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(54) Title: ANTI-HDAC11 ANTIBODIES

(57) Abstract: The present invention relates to an antibody specifically recognizing histone deacetylase 11 (HDAC11), wherein said antibody recognizes native HDAC11 or native and denatured HDAC11. The present invention also relates to a cell line producing said antibody. It further relates to methods for identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor, for identifying HDAC11 recruitment sites, for identifying a subject afflicted with cancer, for identifying an inhibitor of HDAC11 recruitment, and for identifying an HDAC11 binding compound, said methods making use of the antibody of the present invention. The invention further relates to the use of the antibody of the present invention in said methods and to devices and kits comprising the antibody of the present invention.



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anti-HDAC11 antibodies

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The present invention relates to an antibody specifically recognizing histone deacetylase 11 (HDAC11), wherein said antibody recognizes native HDAC11 or native and denatured HDAC11. The present invention also relates to a cell line producing said antibody. It further relates to methods for identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor, for identifying HDAC11 recruitment sites, for identifying a subject afflicted with cancer, for identifying an inhibitor of HDAC11 recruitment, and for identifying an HDAC11 binding compound, said methods making use of the antibody of the present invention. The invention further relates to the use of the antibody of the present invention in said methods and to devices and kits comprising the antibody of the present invention.

Histone deacetylases (HDAC, EC number 3.5.1) are a group of hydrolases removing the acetyl group from an ϵ -N-acetyl lysine amino acid of a histone. Depending on sequence identity and domain organization, HDACs have been classified into class I (including HDAC1 - 3 and 8), class IIa (HDAC4, 5, 7, 9), class IIb (HDAC6 and 10), class III (including sirtuins) and class IV (HDAC11) (Dokmanovic et al., 2007, Mol Cancer Res October 5; 981-989). It has become clear over recent years that HDACs are not restricted to histones as substrates and that they have functions in several regulative pathways of the cell (Choudhary, C, Kumar, C, Gnad, F, Nielsen, ML, Rehman, M, Walther, TC et al., (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325: 834-40.).

HDAC inhibitors have been traditionally used in psychiatry and neurology as mood stabilizers and anti-epileptics (e.g. valproic acid). More recently, they have been studied for their effects in neurodegenerative diseases, but also in a supportive manner in cancer therapy (Bolden, JE, Peart, MJ and Johnstone, RW, (2006) Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 5: 769-84.). Despite the fact that several non-

histone substrates have been identified for the various HDAC, information is scarce as to which molecular pathways mediate the effects of HDAC inhibitor treatment in cancer; even less is known as to if all cancer patients or just certain cohorts would benefit from HDAC treatment (Witt, O, Deubzer, HE, Milde, T and Oehme, I, (2009) HDAC family: What are the cancer relevant targets? Cancer Lett 277: 8-21.). Moreover, it is unknown, how a potential cohort of patients who would benefit from treatment with a HDAC inhibitor could be identified (Witt, O, Deubzer, HE, Milde, T and Oehme, I, (2009) HDAC family: What are the cancer relevant targets? Cancer Lett 277: 8-21.).

- 10 HDAC1, -2, and 8 have been suggested to play a role in reduction of multi-drug resistance, apoptosis, and differentiation of tumor cells (see Oehme 2009, Expert Opin Investig Drugs 18: 1605-1617; Witt 2009, Curr Pharm Des 15:436-447; Keshelva 2007, J Natl Cancer Inst 99: 1107-1119; Oehme 2009, Clin Cancer Res 15: 91-99; Witt 2009, Cancer Lett 277: 8-21). HDAC10 has been merely reported to be involved in deacetylation and thioredoxin regulation (Lai 2010, J Biol Chem 285: 7187-7196; Lee 2010, Mol Cells 30: 107-112).

- HDAC11 is the most recently identified HDAC family member with, as yet, poorly understood function. The catalytic domain of HDAC11 is located at the amino terminus, and contains residues conserved in class I and IIa/b HDACs (Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. J Biol Chem. 2002;277(28):25748-25755). Yet, amino acid sequences are so dissimilar that HDAC11 was not grouped into classes I or IIa/b, but designated as a separate novel class IV (Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol. 2008;9(3):206-218). Phylogenetic analysis has shown that HDAC11 is most closely related to HDACs 3 and 8 (de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003;370:737-749). HDAC11 was shown to negatively regulate IL10 expression in human antigen-presenting cells (Villagra A, Cheng F, Wang HW, Suarez I, Glozak M, Maurin M, Nguyen D, Wright KL, Atadja PW, Bhalla K, Pinilla-Ibarz J, Seto E, Sotomayor EM. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol. 2009;10(1):92-100.) and to regulate OX40 ligand expression in Hodgkin lymphoma (Buglio D, Khaskhely NM, Voo KS, Martinez-Valdez H, Liu YJ, Younes A. HDAC11 plays an essential role in regulating

OX40 ligand expression in Hodgkin lymphoma. Blood 2011;117(10):2910-2917.). So far, HDAC11 has not been detected in any of the multi-protein HDAC complexes (Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. J Biol Chem. 2002;277(28):25748-25755), but was shown to interact with the Cdt1 replication licensing factor (Glozak MA, Seto E. Acetylation/deacetylation modulates the stability of DNA replication licensing factor Cdt1. J Biol Chem. 2009;284:11446-11453). Transfected into 293 cells, HDAC11 co-immunoprecipitates with HDAC6, suggesting they share common binding partners or associate directly (Gao et al., loc. cit.). Antibodies are an indispensable tool for elucidating the role of HDAC11 in different tissues. Anti-HDAC11 antibodies available at present were found to give results with varying consistency in most of the assays tested. There is thus a need in the art for reliable anti-HDAC11 antibodies. Specifically, antibodies recognizing the native form of HDAC11 are required.

Therefore, the technical problem underlying the present invention is to provide means and methods for complying with the aforementioned needs. Thus, the present invention relates to an antibody specifically recognizing histone deacetylase 11 (HDAC11), wherein said antibody recognizes native HDAC11 or native and denatured HDAC11.

As used herein, the term "histone deacetylase" or "HDAC" relates to a member of the family of enzymes removing acetyl groups from an ϵ -N-acetyl lysine amino acid on a histone (EC 3.5.1) and, preferably, thereby controlling gene expression. The term "histone deacetylase 11" or "HDAC11" relates to members of the family of HDAC enzymes similar to the polypeptide encoded by the human HDAC11 gene (human gene: SEQ ID NO: 1, Genbank Acc No: NC_000003.11 GI:224589815; transcript variant 1 of the human gene: SEQ ID NO:2, NM_024827.3 GI:227430412; transcript variant 2 of the human gene: SEQ ID NO:3, NM_001136041.2 GI:227430411) classified as the hitherto sole members of class IV of histone deacetylases. Preferably, HDAC11 relates to the human HDAC11 isoform 1 and / or isoform 2 polypeptides (isoform 1: SEQ ID NO: 4, Genbank Acc No: NP_079103.2 GI:217272877; isoform 2: SEQ ID NO:5, Genbank Acc NO: NP_001129513.1 GI:209862883). It is understood by the skilled person that HDAC11 binds to proteins other than histones in order to fulfill its function, e.g. HDAC6 or proteins recruiting HDAC11 to its recruitment sites.

The terms "specifically recognizing", as used herein, relates to binding of an antibody specific to HDAC11. Specific recognition, preferably, requires binding to HDAC11 with an affinity at least 5fold, at least 10fold, at least 25fold, at least 50fold higher, or at least 100fold higher than for polypeptides or peptides which are not variants or peptide fragments of HDAC11. More preferably, specifically recognizing relates to a lack of cross reaction with other polypeptides or peptides which are not variants or peptide fragments of HDAC11, i.e. to binding to any of said other polypeptides or peptides with negligible affinity. Specific binding can be tested by various well known techniques.

The term "native" HDAC11, as used herein, relates to a non-denatured HDAC11 molecule. Preferably, the native HDAC11 has the conformation, preferably tertiary structure, assumed by HDAC11 in the normal living cell, as can be isolated by the methods described herein below. More preferably, the native HDAC11 is the polypeptide having at least one enzymatic or binding activity of HDAC11, most preferably having all measurable enzymatic and binding activities of HDAC11. It is understood by the skilled artisan that the binding activity of HDAC11 does not necessarily relate to DNA binding but may, preferably, also relate to binding of other interaction partners, e.g. polypeptides or other molecules, said other interaction partners themselves binding to DNA.

The term "denatured" HDAC11, as used herein, relates to an HDAC11 molecule wherein the tertiary structure has been caused at least partially to unfold, e.g. by treatment with one of heat, detergent, alkali, and acid. Preferably, in the the denatured HDAC11 some of its original properties, preferably its biological activity, i.e. enzymatic and / or binding activities, are diminished or eliminated. More preferably, in the the denatured HDAC11 all of its biological activities, i.e. enzymatic and / or binding activities, are diminished. Most preferably, in the denatured HDAC11 all of its biological activities, i.e. enzymatic and / or binding activities, are eliminated. Preferably, in the denatured HDAC11 the amino acid chain of HDAC11 is essentially linear as is e.g. induced by heating to more than 90°C in the presence of at least 0.1% sodium dodecylsulfate as described herein below.

The term "antibody" as used in this specification refers to a molecule from the subgroup of gamma globulin proteins which is also referred to as the immunoglobulins (Ig). Antibodies can, preferably, be of any subtype, i.e. IgA, IgD, IgE, IgM or, more preferably, IgG. Antibodies against polypeptides encoded by target genes of the invention can be prepared

by well known methods using a purified polypeptide or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either by proteolytic digestion from polypeptides encoded by target genes or may be synthetic peptides. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a human or humanized antibody or primatized, chimerized or fragment thereof. Also comprised as antibodies by the present invention are a bispecific antibody, a synthetic antibody, an antibody fragment, such as Fab, Fv or scFv fragments etc., or a chemically modified derivative of any of these.

Antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Preferably, the antibody of the present invention is a monoclonal antibody, which can, e.g. be prepared by the techniques originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. More preferably, the antibody of the present invention is an antibody recognizing an epitope comprising an amino acid sequence as depicted in Fig. 2a) (SEQ ID NO:6), Fig. 2b) (SEQ ID NO:7), Fig. 2c) (SEQ ID NO:8), or Fig. 2d) (SE ID NO:9); or the antibody of the present invention is an antibody recognizing a discontinuous and/or conformational epitope of HDAC11. Most preferably, the antibody of the present invention is an antibody produced by one of the cell lines deposited at the Leibnitz-Institut DSMZ-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (DSMZ) under No DSM ACC3170 and No DSM ACC3171.

The antibody of the present invention recognizes native HDAC11. It is, however, also envisaged by the present invention that the antibody recognizes native and denatured HDAC11. In a preferred embodiment, the antibody of the present invention recognizes HDAC11 in immune cytochemistry. Preferably, the antibody of the present invention recognizes HDAC11 also in immune histochemistry. The term "cytochemistry" relates to the biochemistry of cells, especially to the macromolecules determining cell structure and function. The term "immune cytochemistry" or "immunocytochemistry" relates to determining the presence and / or the amount of at least one macromolecule in a cell of interest by immunological means, e.g. by use of an antibody specific for said macromolecule. The term "immune histochemistry" or immunohistochemistry" refers to

detecting a macromolecule (e.g., proteins) in cells of a tissue section by immunological means, e.g. by use of an antibody specific for said macromolecule.

In a further preferred embodiment, the antibody of the present invention recognizes HDAC11 in chromatin immunoprecipitation. The term "chromatin immunoprecipitation" or "ChIP" is understood by the skilled artisan and relates to a method wherein a protein of interest and associated sheared chromatin are bound by an agent specific for said protein, preferably an antibody, and by this means isolated, allowing a detection of polynucleotides associated with said protein of interest. More preferably, the ChIP is native ChIP, wherein native chromatin sheared mechanically and / or digested by micrococcal nuclease is used. It is understood by the skilled artisan that the protein of interest is required to be native in native ChIP, since at least binding of the protein of interest to said polynucleotide or to a protein mediating its recruitment is required for the method to work.

The definitions made above apply mutatis mutandis to the following:

The present invention also relates to a cell line producing the monoclonal antibody of the present invention.

As used herein, the term "cell line" relates to cells, preferably mammalian cells, more preferably mouse or rat cells, grown in tissue culture and consisting of, preferably, clonal descendants of a single cell. Preferably, the cell line is a hybridoma cell line, more preferably a mouse hybridoma cell line, even more preferably a mouse hybridoma cell line prepared by fusion of mouse cell line Sp 2/0-AG14 and mouse lymphocytes, most preferably a hybridoma cell line deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) under No DSM ACC3170 and No DSM ACC3171. Preferably, the hybridoma cell line is obtained from lymphocytes obtained from a mammal, preferably a non-human mammal, immunized with HDAC11, more preferably immunized with a polypeptide having an amino acid as depicted in Fig. 1 (SEQ ID NO:4). Preferably, the cell line produces an antibody of the present invention. More preferably, the cell line secretes an antibody of the present invention into the culture medium at a concentration of at least 25 µg/ml, at least 50 µg/ml, at least 75 µg/ml, at least 100 µg/ml, at least 125 µg/ml, or at least 150 µg/ml.

The present invention further relates to a method for identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor, comprising (a) contacting a sample from said subject with the antibody of the present invention, (b) determining the amount of HDAC11/antibody complexes formed in the mixture of (a), and
5 (c) comparing said amount with a reference amount, thereby identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor.

The method for identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor, preferably, is an in vitro method. Moreover, it may comprise
10 steps in addition to those explicitly mentioned above. For example, further steps may relate to cell and/or compound pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or assisted by automation. Preferably, steps a) and/or b) and/or c) or parts thereof may in total or in part be assisted by automation, e.g., by a suitable robotic equipment for the determining the amount of HDAC11/antibody
15 complexes in step b), or a computer-implemented calculation or comparison step in step c). Preferably, the method for identifying a subject susceptible to treatment with an HDAC11 inhibitor is comprised in a method for initiating treatment in a subject afflicted with cancer with an HDAC11 inhibitor, comprising the further step of initiating treatment with a pharmaceutical composition comprising at least one HDAC11 inhibitor in case said subject
20 afflicted with cancer was identified as being susceptible to treatment with an HDAC11 inhibitor. Also preferably, the method for identifying a subject susceptible to treatment with an HDAC11 inhibitor is comprised in a method of treatment of a subject afflicted with cancer with an HDAC11 inhibitor, comprising the further step of initiating treatment with a pharmaceutical composition comprising at least one HDAC11 inhibitor in case said subject
25 afflicted with cancer was identified as being susceptible to treatment with an HDAC11 inhibitor.

The term “identifying” as used herein refers to assessing the probability according to which a subject afflicted with cancer is susceptible to treatment with an HDAC11 inhibitor.

30 As will be understood by those skilled in the art, such an assessment is usually not intended to be correct for 100% of the subjects to be identified. The term, however, requires that a statistically significant portion of subjects afflicted with cancer can be correctly identified to be susceptible to treatment with an HDAC11 inhibitor. Whether a portion is statistically significant can be determined without further ado by the person

skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc.. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 5 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the present invention allows that the identification will be correct for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

10 "Cancer" in the context of this invention refers to a disease of an animal, including man, characterized by uncontrolled growth by a group of body cells ("cancer cells"). This uncontrolled growth may be accompanied by intrusion into and destruction of surrounding tissue and possibly spread of cancer cells to other locations in the body (metastasis). Preferably, the cancer is selected from the list consisting of acute lymphoblastic leukemia, 15 acute myeloid leukemia, adrenocortical carcinoma, aids-related lymphoma, anal cancer, appendix cancer, astrocytoma, atypical teratoid, basal cell carcinoma, bile duct cancer, bladder cancer, brain stem glioma, breast cancer, burkitt lymphoma, carcinoid tumor, cerebellar astrocytoma, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, colon cancer, colorectal cancer, craniopharyngioma, endometrial 20 cancer, ependymoblastoma, ependymoma, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal stromal tumor, gestational trophoblastic tumor, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular melanoma, 25 kaposi sarcoma, laryngeal cancer, medulloblastoma, medulloepithelioma, melanoma, merkel cell carcinoma, mesothelioma, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, ovarian epithelial 30 cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, papillomatosis, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pituitary tumor, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sézary syndrome, small cell

lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, testicular cancer, throat cancer, thymic carcinoma, thymoma, thyroid cancer, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, waldenström macroglobulinemia, and wilms tumor. More preferably, the cancer is a neuroblastoma.

The term "subject afflicted with cancer" as referred to herein encompasses animals, preferably mammals, and, more preferably, humans. Preferably, said subject is afflicted with a cancer as specified herein above. Subjects afflicted with cancer can be identified by the accompanying symptoms known for the disease. These symptoms are known in the art.

The term "HDAC11 inhibitor" encompasses compounds which are capable of specifically inhibiting at least one biological activity of HDAC11. Biological activities of HDAC11 which are preferably inhibited by the inhibitor according to the invention are specified herein above. The inhibitor may directly or indirectly inhibit the at least one biological activity of HDAC11. Also included are compounds developing inhibitory activity after transformation in or by the cell, like, e.g. prodrugs or polynucleotides encoding inhibitory polypeptides.

A direct inhibition is achieved by a compound which binds to HDAC11 and thereby inhibits the said biological activity. Compounds which directly inhibit HDAC11 in this sense are, preferably, compounds which block the interaction of HDAC11 with other proteins or with its ligands. Alternatively, but nevertheless preferred, a direct inhibitor of HDAC11 may induce an allosteric change in the conformation of the HDAC11 polypeptide. The allosteric change may subsequently block the interaction of HDAC11 with other proteins or with its ligands and, thus, interfere with the biological activity of HDAC11. Compounds which are suitable as direct inhibitors of HDAC11 encompass antibodies, aptameres, soluble mutants or variants of the HDAC11 polypeptide, and a small molecule antagonist. Small molecule antagonists of HDAC11 activity are known to the skilled artisan and are selected from hydroxamic acids (e.g. Trichostatin A), cyclic peptides (such as Trapoxin B, HC toxin) and depsipeptides, benzamides (e.g. entinostat, mocetinostat (MGCD0103; Fournel M, Bonfils C, Hou Y, Yan PT, Trachy-Bourget MC, Kalita A, Liu J, Lu AH, Zhou NZ, Robert MF, Gillespie J, Wang JJ, Ste-Croix H, Rahil J, Lefebvre S, Moradei O, Delorme D, Macleod AR, Besterman JM, Li Z. MGCD0103, a

novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity in vitro and in vivo. Mol Cancer Ther. 2008; 7(4):759-68)), or aliphatic acids (such as phenylbutyrate or valproic acid). The small molecule antagonists of HDAC11 known at present are inhibitors of the deacetylase activity of HDAC11. It is known to the skilled person that the known inhibitors of HDAC11 are not specific for HDAC11, though molecular modeling has been proposed for the development of isoform-selective HDAC inhibitors (Sundarapandian et al., Molecular Modeling Study on Tunnel Behavior in Different Histone Deacetylase Isoforms. PLoS One. 2012;7(11):e49327)..

The term "treatment with an HDAC11 inhibitor" relates to a treatment comprising the application of at least one HDAC11 inhibitor to a subject. It is to be understood that the term "treatment with an HDAC11 inhibitor" may relate to the treatment with an HDAC11 inhibitor alone or in combination with other one or more pharmaceutically active compounds, i.e. the treatment preferably is an HDAC11 inhibitor single therapy, or, more preferably, a co-treatment comprising the application of at least one second compound, said second compound, most preferably, not being an HDAC11 inhibitor.

As used herein, a "subject afflicted with cancer being susceptible to treatment with an HDAC11 inhibitor" is a subject wherein treatment with an HDAC11 inhibitor, preferably in monotherapy or in combination with, e.g. chemotherapy, leads to an amelioration of the diseases or disorders referred to herein or the symptoms accompanied therewith to a significant extent. Whether an amelioration is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools described herein above. Said amelioration as used herein also includes an entire restoration of the health with respect to the diseases or disorders referred to herein.

The term "contacting" as used in the context of the methods of the present invention is understood by the skilled person. Preferably, the term relates to bringing an antibody of the present invention in physical contact with a sample and thereby, e.g. allowing the sample and the composition to interact. Contacting may further involve lysing cells present in the sample and/or the extraction or purification of DNA and / or RNA and / or proteins.

The term "sample" refers to a sample of a body fluid, to a sample of separated cells or to a sample from a tissue or an organ or to a sample of wash/rinse fluid obtained from an outer

or inner body surface. Samples can be obtained by well known techniques and include, preferably, scrapes, swabs or biopsies. Samples can be obtained by use of brushes, (cotton) swabs, spatula, rinse/wash fluids, punch biopsy devices, puncture of cavities with needles or surgical instrumentation. Tissue, organ or tumor samples may be obtained from any
5 tissue or organ by, e.g., biopsy or other surgical procedures. Preferably, a sample is a tissue or a tumor obtained from brain, bone, skeletal or smooth muscle, the digestive tract, oropharynx, tongue, oesophagus, stomach, duodenum, ileum, colon, rectum, lung, liver, spleen, kidney, bladder, skin, a lymph node, ovary, oviduct, uterus, vagina, prostate, or testis. More preferably, the sample is a sample from a brain tumor, most preferably a
10 neuroblastoma. Separated cells may be obtained from the body fluids or the tissues or organs by separating techniques such as filtration, centrifugation or cell sorting. Preferably, cell, tissue or organ samples are obtained from those cells, tissues or organs, which are known or suspected to contain HDAC11. Preferably, the sample is known or suspected to be comprising HDAC11.

15 The term "HDAC11/antibody complex" is understood by the skilled person and relates to a complex formed between at least one molecule of the HDAC11 polypeptide and at least one molecule of the antibody of the present invention. Preferably, the HDAC11/antibody complex is a complex between HDAC11 and an antibody according to the present
20 invention recognizing native, but not denatured HDAC11, meaning, preferably, that the amount of said native HDAC11/antibody complex is a measure for HDAC11 activity in a sample.

The term "determining" relates to the quantification of the amount of HDAC11/antibody
25 complex present in a sample, i.e. measuring the amount or concentration of said HDAC11-antibody complex, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly. The determining of the amount of immune complexes can be accomplished in a variety of ways known to the skilled person, e.g. gel filtration chromatography followed by western blotting, co-immunoprecipitation, ELISA, or the
30 like. In accordance with the present invention, determining the amount of the HDAC11-antibody complexes can be achieved by all known means for determining the amount of a polypeptide or peptide in a sample, provided that they are adapted to specifically detect the HDAC11-antibody complexes of the present invention. Preferably, detection agents are to be used which specifically bind to and, thus, allow for the detection of the HDAC11-

antibody complexes. Detection agents, preferably, encompass antibodies or fragments thereof that specifically bind to the complexes, aptameres, anticalins, or Designed Ankyrin Repeat Proteins (DARPs) that specifically bind to the complexes. Preferably, double-specificity immunoassays are applied, i.e. assays wherein the presence or the intensity of a signal will depend on the presence of both kinds of molecules comprised in the HDAC11-antibody complexes, i.e. the HDAC11 polypeptide and the anti-HDAC11 immunoglobulin. Said means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative of the presence or absence of the HDAC11-antibody complexes. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse-proportional) to the amount of HDAC11-antibody complexes present in a sample. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-spectrometers, NMR-analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on multi parameter biochip platforms), CBA (an enzymatic Cobalt Binding Assay), and latex agglutination assays.

The term “amount” as used herein encompasses the absolute amount of the HDAC11/antibody complexes referred to herein, the relative amount or concentration of the HDAC11/antibody complexes referred to herein as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the HDAC11/antibody complexes referred to herein by measurements, e.g., expression levels determined from biological read out systems in response to the polypeptides referred to herein or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

“Comparing” as used herein encompasses comparing the amount of the HDAC11/antibody complexes referred to herein which are comprised by the sample to be analyzed with an amount of the said HDAC11/antibody complexes in a suitable reference sample as specified elsewhere herein. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount of the

HDAC11/antibody complexes as referred to herein is compared to an absolute reference amount of said HDAC11/antibody complexes; a concentration of the HDAC11/antibody complexes as referred to herein is compared to a reference concentration of said HDAC11/antibody complexes; an intensity signal obtained from the HDAC11/antibody complexes as referred to herein in a test sample is compared to the same type of intensity signal of said HDAC11/antibody complexes in a reference sample. The comparison referred to in the methods of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount or ratio may be compared to values corresponding to suitable reference values which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison by means of an expert system. Accordingly, the result of the identification referred to herein may be automatically provided in a suitable output format.

The terms "reference value" or "reference amount" as used herein refer to an amount of HDAC11/antibody complexes, which allows assessing if being susceptible to HDAC11 inhibitor treatment or not being susceptible to HDAC11 inhibitor treatment is to be assumed for the subject from which the sample is derived. A suitable reference value may be determined from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the sample.

Reference amounts can, in principle, be calculated for a group or cohort of subjects as specified herein based on the average or mean values for a given HDAC11/antibody complex by applying standard methods of statistics. In particular, accuracy of a test such as a method aiming to diagnose an event, or not, is best described by its receiver-operating characteristics (ROC) (see especially Zweig 1993, Clin. Chem. 39:561-577). The ROC graph is a plot of all of the sensitivity versus specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed. The clinical performance of a diagnostic method depends on its accuracy, i.e. its ability to correctly allocate subjects to a certain prognosis or diagnosis. The ROC plot indicates the overlap between the two distributions by plotting the sensitivity versus 1-specificity for the complete range of thresholds suitable for making a distinction. On the y-axis is sensitivity, or the true-positive fraction, which is defined as the ratio of number of true-positive test results to the product of number of true-positive and number of false-negative test results.

This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected subgroup. On the x-axis is the false-positive fraction, or 1-specificity, which is defined as the ratio of number of false-positive results to the product of number of true-negative and number of false-positive results. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of the event in the cohort. Each point on the ROC plot represents a sensitivity/-specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot that passes through the upper left corner, where the true-positive fraction is 1.0, or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two extremes. If the ROC plot falls completely below the 45° diagonal, this is easily remedied by reversing the criterion for "positivity" from "greater than" to "less than" or vice versa. Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test. Dependent on a desired confidence interval, a threshold can be derived from the ROC curve allowing for the diagnosis or prediction for a given event with a proper balance of sensitivity and specificity, respectively. Accordingly, the reference to be used for the methods of the present invention can be generated, preferably, by establishing a ROC for said cohort as described above and deriving a threshold amount there from. Dependent on a desired sensitivity and specificity for a diagnostic method, the ROC plot allows deriving suitable thresholds.

Preferably, the reference amount as used herein is derived from samples for which it is known if their donors responded to treatment or not. This reference amount level may be a discrete figure or may be a range of figures. Evidently, the reference level or amount may vary between individual species of HDAC11/antibody complexes. The measuring system therefore, preferably, is calibrated with a sample or with a series of samples comprising known amounts of HDAC11/antibody complex or HDAC11/antibody complexes. It is understood by the skilled person that in such case the amount of HDAC11/antibody complex will preferably be expressed as arbitrary units (AU). Thus, preferably, the amounts of HDAC11/antibody complex or HDAC11/antibody complexes is or are

determined by comparing the signal obtained from the sample to signals comprised in a calibration curve.

The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age, gender, or subpopulation. Thus, a suitable reference amount may be determined by the methods of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. Moreover, a threshold amount can be preferably used as a reference amount. Preferably, an amount of HDAC11/antibody complexes which is above the threshold amount is indicative of a subject susceptible to treatment with an HDAC11 inhibitor; and an amount of HDAC11/antibody complexes which is equal or below the threshold amount will be indicative for a subject not susceptible to treatment with an HDAC11 inhibitor. It is to be understood that the aforementioned amounts may vary due to statistics and errors of measurement. Preferably, the threshold amount is equal to the detection limit of the method applied to detect the HDAC11/antibody complexes, i.e. a detectable amount of HDAC11/antibody complexes is indicative of a subject susceptible to treatment with an HDAC11 inhibitor, and an amount of HDAC11/antibody complexes below the detection limit is indicative of a subject not susceptible to treatment with an HDAC11 inhibitor.

The present invention also relates to a method for identifying HDAC11 recruitment sites, comprising (a) contacting a mixture comprising HDAC11 and polynucleotides with an antibody of the present invention, and (b) detecting polynucleotides specifically bound to HDAC11/antibody complexes formed in the mixture of (a), thereby identifying HDAC11 recruiting sites.

The present invention also relates to a method for identifying a subject afflicted with cancer, comprising (a) contacting a sample from said subject comprising HDAC11 and polynucleotides with the antibody of the present invention, and (b) detecting polynucleotides specifically bound to HDAC11/antibody complexes formed in the mixture of (a), and (c) comparing the polynucleotides specifically bound to HDAC11/antibody complexes to a reference, thereby identifying a subject afflicted with cancer.

The method for identifying HDAC11 recruitment sites and the method for identifying a subject afflicted with cancer, preferably, are in vitro methods. Moreover, they may

comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to cell and/or sample pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or, preferably, assisted by automation. Preferably, all steps or parts thereof may in total or in part be assisted by automation, e.g.,
5 by a suitable robotic equipment for detecting polynucleotides. Preferably, the method for identifying a subject afflicted with cancer is comprised in a method for initiating treatment in a subject afflicted with cancer, comprising the further step of initiating treatment in case said subject was identified as being afflicted with cancer. Also preferably, the method for identifying a subject afflicted with cancer is comprised in a method of treatment of a
10 subject afflicted with cancer, comprising the further step of initiating treatment in case said subject was identified as being afflicted with cancer.

As used herein, the term "HDAC11 recruitment site" relates to a section of a genome bound by HDAC11. It is to be understood that binding by HDAC11 does not necessarily
15 relate to direct binding between HDAC11 and DNA. Preferably, binding relates to any kind of interaction leading to an accumulation of HDAC11 in the vicinity of a section of the genome. Thus, indirect binding of HDAC11 mediated by one or more macromolecules of the cell, e.g. macromolecular HDAC11 binding compounds as specified herein below, e.g. HDAC11 binding proteins, is also encompassed by the term binding. Given the role of
20 HDAC11 in transcription activation and/ or repression, it is to be understood that the pattern of HDAC11 recruitment sites is, preferably, dependent on the kind and state of cell or cells analyzed. E.g., preferably, in a tumor cell HDAC11 may be recruited to at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 15, at least 20 HDAC11 recruitment sites not used
25 by a normal cell. Also preferably, in a tumor cell HDAC11 is not recruited to at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 15, at least 20 HDAC11 recruitment sites used by a normal cell. I.e., preferably, tumor cells and normal cell may have differential patterns of HDAC11 recruitment sites.

30 As used herein, the term "mixture" relates to a composition comprising at least the compounds described. It is, however, clear from the above that the mixture preferably comprises macromolecules, more preferably polypeptides, in addition to HDAC11 and polynucleotides. Most preferably, the mixture comprises chromatin, in which case the

polynucleotides can be comprised in the chromatin. The skilled artisan knows that the mixture may also comprise low-molecular weight substances mediating stabilization of polypeptides and / or their interactions and providing an environment suitable for the macromolecules to interact, like salts, buffer compounds, and the like.

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The term "detecting polynucleotides specifically bound to HDAC11/antibody complexes", as used herein, relates to identifying polynucleotide molecules, preferably by their nucleotide sequences, bound to HDAC11/antibody complexes formed in the mixture in step a) in a specific manner, specific binding relating to binding under conditions under which the binding affinity of polynucleotides not comprising HDAC11 recruitment sites is very low or negligible. The skilled person knows conditions permitting specific binding, e.g. Lee TI, Johnstone SE, Young RA. Chromatin immunoprecipitation and microarray-based analysis of protein location. *Nat Protoc.* 2006;1(2):729-48; Schmidt D, Wilson MD, Spyrou C, Brown GD, Hadfield J, Odom DT. ChIP-seq: using high-throughput sequencing to discover protein-DNA interactions. *Methods.* 2009;48(3):240-8 or the conditions provided in the Examples below. Methods of detecting polynucleotides are well known in the art and include, e.g. cloning followed by sequencing after ChIP, ChIP-PCR (direct sequencing after PCR amplification), ChIP-Seq (direct sequencing after chromatin immunoprecipitation); ChIP-ChIP (hybridization to microarrays after chromatin immunoprecipitation); ChIP-qRT-PCR (quantitative real-time PCR after chromatin immunoprecipitation), see, e.g. Kaufmann K, Muiño JM, Østerås M, Farinelli L, Krajewski P, Angenent GC. Chromatin immunoprecipitation (ChIP) of plant transcription factors followed by sequencing (ChIP-SEQ) or hybridization to whole genome arrays (ChIP-CHIP). *Nat Protoc.* 2010; 5(3):457-72.

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The sample of the method for identifying a subject afflicted with cancer, preferably, is a sample comprising, more preferably essentially consisting of, tumor cells. Preferably, the sample is a tumor biopsy.

30

The term "reference", as used in conjunction with the method for identifying a subject afflicted with cancer, relates to a compilation of polynucleotide species, preferably polynucleotide sequences, being HDAC11 recruitment sites in normal cells. In such case, detecting only the polynucleotides comprised in said compilation in a sample from a subject is indicative of a subject not afflicted with cancer, whereas detecting at least one, at

least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 15, at least 20 polynucleotides not comprised in said compilation is indicative of a subject afflicted with cancer. It is, however, also envisaged by the method for identifying a subject afflicted with cancer that the reference is a compilation of polynucleotide species, preferably polynucleotide sequences, being HDAC11 recruitment sites in cells known to be tumor cells, but not in normal cells. In such case, detecting at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least 15, at least 20 polynucleotides comprised in said compilation in a sample from a subject is indicative of a subject afflicted with cancer, whereas not detecting a significant number of polynucleotides comprised in said compilation is indicative of a subject not afflicted with cancer.

The present invention also relates to a method for identifying an inhibitor of HDAC11 recruitment, comprising (a) contacting a mixture comprising HDAC11 and polynucleotides with a compound suspected to be an inhibitor of HDAC11 recruitment, (b) contacting the mixture of (a) with the antibody of any one of claims 3 to 5, and (c) determining the polynucleotide content of HDAC11/antibody complexes formed in the mixture of (a), thereby identifying an inhibitor of HDAC11 recruitment.

The method for identifying an inhibitor of HDAC11 recruitment, preferably, is an in vitro method. Moreover, the method may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to cell and/or compound pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or, preferably, assisted by automation. Preferably, all steps or parts thereof may in total or in part be assisted by automation, e.g., by a suitable robotic equipment for determining the polynucleotide content of HDAC11/antibody complexes.

As used herein, an "inhibitor of HDAC11 recruitment" is an HDAC11 inhibitor as described herein above inhibiting the interaction of HDAC11 with chromatin and / or chromatin components, or with other compounds, preferably polypeptides, said compounds mediating accumulation of HDAC11 in the vicinity of a section of the genome. It is thus clear that small molecule HDAC11 inhibitors specifically inhibiting the deacetylase activity of HDAC11 described herein above are not inhibitors of HDAC11 recruitment. It

is also clear for the skilled person that an antibody of the present invention may be, but need not necessarily be, an inhibitor of HDAC11 recruitment, and that the antibodies being inhibitors of HDAC11 recruitment are identified by the method for identifying an inhibitor of HDAC11 recruitment.

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The "compound suspected to be an inhibitor of HDAC11 recruitment" of the present invention can be of any molecular species, e.g. a small molecule compound, a polypeptide or protein, a poly- or oligonucleotide, an antibody, and the like. Preferably, the compound is able to penetrate a cell either by itself or in combination with other chemical or biological agents, such as penetratins, lipofection or other transfection reagents, recombinant viruses or bacteria. Preferably, the compound is a polypeptide, more preferably a protein, most preferably an antibody. Also included are compounds developing inhibitory activity after transformation in or by the cell, like, e.g. prodrugs or polynucleotides encoding inhibitory polypeptides. The term "suspected to be an inhibitor of HDAC11 recruitment" relates to the absence of knowledge to the contrary, i.e., preferably, any substance not known to be ineffective in inhibiting HDAC11 recruitment can be a compound suspected to be an inhibitor of HDAC11 recruitment.

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The skilled artisan knows that identifying a specific compound as an inhibitor of HDAC11 recruitment according to the method of the present invention can be accomplished by determining that the HDAC11/antibody complexes formed in the presence of said compound do not comprise significant amounts of polynucleotides. It is, however, also envisaged by the method for identifying an inhibitor of HDAC11 recruitment, that the polynucleotide content of the HDAC11/antibody complexes formed in the presence of said compound is compared to the polynucleotide content of the HDAC11/antibody complexes formed in the absence of said compound, wherein a decreased polynucleotide content of the HDAC11/antibody complexes formed in the presence of said compound being indicative of said compound being an inhibitor of HDAC11 recruitment.

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The present invention also relates to a method for identifying an HDAC11 binding compound, comprising (a) providing a mixture comprising HDAC11 and a compound suspected to be an HDAC11 binding compound, (b) contacting the mixture of (a) with the antibody of the present invention, and (c) determining the amount of compound suspected

to be an HDAC11 binding compound in HDAC11/antibody complexes formed in the mixture of (a), thereby identifying an HDAC11 binding compound.

The method for identifying an HDAC11 binding compound, preferably, is an in vitro method. Moreover, the method may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate to cell and/or compound pre-treatments or evaluation of the results obtained by the method. The method may be carried out manually or, preferably, assisted by automation. Preferably, all steps or parts thereof may in total or in part be assisted by automation, e.g., by a suitable robotic equipment for determining the amount of compound suspected to be an HDAC11 binding compound.

The "HDAC11 binding compound" of the present invention can be of any molecular species, e.g. a small molecule compound, a polypeptide or protein, a poly- or oligonucleotide, an antibody, and the like. Preferably, the compound is able to penetrate a cell either by itself or in combination with other chemical or biological agents, such as penetratins, lipofection or other transfection reagents, recombinant viruses or bacteria. Preferably, the compound is a polypeptide, more preferably a protein. The term "suspected to be an HDAC11 binding compound" relates to the absence of knowledge to the contrary, i.e., preferably, any substance not known to be ineffective in binding HDAC11 can be a compound suspected to be an HDAC11 binding compound. Methods of determining the amount of a specific compound comprised in HDAC11/antibody complexes will depend on the nature of said compound. The skilled artisan knows how to select the appropriate method of determining a compound, said methods preferably including, e.g. mass spectrometry and other spectrometric methods, preferably for small molecule compounds, quantitative PCR and other amplification methods, preferably for polynucleotides, and immunological or mass spectrometry methods, preferably for polypeptides.

The present invention also relates to the use of the antibody of present invention for identifying HDAC recruitment sites, for identifying an inhibitor of HDAC11 recruitment, or for identifying HDAC11 binding compounds.

The present invention further relates to a device comprising the antibody of the present invention.

Preferably, the device comprises an analyzing unit comprising a) the antibody of the present invention for determining the amount of HDAC11 in a sample and b) an evaluation unit comprising a data processor having tangibly embedded an algorithm for comparing the amount determined by said analyzing unit to a reference amount and which is capable of generating an output file containing a result or diagnosis established based on said comparison. The term “device” as used herein relates to a system comprising the aforementioned units operatively linked to each other as to allow the determination according to the methods of the invention. The analyzing unit, preferably, comprises said antibodies in immobilized form on a solid support which is to be contacted to the sample wherein the amount is to be determined. Moreover, the analyzing unit can also comprise a detector, which determines the amount of antibody which is specifically bound to HDAC11. The determined amount can be transmitted to the evaluation unit. Said evaluation unit comprises a data processing element, such as a computer, with an implemented, tangibly embedded algorithm for carrying out a comparison between the determined amount and a suitable reference. Suitable references depend on the method the device is used in and are described elsewhere herein. The results may be given as output of parametric diagnostic raw data, preferably, as absolute or relative amounts. It is to be understood that these data will need interpretation a human being, preferably by a clinician. However, also envisaged are expert system devices wherein the output comprises processed diagnostic raw data the interpretation of which does not require human intervention and may directly serve as an aid for diagnosis.

Finally, the invention relates to a kit for carrying out at least one of the methods of the present invention, wherein said kit comprises instructions for carrying out said method, an antibody of the present invention, and standards for a reference.

The term “kit” as used herein refers to a collection of the aforementioned components, preferably, provided in separately or within a single container. The container also comprises instructions for carrying out the method of the present invention. These instructions may be in the form of a manual or may be provided by a computer program code, which is capable of carrying out the comparisons referred to in the methods of the present invention and to establish a diagnosis accordingly when implemented on a computer or a data processing device. The computer program code may be provided on a data storage medium or device such as an optical storage medium (e.g., a Compact Disc) or

directly on a computer or data processing device. Moreover, the kit may, preferably, comprise standards for reference amounts as described elsewhere herein in detail.

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

10 Figure Legends:

Fig. 1 represents the amino acid sequence of HDAC11 used as an antigen for mice immunization (SEQ ID NO:4).

- 15 Fig. 2 represents the amino acid sequence of the epitope recognized by the antibody produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3170 (SEQ ID NO:6).

- Fig. 3 shows a western blot analysis of HDAC11 protein in human neuroblastoma BE(2)-C
20 cell line. Cells were transfected either with empty vector or plasmid that encodes wild type HDAC11 flanked by Myc tag. After 48 hours, the cells were harvested and lysed in urea-lysis-buffer. Whole cell lysate of 50µg was loaded per lane, transferred on PVDF membrane. The upper panel details the western blot analysis using purified antibody produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3170.

- 25 Fig. 4 shows a western blot analysis in human neuroblastoma BE(2)-C cell line as in Fig. 3 where the antibody binding was diminished by a blocking peptide.

- Fig. 5 shows immunocytochemical analysis of HDAC11 protein in human neuroblastoma
30 BE(2)-C cell line using the antibody produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3170.

FIG. 6 shows immunocytochemical analysis of HDAC11 protein in human neuroblastoma BE(2)-C cell line using the antibody produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3171.

- 5 Fig. 7: Chromatin-Immunoprecipitation (ChIP) using antibodies produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3170 and No DSM ACC3171. ChIP-PCR shows HDAC11 protein recruitment to the *BMP4* promoter site in BE(2)-C neuroblastoma cells. No HDAC11-recruitment was observed to the *Rassf1a* gene and to the house keeping genes *Gapdh* and *Actb*.

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- Fig 8: Summary of characteristic and applications of the antibodies produced by the hybridoma cell line deposited at DSMZ under No DSM ACC3170 and No DSM ACC3171. Abbreviations IC: Immunocytochemistry, IHC: Immunohistochemistry, WB: Western Blot, ChIP: Chromatin Immunoprecipitation; n.d.: not determined, - : antibody not suited for application, + : antibody suited for application, +++ : antibody preferred for application.
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Fig. 9: Plasmid map of the pQcH6-HDAC11 vector

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

Examples

25 **Example 1: HDAC11 antigen production**

HDAC11 Antigen preparation and purification

- The recombinant histidine-tagged HDAC11 variant 1 protein (amino acids 1-347) was used as an antigen. pENTR™221 clone encoding HDAC11 variant 1 (Ultimate™ ORFCard for Clone ID IOH9974) was purchased from Invitrogen. Antigen encoding cDNA corresponding to the full-length sequence of human *HDAC11 variant 1*, Genbank accession number NM_024827.3, was cloned via TA-vector (Invitrogen) into the pQcH6-vector to yield the expression vector pQcH6-HDAC11 as depicted in Figure 9 (original vector pQE8 tagged with six histidine residues at C-terminus, Qiagen), according to the
- 30

manufacturer's instructions. The construct was confirmed by sequencing and transformed into competent *Escherichia coli* M15[pREP4] host strain (Qiagen).

The recombinant HDAC11 protein fused to a hexa-histidine tag was expressed by competent M15 bacteria and purified by Ni-NTA-agarose affinity chromatography column according to the manufacturer's conditions (Qiagen). Shortly, the bacterial pellet was resuspended in buffer containing 6M guanidine hydrochloride, 100mM NaH₂PO₄ x 2H₂O, 10mM Tris/HCl and slowly loaded onto the Ni-NTA-Agarose column. The protein was eluted with a pH gradient in the buffer containing 8M urea, 100mM NaH₂PO₄ x 2H₂O, 10mM Tris/HCl; the pH value was adjusted to 8.0, 6.3, 5.9, and 4.5, respectively. After elution, the pH was adjusted by NaOH to the value of 7. The purified recombinant HDAC11 protein fused to a hexa-histidine tag was further used as antigen for mice immunization.

Example 2: Generation of hybridomas

Mice immunization

Two female BALB/c mice were immunized with recombinant HDAC11 protein of whole HDAC11 variant 1 sequence (Sequence: Accession number NP_079103.2) fused to a hexa-histidine tag by s.c. injections according to the standard protocols. The injection sites were situated in the left and right leg. The immunization scheme was as follows: the primary dose of 10µg of antigen in Freund's adjuvant Complete for stimulating the animals' immune system (Sigma) was followed by a dose of 10µg of antigen with Freund's adjuvant Incomplete (Sigma). The immunization followed with two to five doses of 10µg of antigen in 4M urea buffer. Blood taken from the submandibular vein was used for an assessment of antiserum titre via enzyme-linked immunosorbent assay (direct binding ELISA). Test bleeds were additionally evaluated by immunocytochemistry and western blot analyses.

Fusion

The monoclonal antibodies were raised from successfully immunized mice according to the technique developed by Köhler and Milstein (Kohler, G. and C. Milstein (1975). "Continuous cultures of fused cells secreting antibody of predefined specificity." Nature 256(5517): 495-497). The hybridoma fusion of mouse lymphocytes from lymph nodes and

mouse Sp2/0-AG14 myeloma cells (Kohler, G. and C. Milstein (1976). "Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion." Eur J Immunol 6(7): 511-519.) was done by use of polyethylene glycol (PEG). Hybridomas were grown in RPMI1640 medium supplemented by 10% fetal calf serum and 1xHAT (hypoxanthine-aminopterin-thymidine). Growing hybridoma clones were screened by ELISA. Positive clones were further assessed for their HDAC11 specificity via western blotting and immunocytochemistry. We tested 264 clones from the fusion of the first mouse and 534 clones from the second mouse. We selected 15 clones and these were cryoprotected. The four out of 15 cryoprotected clones were further subcloned by limited dilution in order to obtain monoclonality. We obtained two clones producing monoclonal anti-HDAC11 antibodies: the antibodies are antibodies produced by cell lines deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) under No DSM ACC3170 and DSM ACC3171. Antibody isotyping was assessed by ELISA. The antibodies of IgG isotype were purified by affinity chromatography.

Example 3: Monoclonal antibody purification

Hybridoma cells' supernatant containing antibody was purified using protein A/G sepharose (GE Healthcare) affinity chromatography and eluted into the buffer containing 100mM citric acid of pH6 for antibody isotype IgG_{1,3} and pH 3.2 for antibody isotype IgG_{2a,2b}, respectively.

Example 4: Epitope mapping and permutation analysis

Antibody specificity was addressed by epitope mapping and permutation analysis performed by PEPperPRINT GmbH (Heidelberg, Germany). It was found that (numbers relate to the sequence of SEQ ID NO:4 (Fig. 2a)) L₁₁ (alternatively F or I) and E₂ also contribute to antibody recognition, whereas position M₁₀ is of lower importance and can be exchanged by A, F, G, I, L, N, Q, S, T, V, W and Y. Positions K₄ (replaceable by R, F, M), S₇ (replaceable by T), D₈ (replaceable by E) and L₁₁ (replaceable by F or I) can only be replaced by few selected amino acids predominantly in line of a conservative exchange. E₂, E₃, L₅, L₆ and S₉ cannot be replaced at all and are essential part of the DSM ACC3170 antibody epitope. With linear 13 and 15mer peptides of histone deacetylase 11 isoform 1, identified the sequence KLLSDSM was identified as minimal consensus motif recognized

by the DSM ACC3170 antibody epitope. Mappings of monoclonal DSM ACC3171 antibody against the target protein HDAC11 did not reveal any linear epitope with 10, 12, and 15mer peptides of HDAC11. The results are in consistency with the negative outcome of Western blotting experiments. Therefore, monoclonal DSM ACC3171 antibody
5 apparently recognizes a conformational and/or a discontinuous epitope with low affinity of partial epitopes.

Example 5: Immunocytochemistry

For detection and cellular localization studies of the HDAC11 protein, we performed
10 immunocytochemistry (ICC) according to Abcam protocol with minor modifications (Cambridge, UK). Cells were grown on poly-L-lysine coated slides (Thermo Scientific), rinsed shortly with phosphate buffer saline (PBS), fixed with 2% paraformaldehyde for 60 minutes at room temperature, and permeabilized for 15 min in PBS containing 0.1% Triton X-100. Cells were incubated in blocking solution containing 10% goat serum, 0.25%
15 bovine serum albumin, 0.1% Triton X-100, PBS for 60 min at room temperature to block unspecific binding of antibodies. Primary antibody incubation was done overnight at 4°C followed by three PBS-washing steps and incubation with secondary anti mouse IgG antibody conjugated to Cy3 fluorophore (Dianova) for 60 min at room temperature in the dark humidified chamber. The slides were washed three times with PBS, incubated with
20 2.5 µg/ml solution of 4',6-diamidin-2-phenylindol in PBS to visualize cell nuclei, and then rinsed three times with PBS and mounted with Fluoromount mounting medium (Sigma).

Example 6: Western blot analysis

The whole cell lysates were prepared by cells lysis in urea-containing buffer followed by
25 protein separation by electrophoresis on 15% denaturing polyacrylamid-SDS gels and blotted on PVDF membranes. The membranes were incubated with hybridomas supernatants containing primary antibody overnight at 4°C followed by the incubation with the secondary antibody goat anti mouse IgG horse radish peroxidase conjugated (Sigma) diluted 1:5000 in 5% non-fat dry milk/PBS. The signals were detected with ECL Plus
30 (Thermo Scientific), according to the manufacturer's instructions.

Example 7: Chromatin Immunoprecipitation

- 5 ChIP was performed according to the standard procedure described in the ChIP Assay Kit (Upstate/ Milipore, Catalog # 17-229). Cells were fixed with 1% formaldehyde for 10 minutes at room temperature and quenched with 125mM glycine for 5 minutes at room temperature, harvested and lysed with lysis buffer containing 1% sodium dodecyl sulfate (SDS). Cell lysates were sonicated using Covaris S2 device (Covaris, Inc. USA).
- 10 Chromatin complexes containing HDAC11 protein were immunoprecipitated, eluted with buffer containing 1%SDS, 0.1M sodium hydrogen carbonate (NaHCO_3) for two times 15 minutes at room temperature. Chromatin complexes were de-crosslinked with 5M sodium chloride for overnight at 65°C and genomic DNA was isolated. Genomic DNA served as a template for qPCR analysis.

PCT

Print Out (Original in Electronic Form)
(This sheet is not part of and does not count as a sheet of the international application)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.235 MT/FOP 20020701/0.20.5.20
0-2	International Application No.	
0-3	Applicant's or agent's file reference	DK11023PC
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	5, 6, 23, 23, 23, 25, 26, 26
1-2	line	21-23, 27, 3, 7, 13, 13, 1, 4
1-3	Identification of deposit	
1-3-1	Name of depositary institution	DSMZ DSMZ-Deutsche Sammlung von Mikroor- ganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Inhoffenstr. 7B, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	26 April 2012 (26.04.2012)
1-3-4	Accession Number	DSMZ ACC3171
1-4	Additional Indications	HDAC11#170/9
1-5	Designated States for Which Indications are Made	All designations
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	5, 6, 22, 22, 22, 23, 23, 25, 25, 26
2-2	line	21-23, 26, 16, 24, 31, 6, 12, 13, 31
2-3	Identification of deposit	
2-3-1	Name of depositary institution	DSMZ DSMZ-Deutsche Sammlung von Mikroor- ganismen und Zellkulturen GmbH
2-3-2	Address of depositary institution	Inhoffenstr. 7B, D-38124 Braunschweig, Germany
2-3-3	Date of deposit	26 April 2012 (26.04.2012)
2-3-4	Accession Number	DSMZ ACC3170
2-4	Additional Indications	HDAC11#35/47
2-5	Designated States for Which Indications are Made	All designations

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0-4	This form was received with the international application: (yes or no)	yes
0-4-1	Authorized officer	Pasche, Constantinus

Claims

1. An antibody specifically recognizing histone deacetylase 11 (HDAC11), wherein said antibody recognizes native HDAC11 or native and denatured HDAC11.
2. The antibody of claim 1, wherein the antibody is a monoclonal antibody, preferably a monoclonal antibody recognizing an epitope comprising an amino acid sequence as depicted SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9.
3. The antibody of claim 1 or 2, wherein the antibody is an antibody produced by a cell line deposited at the Leibnitz-Institut DSMZ-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (DSMZ) under No DSM ACC3170 or No DSM ACC3171.
4. A cell line producing the monoclonal antibody of claim 2 or 3.
5. The cell line of claim 4, wherein the cell line is a cell line deposited at the DSMZ under No DSM ACC3170 or No DSM ACC3171.
6. A method for identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor, comprising
 - (a) contacting a sample from said subject with the antibody of any one of claims 1 to 3,
 - (b) determining the amount of HDAC11/antibody complexes formed in the mixture of (a), and
 - (c) comparing said amount with a reference amount, thereby identifying a subject afflicted with cancer as being susceptible to treatment with an HDAC11 inhibitor.
7. A method for identifying HDAC11 recruitment sites, comprising
 - (a) contacting a mixture comprising HDAC11 and polynucleotides with the antibody of any one of claims 1 to 3, and
 - (b) detecting polynucleotides specifically bound to HDAC11/antibody complexes formed in the mixture of (a), thereby identifying HDAC11 recruitment sites.

8. A method for identifying a subject afflicted with cancer, comprising
- (a) contacting a sample from said subject comprising HDAC11 and polynucleotides with the antibody of any one of claims 1 to 3, and
 - 5 (b) detecting polynucleotides specifically bound to HDAC11/antibody complexes formed in the mixture of (a), and
 - (c) comparing the polynucleotides specifically bound to HDAC11/antibody complexes to a reference, thereby identifying a subject afflicted with cancer.
- 10 9. A method for identifying an inhibitor of HDAC11 recruitment, comprising
- (a) contacting a mixture comprising HDAC11 and polynucleotides with a compound suspected to be an inhibitor of HDAC11 recruitment,
 - (b) contacting the mixture of (a) with the antibody of any one of claims 1 to 3, and
 - 15 (c) determining the polynucleotide content of HDAC11/antibody complexes formed in the mixture of (a), thereby identifying an inhibitor of HDAC11 recruitment.
10. A method for identifying an HDAC11 binding compound, comprising
- 20 (a) providing a mixture comprising HDAC11 and a compound suspected to be an HDAC11 binding compound,
 - (b) contacting the mixture of (a) with the antibody of any one of claims 1 to 3, and
 - (c) determining the amount of compound suspected to be an HDAC11 binding compound in HDAC11/antibody complexes formed in the mixture of (a), thereby identifying an HDAC11 binding compound.
- 25 11. Use of the antibody of any one of claims 1 to 3 for identifying HDAC recruitment sites.
12. Use of the antibody of any one of claims 1 to 3 for identifying an inhibitor of
- 30 HDAC11 recruitment.
13. Use of the antibody of any one of claims 1 to 3 for identifying HDAC11 binding compounds.

14. A device comprising the antibody of any one of claims 1 to 3.
15. A kit for carrying out a method of any one of claims 6 to 10, wherein said kit comprises instructions for carrying out said method, an antibody of any one of claims
5 1 to 3, and standards for a reference.

```
1  mlhttqlyqh vpetrwpivy sprynitfmg leklhpfdag kwgkvinflk eeklstdsml
61  veareaseed llvvhtrryl nelkwsfava titeippvif lpnflvqrkv lrplrtqtgg
121 timagklave rgwainvggg fhhcssdrvg gfcayaditl aikflferve gisratiidl
181 dahqgngher dfmddkrvyi mdvynrhiyp gdrfakqair rkvelewgte ddeyldkver
241 nikkslqehl pdvvvynagt dilegdrllg lsispagivk rdelvfrmvr grrvpilmvt
301 sggyqkrtar iiadsilnlf glgligpesp svsaqnsdtp llppavp
```

Fig.1

a)
KEEKLLSDSML

b)
EKLLSDSM

c)
KLLSDSM

d)
EE(K/R/F/M)LL(S/T)(D/E)S(M/A/F/G/I/L/N/Q/S/T/V/W/Y)(L/F/I)

Fig. 2

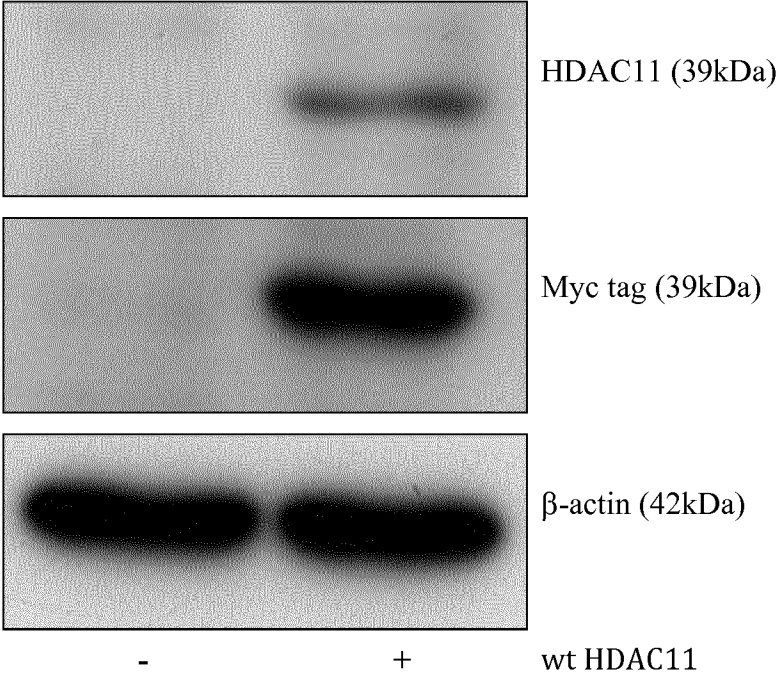


Fig. 3

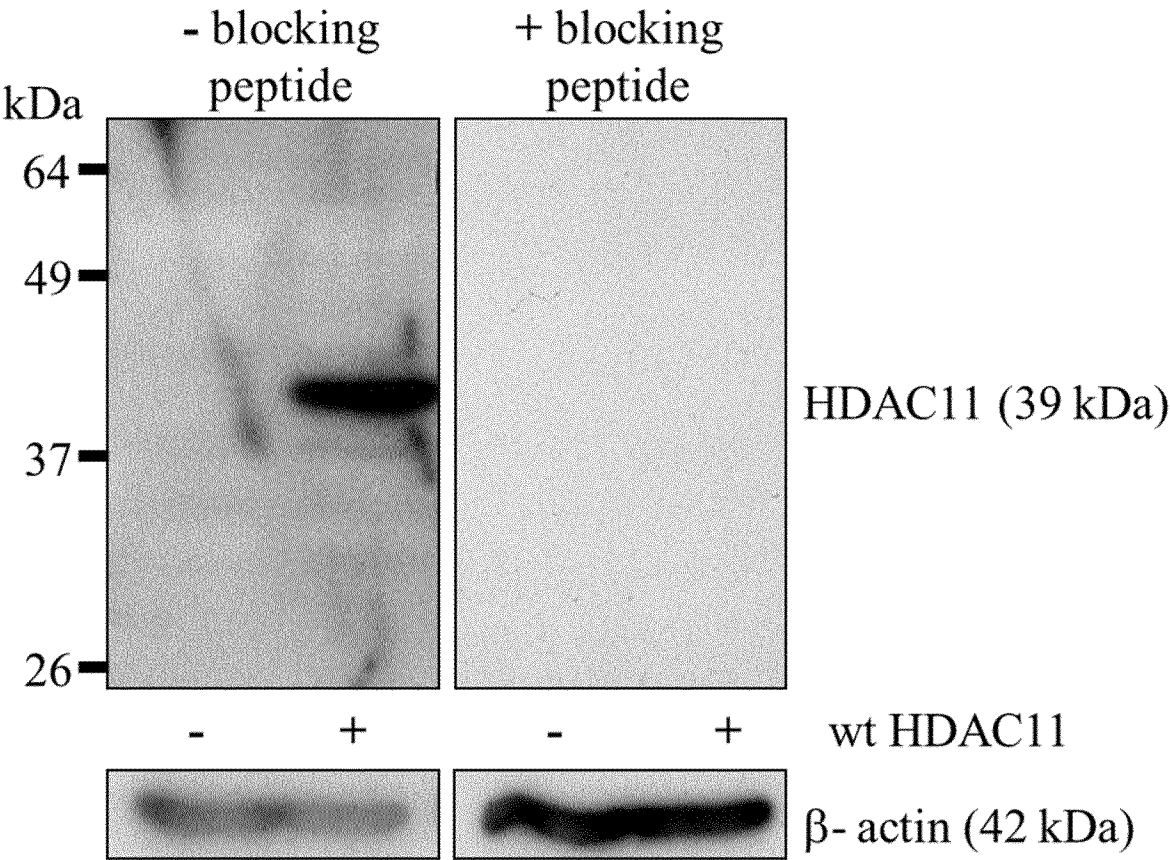


Fig. 4

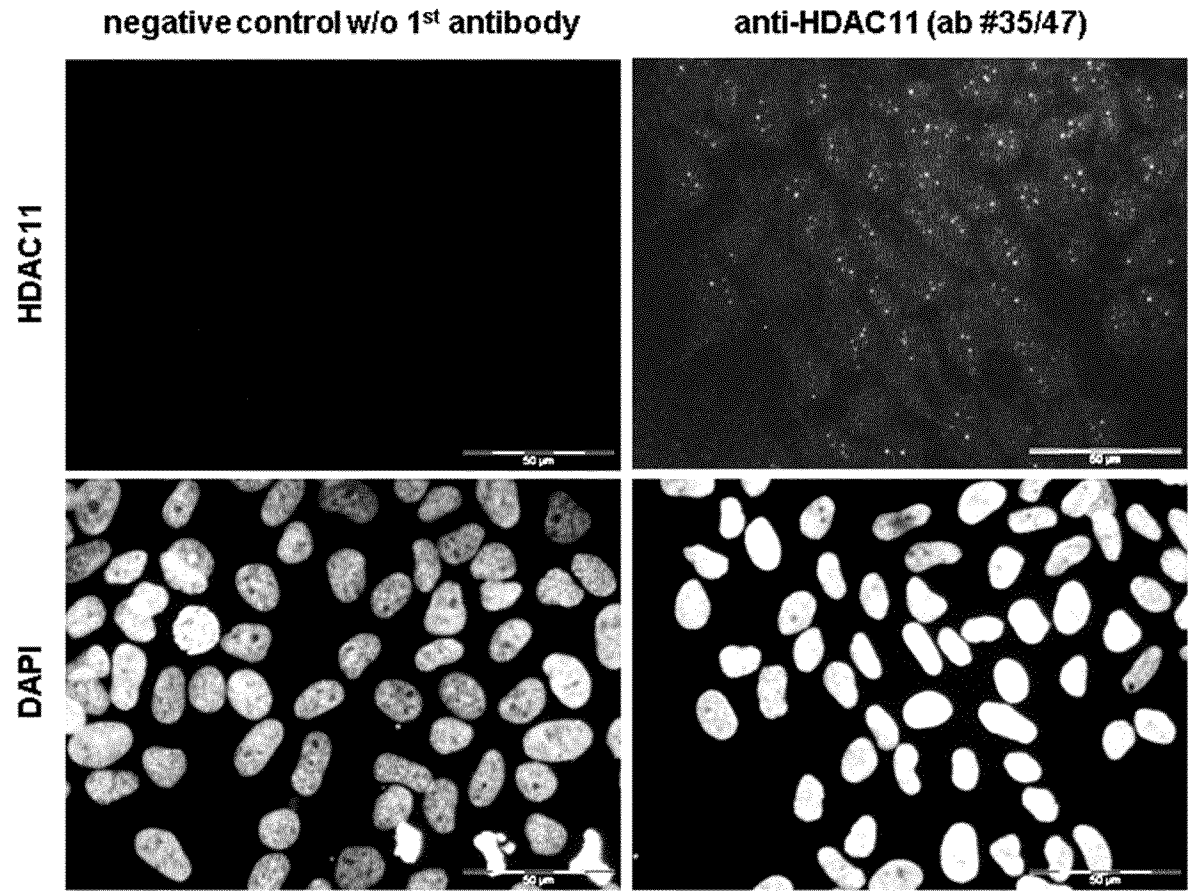


Fig. 5

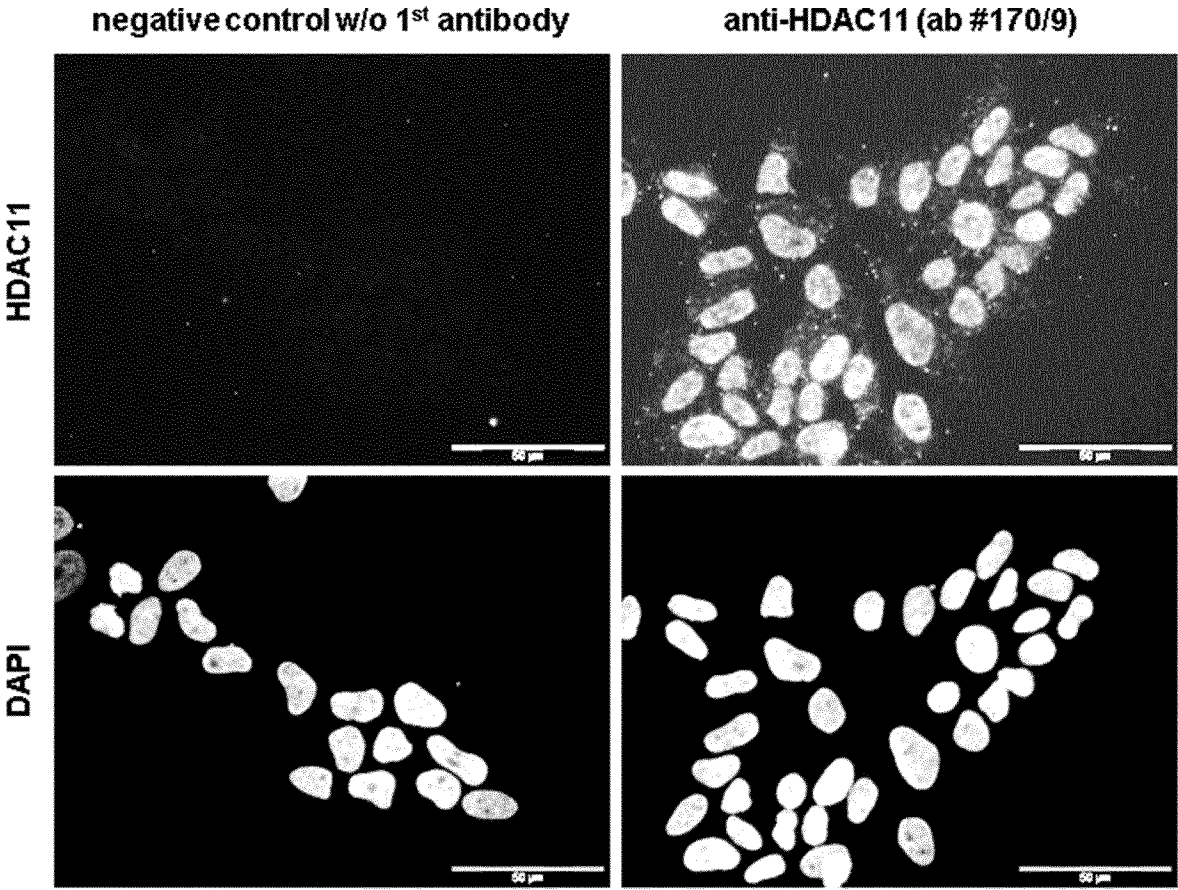


Fig. 6

HDAC11 recruitment to distinct gene promoters

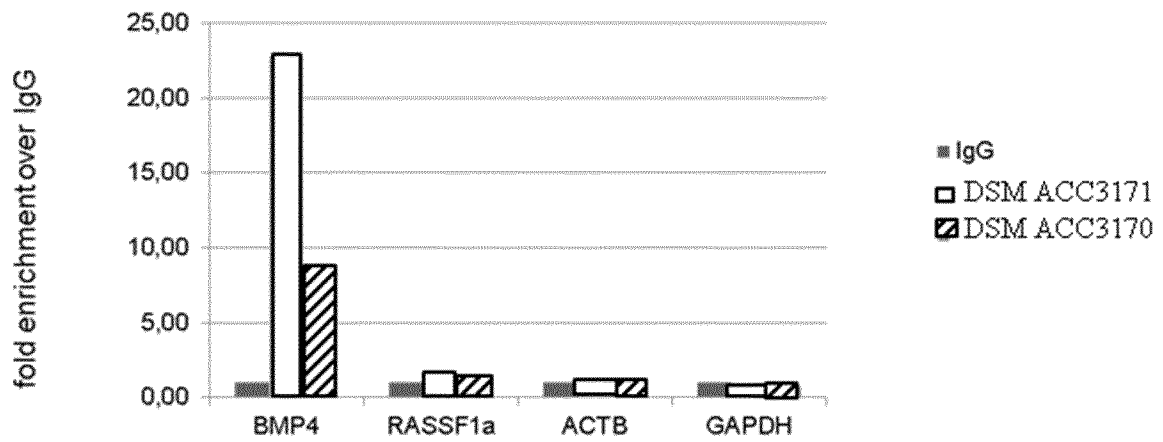


Fig. 7

Antibody ID	Isotype	Epitope	Application			
			IC	IHC	WB	ChIP
DSM ACC3170	IgG _{2a}	EEKLLSDSM	+	n.d.	+	+
DSM ACC3171	IgG ₁	conformational discontinuous	+++	-	-	+

Fig. 8

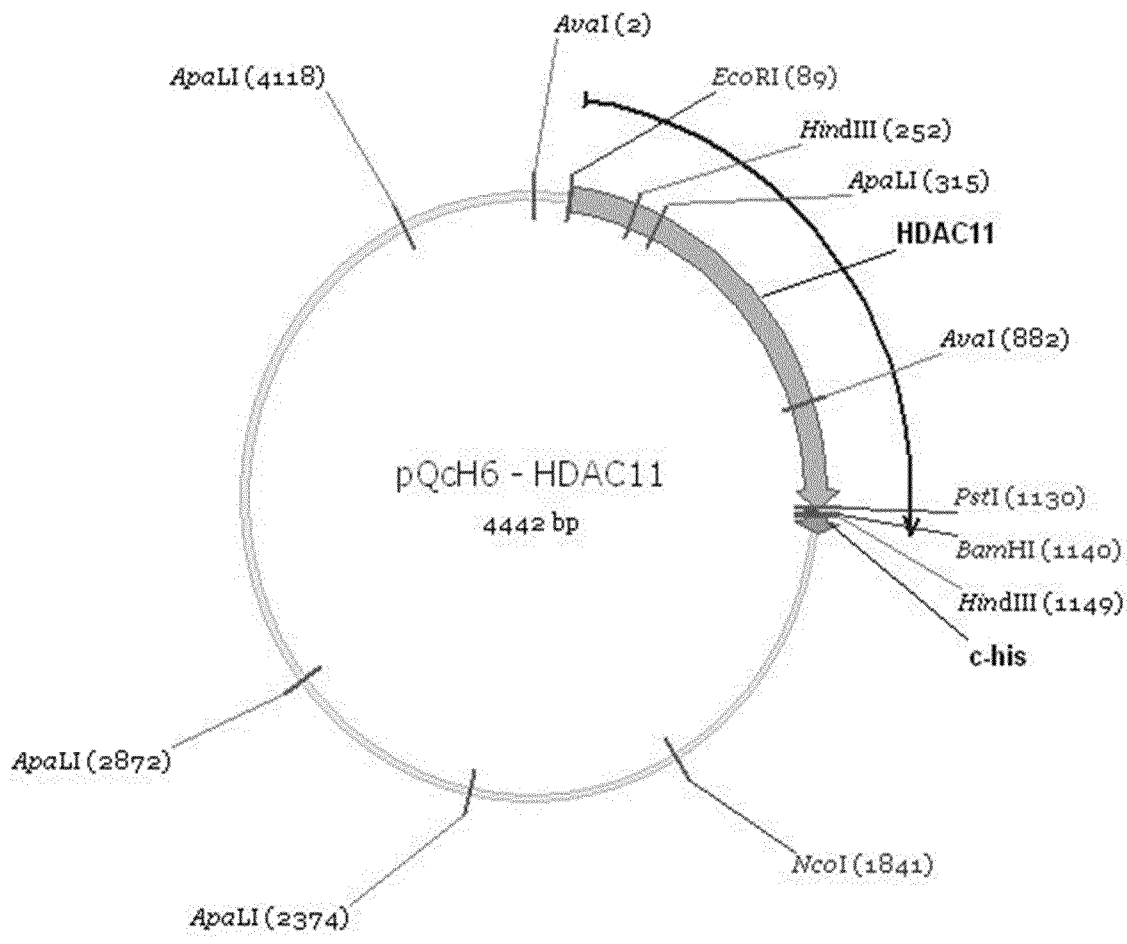


Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077750

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/40
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/071079 A1 (ANGELETTI P IST RICHERCHE BIO [IT]; FILOCAMO GESSICA [IT]; STEINKUHLER) 4 August 2005 (2005-08-04) e.g. claim 15; ; page 28, penultimate and last paragraph; example 1; the whole document ----- -/--	1,11,14, 15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

27 February 2014

Date of mailing of the international search report

07/03/2014

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Authorized officer

Gruber, Andreas

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/077750

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	TOROPAINEN S ET AL: "The Down-regulation of the Human MYC Gene by the Nuclear Hormone 1alpha,25-dihydroxyvitamin D3 is Associated with Cycling of Corepressors and Histone Deacetylases", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 400, no. 3, 16 July 2010 (2010-07-16), pages 284-294, XP027125515, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2010.05.031 [retrieved on 2010-05-21]	1-5,14,15
Y	e.g. fig. 5 and legend thereto; page 293, left-hand column, section entitled "ChIP assays"; the whole document	9,10,12,13
X	LAI X ET AL: "Advantages of Promoting Interleukin-10 by Silence of Histone Deacetylase 11 in Inducing Tolerance in Orthotopic Liver Transplantation in Rats", TRANSPLANTATION PROCEEDINGS, vol. 43, no. 7, 2011, pages 2728-2732, XP028287105, ISSN: 0041-1345, DOI: 10.1016/J.TRANSPROCEED.2011.05.044 [retrieved on 2011-07-28] e.g. page 2729, right-hand column, section "Western Blotting"; the whole document	1,14,15
A	WO 2007/038073 A2 (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; CULVER KENNETH WAYNE [US]) 5 April 2007 (2007-04-05) the whole document	1-15
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077750

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	----- HEDWIG E. DEUBZER ET AL: "HDAC11 is a novel drug target in carcinomas", INTERNATIONAL JOURNAL OF CANCER, vol. 132, no. 9, 1 October 2012 (2012-10-01), pages 2200-2208, XP055104654, ISSN: 0020-7136, DOI: 10.1002/ijc.27876	6-8,11
Y	e.g. abstract; paragraph bridging page 2203 and 2204; the whole document	9,10,12, 13
T	----- "Anti-HDAC11 antibody ab18973", 27 February 2014 (2014-02-27), XP055104671, Retrieved from the Internet: URL: http://www.abcam.com/HDAC11-antibody-a b18973.pdf [retrieved on 2014-02-27] the whole document	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/077750

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			WO 03014340 A2 20-02-2003
