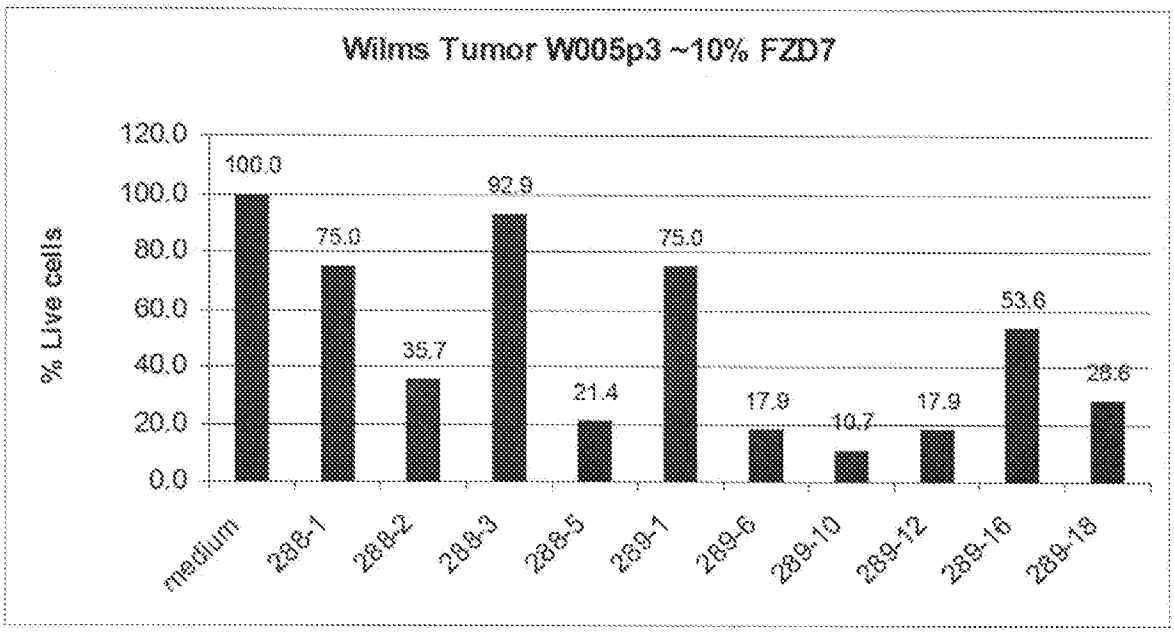

Wilms Tumor W005p3 ~10% FZD7
% Live cells
120.0
100.0
80.0
60.0
40.0
20.0
0.0
100.0
75.0
35.7
92.9
21.4
75.0
17.9
10.7
17.9
53.6
28.6
medium
288-1
288-2
288-3
288-5
289-1
289-6
289-10
289-12
289-16
289-18

Figure 4A

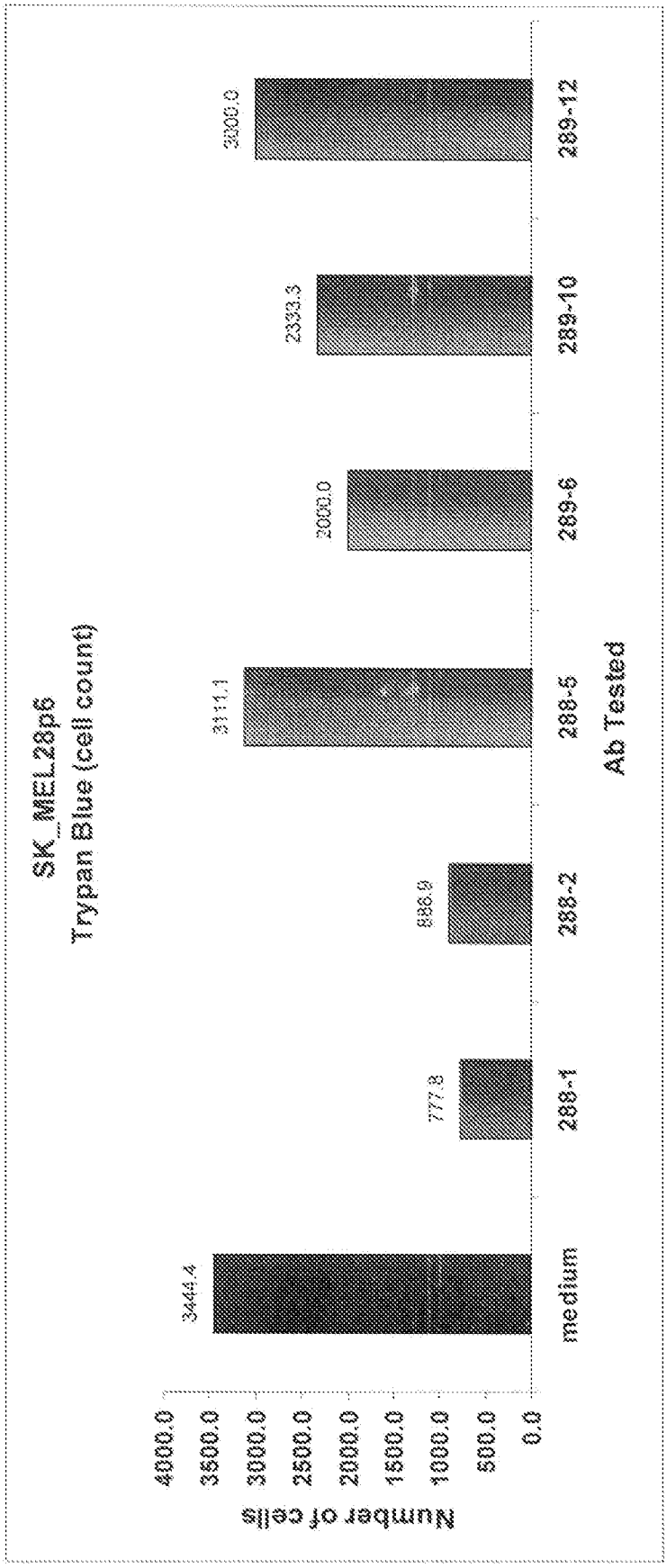

SK_MEL28p6
Trypan Blue (cell count)
Number of cells
4000.0
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
3444.4
777.8
888.9
3111.1
2000.0
2333.3
3000.0
medium
288-1
288-2
288-5
289-6
289-10
289-12
Ab Tested

Figure 4B

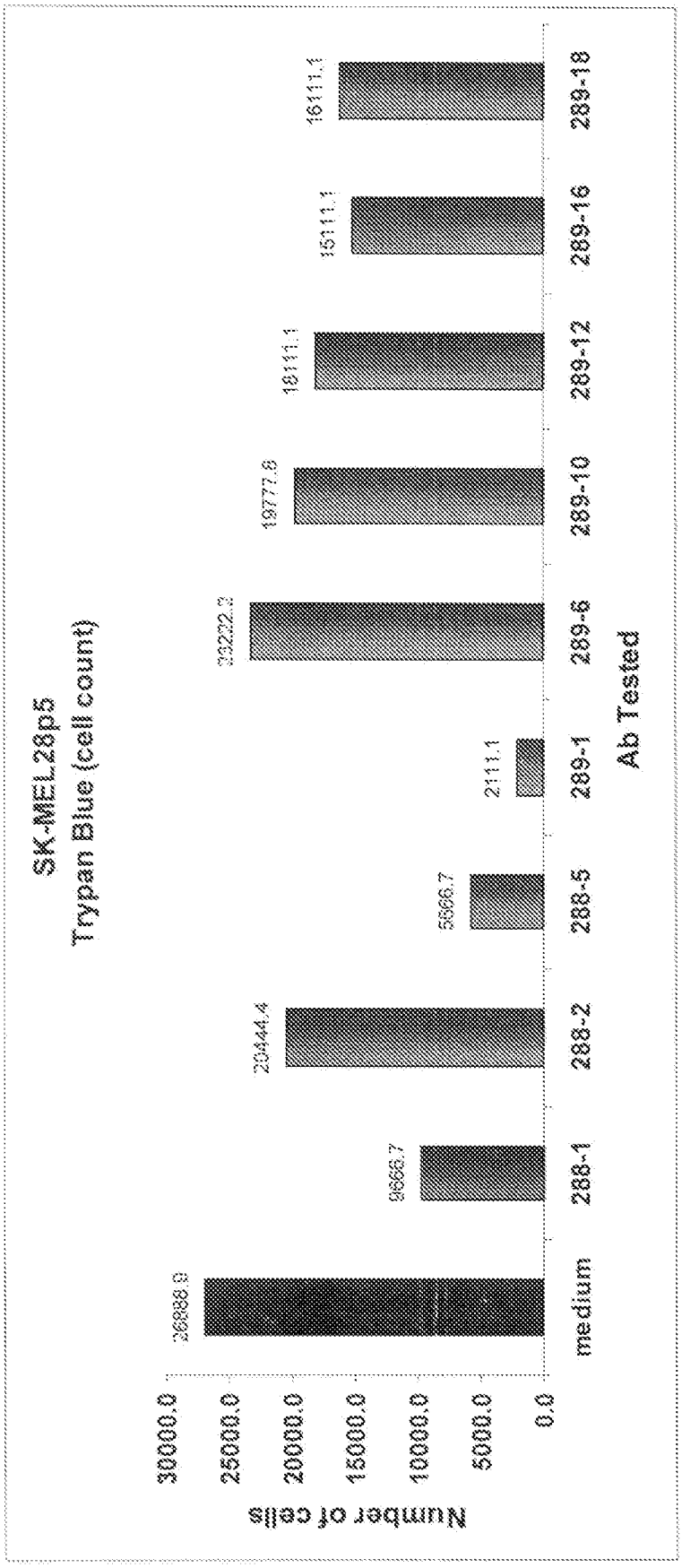


Figure 4B (cont.)

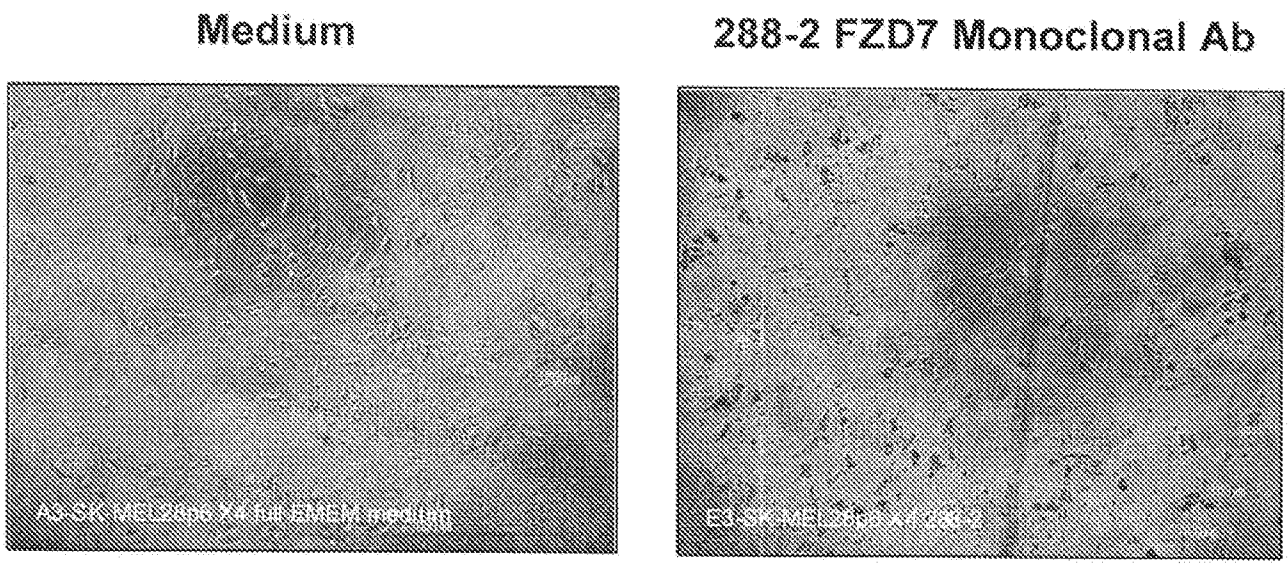


Figure 4C

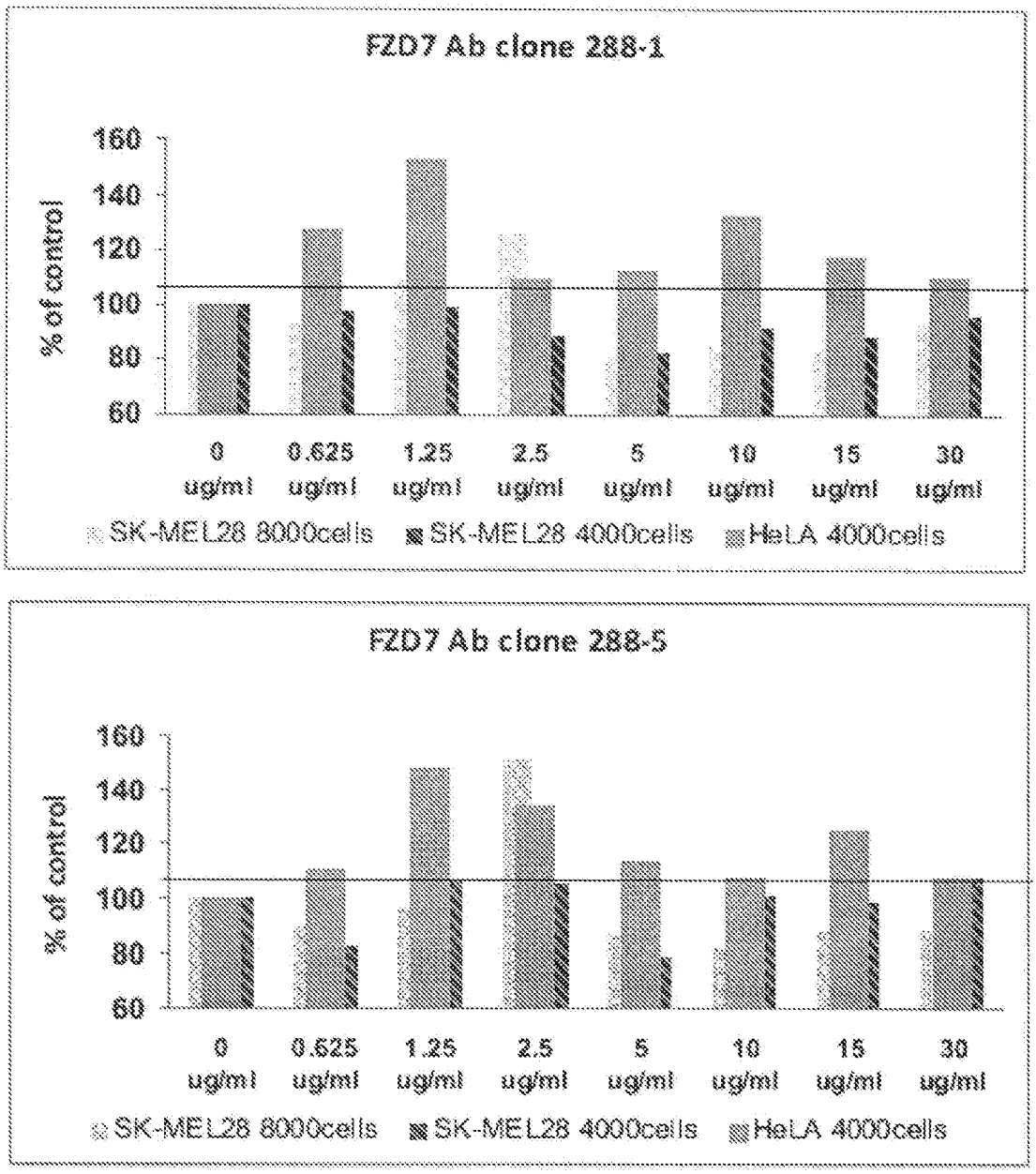

FZD7 Ab clone 288-1
% of control
160
140
120
100
80
60
0 ug/ml
0.625 ug/ml
1.25 ug/ml
2.5 ug/ml
5 ug/ml
10 ug/ml
15 ug/ml
30 ug/ml
SK-MEL28 8000cells
SK-MEL28 4000cells
HeLA 4000cells
FZD7 Ab clone 288-5
% of control
160
140
120
100
80
60
0 ug/ml
0.625 ug/ml
1.25 ug/ml
2.5 ug/ml
5 ug/ml
10 ug/ml
15 ug/ml
30 ug/ml
SK-MEL28 8000cells
SK-MEL28 4000cells
HeLA 4000cells

Figure 5A

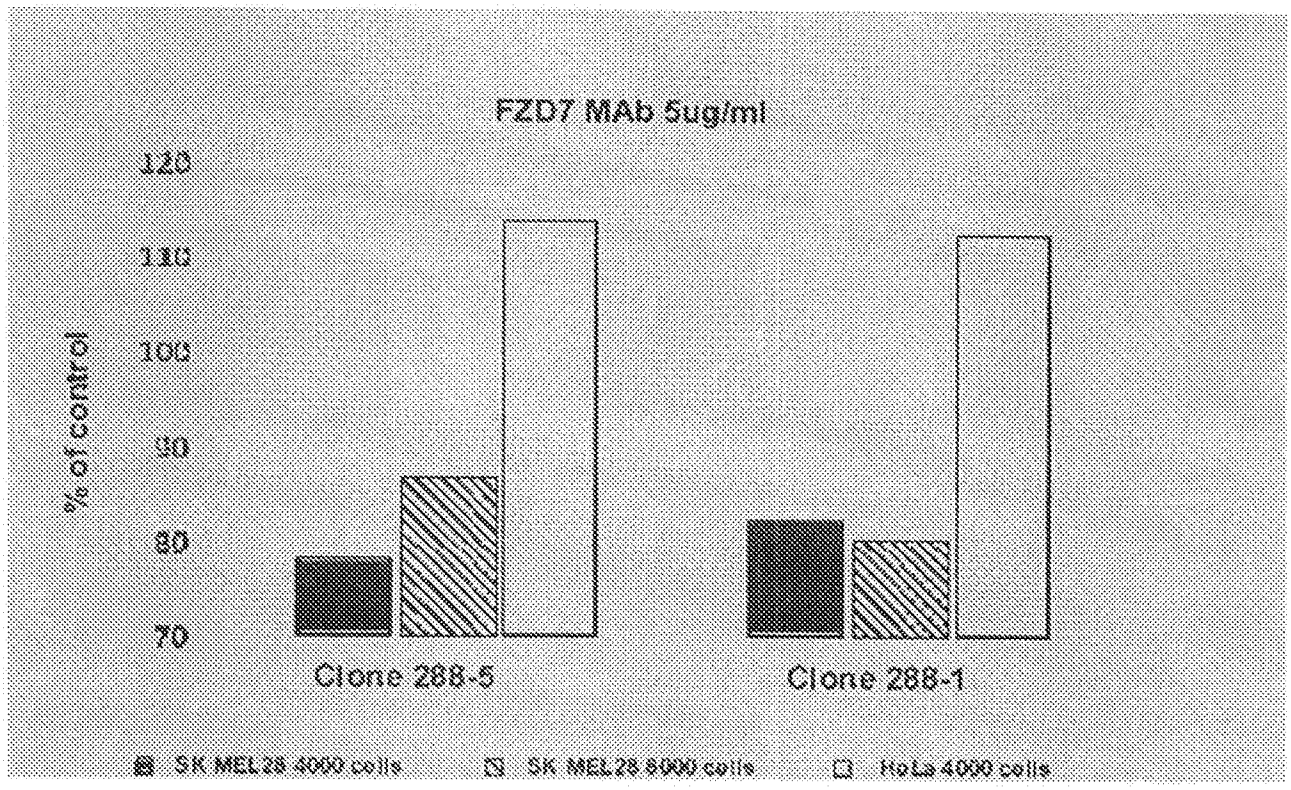


Figure 5B

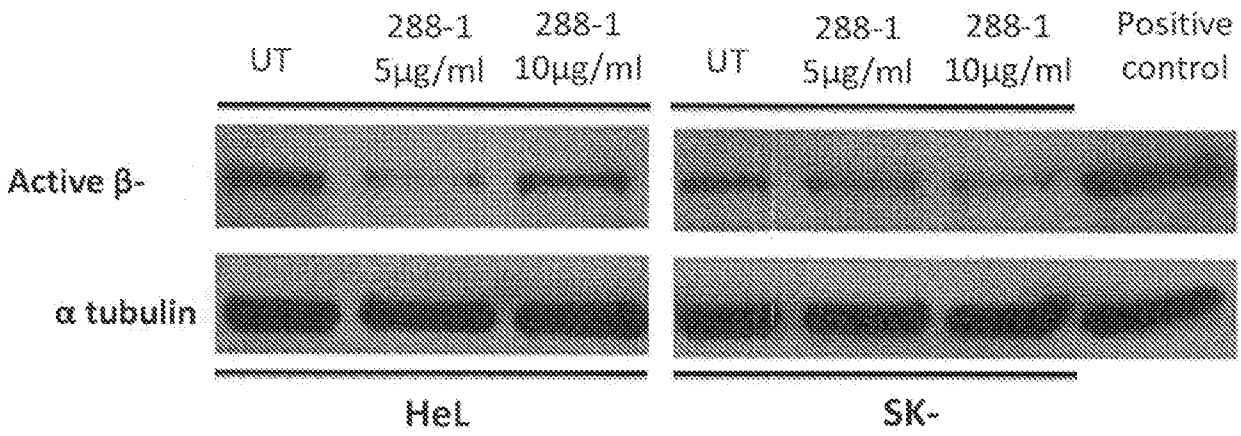


Figure 6

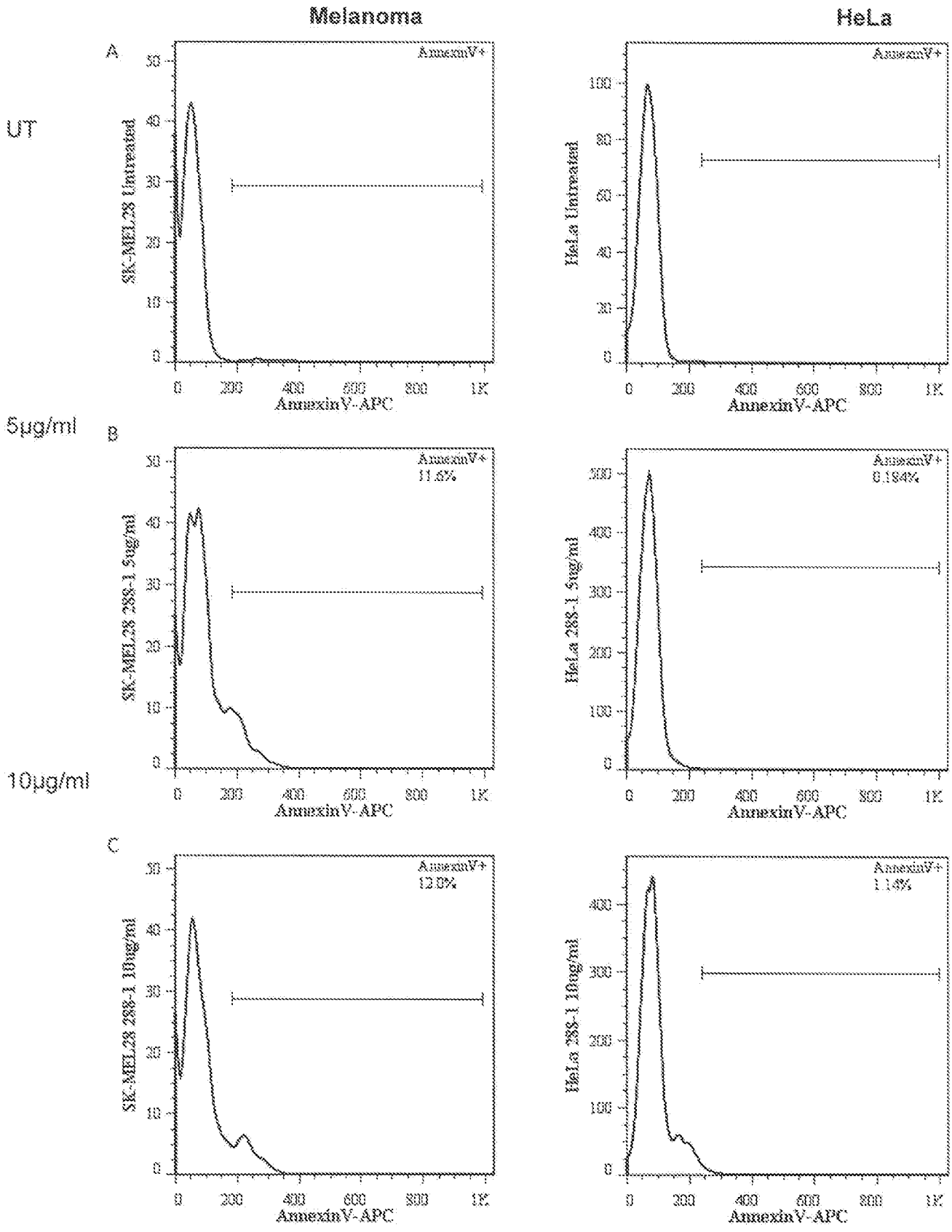
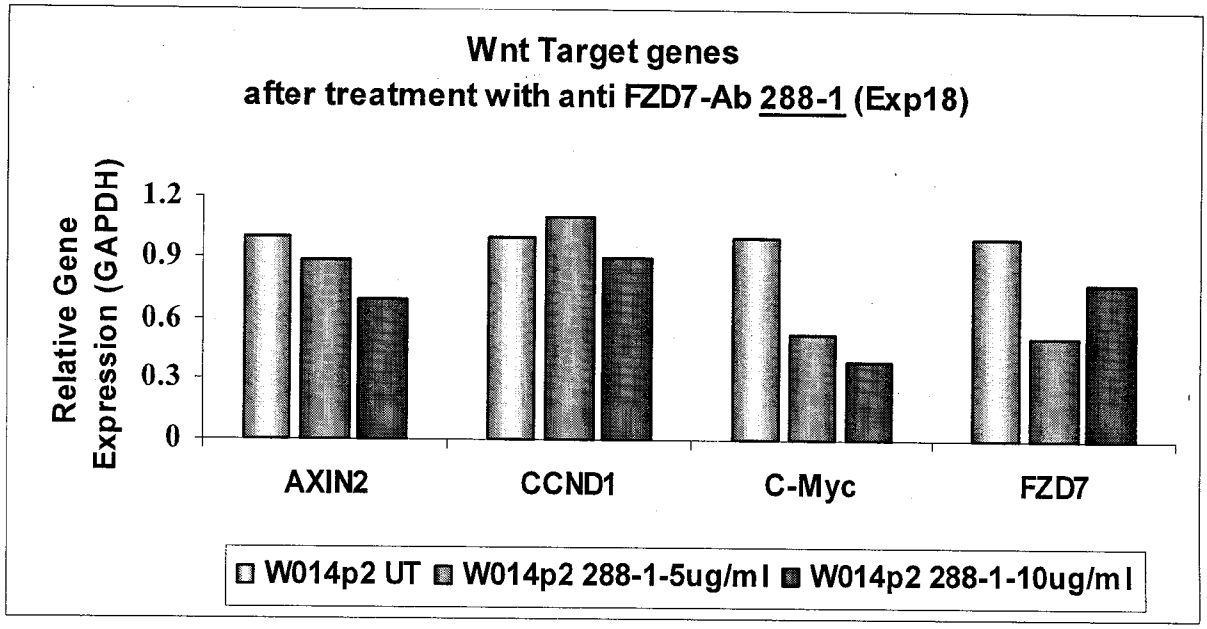

Melanoma
HeLa
A
UT
AnnexinV+
SK-MEL28 Untreated
HeLa Untreated
AnnexinV-APC
5µg/ml
B
AnnexinV+
11.6%
SK-MEL28 288-1 5ug/ml
AnnexinV+
0.184%
HeLa 288-1 5ug/ml
10µg/ml
C
AnnexinV+
12.0%
SK-MEL28 288-1 10ug/ml
AnnexinV+
1.14%
HeLa 288-1 10ug/ml

Figure 7A


Wnt Target genes
after treatment with anti FZD7-Ab 288-1 (Exp18)
Relative Gene Expression (GAPDH)
1.2
0.9
0.6
0.3
0
AXIN2
CCND1
C-Myc
FZD7
W014p2 UT
W014p2 288-1-5ug/ml
W014p2 288-1-10ug/ml

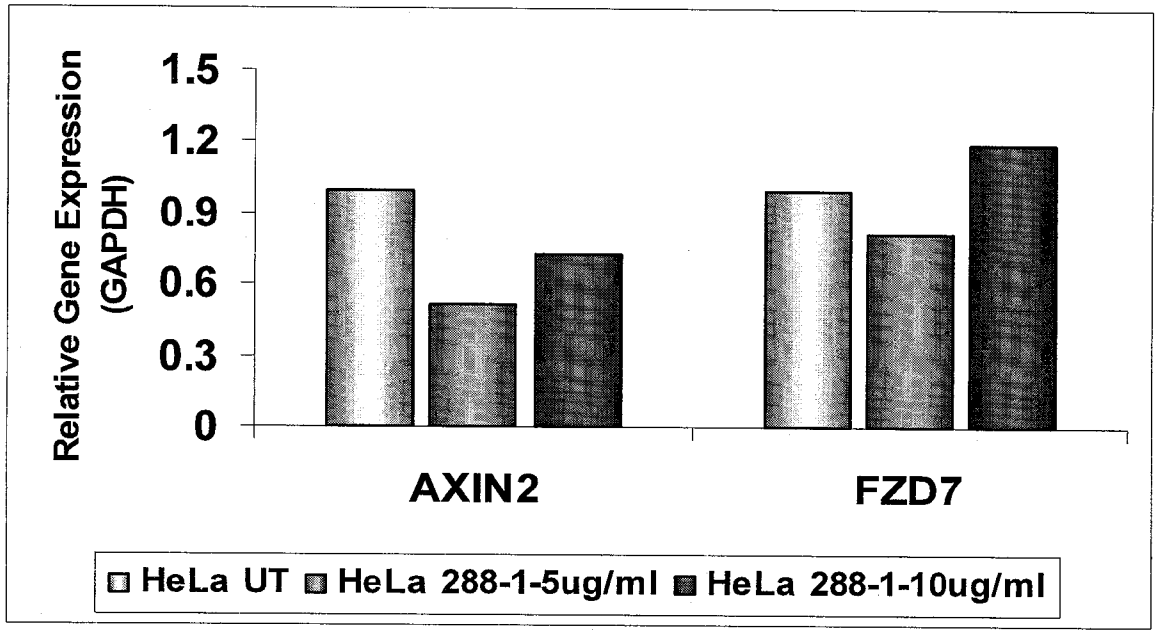
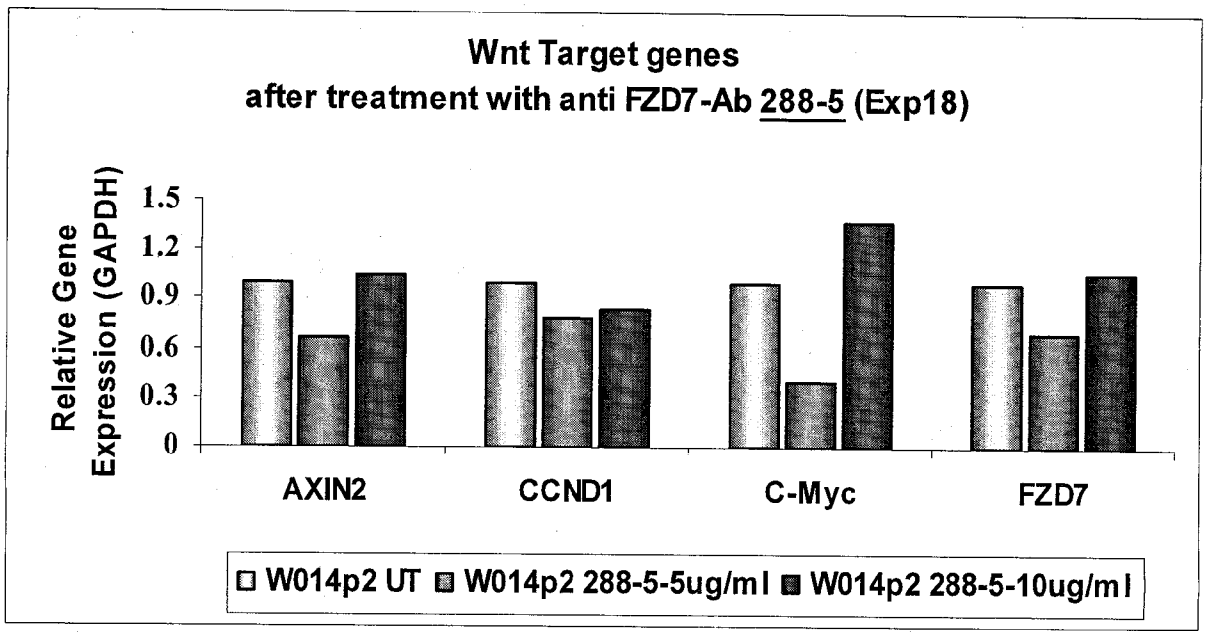

Relative Gene Expression (GAPDH)
1.5
1.2
0.9
0.6
0.3
0
AXIN2
FZD7
HeLa UT
HeLa 288-1-5ug/ml
HeLa 288-1-10ug/ml

Figure 7B



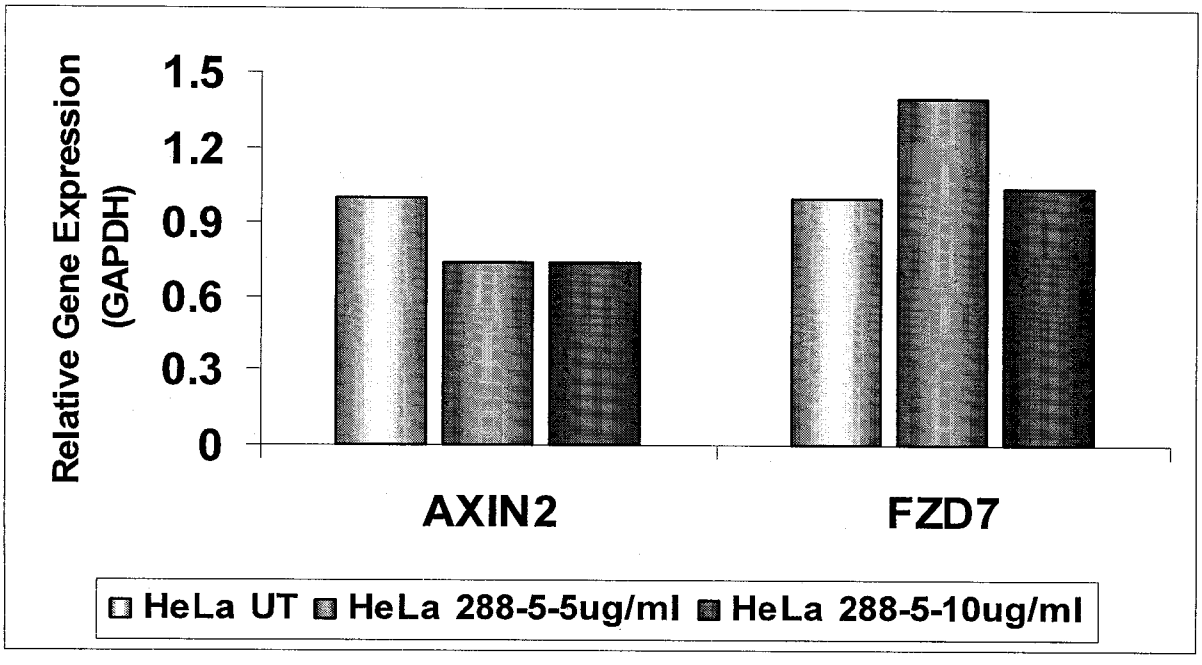


Figure 7B (cont.)

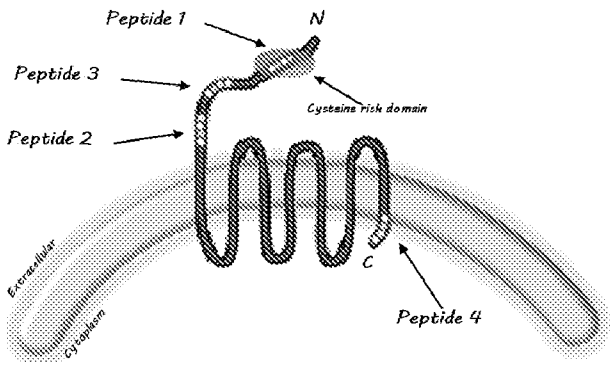


Figure 8

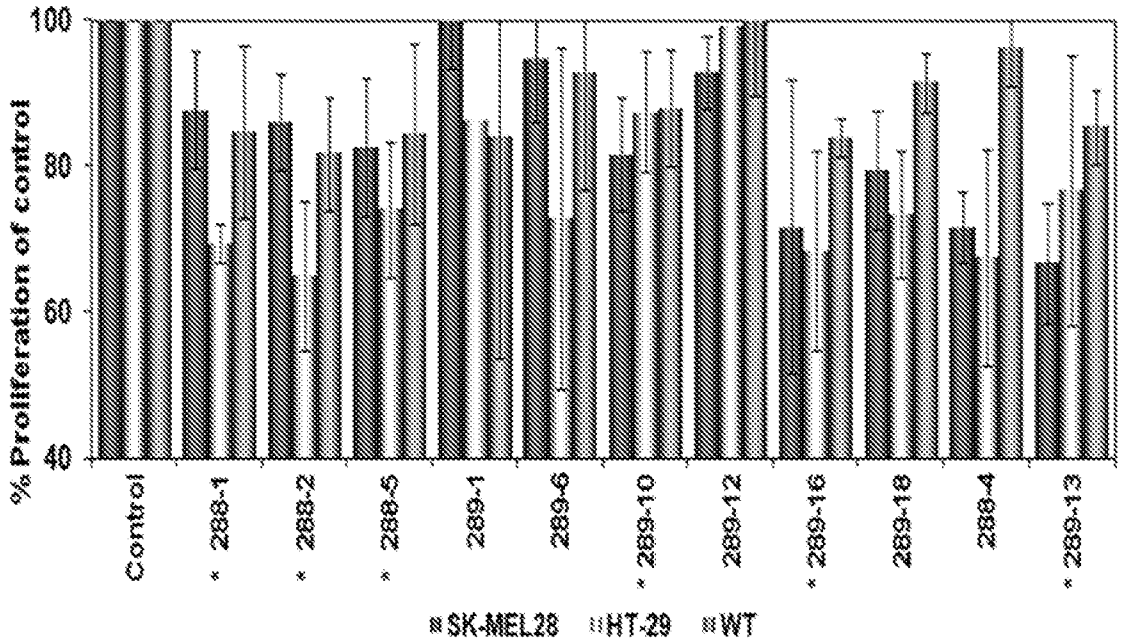


Figure 9



Figure 10

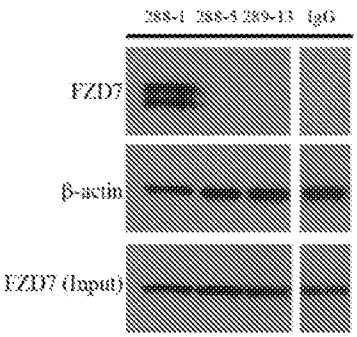


Figure 11A

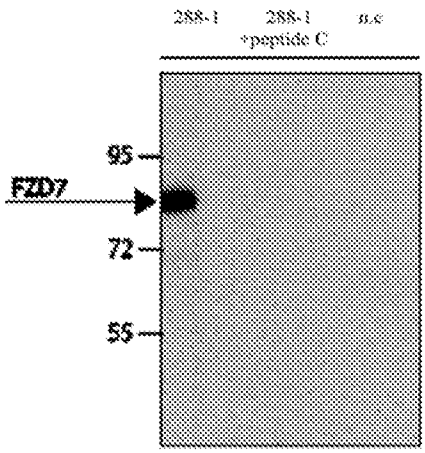


Figure 11B

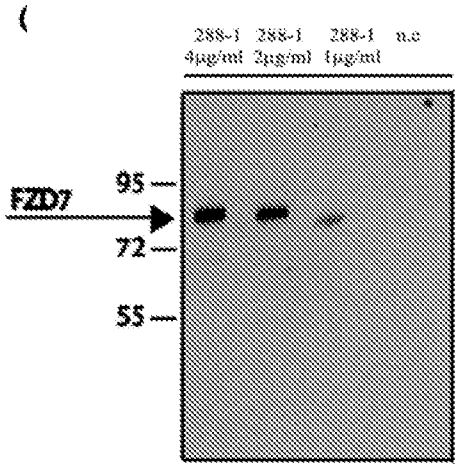


Figure 11C

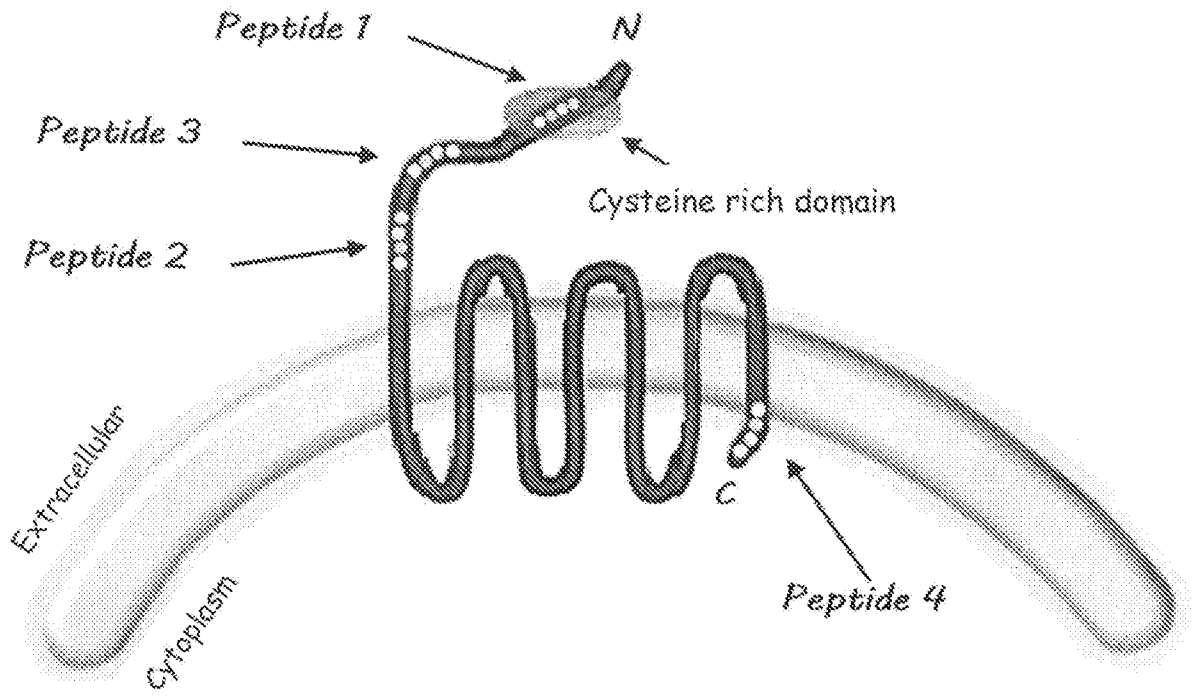


Figure 8

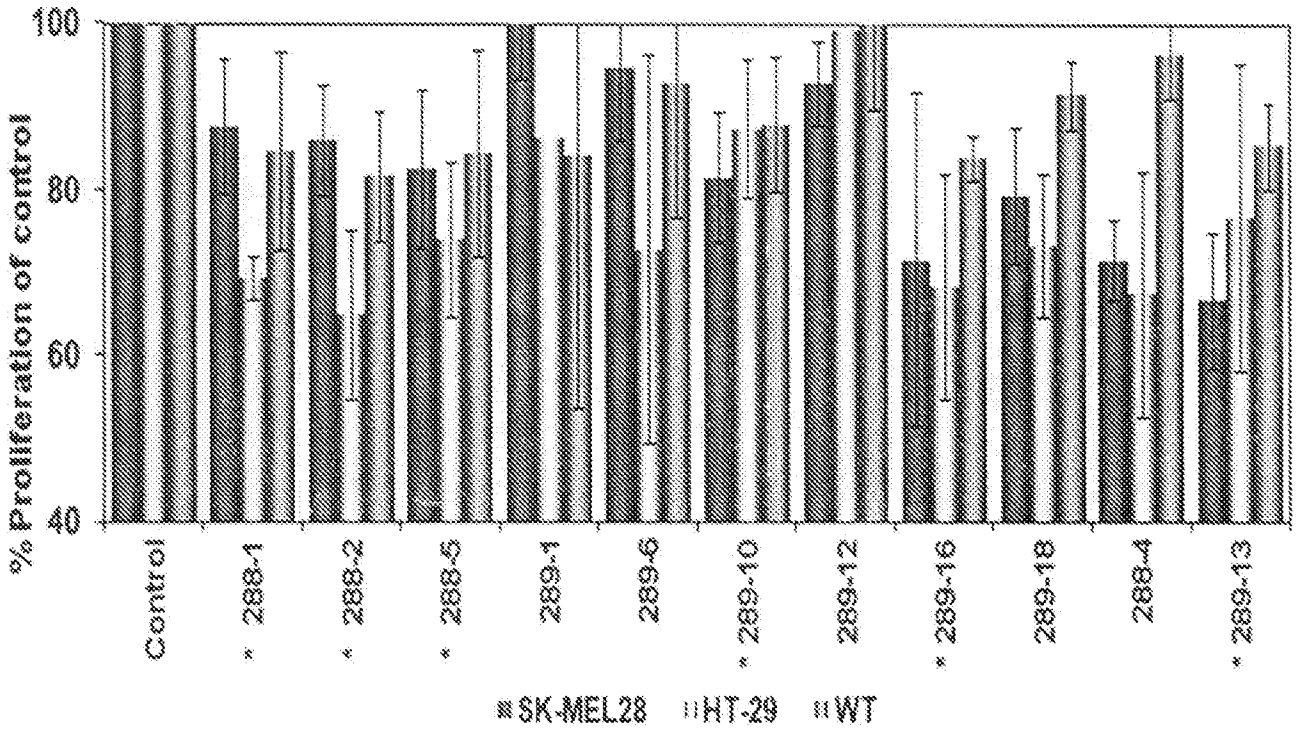


Figure 9

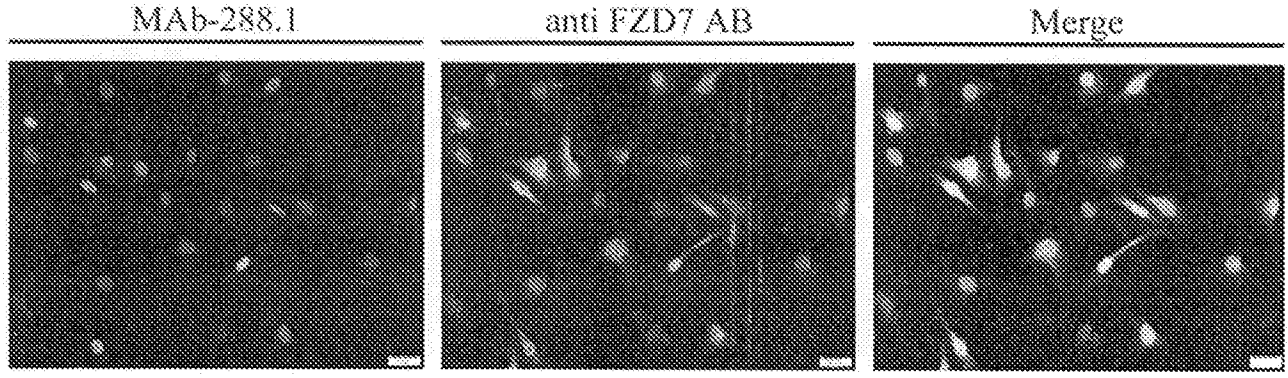


Figure 10

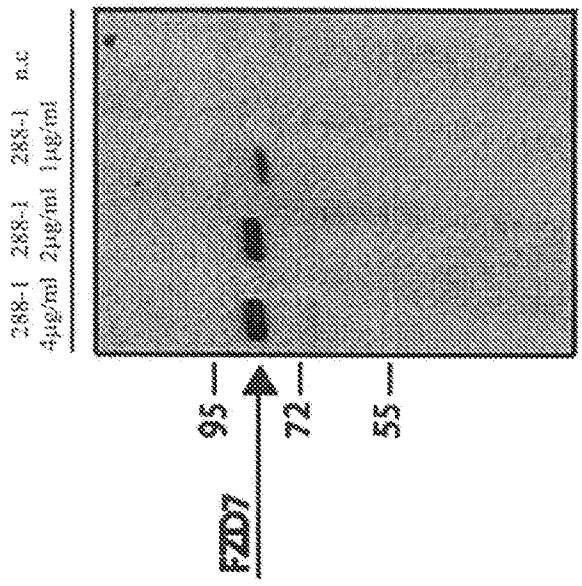


Figure 11C

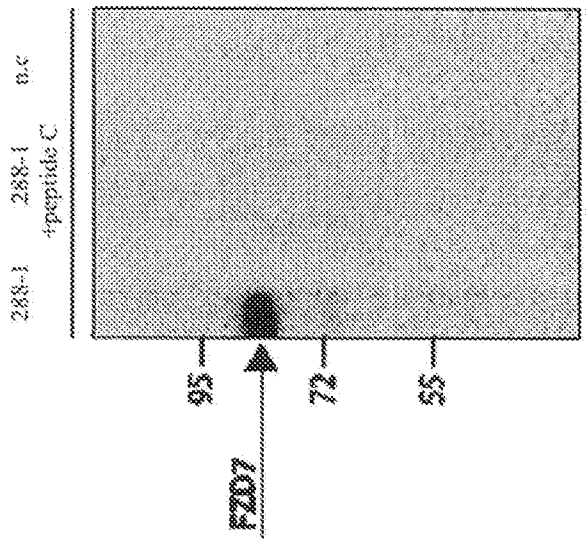


Figure 11B

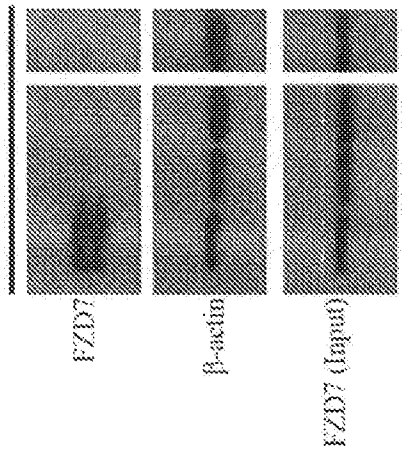


Figure 11A

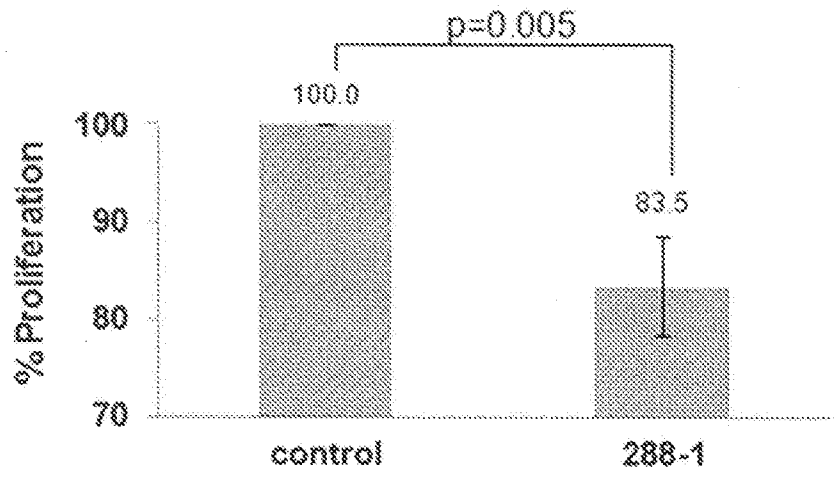


Figure 12

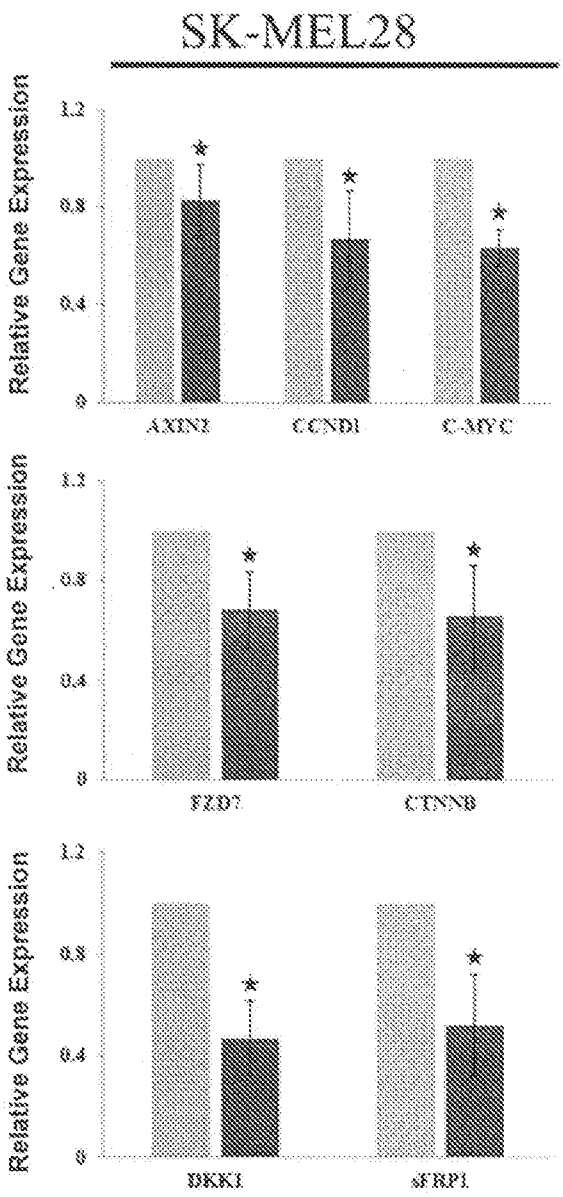


Figure 13A

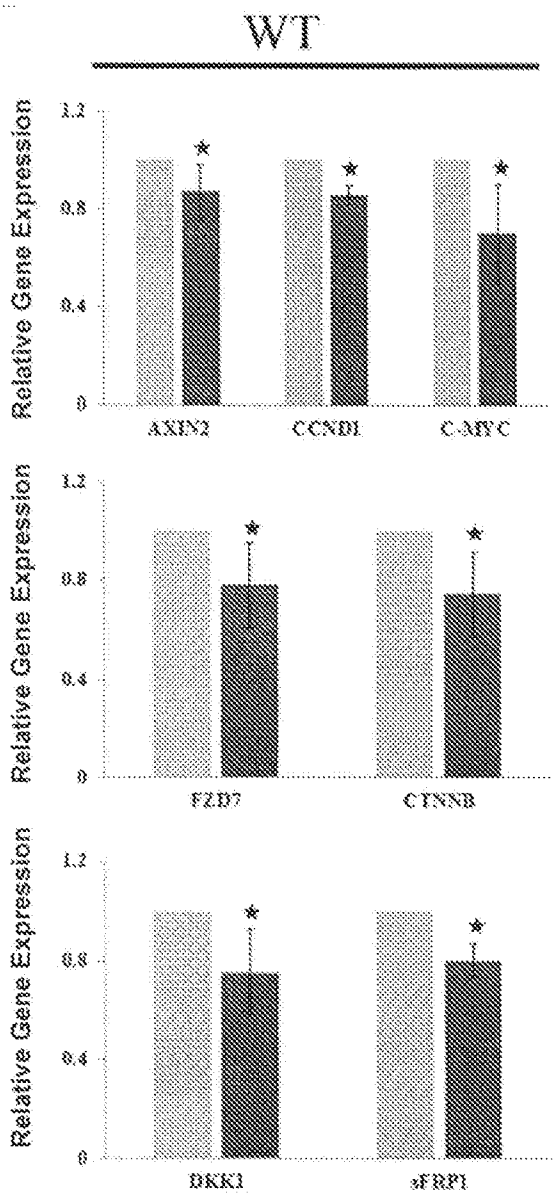


Figure 13B

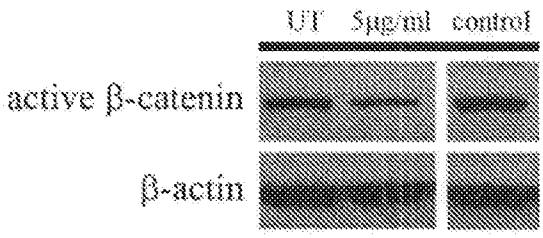


Figure 13C

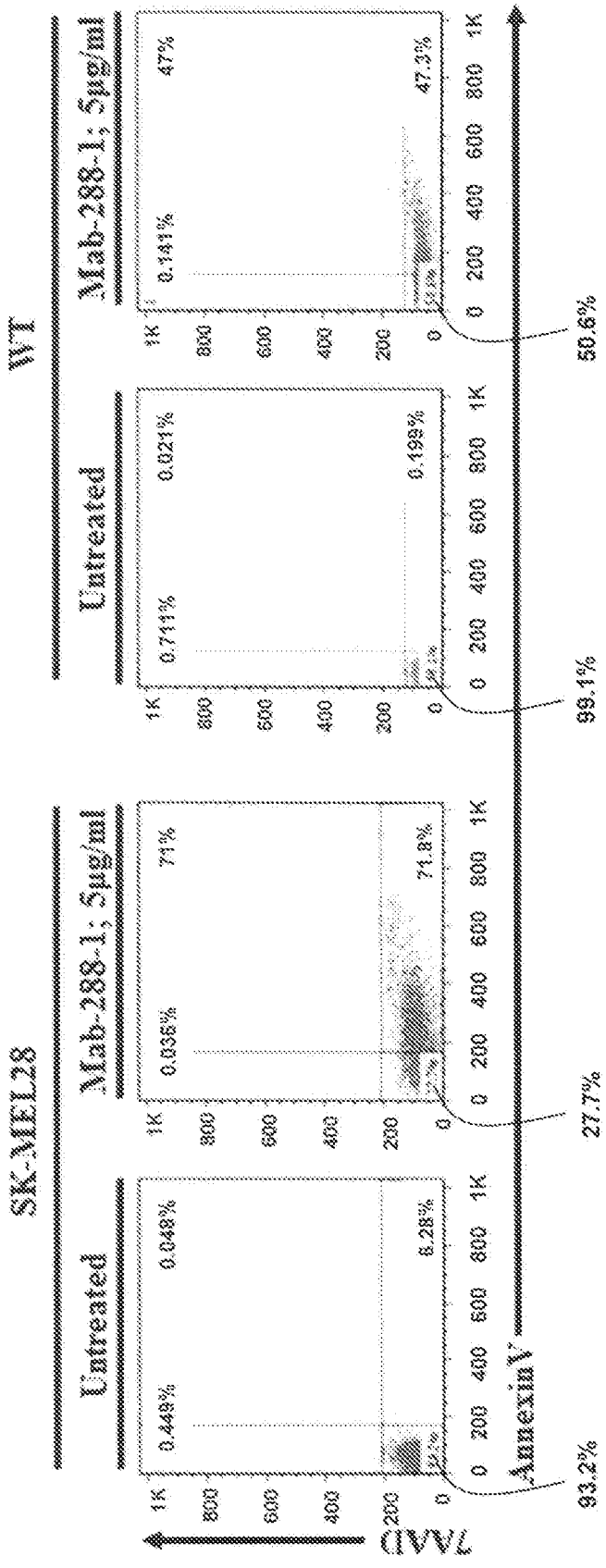

WT
SK-MEL28
Untreated
Mab-288-1; 5µg/ml
Untreated
Mab-288-1; 5µg/ml
0.448%
0.048%
6.28%
0.838%
71%
71.8%
0.711%
0.021%
0.198%
0.141%
47%
47.3%
93.2%
27.7%
99.1%
50.6%
PI
AnnexinV

Figure 14A

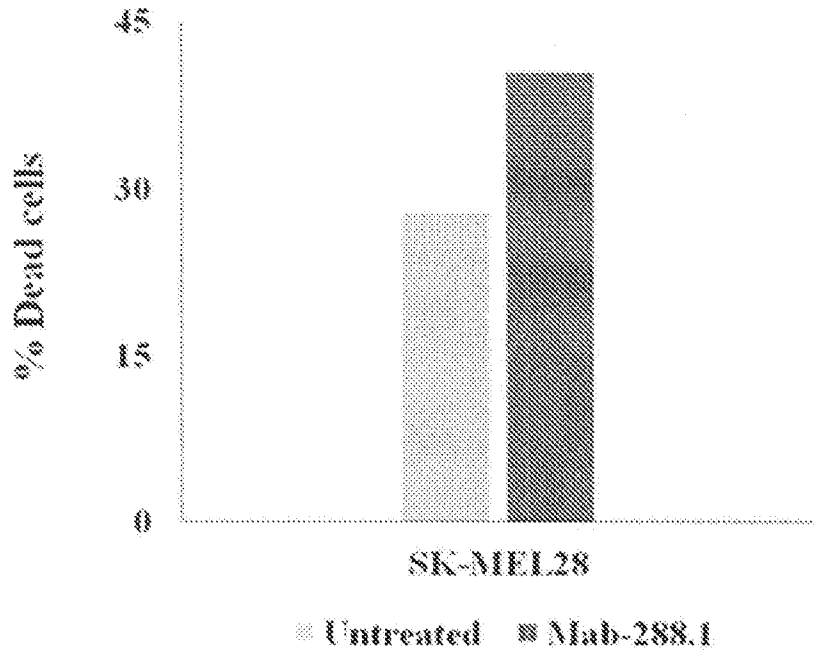


Figure 14B

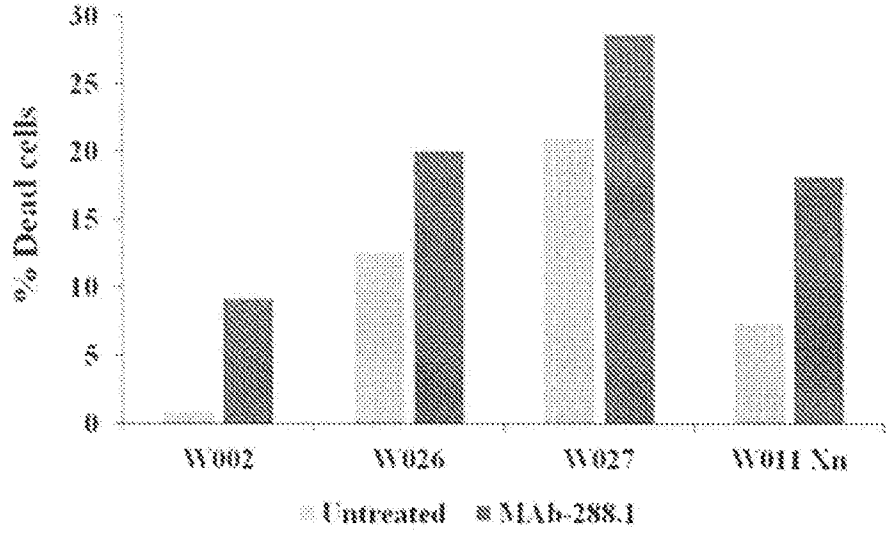


Figure 14C

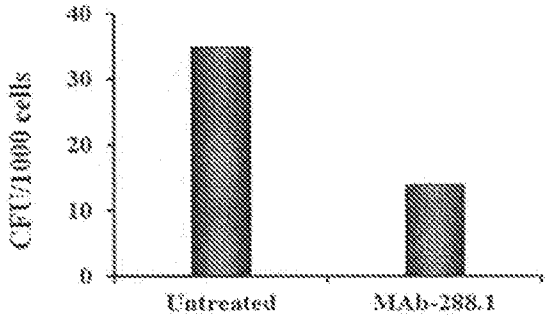


Figure 15A

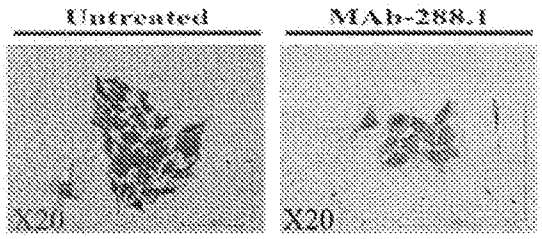


Figure 15B

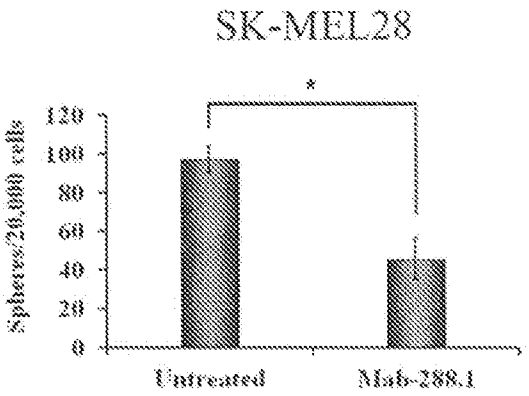


Figure 15C

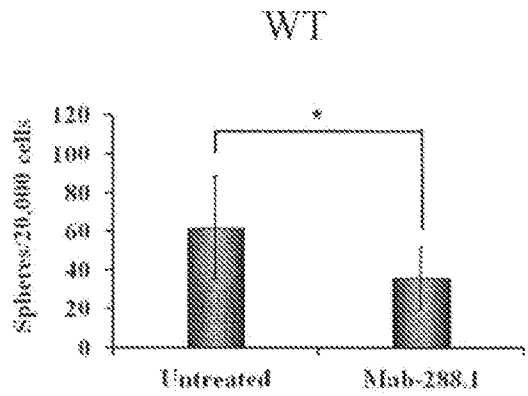


Figure 15D

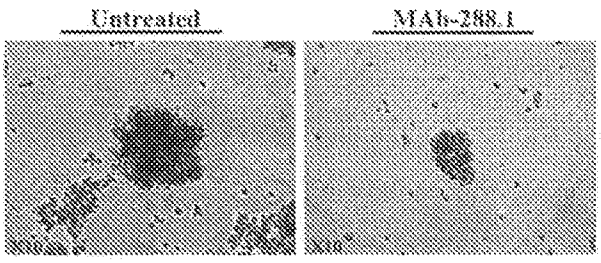


Figure 15E

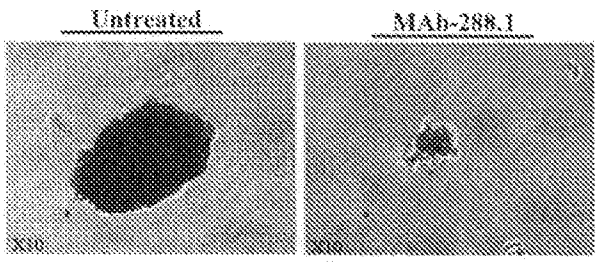


Figure 15F

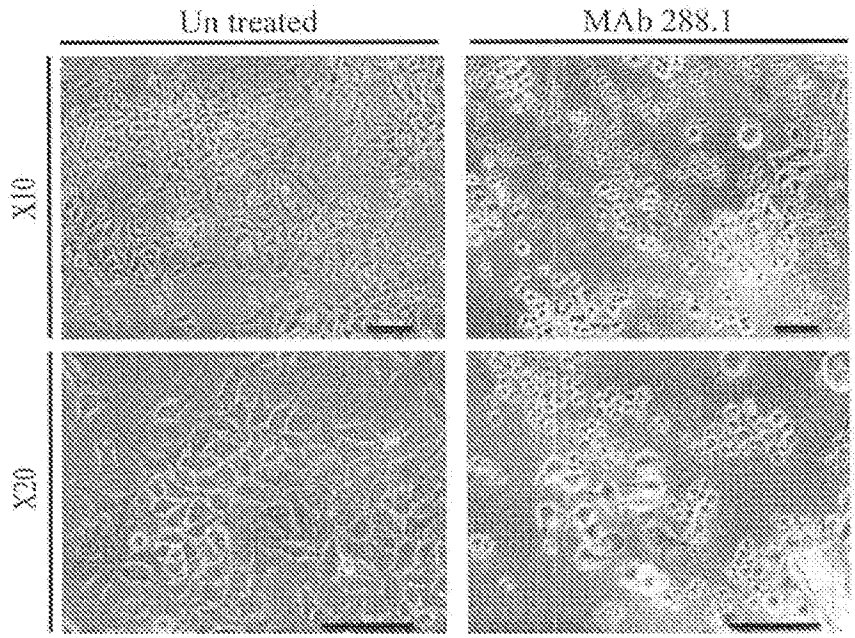


Figure 16A

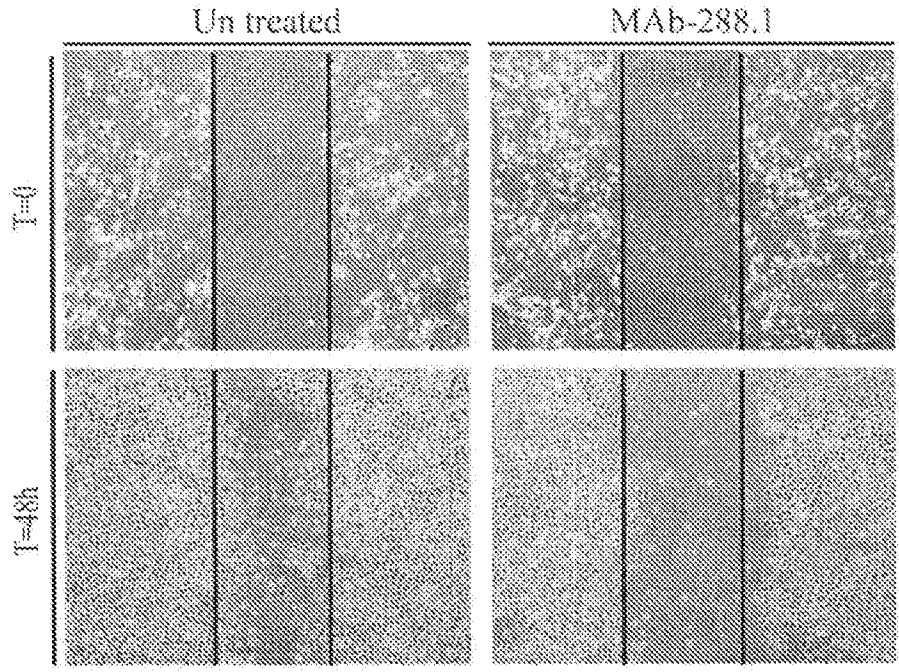


Figure 16B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050009

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2015.01) C07K 16/28, C07K 16/30, A61K 39/395, A61P 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2015.01) C07K, A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: THOMSON INNOVATION, CAPLUS, BIOSIS, EMBASE, MEDLINE, Google Scholar
Search terms used: Frizzled-7, FZD7, antibody, inventor's name

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PODE-SHAKKED, N., et al. Resistance or sensitivity of Wilms' tumor to anti-FZD7 antibody highlights the Wnt pathway as a possible therapeutic target. Oncogene, 2011, 30.14: 1664-1680. doi:10.1038/onc.2010.549 07 Apr 2011 (2011/04/07) page 1665 left column second para- page 1667 right column first para. Figure 1	1-52
X	WO 2011004379 A1 TEL HASHOMER MEDICAL RES INFRA[IL]; DEKEL BENJAMIN [IL]; PODE-SHAKKED NAOMI[IL]; KESHET GILMOR IRIS 13 Jan 2011 (2011/01/13) examples 1, 4	1-52
X	WO 2007053577 A2 GURNEY AUSTIN[US]; LEWICKI JOHN[US]; SATYAL SANJEEV [US]; HOEY TIMOTHY 10 May 2007 (2007/05/10) para 0064, 0164-0165	1-52

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 Apr 2015	16 Apr 2015
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer HOROWITZ Anat Telephone No. 972-2-5651689

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050009

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. [X] forming part of the international application as filed:

[X] in the form of an Annex C/ST.25 text file.

[ ] on paper or in the form of an image file.

b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

[ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

[ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL2015/050009

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

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

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2015/050009

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PODE SHAKKED, Naomi, et al. Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population. Journal of cellular and molecular medicine, 2009, 13.8b: 1792-1808. DOI: 10.1111/j.1582-4934.2008.00607.x Retrieved from the internet: http://onlinelibrary.wiley.com/doi/10.1111/j.1582-4934.2008.00607.x/full 16 Dec 2008 (2008/12/16) abstract, page 1800	1-52

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2015/050009

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2011004379 A1	13 Jan 2011	WO 2011004379 A1	13 Jan 2011
WO 2007053577 A2	10 May 2007	WO 2007053577 A2	10 May 2007
		WO 2007053577 A3	06 Dec 2007
		AU 2006308870 A1	10 May 2007
		AU 2006308870 B2	16 Aug 2012
		AU 2006344359 A1	13 Dec 2007
		AU 2006344359 B2	15 Aug 2013
		CA 2628116 A1	13 Dec 2007
		CA 2628221 A1	10 May 2007
		CN 101356679 A	28 Jan 2009
		CN 102827287 A	19 Dec 2012
		EP 1961065 A2	27 Aug 2008
		EP 1961065 A4	11 Nov 2009
		EP 1978993 A2	15 Oct 2008
		EP 1978993 A4	18 Nov 2009
		EP 2479192 A1	25 Jul 2012
		EP 2481756 A2	01 Aug 2012
		EP 2481756 A3	08 Aug 2012
		EP 2500360 A2	19 Sep 2012
		EP 2500360 A3	12 Dec 2012
		EP 2511298 A2	17 Oct 2012
		EP 2511298 A3	13 Feb 2013
		JP 2009515513 A	16 Apr 2009
		JP 5368798 B2	18 Dec 2013
		JP 2009513708 A	02 Apr 2009
		JP 2013039127 A	28 Feb 2013
		JP 2013241409 A	05 Dec 2013
		US 2007117751 A1	24 May 2007
		US 7723477 B2	25 May 2010
		US 2010317098 A1	16 Dec 2010

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IL2015/050009

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
		US 8324361 B2	04 Dec 2012
		US 2013273044 A1	17 Oct 2013
		US 8765913 B2	01 Jul 2014
		US 2007116701 A1	24 May 2007
		US 2014349399 A1	27 Nov 2014
		WO 2007133250 A2	22 Nov 2007
		WO 2007133250 A3	26 Mar 2009
		WO 2007142711 A2	13 Dec 2007
		WO 2007142711 A3	18 Dec 2008

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

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

Invention/s 1	An isolated antibody or antigen-binding portion thereof that binds to the cytoplasmic portion and optionally, the transmembrane portion of Frizzled receptor, pharmaceutical composition comprising the same, method of reducing the tumorigenicity of a tumor that comprises cancer stem cells in a subject, method for treating a tumor in a subject, said methods comprising the step of administering a therapeutically effective dose of said isolated antibody.	Claim/s 1-3,8-20,25-40,45-52
Invention/s 2	Claims Nos. 4, 21, 41(all) 8-17, 25-37, 45-52 (in part) ; An isolated antibody or antigen-binding portion thereof that binds to an epitope of Frizzled receptor comprising amino acid of SEQ ID NO:2, , pharmaceutical composition comprising the same, method of reducing the tumorigenicity of a tumor that comprises cancer stem cells in a subject, method for treating a tumor in a subject, said methods comprising the step of administering a therapeutically effective dose of said isolated antibody, method of detecting a tumor in a subject using said isolated antibody.	Claim/s 4,8-17,21,25-37,41,4
Invention/s 3	Claims Nos. 5, 22, 42 (all) 8-17, 25-37, 45-52 (in part); An isolated antibody or antigen-binding portion thereof that binds to an epitope of Frizzled receptor comprising amino acid of SEQ ID NO:3, pharmaceutical composition comprising the same, method of reducing the tumorigenicity of a tumor that comprises cancer stem cells in a subject, method for treating a tumor in a subject, said methods comprising the step of administering a therapeutically effective dose of said isolated antibody, method of detecting a tumor in a subject using said isolated antibody.	Claim/s 5,8-17,22,25-37,42,4
Invention/s 4	Claims Nos. 6-7, 23-24, 43-44 (all) 8-17, 25-37, 45-52 (in part); An isolated antibody or antigen-binding portion thereof that binds to an epitope of Frizzled receptor comprising amino acid of SEQ ID NO:4, pharmaceutical composition comprising the same, method of reducing the tumorigenicity of a tumor that comprises cancer stem cells in a subject, method for treating a tumor in a subject, said methods comprising the step of administering a therapeutically effective dose of said isolated antibody, method of detecting a tumor in a subject using said isolated antibody.	Claim/s 6-17,23-37,43-52