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(54) **Title**: MATERIALS AND METHODS RELATING TO PANCREATIC CANCER

(57) **Abstract**: The present invention concerns materials and methods relating to pancreatic cancer and personalised medicine as applied to pancreatic cancer. Particularly, the invention relates to materials and methods for the determination of significantly modulated protein phosphorylation and/or expression as well as the activity of signaling pathways collectively providing a tumour profile that can guide selection of the most appropriate treatment regime based on the likelihood of tumour recurrence; or the identity of activated drug targets in pancreatic cancer tissue.

Materials and Methods relating to Pancreatic Cancer

Field of the Invention

The present invention concerns materials and methods relating to pancreatic cancer and personalised medicine as applied to pancreatic cancer. Particularly, the invention relates to materials and methods for the determination of significantly modulated protein phosphorylation and/or expression as well as the activity of signaling pathways collectively providing a tumour profile that can guide selection of the most appropriate treatment regime based on the likelihood of tumour recurrence or the identity of activated drug targets in pancreatic cancer tissue.

Background of the Invention

Protein phosphorylation is a common process modulating the activity of oncogenic and tumor suppressor proteins [1-3]. In many cases, phosphorylation results in switch-like changes in protein function due to modulation of protein folding, substrate affinity, stability, and activity of its substrates, in turn affecting signaling pathways controlling cell proliferation, migration, differentiation, and apoptosis. Dysregulation of phosphorylation can thus contribute to the cancer phenotype [4] and provides a potential source of new drug targets, diagnostic and prognostic biomarkers that significantly cannot be measured using genomic methods. Pancreatic cancer is one of the most aggressive malignant neoplasms with a median survival of 6 months post-diagnosis. In part this is a result of the fact that a significant proportion of patients are diagnosed at an advanced stage where treatment options are very limited [5]. As is the case for other cancers, molecular targeting therapy is promising for treatment of advanced or recurrent pancreatic cancer [6]. Although a variety of molecular targeting drugs have been available in the last decade and many others are also expected in the next few years, a breakthrough is still required for prediction of drug effects and drug selection. For example, sorafenib, a multi-kinase inhibitor acting on hyperactive vascular endothelial growth factor receptor,

platelet-derived growth factor receptor and Raf, has proven efficacy in some patients with advanced hepatocellular carcinoma [7], but response rates remain frustratingly low as there are currently no pathway activity tests that can predict its effect in an individual patient before starting treatment.

It has long been recognised that chemotherapy, even with highly selective molecular targeting medicines will ultimately fail due to acquired resistance. Typically this is driven by the switching from one oncogenic pathway to another under the selective pressure of the drug treatment. As an example, the V600E mutation of B-Raf is a common feature in aggressive melanoma leading to hyperactivation of the Raf signalling pathway. Highly selective inhibitors of V600E B-Raf were rapidly developed and approved based on dramatic initial treatment response. However, the vast majority of patients ultimately relapse, despite B-Raf signalling being silenced, through a range of different mechanisms involving aberrant dimerization, Raf isoform switching and alternative activation of MEK and ERK. A proposed solution for such patterns of acquired resistance is the administration of multiple molecular targeting drug combinations which each may not be sufficient to kill the tumour, but which collectively act to block evolving resistance. This strategy has been termed 'synthetic lethality'.

Summary of the Invention

The inventors have recognised a need for a reliable and time and cost-effective means for defining the optimal drug combination for treating pancreatic cancer and for the prediction of and monitoring for drug resistance in such tumours.

Accordingly, the inventors set out to establish an analytical approach to help drug selection, where expression and activity of multiple drug targets are comprehensively assessed on a case-by-case basis. Phosphorylation is a key event modulating protein activity, therefore measuring protein phosphorylation is a useful indicator of activation status.

There are hundreds of anti-cancer drug targets and thousands of oncogenic signaling proteins and measuring expression and activation status of all of these using immunohistochemistry (IHC), the current gold standard analysis, to guide optimal treatment selection is not feasible. Reverse phase protein microarrays (RPMA) have the potential to offer broader coverage than IHC but have limitations due to a currently small repertoire of phosphorylation site-specific antibodies and poor specificity/cross reactivity. Since the prime regulatory processes controlling oncoprotein activity are post-translational modifications, genomics-based technologies cannot provide an alternative solution. Previously liquid chromatography-mass spectrometry (LC-MS/MS) based proteomic approaches have been developed to identify and quantify thousands of proteins and their phosphorylation sites [8, 9] and the inventors have now successfully adapted and applied these methods to the analysis of oncogenic signalling pathways to identify the optimal drug targets expressed within an individual tumour.

The inventors have developed a new LC-MS/MS based proteomic workflow to overcome many of the technical and bio-informatic difficulties involved in effectively identifying and quantifying activated proteins, activated signaling pathways, and activated drug targets, at a global or system wide level on a case by case basis. In specific terms, the inventors provide a high-density phospho-proteomic workflow applicable to experimental cancer cell lines, xenograft tumour tissue and clinical tissue using isotopic and/or isobaric mass tag labelling enabling the analysis of multiple samples simultaneously [10, 11]. Preferably two or more samples are analysed simultaneously. Most preferably at least 10 samples can be analysed together. Samples may be paired tissues from the tumour and adjacent healthy tissue from individual patients or from more than one patient, e.g. at least two, at least three, or at least 4. Most preferably paired tumour and healthy tissues from 5 patients are analysed together in a single 10-plex experiment.

It is a particular feature of the present invention that, given the large amount of data generated for each individual patient, a system for data storage, retrieval and analysis is provided. In particular

the inventors provide a database and suite of data analysis tools to extract relevant biological information from their complex dataset.

Specifically, the inventors have applied their global phospho-proteomic workflow (SysQuant) to compare cancerous and non-cancerous pancreatic tissue. This phosphoproteomic workflow allows simultaneous measurements of multiple phosphoproteins and provides rapid measure of signaling pathway activity in a sample. This workflow has enabled the inventors to identify signaling pathways and drug targets that show significant modulation in expression and activity between cancerous and non-cancerous tissue types at an average level across all pancreatic cancer cases to determine common drivers of the pancreatic cancer phenotype. The inventors were also able to interrogate the entire database to identify different combinations of molecular events contributing to the cancer phenotype which were unique to an individual case or subgroups. Accordingly, this workflow provides for the first time a way of not only diagnosing pancreatic cancer, but more importantly stratifying patients into different treatment regimens based on the activation status of these newly determined targets on a case by case basis.

In addition, measuring the phosphopeptide molecular profile allows for the first time a prognostic tool for pancreatic cancer. Hierarchal clustering of phosphopeptide abundance separated patients into groups based on recurrence and non-recurrence. This led to the identification of many prognostic phosphopeptide and thus their respective phosphoprotein markers which form independent aspects of the present invention.

The approach taken by the inventors allowed simultaneous measurement of more than 5000 phosphorylation sites of more than 2000 proteins in tumor versus background pancreatic tissue from patients with pancreatic head adenocarcinoma. Many of these were determined to be modulatory phosphorylation sites known to affect activity of drug targets such as FYN, GSK3α/β, HDAC1/2, the RAF kinases, MAPKs (p38 and ERK2), AKT, PKCs, Casein Kinases and others.

The inventors determined the relative abundance of proteins in tumor (T) compared to non-tumor (NT) tissue, using median $\log_2$ T/NT ratios

of the non-phosphorylated peptides unique to each protein as surrogates to calculate the relative abundance of the respective proteins.

From this information, they found it was possible to develop a predictive algorithm to assign tissue samples to tumour or non-tumour phenotype, i.e. as a diagnostic aid. Further, they found that the differentially activated pathway proteins can be used as therapeutic targets. That is, drugs may be developed which are capable, either directly or indirectly, of regulating the expression, activation or inhibition of the proteins of interest as appropriate towards those levels found in normal healthy tissue.

Having created a comprehensive database of individual phosphorylation site status across thousands of proteins, the invention provides for the first time the means for a number of additional analyses to be performed. For example, the ability to predict the likelihood and potential timing of tumour recurrence provides a major benefit in designing the optimal treatment strategy. Using hierarchical clustering analysis of the data, the inventors were surprisingly able to categorise tumours into recurrent and non-recurrent phenotypes independently of any other clinical data. Even more surprisingly, a subset of protein phosphorylation sites were highly correlated with recurrence and each of these represents a novel therapeutic target or marker in pancreatic cancer. Thus, the inventors also provide new therapeutic targets to enable the development of molecular targeting drugs for the treatment of pancreatic cancer.

In a yet further aspect of the present invention, one or more of the regulated protein phosphorylation sites associated with the recurrent pancreatic cancer phenotype represent novel biomarkers for the diagnosis and prognosis of recurrent pancreatic cancer. In accordance with this aspect of the invention means of detecting and/or quantifying phosphorylation at the one or more sites are provided. Such methods include but are not limited to immunohistochemistry, Western blotting, ELISA and mass spectrometry.

To ascertain relative activation status of kinases, other enzymes and other classes of proteins in tumor compared to non-tumor tissue in each case, the inventors used relative abundance of phosphopeptides containing phosphorylation sites known to either induce enzyme activation or inhibition. Table 15 provides all phosphopeptides displaying $\log_2$ T/NT ratios ≥1 or ≤ -1 that contain phosphorylation sites that are known to either induce activation or inhibition of the phosphorylated enzyme, in each case.

In addition to determining which proteins and phosphopeptides demonstrated significant differences in abundance between tumor and non-tumor tissue when averaged across all cases, the inventors have also determined which phosphopeptides were highly modulated within each individual patient and provide herein markers and targets for the diagnosis and prognosis, including prediction of recurrence and drug resistance, of pancreatic cancer.

For example, the inventors have determined the relative activation status of; Glycogen synthase kinase-3 alpha and beta, Histone deacetylase 1 and 2, RAF proto-oncogene serine/threonine-protein kinase, Serine/threonine-protein kinase A-Raf, Dual specificity mitogen-activated protein kinase kinase 6, Mitogen-activated protein kinase 14 (p38 MAPK), and over 20 others (see e.g. Table 4 and Table 15).

The inventors further provide examples which demonstrate how their LC-MS workflow, can simultaneously measure the abundance and activity of 1000's of signaling and structural proteins in tumor tissue relative to non-tumor tissue, and show how such measurements can be used to better understand the molecular events leading to cancer and therefore guide selection of the most suitable inhibitory agents to treat a patient on an individual basis using one, or a combination of approved or experimental molecular targeting medicines. Critically, the inventors have demonstrated using hierarchal clustering of phosphopeptide $\log_2$ T/NT ratios that they can identify those patients more likely to show recurrence of pancreatic cancer compared to those patients less likely to show recurrence at the same time point.

Accordingly, at its most general, the invention provides materials and methods for the diagnosis, prognosis and treatment (including the selection of targeted therapies) of pancreatic cancer arising from the identification of signaling pathways and drug targets that show significant modulation in expression and activity between cancerous and non-cancerous tissue types. The data provided herein shows the molecular events driving the cancer phenotype on a case by case basis and for the first time provides the means for clinicians to predict not only the most effective targeted therapy, but also predict likelihood of recurrence of pancreatic cancer.

In a first aspect, there is provided a pancreatic tumor classification system comprising a pancreatic tumour classification apparatus and an information communication terminal apparatus, said pancreatic tumor classification apparatus including a control component and a memory component, said apparatuses being communicatively connected to each other via a network;

(1) wherein the information communication terminal apparatus includes

(1a) a protein data sending unit that transmits the protein data derived from a pancreatic tumor sample of a subject to the pancreatic tumor classification apparatus;

(1b) a result-receiving unit that receives the result of the pancreatic tumor classification of the subject transmitted from the pancreatic tumour classification apparatus;

(2) wherein the pancreatic tumor classification apparatus includes

(2a) a protein data-receiving unit that receives protein data derived from the pancreatic tumor sample of the subject transmitted from the information communication terminal apparatus;

(2b) a data comparison unit which compares the data from the data-receiving unit with the data stored in the memory unit;

(2c) a classifier unit that determines the class (e.g. molecular phenotype) of the pancreatic tumour of the subject, based on the results of the data comparison unit; and

(2d) a classification result-sending unit that transmits the classification result of the subject obtained by the classifier unit to the information communication terminal apparatus; and

wherein the memory unit contains protein expression level and/or phosphorylation data of at least one (preferably a plurality) proteins selected from Tables 2, 3, 4, 11, 12, 13 and/or 15.

The memory unit may contain protein expression level and/or phosphorylation data of at least one or a plurality of proteins selected from each of Tables 2, 3, 4, 11, 12, 13 and/or 15. That is, the memory unit may contain data from two more proteins from Table 2 in combination with data from two more, three or more, four or more, five or more proteins from Table 3, 4, 11, 12, 13 and/or 15; or any combination thereof. This combination of proteins from Tables 2, 3, 4, 11, 12, 13 and/or 15 is applicable to each and every aspect of the invention described herein.

The data derived from the pancreatic tumor sample of the subject is preferably expression level data and/or phosphorylation status data, such as that obtained from methods described herein e.g. LC-MS/MS and other proteomic approaches. The data may be derived just from the tumor (or suspected tumor) sample, but in preferred embodiments, a second data set derived from non-tumor (background) pancreatic tissue of the same subject may also be provided.

The protein data received by the data-receiving unit may be the actual protein or phosphoprotein levels, or it may be peptide or phosphopeptide levels from which the protein or phosphoprotein levels can be calculated. The peptide or phosphopeptide is unique to the at least one (preferably plurality) protein or phosphoprotein. In some embodiments it is preferable to use multiple, i.e. 2, 3, 4, or 5 peptides which are all unique to said protein. Where multiple peptides are used, data may be collated and optionally a median value used in the data comparison step.

The memory unit preferably includes data sets relating to protein expression levels and/or phosphoprotein levels representative of pancreatic tumor. In a preferred embodiment, the protein expression levels and/or phosphoprotein levels are derived from actual peptide or phosphopeptide levels in the sample. This is particularly so if the data has been obtained using proteomic methods such as the LC-MS/MS method described herein. The data sets may provide a representative (e.g. average) level of protein expression levels or phosphoprotein levels found in pancreatic tumors from a collection of data sets, e.g. as provided herein by Table 12. Alternatively, it may be preferable for the data sets to include a value representing a ratio of the protein expression level or phosphoprotein level as compared to the protein expression level or phosphoprotein level of background (i.e. non-tumor) tissue obtained from the same source. By way of example, this value is presented herein as Log2 T/NT.

In addition to confirming that the sample is a pancreatic tumor, the data sets held in the protein data-storing unit allow the system to classify the tumor into recurrence or non-recurrence classes. By inputting the data representative of phosphoprotein levels of the pancreatic tissue sample taken from a subject, and optionally, data representative of phosphoprotein levels of background pancreatic tissue taken from the same subject, the data comparison unit may compare this data with a data set including at least data relating to a plurality of proteins selected from Table 11 held in the memory unit.

In one embodiment, there is provided a method of predicting the likelihood of recurrence of a pancreatic tumor in a subject after treatment, said method comprising detecting the level of phosphorylation of at least one protein selected from the group consisting of Homeodomain-interacting protein kinase I (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and myosin light chain kinase, smooth muscle (MLCK) in a tumour sample obtained from said subject, wherein elevated levels of phosphorylation compared to background (non-tumor) levels is indicative of the likelihood of tumor recurrence.

In this way, the system can compare the phosphoprotein levels obtained from pancreatic tumor sample with phosphoprotein levels representative of a tumor recurrence phenotype for the same protein and thereby classify the tumor as either a tumor with likelihood of recurrence or likelihood of non-recurrence.

In a preferred embodiment the comparison of phosphoprotein levels may also provide a prediction of timing of tumor recurrence, e.g. between 8 and 33 months, between 10 and 20 months or between 15 and 17 months after removal of the tumors.

The pancreatic tumor classification system described above may also be used to classify a pancreatic tumor based on drug susceptibility. In this embodiment, the memory unit may contain, at least phosphoprotein data of a plurality of proteins selected from Table 15 or Table 4.

For example, the inventors have determined those phosphoproteins which are up-regulated or down-regulated in pancreatic tumor (and/or have differences in phosphorylation status) compared to normal pancreatic tissue, and from these have identified those that contain phosphorylation sites that are known to either induce activation or inhibition of the phosphorylated protein (e.g. enzyme). (See Table 15 and Table 4).

Accordingly, by comparing the phosphoprotein levels of a pancreatic tumor sample with the phosphoprotein levels of a plurality of proteins selected from Table 15 and/or Table 4, it is possible for the system to classify the tumor on the basis of drug susceptibility. The drugs may be selected from GSK2141795, GSK2141796, GSK214179, Dasatinib, AEZS-131, Vorinostat, and Sorafenib.

In some cases, the phosphoprotein levels of the sample are compared with those for one or more, two or more, three or more, or all of the following proteins: Glycogen Synthase kinase-3 alpha and beta, Histone deacetylase I and 2, RAF proto-oncogene serine/threonine-protein kinase, serine/threonine-protein kinase A-Raf, Dual

specificity mitogen-activated protein kinase kinase 6, mitogen-activated protein kinase 14 (p38 MAPK).

The pancreatic tumor classification system may be used to determine tumor or non-tumor phenotype of the sample obtained from the subject where the memory unit contains data relating to protein expression levels of a plurality of proteins selected from Table 12 or Table 2.

As a result, the system can compare the expression levels of proteins determined from the sample with expression levels held in the memory unit that are representative of pancreatic tumor. In this way, the sample can be identified as tumor or non-tumor.

Although the inventors acknowledge that the system may be used to perform independent classification of phenotypes, i.e. tumor v non-tumor, recurrence phenotype v non-recurrence phenotype, drug susceptibility profile, and primary tumour v secondary (metastatic tumor), it is preferred that the data contained within the memory unit of the system will allow a sample to be classified as multiple phenotypes, e.g. tumor, predicted recurrence and drug susceptibility profile.

In a preferred embodiment, the system further comprises the means to add the inputted data via the data sending unit to the stored data already held in the memory unit so that this new data can be included in the analysis performed by the determining unit. In this way the data representative of pancreatic tumor molecular phenotypes is constantly updated.

In a preferred embodiment, the pancreatic tumor classification system is connected to an apparatus for determining protein expression levels or protein phosphorylation levels in a pancreatic tumor sample and feeding this data to the protein data sending unit.

Ideally the apparatus can process multiple samples using LC-MS/MS as described herein.

In accordance with this first aspect of the invention, there is also provided a pancreatic tumor cellular classification program that makes an information processing apparatus including a control

component and a memory component execute a method of determining and/or classifying the pancreatic tumor of a subject, the method comprising:

(i) a comparing step of comparing data based on the protein expression levels and/or protein phosphorylation levels of at least one (preferably a plurality) protein selected from Tables 2, 3, 4, 11, 12, 13 and/or 15 obtained of a subject with the protein expression level data and/or the protein phosphorylation data stored in the memory component; and

(ii) a classifying step for classifying the pancreatic tumor cells of said subject, based on the comparison calculated at the comparing step; and wherein said tumor is classified into phenotypes including tumor, non-tumor; tumor recurrence, tumor non-recurrence; primary tumour, secondary (metastatic tumor) and/or drug susceptibility.

In accordance with this aspect of the invention, there is also provided a computer-readable recording medium, comprising the pancreatic tumour classification program described above recorded thereon.

The data representing protein expression levels and/or protein phosphorylation levels (i.e. amount of phosphorylated protein) may be derived from peptide levels and/or phosphopeptide levels in the sample where said peptides and/or phosphopeptides are each unique to a particular protein selected from the specified Tables. Example peptides and phosphopeptides are provided in the Tables for each protein. However, it will be appreciated that other peptides and phosphopeptides may be designed which will also be unique for the protein from which they are derived, e.g. by proteolytic enzyme digestion such as trypsin, aspN, gluC and other such enzymes well known in the art.

In respect of all aspects of the invention described herein, the sample from which the protein data is derived may be obtained from a subject already diagnosed with pancreatic cancer or it may be obtained from a subject suspected of having pancreatic cancer.

Accordingly, with regard to the latter, the classification of the cancer may also include the diagnosis.

In a second aspect of the invention, there is provided a method of diagnosing pancreatic cancer in a subject comprising determining the modulation of one or more, or a plurality of proteins and/or phosphorylation sites selected from Table 12 and/or Table 2, Table 15 and/or Table 3 in a biological sample obtained from said subject, wherein

(a) the presence of said one or more, or plurality of proteins in said sample is indicative of the subject having pancreatic cancer;

(b) the amount (concentration) of said one or more, or plurality of proteins as compared to a reference amount for said one or more, or plurality of proteins is indicative of the subject having pancreatic cancer;

(c) a change in amount (concentration) of said one or more, or plurality of proteins as compared to a reference amount for said one or more, or plurality of proteins is indicative of the subject having pancreatic cancer; or

(d) a change in phosphorylation status of said one or more, or plurality of proteins as compared to a reference status for said one or more, or plurality of proteins is indicative of the subject having pancreatic cancer.

In a third aspect, the invention provides a method of classifying a pancreatic tumour into molecular phenotypes selected from the group consisting of tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumor and secondary (metastatic) tumor, said method comprising

(1) determining expression levels and/or protein phosphorylation level of a plurality of proteins in a biological sample obtained from said subject;

(2) producing an expression level and/or phosphoprotein profile for said sample;

(3) comparing said subject profile with a reference profile representative of the pancreatic tumour molecular phenotype(s);and

(4) determining the molecular phenotype of pancreatic tumour based on the comparison between the subject profile and the reference profile;

wherein the plurality of proteins are selected from a biomarker panel as represented by Table 2, 3, 4, 11, 12, 13 and/or 15.

For this and all other aspects of the invention, the reference protein expression levels and/or protein phosphorylation level profile may be determined from non-tumor pancreatic tissue from the same subject. In this way, the difference in protein expression levels and/or protein phosphorylation levels may be used to determine the molecular phenotype of the pancreatic tumor. Alternatively, the reference levels may be a database comprising data representing expression levels and/or phosphorylation levels for the proteins of interest as selected from any one or more of Tables 2, 3, 4, 11, 12, 13 and 15. Ideally, the reference levels are provided by a pancreatic tumor classification system according to the first aspect. The data representing expression levels and/or protein levels may be a collection of data obtained from multiple tumor samples and presented as an average or range. The data may relate to the levels of specific peptides and/or phosphopeptides each being unique to a protein of interest.

In a fourth aspect of the invention, there is provided a method of selecting a treatment regime for a subject suffering from pancreatic cancer, said method comprising

(1) determining expression levels and/or phosphorylation of one or more, or a plurality of proteins in a biological sample obtained from said subject;

(2) comparing said expression levels and/or phosphorylation status with reference expression levels and/or phosphorylation levels for said one or more, or plurality of proteins, said reference levels representative of pancreatic tumour molecular phenotypes selected from tumor, non-tumor; tumor recurrence, tumor non-recurrence; primary tumor, secondary (metastatic) tumor and/or drug susceptibility;

(3) determining the molecular of pancreatic tumour based on the comparison between the expression levels and/or phosphorylation levels of the proteins in the biological sample and the reference expression levels; and

(4) selecting a treatment regime on the basis of the molecular phenotype of pancreatic tumour,

wherein the plurality of proteins are selected from a biomarker panel as represented by Table 2, 3, 4, 11, 12, 13 and/or 15.

The biological sample is preferably a sample of the pancreatic tumor (e.g. a biopsy), but it is envisaged that for this and other aspects of the invention, the biological sample could be any fluid or solid sample of the subject that was capable of providing a representation of the proteins regulated in pancreatic tumor. For example, biological markers as identified herein may be determined and their amount or concentration, or phosphorylation status, quantified from a blood or urine sample from the subject, thereby avoiding the need for a biopsy.

The method may, for example, allow the user to determine whether the pancreatic sample obtained from the subject is tumor, has a likelihood of recurrence, (i.e. between 8 and 33 months, between 10 and 20 months or between 15 and 17 months after removal of the tumor) and/or what drug targets are present in the tumor.

For example, by comparison with the reference expression levels, the method may identify a plurality of up-regulated proteins selected from Table 12, or more preferably selected from Table 2. In still preferred embodiments, these up-regulated proteins include at least Homeodomain-interacting protein kinase-1 and/or Mucin 1; optionally in combination with any one, two, three, four or more further proteins selected from Table 12 and/or 2. The presence of these up-regulated proteins as compared to the reference level will indicate that the sample is pancreatic tumor.

Likewise, the method may determine those proteins with phosphorylation sites which are significantly regulated compared to references levels, i.e. by comparing the levels of a plurality of phosphorylated proteins with reference levels selected from Table 3, 11, 4, 13 and/or 15. This comparison allows the sample to be classified into the phenotype tumor with a likelihood of recurrence or the phenotype tumor with a non-likelihood of recurrence. For example, the plurality of proteins with regulated phosphorylation sites may be selected from Table 11 or, more preferably, from Table 11A (up-regulated phosphorylation in recurrent tumors) and Table 11B (down-regulated phosphorylation in recurrent tumors).

In fact, the results obtained by the present inventors suggest that the up-regulation in phosphorylation of Dual specificity mitogen-activated protein kinase kinase 2 alone may be sufficient to predict the likelihood of recurrence in a tumor between 8 and 33 months, between 10 and 20 months or between 15 and 17 months after removal of the tumor. Accordingly, the determination of increased phosphorylation of Dual specificity mitogen-activated protein kinase kinase 2 in a biological sample obtained from a subject in order to predict likelihood of recurrence of pancreatic tumor forms a further aspect of the invention. In some cases, the increased phosphorylation may be determined at Threonine 394 of Dual specificity mitogen-activated protein kinase kinase 2. The method may involve determination of increased phosphorylation at this site only, e.g. by immunohistochemistry, or it may include determination at this site in combination with other phosphorylation sites. The method may further include determination of increase or decrease in phosphorylation of sites on one or more further proteins selected from Table 11.

In a further embodiment of this fourth aspect of the invention, the method allows the determination of drug susceptibility for said tumor under test. The inventors have determined from their analysis of the phosphopeptide data that tumors can be classified with respect to the signaling pathways that are affected compared to non-tumor and consequently personalised treatment regimes can be

designed based on the drug targets most susceptible in the tumor. In particular, Table 15 provides those proteins (enzymes) which contain phosphorylation sites known to either induce activation or inhibition of the protein (enzyme). Thus, the method may identify a plurality of proteins selected from Table 15 which have been regulated (up- or down-regulated) and thus provide information as to the signalling pathways affected in the tumor. This information allows the clinician to determine a personalised drug treatment regime for said subject by selecting those drugs known to target the particular proteins in said signalling pathways. The drugs may be selected from the group consisting of Dasatinib, Sorafenib, Vorinostat, Temsirolimus, AEZS-131 and GSK2141795.

In a preferred embodiment, the plurality of proteins selected from Table 15 include Tyrosine-protein kinase (Fyn), Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, and/or RAC-alpha serine/threonine-protein kinase.

Table 15 and Table 4 provide details of those peptides which contain phosphorylation sites which are known to inhibit or activate the protein when phosphorylated. The proteins containing these sites have been identified by the inventors as being either up or down regulated in tumor as compared to background (normal) tissue. As a result, these sites can be used as markers for pancreatic tumor and depending of which proteins are regulated in the particular sample, can be used to select the drug combination used to treat the subject to inhibit the growth or recurrence of the tumor.

In a still further preferred embodiment, the plurality of proteins is selected from the group consisting of Integrin Beta-4; Catenin alpha- 1, Junctional adhesion molecule A (JAM-A); Tyrosine protein kinase Fyn; Mitogen-activated protein kinase 1 (MAPK1); RAC-alpha serine/threonine-protein kinase (AKT1); Glycogen synthase kinase-3 alpha.

The biological sample obtained from said subject is preferably a biopsy sample taken from an individual suspected of having pancreatic cancer. The method may be performed on a number of biopsy samples from said subject over a period of time so as to monitor the effectiveness of the drug treatment.

In a preferred embodiment, the steps of comparing expression levels and/or phosphorylation levels and determining the molecular phenotype of tumour may be carried out using the pancreatic tumour classification system according to the first aspect.

The inventors have used an adapted liquid chromatography-mass spectrometry (LC-MS/MS) method to perform the proteomic analysis of the pancreatic tumor samples. While this may be a preferred method, now that specific biomarkers have been determined by the inventors, i.e. those proteins that are significantly up-or down-regulated in tumor as opposed to non-tumor, other standard methods may be adopted for determining these markers in a sample. Indeed, the inventors have determined a number of markers which are so significantly modulated in tumor tissue that they can act as individual markers thereby avoiding the analysis of multiple markers.

Accordingly, the method of this and other aspects of the invention, for determining the amount of the one or more, or plurality of proteins in the biological sample may be achieved using any suitable method. The determination may involve direct quantification of the protein mass or concentration. The determination may involve indirect quantification, e.g. using an assay that provides a measure that is correlated with the amount (e.g. concentration) of the protein. In certain cases of the method of this and other aspects of the invention, determining the amount of the one or more, or plurality of proteins comprises:

contacting said sample with a specific binding member(s) that selectively and independently binds to the one or more, or plurality of proteins; and

detecting and/or quantifying a complex formed by said specific binding member(s) and the one or more, or plurality of proteins.

The specific binding member may be an antibody or antibody fragment that selectively binds to the protein biomarker. It is preferable that the antibody is labelled for detection. For example, a convenient assay format for determination of a protein concentration is an ELISA. The determination may comprise preparing a standard curve using standards of known concentration for the peptide concentration and comparing the reading obtained with the sample from the subject with the standard curve thereby to derive a measure of the protein biomarker concentration in the sample from the subject. A variety of methods may suitably be employed for determination of protein amount (e.g. concentration), non-limiting examples of which are: Western blot, ELISA (Enzyme-Linked Immunosorbent assay), RIA (Radioimmunoassay), Competitive EIA (Competitive Enzyme Immunoassay), DAS-ELISA (Double Antibody Sandwich-ELISA), liquid immunoarray technology (e.g. Luminex xMAP technology or Becton-Dickinson FACS technology), immunocytochemical or immunohistochemical techniques, techniques based on the use of protein microarrays including reverse protein microarrays and reverse phospho-protein arrays that include specific antibodies, "dipstick" assays, affinity chromatography techniques and ligand binding assays. The specific binding member may be an antibody or antibody fragment that selectively binds a protein biomarker. Any suitable antibody format may be employed. A further class of specific binding members contemplated herein in accordance with any aspect of the present invention comprises aptamers (including nucleic acid aptamers and peptide aptamers). Advantageously, an aptamer directed to the protein biomarker may be provided using a technique such as that known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment), described in U.S. Pat. Nos. 5,475,096 and 5,270,163.

In some cases of the method in accordance with this and other aspects of the invention, the determination of the amount of the protein biomarkers selected from the referenced Tables may comprise

measuring the level of a peptide unique to said protein by mass spectrometry. Techniques suitable for measuring the level of a peptides by mass spectrometry are readily available to the skilled person and include techniques related to Selected Reaction Monitoring (SRM) and Multiple Reaction Monitoring (MRM)isotope dilution mass spectrometry including SILAC, AQUA (as disclosed in WO 03/016861; the entire contents of which is specifically incorporated herein by reference) and TMTcalibrator (as disclosed in WO 2008/110581; the entire contents of which is specifically incorporated herein by reference). WO 2008/110581 discloses a method using isobaric mass tags to label separate aliquots of all proteins in a reference sample which can, after labelling, be mixed in quantitative ratios to deliver a standard calibration curve. A patient sample is then labelled with a further independent member of the same set of isobaric mass tags and mixed with the calibration curve. This mixture is then subjected to tandem mass spectrometry and peptides derived from specific proteins can be identified and quantified based on the appearance of unique mass reporter ions released from the isobaric mass tags in the MS/MS spectrum.

By way of a reference level, the marker protein(s) as selected from Table 2, 3, 4, 11, 12, 13 and/or Table 15 may be used. In some cases, when employing mass spectrometry based determination of protein markers, the methods of the invention comprises providing a calibration sample comprising at least two different aliquots comprising the marker peptide(s), each aliquot being of known quantity and wherein said biological sample and each of said aliquots are differentially labelled with one or more isobaric mass labels. Preferably, the isobaric mass labels each comprise a different mass spectrometrically distinct mass marker group.

Accordingly, in a preferred embodiment of the invention, the method comprises determining a change in expression level or phosphorylation level of one or more, or a plurality of the marker proteins selected from Table 2, 3, 4, 11, 12, 13 and/or Table 15 by Selected Reaction Monitoring using one or more determined transitions for the known protein marker derived peptides; comparing

the peptide levels in the sample under test with peptide levels previously determined to represent pancreatic cancer based on changes in expression of said one or more, or plurality of marker proteins. The comparison step may include determining the amount of the marker peptides from the sample under test with known amounts of corresponding synthetic peptides. The synthetic peptides are identical in sequence to the peptides obtained from the sample, but may be distinguished by a label such as a tag of a different mass or a heavy isotope.

One or more of these synthetic marker peptides (with or without label) form a further aspect of the present invention. These synthetic peptides may be provided in the form of a kit for the purpose of diagnosing pancreatic cancer in a subject; or for the purpose of classifying a pancreatic sample from a subject into a molecular phenotype selected from tumor, non-tumor, likelihood or recurrence, likelihood of non-recurrence, drug susceptibility, primary tumor, or secondary (metastatic tumor); or for selecting a treatment regimen for said subject.

In preferred embodiments with respect to this and other aspects of the invention, the one or more proteins, or plurality of proteins includes Mucin-1 and/or Homeodomain-interacting protein kinase-1; optionally in combination with one, two, three or four further proteins selected from Table 2, 3, 4, 11, 12, 13 and/or 15, preferably Table 12 and/or Table 2.

Other suitable methods for determining levels of protein expression include surface-enhanced laser desorption ionization-time of flight (SELDI-TOF) mass spectrometry; matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, including LS/MS/MS; electrospray ionization (ESI) mass spectrometry; as well as the preferred SRM and TMT-SRM.

In a further aspect of the invention, there is provided a kit for use in carrying out the methods described above, in particular classifying pancreatic cancer into molecular phenotypes selected

from the group consisting of tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor for a sample obtained from a subject.

In all embodiments, the kit allows the user to determine the presence, level (up- or down-regulation) of protein expression and/or phosphorylation status of a plurality of analytes selected from a plurality of marker proteins or fragments thereof provided in Table 2, 3, 4, 11, 12, 13 and/or 15 and antibodies against said marker proteins in a sample under test; the kit comprising

(a) a solid support having a plurality of binding members, each being independently specific for one of said plurality of analytes immobilised thereon;

(b) a developing agent comprising a label; and, optionally

(c) one or more components selected from the group consisting of washing solutions, diluents and buffers.

The binding members may be as described above.

In one embodiment, the kit may provide the analyte in an assay-compatible format. As mentioned above, various assays are known in the art for determining the presence or amount of a protein, antibody or nucleic acid molecule in a sample. Various suitable assays are described below in more detail and each form embodiments of the invention.

The kit may additionally provide a standard or reference which provides a quantitative measure by which determination of an expression level of one or more marker proteins can be compared. The standard may indicate the levels of the two or more biomarkers which indicate pancreatic cancer.

The kit may also comprise printed instructions for performing the method.

In a preferred embodiment, the kit may be for performance of a mass spectrometry assay and may comprise a set of reference peptides

derived from proteins set out in Table 2, 3, 4, 11, 12, 13 and/or 15 (e.g. SRM peptides) in an assay compatible format wherein each peptide in the set is uniquely representative of each of the plurality of marker proteins. Preferably two , three, four or five or more such unique peptides are used for each protein for which the kit is designed, and wherein each set of unique peptides are provided in known amounts which reflect the levels of such proteins in a standard preparation of said sample. Optionally the SRM peptides are phosphopeptides representing differentially phosphorylated sites within the target proteins set out in Table 13, 3, 11 and/or Table 14. Optionally the kit may also provide protocols and reagents for the isolation and extraction of proteins from said sample, a purified preparation of a proteolytic enzyme such as trypsin and a detailed protocol of the method including details of the precursor mass and specific transitions to be monitored. The peptides may be synthetic peptides and may comprise one or more heavy isotopes of carbon, nitrogen, oxygen and/or hydrogen.

The classification methods as provided herein also include determination of protein modulation as a result of phosphorylation. The inventors have shown that a number of proteins are induced or inhibited in pancreatic cancer tissue as opposed to background tissue. Accordingly, the invention provides a method comprising determining the phosphorylation status of one or more, or a plurality of proteins selected from Table 13, 3, 11 and/or Table 14 in a sample obtained from a subject suspected of having pancreatic cancer.

Preferably said one or more or plurality of proteins are selected from the group consisting of integrin beta-4, Catenin alpha-1, Junctional adhesion molecule A (JAM-A), Tyrosine protein kinase Fyn; Mitogen-activated protein kinase 1 (MAPK1); RAC-alpha serine/threonine-protein kinase (AKT1); Glycogen synthase kinase-3 alpha.

In a preferred embodiment, the protein is Dual specificity mitogen-activated protein kinase kinase 2. In particular, the inventors have

determined that phosphorylation of Dual specificity mitogen-activated protein kinase kinase 2 at phospho-T394 was increased in tumor cases compared to background (non-tumor) and have shown that phosphorylation at this site correlates positively with recurrence of tumor at median 16.5 months (Figure 4).

Table 11, 15 and/or Table 4 provide a list of other phosphorylation sites on proteins which are regulated in pancreatic tumor samples as compared to non-tumor. Each of these sites provides a marker for classifying pancreatic tumor with respect to likelihood of recurrence and drug susceptibility. Accordingly, each phosphorylation site forms an aspect of the present invention either alone or in combination for use in classifying pancreatic tumor with respect to likelihood and timing of recurrence and/or drug susceptibility.

By way of example, there is provided a method of predicting susceptibility of a pancreatic tumor to treatment with Dasatinib (BMS-354825 - Sprycel™) comprising determining the level of phospho-S21 on Tyrosine-protein kinase Fyn, wherein an up-regulation of this protein is indicative that the pancreatic tumor will be susceptible to treatment with Dasatinib (Table 4).

Further there is provided a method of predicting susceptibility of a pancreatic tumor to treatment with AEZS-131 (Aeterna Zentaris Inc) and/or SCH772984 (Merck) comprising determining the level of phospho-T185 and/or phospho-Y187 on Mitogen-activated protein kinase 1 (MAPK1); and additionally or alternatively phospho-T202 and/or phospho-Y204 of Mitogen-activated protein kinase 3 (MAPK3/ERK1), wherein an up-regulation of this protein phosphorylation is indicative that the pancreatic tumor will be susceptible to treatment with AEZS-131 and/or SCH772984. For further examples, see Table 4.

Determining phosphorylation of proteins is standard in the art. For example, antibodies that have specificity for a particular phosphorylation motif can be raised in a host animal and used for

subsequent detection of the relevant motif in tissues *in situ* using immunohistochemistry or following extraction of the target protein from the tissue or body fluid using Western blotting or enzyme-linked immunosorbent assay (ELISA). Other antibody-based detection methods are well known to the skilled practitioner and include bead-suspension arrays, planar arrays, radio-immunoassays and immunoprecipitation linked to mass spectrometry. However, it is normally necessary to use phosphoprotein specific antibodies in a two-step process where the target protein is first enriched prior to detection. This is due to the commonality of epitopes recognised by such antibodies within multiple substrates of a particular kinase. In other words, the way a kinase recognises phosphorylation sites within its substrates is similar to the epitope recognised by an antibody being a conserved sequence of 4-8 amino acids.

In some cases phosphorylation of proteins can be monitored by providing a radioactive isotope of phosphorous, typically P32 in a growth medium or dietary supplement for experimental animals. After a defined period of metabolic labelling the incorporation of P32 in specific proteins can be followed by detection the radioactive signal using standard protein separation methods such as gel electrophoresis and liquid chromatography.

In a preferred embodiment, the plurality of proteins selected from Table 13, Table 3, and/or Table 11 include Integrin Beta-4; Catenin alpha- 1, Junctional adhesion molecule A (JAM-A); Tyrosine protein kinase Fyn; Mitogen-activated protein kinase 1 (MAPK1); RAC-alpha serine/threonine-protein kinase (AKT1); Glycogen synthase kinase-3 alpha.

In a further aspect of the invention, a method is provided for classifying a pancreatic tumor sample into one or more molecular phenotypes comprising

(1) determining the protein expression levels of one or more, or a plurality of proteins selected from Table 12 and/or Table 2, for both a pancreatic tumor sample and a pancreatic non-tumor sample taken from a subject

and/or

(2) determining the up or down regulation of one or more, or a plurality of phosphoproteins selected from Table 3, Table 13 and/or Table 11 in a pancreatic tumor sample and a pancreatic non-tumor sample taken from a subject,

(3) comparing said protein expression levels of the tumor sample with the non-tumor sample; and/or comparing the up or down regulation of phosphoproteins in the tumor sample with the non-tumor sample

(4) applying predictive algorithm

$$log_2(_iT/_{iNT})$$

(where i is subject sample, T = tumour and NT = non-tumour)

to produce a prediction value that for said protein expression level and/or phosphoprotein level for said subject;

(5) classifying said pancreatic tumor sample into a molecular phenotype by reference to a database comprising values predictive of said phenotypes, wherein said database comprises predictive values for one or more or a plurality of proteins selected from Table 2, 3, 4, 11, 12, 13 and/or 15; and wherein the molecular phenotype is selected from tumor, non-tumor; tumor recurrence, tumor non-recurrence; drug susceptibility; primary and/or secondary tumor.

In a preferred embodiment the protein marker is considered modulated (either by up-regulated or down-regulated expression or phosphorylation) if the $\log_2$ T/NT ratio is ≥ 1 or ≤ -1.

In a preferred embodiment, the classification is carried out by a pancreatic tumor classification system according to the first aspect.

Preferably the above method may be used to determine the prognosis of a subject with pancreatic cancer. In this respect, prognosis includes the determination or early, late or no recurrence following surgical removal, radiological or chemotherapy treatment. For

example the method may compare the expression and phosphorylation values with values for one or more or a plurality of proteins selected from Tables 11, 3, and/or 13.

In preferred embodiment, the one or more or plurality of proteins includes Dual specificity mitogen-activated protein kinase kinase 2.

In respect of this and other aspects of the invention, the total protein content of a surgically-resected tumor or a tumor biopsy is extracted and subjected to phosphoproteomic analysis by methods known in the art and/or described herein. The relative abundance of each phosphopeptide detected by such analysis is recorded in a database (e.g. using a system according to the first aspect) and the total profile is compared with known cases of recurrent and non-recurrent pancreatic cancer using methods such as Agglomerative Clustering. By this "bottom up" approach: each observation starts in its own cluster, and pairs of clusters are merged as one moves up the hierarchy. At the end of the Agglomerative Clustering process the tumor being analysed will have been clustered into a group representing its likelihood of recurrence. In a preferred embodiment, the database also carries sufficient numbers of samples with specific times of recurrence post-surgery or initial treatment to also assign a likely time of recurrence to the individual patient with a recurrent tumor profile. The likely time of recurrence is between 8 and 33 months, between 10 and 20 months or between 15 and 17 months after removal of the tumor.

In a further aspect of the invention, there is provided a method selecting a treatment regimen for a subject with pancreatic cancer, said method comprising

(1) determining phosphoprotein levels of one or more, or a plurality of protein markers selected from Table 15 and/or Table 4,

(2) comparing said determination with a previously determined reference representative of drug susceptibility, and

(3) selecting a drug treatment regime for said subject based on the drug susceptibility of said tumor.

In a preferred embodiment, the drug target is a particular protein carrying a differential phosphorylation site, or it is an upstream kinase or phosphatase responsible for such differential phosphorylation.

In a preferred embodiment, the plurality of proteins selected from Table 15 and/or Table 4 include Tyrosine-protein kinase Fyn, Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, and/or RAC-alpha serine/threonine-protein kinase.

Preferably the drugs are selected from the group consisting of Dasatinib, Sorafenib, Vorinostat, Temsirolimus, AEZS-131 and GSK2141795.

For all aspects of the invention, the determination step is preferably carried out by liquid chromatography-mass spectrometry (LC-MS/MS).

In a still further aspect of the invention a method for improving the design of molecular targeting drugs is provided wherein the methods and systems of the invention are used to analyse the performance of novel compounds in modulating the oncogenic pathway on the proteins selected from Tables 2, 3, 12, 11, 13, 14 and/or 15.

Accordingly, the invention further provides a method of testing the effectiveness of a molecular targeting drug comprising

obtaining a sample of pancreatic tumor from a subject; said tumor having been in contact with the molecular targeting drug under test, e.g. by administration to said subject prior to the sample being obtained;

extracting proteomic data from said sample, e.g. relative abundance of proteins or phosphorylated proteins;

comparing said proteomic data with reference data, e.g. data obtained from a sample of the same tumor prior to contact with the molecular targeting drug under test;

wherein a change in the proteomic data between the sample taken after contact with the molecular targeting drug and the sample taken prior to contact with the molecular targeting drug is indicative of the effectiveness of the molecular targeting drug in treating pancreatic tumor; and

wherein the proteomic data comprises relative abundance levels of a plurality of phosphoproteins selected from Table 15 and/or Table 4.

The proteomic data may be obtained by measuring the relative abundance (e.g. up-regulated or down-regulated) of phosphopeptides unique to each of the plurality of proteins. Preferably the phosphopeptides are selected from Table 15 and/or Table 4.

By way of example, human pancreatic cancer-derived cell lines are exposed to a candidate therapeutic compound at different concentrations, including a vehicle control, or for different periods of time. Following exposure to the candidate therapeutic compound, cells are lysed and total proteins extracted. Preferably the proteins are digested using a proteolytic enzyme such as trypsin and labelled, e.g. using an isobaric mass tag. Preferably the isobaric mass tags are Tandem Mass Tags (Thermo Scientific). Labelled peptides from several cell lines may be mixed together prior to analysis by LC-MS/MS. Preferably one or more reference labelled peptides (e.g. selected from Table 15 and/or Table 4) representing known targets of the candidate drugs may be included to provide a quantitative internal standard. Following LC-MS/MS analysis the relative abundance of one or more, and preferably all phosphopeptides in each treated sample are submitted to analysis in a system according to the first aspect e.g. the SysQuant database, and subjected to Agglomerative Heirarchical Clustering to obtain a treatment phenotype. Compounds achieving a positive treatment phenotype may be prioritised for further development.

It is to be understood that the methods of this aspect of the invention may be applied to any aspect of the drug development

process including xenograft tumors and tumors taken from human subjects participating in clinical trials.

It is further to be understood that the methods of this aspect of the invention may also be applied to the determination of the most effective molecular targeting medicines in a patient with a pancreatic tumor based on preparation of primary tumour cell cultures from the resected tumor, exposure of primary cell cultures to different molecular targeting drugs and analysis of the relative levels of phosphoproteins using the methods described herein, e.g. inventors' SysQuant methods.

Preferably the proteins (or their unique peptides) include one or more of, or a plurality of, Tyrosine-protein kinase Fyn, Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, Intergrin beta 4, Catenin alpha-1, Junctional adhesion molecule A (JAM-A); Mitogen-activated protein kinase 1 (MAPK1); Glycogen synthase kinase-3 alpha; Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); Myosin light chain kinase, smooth muscle (MLCK) and/or RAC-alpha serine/threonine-protein kinase (AKT1).

In a further aspect of the invention the methods and systems of the invention e.g. the SysQuant database, may be applied to the analysis of recurrent pancreatic cancer. When a new tumour is identified in a patient that has previously received treatment for pancreatic cancer, a so-called recurrent tumor, or a new tumor is found in the pancreas of patients that have previously been treated for a tumor elsewhere in the body, a so-called metastatic tumor, it is important to identify the mechanism of resistance and potential new targets for treatment in the recurrent or metastatic tumor. Accordingly, the methods of the present invention may be utilised in the analysis of

protein and phosphorylation site changes in the recurrent or metastatic tumor.

The invention also provides the use of a plurality of biomarkers selected from Table 2, 3, 4, 11, 12, 13 and/or 15 for determining the molecular phenotype of a pancreatic tumor in a subject, wherein said molecular phenotype is selected from the group consisting of tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor.

Preferably the biomarkers are selected from Table 2 and/or Table 12 and the molecular phenotype is selected from tumor or non-tumor.

In particular, the biomarkers may comprise Mucin-1, Intergrin beta 4, and/or Homeodomain-interacting protein kinase 1.

In a further embodiment, the biomarkers are selected from Table 3, 11 and/or Table 13 and the molecular phenotype is selected from tumor recurrence or tumor non-recurrence, e.g. Dual specificity mitogen-activated protein kinase kinase 2.

In a still further embodiment, the biomarkers are selected from Table 4 and/or 15 and the molecular phenotype is selected from drug susceptibility. For example, the biomarkers may include one or more of, or a plurality of, Tyrosine-protein kinase Fyn, Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, Intergrin beta 4, Catenin alpha-1, Junctional adhesion molecule A (JAM-A); Mitogen-activated protein kinase 1 (MAPK1); Glycogen synthase kinase-3 alpha; and/or RAC-alpha serine/threonine-protein kinase (AKT1).

The inventors have determined a number of protein kinases which are consistently differentially expressed in tumor versus non-tumor

patients. Accordingly, the invention provides a number of novel therapeutic targets for pancreatic cancer. In addition, the invention provides methods of treating subjects with pancreatic cancer using kinases inhibitors. In one embodiment, the invention provides a method of treating pancreatic cancer in a subject, said method comprising administering a compound effective in inhibiting the kinase activity of one or more proteins selected from HIPK1; MRCK alpha; and MLCK.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures and tables described above. The present invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or is stated to be expressly avoided. All documents mentioned in this specification are incorporated herein by reference in their entirety for all purposes.

Brief Description of the Figures and Tables

Figure 1 Venn diagrams demonstrate the number of; **A.** unique phosphopeptides, **B.** unique non-phosphopeptides, and **C.** unique total peptides identified in the TiO2, IMAC, and/or non-enrich arm of the SysQuant workflow, across all three TMT8plex samples in total (TMT8plex-ALL) and individually per TMT8plex (TMT8plex 1, TMT8plex 2, TMT8plex 3). **1.D** demonstrates the level of overlap the inventors observed for peptide identifications from analytical run 1, analytical run 2, and analytical run 3 (including time dependent rejection list compiled from identifications from run 1 and 2).

Figure 2A: PC1 and PC2 Score plot of the first two principal components describing 13.6% (PC1) and 10.6% (PC2) of the total variance in the data. The circle depicts the T2 hotelling space based on 95% confidence. 2B: PC2 and PC3 Score plot of the next principal components describing 10.6% (PC2) and 14.4% (PC3) of the total variance in the data.

Figure 3: Hierarchal cluster analysis was performed on $\log_2$ T/NT values of all 5409 phosphopeptides quantified in this study.

Phosphopeptides are clustered in rows and cases are clustered in columns. **3A**: focusses on regions of the cluster map which contain phosphopeptides demonstrating lower levels (GREEN) in tumor tissue from patients with recurrence, but higher levels (RED) in tumor from patients with no recurrence. The red arrows indicate phosphopeptides that correlate best with recurrence. **3B**: focusses on regions of the cluster map which contain phosphopeptides demonstrating the inverse of **3A**. **3C**: phosphopeptides demonstrating lower levels in tumor from all cases (upper panel), and higher levels in tumor from all cases (lower panel). **3D**: Pearson's correlation coefficients were calculated across all cases and hierarchal clustering was performed on these values. The table indicates presence or absence of lymph node metastases and recurrence in each case.

Figure 4. All log2 T/NT ratios of phospho-peptides containing phospho-T394 of Dual specificity mitogen-activated protein kinase kinase 2 were summed and displayed on the table and plotted on the bar chart. Patients with recurrence (median 16.5 month follow up) were grouped with patients with no recurrence at time of last examination. Time of examination, time of recurrence, time of tissue storage in -80°C freezer and presence of lymph node mets are displayed in the table.

Figure 5. Volcano plots showing $-\log_{10}$ P-values in relation to $\log_2$ T/NT ratios for; (A) proteins and (B) phosphopeptides measured in the IMAC, (C) phosphopeptides measured in the TiO_2 and (**D**) phosphopeptides measured in the Non-enriched arm of the SysQuant workflow. Red circles point out biologically significantly phosphopeptides as they demonstrate $\log_2$ T/NT ratios ≥ 0.75 or ≤ -0.75 and have p-values ≤ 0.05. **E**: is a Venn diagram illustrating the distribution of the 635 phosphopeptides across the three arms of the workflow that were significantly modulated.

Figure 6.A: shows a STRING protein interaction network built using accession numbers from all proteins with significantly regulated phosphopeptides. In total there were 635 significantly modulated phosphopeptides from 408 proteins in the illustrated network. **B**: shows the same STRING network but highlights in RED those proteins

involved in the KEGG Tight Junction signaling pathway. The phosphopeptides from the Tight Junction proteins are also listed. **C**: highlights in RED those proteins associated to the GO biological process 'Regulation of RAS protein signal transduction' and there phosphopeptides are listed in the table.

Figure 7. Signaling pathways modulated in pancreatic cancer tissue. (**A**) This schema summarizes all proteins identified as phosphorylated from the following KEGG signaling pathways; Tight Junction, Adherens Junction and Focal Adhesion. Red stars indicate those proteins identified as phosphorylated in any of 12 cases. Proteins highlighted by coloured circles are known drug targets. (**B**) Phosphopeptides from case 1 (Fig. 4B) demonstrating $\log_2$ T/NT ratios ≥ 1 or ≤ -1, were from proteins matched with greatest significance (based on Benjamini) by the DAVID Bio-informatic resource to the Tight Junction and Adherens Juntion signaling pathways from KEGG. Red stars indicate proteins yielding phosphopeptides with $\log_2$ T/NT ratios ≥ 1 or ≤ -1 from case 1, and coloured circles indicate most suitable drug target, which in case 1 is FYN. (**C**) Phosphopeptides from case 10 demonstrating $\log_2$ T/NT ratios ≥ 1 or ≤ -1, were from proteins matched with greatest significance (based on Benjamini) by the DAVID Bio-informatic resource to the Tight Junction and Focal Adhesion signaling pathways from KEGG. Red stars indicate proteins yielding phosphopeptides with $\log_2$ T/NT ratios ≥ 1 or ≤ -1 from case 10, and coloured circles indicate most suitable drug target, which in case 10 appears to be AKT1 and MAPK1.

Figure 8A: This MA-plot shows the logarithmized ratios vs. the logarithmized intensities over the complete non-normalized data set. **Figure 8B**: This MA-plot shows the same as **Figure 8A**, but the data are normalized by sum-scaling and therefore better zero-centred.

Table 1: Number of peptide spectrum matches, number of unique peptides and number of phosphorylation sites identified in each TMT8plex and in total.

Table 2: Top 12 proteins significantly up-regulated in tumor compared to background tissue, on average over all 12 cases. Log_2 T/NT ratios of the non-phosphorylated peptides from each protein were used as surrogates to calculate the relative abundance of the respective proteins. Log2 T/NT ratios of the non-phosphorylated peptides were averaged over three arms of the workflow (IMAC, TiO2, Non-enrich).

Table 3: Significantly regulated phosphopeptides in tumor compared to background tissue, on average over all 12 cases. All phosphopeptides are from proteins involved in KEGG signaling pathways; Tight Junction, Focal Adhesion, Vascular Smooth Muscle Contraction, Rearrangement of Actin Cytoskeleton. Here we display the p values and Log_2 T/NT ratios for protein and phosphopeptide.

Table 4: Displays examples of peptides that contain activator and inhibitor phosphorylation sites on proteins known to be anti-cancer drug targets. The phosphorylated residue in each peptide sequence is underlined. The log_2 T/NT ratios were median values calculated from all three arms of the workflow, and all ratios ≥ 1 or ≤ -1 were highlighted in bold text. Peptides in red contain activator phosphorylation sites, while peptides in blue contain inhibitor phosphorylation sites. Peptides in black contain phosphorylation sites with no known function.

Table 5: Characteristics of fourteen cases of pancreatic head ductal adenocarcinoma were selected from Institute of Liver Studies BioBank for use in this study.

Table 6: Tumor stage and recurrence of each case under study. Yellow cases showed recurrence between 8 & 33 months (median follow-up period 16.5 months) after tumor removal. The difference between stage **IIA** and **IIB** is the presence (IIB) or absence (IIA) of lymph node metastasis.

Table 7: Clinical information (e.g. time of recurrence) for each case under test.

Table 8: Protein amounts from each sample used for the SysQuant workflow in this study.

Table 9: Peptides are labelled with different tandem mass tags (TMT). Table 9 shows which TMT8plex tag is used to label which sample within each of the three TMT8plex samples analysed in this study.

Table 10: All three of the TMT8plex samples were separated into 3 aliquots. All nine aliquots of TMT labelled peptides were then separated by SCX-HPLC into 12 fractions each. For each of the three TMT8plex samples, 12 fractions were enriched for phosphopeptides using IMAC, 12 fractions enriched for phosphopeptides using TiO_2, and 12 fractions were not enriched for phosphopeptides but instead analysed directly by LC-MS/MS to determine relative protein abundance for normalisation purposes.

Table 11: Phosphopeptides displaying high (Log2 T/NT ≥0.7) and low (log2 T/NT ≤ -0.7) levels in tumour versus non-tumor from the cases with recurrence that clustered together in Figure 3D.

Table 12: Significantly regulated proteins in tumor versus non-tumour (150 proteins). T.test p-values and average log2 T/NT ratios across 12 cases as well as Log2 T/NT ratios for each case are provided.

Table 13: Accession numbers of proteins involved in signaling pathways (Kegg pathways shown in column entitled 'term') which also yielded phosphopeptides demonstrating log2 T/NT ratios of ≥ 1, or ≤ -1 (more than 2 fold up/down-regulated) from each case. Information such as p values and Benjamini probabilities are also shown.

Table 14: *Case 1* - Phosphopeptides from case 1 displaying log_2 T/NT ratios ≥1 or ≤ -1, from proteins involved in the following KEGG signaling pathways Tight Junction, Adherens Junction and Focal Adhesion

Table 15: *Case by case* – Phosphopeptides displaying log_2 T/NT ratios ≥1 or ≤ -1 at sites known to either induce activation or inhibition of the phosphorylated enzyme.

Abbreviations and Definitions

HPLC = high pressure liquid chromatography

SCX = strong cation exchange

TiO_2 = titanium dioxide

IMAC = immobilised metal affinity chromatography

T = tumor

NT = non-tumor

LC-MS = liquid chromatography – mass spectrometry

STRING = Search Tool for the Retrieval of Interacting Genes/Proteins

GO = Gene Ontology

KEGG = Kyoto Encyclopedia of Genes and Genomes

TMT = Tandem mass tags

The phenotype "tumor" in the context of the present invention shall mean neoplastic cells resulting in abnormal proliferation (malignant growth) as a result of carcinoma of the pancreas, in particular pancreatic head adenocarcinoma.

The phenotype "non-tumor" in the context of the present invention shall mean normal, non-neoplastic or benign neoplastic pancreatic cells. It will be understood that such cells may be obtained from abnormal growth, but such growth is not malignant, e.g. cyst.

The phenotype "likelihood of recurrence" shall mean the likelihood of the tumor reappearing between 8 and 30 months following removal by e.g. surgery.

The phenotype "likelihood of non-recurrence" shall mean the likelihood of the tumor not reappearing following removal by e.g. surgery.

The phenotype "drug susceptibility" in the context of the present invention shall mean a pancreatic tumor presenting a molecular profile indicative of modulation of a cell signalling pathway comprising one or more molecular drug targets. The drug targets may be selected from FYN, GSK3α/β, HDAC1/2, the RAF kinases, MAPKs (p38 and ERK2), AKT, PKCs, Casein Kinases.

The phenotype "primary tumor" shall mean tumor originating from the pancreas.

The phenotype "secondary tumor" or "metastatic tumor", shall mean a pancreatic tumor that is formed by cancer cells originating from a tumor located elsewhere in the subject.

The term "plurality" may mean more than one, more than two, more than three, more than four, more than five, more than 10, more than 15, more than 20, more than 25, more than 30 proteins, peptides, phosphoproteins or phosphopeptides selected from one or more referenced Table.

The term "plurality" may also mean more than one protein, peptide, phosphoprotein, phosphopeptide as expressed as a percentage of the reference Table. For example, a plurality may include 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 85%, 90%, 95% of the proteins, peptides, phosphoproteins or phosphopeptides provided in the referenced Table.

In both cases, where the plurality is selected from a referenced Table, it is envisaged that any combination of the proteins, peptides, phosphoproteins, or phosphopeptides will form embodiments of the present invention. For example, with respect to Table 2 where 12 proteins are listed, it is contemplated that the plurality of proteins may comprise Homeodomain-interacting protein kinase 1 with one or more, two or more, three or more etc of the remaining proteins listed in Table 2. This would be true for each of the proteins independently, i.e. Mucin-1 may be combined with one or more, two or more, three or more etc of the remaining proteins listed in Table 2.

By way of example, such combinations can be expressed mathematically notation "combination":

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$

This can be expressed in the form ${}^{n}C_{k}$ (i.e. "n choose k")
In the case of Table 12, n=12 (the total of the table) and k is the number in a chosen subset.

All combinations of two or more markers from Tables 2, 3, 4, 11, 12, 13, 14 and/or 15 are specifically contemplated herein, i.e. for Table 2 all 66 possible pairs (${}^{12}C_{2}$), all 220 possible combinations of 3 markers (${}^{12}C_{3}$), all 495 possible combinations of 4 markers (${}^{12}C_{4}$), all 792 possible combinations of 5 markers (${}^{12}C_{5}$), all 924 possible combinations of 6 markers (${}^{12}C_{6}$), etc.

The term "protein" shall be construed to include the full length protein or any form of the protein, e.g. translational splice variants, isoforms, glycosylated forms, phosphorylated forms or comprising other post-translational modifications. For the proteins referenced in the Tables, Uniprot-IDs are provided allowing full details of the protein including its sequence to be obtained. It is understood in the art that each Uniprot-ID has a history log that allows the specific sequence associated with said Uniprot-ID on any given date such as the date of the present invention can be readily determined irrespective of subsequent modification or revision. This information and data is incorporated herein by reference.

Accordingly, a change in expression level of a protein may mean the up- or down-regulation of the expression of the protein in all its forms, or it may mean the up- or down-regulation of a particular form of the protein, e.g. isoform, splice variant etc.

The term "relative abundance" shall mean the level, amount or concentration of a protein as compared to a reference level, i.e. from a database or from levels obtained from a different/background sample. The relative abundance of a protein may be obtained from measuring the level, amount or concentration of one or more, preferably two, three, four or five peptides unique to said protein

and comparing the level, amount or concentration with the same peptides in the reference sample. This provides relative abundance levels for each peptide. A median average may then be taken to illustrate the level, amount or concentration of the protein itself.

The term "peptide" shall mean an amino acid sequence derived from a full length protein. The peptide will comprise enough amino acids such that its sequence is unique to the protein from which it is derived. This may be as few as at least 4, 5, 6, 7, 8, 9 or 10 amino acids in length, more preferable between 4 and 50, 40, 35, 30, 25 or 20 amino acids, or between 5 and 50, 45, 40, 35, 30, 25 or 20 amino acids or between 5 and 50, 45, 40, 35, 30, 25, or 20 amino acids. The peptide may be made synthetically, or it may be the result of proteolytic enzyme digestion, e.g. trypsin of the full length protein.

The term "phosphoprotein" shall mean any protein which has been phosphorylated at a phosphorylation site e.g. serine, tyrosine or threonine. Herein, such sites are denoted as 'phospho-Xyyy' where X represents the one or three letter amino acid code and y represents integers defining the residue location within the Uniprot-ID of the relevant phosphoprotein.

The term "phosphopeptide" shall mean a peptide sequence which comprises one or more, preferably one, phosphorylated site, e.g. serine, tyrosine or threonine.

A change in the level or phosphorylation status of a phosphoprotein or phosphopeptide derived from a phosphoprotein does not necessarily mean a change in the amount (concentration) of the protein itself, but rather a change in the phosphorylated form of said protein, perhaps at a specific site.

Materials and Methods

Twelve cases of pancreatic head ductal adenocarcinoma were selected (Table 5). Case selection is described in Supplemental methods below. Briefly, 12 tumor (T) versus 12 non-tumor (NT) pancreatic tissue specimens were analysed using the SysQuant workflow. Tissue samples were taken from the pancreatic tumor masses, while NT

samples were taken from the same pancreas at a distal site from the tumor mass. All tissue samples were frozen within 30 minutes of surgical resection and stored at -80°C until analysis by SysQuant (median time of storage 18.5 months (range 4-28 months)). Details of experiments are described in Supplemental Methods below. In summary, this entailed protein extraction from tissue specimens, trypsin digestion of proteins into peptides, TMT 8-plex labelling of peptides (tumor and non-tumor tissue from 4 cases per TMT 8-plex) followed by mixing to form a single 8-plex sample mixture (See Table 9). Each TMT 8-plex sample was then split into three independent aliquots, each of which was further split into 12 fractions by strong cation exchange (SCX) chromatography (Table 10). The first set of 12 SCX fractions were then analysed directly by nano-flow HPLC-MS/MS using duplicate data dependent acquisition runs followed by a third run using time dependent rejection of all features identified in runs 1 & 2. The remaining two sets of 12 fractions were first enriched for phosphopeptides using either IMAC or TiO_2 (Table 10). The resulting 24 phosphopeptide enriched fractions were submitted to the same nano-flow HPLC-MS/MS analysis. In total 108 separate nano-flow HPLC-MS/MS runs were performed for each TMT 8-plex sample. Raw MS data were searched against the human UniProtKB/Swiss-Prot database using Mascot and Sequest (via Proteome Discoverer). Peptide spectrum matches (PSMs) were rejected if they were identified with only low confidence (≥ 5% FDR), showed ≤ 75% phospho-RS probability score, and had missing quantification channels (e.g. not all peaks for isobaric tags were visible in spectra). Raw intensity values of isobaric tags from PSMs passing filters were used for quantification, and these values were normalised using sum-scaling to reduce potential experimental/systematic bias. As a first step, $\log_2$ ratios were calculated from isobaric tag intensities, showing the regulation between T over NT for all and for each case. A phosphopeptide T/NT $\log_2$ ratio is the median T/NT $\log_2$ ratio from all PSMs unique to that specific peptide sequence. A protein T/NT $\log_2$ ratio is the median T/NT $\log_2$ ratio from all unique non-phosphorylated peptides unique to that specific protein. For the data analysis a one sided t-test

(one-sample location test) was used to calculate p-values. P-values were plotted against $\log_2$ T/NT ratios on Volcano plots to detect any significant regulation over all cases. At the protein level, annotation using GO-terms (http://www.geneontology.org/), KEGG-pathways (http://www.genome.jp/kegg/) and Drugbank (http://www.drugbank.ca/) information were added, and also mapped to pathways using resources such as DAVID (http://david.abcc.ncifcrf.gov/) and STRING (http://string-db.org/). At the phosphorylation site level annotation using PhosphoSitePlus (www.phosphosite.org) were added, including known functional and biological/pathological role of the phosphorylation site. Principal component analysis (PCA) and Projection to Latent Structure (PLS) were used to model / investigate the multivariate dataset and identify outliers and groups/clusters, from all peptide ratios (phospho and non-phospho peptides) from all arms of the workflow (IMAC, TiO_2 and non-enriched). Finally hierarchal clustering were performed to build a hierarchy of clusters at the case/specimen level in relation to phosphopeptide relative abundance between T and NT tissue types, and also in relation to the protein relative abundance. The SysQuant workflow, combining phosphoproteomic sample preparation, LC-MS/MS analysis, and bioinformatics analysis, was used to identify important molecular events the inventors believe contribute to pancreatic cancer in the cases analysed here.

Supplemental Methods

Frozen Clinical Tissue. Ethical aspects and research protocol were approved by the BioBank Committee of the Institute of Liver Studies, King's College Hospital. Twelve cases of pancreatic head ductal adenocarcinoma were selected in the database of BioBank at the Institute of Liver Studies (Table 5). Initially cases 2 and 3 were selected but later found to have too little protein extracted for this workflow. Therefore two additional cases were selected (Cases 13 and 14) to increase the number back to twelve. Small pieces of tissue were snap frozen from Whipple's specimens and stored in a BioBank freezer (for at least 2 years). This process of tissue sampling was completed within 30 min. Paired samples of cancer (tumor) and background (non-tumor) were used for each case. Table 6

describes tumor grade and whether recurrence was present at median follow up of 16.5 months (Range between 8 & 33 months).

Tissue cell lysis. Frozen clinical tissue samples were pulverized then ground into a fine powder using a Pestle and Mortar in the presence of liquid nitrogen. The powder was then transferred to eppendorf tubes containing 1.3 mL of ice cold lysis buffer (8M urea, 75 mM NaCl, 50 mM Tris-pH 8.2, one tablet of protease inhibitors cocktail (complete mini, Roche) per 10 mL of lysis buffer, and one tablet of phosphatase inhibitor cocktail (Roche) per 10 mL of lysis buffer). Samples were then sonicated at 20% Amplitude for 20 x 1 second, pulsing on and off, on ice (4°C). Following centrifugation at 12,500g for 10 min at 4°C, the protein concentration of each sample were then determined using the Bradford protein assay and microplate luminometer. Protein amounts used for this workflow for each TMT 8-plex are shown in Table 7.

In-Solution Trypsin Digestion.

Reduction, alkylation of cysteines, and digestion was performed on lysates by following the Villén and Gygi, Nature Protocol, approach [Villén, J., Gygi S. The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry. Nature Protocols. **3**, 1630 (2008)]. The digested samples were spun for 10 minutes at 2,500g and de-salted on 100mg SepPak tC18 cartridges (Waters, Milford, MA, USA). Peptides were eluted with 50% ACN/0.1% TFA and lyophilised.

TMT Labelling. Digested peptides from all samples were separately re-suspended in 200mM TEAB/10%ACN, mixed with their respective TMT8plex reagent (15mM final concentration), as shown in labelling design below, and left to incubate for 1 hour at room temperature. The TMT reactions were then terminated with 0.25% hydroxylamine for 15 minutes. Samples were pooled into three TMT8plex (labelling design shown below) and left to incubate for another 15 minutes. Each TMT8plex sample were acidified and the acentonitrile concentration diluted to below 5%, then divided into three aliquots

each of which were desalted on a 200mg SepPak tC18 cartridge, eluted, then lyophilized. Labeling design shown in Table 8.

SCX-HPLC.

All 9 aliquots of lyophilized peptides (Table 9) were re-suspend in SCX buffer C, then separated into 12 fractions by SCX-HPLC. The fractionation was carried out using a polySULFOETHYL-A column (PolyLC) and our SCX HPLC system (Waters Alliance 2695) according to the Villén and Gygi, Nature Protocol26, approach.

Buffer A: 0,1% TFA in water.

Buffer C: 7 mM KH2PO4, pH 2.65, 30% ACN (vol/vol).

Buffer D: 7 mM KH2PO4, 350 mM KCl, pH 2.65, 30% ACN (vol/vol).

Immobilized Metal-Affinity Chromatography (IMAC) and TiO_2.

Phosphopeptides were enriched by IMAC (Thermo Scientific Pierce product code 88300) or TiO_2 (Thermo Scientific Pierce product code 88301), in accordance with manufacturer's instructions.

Graphite Spin Columns. Following phosphopeptide enrichment, peptides were purified using graphite spin columns (Thermo Scientific Pierce product code 88302), according to manufacturer's instructions.

Liquid Chromatography Mass spectrometry (LC-MS). Peptides from all 108 fractions were re-suspended in 35 µl of 2% ACN, 0.1% FA, then 8µL of each sample were injected onto a 0.1 × 20 mm pre-column self-packed with ReproSil C18, 5 µm (Dr. Maisch), using the Thermo Scientific Proxeon EASY-nLC II system. Peptides were then resolved using an increasing gradient of 0.1% formic acid in acetonitirile (10 to 25% over 90 minutes) through a 0.075 × 150 mm self-packed column with ReproSil C18, 3 µm (Dr. Maisch) at a flow rate of 300nL/min. Mass spectra were acquired on a Thermo Scientific LTQ Orbitrap Velos throughout the chromatographic run (115 minutes), using 10 higher collision induced dissociation (HCD) FTMS scans at 15000 resolving power @ 400 *m/z*, following each FTMS scan (2 x µScans at 30000 resolving power @ 400 *m/z*). HCD was carried out on 10 of the most intense ions from each FTMS scan then put on a dynamic exclusion list for 30secs (10 ppm *m/z* window). AGC ion injection target for each FTMS1 scan were 1000000 (500ms max

injection time). AGC ion injection target for each HCD FTMS2 scan were 50000 (500ms max ion injection time). Each sample were analysed by three LC-MSMS analytical repeats, where the third analytical repeat used a time dependent rejection list, rejecting all peptide ions that were identified as peptides, with 1%FDR, in one of the first two analytical repeats.

Peptide identification and quantification.

Proteome Discoverer

In total there were 324 Raw data files (3 x TMT8plex sample X3 aliquots X12 fractions X3 analytical repeats), where there were 108 raw data files belonging to each TMT8plex. All 108 raw data files from the first TMT8plex sample were combined for a Mudpit search using Proteome Discoverer, as described below. This was also carried out for the second and third TMT8plex samples.

Raw data were submitted to the Thermo Scientific Proteome Discoverer 1.3 software, using the Spectrum Files node. Spectrum selector was set to its default values, while the Mascot node was set up to search data against the uniprot_sprot database, taxonomy *homo sapiens*. This node was programmed to search for tryptic peptides (two missed cleavages) with static modifications of carbamidomethyl (C), TMT6plex (K), and TMT6plex (N-Term). Dynamic modifications were set to deamidation (N/Q), oxidation (M), and phosphorylation of STY. Precursor mass tolerance was set to 20ppm and fragment (b and y ions) mass tolerance to 20mmu. Spectra were also searched against SEQUEST, using the same database, modifications, and tolerances as the Mascot node. Spectra were also search using the PhosphoRS2.0 (fragment mass tolerance of 20mmu, considering neutral loss peaks for CID and HCD) and Percolator nodes.

The reporter ions quantifier node was set up to measure the raw intensity values of TMT8plex mono-isotopic ions, from all identified PSMs, at; 126.12773 *m/z* (126), 127.12476 *m/z* (127e), 127.13108 *m/z* (127), 128.13444 *m/z* (128), 129.13147 *m/z* (129e), 129.13779 *m/z* (129), 130.14115 *m/z* (130), 131.13818 *m/z* (131), using a tolerance of 20ppm after centroiding. No filters were applied at this stage

using Proteome Discoverer, therefore all raw intensity values were exported to excel for later processing and filtering using in house software.

Bioinformatics

Statistical analysis was performed to investigate relevant regulations with respect to the disease group of T (*pancreatic tumor tissue*) and NT (*non-tumor tissue*) from 12 patients.

Accuracy and precision of mass spectrometry quantification approaches can suffer from issues such as Experimental bias, Systematic errors, Random Errors (Heterogeneity of Variance), and missing quantification values. To improve accuracy and precision the inventors assessed the quality of their data, then filtered and normalised as described below.

MS quality - Data Filtering and normalisation:

All spectra which did not include all TMT-8 plex reporter intensities were deleted. For nomalisation a sum-scaling was performed. Due to differences between samples it is advisable to normalize data before further processing. The effects of the normalization can be observed by the follow maplots (http://en.wikipedia.org/wiki/MA_plot).

Statistics

As first step log2 ratios are calculated, which show the regulations T (*pancreatic tumor tissue*) over NT (*non-tumor tissue*) for all and for each patient. For protein ratios all peptides which are not phosphorylated were used and combined with the median.

The ratios were calculated:

$$\log_2(T/NT)$$

Where i = patient 1,4,5,6,7,8,9,10,11,12,13,14

For the data analysis a one sided t-test (or one-sample location test) will be used [http://en.wikipedia.org/wiki/T_test]. A one side

t-test is able to detect significant regulations in the subject of the question.

P-values and log2 ratios can be observed in the attached list of interest (Table 5). Significant p-values were highlighted in red. Annotation with GO-terms, KEGG-pathways and Drugbank info were added at the protein level, and annotation from phosphosite plus were added at the phosphorylation site level.

For the phosphopeptide ratios all peptides which have a probability in the phospho-RS utility in the Proteome Discoverer from over 75% in any phosphorylation position was used.

Results and Discussion

In total the inventors have identified 6,543 unique phosphopeptides (6,284 unique phosphorylation sites), from 2,101 protein groups (Table 1). Figure 1 shows identified peptide (phosphorylated and non-phosphorylated) distribution over all the three arms (Non-enriched, TiO_2, IMAC) of the SysQuant workflow for each TMT 8-plex. Figure 1 also illustrates the number of peptides detected for each of the three analytical repeats per sample. When results from each of the parallel components (TiO_2, IMAC, non-enriched) are compared the benefits of a combined approach are apparent. The largest total number of phospho-peptides was seen using IMAC enrichment which accounted for 79% of all unique phosphopeptides identified. However, the TiO_2 fractions uniquely identified nearly 19% of the total which would be missed using a single phospho-peptide enrichment strategy (Figure 1:TMT8plex-ALL:a). The same is true for the three analytical runs performed on each sample. If a single data dependent run was performed only 20,318 unique peptides are seen (Figure 1:TMT8plex-ALL:d). A second data-dependent run adds 5,868 peptides whilst the use of the time dependent rejection list in run 3 allowed a further 3257 peptides to be identified overall. Collectively (run 2&3) this represents an additional 45% over run 1 alone and 31% of the total number of unique peptides. Importantly the peptides identified in the third run are generally of lower abundance.

PLS/PCA

PLS demonstrated that there are no outliers in this dataset. PLS PC1 and PC2 show that there are three clusters IMAC, TiO_2 and TotalProtein (i.e. non-enriched arm of workflow), as shown in Figure 2A. PC1 and PC2 Score plot of the first two principal components describing 13.6% (PC1) and 10.6% (PC2) of the total variance in the data. The circle depicts the T2 hotelling space based on 95% confidence. All samples were in the border of the model. PC1 refers to the enrichment, PC2 refers to the patient. TotalProtein (non-enriched peptides) has a cluster which is different to the enrichment arms of the workflow, IMAC and TiO_2 (Figure 2A). PC2 and PC3 Score plot of the next principal components describing 10.6% (PC2) and 14.4% (PC3) of the total variance in the data (Figure 2B). In PC3 PLS can split T and NT in two clusters. TotalProtein (non-enriched peptides) has its own cluster, but it can also be separated into the classes T and NT. Only in patient 12 were no differences in T compared to NT observed. PLS/PCA confirm that the experiment is successful, and that there are significant differences between T and NT. Differences between TiO_2, IMAC and Totalprotein (non-enriched) exists, but TiO_2 and IMAC have a nearly equal correlation.

Hierarchal Cluster Analysis

Hierarchal cluster analysis was used to cluster cases which demonstrate similar profile in the relative abundance of these 5409 phosphopeptides in T relative to NT (Figure 3A-3C show particular regions of interest). Using all 5409 unique phospho-peptides the 12 patients could be clustered into three independent groups. One cluster contained cases 5, 9, 1, and 14, a second cluster contained cases 7, 6, 12, 4 and 13, while cases 8, 10, and 11 separated to a third cluster and were less closely related to each other than members of the other two clusters . Interestingly, when the clinical history of the 12 patients was un-blinded, the inventors found that cases 5, 9, 1, and 14 were patients that suffered tumor recurrence between 8 & 33 months (median follow-up period 16.5 months) after removal of the tumors analysed in this study, whereas cases 7, 6, 12, 4 and 13, were patients with no recurrence in this same time period. For more details on patient history refer to Tables 6 and 7.

Of the three outliers two were from patients with subsequent recurrence (Cases 10 and 11) and one was from a non-recurrent patient (Case 8). It is interesting that 2 out of the 3 outliers had less advanced stage IIA (pT3N0M0) compared to the recurrent (4/4 stage IIB, pT3N1M0) and non-recurrent (4/5 stage IIB, pT3N1M0) clusters. Further refining of the cluster analysis was performed by clustering on Pearson's correlation coefficients. The Pearson's correlation coefficients were obtained by comparing all phospho-peptide log2 T/NT values across all cases (Figure 3D). This refinement of cluster analysis better separates the recurrent and non-recurrent cases.

Hierarchal cluster analysis clearly separated patients into groups dependent on recurrence and no recurrence therefore the inventors were particularly interested in identifying those phosphopeptides whose abundance correlated positively and inversely with recurrence as these may prove useful prognostic markers and help forecast the likelihood of recurrence in new patients after analysis of their resected T & NT tissue. These phosphopeptides can be viewed in Table 11. Table 11 displays all phosphopeptides displaying high (log2 T/NT $\geq$0. 7) and low (log2 T/NT $\leq$ -0. 7) levels in tumor versus non-tumor from cases with recurrence that clustered together in Figure 3D. The combined list of phosphopeptides in Table 11 provides useful prognostic markers helping clinicians predict patients who will go on to present recurrence before 31 months after surgery.

In addition to the differences in global profiles between T and NT there are many individual phosphorylation site changes of particular interest. As an example, the relative abundance profile of the phosphopeptides containing phospho-T394 of Dual specificity mitogen-activated protein kinase kinase 2, as seen on Figure 2B (highlighted with a red arrow), and on Figure 4 correlate positively with patients who suffered tumor recurrence at median 16.5 months. They were substantially increased in T relative to NT in all cases showing recurrence, and down or only slightly increased in T relative to NT in all cases that did not show recurrence (Figure 4). This kinase is part of the RAS/RAF/MEK/ERK signaling pathway known

to be down stream of RAS and RAF, but upstream of ERK1/2. K-RAS gene is mutated to an oncogenic form in most pancreatic tumors, most commonly in the form of $K\text{-}RAS^{G12D}$ [12]. Unfortunately no K-RAS peptides were detected in this study. However, measurement of phospho-T394 on Dual specificity mitogen-activated protein kinase kinase 2, which is downstream of K-RAS, may prove to be an important prognostic marker assisting prediction of time of recurrence. The UniProtKB/Swiss-Prot database the inventors used to search peptides does not contain K-RAS point mutations, explaining the lack of detected K-RAS peptides in this study. This emphasises the need for a database containing known oncogenic point mutations. Other RAS signaling proteins were identified to show significantly modulated phosphopeptides as seen in the STRING map (see below).

The inventors also performed hierarchal cluster analysis to cluster cases which demonstrate similar profile in the relative abundance of protein in T relative to NT, however the correlation between clusters and recurrence/non-recurrence was less obvious, suggesting that total levels of protein expression change less dramatically than phosphorylation and signifying the importance of our phosphopeptide analysis as a prognostic tool.

Significantly regulated protein expression

The inventors determined the relative abundance of proteins in tumor compared to non-tumor tissue, using median $\log_2$ T/NT ratios of the non-phosphorylated peptides unique to each protein as surrogates to calculate the relative abundance of the respective proteins. A one sided t-test was used to calculate p-values and these were plotted against $\log_2$ T/NT ratios on a volcano plot to detect significant (Log_2 T/NT ≥ 0.3 or ≤ -0.3 and $p \leq 0.05$) regulations over all cases (Figure 5A). In total there were 150 proteins significantly regulated based on Log_2 T/NT ≥ 0.3 or ≤ -0.3 and $p \leq 0.05$ (Table 12). Table 2 displays the 12 most significantly upregulated proteins in tumor compared to non-tumor tissue, and also provides a description of any known function of each protein or association with cancer [13-31]. Overexpression of Mucin-1 is often associated with cancer and the inventor also found Mucin-1 to be significantly up-regulated

in pancreatic tumor tissue. Interestingly the inventors found more significant up-regulated proteins than Mucin-1, some of which may prove to be more specific markers of pancreatic cancer, perhaps even new therapeutic targets e.g. Homeodomain-interacting protein kinase 1.

The inventors selected all accession numbers of significantly modulated proteins and uploaded these to the DAVID Bio-informatic resource to identify those KEGG signalling pathways most significantly modulated. The Focal Adhesion KEGG signaling pathway was most significantly modulated giving a Benjamini score of 1.0E-3. Significantly modulated Focal Adhesion proteins included; Talin-1, Filamin-A, Filamin-C, Vinculin, Filamin B, Fibronectin, Focal adhesion kinase 1, Zyxin, Talin-2, Protein phosphatase 1 regulatory subunit 12A, and Myosin light chain kinase, smooth muscle (Table 12). In fixed or immobile cells, focal adhesions are quite stable under normal conditions, while less so in motile cells, where focal adhesions are constantly assembled and disassembled as the cell establishes new contacts at its leading edge, breaking old contacts at its trailing edge.

Hepatoma derived growth factor was also upregulated in most tumor specimens and this was significant based on p-value ($p \leq 0.05$).

Of particular interest to the inventors was the determination that Myosin light chain kinase (MLCK) is significantly overexpressed in tumor compared to non-tumor tissue (median log2 T/NT = 0.5 & p-value = 2.95E - 02). MLCK is a Ca2+/calmodulin-dependent protein kinase that regulates a variety of cellular functions, such as, muscle contraction and cell migration, via phosphorylation of myosin light chain proteins. Since tumor cell migration is a key step in tumor spread, myosin light chain kinase (MLCK) may be regarded as a therapeutic target for preventing tumor spread. In fact, MLCK activation and expression have been found to be positively related with metastatic propensity.

Significantly regulated phosphopeptides

Log_2 T/NT ratios of the phosphorylated peptides were used to calculate the relative level of phosphorylation at specific unique phosphorylation sites. The inventors used t-tests to calculate p-values and these were plotted against log_2 T/NT ratios on volcano plots for IMAC, TiO_2, and Non-enriched arms of the workflow, to detect significant (Log_2 T/NT ≥ 0.75 or ≤ -0.75 and $p \leq 0.05$) regulations over all cases, as shown in Figure 5B-5D. Of the 6,543 phosphopeptides identified in this study, 5409 were quantifiable (Data not shown). Of the quantifiable peptides, 635 showed significant regulation (Figure 5B-5D).

The inventors selected all 408 unique accession numbers of those proteins yielding phosphopeptides (635) with significant differential abundance between tumor compared to non-tumor tissue and uploaded the accession numbers to STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*). STRING matched these proteins to the Tight Junction KEGG Signaling pathway with greatest significance giving a p-value of 2.50E-5 after matching 14 of the 408 proteins to the pathway. The inventors also used STRING to identify which GO terms (Biological process, molecular function, and cellular component) these 408 proteins were most strongly associated to. Actin filament based process (n=29; p-value=4.47E-8), Actin binding (n=40; p-value=2.59E-18), and Cytoskeleton (n=77; p-value=2.66E-13) were the GO terms matched with greatest significance. The inventors also used STRING to identify which out of the 408 proteins were associated with the GO biological process 'Regulation of RAS protein signal transduction', as RAS is known to be an important onco-protein in pancreatic cancer. 16 of the 408 proteins were matched to this GO biological process with a p-value of 1.06E-2, while 10 of these 16 could be mapped to the STRING network (Figure 6C).

Phosphorylation of protein kinases

Of particular interest to the inventors was the observation that the phosphopeptides from Serine/Threonine-protein kinase MRCK alpha (see Table 11a) were significantly elevated in tumor compared to non-tumor. This was particulary so for those containing phosphorylation

site S1629. MRCK alpha is an important downstream effector of the Rho GTPase, CDC42, and plays a critical role in the regulation of cytoskeleton reorganization, formation of cell protrusion, and promotes cell migration. Further information can be found in Britton et al PLOS ONE March 2014; Vol. 9, Issue 3 e90948, the contents of which are hereby incorporated by reference in their entirety.

Accordingly, MRCK alpha is provided as an important therapeutic target for pancreatic cancer and kinase inhibitors of MRCK alpha as potential therapeutics.

Case by Case

In addition to determining which proteins and phosphopeptides demonstrated significant differences in abundance between tumor and non-tumor tissue when averaged across all cases, the inventors also wanted to determine which phosphopeptides were highly modulated on a case by case basis. Accession numbers of proteins which yielded phosphopeptides demonstrating $\log_2$ T/NT ratios of ≥ 1, or ≤ -1 (More than 2 fold up/down- regulated), were selected from case 1. These accession numbers were then uploaded to the DAVID Bioinformatic resource which identified KEGG signaling pathways most modulated for case 1. The inventors repeated this approach for each case, then selected KEGG signaling pathways that demonstrated significance, based on p values, and on Benjamini scores on a case by case basis (Table 13). All those KEGG pathways in Table 12 with Benjamini scores ≤ 0.05 were highlighted in Yellow. Based on p values from the DAVID Bioinformatic output, tight junction signaling pathway was determined to be modulated between tumor compared to non-tumor in all cases (12/12 cases), followed by adherens junction signaling (10/12 cases) and focal adhesion signaling (10/12). Figure 7, shows the three signaling pathways and the rectangles marked with red stars indicate those proteins the inventors identified as phosphorylated across all 12 cases. Table 3 displays all phosphopeptides displaying significant regulation that belong to proteins involved in Tight Junction and Focal Adhesion signaling pathways, as well as other signaling pathways (Regulation of Actin

Cytoskeleton and Vascular smooth muscle contraction) found to be significantly modulated.

Table 14 shows all phosphopeptides demonstrating $\log_2$ T/NT ratios of ≥ 1, or ≤ -1, from case 1, that belong to proteins involved in tight junction, adherens junction, and focal adhesion KEGG signaling pathways. These are also mapped to Figure 7B

Integrin beta-4 - The doubly phosphorylated peptide containing the Integrin beta-4 phosphorylation sites S1483 and S1486, was elevated more than two fold in the tumor tissue compared to non-tumor tissue of case 1. In fact this phosphopeptide was found to be significantly elevated in tumor tissue compared to non-tumor in general across all measured cases (data not shown). Integrin beta-4 phosphorylation has been associated with the disassembly of cell anchoring junctions, such as hemidesmosomes at the trailing edge of migrating cells [32, 33]. Such phosphorylation events have been shown to be induced by Fyn (primarily at Tyrosine residues), PKC (primarily at Serine residues), and other kinases [32].

Catenin alpha-1 - The peptide containing Catenin alpha-1 phosphorylation site S655 was elevated more than two fold in tumor tissue compared to non-tumor, in case 1. In fact, the singly phosphorylated peptide containing phospho-S655 was significantly elevated in tumor tissue on average across all cases (Data not shown). Phosphorylation at S641, S655, and S658, was elevated in tumor tissue of all but three cases, two of those three being stage IIA. Interestingly phosphorylation of catenin alpha-1 at S641 has been shown to lead to dissociation between catenin alpha-1 and catenin beta-1 (beta catenin), leading to increased transcriptional activation of beta-catenin and tumor cell invasion [34].

Junctional adhesion molecule A (JAM-A) - The peptide containing JAM-A phosphorylation site S284 was decreased more than two fold in tumor tissue compared to non-tumor, in case 1 and was found to be significantly decreased in tumor tissue compared to non-tumor across all cases (Data not shown). Phosphorylation of JAM-A at S284 is found to be a critical step in the formation and maturation of tight junctions [35]. Here the inventors observe that this phosphorylation

event is significantly decreased in tumor tissue an event that could favour epithelial to mesenchymal transition (EMT) of the cells and consequently metastatic spread.

Phosphorylation events to indicate activity status of drug targets and other enzymes

To ascertain relative activation status of enzymes in tumor compared to non-tumor tissue in each case, the inventors used relative abundance of phosphopeptides containing phosphorylation sites known to either induce enzyme activation or inhibition. Table 4 and Table 15 short lists all phosphopeptides displaying $\log_2$ T/NT ratios ≥1 or ≤ -1 that contain phosphorylation sites that are known to either induce activation or inhibition of the phosphorylated enzyme, in each case.

Tyrosine-protein kinase Fyn - The relative abundance of the peptide containing phospho-S21 of the Tyrosine-protein kinase Fyn is elevated more than two fold in tumor tissue compared to non-tumor tissue of case 1 (Table 4). Phosphorylation of Fyn at serine 21 is reported to activate Fyn kinase [36]. This suggests therefore, that Fyn is more active in the tumor tissue compared to non-tumor tissue of case 1. Interestingly, phospho-serine 21 of Fyn is detected in all 12 cases, but it is only in case 1 that the inventors observed such relatively high levels in tumor compared to non-tumor. Inversely, the tumor tissue of case 7 shows greater than two fold lower abundance of this phosphopeptide compared to non-tumor tissue. As Fyn is a target of the approved kinase inhibitor Dasatinib this new data suggests that measurement of the peptide containing phospho-S21 using the workflow methods described herein may be an attractive predictive marker for Dasatinib.

Mitogen-activated protein kinase 1 (MAPK1) - The relative abundance of the peptide containing phospho-T185 and phospho-Y187 of the MAPK1 is elevated more than two fold in tumor tissue compared to non-tumor tissue of cases 5, 8, and 10 (Table 4). Phosphorylation of MAPK1 at T185 and/or Y187 is reported to activate MAPK1 [3 7]. This suggests therefore, that MAPK1 is more active in the tumor tissue compared to non-tumor tissue of cases 5, 8, and 10. Inversely, the tumor tissue

of cases 4 and 11 shows more than two fold less of this phospho-T185 and phospho-Y187 containing phosphopeptide, compared to non-tumor tissue. MAPK1 is an anti-cancer drug target (AEZS-131 and SCH772984) and is also down-stream of many other anti-cancer drug targets (Anti-HER TKIs, Anti-MEK KIs), therefore this new data suggests that measurement of the peptide containing phospho-T185 and phospho-Y187 using our workflow may be a predictive marker for these targeted anti-cancer therapies. The inventors have also measured the singly phosphorylated peptides containing phospho-T185 or phospho-Y187, as well as the MAPK2 doubly and singly phosphorylated peptides containing phospho-T202 and phospho-Y204. The workflow methods described herein can easily determine whether MAPK2 is phosphorylated on T202 and/or Y204 and/or MAPK1 is phosphorylated on T185, and/or Y187, yielding critical signaling pathway activation status information.

RAC-alpha serine/threonine-protein kinase (AKT1) – The relative abundance of the singly phosphorylated peptides containing phospho-S124 and the doubly phosphorylated peptide containing phospho-S124 and phospho-S129 of AKT1 are elevated more than two fold in tumor tissue compared to non-tumor tissue of cases 4, 7, 10, and 13 (Table 4). Phosphorylation of AKT1 at S124 and/or S129 is reported to activate AKT1 [38, 39]. This suggests that AKT1 is more active in the tumor tissue compared to non-tumor tissue of cases 4, 7, 10, and 13. Therefore, anti-AKT kinase inhibitors may be effective in these patients. Interestingly, Case 10 also demonstrated elevated MAPK1 activity suggesting this patient may be a candidate for dual AKT1 & MAPK1 inhibitor treatment, as such combination strategies have proven efficacy in pancreatic cancer cell lines and xenograft models [12]. Inversely, the relative lower abundance of phosphopeptides containing these activator phosphorylation sites suggests AKT1 is less active in the tumor tissue compared to non-tumor tissue of cases 1, 6, 8, 9, 11, and 14. AKT1 is an anti-cancer drug target therefore, the inventor's data suggests that measurement of the peptides containing phospho-S124 and phospho-S129 using the workflow methods described herein may be an attractive predictive marker for these targeted anti-cancer therapies.

Glycogen synthase kinase-3 alpha - The peptide containing the Glycogen synthase kinase-3 alpha phosphorylation site Y279 increased more than two fold in the tumor tissue compared to non-tumor tissue of cases 1, 6, 13, and 14 (Table 15). Phosphorylation of Y279 causes activation of GSK3a which then induces cell survival, and reduces glycogen production [40]. GSK3a expression was measured in 8 out of 12 cases and shown to be significantly over expressed on average in tumor.

Using the approach where one measures the relative abundance of phosphopeptides containing activator or inhibitor phosphorylation sites, the inventors were able to determine the relative activation status of; Glycogen synthase kinase-3 alpha and beta, Histone deacetylase 1 and 2, RAF proto-oncogene serine/threonine-protein kinase, Serine/threonine-protein kinase A-Raf, Dual specificity mitogen-activated protein kinase kinase 6, Mitogen-activated protein kinase 14, and over 20 others (Table 4 and Table 15).

Notably, the most significantly enriched signalling pathways principally belong to cytoskeletal dynamics and cell adhesion, pathways that are usually deregulated during cell motility and metastatic spreading, highlighting the importance of these proteins in a highly metastatic disease such as pancreatic cancer and demonstrating the validity of the inventors' approach. Many other interesting molecular events, independent of the mentioned KEGG signaling pathways, were also observed in this experiment including the consistent and significant reduction in phosphorylation sites of the Microtubule-associated protein Tau, in all tumor tissue (data not shown), the inverse is known to cause pathology associated with Alzheimer's disease. Also, the activator phosphorylation site, S389 on Casein kinase I isoform epsilon, was significantly elevated on average in tumor tissue.

In conclusion, the inventors provide examples which demonstrate how their LC-MS workflow, can simultaneously measure the abundance and activity of 1000's of signaling and structural proteins in tumor tissue relative to non-tumor tissue, and show how such measurements can be used to better understand the molecular events leading to cancer, and therefore the most suitable inhibitory agents, to treat

a patient on a case by case basis. Critically, the inventors have demonstrated using hierarchal clustering of phosphopeptide $\log_2$ T/NT ratios that they can identify those patients more likely to show recurrence at a median follow up of 16.5 months compared to those patients less likely to show recurrence at this time point.

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Claims

What is claimed is

1. Use of a plurality of biomarkers selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15 for determining the molecular phenotype of a pancreatic tumor in a subject, wherein said molecular phenotype is selected from the group consisting of tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor; and wherein said plurality of biomarkers at least comprises a biomarker selected from the group consisting of Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

2. Use according to claim 1 wherein said plurality of biomarkers at least comprises two biomarkers selected from the group consisting of Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

3. Use according to claim 1 wherein said plurality of biomarkers at least comprises Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

4. A pancreatic tumour classification system comprising a pancreatic tumour classification apparatus and an information communication terminal apparatus, said pancreatic tumour classification apparatus including a control component and a memory component, said apparatuses being communicatively connected to each other via a network;

(1) wherein the information communication terminal apparatus includes

(1a) a protein data sending unit that transmits protein data derived from a pancreatic tumor sample of a subject to the pancreatic tumor classification apparatus;

(1b) a result-receiving unit that receives the result of the pancreatic tumour classification of the subject transmitted from the pancreatic tumor classification apparatus;

(2) wherein the pancreatic tumour classification apparatus includes

(2a) a protein data-receiving unit that receives protein data derived from the pancreatic tumor sample of the subject transmitted from the information communication terminal apparatus;

(2b) a data comparison unit which compares the data from the data-receiving unit with the data stored in the memory unit;

(2c) a determining unit that determines the molecular phenotype of the pancreatic tumor of the subject, based on the results of the data comparison unit; and

(2d) a classification result-sending unit that transmits the classification result of the subject obtained by the determining unit to the information communication terminal apparatus; and

wherein the memory unit contains protein expression level data and/or protein phosphorylation level data of a plurality of proteins selected from Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15.

5. A pancreatic tumor classification system according to claim 4, wherein the determining unit classifies the pancreatic tumor of the subject into molecular phenotypes including tumor, non-tumor; tumor recurrence, tumor non-recurrence; primary tumor, secondary (metastatic) tumor and/or drug susceptibility.

6. A pancreatic tumor classification system according to claim 3 or claim 5 wherein the protein expression level and/or protein phosphorylation level data of the memory unit is representative of multiple data sets derived from pancreatic tumour samples.

7. A pancreatic tumor classification system according to claim 6 wherein the multiple data sets include a value representing the protein expression level or protein phosphorylation level relative to the protein expression level or protein phosphorylation level of corresponding pancreatic non-tumor sample from the same subject.

8. A pancreatic tumor classification system according to any one of the preceding claims wherein the memory unit contains protein phosphorylation data of a plurality of proteins selected from Table 11A and 11B, and wherein the classification results in predicting tumor recurrence or tumor non-recurrence of the sample.

9. A pancreatic tumor classification system according to any one or claims 4 to 7 wherein the memory unit contains protein phosphorylation data of a plurality of proteins selected from Table 15 and/or Table 4, and wherein the classification results in predicting drug susceptibility of the pancreatic tumor.

10. A pancreatic tumor classification system according to any one of claims 4 to 7 wherein the memory unit contains protein expression levels of a plurality of proteins selected from Table 12 and/or Table 2, and wherein the classification results in predicting tumor or non-tumor phenotype of the sample.

11. A pancreatic tumor classification system according to any one of the preceding claims connected to an apparatus for determining the protein expression level or protein phosphorylation levels in a pancreatic tumor sample.

12. A pancreatic tumor classification system according to claim 11 wherein said apparatus can process multiple samples using liquid chromatography-mass spectrometry (LC-MS/MS).

13. A pancreatic tumor classification program that makes an information processing apparatus including a control component and a memory component execute a method of determining and/or classifying a pancreatic tumor of a subject, the method comprising

a comparing step of comparing data based on the protein expression levels and/or protein phosphorylation levels of a plurality of proteins selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15 obtained from a tissue sample of a subject suffering from or suspected to be suffering from pancreatic cancer, with the protein data stored in the memory component; and

a classifying step for classifying the pancreatic tumor of said subject, based on the comparison calculated at the comparing step;

wherein said tumor is classified into phenotypes including tumor, non-tumor; tumor recurrence, tumor non-recurrence; primary tumor, secondary (metastatic) tumor, and/or drug susceptibility.

14. A computer-readable recording medium, comprising the pancreatic tumor cellular classification program according to claim 13 recorded thereon.

15. A method of diagnosing pancreatic cancer in a subject comprising determining the modulation of a plurality of proteins selected from Table 12, Table 2, and/or Table 3 in a biological sample obtained from said subject, wherein

(a) the presence of said plurality of proteins in said sample is indicative of the subject having pancreatic cancer;
(b) the amount (concentration) of said plurality of proteins as compared to a reference amount for said plurality of proteins is indicative of the subject having pancreatic cancer;

(c) a change in amount (concentration) of said plurality of proteins as compared to a reference amount for said plurality of proteins is indicative of the subject having pancreatic cancer; or

(d) a change in phosphorylation status of said plurality of proteins as compared to a reference status for said plurality of proteins is indicative of the subject having pancreatic cancer.

16. A method according to claim 15 wherein the plurality of proteins comprises the proteins provided in Table 2 and/or Table 3.

17. A method according to claim 15 or claim 16 wherein the comparison with a reference amount or reference status is obtained using a pancreatic tumor classification system according to any one of claims 3 to 7.

18. A method for classifying a pancreatic tumor into a molecular phenotype, said method comprising

(1) determining expression levels and/or protein phosphorylation levels for a plurality of proteins in a biological sample obtained from said subject in order to produce a protein expression and/or protein phosphorylation profile of said sample;

(2) comparing said profile with reference protein expression and/or protein phosphorylation profile for said plurality of proteins, said reference profile being representative of pancreatic tumour phenotypes selected from tumor, non-tumor; tumor recurrence, tumor non-recurrence; drug susceptibility; primary and/or secondary tumor

(3) classifying the pancreatic tumour into a phenotype based on the comparison between the sample profile and the reference profile;

wherein the plurality of proteins are selected from a biomarker panel as represented by Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15.

19. A method according to claim 18 wherein the reference profile is obtained from the protein expression levels and/or protein phosphorylation levels of a non-tumor pancreatic sample obtained from the same subject.

20. A method according to claim 18 wherein the reference profile is derived from the protein expression levels and/or protein phosphorylation levels of previously obtained pancreatic tumor samples.

21. A method according to claim 20 wherein the step of comparing said profile with the reference profile is carried out using a pancreatic tumor classification system according to any one of claims 3 to 7.

22. A method according to any one of claims 18 to 21 wherein the pancreatic tumor classification is predicting tumor recurrence or tumor non-recurrence and the plurality of proteins is selected from Table 11; wherein tumor recurrence is predicted where a plurality of proteins selected from Table 11A show increase phosphorylation relative to normal and a plurality of proteins selected from Table 11B show decrease phosphorylation relative to normal.

23. A method according to claim 22 wherein said plurality of proteins includes Dual specificity mitogen-activated protein kinase 2.

24. A method according to any one of claims 18 to 21 wherein the pancreatic tumor classification is between tumor and non-tumor and the plurality of proteins is selected from Table 12 and/or Table 2.

25. A method according to claim 18 or claim 19 wherein the pancreatic tumor classification is drug susceptibility and the plurality of proteins is selected from Table 15 and/or Table 4.

26. A method according to claim 25 wherein the drug is selected from the group consisting of Dasatinib, Sorafenib, Vorinostat, Temsirolimus, AEZS-131 and GSK2141795.

27. A method of selecting a treatment regime for a subject suffering from pancreatic cancer, said method comprising

(1) obtaining protein expression levels and/or protein phosphorylation levels of a plurality of proteins in a pancreatic tumor of said subject so as to produce an expression level and/or protein phosphorylation profile of said tumor;

(2) comparing said profile with a reference profile, said reference profile being representative of pancreatic tumor phenotypes selected from tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor;

(3) classifying the pancreatic tumor of the subject into a phenotype based on the comparison between the tumor profile and the reference profile; and

(4) selecting a treatment regime according to phenotype of the pancreatic tumor of the subject;

wherein the plurality of proteins are selected from a biomarker panel as represented by Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15.

28. A method according to claim 27 wherein the plurality of proteins are selected from Table 15 and/or Table 4 and the treatment regime is selected on the determination of drug susceptibility phenotype characterised by the increase or decrease in phosphorylation levels of tyrosine-protein kinase Fyn, Mitogen-activated protein kinase 1 (MAPK1), Mitogen-activated protein kinase 3 (MAPK3); RAC-alpha serine/threonine-protein kinase (AKT1)and/or Glycogen synthase kinase-3 alpha.

29. A method for classifying a pancreatic tumor sample into one or more molecular phenotypes comprising

(1) determining the protein expression levels of one or more proteins selected from Table 12 and/or Table 2, for both a pancreatic tumor sample and a pancreatic non-tumor sample taken from a subject

and/or

(2) determining an increase or decrease in phosphorylation of one or more proteins selected from Table 3, Table 13 and/or Table 11A and/or Table 11B in a pancreatic tumor sample and a pancreatic non-tumor sample taken from a subject,

(3) comparing said protein expression levels of the tumor sample with the non-tumor sample; and/or comparing the increase or decrease in phosphorylation in the tumor sample with the non-tumor sample

(4) applying predictive algorithm

$$log_2(T/_iNT)$$

(where i is subject sample, T = tumour and NT = non-tumour)

to produce a prediction value that for said protein expression level and/or phosphorylation level for said subject;

(5) classifying said pancreatic tumor sample into a molecular phenotype by reference to a database comprising values predictive of said phenotypes;

wherein said database comprises predictive values for one or more of proteins selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15; and

wherein the molecular phenotype is selected from tumor, non-tumor; tumor recurrence, tumor non-recurrence; drug susceptibility; primary and/or secondary tumor.

30. A method according to claim 29 wherein the protein is considered to increase or decrease if the $\log_2$ T/NT ratio is ≥ 1 or ≤ -1.

31. A method according to claim 29 or claim 30 wherein the classification is carried out by a pancreatic tumor classification system according to any one of claims 3 to 7.

32. A method according to any one of the preceding claims wherein the step of determining protein expression levels or protein phosphorylation levels of the one or more, or plurality of proteins in said sample is performed by mass spectrometry.

33. A method according to any one of claims 1 to 31 wherein said step of determining protein expression levels or protein phosphorylation levels of the one or more or plurality of proteins is performed by Selected Reaction Monitoring using one or more transitions for protein derived peptides or phosphopetides; and comparing the peptide or phosphopeptide levels in the sample under test with peptide or phosphopeptide levels previously determined to represent a molecular phenotype.

34. A method according to claim 33 wherein comparing the peptide levels includes determining the amount of protein derived peptides from the pancreatic sample with known amounts of corresponding synthetic peptides, wherein the synthetic peptides are identical in sequence to the peptides obtained from the sample except for a label.

35. A method according to claim 34 wherein the label is a tag of a different mass or a heavy isotope.

36. Use of a plurality of biomarkers selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15 for determining the molecular phenotype of a pancreatic tumor in a subject, wherein said molecular phenotype is selected from the group consisting of tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor.

37. Use according to claim 36 wherein the biomarkers are selected from Table 2 and/or Table 12 and the phenotype is selected from tumor or non-tumor.

38. Use according to claim 37 wherein the biomarkers comprise Mucin-1, Intergrin beta 4, and/or Homeodomain-interacting protein kinase 1.

39. Use according to claim 36 wherein the biomarkers are selected from Table 3, 11A, 11B and/or Table 13 and the phenotype is selected from tumor recurrence or tumor non-recurrence.

40. Use according to claim 39 wherein the biomarker comprises dual specificity mitogen-activated protein kinase kinase 2.

41. Use according to claim 36 wherein the biomarkers are selected from Table 4 and/or 15 and the phenotype is selected from drug susceptibility.

42. Use according to claim 41 wherein the biomarkers comprise and one or more of Tyrosine-protein kinase Fyn, Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, Intergrin beta 4, Catenin alpha-1, Junctional adhesion molecule A (JAM-A); Mitogen-activated protein kinase 1 (MAPK1); Glycogen synthase kinase-3 alpha; and/or RAC-alpha serine/threonine-protein kinase (AKT1).

43. A method of classifying a pancreatic tissue sample into phenotype selected from tumor, non-tumor, recurrence, non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor by determining the amount of one or more, or plurality of marker proteins comprising:

contacting said sample with a specific binding member(s) that selectively and independently binds to the one or more proteins; and

detecting and/or quantifying a complex formed by said specific binding member(s) and the one or more proteins;

classifying the pancreatic tissue sample based on the detection or quantity of said complex;

wherein said one or more proteins are selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15.

44. A method according to claim 43 wherein the specific binding member(s) is an antibody or antibody fragment specific for the protein marker.

45. A method according to claim 43 wherein the specific binding member is an aptamer.

46. A method according to any one of claims 43 to 45 wherein the binding member is immobilised on a solid support.

47. A solid support comprising a plurality of binding members each capable of specifically and selectively binding to one of said plurality of proteins or nucleic acid sequences encoding said proteins; wherein said proteins are selected from Table 2, 3, 4, 11A, 11B, 12, 13 and/or 15.

48. A synthetic peptide or a plurality of synthetic peptides each having a sequence identical to a fragment of one of a plurality of marker proteins selected from Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15, said fragment resulting from digestion of the protein by trypsin, ArgC, AspN or Lys-C digestion.

49. A synthetic peptide or a plurality of synthetic peptides according to claim 48 wherein said plurality of marker proteins includes at least one marker protein selected from the group consisting of Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

50. A synthetic peptide according to claim 48 or claim 49 further comprising a label.

51. A synthetic peptide according to claim 50 wherein the label is a heavy isotope.

52. A synthetic peptide according to claim 50 or claim 51 for use in Selective Reaction Monitoring.

53. A kit for use in classifying a pancreatic tissue sample into a molecular phenotype selected from tumor, non-tumor, tumor recurrence, tumor non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor, said kit allowing the user to determine the up- or down-regulation of one or more analytes selected from proteins of Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15, one or more antibodies against said proteins and one or more nucleic acid molecules encoding said proteins or fragments thereof, in a sample under test; the kit comprising

(a) a solid support having a plurality of binding members, each capable of binding to one of the analytes immobilised thereon;

(b) a developing agent comprising a label; and, optionally

(c) one or more components selected from the group consisting of washing solutions, diluents and buffers.

54 A kit according to claim 53 wherein said proteins of Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15 comprise at least one protein selected from the group consisting of Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

55. A kit for classifying a pancreatic tissue sample into a molecular phenotype selected from tumor, non-tumor, tumor recurrence, tumor non-recurrence, drug susceptibility, primary tumour and/or secondary (metastatic) tumor, said kit allowing the user to determine an increase or decrease in expression levels and/or phosphorylation levels of a plurality of marker proteins

selected from Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15, in a sample under test; the kit comprising

(a) a set of reference peptides and/or reference phosphopeptides in an assay compatible format wherein each peptide and/or phosphopeptide in the set is uniquely representative of one of the plurality of marker proteins provided in any one of Tables 2, 3, 4, 11A, 11B, 12, 13 and/or 15; and,

optionally

(b) one or more components selected from the group consisting of washing solutions, diluents and buffers.

56. A kit according to claim 55 where the level of protein phosphorylation is determined using reference phosphopeptides representing differentially phosphorylated sites within the marker proteins set out in Table 3, 4, 11A, 11B, 13 and/or 15.

57. A kit according to claim 56 wherein the reference phosphopeptides are unique to one or more of the group consisting of Tyrosine-protein kinase Fyn, Tyrosine-protein kinase CSK (Src), RAF proto-oncogene serine/threonine-protein kinase, Histone deacetylase 1, Histone deacetylase 2, Rapamucin-insensitive companion of mTOR (RICTOR); ERK1 mitogen-activated protein kinase, ERK2 mitogen-activated protein kinase, Intergrin beta 4, Catenin alpha-1, Junctional adhesion molecule A (JAM-A); Mitogen-activated protein kinase 1 (MAPK1); Glycogen synthase kinase-3 alpha; Dual specificity mitogen-activated protein kinase kinase 2; and/or RAC-alpha serine/threonine-protein kinase (AKT1).

58. A method for predicting the likelihood of recurrence of a pancreatic tumor in a subject after treatment comprising detecting the level of phosphorylation at phospho T-394 of Dual specificity mitogen-activated protein kinase kinase 2 in a tumor sample of said subject, where elevated levels of phosphorylation at T-394 compared to background (non-tumor) levels is indicative of the likelihood of tumor recurrence.

59. A method according to claim 58 where recurrence is between 2 and 33 months post-surgery.

60. A method according to claim 58 or claim 59 wherein the level of phosphorylation at phospho-T394 of Dual specificity mitogen-activated protein kinase kinase 2 is determined using immunohistochemistry.

61. A method of predicting the likelihood of recurrence of a pancreatic tumor in a subject after treatment, said method comprising detecting the level of phosphorylation of at least one protein selected from the group consisting of Homeodomain-interacting protein kinase I (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and myosine light chain kinase, smooth muscle (MLCK) in a tumour sample obtained from said subject, wherein elevated levels of phosphorylation compared to background (non-tumor) levels is indicative of the likelihood of tumor recurrence.

62. A method of predicting susceptibility of a pancreatic tumor to treatment with Dasatinib (BMS-354825 - Sprycel™) comprising determining the level of phospho-S21 on Tyrosine-protein kinase Fyn, wherein an up-regulation of this protein is indicative that the pancreatic tumor will be susceptible to treatment with Dasatinib.

63. A method of predicting susceptibility of a pancreatic tumor to treatment with AEZS-131 (Aeterna Zentaris Inc) and/or SCH772984 (Merck) comprising determining the level of phospho-T185 and/or Y187 on Mitogen-activated protein kinase 1 (MAPK1), wherein an up-regulation of this protein is indicative that the pancreatic tumor will be susceptible to treatment with AEZS-131 and/or SCH772984.

64. A method according to claim 62 or claim 63 wherein the step of determining the level of phosphorylation is by immunohistochemistry.

65. A method of diagnosing pancreatic tumor in a subject comprising

(a) determining the level of expression of MLCK;

(b) determining the level of phosphorylation of MRCK alpha; or

(c) determining the level of phosphorylation of HIPK1; wherein an increase in the level of expression of MLCK compared to normal tissue is indicative of the subject having pancreatic tumor, and wherein an increase in phosphorylation of MRCK alpha and HIPK1 compared the level of phosphorylation in normal tissue is indicative of the subject having pancreatic tumor.

66. A method of diagnosing pancreatic tumor in a subject comprising determining the level of phosphorylation at phospho-S655 of Catenin alpha-1 in a biological sample obtained from said subject, where an increase in phosphorylation level is indicative of the subject having pancreatic tumor.

67. A method according to claim 66 further determining the level of phospho-S641, S655 and/or S658 of Catenin alpha-1.

68. A method of diagnosing pancreatic tumor in a subject comprising determining the level of phosphorylation at phospho-S1483 of Integrin beta-4 in a biological sample obtained from said subject, where an increase in phosphorylation level is indicative of the subject having pancreatic tumor.

69. A method according to claim 68 further determining the level of phospho-S1486 of Integrin beta-4.

70. A method of diagnosing pancreatic tumor in a subject comprising determining the level of phosphorylation at phospho-S284 of Junctional adhesion molecule A (JAM-A) in a biological sample obtained from said subject, where a decrease in phosphorylation level is indicative of the subject having pancreatic tumor.

71. A method according to any one of claims 65 to 70 wherein the level of phosphorylation is determined using immunohistochemistry.

72. A kit for performing a method according to any one of claims 65 to 71 comprising an antibody or antibody fragment capable of specifically binding to the phosphorylated site on said protein.

73. A kit according to claim 72 wherein the antibody or antibody fragment is labelled to aid in detection and quantification.

74. A method of treating a subject having pancreatic cancer; said method comprising administration of a kinase inhibitor capable of inhibiting the activity of protein kinase selected from the group consisting of Homeodomain-interacting protein kinase 1 (HIPK1); Serine/threonine-protein kinase MRCK alpha (MRCK alpha); and Myosin light chain kinase, smooth muscle (MLCK).

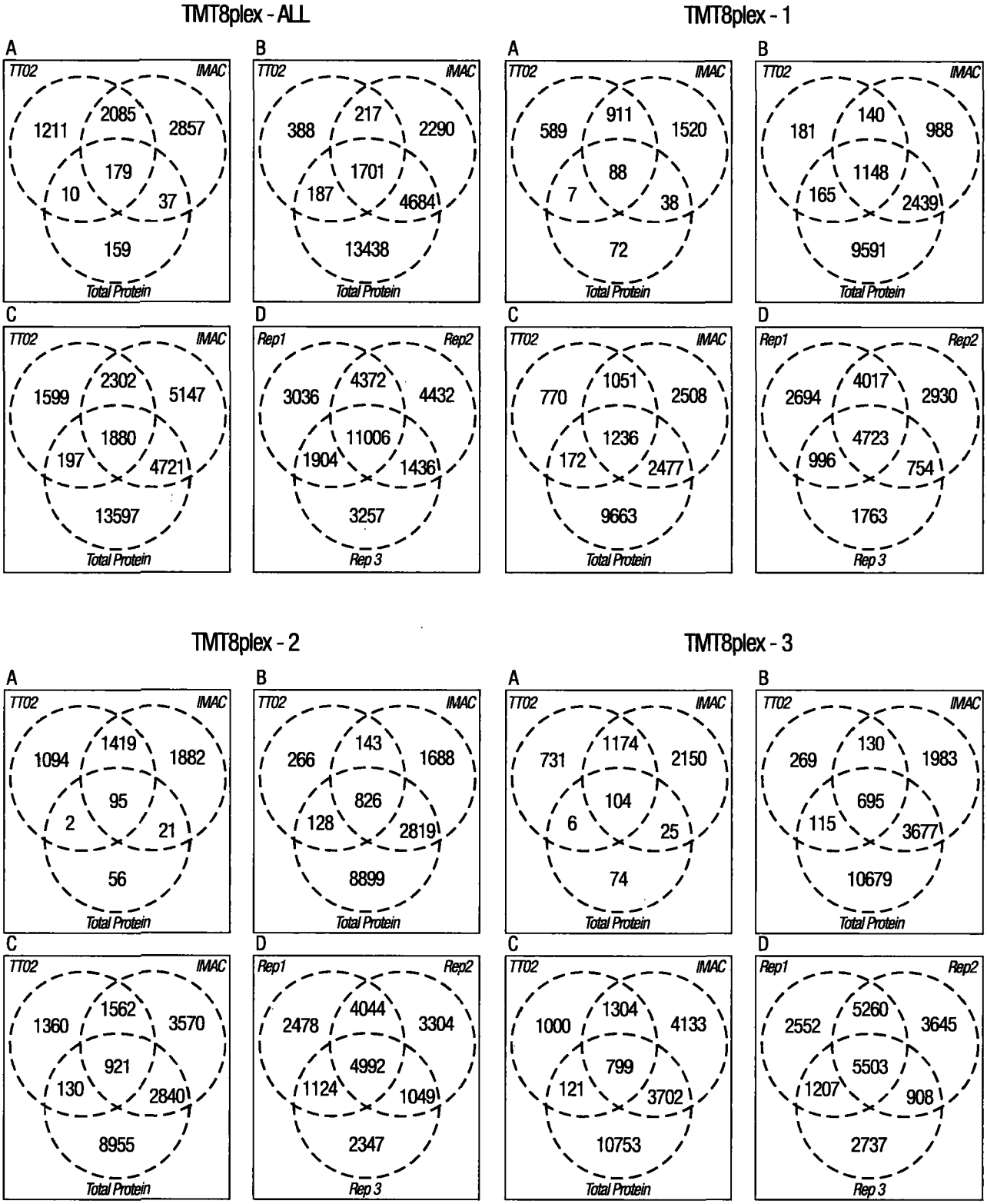
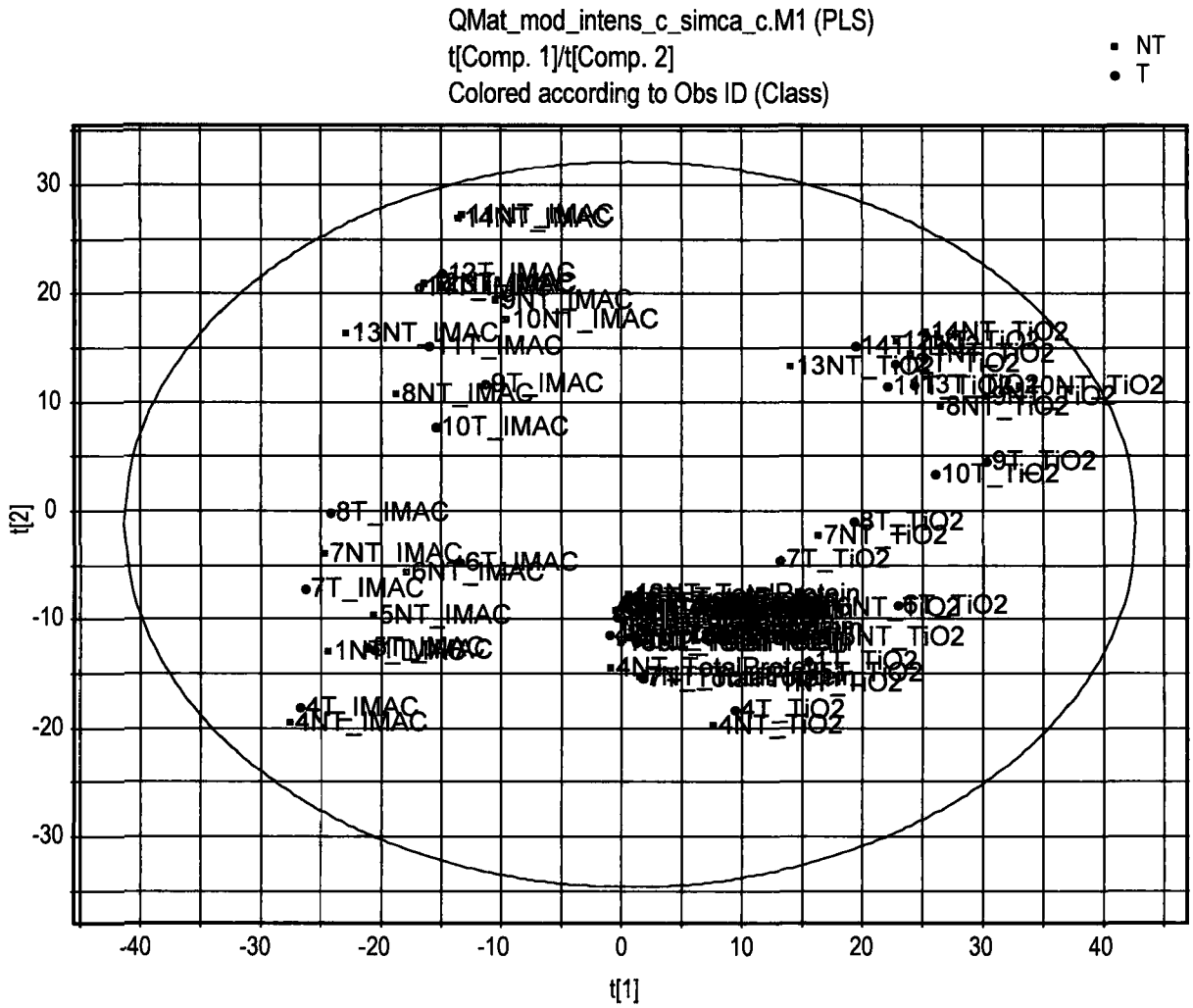

TMT8plex - ALL
A
TT02
IMAC
1211
2085
2857
179
10
37
159
Total Protein
B
TT02
IMAC
388
217
2290
1701
187
4684
13438
Total Protein
C
TT02
IMAC
1599
2302
5147
1880
197
4721
13597
Total Protein
D
Rep1
Rep2
3036
4372
4432
11006
1904
1436
3257
Rep 3
TMT8plex - 1
A
TT02
IMAC
589
911
1520
88
7
38
72
Total Protein
B
TT02
IMAC
181
140
988
1148
165
2439
9591
Total Protein
C
TT02
IMAC
770
1051
2508
1236
172
2477
9663
Total Protein
D
Rep1
Rep2
2694
4017
2930
4723
996
754
1763
Rep 3
TMT8plex - 2
A
TT02
IMAC
1094
1419
1882
95
2
21
56
Total Protein
B
TT02
IMAC
266
143
1688
826
128
2819
8899
Total Protein
C
TT02
IMAC
1360
1562
3570
921
130
2840
8955
Total Protein
D
Rep1
Rep2
2478
4044
3304
4992
1124
1049
2347
Rep 3
TMT8plex - 3
A
TT02
IMAC
731
1174
2150
104
6
25
74
Total Protein
B
TT02
IMAC
269
130
1983
695
115
3677
10679
Total Protein
C
TT02
IMAC
1000
1304
4133
799
121
3702
10753
Total Protein
D
Rep1
Rep2
2552
5260
3645
5503
1207
908
2737
Rep 3

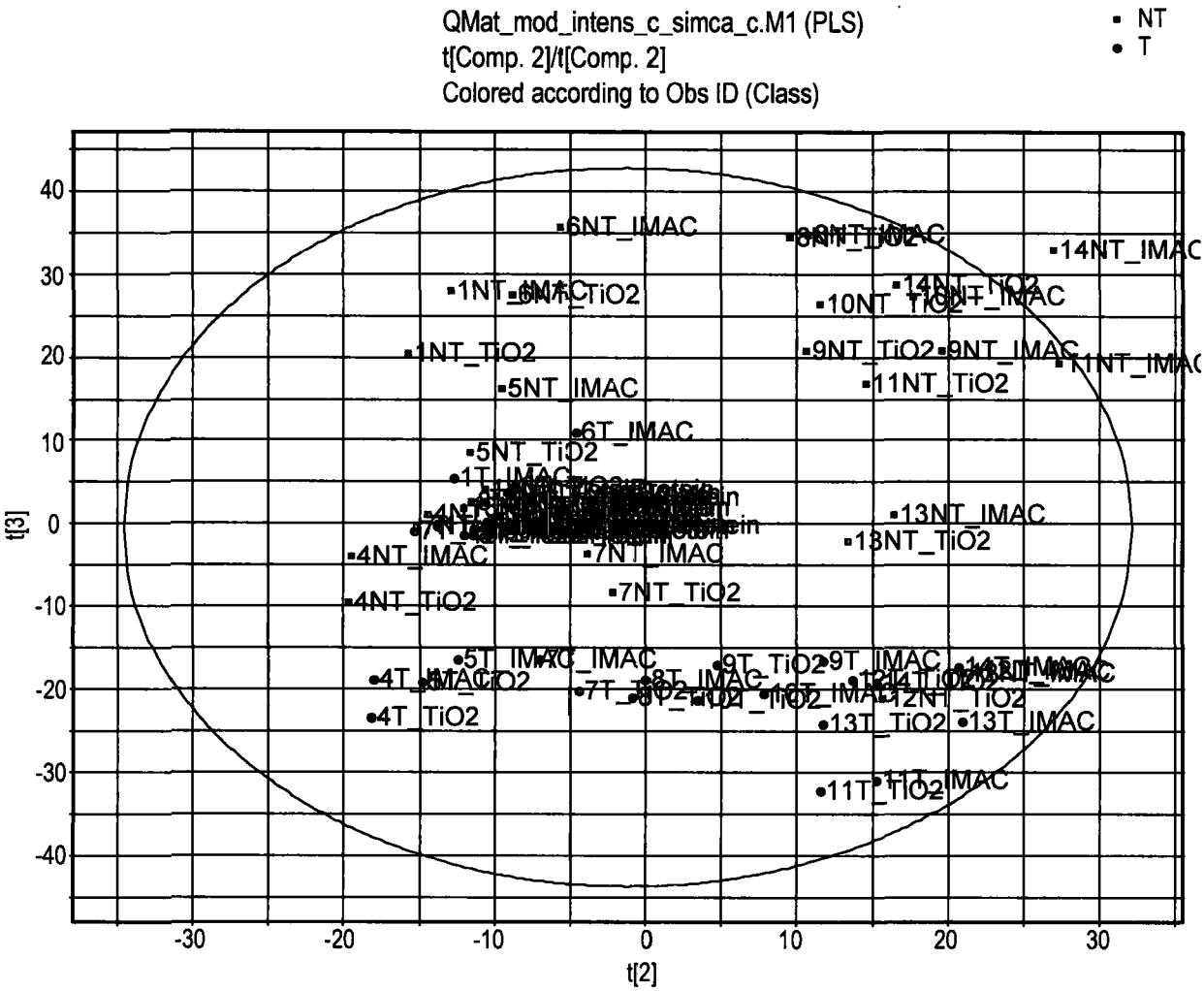
FIG. 1



R2X[1] = 0,136035 R2X[2] = 0,105612

Ellipse: Hotelling T2 (0,95)

FIG. 2a



R2X[2] = 0,105612 R2X[3] = 0,143677

Ellipse: Hotelling T2 (0,95)

FIG. 2b

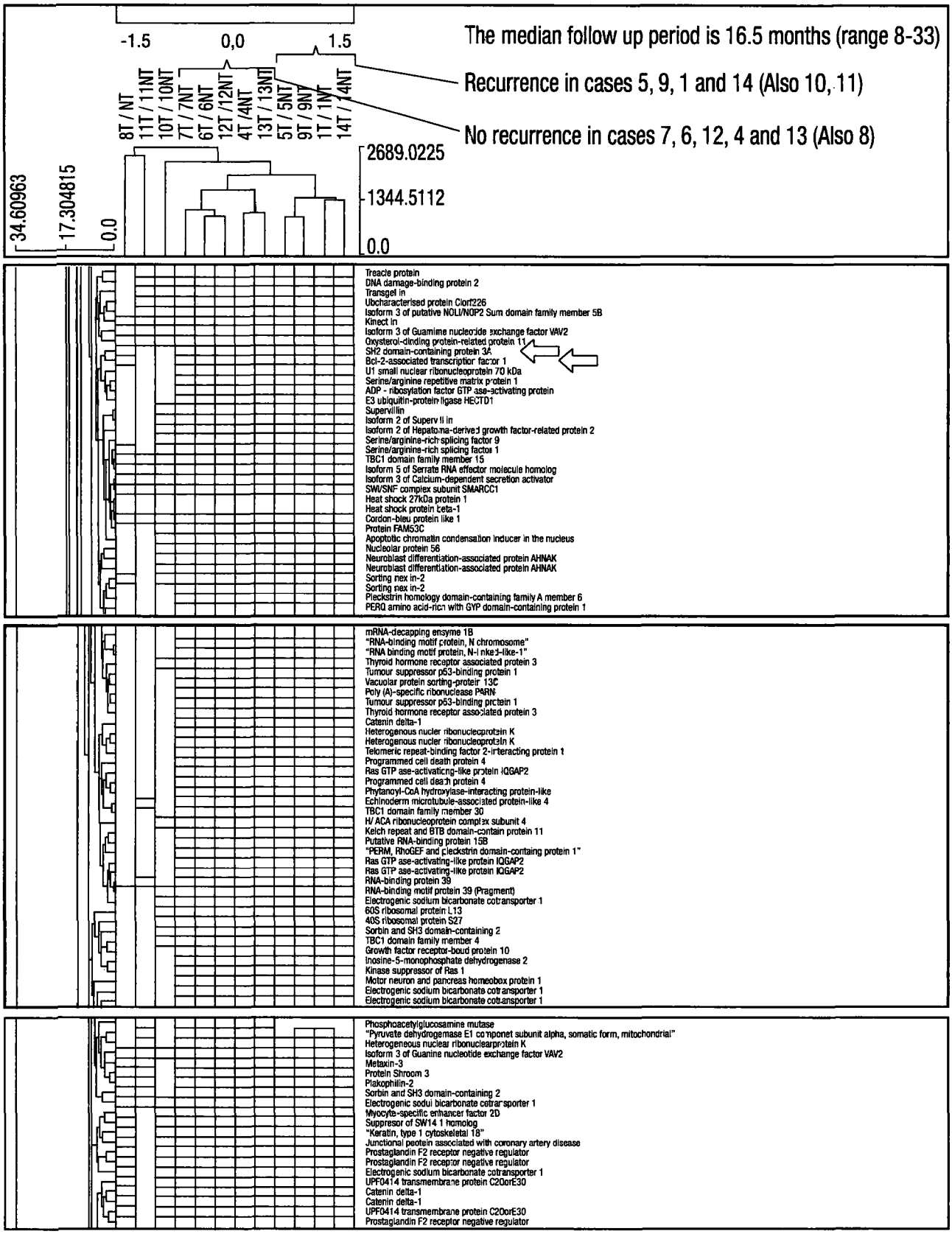

-1.5
0,0
1.5
The median follow up period is 16.5 months (range 8-33)
Recurrence in cases 5, 9, 1 and 14 (Also 10, 11)
No recurrence in cases 7, 6, 12, 4 and 13 (Also 8)
8T / NT
11T / 11NT
10T / 10NT
7T / 7NT
6T / 6NT
12T /12NT
4T /4NT
13T / 13NT
5T / 5NT
9T / 9NT
1T / 1NT
14T / 14NT
34.60963
17.304815
0.0
2689.0225
1344.5112
0.0
Treacle protein
DNA damage-binding protein 2
Transgelin
Uncharacterised protein C1orf226
Isoform 3 of putative NOL1/NOP2 Sun domain family member 5B
Kinectin
Isoform 3 of Guanine nucleotide exchange factor VAV2
Oxysterol-binding protein-related protein 11
SH2 domain-containing protein 3A
Bcl-2-associated transcription factor 1
U1 small nuclear ribonucleoprotein 70 kDa
Serine/arginine repetitive matrix protein 1
ADP - ribosylation factor GTP ase-activating protein
E3 ubiquitin-protein ligase HECTD1
Supervillin
Isoform 2 of Supervillin
Isoform 2 of Hepatoma-derived growth factor-related protein 2
Serine/arginine-rich splicing factor 9
Serine/arginine-rich splicing factor 1
TBC1 domain family member 15
Isoform 5 of Serrate RNA effector molecule homolog
Isoform 3 of Calcium-dependent secretion activator
SWI/SNF complex subunit SMARCC1
Heat shock 27kDa protein 1
Heat shock protein beta-1
Cordon-bleu protein like 1
Protein FAM53C
Apoptotic chromatin condensation inducer in the nucleus
Nucleolar protein 56
Neuroblast differentiation-associated protein AHNAK
Neuroblast differentiation-associated protein AHNAK
Sorting nexin-2
Sorting nexin-2
Pleckstrin homology domain-containing family A member 6
PERQ amino acid-rich with GYP domain-containing protein 1
mRNA-decapping enzyme 1B
"RNA-binding motif protein, N chromosome"
"RNA binding motif protein, N-linked-like-1"
Thyroid hormone receptor associated protein 3
Tumour suppressor p53-binding protein 1
Vacuolar protein sorting-protein 13C
Poly (A)-specific ribonuclease PARN
Tumour suppressor p53-binding protein 1
Thyroid hormone receptor associated protein 3
Catenin delta-1
Heterogenous nuclear ribonucleoprotein K
Heterogenous nuclear ribonucleoprotein K
Telomeric repeat-binding factor 2-interacting protein 1
Programmed cell death protein 4
Ras GTP ase-activating-like protein IQGAP2
Programmed cell death protein 4
Phytanoyl-CoA hydroxylase-interacting protein-like
Echinoderm microtubule-associated protein-like 4
TBC1 domain family member 30
H/ ACA ribonucleoprotein complex subunit 4
Kelch repeat and BTB domain-contain protein 11
Putative RNA-binding protein 15B
"PERM, RhoGEF and pleckstrin domain-containg protein 1"
Ras GTP ase-activating-like protein IQGAP2
Ras GTP ase-activating-like protein IQGAP2
RNA-binding protein 39
RNA-binding motif protein 39 (Fragment)
Electrogenic sodium bicarbonate cotransporter 1
60S ribosomal protein L13
40S ribosomal protein S27
Sorbin and SH3 domain-containing 2
TBC1 domain family member 4
Growth factor receptor-boud protein 10
Inosine-5-monophosphate dehydrogenase 2
Kinase suppressor of Ras 1
Motor neuron and pancreas homeobox protein 1
Electrogenic sodium bicarbonate cotransporter 1
Electrogenic sodium bicarbonate cotransporter 1
Phosphoacetylglucosamine mutase
"Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial"
Heterogeneous nuclear ribonucleoprotein K
Isoform 3 of Guanine nucleotide exchange factor VAV2
Metaxin-3
Protein Shroom 3
Plakophilin-2
Sorbin and SH3 domain-containing 2
Electrogenic sodium bicarbonate cotransporter 1
Myocyte-specific enhancer factor 2D
Suppresor of SW14 1 homolog
"Keratin, type 1 cytoskeletal 18"
Junctional peotein associated with coronary artery disease
Prostaglandin F2 receptor negative regulator
Prostaglandin F2 receptor negative regulator
Electrogenic sodium bicarbonate cotransporter 1
UPF0414 transmembrane protein C20orf30
Catenin delta-1
Catenin delta-1
UPF0414 transmembrane protein C20orf30
Prostaglandin F2 receptor negative regulator

FIG. 3a

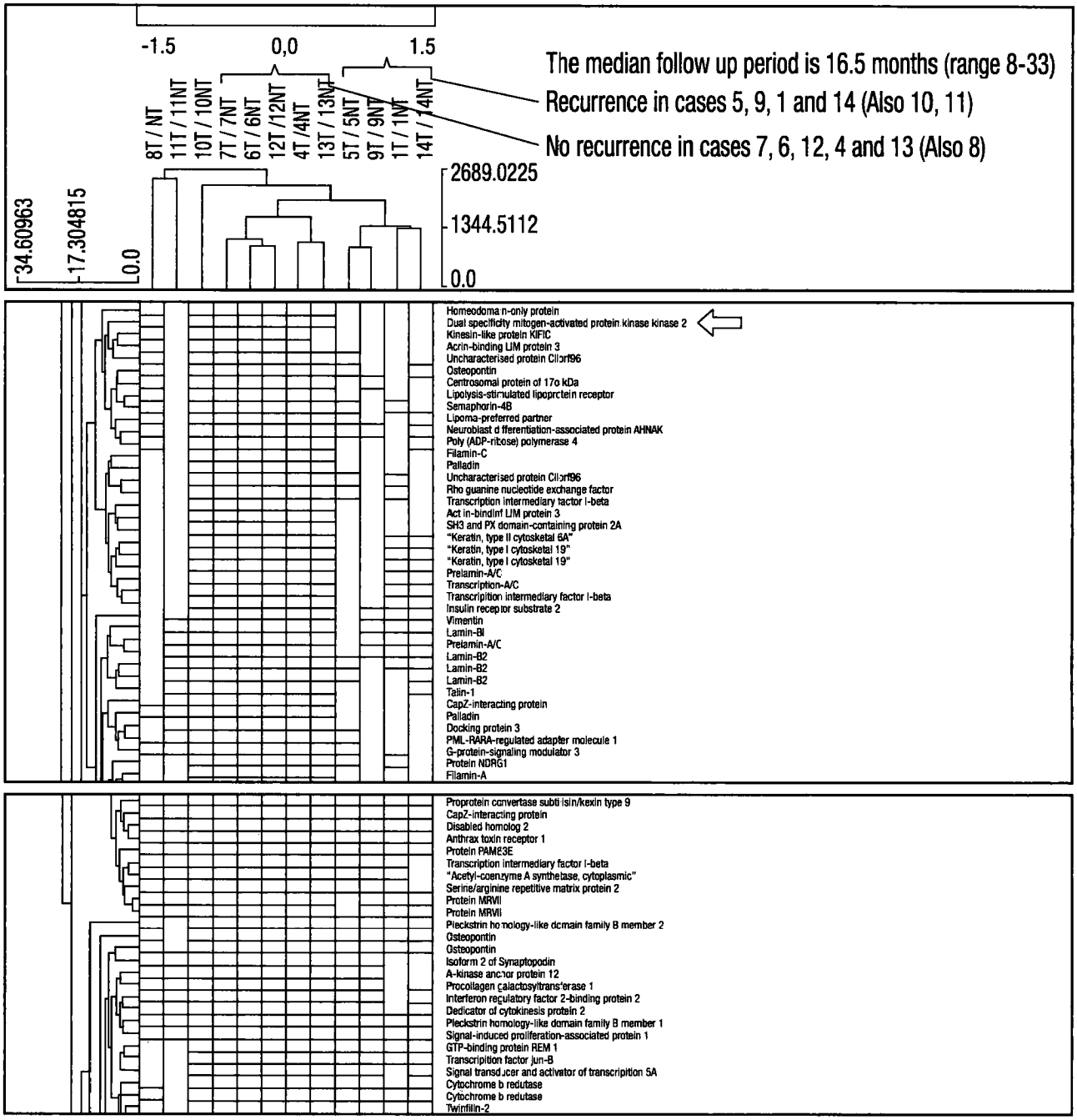
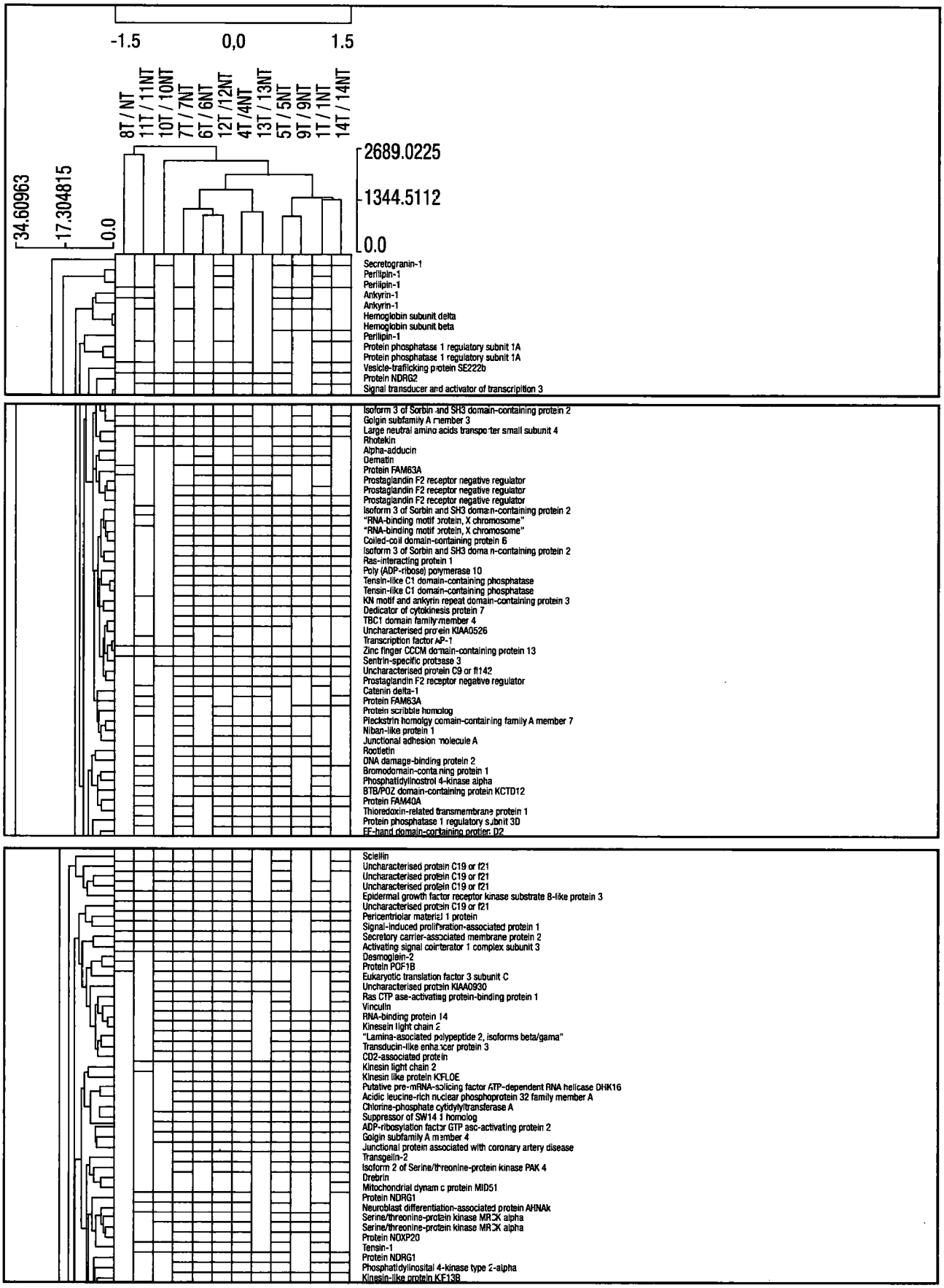

-1.5
0,0
1.5
8T / NT
11T / 11NT
10T / 10NT
7T / 7NT
6T / 6NT
12T /12NT
4T /4NT
13T / 13NT
5T / 5NT
9T / 9NT
1T / 1NT
14T / 14NT
34.60963
17.304815
0.0
2689.0225
1344.5112
0.0
The median follow up period is 16.5 months (range 8-33)
Recurrence in cases 5, 9, 1 and 14 (Also 10, 11)
No recurrence in cases 7, 6, 12, 4 and 13 (Also 8)
Homeodomain-only protein
Dual specificity mitogen-activated protein kinase kinase 2
Kinesin-like protein KIFIC
Acrin-binding LIM protein 3
Uncharacterised protein C1orf96
Osteopontin
Centrosomal protein of 170 kDa
Lipolysis-stimulated lipoprotein receptor
Semaphorin-4B
Lipoma-preferred partner
Neuroblast differentiation-associated protein AHNAK
Poly (ADP-ribose) polymerase 4
Filamin-C
Palladin
Uncharacterised protein C1orf96
Rho guanine nucleotide exchange factor
Transcription intermediary factor 1-beta
Actinin-binding LIM protein 3
SH3 and PX domain-containing protein 2A
"Keratin, type II cytoskeletal 6A"
"Keratin, type I cytoskeletal 19"
"Keratin, type I cytoskeletal 19"
Prelamin-A/C
Transcription-A/C
Transcription intermediary factor 1-beta
Insulin receptor substrate 2
Vimentin
Lamin-B1
Prelamin-A/C
Lamin-B2
Lamin-B2
Lamin-B2
Talin-1
CapZ-interacting protein
Palladin
Docking protein 3
PML-RARA-regulated adapter molecule 1
G-protein-signaling modulator 3
Protein NDRG1
Filamin-A
Proprotein convertase subtilisin/kexin type 9
CapZ-interacting protein
Disabled homolog 2
Anthrax toxin receptor 1
Protein PAM83E
Transcription intermediary factor 1-beta
"Acetyl-coenzyme A synthetase, cytoplasmic"
Serine/arginine repetitive matrix protein 2
Protein MRVI1
Protein MRVI1
Pleckstrin homology-like domain family B member 2
Osteopontin
Osteopontin
Isoform 2 of Synaptopodin
A-kinase anchor protein 12
Procollagen galactosyltransferase 1
Interferon regulatory factor 2-binding protein 2
Dedicator of cytokinesis protein 2
Pleckstrin homology-like domain family B member 1
Signal-induced proliferation-associated protein 1
GTP-binding protein REM 1
Transcription factor jun-B
Signal transducer and activator of transcription 5A
Cytochrome b reductase
Cytochrome b reductase
Twinfilin-2

FIG. 3b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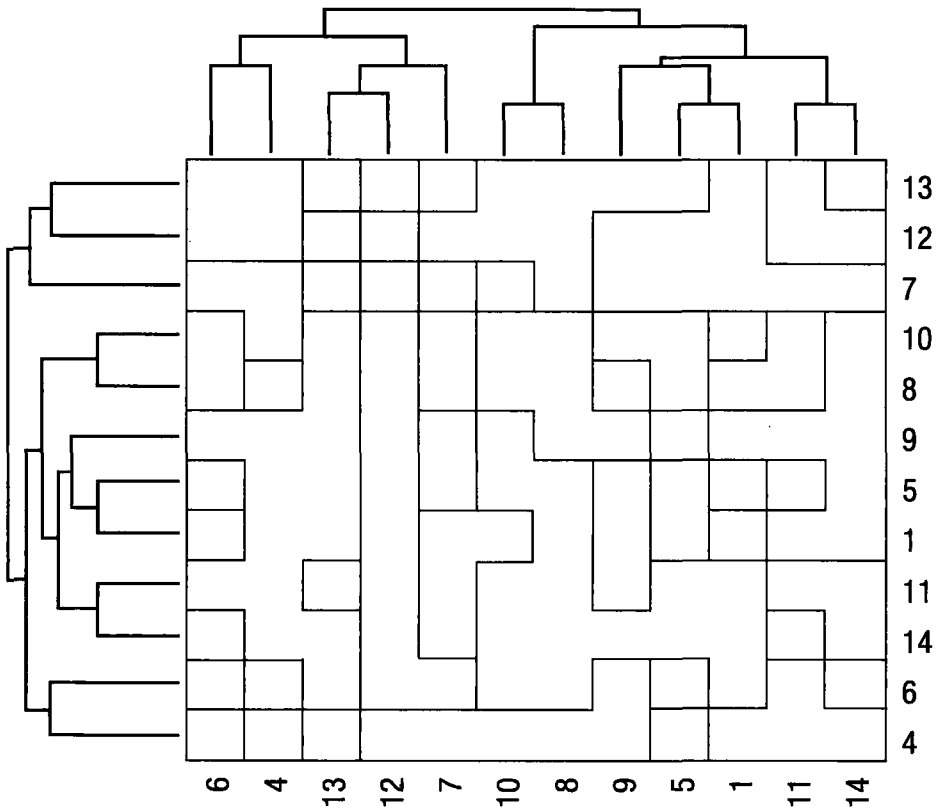
8T / NT
11T / 11NT
10T / 10NT
7T / 7NT
6T / 6NT
12T /12NT
4T /4NT
13T / 13NT
5T / 5NT
9T / 9NT
1T / 1NT
14T / 14NT
34.60963
17.304815
0.0
2689.0225
1344.5112
0.0
Secretogranin-1
Perilipin-1
Perilipin-1
Ankyrin-1
Ankyrin-1
Hemoglobin subunit delta
Hemoglobin subunit beta
Perilipin-1
Protein phosphatase 1 regulatory subnit 1A
Protein phosphatase 1 regulatory subnit 1A
Vesicle-trafficking protein SE222b
Protein NDRG2
Signal transducer and activator of transcription 3
Isoform 3 of Sorbin and SH3 domain-containing protein 2
Golgin subfamily A member 3
Large neutral amino acids transporter small subunit 4
Rhotekin
Alpha-adducin
Dematin
Protein FAM63A
Prostaglandin F2 receptor negative regulator
Prostaglandin F2 receptor negative regulator
Prostaglandin F2 receptor negative regulator
Isoform 3 of Sorbin and SH3 domain-containing protein 2
"RNA-binding motif protein, X chromosome"
"RNA-binding motif protein, X chromosome"
Coiled-coil domain-containing protein 6
Isoform 3 of Sorbin and SH3 domain-containing protein 2
Ras-interacting protein 1
Poly (ADP-ribose) polymerase 10
Tensin-like C1 domain-containing phosphatase
Tensin-like C1 domain-containing phosphatase
KN motif and ankyrin repeat domain-containing protein 3
Dedicator of cytokinesis protein 7
TBC1 domain family member 4
Uncharacterised protein KIAA0526
Transcription factor AP-1
Zinc finger CCCM domain-containing protein 13
Sentrin-specific protease 3
Uncharacterised protein C9 or f142
Prostaglandin F2 receptor negative regulator
Catenin delta-1
Protein FAM63A
Protein scribble homolog
Pleckstrin homolgy domain-containing family A member 7
Niban-like protein 1
Junctional adhesion molecule A
Rootletin
DNA damage-binding protein 2
Bromodomain-containing protein 1
Phosphatidylinostrol 4-kinase alpha
BTB/POZ domain-containing protein KCTD12
Protein FAM40A
Thioredoxin-related transmembrane protein 1
Protein phosphatase 1 regulatory subnit 3D
EF-hand domain-containing protier D2
Sciellin
Uncharacterised protein C19 or f21
Uncharacterised protein C19 or f21
Uncharacterised protein C19 or f21
Epidermal growth factor receptor kinase substrate 8-like protein 3
Uncharacterised protein C19 or f21
Pericentriolar material 1 protein
Signal-induced proliferation-associated protein 1
Secretory carrier-associated membrane protein 2
Activating signal cointerator 1 complex subunit 3
Desmoglein-2
Protein POF1B
Eukaryotic translation factor 3 subunit C
Uncharacterised protein KIAA0930
Ras GTP ase-activating protein-binding protein 1
Vinculin
RNA-binding protein 14
Kinesin light chain 2
"Lamina-associated polypeptide 2, isoforms beta/gama"
Transducin-like enhancer protein 3
CD2-associated protein
Kinesin light chain 2
Kinesin like protein KIFLOE
Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16
Acidic leucine-rich nuclear phosphoprotein 32 family member A
Chlorine-phosphate cytidylyltransferase A
Suppressor of SW14 1 homolog
ADP-ribosylation factor GTP ase-activating protein 2
Golgin subfamily A member 4
Junctional protein associated with coronary artery disease
Transgelin-2
Isoform 2 of Serine/threonine-protein kinase PAK 4
Drebrin
Mitochondrial dynamic protein MID51
Protein NDRG1
Neuroblast differentiation-associated protein AHNAk
Serine/threonine-protein kinase MRCK alpha
Serine/threonine-protein kinase MRCK alpha
Protein NOXP20
Tensin-1
Protein NDRG1
Phosphatidylinositol 4-kinase type 2-alpha
Kinesin-like protein KIF13B

FIG. 3c



	Case											
	6	4	13	12	7	10	8	9	5	1	11	14
Recurrence	-	-	-	-	-	+	-	+	+	+	+	+
LN metastasis	+	+	+	+	+	-	-	+	-	+	+	+

FIG. 3d

Protein	Global phos	Peptide	Time in freezer (Months)	7	18	23	28	24	8	13	19	20	21	3	12	
			Time of assesment of recurrence/non-recurrence (Months)	11	19	5	31	17	2	10	19	13	23	8	16	
			Recurrence	+	+	+	+	+	+	-	-	-	-	-	-	
			Lymph node mets	+	-	+	-	+	+	+	+	+	-	+	+	
				1T/1NT	5T/5NT	9T/9NT	10T/10 NT	11T/11 NT	14T/14 NT	4T/4NT	6T/6NT	7T/7NT	8T/8NT	12T/12 NT	13T/13 NT	
MEK2	T394	P1 - LNQPGtPTR		1.79	1.98	1.84	2.09	0.96	2.00	-0.53	1.92	-0.60	-0.05	-0.39	0.16	
MEK2	T394	P2 - LNQPGtPTRTAV		3.41	2.14	2.12	1.21	4.26	2.39	-0.13	0.22	0.31	-1.14	-0.70	-0.99	
MEK2	T394	P3 - TLRLNQPGtPTR		NA	NA	0.38	0.67	NA	NA	NA	NA	0.61	1.16	NA	NA	
MEK2	T394	Sum		**5.20**	**4.12**	**4.34**	**3.97**	**5.22**	**4.39**	**-0.66**	**2.14**	**0.32**	**-0.03**	**-1.09**	**-0.83**	

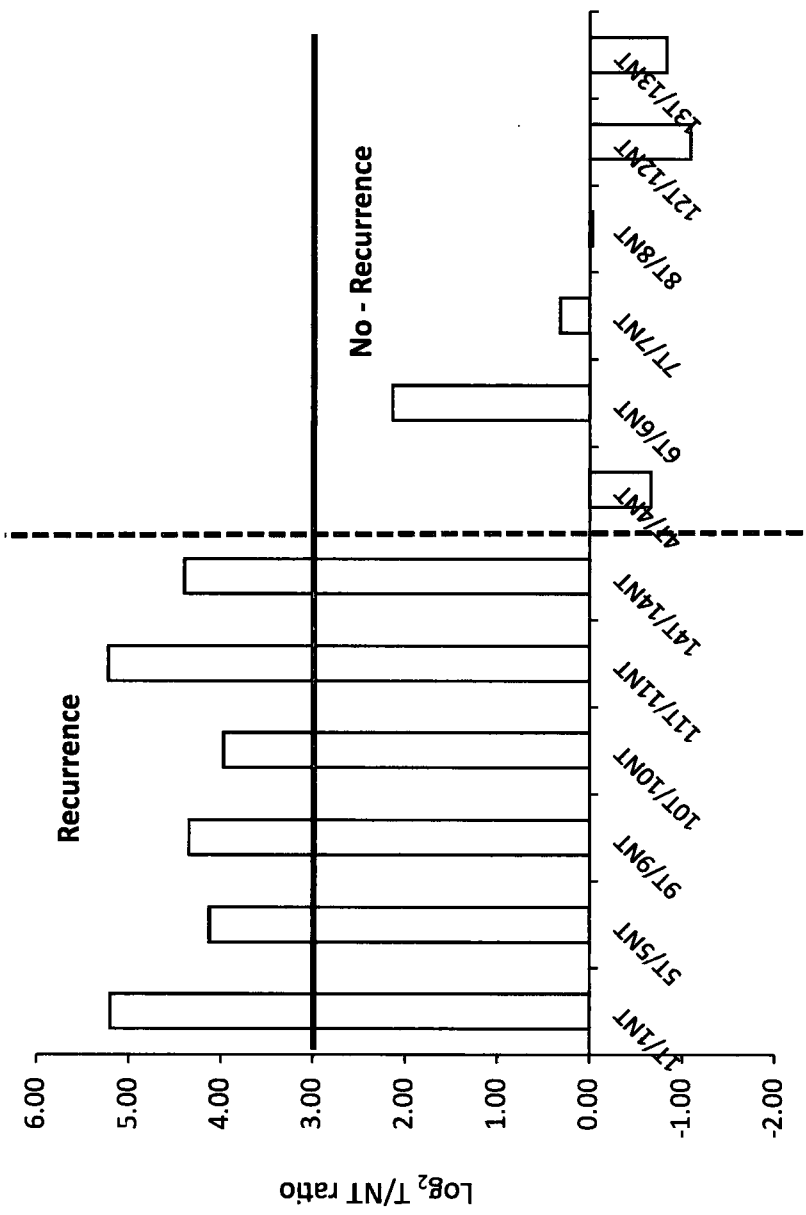


FIG. 4

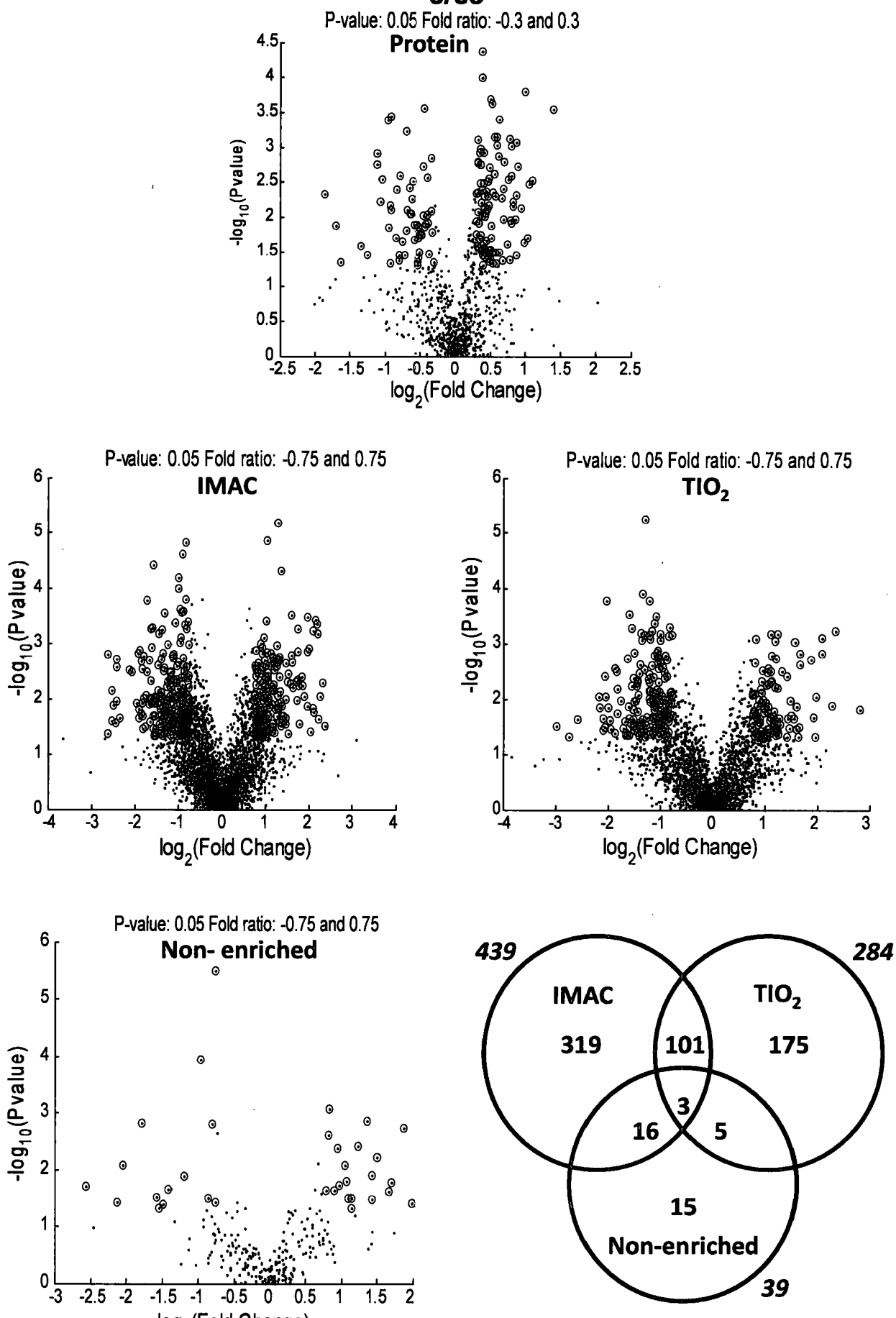

P-value: 0.05 Fold ratio: -0.3 and 0.3
Protein
-log10(Pvalue)
log2(Fold Change)
P-value: 0.05 Fold ratio: -0.75 and 0.75
IMAC
-log10(Pvalue)
log2(Fold Change)
P-value: 0.05 Fold ratio: -0.75 and 0.75
TIO2
-log10(Pvalue)
log2(Fold Change)
P-value: 0.05 Fold ratio: -0.75 and 0.75
Non- enriched
-log10(Pvalue)
log2(Fold Change)
439
284
IMAC
319
101
TIO2
175
3
16
5
15
Non-enriched
39

FIG. 5

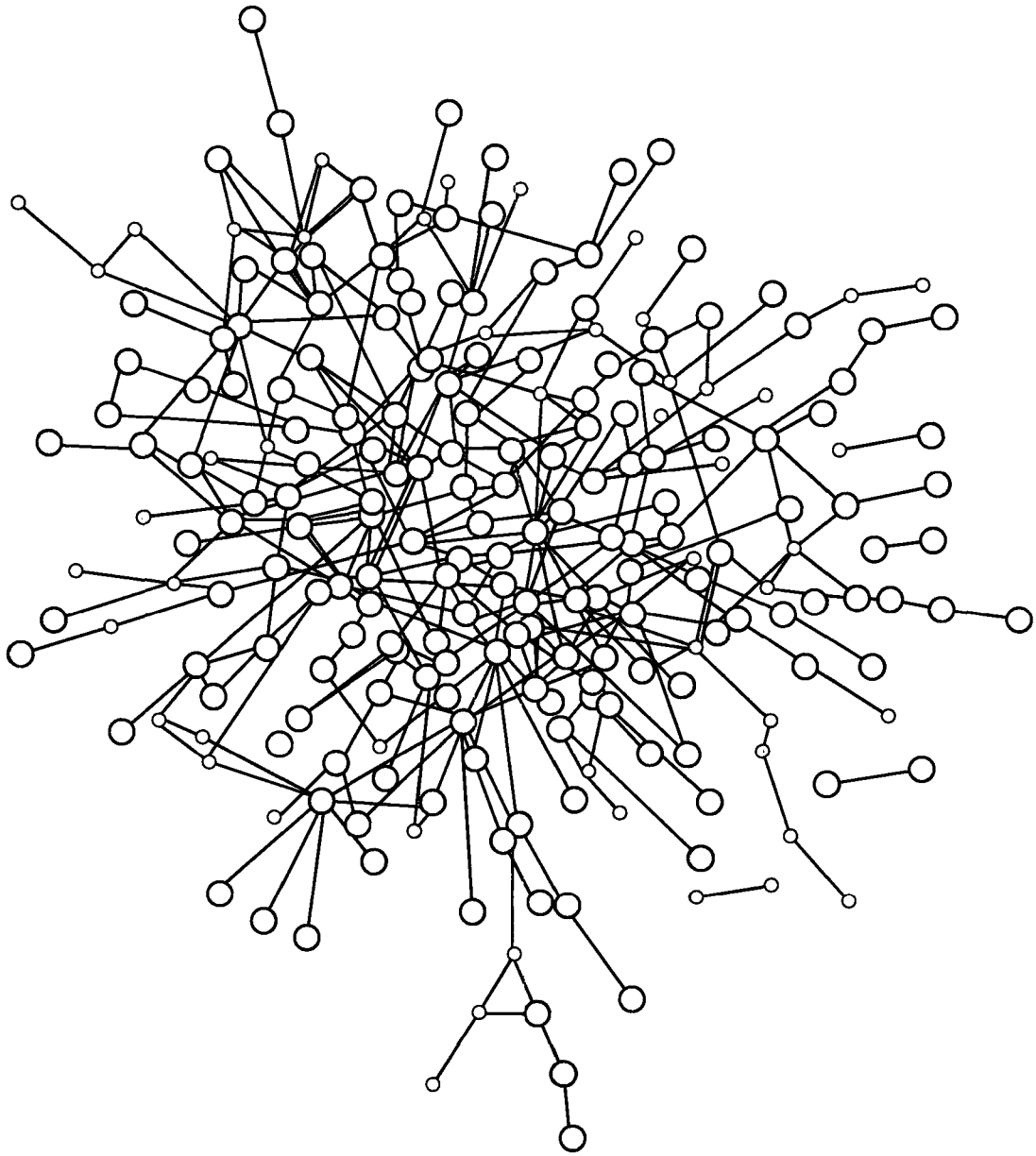
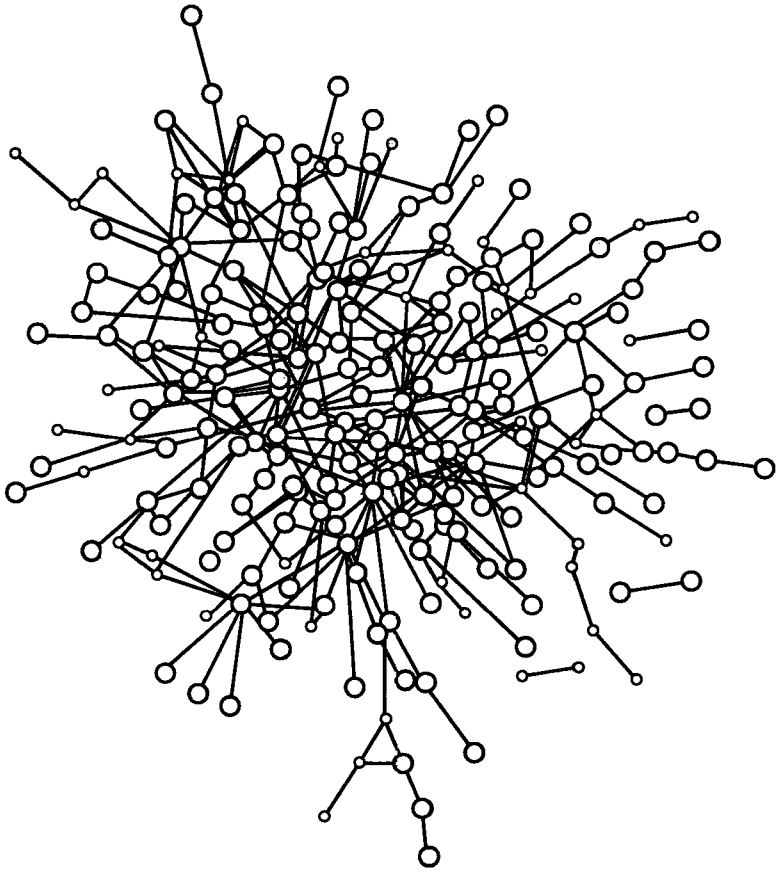
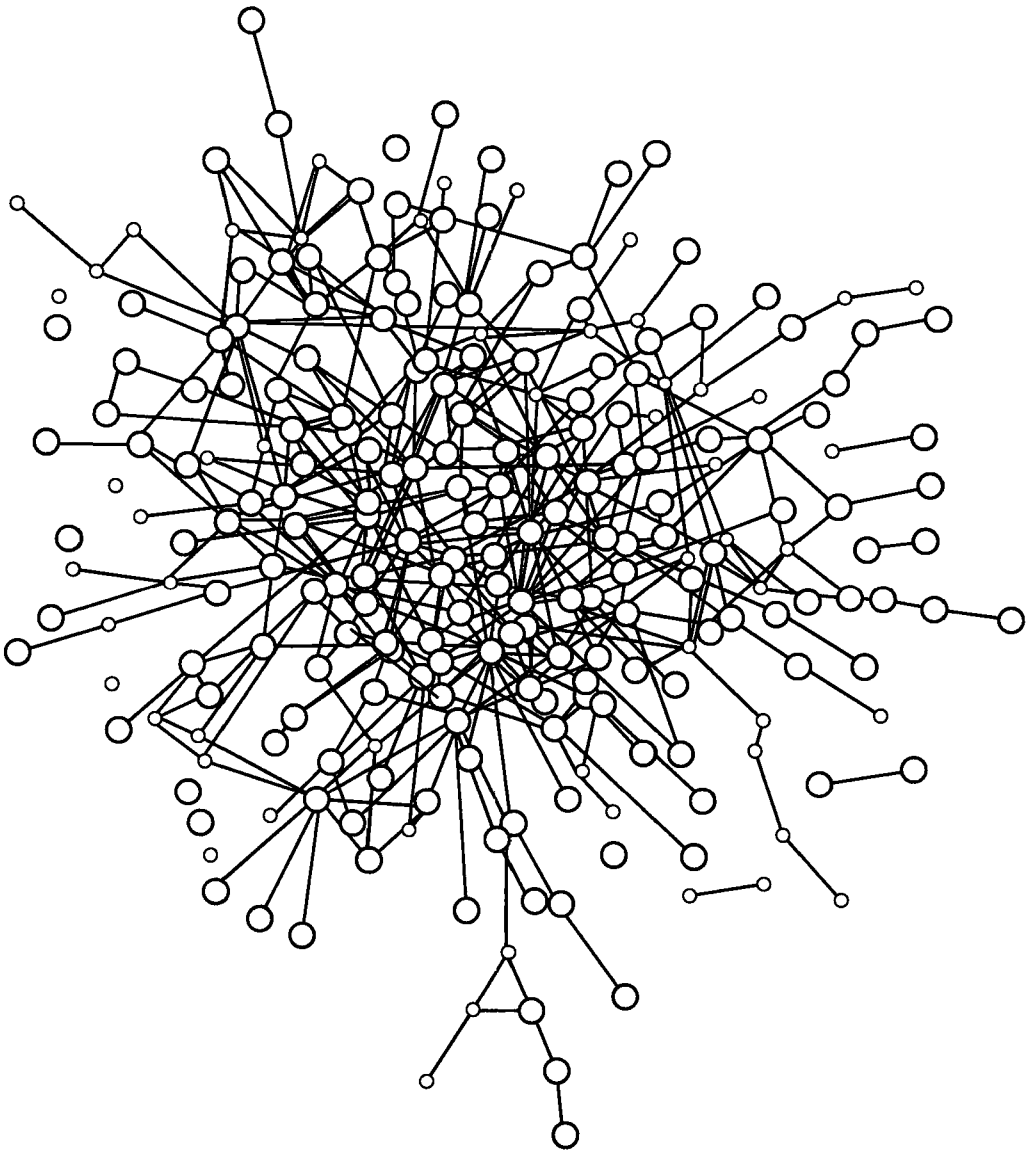


FIG. 6a



TIGHT JUNCTION PROTEINS			IMAC	TiO2	Non-enrich	IMAC	TiO2	Non-enrich
			Phos pep			Phos pep		
Uniprot-ID	Protein	Global	t.test p-values	t.test p-values	t.test p-values	log2 T/NT	log2 T/NT	log2 T/NT
P55196	Afadin	S1721	0.002	0.006	NA	-1.107	-1.538	NA
P55196	Afadin	S1182	0.009	0.033	NA	-0.881	-0.890	NA
P35221	Catenin alpha-1	S431;S435	0.745	0.049	NA	0.205	1.428	NA
Q9P2M7	Cingulin	S149	0.021	0.211	NA	-1.104	-1.281	NA
E9PCJ3	Erythrocyte membrane protein band 4.1-like 1	S84	0.030	NA	NA	-1.189	NA	NA
Q8NI35	InaD-like protein	S645	0.028	NA	NA	-0.932	NA	NA
Q9Y624	JunctIonal adhesion molecule A	S284	0.000	NA	NA	-1.007	NA	NA
P35580	Myosin-10	S1954	0.001	NA	NA	2.098	NA	NA
P35749	Myosin-11	S1954	0.001	NA	NA	2.098	NA	NA
P35749	Myosin-11	S1954	0.020	0.088	NA	0.962	1.687	NA
P35749	Myosin-11	S1954	0.016	0.045	NA	1.247	1.216	NA
P35749	Myosin-11	S1487	0.005	NA	NA	0.870	NA	NA
P35749	Myosin-11	S1487	0.001	NA	NA	2.098	NA	NA
P35749	Myosin-11	S1487	0.020	0.088	NA	0.962	1.687	NA
P35749	Myosin-11	S1487	0.016	0.045	NA	1.247	1.216	NA
P35749	Myosin-11	S1487	0.005	NA	NA	0.870	NA	NA
Q7Z406	Myosin-14	S1504	0.000	NA	0.033	2.179	NA	1.431
P35579	Myosin-9	S1480	0.001	NA	NA	2.098	NA	NA
Q05655	Protein kinase C delta type	S645	0.010	NA	NA	-0.769	NA	NA
Q01082	Spectrin beta chain, brain 1	S2161	0.364	0.029	NA	-0.821	-1.395	NA
Q01082	Spectrin beta chain, brain 1	S2161;S2165;S2169	0.028	NA	NA	-0.938	NA	NA
Q01082	Spectrin beta chain, brain 1	S2165;S2169	NA	0.014	NA	NA	-1.985	NA
Q14247	Src substrate cortactIn	T401;S405	NA	0.019	NA	NA	-0.864	NA
O95049	Tight junctIon protein ZO-3	S605	0.011	NA	NA	0.825	NA	NA

FIG. 6b



RAS SIGNAL TRANSDUCTION PROTEINS			IMAC	TiO2	Non-enrich	IMAC	TiO2	Non-enrich
			Phos pep			Phos pep		
Uniprot-ID	Protein	Global	t.test p-values	t.test p-values	t.test p-values	log2 T/NT	log2 T/NT	log2 T/NT
Q9P107	GEM-interac3ng protein	S437	0.002	0.061	NA	1.460	0.863	NA
Q9P107	GEM-interac3ng protein	S437	0.010	0.009	NA	1.471	1.970	NA
Q99569	Plakophilin-4	S461	0.034	NA	NA	0.904	NA	NA
Q14160	Protein scribble homolog	S1475	0.009	NA	NA	-1.541	NA	NA
Q14160	Protein scribble homolog	S1475	NA	0.046	NA	NA	-1.399	NA
Q14160	Protein scribble homolog	S504	0.000	0.002	NA	-0.940	-1.028	NA
Q14160	Protein scribble homolog	S835	0.037	NA	NA	-0.796	NA	NA
Q5U651	Ras-interac3ng protein 1	S328	0.002	0.045	NA	-0.902	-0.656	NA
O15085	Rho guanine nucleo3de exchange factor 11	S251	0.018	NA	NA	0.808	NA	NA
Q9NZN5	Rho guanine nucleo3de exchange factor 12	S1327	0.024	NA	NA	-0.893	NA	NA
Q96PE2	Rho guanine nucleo3de exchange factor 17	S420	0.006	0.005	NA	1.012	0.665	NA
Q96PE2	Rho guanine nucleo3de exchange factor 17	S735	0.002	0.591	NA	1.294	0.052	NA
Q96FS4	Signal-induced prolifera3on-associated protein 1	S908;S912	0.000	0.883	NA	1.033	-0.323	NA
P05412	Transcrip3on factor AP-1	S73	0.001	0.000	0.066	-1.455	-1.584	-0.816

FIG. 6c

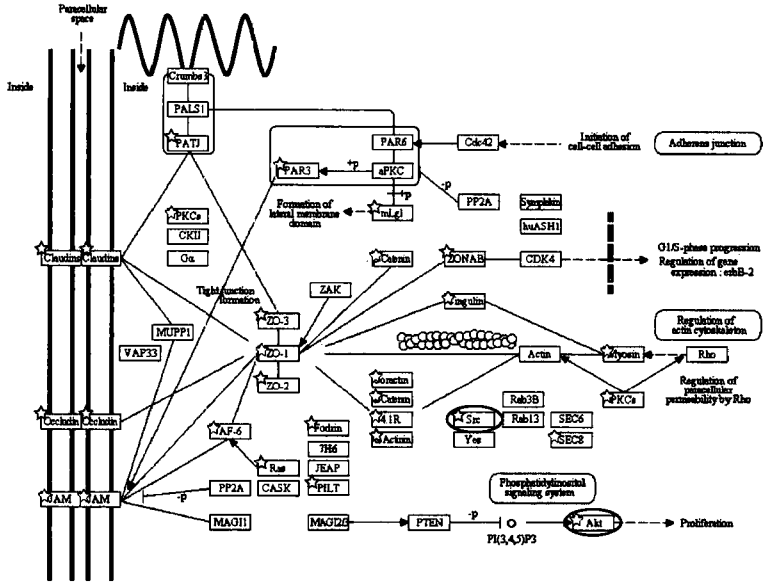
AKT Inhibitors
API-2
Perifosine
ErPC
ErPC3
MK-2206
KP372-1
GSK2141795
GSK690693
Enzastaurin
PBI-05204
XL-418

SRC family
Inhibitors
Dasatinib
Saracatinib
Bosutinib

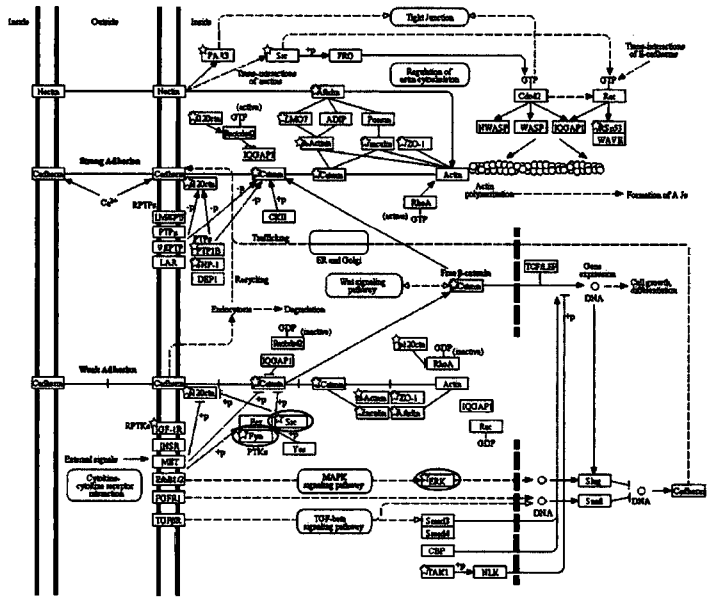
ERK Inhibitors
AEZS-131
SCH772984

Raf Inhibitors
Sorafenib
Regorafenib
PLX5568
AZ628
RAF265

Tight Junction Signaling



Adherens Junction Signaling



Focal Adhesion Signaling

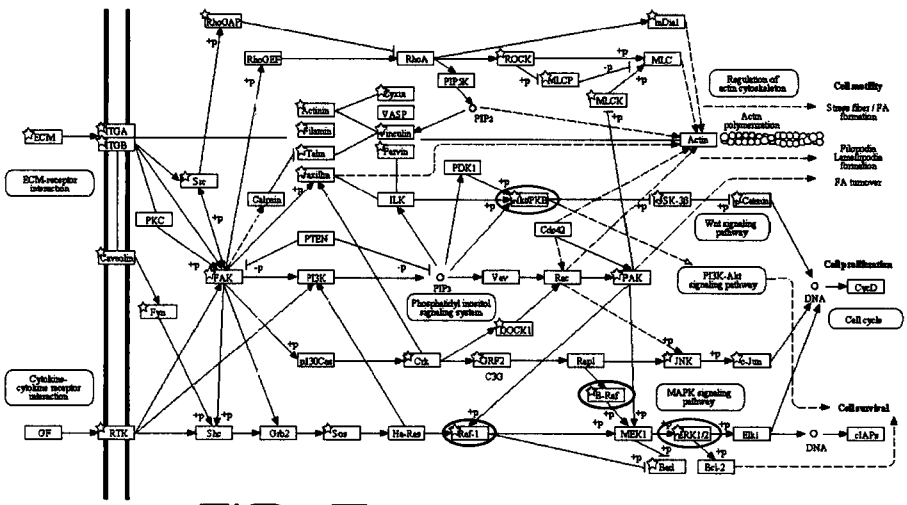
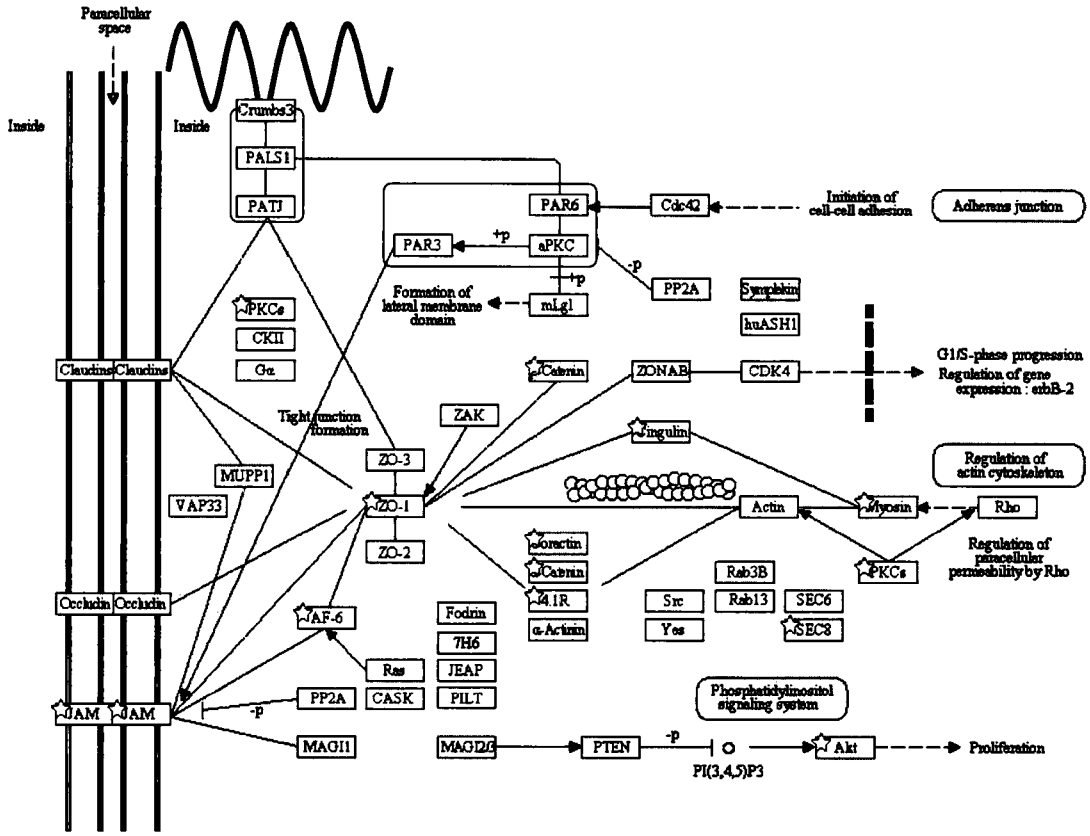


FIG. 7a

Tight Junction Signaling Pathway



Adherens Junction Signaling Pathway

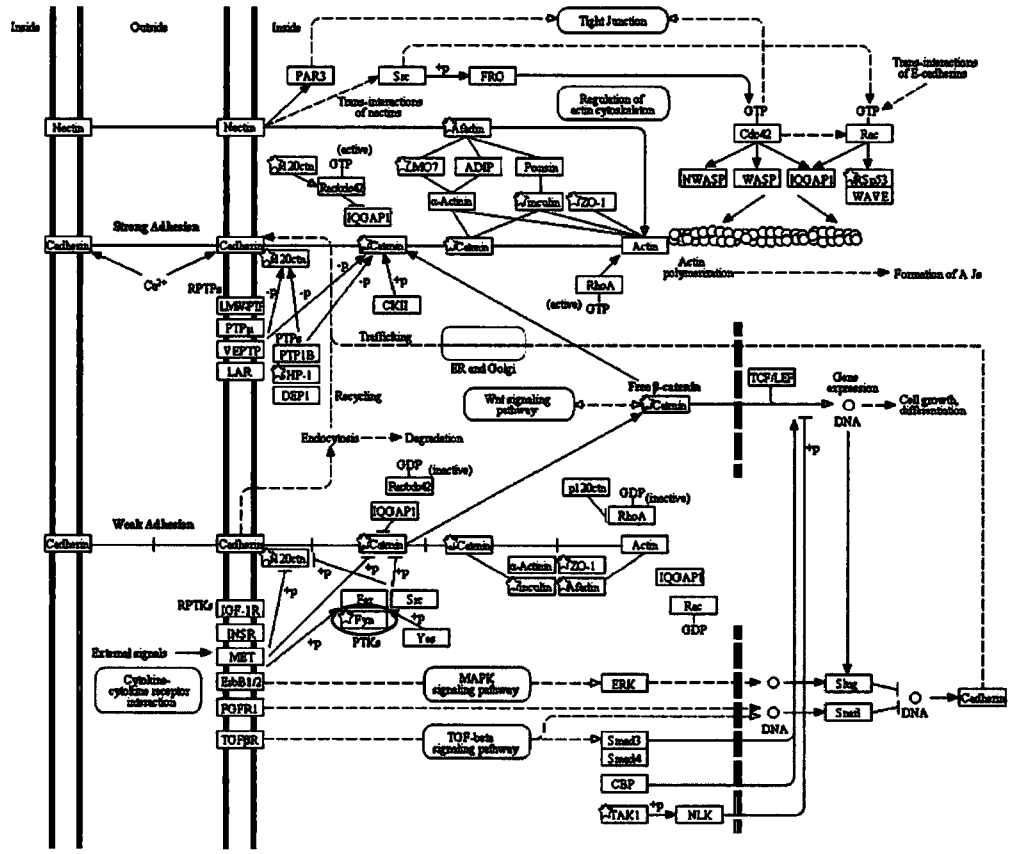
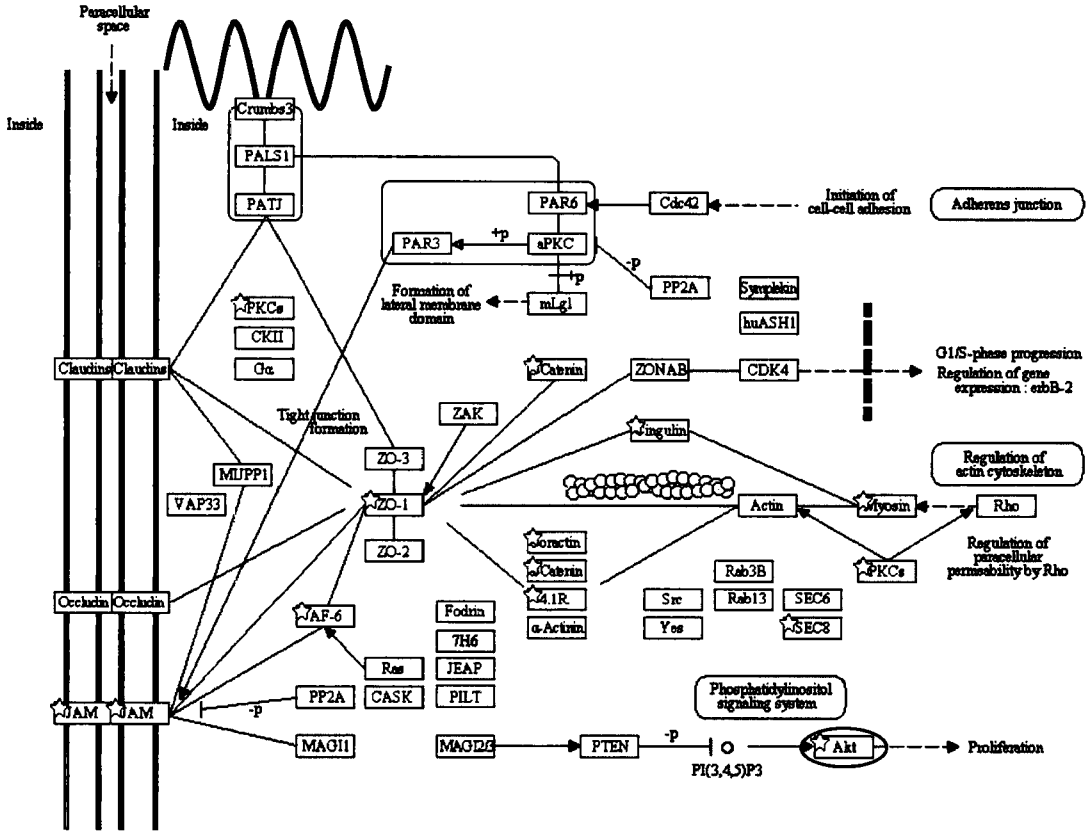


FIG. 7b

Tight Junction Signaling Pathway



Focal Adhesion

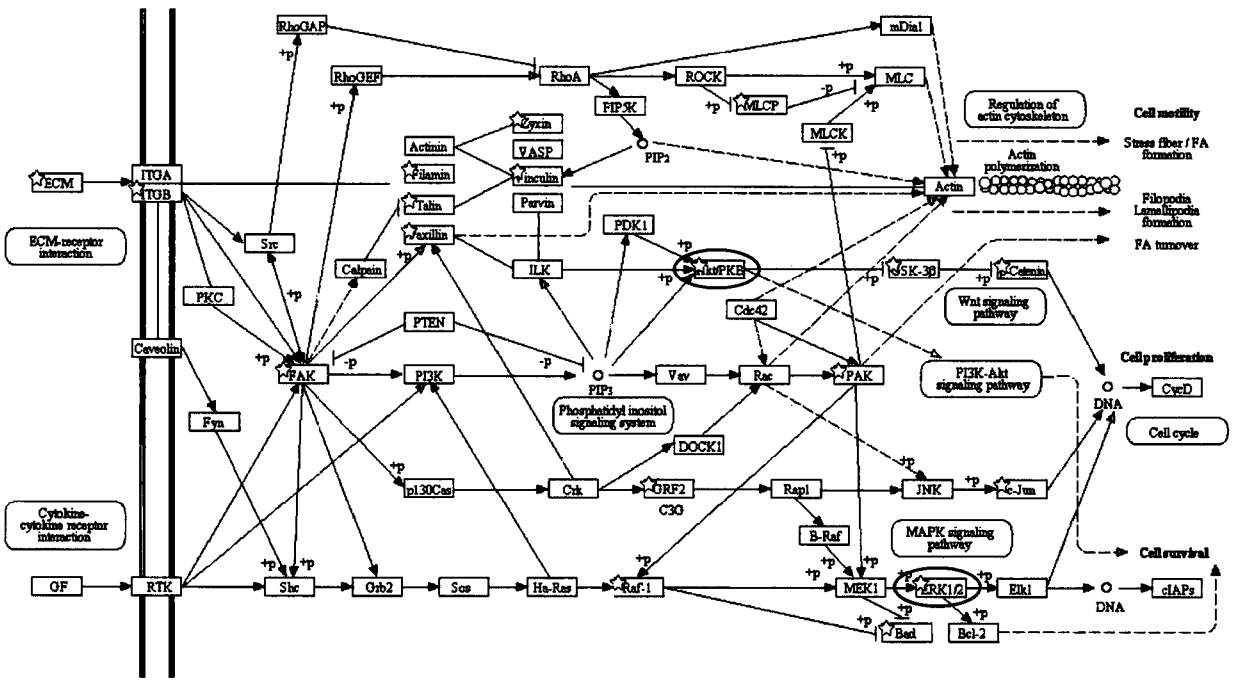


FIG. 7c

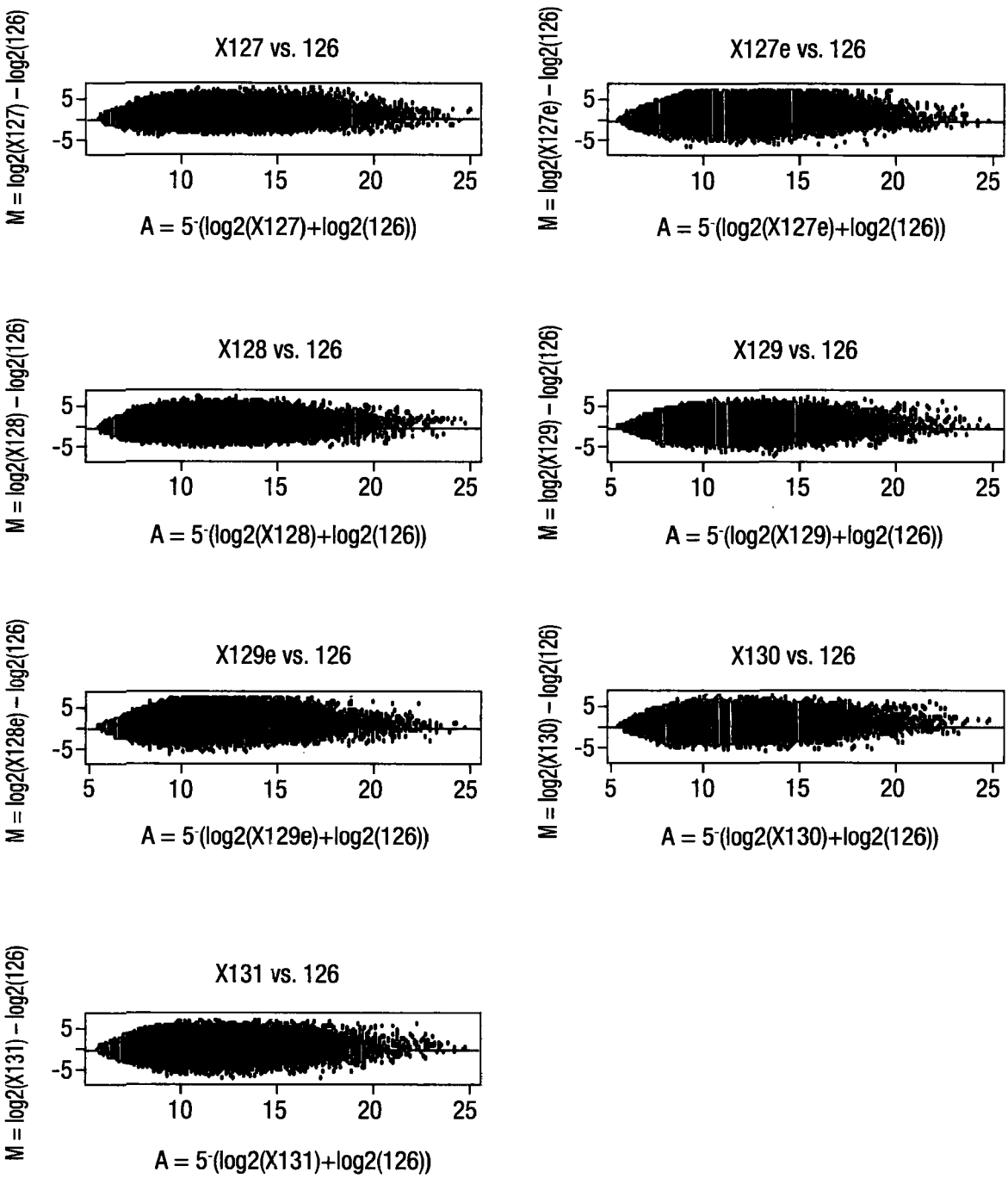

X127 vs. 126
M = log2(X127) – log2(126)
A = 5-(log2(X127)+log2(126))
X127e vs. 126
M = log2(X127e) – log2(126)
A = 5-(log2(X127e)+log2(126))
X128 vs. 126
M = log2(X128) – log2(126)
A = 5-(log2(X128)+log2(126))
X129 vs. 126
M = log2(X129) – log2(126)
A = 5-(log2(X129)+log2(126))
X129e vs. 126
M = log2(X128e) – log2(126)
A = 5-(log2(X129e)+log2(126))
X130 vs. 126
M = log2(X130) – log2(126)
A = 5-(log2(X130)+log2(126))
X131 vs. 126
M = log2(X131) – log2(126)
A = 5-(log2(X131)+log2(126))

FIG. 8a

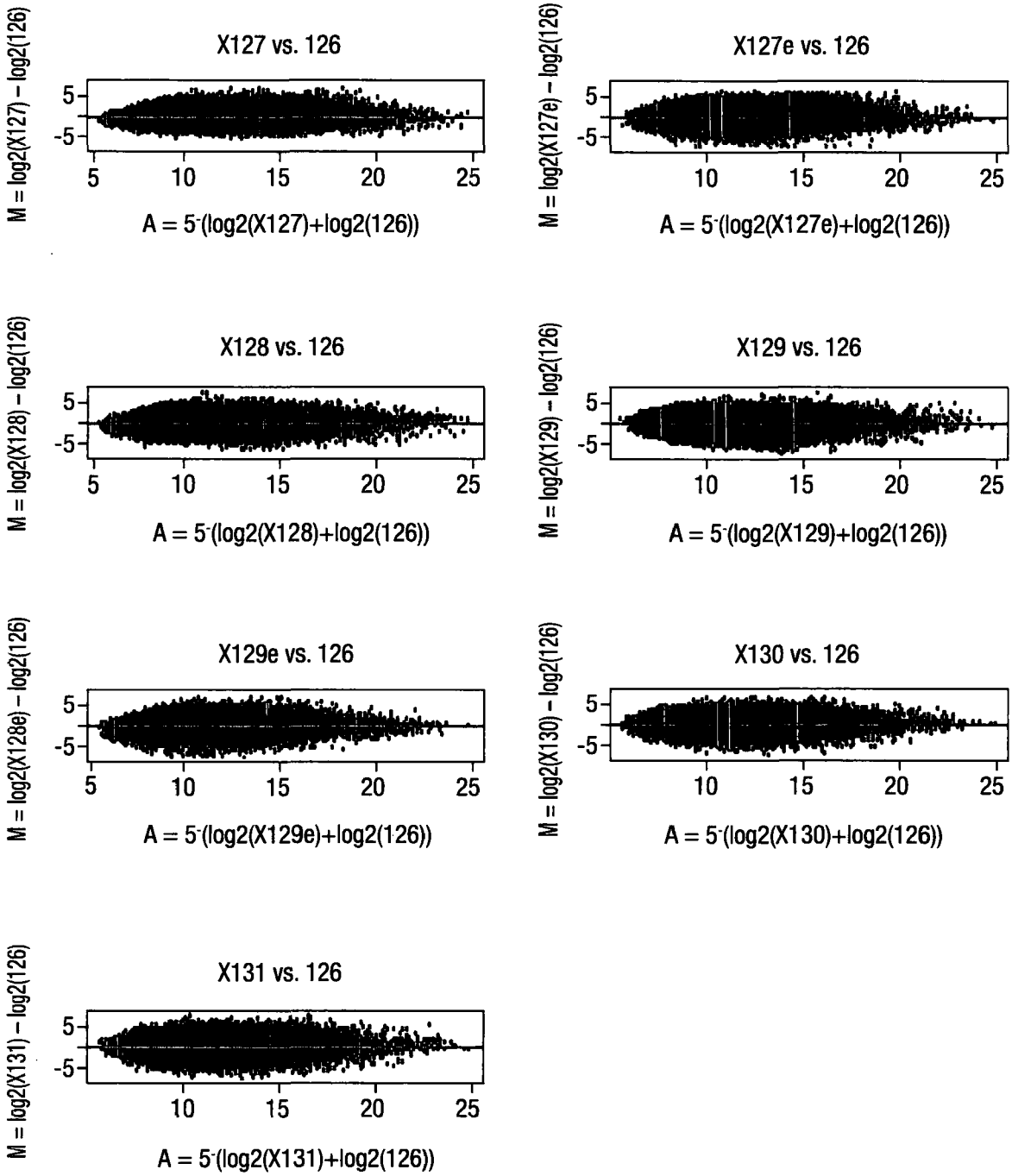

X127 vs. 126
M = log2(X127) - log2(126)
A = 5-(log2(X127)+log2(126))
X127e vs. 126
M = log2(X127e) - log2(126)
A = 5-(log2(X127e)+log2(126))
X128 vs. 126
M = log2(X128) - log2(126)
A = 5-(log2(X128)+log2(126))
X129 vs. 126
M = log2(X129) - log2(126)
A = 5-(log2(X129)+log2(126))
X129e vs. 126
M = log2(X128e) - log2(126)
A = 5-(log2(X129e)+log2(126))
X130 vs. 126
M = log2(X130) - log2(126)
A = 5-(log2(X130)+log2(126))
X131 vs. 126
M = log2(X131) - log2(126)
A = 5-(log2(X131)+log2(126))

FIG. 8b

	# PSM (phos)	# PSM (non-phos)	# PSM (phos + non- phos)	# Unique peptides (phos)	# unique peptides (Non-phos)	# unique peptides (phos + non-phos)	# phospho-sites Mascot + Sequest
Σ TMT8plex - 1	21428	88911	110339	3245	14673	17918	3161
Σ TMT8plex - 2	29300	88568	117868	4569	14769	19338	4426
Σ TMT8plex - 3	25914	102303	128217	4264	17548	21812	4184
Σ TMT8plex 1+2+3	**76642**	**279782**	**356424**	**6543**	**22909**	**29452**	**6284**

Table. 1

Uniprot-ID	Protein	t.test p-values	log2 (T/NT)	Function	Role in cancer	References
P14618	Pyruvate kinase isozymes M1/M2	4.20E-05	0.383	Glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP	In addition to aerobic glycolysis, regulates gene transcription. Isoform M2 phosphorylates histone H3 at T11, which is related to expression of cyclin D1 and c-Myc, tumor cell proliferation, cell-cycle progression, and brain tumorigenesis.	Yang W, et al. Cell 2012. Christofk HR, et al. Nature 2008.
Q86Z02	Homeodomain-interacting protein kinase 1	1.59E-04	1.002	Belongs to the Ser/Thr family of protein kinases and HIPK subfamily. Phosphorylates p53, DAXX, and MYB. Prevents MAP3K5-JNK activation in the absence of TNF.	Known to be upregulated in many tumor cell lines. Involved in tumourigenesis and tumour growth by its oncogenic and anti-apoptotic function.	Kondo S, et al. Proc Natl Acad Sci USA 2003. Lee D, et al. EMPO Rep 2012.
Q14847	LIM and SH3 domain protein 1	2.01E-04	0.496	Plays an important role in the regulation of dynamic actin-based, cytoskeletal activities	Involved in proliferation, invasion and migration of cancer cells.	Zhao L, et al. Gut 2010. Grunewald TG, et al. Br J Cancer 2007.
P37802	Transgelin-2	2.34E-04	0.519	Contains a conserved actin-binding domain also known as the calponin homolog (CH) domain, suggesting a role in cytoskeletal organization.	Overexpressed in various cancers. Higher expression levels were associated with metastasis, advanced clinical stage, and poor survival. But its biological function remains unknown.	Zhang Y, et al. Cancer Sci 2010.
Q92538	Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1	2.84E-04	1.397	Involved in mitosis. Phosphorylated by CDK1. Promotes the activation of ADP-ribosylation factor 5 (ARF5) through replacement of GDP with GTP.	Unknown.	Morohashi Y, et al. Biochem J 2010.
P21291	Cysteine and glycine-rich protein α1	3.96E-04	0.628	A cytoskeletal lin-11 isl-1 mec-3 (LIM)-domain protein. Involved in smooth muscle differentiation.	Down-regulated in hepatocellular carcinoma and colorectal cancer. But, its function is unknow.	Miyasaka KY, et al. Proc Natl Acad Sci U S A. 2007. Hirasawa Y, et al. Oncology 2006.
Q8WX93	Palladin	6.97E-04	0.588	Cytoskeletal protein that is required for organization of normal actin cytoskeleton. Roles in establishing cell morphology, motility, cell adhesion and cell-extracellular matrix interactions.	Overexpressed in breast cancer. Involved in cell migration. Plays a key role in the formation of podosomes, actin-rich structures that function in adhesion and matrix degradation.	Goicoechea SM, et al. Oncogene 2009.
Q14195-2	Isoform LCRMP-4 of Dihydropyrimidinase-related protein 3	7.01E-04	0.555	Necessary for signaling by class 3 semaphorins and subsequent remodeling of the cytoskeleton. Plays a role in axon guidance and cell migration	Unknown.	Weitzdoerfer R, et al. J Neural Transm Suppl. 2001.

Table. 2

Q9NR12	PDZ and LIM domain protein 7	7.39E-04	0.778	PDZ domain binds actin-binding proteins such as β-tropomyosin, while LIM domains interact with proteins involved in mitogenic or insulin signaling such as protein kinases. Involved in bone morphogenesis.	Promotes cell survival and chemoresistance by suppressing p53-mediated apoptosis. Elicited p53 degradation by inhibiting MDM2 self-ubiquitination and increasing its ubiquitin ligase activity toward p53 in cells.	Jung CR, et al. J Clin Invest 2010.
P26038	Moesin	7.62E-04	0.334	A membrane-cytoskeleton linking protein, belongs to the ERM (ezrin, radixin and moesin) family. Participates in various signalling pathways and play a crucial role in cell morphology, adhesion and motility.	Involved in actin filament remodelling and epithelial mesenchymal transition.	Haynes J, et al. Mol Biol Cell. 2011.
P15941	Mucin-1	8.57E-04	0.873	A transmembrane glycoprotein. The alpha subunit has cell adhesive properties. The beta subunit contains a C-terminal domain which is involved in cell signaling, through phosphorylation and protein-protein interactions.	An anti-adhesion molecule that inhibits cell–cell adhesion. Promoting motility and invasive properties by reducing interactions between integrins and the extracellular matrix. Involved in activation of Wnt and MAP signal pathways, and repression of the p53 gene.	Yonezawa, et al. Pathol Int 2011. Wei X, et al. Cancer Res 2007. Ren J, et al. J Biol Chem 2002.
Q05682	Caldesmon	9.37E-04	0.597	A cytoskeletal protein. Stabilizes actin filaments and involves in myosin-actin interaction. Plays an essential role during cellular mitosis and receptor capping.	Inhibitory effects on cell motility and migration. But phosphorylation at particular sites (i.e., S12) reduces the anti-migratory effect.	Schwappacher R, et al. J Cell Sci 2013. Mayanagi T, et al. J Biol Chem 2008.

Table. 2 (continued)

				TiO2 + IMAC + Non-enrich	IMAC	TiO2	Non-enrich	TiO2 + IMAC + Non-enrich	IMAC	TiO2	Non-enrich
				Protein	Phos pep			Protein	Phos pep		
KEGG	Uniprot	Protein	Global	t.test p-values	t.test p-values	t.test p-values	t.test p-values	log2 T/NT	log2 T/NT	log2 T/NT	log2 T/NT
FA, RAC, VSMC	O14974	Protein phosphatase 1 regulatory subunit 12A	S507	2.31E-02	2.25E-02	NaN	NaN	0.9886	-0.2470	NA	NA
FA, RAC, VSMC	O14974	Protein phosphatase 1 regulatory subunit 12A	S995	2.31E-02	2.38E-02	NaN	NaN	0.9886	-0.2245	NA	NA
VSMC	O15085	Rho guanine nucleotide exchange factor 11	S251	NaN	1.81E-02	NaN	NaN	NA	0.8076	NA	NA
VSMC	O43306	Adenylate cyclase type 6	S576	NaN	1.59E-02	5.05E-02	NaN	NA	-1.1487	-1.1316	NA
VSMC	O43306-2	Isoform 2 of Adenylate cyclase type 6	S576	4.56E-02	1.07E-02	4.00E-02	NaN	-1.6305	-1.2488	-0.8333	NA
VSMC	O43306-2	Isoform 2 of Adenylate cyclase type 6	S576	4.56E-02	1.16E-02	NaN	NaN	-1.6305	-0.7207	NA	NA
FA, RAC	O60610	Protein diaphanous homolog 1	S22	1.56E-01	3.24E-02	NaN	NaN	0.2000	-0.4683	NA	NA
TJ	O95049	Tight junction protein ZO-3	S605	NaN	1.15E-02	NaN	NaN	NA	0.8249	NA	NA
FA	P02452	Collagen alpha-1(I) chain	S176	2.83E-01	NaN	2.65E-03	NaN	-0.1859	NA	-0.9487	NA
FA	P05412	Transcription factor AP-1	S73	NaN	6.74E-04	2.94E-04	6.64E-02	NA	-1.4547	-1.5842	-0.8164
FA	P06241	Tyrosine-protein kinase Fyn	Y420	9.78E-02	1.82E-02	2.58E-02	NaN	0.4737	-0.6782	-0.5255	NA
RAC, VSMC	P10398	Serine/threonine-protein kinase A-Raf	S157	NaN	4.78E-02	NaN	NaN	NA	-0.7991	NA	NA
FA	P10451	Osteopontin	S303;S308	NaN	2.21E-02	2.50E-01	NaN	NA	0.6135	0.1925	NA
TJ, FA	P12931	Proto-oncogene tyrosine-protein kinase Src	Y419	NaN	3.72E-02	7.01E-02	NaN	NA	-0.6782	-0.4745	NA
FA, RAC	P16144	Integrin beta-4	S1483;S1486	5.22E-01	1.74E-01	4.38E-02	NaN	0.1948	1.1656	2.0578	NA
FA, RAC	P18206	Vinculin	S272	4.46E-03	5.71E-01	7.05E-03	NaN	0.3263	0.1297	0.7110	NA
FA, RAC	P18206	Vinculin	S290	4.46E-03	8.44E-02	4.62E-02	NaN	0.3263	1.1502	1.0478	NA
FA, RAC	P18206	Vinculin	S579	4.46E-03	7.24E-03	NaN	NaN	0.3263	0.7051	NA	NA
FA, RAC	P18206	Vinculin	Y822	4.46E-03	4.02E-02	NaN	NaN	0.3263	-0.7723	NA	NA
FA	P21333	Filamin-A	S1459	1.34E-03	5.43E-01	8.63E-02	3.24E-02	0.6182	0.3807	0.9812	1.0915
FA, RAC	P26010	Integrin beta-7	T797	NaN	7.29E-03	NaN	NaN	NA	0.6350	NA	NA
RAC	P26038	Moesin	S576	7.62E-04	8.55E-03	NaN	NaN	0.3340	0.7557	NA	NA
TJ	P35221	Catenin alpha-1	S655	1.24E-01	7.45E-01	4.86E-02	NaN	-0.2074	0.2050	1.4275	NA
TJ, RAC	P35579	Myosin-9	S1480	5.93E-03	5.97E-04	NaN	NaN	0.2877	2.0980	NA	NA
TJ, RAC	P35580	Myosin-10	S1939	2.18E-02	1.19E-02	8.92E-01	NaN	0.2259	-0.5713	-0.1338	NA
TJ, VSMC	P35749	Myosin-11	S1954	2.75E-02	1.55E-02	4.50E-02	NaN	0.3431	1.2465	1.2156	NA
TJ, VSMC	P35749	Myosin-11	S1954	2.75E-02	2.01E-02	8.83E-02	NaN	0.3431	0.9621	1.6869	NA
TJ, VSMC	P35749	Myosin-11	S1954	2.75E-02	1.74E-02	9.48E-02	NaN	0.3431	0.6778	1.0375	NA
TJ, VSMC	P35749	Myosin-11	S1954	2.75E-02	4.78E-03	NaN	NaN	0.3431	0.8695	NA	NA
RAC, VSMC	P36507	Dual specificity mitogen-activated protein kinase kinase 2	T394	3.97E-01	1.17E-02	1.91E-02	NaN	-0.1358	0.5546	0.8362	NA
RAC, VSMC	P36507	Dual specificity mitogen-activated protein kinase kinase 2	T394	3.97E-01	NaN	2.38E-02	NaN	-0.1358	NA	0.6415	NA
FA, RAC	P49023	Paxillin	S106	NaN	4.87E-02	4.19E-01	NaN	NA	-0.5612	-0.0669	NA
RAC	P53667	LIM domain kinase 1	S298	NaN	7.75E-03	2.56E-01	NaN	NA	0.8768	0.7315	NA

Table. 3

TJ	P55196	Afadin	S1182	5.11E-01	8.59E-03	3.28E-02	NaN	0.1801	-0.8806	-0.8898	NA
TJ	P55196	Afadin	S1182	5.11E-01	1.16E-02	4.50E-01	NaN	0.1801	-0.6459	-0.3325	NA
TJ	P55196	Afadin	S1721	5.11E-01	1.58E-03	5.75E-03	NaN	0.1801	-1.1066	-1.5385	NA
TJ, VSMC	Q05655	Protein kinase C delta type	S645	5.08E-01	9.93E-03	NaN	NaN	-0.0412	-0.7690	NA	NA
RAC	Q13576	Ras GTPase-activating-like protein IQGAP2	S16	3.86E-01	2.04E-03	9.01E-03	NaN	0.1666	-0.6374	-2.1648	NA
RAC	Q13576	Ras GTPase-activating-like protein IQGAP2	S16	3.86E-01	9.74E-03	NaN	NaN	0.1666	-1.4953	NA	NA
RAC	Q13576	Ras GTPase-activating-like protein IQGAP2	S16	3.86E-01	9.75E-03	NaN	NaN	0.1666	-1.3541	NA	NA
TJ	Q13813	Spectrin alpha chain, brain	S1217	3.35E-01	5.40E-01	3.90E-02	NaN	-0.1001	-0.2181	-1.3428	NA
TJ	Q14247	Src substrate cortactin	T401;S405	3.23E-01	NaN	1.92E-02	NaN	0.0531	NA	-0.8638	NA
FA	Q14315	Filamin-C	S2233	2.38E-03	3.08E-04	5.89E-04	1.39E-03	0.5615	1.5875	2.3520	1.3572
VSMC	Q14573	Inositol 1,4,5-trisphosphate receptor type 3	S1832	5.61E-01	4.81E-03	1.15E-01	6.38E-02	0.1397	0.5481	0.3586	1.1922
TJ, VSMC	Q3MNF1	NA		6.29E-02	1.97E-02	7.96E-02	NaN	0.3760	0.9201	1.1924	NA
TJ	Q5JTD0	Tight junction-associated protein 1	T422	NaN	4.49E-02	NaN	NaN	NA	0.7021	NA	NA
TJ	Q6P1M3	Lethal(2) giant larvae protein homolog 2	S1013	3.68E-01	1.50E-02	NaN	NaN	-0.5227	-0.5448	NA	NA
TJ, RAC	Q7Z406	Myosin-14	S1504	2.03E-02	4.50E-04	NaN	3.33E-02	0.3493	2.1786	NA	1.4309
TJ	Q8NI35	InaD-like protein	S645	4.68E-01	2.82E-02	NaN	NaN	0.6231	-0.9323	NA	NA
FA	Q92934	Bcl2 antagonist of cell death	S118	NaN	2.75E-03	NaN	NaN	NA	-1.3183	NA	NA
FA	Q92934	Bcl2 antagonist of cell death	S134	NaN	1.90E-01	1.79E-02	NaN	NA	0.6779	0.7760	NA
VSMC	Q96A00	Protein phosphatase 1 regulatory subunit 14A	S128;S136	7.28E-01	5.35E-02	2.11E-02	NaN	-0.0997	-1.2981	-0.8118	NA
VSMC	Q96A00	Protein phosphatase 1 regulatory subunit 14A	S128;S136	7.28E-01	1.20E-02	NaN	NaN	-0.0997	-1.5386	NA	NA
TJ	Q9H4G0	Band 4.1-like protein 1	S510	9.68E-01	2.87E-03	1.58E-01	NaN	0.1569	-1.8342	-3.3910	NA
TJ	Q9H4G0	Band 4.1-like protein 1	S541;S544	9.68E-01	2.83E-02	2.59E-02	NaN	0.1569	-0.4004	-1.3756	NA
TJ	Q9H4G0	Band 4.1-like protein 1	S784	9.68E-01	4.85E-03	NaN	NaN	0.1569	-1.2407	NA	NA
TJ	Q9H4G0	Band 4.1-like protein 1	S820	9.68E-01	1.68E-04	NaN	NaN	0.1569	-1.7144	NA	NA
RAC, VSMC	Q9NZN5	Rho guanine nucleotide exchange factor 12	S1327	6.82E-01	1.01E-02	7.07E-02	NaN	0.1252	-0.6148	-1.5399	NA
RAC, VSMC	Q9NZN5	Rho guanine nucleotide exchange factor 12	T703	6.82E-01	2.42E-02	NaN	NaN	0.1252	-0.8934	NA	NA
TJ	Q9P2M7	Cingulin	S149	NaN	2.09E-02	2.11E-01	NaN	NA	-1.1041	-1.2806	NA
RAC	Q9Y2I7	1-phosphatidylinositol-3-phosphate 5-kinase	S307;S312	NaN	4.38E-02	2.49E-01	NaN	NA	0.6748	0.8822	NA
FA	Q9Y490	Talin-1	S1201	1.06E-03	4.04E-01	7.01E-01	3.26E-02	0.3681	-0.0394	-0.1647	1.1455
FA	Q9Y490	Talin-1	S1225	1.06E-03	3.57E-02	2.13E-01	NaN	0.3681	-0.6687	-0.8313	NA
FA	Q9Y490	Talin-1	S620	1.06E-03	1.08E-02	NaN	NaN	0.3681	1.7192	NA	NA
FA	Q9Y4G6	Talin-2	T1843	2.00E-02	1.42E-02	NaN	NaN	0.5179	-0.5952	NA	NA
TJ	Q9Y624	Junctional adhesion molecule A	S284	NaN	6.59E-05	NaN	NaN	NA	-1.0066	NA	NA
VSMC	Q9Y6F6	Protein MRVI1	S657	NaN	1.67E-02	2.19E-01	NaN	NA	0.4638	0.3463	NA
VSMC	Q9Y6F6	Protein MRVI1	S657	NaN	1.82E-02	NaN	NaN	NA	0.4092	NA	NA

Table. 3 (continued)

Peptide log_2 ratios															
TiO2 + IMAC + TotalProtein												Name	Sequence	Global Position	DrugBank
1T / 1NT	4T / 4NT	5T / 5NT	6T / 6NT	7T / 7NT	8T / 8NT	9T / 9NT	10T / 10NT	11T / 11NT	12T / 12NT	13T / 13NT	14T / 14NT				
-	-	-	-	**-1.136**	0.414	0.386	0.642	-0.71	0.088	0.567	0.483	**Fyn**	**dGsLNQSSGYR**	**S21**	Dasatinib
1.149	0.076	-0.792	-0.494	0.086	0.801	-0.099	0.615	-	-	-	-	**Fyn**	**ITEERDGsLNQSSGYR**	**S21**	Dasatinib
-	-	-	-	-0.749	-0.237	0.375	-0.994	-0.626	-0.195	-0.736	-0.796	Fyn	IIEDNEyTAR	Y420	Dasatinib
-	-	-	-	-	-	-	-	0.769	0.091	**-1.227**	-0.484	Src	IFGGFNsSDTVTSPQR	S69	Dasatinib
-	-	-	-	-	-	-	-	-0.626	-0.195	-0.736	-0.796	**Src**	**IIEDNEyTAR**	**Y419**	Dasatinib
0.534	0.188	-0.555	-0.406	-	-	-	-	-	-	-	-	Abl2	gAQASSGsPALPR	S620	Dasatinib
-	-	-	-	-0.155	-0.453	-0.734	-0.121	-0.971	0.18	0.605	**-1.251**	**Raf1**	**STsTPNVHMVSTTLPVDSR**	**S259**	Sorafenib
-	-	-	-	-0.137	0.46	-0.846	1.485	**-1.712**	0.354	1.913	0.269	Raf1	SAsEPSLHR	S621	Sorafenib
-	-	-	-	-0.359	-0.167	-0.223	0.261	0.649	-0.325	0.381	0.48	**B-Raf**	**SAsEPSLNR**	**S729**	Sorafenib
-1.293	0.08	-0.292	-0.147	-	-	-	-	-	-	-	-	HDAC1	iSIcsSDkR	S409	Vorinostat
-	-	-	-	-0.622	**-1.042**	0.216	**-1.565**	-0.636	-0.041	**1.656**	-0.585	**HDAC1**	**iAcEEEFsDsEEEGEGGRk**	**S421;S423**	Vorinostat
-	-	-	-	0.056	**1.433**	0.055	-0.254	-	-	-	-	**HDAC2**	**iAcDEEFsDSEDEGEGGRR**	**S422**	Vorinostat
-	-	-	-	0.014	-0.13	0.272	0.458	-0.367	-0.408	1.421	0.873	**HDAC2**	**iAcDEEFsDsEDEGEGGRR**	**S422;S424**	Vorinostat
-0.554	**1.034**	**-1.089**	0.486	-0.895	-0.421	-0.682	**-1.747**	-	-	-	-	RICTOR	hIEDTGsTPsIGENDLk	S1174;S1177	Temsirolimus
0.145	0.438	0.306	-0.195	-	-	-	-	0.124	0.034	1.242	0.093	RPTOR	SVSsYGNIR	S722	Temsirolimus
-	-	-	-	-0.146	-0.269	-0.734	0.321	-	-	-	-	**ERK1**	**iADPEHDHTGFLtEyVATR**	**T202;Y204**	AEZS-131
-0.115	**-1.927**	**1.046**	-0.508	0.037	**1.437**	-0.428	**1.285**	**-1.074**	-0.019	-0.809	0.069	**ERK2**	**vADPDHDHTGFLtEyVATR**	**T185;Y187**	AEZS-131
-1.036	**1.305**	-0.269	**-1.099**	**1.119**	**-2.86**	**-1.263**	**1.256**	-	-	-	-	**Akt1**	**SGsPSDNsGAEEMEVSLAkPk**	**S124;S129**	GSK2141795
-1.162	**1.587**	-0.31	-0.38	-	-	-	-	-	-	-	-	**Akt1**	**SGsPSDNSGAEEmEVSLAkPk**	**S124**	GSK2141795
-1.283	**1.688**	-0.178	-0.029	-0.004	**-1.572**	-0.127	-0.208	-0.806	0.171	**1.482**	-0.838	**Akt1**	**SGsPSDNSGAEEMEVSLAkPk**	**S124**	GSK2141795

Table. 4

Case #	Sample name	BioBank #	Tissue Type	Tissue weight (mg)
1	1T	14981	Pancreatic cancer	362
1	1NT	14980	Background pancreas	198
2	*2T*	*14837*	*Pancreatic cancer*	*102*
2	*2NT*	*14836*	*Background pancreas*	*128*
3	*3T*	*14786*	*Pancreatic cancer*	*135*
3	*3NT*	*14785*	*Background pancreas*	*56*
4	4T	14938	Pancreatic cancer	458
4	4NT	14987	Background pancreas	231
5	5T	11967	Pancreatic cancer	204
5	5NT	11966	Background pancreas	223
6	6T	11946	Pancreatic cancer	204
6	6NT	11945	Background pancreas	136
7	7T	11250	Pancreatic cancer	303
7	7NT	11251	Background pancreas	240
8	8T	10652	Pancreatic cancer	315
8	8NT	10653	Background pancreas	273
9	9T	10619	Pancreatic cancer	247
9	9NT	10618	Background pancreas	223
10	10T	10666	Pancreatic cancer	436
10	10NT	10665	Background pancreas	489
11	11T	14894	Pancreatic cancer	145
11	11NT	14896	Background pancreas	150
12	12T	16195	Pancreatic cancer	113
12	12NT	16194	Background pancreas	110
13	13T	14784	Pancreatic cancer	202
13	13NT	14783	Background pancreas	190
14	14T	14950	Pancreatic cancer	190
14	14NT	14949	Background pancreas	120

Table. 5

1pT3N1	Stage IIB
4pT3N1	Stage IIB
5pT3N0	**Stage IIA**
6pT3N1	Stage IIB
7pT3N1	Stage IIB
8pT3N0	**Stage IIA**
9pT3N1	Stage IIB
10pT3N0	**Stage IIA**
11pT3N1	Stage IIB
12pT3N1	Stage IIB
13pT3N1	Stage IIB
14pT3N1	Stage IIB

Table. 6

Clinical information (e.g. time of recurrence)	Case #
Liver metastasis at 11 mo	1
No recurrence for 10 mo	4
Local recurrence at 19 mo	5
No recurrence for 19 mo	6
No recurrence for 13 mo	7
No recurrence for 23 mo	8
Liver metastasis at 5 mo	9
Lung metastasis at 31 mo	10
Local recurrence, peritoneal disease at 17 mo	11
No recurrence for 8 mo	12
No recurrence for 16 mo	13
Local recurrence, peritoneal disease at 2 mo	14

Table. 7

Sample-Name	µg	µg 8plex
1T	3900	**31200**
1NT	3900	
4T	3900	
4NT	3900	
5T	3900	
5NT	3900	
6T	3900	
6NT	3900	
10NT	3900	**29800**
10T	3900	
9NT	3900	
9T	3900	
8NT	3900	
8T	3900	
7NT	3200	
7T	3200	
11T	3900	**25720**
11NT	3900	
12T	2800	
12NT	2800	
13T	2560	
13NT	2560	
14T	3600	
14NT	3600	

Table. 8

TMT reporter mass:	126	127e	127	128	129e	129	130	131
TMT8plex-1	1T	1NT	4T	4NT	5T	5NT	6T	6NT
TMT8plex-2	10NT	10T	9NT	9T	8NT	8T	7NT	7T
TMT8plex-3	11T	11NT	12T	12NT	13T	13NT	14T	14NT

Table. 9

Sample/aliquot	SCX-HPLX	Enrichment method
TMT8plex 1a	12 x fractions	for TiO_2
TMT8plex 1b	12 x fractions	for IMAC
TMT8plex 1c	12 x fractions	for total protein
TMT8plex 2a	12 x fractions	for total protein
TMT8plex 2b	12 x fractions	for IMAC
TMT8plex 2c	12 x fractions	for TiO_2
TMT8plex 3a	12 x fractions	for TiO_2
TMT8plex 3b	12 x fractions	for IMAC
TMT8plex 3c	12 x fractions	for total protein

Table. 10

					Phosphopeptide log2ratios MEDIAN (TiO2 + IMAC + Non-enrich)											
				Time in freezer (Months)	7	18	23	24	8	13	19	20	3	12	21	28
				Time of assesment of recurrence/non-recurrence (Months)	11	19	5	17	2	10	19	13	8	16	23	31
				Recurrence	+	+	+	+	+	-	-	-	-	-	-	+
				Lymph node mets	+	-	+	+	+	+	+	+	+	+	-	-
					1T/1NT	5T/5NT	9T/9NT	11T/11NT	14T/14NT	4T/4NT	6T/6NT	7T/7NT	12T/12NT	13T/13NT	8T/8NT	10T/10NT
Uniprot-ID	Protein	Global	Peptide sequence	GetLocalizedSites	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio
O14639	Actin-binding LIM protein 1	S452	.STsQGSINSPVYSR	S3: 99.6	1.12	3.57	2.38	1.07	3.27	-0.15	2.27	0.13	0.14	2.06	3.02	2.70
O94929	Actin-binding LIM protein 3	S388	.qGmsPTFSR	S4: 99.5	1.84	3.03	2.81	2.55	2.34	-0.64	0.99	0.18	-0.30	1.07	1.39	1.34
O94929	Actin-binding LIM protein 3	S388	.qGMsPTFSR	S4: 100.0	1.68	1.68	1.77	2.76	1.67	-0.38	-0.33	0.43	-0.48	1.61	0.76	0.95
O95999	B-cell lymphoma/leukemia 10	S138	.SNsDESNFSEkLR	S3: 100.0	1.04	1.29	1.95	0.91	2.15	-0.59	0.85	0.66	0.13	0.25	2.00	2.49
Q5SW79	Centrosomal protein of 170 kDa	S1160	.lGsLSAR	S3: 100.0	1.36	1.75	1.74	1.48	1.70	0.22	1.75	-0.82	-0.13	0.49	2.09	0.00
Q5SW79	Centrosomal protein of 170 kDa	S1160;S1165	.lGsLSARsDSEATISR	S3: 99.4; S8: 98.8	1.56	2.06	1.06	3.89	1.48	0.53	0.82	0.49	-0.20	0.26	-0.11	0.23
Q5VZ89	DENN domain-containing protein 4C	S1089	.STsLSALVR	S3: 100.0	0.74	2.33	1.41	1.45	2.33	-0.10	1.82	-0.40	-0.40	0.84	1.68	0.72
P98082	Disabled homolog 2	S723	.qVsLPVTk	S3: 100.0	1.01	1.09	1.09	1.09	0.73	-0.33	0.09	-0.04	-0.41	-0.64	1.84	-0.78
Q7L591	Docking protein 3	S330	.aTsLPSLDTPGELR	S3: 100.0	2.20	1.28	2.52	0.91	2.09	0.80	1.14	-0.35	-1.01	-0.26	2.09	-0.61
P36507	Dual specificity mitogen-activated protein kinase kinase 2	T394	.lNQPGtPTR	T6: 100.0	1.79	1.98	1.84	0.96	2.00	-0.53	1.92	-0.60	-0.39	0.16	-0.05	2.09
P36507	Dual specificity mitogen-activated protein kinase kinase 2	T394	.lNQPGtPTRTAV	T6: 100.0	3.41	2.14	2.12	4.26	2.39	-0.13	0.22	0.31	-0.70	-0.99	-1.14	1.21
Q32P44	Echinoderm microtubule-associated protein-like 3	S176	.aIsSANLLVR	S3: 100.0	1.22	2.34	1.81	2.43	2.85	-0.24	1.40	0.11	-0.74	0.88	2.36	1.60
P21333	Filamin-A	S1459	.cSGPGLsPGmVR	S7: 100.0	1.94	1.44	1.52	2.86	1.47	0.36	0.95	-1.87	-0.94	-0.39	2.83	0.47
Q14315	Filamin-C	S2233	.lGsFGSITR	S3: 100.0	1.47	1.93	2.19	2.67	2.52	1.62	0.52	0.58	-0.40	2.62	2.35	0.53
Q9P107	GEM-interacting protein	S437	.SLDsPTSSPGAGTR	S4: 100.0	1.76	1.61	1.46	2.27	1.97	0.28	1.15	-0.15	-0.49	0.20	1.92	0.72
Q9Y4H4	G-protein-signaling modulator 3	S35;S39	.sAPPsPPPPGTR	S1: 100.0; S5: 100.0	1.98	1.44	2.08	1.27	2.14	0.73	0.10	-0.08	-0.63	0.62	0.58	-0.38
Q9BPY8	Homeodomain-only protein	S70	.SEGLPSEcRsVTD	S10: 100.0	2.39	1.72	1.86	4.35	3.26	0.27	2.79	-0.67	-0.98	0.62	-0.89	-0.18
Q9Y4H2	Insulin receptor substrate 2	S577	.TYsLTTPAR	S3: 100.0	0.78	1.78	2.34	3.63	1.14	-0.33	0.38	0.00	-0.18	0.46	1.94	0.10
Q8N3V7-2	Isoform 2 of Synaptopodin	S894	.rGsLPAEAScTT	S3: 100.0	1.56	0.89	0.90	3.05	1.68	0.04	0.87	-0.98	-0.47	-0.19	-2.26	-0.72
Q15149-4	Isoform 4 of Plectin	S21	.TSsEDNLYLAVLR	S3: 100.0	1.38	2.63	3.87	6.14	3.72	0.45	1.06	1.50	-0.05	1.55	2.83	3.20
Q15149-4	Isoform 4 of Plectin	S4249	.SSsVGSSSSYPISPAVSR	S3: 100.0	1.20	2.29	1.64	2.18	4.12	-0.07	2.36	0.02	-0.44	2.13	2.45	1.06
Q9P266	Junctional protein associated with coronary artery disease	S757	.SLsPSSNSAFSR	S3: 100.0	1.30	1.41	0.96	1.65	0.98	-0.11	1.81	-0.05	-0.33	0.44	1.85	0.21
P08727	Keratin, type I cytoskeletal 19	S13	.qSSATsSFGGLGGGSVR	S6: 99.6	1.17	2.27	3.25	4.23	0.81	-0.29	1.22	-0.35	0.09	1.64	2.06	0.90
O43896	Kinesin-like protein KIF1C	S1092	.qRsAPDLkESGAAV	S3: 100.0	1.90	1.77	2.23	4.53	2.29	-0.21	-0.69	0.81	-0.82	1.79	-0.40	1.63

Table. 11A

Q86X29	Lipolysis-stimulated lipoprotein receptor	S643	.nLALsRESLVV	S5: 99.6	2.55	1.36	3.18	3.83	2.03	0.76	0.30	-0.97	-0.21	0.06	-0.57	-0.54
Q86X29	Lipolysis-stimulated lipoprotein receptor	S643;S646	.nLALsREsLVV	S5: 100.0; S8: 100.0	2.61	2.84	4.53	5.46	2.19	0.69	0.79	0.70	0.67	1.88	1.43	3.09
Q93052	Lipoma-preferred partner	S609	.vLTAkAsTDL	S7: 99.7	2.36	2.27	2.84	3.20	1.00	-0.26	-0.53	-0.73	-0.76	-0.20	0.25	-0.69
Q7Z406	Myosin-14	S1504	.aLsLTR	S3: 100.0	2.71	2.48	2.20	1.99	2.26	-1.14	1.90	1.09	0.64	3.17	4.93	2.75
Q09666	Neuroblast differentiation-associated protein AHNAK	T5824	.fGtFGGLGSk	T3: 100.0	1.59	1.23	1.21	3.71	1.14	-1.02	-0.95	-0.22	-0.01	0.08	-0.02	-0.37
Q8WX93	Palladin	S893	.iAsDEEIQGTkDAVIQDLER	S3: 100.0	1.40	1.60	2.27	2.86	1.76	1.19	-0.14	-0.32	-1.31	0.52	1.49	1.43
Q15149	Plectin	S4386	.SSsVGSSSSYPISPAVSR	S3: 100.0	1.20	2.29	1.64	2.18	4.12	-0.07	2.36	0.02	-0.44	2.13	2.45	1.06
Q96QH2	PML-RARA-regulated adapter molecule 1	S382;S388	.TSsEPEFNsLPR	S3: 100.0; S9: 100.0	2.00	1.08	3.17	1.02	1.64	0.53	1.28	-0.17	-0.84	1.05	2.05	-1.15
P02545	Prelamin-A/C	S12	.sGAQASSTPLSPTR	S1: 100.0	1.10	1.21	0.98	1.50	3.17	-0.04	0.80	-0.45	-0.06	0.80	2.66	0.57
P02545	Prelamin-A/C	S392	.ISPsPTSQR	S4: 100.0	1.99	1.55	1.35	2.98	0.74	-0.81	1.27	-0.66	-0.49	0.59	0.30	0.31
P02545	Prelamin-A/C	S632	.SVGGsGGGSFGDNLVTR	S5: 100.0	1.11	1.08	1.14	2.21	1.42	-0.67	1.07	-1.32	-0.58	0.25	2.49	-0.48
P02545	Prelamin-A/C	S636	.SVGGSGGGsFGDNLVTR	S9: 100.0	1.51	1.54	2.30	3.18	1.54	-0.49	0.93	-0.58	-0.65	0.81	3.07	-0.72
Q2M2I3	Protein FAM83E	S351	.vSPATPGPALsDILR	S11: 100.0	0.96	1.44	1.04	0.74	1.04	-0.40	-0.01	0.59	0.80	0.49	1.50	-0.75
Q9Y6F6	Protein MRVI1	S657	.SmsLTLGk	S3: 100.0	0.81	1.37	0.90	1.26	0.96	0.35	0.45	-0.35	-0.08	0.09	1.04	-0.61
Q9Y6F6	Protein MRVI1	S657	.SMsLTLGk	S3: 100.0	0.76	1.50	0.72	1.11	0.94	0.22	0.49	-0.17	-0.03	0.19	1.72	-0.35
Q92597	Protein NDRG1	S332;S333	.TASGssVTSLDGTR	S5: 99.5; S6: 99.5	0.95	2.42	3.26	3.19	2.67	-0.23	2.38	0.14	0.91	0.53	3.82	3.22
Q92597	Protein NDRG1	S333	.TASGSsVTSLDGTR	S6: 99.6	1.06	1.08	2.12	2.55	1.80	0.00	1.48	-0.31	0.62	0.49	3.61	1.29
Q92597	Protein NDRG1	S333;S336	.TASGSsVTsLDGTR	S6: 98.8; S9: 99.4	0.76	0.92	1.82	1.32	1.91	0.74	1.31	0.12	1.64	2.88	3.16	0.76
P29590	Protein PML	T867	.aEGVStPLAGR	T6: 99.6	1.30	2.22	2.39	1.39	1.64	-0.26	0.87	0.03	-0.18	1.06	2.02	1.26
Q9BXF6	Rab11 family-interacting protein 5	S307	.TYsDEANQmR	S3: 99.4	1.99	1.99	1.49	2.75	2.53	-0.27	2.32	0.05	-0.44	0.96	4.18	2.31
Q96PE2	Rho guanine nucleotide exchange factor 17	S420	.SPsFGAGEGLLR	S3: 100.0	0.90	1.92	1.29	1.66	3.09	0.68	0.20	-0.35	-0.05	1.14	1.56	0.89
Q92974	Rho guanine nucleotide exchange factor 2	S174	.iLSQsTDSLNmR	S5: 99.5	1.74	1.77	1.80	1.34	1.82	0.64	0.78	0.42	-0.36	0.99	2.02	0.41
Q92974	Rho guanine nucleotide exchange factor 2	S174	.iLSQsTDSLNMR	S5: 100.0	0.90	1.14	1.99	1.99	3.49	0.27	-0.81	0.84	0.08	0.91	1.37	0.47
Q9UQ35	Serine/arginine repetitive matrix protein 2	S24	.nLsLVR	S3: 100.0	0.93	1.20	0.90	1.34	2.36	0.33	1.20	-0.48	0.44	0.37	1.65	-0.46
Q5VT25	Serine/threonine-protein kinase MRCK alpha	S1629	.SmsASSGLSAR	S3: 100.0	0.92	1.41	1.05	0.91	2.45	0.29	1.13	0.14	-0.32	1.61	3.11	1.09
Q5TCZ1	SH3 and PX domain-containing protein 2A	S420	.aQlsSPNLR	S4: 100.0	1.54	2.67	2.92	3.20	1.76	0.85	0.98	0.30	-1.13	1.24	3.18	1.13
P53814	Smoothelin	S301	.SLsVLSPR	S3: 100.0	1.30	0.95	1.11	1.19	1.34	1.33	-0.89	0.72	0.05	1.79	0.03	-1.25
Q9HBL0	Tensin-1	S1119	.SGsLGQPSPSAQR	S3: 100.0	0.87	2.09	0.83	1.33	2.61	-0.84	1.80	-0.63	-0.27	0.91	1.46	2.14
Q9Y2W1	Thyroid hormone receptor-associated protein 3	S682	.iDlsPSTFR	S4: 99.6	1.12	0.99	0.85	1.07	1.14	0.58	0.81	0.09	-0.30	0.72	2.48	0.94
Q13263	Transcription intermediary factor 1-beta	S473	.sGEGEVSGLmR	S1: 100.0	1.58	4.42	3.01	3.15	2.72	0.40	1.69	-0.27	-0.31	1.54	2.50	0.76
Q13263	Transcription intermediary factor 1-beta	S473	.sGEGEVSGLMR	S1: 100.0	0.85	2.72	2.71	2.68	1.77	0.29	0.54	-0.09	-0.36	1.13	1.84	-0.36
O43294	Transforming growth factor beta-1-induced transcript 1 protein	S418	.cVsALGR	S3: 100.0	1.17	1.32	0.71	3.39	1.19	0.02	0.94	-0.57	-0.46	0.45	1.25	0.27
Q7Z7L8	Uncharacterized protein C11orf96	S425	.IRNsLDSSDSDSAL	S4: 100.0	1.85	0.94	1.66	3.07	2.08	-0.05	-0.51	1.33	-0.82	-0.51	-0.24	0.35
Q8IVT2	Uncharacterized protein C19orf21	S394	.aLsSDSILSPAPDAR	S3: 100.0	4.39	1.59	1.55	3.12	1.63	-0.39	0.25	0.88	0.33	2.74	1.88	-1.33
P08670	Vimentin	S55	.SLYAsSPGGVYATR	S5: 100.0	1.22	2.03	1.36	2.87	3.71	-0.74	0.84	-0.10	-0.39	0.53	2.54	1.02
P08670	Vimentin	S73	.SsVPGVR	S2: 100.0	1.18	1.33	0.96	2.12	2.97	-1.87	2.07	-1.75	-1.67	-0.51	2.91	-0.62
Q13303	Voltage-gated potassium channel subunit beta-2	S9	.mYPESTTGsPAR	S9: 98.9	0.91	0.72	1.68	1.77	1.67	0.89	1.11	0.13	-0.48	-0.34	0.22	0.01

Table. 11A (continued)

P23396	40S ribosomal protein S3	T221	.dEIUPTPISEQk	T7: 100.0	-2.43	-2.21	-2.17	-4.46	-5.21	0.16	-1.00	-0.56	0.75	2.18	-1.94	-3.01
P62753	40S ribosomal protein S6	S235;S236	.tLssIRASTSk	S3: 100.0; S4: 100.0	-2.64	-0.77	-1.61	-1.74	-2.82	0.09	-0.08	0.33	0.20	0.70	-1.33	-0.59
P35611	Alpha-adducin	S338	.SKSPGsPVGEGTGSPPk	S6: 100.0	-0.99	-1.46	-0.97	-1.52	-1.61	-1.24	-0.73	-1.54	0.12	-1.67	-2.45	-1.75
Q9H4G0	Band 4.1-like protein 1	S510	.hQAsINELk	S4: 100.0	-1.64	-1.82	-2.71	-2.87	-4.42	-0.40	-1.11	0.09	1.11	-1.61	-2.74	-4.86
Q9H4G0	Band 4.1-like protein 1	S820	.gGFsETRIEk	S4: 100.0	-1.71	-2.18	-1.31	-2.20	-1.98	-0.95	-0.75	-0.73	-0.06	-0.69	-3.47	-2.24
P27824	Calnexin	S583	.aEEDEILNRsPR	S10: 100.0	-1.48	-1.43	-1.68	-2.39	-2.67	-0.59	-0.65	-0.01	0.38	-0.10	-1.34	-0.90
O60716	Catenin delta-1	S352	.gSLASLDSLNk	S8: 100.0	-0.89	-0.84	-1.14	-1.97	-1.81	0.09	-0.17	-0.29	0.62	0.70	-0.58	-0.26
Q53SF7	Cordon-bleu protein-like 1	S294;T298	.dQTAsAPAPLVNk	S5: 100.0; T9: 100.0	-1.85	-1.08	-1.97	-3.33	-2.39	0.77	-1.22	0.33	0.32	1.52	-2.35	-1.62
P15924	Desmoplakin	S2209	.SmsFQGIR	S3: 100.0	-1.54	-1.22	-1.72	-2.91	-2.71	-0.39	-0.41	-0.62	0.19	0.58	-1.63	-2.18
P15924	Desmoplakin	S2821;S2825	.gLPSPYNMSsAPGsR	S10: 100.0; S14: 100.0	-1.46	-0.76	-1.01	-1.89	-2.74	0.25	-0.27	-0.58	0.60	2.28	-0.51	-1.58
Q92466	DNA damage-binding protein 2	S26	.SRsPLELEPEAk	S3: 100.0	-0.76	-0.71	-1.22	-0.86	-1.66	0.92	-0.11	0.54	0.21	2.54	-1.15	-0.22
Q92685	Dol-P-Man:Man(5)GlcNAc(2)-PP-Dol alpha-1,3-mannosyltransferase	S13	.gRSGsAAQAEGLck	S5: 99.7	-2.46	-1.85	-3.44	-3.90	-3.57	-0.89	-0.41	-0.03	0.17	-0.22	-1.26	-0.67
Q8IUH5D	Doublecortin domain-containing protein 2	S270	.STVGSDINSsPQPLkR	S10: 100.0	-1.01	-1.89	-1.79	-1.54	-1.81	0.06	1.11	-1.56	0.85	-1.05	-0.31	-1.46
Q8HC35	Echinoderm microtubule-associated protein-like 4	S200;T201	.IPrsPKLIPk	S3: 100.0; T4: 100.0	-1.73	-1.21	-1.71	-3.10	-2.82	0.17	-1.34	0.21	0.23	0.02	-2.15	-2.22
Q9Y6R1	Electrogenic sodium bicarbonate cotransporter 1	S223	.sLADIGk	S1: 100.0	-3.38	-1.92	-3.08	-3.72	-4.54	-0.46	0.16	-1.41	0.57	0.28	-4.39	-4.42
Q9Y6R1	Electrogenic sodium bicarbonate cotransporter 1	S223	.SNLRsLADIGk	S5: 100.0	-3.34	-3.15	-2.92	-3.04	-4.36	-2.02	-0.39	-0.05	0.99	-0.11	-3.10	-3.27
Q9Y6R1	Electrogenic sodium bicarbonate cotransporter 1	S232;S233	.SLADIGkTVssASR	S10: 100.0; S11: 100.0	-0.73	-2.22	-2.33	-1.41	-2.73	-0.43	0.11	-0.45	0.95	-0.30	-3.12	-3.30
P13639	Elongation factor 2	T57	.aGETRFtDTR	T7: 99.4	-1.98	-0.80	-1.85	-3.36	-1.38	-0.11	1.62	-0.05	0.31	2.56	-0.84	-0.11
Q04637	Eukaryotic translation initiation factor 4 gamma 1	S1185	.sFSKEVEER	S1: 100.0	-1.88	-1.14	-2.42	-4.28	-2.77	-0.31	-0.02	0.85	0.84	1.14	-1.83	-0.89
O60841	Eukaryotic translation initiation factor 5B	S214	.nMPGPNIsGNEDDDASF	S9: 100.0	-0.75	-0.91	-1.48	-3.61	-1.98	1.28	-0.02	-0.05	0.53	1.36	0.61	-0.54
Q9Y4F1	FERM, RhoGEF and pleckstrin domain-containing protein 1	S23;T24	.tGAPENSGsIsLER	S10: 100.0; T11: 100.0	-1.59	-1.43	-1.12	-3.06	-2.16	-0.31	0.51	-0.75	0.70	1.25	-0.81	-0.94
Q9NQX3	Gephyrin	S305	.aSHsAVDITk	S4: 100.0	-1.44	-0.95	-2.85	-2.77	-2.31	-0.56	-0.80	-0.04	0.95	-0.10	-0.92	-0.11
P09210	Glutathione S-transferase A2	S202	.fLQPGsPR	S6: 100.0	-2.45	-1.29	-3.14	-1.92	-4.51	-0.14	-0.52	-1.75	-0.15	0.20	-3.92	-2.82
Q13322	Growth factor receptor-bound protein 10	S104	.SIQPQVsPR	S7: 100.0	-0.85	-1.44	-1.01	-1.33	-2.48	0.40	-0.31	-1.01	0.54	-0.10	-2.26	-1.90
P12268	Inosine-5-monophosphate dehydrogenase 2	S160	.lVGIISR	S7: 100.0	-0.77	-1.18	-2.20	-2.46	-1.72	0.62	-0.43	-0.57	0.06	-0.46	-1.80	-2.19
Q8IZK5-3	Isoform 3 of CKLF-like MARVEL transmembrane domain-containing protein 1 T179		.dVDSRPEIQRLDt	T13: 100.0	-1.32	-1.29	-0.87	-0.97	-2.27	-0.47	0.04	0.08	0.15	0.08	-0.77	-1.04
O94875-3	Isoform 3 of Sorbin and SH3 domain-containing protein 2	S374	.SFTSSPSsPSR	S9: 97.8	-1.10	-1.14	-1.04	-2.71	-0.78	-0.93	0.41	-0.10	0.18	-1.61	-1.20	-0.89
P05787	Keratin, type II cytoskeletal 8	S274	.aQVEDIANRsR	S10: 100.0	-2.16	-1.56	-1.20	-3.34	-3.71	-0.64	0.03	-0.23	0.76	1.53	-1.99	-1.50
Q8IZK5-3	Isoform 3 of CKLF-like MARVEL transmembrane domain-containing protein 1 T179	S517;S521	.dHYQkETGsAPGsPR	S9: 100.0; S13: 100.0	-1.03	-1.26	-1.98	-2.46	-2.63	0.00	0.09	0.01	0.20	0.84	-1.51	-0.92
P11137	Microtubule-associated protein 2	S1782	.vDHGAEITQsPGR	S11: 100.0	-1.84	-1.06	-0.91	-3.18	-2.64	-0.89	1.47	-0.34	0.63	-0.23	-1.86	-0.35
P50219	Motor neuron and pancreas homeobox protein 1	S77;S79	.IRAsPsPPR	S5: 100.0; S7: 100.0	-0.71	-1.82	-2.12	-1.56	-2.98	0.69	1.10	-1.63	0.40	-0.06	-2.70	-2.94
Q96TA1	Niban-like protein 1	S696	.aAPEASSPPAsPLQHLLPGk	S11: 100.0	-1.06	-0.85	-1.35	-1.73	-1.43	-0.16	-1.05	-0.22	-0.27	-0.31	-1.45	-0.94

Table. 11B

Q6KC79	Nipped-B-like protein	S2658	.aTSLGGGSpK	S10: 100.0	-1.69	-1.21	-2.17	-2.15	-0.95	1.24	-0.18	1.10	0.19	-1.31	-2.83	0.82	
P19338	Nucleolin	S563	.IELQGPRGsPNAR	S9: 100.0	-1.62	-1.07	-1.55	-1.88	-1.03	0.23	0.31	0.57	0.37	-1.02	0.96	0.65	
Q8TEW8	Partitioning defective 3 homolog B	S780	.gsNEsFRAAIDk	S5: 100.0	-1.58	-0.95	-0.93	-2.92	-2.35	-0.62	-1.17	-0.60	0.36	0.36	-2.03	-0.76	
Q99959	Plakophilin-2	S251	.smGNLLEk	S1: 100.0	-1.51	-1.31	-1.36	-3.09	-2.55	0.14	-0.52	-0.72	0.30	1.27	-1.90	-2.63	
Q6IQ23	Pleckstrin homology domain-containing family A member 7	S556	.SksmLEVPR	S3: 99.6	-1.04	-1.56	-2.03	-1.62	-1.80	-0.92	-1.15	0.94	0.95	-0.47	-1.91	-1.14	
Q6IQ23	Pleckstrin homology domain-containing family A member 7	S604	.sVDISLGDSPR	S1: 100.0	-1.22	-1.39	-1.23	-1.72	-1.68	-0.10	-1.02	0.30	-0.17	-0.24	-1.62	-2.16	
Q53EL6	Programmed cell death protein 4	S457	.rFvsEGDGGR	S4: 100.0	-1.13	-1.07	-2.58	-2.40	-2.05	-0.73	-0.47	-0.32	0.55	0.57	-2.80	-1.25	
Q9P2B2	Prostaglandin F2 receptor negative regulator	S875	.lmsmEmD	S3: 100.0	-1.51	-1.57	-2.85	-2.27	-1.15	-0.15	0.20	-0.09	0.41	0.67	-0.48	-0.26	
Q9P2B2	Prostaglandin F2 receptor negative regulator	S875	.lmsMEmD	S3: 100.0	-1.20	-1.40	-2.24	-1.40	-0.96	-0.41	0.53	0.03	0.64	-0.13	-0.44	0.03	
Q9P2B2	Prostaglandin F2 receptor negative regulator	S875	.lMsmEmD	S3: 100.0	-0.74	-1.39	-1.23	-1.71	-0.91	-0.42	0.95	0.54	0.38	-0.36	-0.62	-0.09	
Q9H8M9	Protein FAM176A	S114	.nVFTsAEELER	S5: 100.0	-1.44	-1.89	-2.72	-3.11	-2.71	0.09	-0.79	-0.88	0.53	-0.40	-2.15	-2.96	
Q8N512	Protein FAM63A	S103	.acSmPQEIPQsPR	S11: 100.0	-1.10	-1.10	-1.76	-0.72	-0.92	-1.30	-1.78	-0.54	-0.56	-0.68	-2.38	-1.37	
Q9UN36	Protein NDRG2	S332;S338	.TAsLTSAAsVDGNR	S3: 100.0; S9: 100.0	-1.28	-1.12	-0.91	-1.73	-0.75	0.73	0.34	0.05	0.93	0.91	-0.57	0.56	
Q96A00	Protein phosphatase 1 regulatory subunit 14A	S126;S136	.qPGLRQPsPSHDGSLsPLQDR	S8: 100.0; S16: 99.2	-2.38	-2.48	-3.04	-2.44	-1.96	0.85	-0.45	-0.20	0.84	0.28	-1.78	-1.70	
Q14671	Pumilio homolog 1	S709	.rDsLTGSSDLYk	S3: 100.0	-0.96	-2.28	-2.91	-2.15	-2.75	-0.51	-0.53	0.09	0.45	-1.78	-1.42	-2.68	
P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mito S232	S232	.YGmGTsVER	S6: 99.5	-1.67	-1.82	-2.07	-3.45	-2.82	0.55	-0.77	-0.31	0.47	0.69	-2.68	-2.81	
P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mito S232	S232	.YGmGTsVER	S6: 99.5	-1.53	-1.41	-1.03	-2.81	-3.02	0.38	-0.57	0.04	0.13	2.82	-0.81	-0.14	
P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mito T231	T231	.YGMGtSVER	T5: 99.5	-1.74	-1.84	-1.89	-2.51	-1.72	0.52	-0.26	0.16	-0.15	2.09	-1.96	-1.79	
Q5TZA2	Rootletin	S1460	.aPSPARPRPVPGsPAR	S12: 100.0	-1.45	-1.23	-0.94	-0.80	-1.80	0.80	0.13	-1.98	0.25	0.68	-0.01	-1.79	
Q8UQ35	Serine/arginine repetitive matrix protein 2	S295;S297	.THTTALAGRsPsPASGR	S10: 100.0; S12: 100.0	-0.93	-0.83	-1.03	-1.36	-1.16	0.06	0.03	-0.18	0.31	0.35	-1.96	-0.14	
P10398	Serine/threonine-protein kinase A-Raf	S157	.qQPYHsVQDLSGCGR	S6: 100.0	-0.77	-1.08	-1.03	-3.05	-1.42	-0.76	-0.33	0.38	0.75	-0.38	0.27	-0.25	
Q13573	SNW domain-containing protein 1	S224;S232	.gPrSPPAPVMHsPSR	S4: 100.0; S12: 100.0	-1.12	-0.88	-1.36	-1.71	-0.84	-0.37	-0.36	0.56	0.29	0.91	-0.76	1.11	
E9PAS5	Sorbin and SH3 domain-containing 2	S13	.rQsSPNLLAAGR	S3: 100.0	-1.02	-2.04	-2.23	-3.17	-3.31	-0.42	0.07	-0.32	0.29	-0.38	-1.11	-2.61	
E9PAS5	Sorbin and SH3 domain-containing 2	S13;S14	.rVQsPNLLAAGR	S4: 100.0; S5: 100.0	-0.82	-1.85	-1.27	-0.72	-0.83	-0.30	0.54	-0.97	0.75	-0.40	-1.42	-2.33	
E9PAS5	Sorbin and SH3 domain-containing 2	S13;S14	.rQssPNLLAAGR	S3: 100.0; S4: 100.0	-0.77	-1.75	-1.84	-1.87	-1.93	-0.84	0.04	-0.99	-0.02	-0.15	-1.09	-2.04	
E9PAS5	Sorbin and SH3 domain-containing 2	S373	.SFTSSPSsPSR	S9: 97.8	-1.10	-1.14	-1.04	-2.71	-0.78	-0.93	0.41	-0.10	0.18	-1.61	-1.20	-0.89	
O60343	TBC1 domain family member 4	S591	.lGSVDsFER	S6: 100.0	-1.04	-1.52	-1.14	-1.99	-2.38	0.35	0.12	-0.56	0.34	-0.10	-1.61	-2.01	
P15374	Ubiquitin carboxyl-terminal hydrolase isozyme L3	S130	.kFLEESVSmsPEER	S10: 99.7	-1.32	-1.48	-1.51	-2.34	-1.84	0.58	-0.83	0.79	0.59	-0.29	-0.04	-1.09	
O60701	UDP-glucose 6-dehydrogenase	S476	.rIPYAPsGEIPk	S7: 100.0	-1.27	-1.32	-1.16	-2.76	-2.67	-0.67	-0.69	0.30	0.66	0.21	-1.15	-1.06	

Table. 11B (continued)

			TiO2+IMAC+ Nonenrich		Protein (patient 1)	Protein (patient 4)	Protein (patient 5)	Protein (patient 6)	Protein (patient 7)	Protein (patient 8)	Protein (patient 9)	Protein (patient 10)	Protein (patient 11)	Protein (patient 12)	Protein (patient 13)	Protein (patient 14)
			Protein(non-phos peptides)		1T/1NT	4T/4NT	5T/5NT	6T/6NT	7T/7NT	8T/8NT	9T/9NT	10T/10NT	11T/11NT	12T/12NT	13T/13NT	14T/14NT
KEGG	Uniprot ID	Protein	t.test p-values	log2 (T/NT)	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2 ratio	log2ratio	log2ratio	log2ratio	log2ratio	log2ratio
Glycolysis/ Gluconeogenesis	P14618	Pyruvate kinase isozymes M1/M2	4.20E-05	0.383	0.256	0.916	0.210	0.321	0.287	0.618	0.784	0.344	0.557	0.197	0.488	0.314
	Q86Z02	Homeodomain - interacting protein kinase 1	1.59E-04	1.002	1.189	0.756	1.171	0.545	0.450	2.508	1.711	0.848	2.997	1.216	0.992	0.767
	Q14847	LIM and SH3 domain protein 1	2.01E-04	0.496	0.492	0.843	0.413	0.904	0.251	1.574	0.513	1.004	0.354	0.020	0.878	0.565
	P37802	Transgelin - 2	2.34E-04	0.519	0.457	1.032	0.363	0.551	0.425	0.864	1.198	0.397	0.354	0.076	1.044	0.122
	P55036	26S proteasome non ATPase regulatory subunit 4	2.77E-04	-0.445	-0.723	-1.280	-0.994	-0.904	-0.323	-0.599	-0.436	-0.334	-0.547	0.191	-0.335	-1.039
	Q92538	Golgi-specific brefeldin A- resistance guanine nucleotide exchange factor 1	2.84E-04	1.397	0.989	0.689	2.121	2.614	0.169	3.464	2.151	2.040	0.975	0.048	1.979	3.281
	P31937	3-hydroxyisobutyrate dehydrogenase, mitochondrial	3.60E-04	-0.913	-0.969	-0.642	-1.056	-0.477	-0.017	-0.797	-1.374	-0.913	-1.517	0.370	-1.079	-0.933
	P21291	Cysteine and glycine - rich protein 1	3.96E-04	0.628	0.638	1.894	0.360	0.279	0.436	0.856	0.680	1.243	0.908	-0.002	1.646	0.725
	O95394	Phosphoacetylglucosamine mutase	4.04E-04	-0.961	-1.293	0.153	-1.731	-1.014	-0.250	-0.961	-1.832	-1.272	-1.319	-0.105	-0.362	-1.151
TJ	Q96A65	Exocyst complex component 4	5.79E-04	-0.694	-0.742	-0.565	-0.647	-0.936	-0.229	-0.783	-0.227	-1.109	NA	NA	NA	NA
	Q8WX93	Palladin	6.97E-04	0.588	0.909	1.470	0.485	0.476	0.041	0.726	1.016	1.077	0.876	-0.295	0.957	0.243
	Q14195-2	Isoform LCRMP - 4 of Dihydropyrimidinase - related protein 3	7.01E-04	0.555	0.677	0.951	0.592	0.854	0.048	0.354	0.721	0.327	0.453	-0.353	0.906	1.028
	Q9NR12	PDZ and LIM domain protein 7	7.39E-04	0.778	0.599	1.162	0.775	0.544	0.127	1.259	1.589	0.022	1.101	-0.101	1.961	1.365
RAC	P26038	Moesin	7.62E-04	0.334	0.256	0.570	0.391	0.525	-0.003	0.630	0.648	0.525	-0.143	-0.011	0.549	0.445
	P15941	Mucin - 1	8.57E-04	0.873	0.873	0.863	0.666	-0.217	1.737	0.101	1.479	1.051	0.523	0.063	1.170	1.664
VSMC	Q05682	Caldesmon	9.37E-04	0.597	1.015	1.280	0.581	0.601	0.025	0.786	0.732	0.673	-0.003	-0.047	1.672	0.656
	Q02818	Nucleobindin - 1	9.66E-04	0.800	1.472	0.494	2.586	1.895	0.207	2.815	2.055	2.244	-0.517	0.066	1.612	1.581
FA	Q9Y490	Talin - 1	1.06E-03	0.368	0.363	1.017	0.251	0.562	-0.240	0.591	0.567	0.521	0.482	-0.125	0.684	0.549
	O433994	Isoform 4 of Tumor D54 protein	1.17E-03	0.409	0.213	0.567	0.280	1.354	0.408	0.648	0.171	0.453	0.669	-0.168	0.419	1.043
Glycolysis/ Gluconeogenesis	P06733	Alpha - enolase	1.18E-03	0.347	0.033	0.906	-0.092	0.590	0.170	0.804	1.027	0.456	0.458	0.158	0.429	0.300
	P53384	Cytosolic Fe-S cluster assembly factor NUBP1	1.22E-03	-1.109	-0.648	-0.090	-1.135	-1.082	0.092	-2.435	-1.592	-1.309	-1.298	0.205	-0.747	-1.312
FA	P21333	Filamin - A	1.34E-03	0.618	0.795	1.436	0.633	0.544	-0.218	0.993	1.020	0.415	0.444	-0.208	1.620	0.767
	P11277	Spectrin beta chain, erythrocyte	1.42E-03	-0.342	-0.105	-1.101	-0.214	-0.058	-0.887	-0.392	-0.098	-1.198	-0.744	-0.039	-0.791	-0.558
	Q15149-4	Isoform 4 of Plectin	1.62E-03	0.325	0.508	0.557	0.250	0.481	-0.200	1.049	0.689	0.419	0.331	0.033	0.955	0.127
	P40763	Signal transducer and activator of transcription 3	1.63E-03	0.697	0.242	0.945	1.336	1.260	-0.057	1.469	0.955	0.673	-0.055	-0.066	0.432	1.008
	Q15149	Plectin	1.63E-03	0.324	0.507	0.557	0.249	0.481	-0.200	1.049	0.689	0.419	0.327	0.033	0.953	0.127
	Q9HBL0	Tensin-1	1.74E-03	0.359	0.098	0.646	0.120	0.606	-0.140	0.546	0.703	0.554	0.682	-0.049	0.734	1.308
	Q6P597	Kinesin light chain 3	1.78E-	-1.114	-2.285	-0.557	-1.133	-1.020	0.338	-1.864	-1.752	-1.925	-1.232	0.288	-0.196	-2.741

Table. 12

			03													
	Q9Y2D5	A-kinase anchor protein 2	1.88E-03	0.894	1.077	0.799	0.703	0.989	NA	NA	NA	NA	NA	NA	NA	NA
	Q8WVM8	Sec1 family domain-containing protein 1	1.89E-03	-0.455	-0.759	-0.355	-1.016	-0.026	-0.215	-0.558	-1.028	-0.455	-1.697	-0.008	-0.128	-1.241
	G3V1V0	Myosin light polypeptide 6	1.93E-03	0.491	0.302	1.708	0.345	0.723	0.140	1.484	0.897	0.815	0.085	-0.049	1.466	0.419
FA	Q14315	Filamin-C	2.38E-03	0.562	0.286	1.602	0.520	0.384	0.176	1.194	1.051	0.577	0.242	-0.147	1.961	0.647
	Q9UN36	Protein NDRG2	2.52E-03	-0.789	-0.586	-0.142	-1.126	-0.786	-0.161	-2.581	-1.170	-2.302	-1.845	0.557	-0.847	-1.295
	Q8WVV4	Protein POF1B	2.53E-03	0.797	0.588	0.783	1.184	1.674	0.010	1.773	0.664	0.571	-0.513	0.863	2.527	0.753
	C9JRL6	Echinoderm microtubule-associated protein-like 2	2.72E-03	-0.395	NA	NA	NA	NA	-0.453	-0.583	-0.395	-0.362	NA	NA	NA	NA
	Q9UK76	Hematological and neurological expressed 1 protein	2.82E-03	0.477	0.951	0.018	0.117	0.327	0.211	0.907	1.477	0.225	0.477	-0.074	1.257	1.009
	Q3SY69	Mitochondrial 10-formyltetrahydrofolate dehydrogenase	2.88E-03	-1.047	-1.335	0.203	-1.243	-0.368	-0.076	-1.679	-2.561	-1.262	-2.471	0.022	-0.199	-1.977
	O43294	Transforming growth factor beta-1-induced transcript 1 protein	2.88E-03	0.752	1.432	0.238	1.586	0.976	-0.003	0.746	0.780	0.250	2.963	0.200	1.243	0.494
	P49840	Glycogen synthase kinase-3 alpha	2.94E-03	1.101	1.189	0.756	1.171	0.545	NA	NA	NA	NA	2.997	1.216	0.992	0.767
	Q13409	Cytoplasmic dynein 1 intermediate chain 2	3.05E-03	0.471	0.687	0.798	-0.392	0.537	-0.182	0.744	0.938	0.433	1.168	0.025	0.902	1.408
	Q9NR45	Sialic acid synthase	3.09E-03	-0.609	-0.591	-0.093	-0.927	-0.522	-0.047	-0.745	-1.125	-0.571	-1.836	0.201	-0.231	-1.602
	Q9NZU5	LIM and cysteine-rich domains protein 1	3.21E-03	0.361	0.400	0.067	0.673	0.799	0.167	0.601	0.675	0.031	0.181	-0.251	0.312	0.842
	P47756	F-actin-capping protein subunit beta	3.22E-03	0.405	0.329	0.593	0.283	0.538	0.038	1.778	0.798	1.056	-0.014	-0.051	0.597	0.762
	Q86TC9	Myopalladin	3.36E-03	1.050	0.732	2.285	1.104	1.528	NA	NA	NA	NA	0.856	-0.238	1.241	1.649
	O43516	WAS/WASL-interacting protein family member 1	3.82E-03	-0.651	-0.521	-1.005	-0.275	-0.638	-0.039	-1.575	-1.462	-1.235	NA	NA	NA	NA
	Q01995	Transgelin	3.85E-03	0.682	0.802	1.819	0.695	0.418	0.683	0.564	0.955	0.509	-0.484	-0.197	1.992	1.372
	Q01518	Adenylyl cyclase-associated protein 1	3.97E-03	0.391	0.865	1.004	0.263	0.506	-0.079	0.954	0.931	0.214	-0.070	-0.118	0.407	0.392
	P16157	Ankyrin-1	4.05E-03	-0.838	0.467	-1.840	-0.780	-1.896	-1.133	-1.436	0.060	-1.736	0.266	-0.065	-2.186	-1.488
	P07951-2	Isoform 2 of Tropomyosin beta chain	4.24E-03	0.440	0.432	1.583	0.383	0.552	0.160	0.475	0.619	0.307	-0.154	-0.061	1.361	0.705
	P13611-2	Isoform V1 of Versican core protein	4.44E-03	0.541	0.641	-0.078	0.777	0.488	-0.004	0.772	0.861	1.635	1.900	-0.414	0.784	0.746
FA,RAC	P18206	Vinculin	4.46E-03	0.326	0.775	0.817	0.196	0.252	-0.055	0.883	0.581	0.315	-0.194	-0.057	0.829	0.369
Glycolysis/ Gluconeogenesis	P00558	Phosphoglycerate kinase 1	4.57E-03	0.307	0.038	0.849	0.323	0.130	0.086	1.036	0.802	0.155	0.454	0.263	0.638	-0.175
	O75387	Large neutral amino acids transporter small subunit 3	4.63E-03	-1.862	NA	NA	NA	NA	-0.834	-1.765	-2.786	-1.960	-1.985	0.159	-1.295	-4.019
	Q96IZ0	PRKC apoptosis WT1 regulator protein	4.82E-03	0.871	0.840	0.330	0.025	1.105	0.438	3.141	1.221	0.672	1.218	-0.175	2.446	0.503
	P06703	Protein S100-A6	4.94E-03	0.470	1.119	0.310	0.543	0.409	0.416	0.839	1.961	0.572	-0.122	-0.057	0.461	0.287
	Q04695	Keratin, type I cytoskeletal 17	4.96E-03	0.572	0.378	0.613	1.414	0.569	-0.367	1.246	1.864	1.015	0.474	0.194	2.079	-0.187
	P01008	Antithrombin-III	5.02E-03	0.420	0.205	-0.182	0.269	0.771	0.752	1.138	0.843	0.836	1.239	-0.213	-0.174	1.241
	Q8IVF2	Protein AHNAK2	5.28E-03	0.662	1.321	0.473	0.734	0.453	0.309	0.848	1.445	-0.376	0.965	0.402	3.307	0.924
	P30050	60S ribosomal protein L12	5.54E-03	-0.613	-1.162	0.357	-1.019	-0.329	0.015	-1.217	-1.663	-0.551	-1.538	0.143	-0.136	-1.673
	O43684	Mitotic checkpoint protein BUB3	5.93E-03	0.828	0.828	1.084	0.828	1.066	0.090	1.591	1.493	0.737	-0.681	0.186	1.592	-0.201
	P09210	Glutathione S-transferase A2	5.99E-03	-1.067	-1.535	0.017	-1.740	-0.787	0.053	-1.587	-2.356	-1.439	-2.597	0.432	0.548	-2.534

Table. 12 (continued)

	Q14980	Nuclear mitotic apparatus protein 1	6.22E-03	0.369	-0.122	0.614	0.093	1.496	0.055	0.263	0.248	0.822	0.568	0.092	1.408	0.538
	O14639	Actin-binding LIM protein 1	6.72E-03	-0.924	-0.363	-0.565	-1.934	-0.544	0.113	-2.831	-1.788	0.522	-2.268	0.039	-0.996	-1.504
	Q96CX2	BTB/POZ domain - containing protein KCTD12	6.77E-03	0.477	0.644	0.269	0.278	0.891	-0.148	0.593	0.663	1.200	-0.364	-0.045	1.008	1.487
	Q7Z2W4	Zinc finger CCCH- type antiviral protein 1	7.06E-03	0.825	1.636	0.838	1.609	0.275	-0.577	1.569	0.707	1.131	1.834	0.407	0.811	-0.688
	Q9UHD8	Septin-9	7.16E-03	0.425	0.481	1.236	0.090	0.694	0.194	-0.188	0.900	-0.304	0.614	0.144	0.751	0.573
	P11532	Dystrophin	7.40E-03	0.938	0.918	0.806	1.088	1.201	-0.250	2.395	1.278	0.781	-1.059	0.102	2.009	1.502
	P52594	Arf-GAP domain and FG repeat-containing protein 1	7.72E-03	0.452	0.081	0.617	-0.105	0.427	0.005	0.293	0.478	0.969	0.524	-0.298	0.966	0.506
	Q9P035	3 - hydroxyacyl - CoA dehydratase 3	7.94E-03	-0.680	0.115	-0.712	-0.938	-0.754	0.291	-0.219	-0.490	-0.791	-1.464	0.331	-0.648	-1.447
	P50440-2	GATM_HUMAN Isoform 2 of Glycine amidinotransferase, mitochondrial	7.94E-03	-0.912	-1.560	-0.239	-1.764	-0.290	-0.084	-0.967	-2.338	-1.201	NA	NA	NA	NA
	O14791	Apolipoprotein L1	8.14E-03	-0.344	-0.080	-0.992	-0.123	-0.069	0.138	-0.492	-1.173	-0.577	-0.924	0.108	-0.524	-0.221
FA	B2ZZ83	Filamin B	8.47E-03	0.326	0.202	0.871	0.248	0.409	-0.032	0.603	0.857	0.275	0.374	0.131	1.653	-0.152
	O15075	Serine/threonine-protein kinase DCLK1	8.64E-03	0.429	0.035	0.636	1.174	0.672	-0.050	0.397	0.794	0.429	NA	NA	NA	NA
	Q53EL6	Programmed cell death protein 4	8.79E-03	-0.625	-0.727	0.086	-0.815	0.262	0.031	-0.811	-2.262	-1.162	-1.644	0.318	-0.743	-1.649
	Q9BZQ8	Protein Niban	8.85E-03	-0.635	-1.097	0.377	-0.997	-0.145	-0.010	-1.125	-0.998	-0.944	-2.389	0.187	-0.210	-1.907
	P05408	Neuroendocrine protein 7B2	9.07E-03	-0.399	-0.437	-0.751	-0.395	-0.193	-0.342	-0.328	-0.403	0.069	-0.677	-0.360	0.326	-1.559
	P41219	Peripherin	9.31E-03	0.383	0.177	0.091	0.443	0.812	0.222	1.519	0.641	1.674	-0.359	-0.054	0.519	1.006
	P62753	40S ribosomal protein S6	9.57E-03	-0.452	-0.904	0.293	-0.938	-0.207	-0.146	-0.227	-1.242	-0.503	-0.819	-0.199	0.205	-1.585
VSMC	Q05682-4	Isoform 4 of Caldesmon	1.06E-02	0.682	1.021	1.233	0.602	0.659	NA	NA	NA	NA	-0.014	-0.047	1.706	0.656
	Q8NHQ9	ATP-dependent RNA helicase DDX55	1.07E-02	0.854	2.040	0.509	1.037	0.260	0.466	2.285	1.824	-0.891	1.042	-0.313	0.869	0.749
	Q96JY6	PDZ and LIM domain protein 2	1.11E-02	0.785	0.735	0.805	1.269	1.876	-0.616	1.092	0.522	0.190	0.198	-0.355	0.512	1.330
	Q9BPU6	Dihydropyrimidinase-related protein 5	1.14E-02	0.305	0.304	-0.423	1.076	1.040	-0.397	1.247	0.034	0.986	0.936	-0.007	0.575	0.928
	P62258	14-3-3 protein epsilon	1.19E-02	-0.384	-0.174	0.382	-0.671	-0.289	-0.255	-0.882	-0.410	-1.060	-1.647	-0.002	0.046	-0.979
	E2QRB5	NCK-associated protein 5-like	1.19E-02	0.800	NA	NA	NA	NA	1.290	0.546	0.843	0.756	NA	NA	NA	NA
	Q14767	Latent-transforming growth factor-beta binding protein 2	1.22E-02	0.337	0.230	0.654	0.502	1.527	-0.393	-0.419	0.415	0.760	1.260	0.051	0.716	1.658
	Q15056	Eukaryotic translation initiation factor 4H	1.24E-02	-0.424	-0.858	0.063	-0.848	0.143	0.094	-0.485	-0.960	-0.526	-0.920	0.129	0.002	-1.431
	O43175	D-3- phosphoglycerate dehydrogenase	1.27E-02	-0.547	-1.777	0.363	-1.633	-0.098	0.210	-0.693	-1.674	-0.791	-1.798	0.250	0.038	-1.409
TJ	P11171	Protein 4.1	1.28E-02	-0.581	0.207	-0.049	-0.736	-0.496	-0.396	-2.049	-0.535	-1.892	0.308	-0.130	-1.187	-0.757
FA,RAC	P02751	Fibronectin	1.32E-02	0.513	1.204	0.526	0.865	0.227	-0.097	1.942	1.638	-0.007	2.760	-0.203	0.748	-0.048
FA,RAC	Q05397	Focal adhesion kinase 1	1.33E-02	-1.696	NA	NA	NA	NA	-0.705	-1.722	-1.671	-2.063	NA	NA	NA	NA
	Q06210	Glucosamine-fructose-6-phosphate aminotransferase [isomerizing] 1	1.34E-02	-0.434	-1.247	-0.159	-1.328	-0.371	0.058	-0.411	-1.551	-0.394	-2.462	0.277	0.273	-2.012
TJ	P16989	DNA-binding protein A	1.35E-02	-0.521	-0.043	0.336	-0.970	-0.139	-0.510	-0.492	-0.536	-1.565	0.091	-0.521	-0.105	-0.833
	Q15751	Probable E3 ubiquitin-protein ligase HERC1	1.41E-02	-0.947	-1.304	-0.563	-1.570	-1.124	NA	NA	NA	NA	-1.922	0.575	-0.449	-0.770
	O95831	Apoptosis-inducing factor 1, mitochondrial	1.52E-02	-0.695	-1.049	0.118	-0.786	-0.493	0.042	-1.029	-1.429	-0.818	-2.542	0.437	0.351	-1.810
	P60468	Protein transport protein Sec61 subunit beta	1.58E-02	-0.527	-1.817	0.124	-0.871	-0.227	-0.460	-1.355	-2.080	-0.673	-2.949	0.096	0.860	-3.369

Table. 12 (continued)

	Q13884	Beta-1-syntrophin	1.63E-02	-0.329	-0.949	0.186	-0.826	-0.055	-0.438	-0.694	-1.512	-0.106	-1.525	0.190	0.326	-0.934
	Q00341	Vigilin	1.72E-02	-0.502	-0.730	0.251	-0.897	-0.304	0.082	-0.624	-1.153	-0.397	-1.831	0.018	0.305	-1.764
	Q13557	Isoform Delta 6 of Calcium/calmodulin-dependent protein kinase type II subunit delta	1.75E-02	0.301	0.064	0.484	0.116	0.473	-0.032	0.805	0.197	0.301	NA	NA	NA	NA
	Q8TAQ2	SWI/SNFcomplex subunit SMARCC2	1.78E-02	-0.483	-0.325	-0.117	-0.435	-0.502	-0.003	-1.146	-0.586	-1.135	-1.231	0.119	0.669	-0.873
FA	Q15942	Zyxin	1.81E-02	0.363	0.721	0.762	0.155	0.645	-0.374	-0.206	0.948	-0.074	1.222	-0.144	0.883	0.533
	C9JD84	Latent-transforming growth factor beta - binding protein 1	1.96E-02	1.029	0.661	0.866	-0.143	0.128	0.314	1.329	2.224	1.029	NA	NA	NA	NA
	Q5TDH0	Protein DDI1 homolog 2	1.98E-02	-0.851	NA	NA	NA	NA	-0.775	-1.497	-0.570	-1.638	0.294	0.129	-2.012	-0.927
	P14625	Endoplasmin	1.98E-02	-0.528	-1.128	0.126	-1.071	-0.344	0.008	-0.038	-1.148	-0.270	-1.872	0.086	0.270	-1.234
FA	Q9Y4G6	Talin-2	2.00E-02	0.518	NA	NA	NA	NA	-0.341	1.023	0.645	0.613	0.847	-0.146	0.737	0.785
TJ, RAC	Q7Z406	Myosin-14	2.03E-02	0.349	0.255	1.364	0.386	0.329	-0.055	0.737	0.610	0.246	-0.194	0.101	1.741	-0.044
	Q8TEH3	DENN domain - containing protein 1A	2.09E-02	-0.578	NA	NA	NA	NA	-0.056	-1.996	-1.421	-1.302	-0.032	0.060	-0.578	-2.003
	P31942	Heterogeneous nuclear ribonucleoprotein H3	2.16E-02	0.447	-0.023	1.080	-0.101	0.802	0.422	2.459	0.058	1.345	-0.459	0.025	1.711	0.651
	P05387	60S acidic ribosomal protein P2	2.21E-02	-0.756	-1.824	0.479	-1.430	-0.134	0.014	-0.587	-1.860	-0.933	-2.680	0.208	0.609	-2.120
FA, RAC, VSMC	O14974	Protein phosphatase 1 regulatory subunit 12A	2.31E-02	0.989	-0.351	1.098	0.136	0.803	NA	NA	NA	NA	1.342	-0.013	1.066	1.401
	P40306	Proteasome subunit beta type-10	2.39E-02	0.408	0.186	0.261	0.100	1.017	0.390	-0.607	1.333	1.324	1.271	-0.240	-0.054	1.289
	P28799	Granulins	2.41E-02	0.747	NA	NA	NA	NA	-0.407	0.498	1.891	1.486	2.622	-0.270	1.122	2.471
	Q13263	Transcription intermediary factor 1-beta	2.58E-02	0.322	0.354	1.204	0.768	1.470	0.237	2.009	0.321	1.081	-0.865	0.090	0.498	-0.204
	Q13202	Dual specificity protein phosphatase 8	2.60E-02	-1.340	-2.445	0.451	-1.969	-0.339	0.140	-1.426	-2.951	-1.218	NA	NA	NA	NA
TJ, VSMC	P35749	Myosin-11	2.75E-02	0.343	0.341	1.727	0.120	0.068	0.215	0.180	0.351	0.145	0.040	-0.082	1.930	0.838
	O00515	Ladinin-1	2.80E-02	0.401	0.366	0.004	-0.117	0.593	0.515	0.721	0.643	-0.321	1.010	0.366	0.957	-0.483
	Q99961	Endophilin-A2	2.85E-02	0.423	NA	NA	NA	NA	-0.068	0.605	0.617	-0.104	0.827	-0.085	0.781	0.601
	P01833	Polymeric immunoglobulin receptor	2.90E-02	0.398	0.464	-0.351	0.005	0.640	-0.221	0.081	2.144	1.453	0.727	0.694	-0.256	1.589
FA, RAC, VSMC	Q15746	Myosin light chain kinase, smooth muscle	2.95E-02	0.497	0.625	1.843	0.267	0.234	0.004	0.789	1.177	-0.671	-0.027	-0.170	2.051	0.954
	Q6Y7W6	PERQ amino acid-rich with GYF domain-containing protein 2	3.02E-02	0.376	NA	NA	NA	NA	0.022	0.205	0.135	0.352	0.903	-0.130	0.928	0.874
	D9YZV3	Tropomyosin 1 (Alpha) isoform 3	3.03E-02	0.490	0.417	1.548	0.361	0.478	NA	NA	NA	NA	-0.154	-0.039	1.258	0.617
	E9PCT1	Serine/arginine repetitive matrix 1	3.18E-02	0.566	NA	NA	NA	NA	NA	NA	NA	NA	1.180	0.633	0.499	0.380
	Q8IYB3	Serine/arginine repetitive matrix protein 1	3.18E-02	0.566	NA	NA	NA	NA	NA	NA	NA	NA	1.180	0.633	0.499	0.380
	B1AHM9	Fibulin 1 (Fragment)	3.22E-02	0.525	NA	NA	NA	NA	-0.061	1.864	2.396	0.300	0.416	-0.034	0.918	0.899
	C9JFC3	Uncharacterized protein	3.22E-02	0.435	NA	NA	NA	NA	-0.332	1.019	1.002	1.268	0.469	-0.154	0.266	1.443
	Q9P2E9	Ribosome-binding protein 1	3.24E-02	-0.512	-1.465	0.203	-1.355	-0.039	0.011	-0.017	-2.052	-0.588	-2.565	0.177	0.598	-2.087
	E9PND2	Cysteine and glycine-rich protein 1 (Fragment)	3.26E-02	0.625	0.123	1.732	0.593	0.672	NA	NA	NA	NA	0.020	0.049	2.299	0.739
	Q86U86	Protein polybromo-1	3.29E-02	0.482	NA	NA	NA	NA	0.237	1.674	0.760	0.726	-0.097	0.192	0.033	0.772
	Q14005	Prointerleukin-16	3.30E-02	0.520	0.416	1.732	0.009	0.913	-0.264	0.915	1.243	0.124	-0.017	-0.105	-0.342	1.730
	P23588	Eukaryotic translation initiation factor 4B	3.46E-02	-0.374	-0.528	0.440	-0.578	-0.012	0.169	-1.210	-1.374	-0.594	-0.582	0.065	0.243	-1.491

Table. 12 (continued)

	Q9H3Q1	Cdc42 effector protein 4	3.47E 02	-1.252	-1.386	1.160	-2.577	-0.234	-0.708	-2.058	-1.737	-1.118	NA	NA	NA	NA
	Q5SW79	Centrosomal protein of 170 kDa	3.51E 02	-0.717	-0.585	-0.850	-1.207	-0.271	NA	NA	NA	NA	NA	NA	NA	NA
	Q96Q06	Perilipin-4	3.53E 02	-0.804	0.045	-1.860	-0.880	-1.716	0.362	-0.979	-1.071	-1.337	-0.061	0.012	-1.475	1.164
	Q92597	Protein NDRG1	3.53E 02	0.398	0.187	0.220	-0.408	0.668	-0.125	3.049	0.963	0.747	1.391	0.037	-0.017	1.002
	E9PP16	Liprin-beta-2	3.55E 02	0.875	NA	NA	NA	NA	0.211	1.011	1.216	0.739	NA	NA	NA	NA
	Q86W92-2	Isoform 2 of Liprin-beta-1	3.55E 02	0.875	NA	NA	NA	NA	0.211	1.011	1.216	0.739	NA	NA	NA	NA
	Q9UBG0	C-type mannose receptor 2	3.59E 02	0.461	0.388	0.729	-0.120	0.654	-0.424	0.445	0.601	0.461	0.601	-0.235	1.241	-0.245
	P08727	Keratin, type I cytoskeletal 19	3.80E 02	0.422	0.198	0.383	0.412	1.078	0.075	0.816	1.428	0.300	-0.839	0.409	2.362	-0.034
	P49590	Probable histidine -- tRNA ligase, mitochondrial	3.92E 02	-0.520	NA	NA	NA	NA	-0.302	-0.349	-1.031	-0.729	NA	NA	NA	NA
	Q92598	Heat shock protein 105 kDa	4.07E 02	0.496	-0.332	0.605	-0.636	0.496	0.250	1.245	0.811	0.128	2.397	-0.102	0.934	0.608
	Q9UDT6	CAP-Glydomain-containing linker protein 2	4.13E 02	0.780	NA	NA	NA	NA	-0.146	2.950	0.835	1.194	0.161	-0.099	1.528	0.801
	Q8TDZ2	Protein-methionine sulfoxide oxidase MICAL1	4.26E 02	0.676	NA	NA	NA	NA	NA	NA	NA	NA	1.067	0.253	0.933	0.418
	Q96PK2	Microtubule-actin cross-linking 1, factor isoform 4	4.30E 02	0.488	0.644	1.034	0.231	0.655	-0.072	1.827	0.248	0.167	-0.491	-0.053	1.125	-0.140
	Q14151	Scaffold attachment factor B2	4.44E 02	-0.535	-0.783	-0.224	-0.535	-0.498	NA	NA	NA	NA	-0.681	0.245	0.278	-0.787
	O94903	Proline synthase co-transcribed bacterial homolog protein	4.53E 02	-0.306	-0.862	0.697	-1.113	-0.066	0.094	-1.565	-1.099	-0.721	-2.063	0.135	0.818	-1.546
VSMC	O43306-2	Isoform 2 of Adenylate cyclase type 6	4.56E 02	-1.630	NA	NA	NA	NA	-0.789	-0.816	-2.445	-2.667	NA	NA	NA	NA
	Q14157	Ubiquitin-associated protein 2 - like	4.57E 02	-0.931	NA	NA	NA	NA	NA	NA	NA	NA	-0.886	-0.093	-0.975	-1.163
	Q8TEW8	Partitioning defective 3 homolog B	4.59E 02	0.581	NA	NA	NA	NA	-0.637	0.451	1.229	1.871	0.604	0.132	0.872	0.558
	Q13951-2	Isoform 2 of Core-binding factor subunit beta	4.62E 02	0.537	0.342	-0.220	0.733	-0.010	0.003	1.684	1.571	0.842	NA	NA	NA	NA
	Q15424	Scaffold attachment factor B1	4.90E 02	-0.535	-0.797	-0.165	-0.535	-0.498	NA	NA	NA	NA	-0.681	0.245	0.278	-0.787
	Q9Y4K4	Mitogen-activated proteinkinase kinase kinase kinase 5	4.93E 02	0.399	0.691	0.285	-0.341	0.048	0.469	0.454	1.004	0.180	2.059	-0.332	0.411	0.029

Table. 12 (continued)

Case	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
T1/NT1	hsa04530:Tight junction	16	4.8338369	**1.67E-07**	Q96A65, Q9H4G0, Q3MNF0, Q3MNF1, P35221, Q05655, P35222, P35579, O43491, P55196, Q9P2M7, P35580, Q7Z406, Q3MIV8, Q9Y624, P31749, P35749, Q14247, Q07157	113	134	5085	5.3731343	1.92E-05	1.92E-05	1.90E-04
T1/NT1	hsa04520:Adherens junction	11	3.3232628	**5.71E-06**	P55196, O43318, P29350, P18206, Q8WWI1, O60716, Q9UQB8, P06241, P35221, P35222, Q07157	113	77	5085	6.4285714	6.57E-04	3.28E-04	0.0065072
T1/NT1	hsa05130:Pathogenic Escherichia coli infection	7	2.1148036	**1.45E-03**	Q92974, P68366, Q9BQE3, P06241, P35222, P19338, Q14247	113	57	5085	5.5263158	0.1539067	5.42E-02	1.6417623
T1/NT1	hsa04660:T cell receptor signaling pathway	9	2.7190332	**2.44E-03**	O43318, P29350, O95999, O14920, Q9BXL7, Q14934, P06241, P36507, P31749	113	108	5085	3.75	0.2445643	6.77E-02	2.7397466
T1/NT1	hsa05416:Viral myocarditis	7	2.1148036	**4.47E-03**	P35580, Q3MNF0, Q7Z406, Q3MNF1, Q04637, P06241, Q3MIV8, P11532, P35749, P35579	113	71	5085	4.4366197	0.4025028	9.79E-02	4.9732348
T1/NT1	hsa04662:B cell receptor signaling pathway	7	2.1148036	**5.85E-03**	P29350, O95999, O14920, Q9BXL7, Q14934, P36507, P31749	113	75	5085	4.2	0.4908272	1.06E-01	6.4670057
T1/NT1	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	7	2.1148036	**6.24E-03**	P02545, P15924, P35221, P17661, Q99959, P35222, P11532	113	76	5085	4.1447368	0.5132938	9.78E-02	6.8841508
T1/NT1	hsa04670:Leukocyte transendothelial migration	8	2.4169184	**1.48E-02**	P55196, P18206, O60716, P26038, Q96FS4, P35221, P35222, Q9Y624	113	118	5085	3.0508475	0.8191351	1.92E-01	15.580936
T1/NT1	hsa04370:VEGF signaling pathway	6	1.8126888	**2.41E-02**	P29474, Q14934, P36507, P04792, P47712, P31749	113	75	5085	3.6	0.9396232	2.68E-01	24.274084
T1/NT1	hsa05221:Acute myeloid leukemia	5	1.510574	**3.81E-02**	O14920, P36507, P42229, P31749, P29590	113	58	5085	3.8793103	0.9884985	3.60E-01	35.743558
T4/NT4	hsa04530:Tight junction	13	4.5454545	**1.18E-06**	Q3MNF1, Q9UDY2, P11171, Q05655, P35222, P35579, Q9P2M7, P55196, P35580, Q5JTD0, Q7Z406, Q9Y624, P31749, P35749	83	134	5085	5.9436252	1.15E-04	1.15E-04	0.0012992
T4/NT4	hsa04660:T cell receptor signaling pathway	8	2.7972028	**1.65E-03**	O43318, P29350, O14920, Q9BXL7, P28482, Q14934, O75791, P31749	83	108	5085	4.5381526	0.1491123	7.76E-02	1.8053893
T4/NT4	hsa04662:B cell receptor signaling pathway	6	2.0979021	**6.89E-03**	P29350, O14920, Q9BXL7, P28482, Q14934, P31749	83	75	5085	4.9012048	0.4920664	2.02E-01	7.358219
T4/NT4	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6	2.0979021	**7.28E-03**	P02545, P15924, P17661, Q99959, P35222, P11532	83	76	5085	4.8367153	0.5114174	1.64E-01	7.76333
T4/NT4	hsa04270:Vascular smooth muscle contraction	7	2.4475524	**8.99E-03**	O43306, Q05682, Q3MNF1, P28482, Q05655, O14974, P35749, Q96A00	83	112	5085	3.8290663	0.5873256	1.62E-01	9.5038909
T4/NT4	hsa05221:Acute myeloid leukemia	5	1.7482517	**1.38E-02**	O14920, P28482, Q01196, P40763, P31749	83	58	5085	5.2814707	0.7432498	2.03E-01	14.221884
T4/NT4	hsa04722:Neurotrophin signaling pathway	7	2.4475524	**1.44E-02**	O14920, P28482, Q05655, Q13554, Q99759, P31749, Q99523	83	124	5085	3.4585115	0.7589093	1.84E-01	14.828775
T4/NT4	hsa04910:Insulin signaling pathway	7	2.4475524	**2.11E-02**	O14920, Q15642, P28482, P13861, P46019, P31749, P10644	83	135	5085	3.1767068	0.8762019	2.30E-01	20.998908

Table. 13

T4/NT4	hsa04010:MAPK signalingpathway	10	3.4965035	**2.70E-02**	Q14315,O43318,Q01201,O14920,P28482, Q14934,P21333,P11831,Q99759,P31749	83	267	5085	2.2945715	0.9314064	2.57E-01	26.090432
T4/NT4	hsa05416:Viral myocarditis	5	1.7482517	**2.70E-02**	P35580,Q7Z406,Q3MNF1,P11532,P35749,P35579	83	71	5085	4.3144409	0.9314481	2.35E-01	26.095508
T4/NT4	hsa04370:VEGF signalingpathway	5	1.7482517	**3.22E-02**	P49023,P29474,P28482,Q14934,P31749	83	75	5085	4.0843373	0.9594031	2.53E-01	30.337414
T4/NT4	hsa04520:Adherens junction	5	1.7482517	**3.50E-02**	P55196,O43318,P29350,P28482,P35222	83	77	5085	3.9782507	0.9694356	2.52E-01	32.533139
T4/NT4	hsa04510:Focal adhesion	8	2.7972028	**4.23E-02**	Q14315,P49023,P28482,P02452,P21333,P35222, O14974,P31749	83	201	5085	2.4384104	0.9855755	2.78E-01	38.013707
T5/NT5	hsa04270:Vascular smoothmuscle contraction	11	3.3742331	**8.26E-05**	Q14573,P63092,Q3MNF0,Q3MNF1,P10398, Q96A00,Q9Y6F6,O43306,Q15746,P28482,P36507, Q3MIV8,P35749,O15085	105	112	5085	4.7563776	0.0088823	8.88E-03	0.0929764
T5/NT5	hsa04530:Tightjunction	10	3.0674847	**1.55E-03**	Q9H4G0,B7Z7H6,Q3MNF0,Q3MNF1,O95049, P35222,P35579,Q9P2M7,P35580,Q7Z406, Q3MIV8,P35749,Q07157	105	134	5085	3.6140725	0.1538314	8.01E-02	1.7264351
T5/NT5	hsa04540:Gapjunction	8	2.4539877	**2.13E-03**	O43306,Q14573,P63092,P68366,Q9BQE3,P28482, P36507,Q07157	105	89	5085	4.35313	0.2057072	7.39E-02	2.3725258
T5/NT5	hsa04510:Focal adhesion	12	3.6809816	**2.48E-03**	Q14315,P22105,P18206,Q15746,Q9Y490,Q92934, P28482,P02452,P21333,Q15942,B2ZZ83,P35222	105	201	5085	2.891258	0.235444	6.49E-02	2.7601375
T5/NT5	hsa05416:Viral myocarditis	7	2.1472393	**3.09E-03**	O43432,P35580,Q3MNF0,Q7Z406,Q3MNF1, Q04637,Q3MIV8,P11532,P35749,P35579	105	71	5085	4.7746479	0.2843357	6.47E-02	3.427805
T5/NT5	hsa05130:Pathogenic Escherichiacoli infection	6	1.8404908	**5.86E-03**	Q92974,P68366,P05783,Q9BQE3,P35222,P19338	105	57	5085	5.0977444	0.4696496	1.00E-01	6.398432
T5/NT5	hsa05221:Acute myeloidleukemia	6	1.8404908	**6.31E-03**	Q92934,P28482,P10398,P36507,P40763,P29590	105	58	5085	5.0098522	0.4949715	9.30E-02	6.8746491
T5/NT5	hsa04720:Longterm potentiation	6	1.8404908	**1.22E-02**	Q14573,Q13522,P28482,P10398,Q13554,P36507	105	68	5085	4.2731092	0.7349611	1.53E-01	12.928771
T5/NT5	hsa04520:Adherens junction	6	1.8404908	**2.01E-02**	P29350,P18206,O60716,P28482,P35222,Q07157	105	77	5085	3.7736549	0.8879411	2.16E-01	20.403138
T5/NT5	hsa05216:Thyroid cancer	4	1.2269939	**2.07E-02**	P28482,P36507,P35222,Q16204	105	29	5085	6.679803	0.8949838	2.02E-01	20.93999
T5/NT5	hsa05213:Endometrial cancer	5	1.5337423	**2.11E-02**	Q92934,P28482,P10398,P36507,P35222	105	52	5085	4.6565934	0.8999753	1.89E-01	21.340372
T5/NT5	hsa05110:Vibrio choleraeinfection	5	1.5337423	**2.69E-02**	P13569,P63092,O43731,P60468,Q07157	105	56	5085	4.3239796	0.9474237	2.18E-01	26.441898
T5/NT5	hsa04912:GnRH signalingpathway	6	1.8404908	**4.94E-02**	O43306,Q14573,P63092,P28482,Q13554,P36507	105	98	5085	2.9650146	0.9958125	3.44E-01	43.497593
T6/NT6	hsa04530:Tightjunction	11	4.0892193	**1.97E-05**	P55196,P35580,Q9H4G0,Q7Z406,Q5JTD0,P35221, P35749,P35579,Q9Y624,P31749,Q14247	75	134	5085	5.5656716	0.001774	1.77E-03	0.0214598
T6/NT6	hsa04910:Insulin signalingpathway	8	2.9739777	**3.25E-03**	Q92934,Q14432,O95685,P10398,P36507,P13861, P31749,P10644	75	135	5085	4.0177778	0.2542474	1.36E-01	3.483869
T6/NT6	hsa05213:Endometrial cancer	5	1.8587361	**6.60E-03**	Q92934,P35221,P10398,P36507,P31749	75	52	5085	6.5192308	0.4491342	1.80E-01	6.9537378
T6/NT6	hsa04670:Leukocyte transendothelial migration	7	2.6022305	**7.05E-03**	P55196,P18206,O60716,Q96FS4,P35221,Q9NRY4, Q9Y624	75	118	5085	4.0220339	0.4709377	1.47E-01	7.4068415
T6/NT6	hsa05221:Acute myeloidleukemia	5	1.8587361	**9.69E-03**	Q92934,Q01196,P10398,P36507,P31749	75	58	5085	5.8448276	0.5838409	1.61E-01	10.054798
T6/NT6	hsa05416:Viral myocarditis	5	1.8587361	**1.93E-02**	P35580,Q7Z406,P11532,P35749,P35579	75	71	5085	4.7746479	0.8265815	2.53E-01	19.085621

Table. 13 (continued)

T6/NT6	hsa05220:Chronic myeloid leukemia	5	1.8587361	**2.31E 02**	Q92934,Q01196,P10398,P36507,P31749	75	75	5085	4.52	0.8779095	2.59E-01	22.446264
T6/NT6	hsa04520:Adherens junction	5	1.8587361	**2.52E 02**	P55196,O43318,P18206,O60716,P35221	75	77	5085	4.4025974	0.8991223	2.49E-01	24.214903
T6/NT6	hsa04810:Regulation of actin cytoskeleton	8	2.9739777	**3.58E 02**	P35580,P18206,Q7Z406,P53667,P10398,Q9NRY4, P36507,P35579	75	215	5085	2.5227907	0.9623427	3.05E-01	32.724673
T6/NT6	hsa05223:Non - small cell lung cancer	4	1.4869888	**4.32E 02**	Q92934,P10398,P36507,P31749	75	54	5085	5.0222222	0.9812867	3.28E-01	38.177535
T7/NT7	hsa04520:Adherens junction	9	3.8793103	**9.44E 06**	P55196,P08069,P29350,P18206,Q8WWI1, O60716,P06241,P18031,Q07157	72	77	5085	8.2548701	8.77E-04	8.77E-04	0.0103315
T7/NT7	hsa04510:Focal adhesion	10	4.3103448	**1.75E 03**	P08069,P18206,Q9Y490,P49841,P49023,P02452, P21333,P06241,Q15942,P31749	72	201	5085	3.5136816	0.150061	7.81E-02	1.8957344
T7/NT7	hsa04530:Tight junction	8	3.4482759	**2.46E 03**	P55196,Q9H4G0,Q7Z406,O95049,P35749,P31749, Q14247,Q07157	72	134	5085	4.2164179	0.2049008	7.36E-02	2.6629666
T8/NT8	hsa04530:Tight junction	20	2.8129395	**2.15E 06**	Q9H4G0,Q96A65,O95049,Q9UDY2,P11171, Q05655,P35579,Q13813,Q8TEW0,P55196, Q9P2M7,P35580,Q5JTD0,Q7Z406,Q9Y2J2, Q9Y624,P31749,P35749,Q07157,Q14247	213	134	5085	3.5631701	2.84E-04	2.84E-04	0.0025155
T8/NT8	hsa03040:Spliceosome	18	2.5316456	**1.49E 05**	P52272,P26368,O75643,P49756,O60231,Q13573, Q13247,Q07955,Q9UKV3,Q08170,Q9Y5S9, P08621,Q13242,Q00839,P61978,P09651,Q15365, P38159	213	126	5085	3.4104628	0.0019674	9.84E-04	0.0174202
T8/NT8	hsa04270:Vascular smooth muscle contraction	15	2.1097046	**1.99E 04**	Q05655,O60237,Q9NZN5,O14974,P47712, Q96A00,Q9Y6F6,Q15746,Q05682,P47901,P28482, P36507,P22694,O15085,P35749	213	112	5085	3.1973089	0.0259813	8.74E-03	0.2326065
T8/NT8	hsa04520:Adherens junction	11	1.5471167	**1.22E 03**	P55196,O43318,P29350,P18206,Q8WWI1, O60716,P28482,Q9UQB8,P18031,Q8TEW0, Q07157	213	77	5085	3.4104628	0.148375	3.94E-02	1.4107526
T8/NT8	hsa04910:Insulin signaling pathway	15	2.1097046	**1.35E 03**	P49841,Q92934,Q15642,O95685,P62753,P35568, P13861,P18031,Q9Y4H2,P54646,P28482,P36507, P22694,P31749,P46019	213	135	5085	2.6525822	0.1636104	3.51E-02	1.5680658
T8/NT8	hsa04660:Tcell receptor signaling pathway	13	1.8284107	**1.64E 03**	O95999,P49841,Q13177,O75791,O15111,O43318, P05412,P29350,Q14934,P28482,Q16539,P36507, P31749	213	108	5085	2.8736307	0.1947729	3.55E-02	1.8981408
T8/NT8	hsa05220:Chronic myeloid leukemia	10	1.4064698	**3.68E 03**	Q13547,Q9UQC2,Q92934,Q92769,P28482, P36507,P46527,P42229,P31749,O15111	213	75	5085	3.1830986	0.385359	6.72E-02	4.2142762
T8/NT8	hsa04662:Bcell receptor signaling pathway	10	1.4064698	**3.68E 03**	P05412,P29350,O95999,P49841,P28482,Q14934, P36507,P31749,O15111,P07948	213	75	5085	3.1830986	0.385359	6.72E-02	4.2142762
T8/NT8	hsa04010:MAPK signaling pathway	21	2.9535865	**7.33E 03**	P10636,P21333,P15336,Q13177,P47712,O15111, O95819,Q14315,Q9UBI6,O43318,P05412,P16949, P28482,Q14934,Q16539,P36507,Q9NYJ8,P04792, P22694,Q99759,P31749	213	267	5085	1.8776705	0.621331	1.14E-01	8.2319478
T8/NT8	hsa04810:Regulation of actin cytoskeleton	18	2.5316456	**7.81E 03**	P02751,P18206,P16144,Q9UQB8,P19634,Q13177, Q9NZN5,O14974,P35579,P35580,Q9UBI6,P15311, Q15746,Q7Z406,Q9Y2X7,P28482,P36507,Q13576	213	215	5085	1.9986898	0.6449799	1.09E-01	8.7539777
T8/NT8	hsa05221:Acute myeloid leukemia	8	1.1251758	**9.77E 03**	Q92934,P28482,P36507,P40763,P42229,P31749, O15111,P29590	213	58	5085	3.2928606	0.7264061	1.22E-01	10.832901
T8/NT8	hsa04370:VEGF signaling pathway	9	1.2658228	**1.22E 02**	Q92934,P29474,P28482,Q14934,Q16539,P36507, P04792,P47712,P31749	213	75	5085	2.8647887	0.8022238	1.37E-01	13.356237
T8/NT8	hsa04722:Neurotrophin signaling pathway	12	1.6877637	**1.38E 02**	Q9Y4H2,P05412,P49841,Q92934,P28482,Q16539, Q05655,P36507,P35568,Q99759,P31749,Q99523	213	124	5085	2.3103135	0.8397247	1.42E-01	14.952809

Table. 13 (continued)

T8/NT8	hsa04664:Fc epsilon RI signaling pathway	9	1.2658228	**1.53E-02**	Q9UQC2, P30273, P28482, Q16539, Q05655, P36507, P47712, P31749, P07948	213	78	5085	2.7546046	0.8685133	1.44E-01	16.429408
T8/NT8	hsa04510:Focal adhesion	16	2.2503516	**1.97E-02**	P02751, P18206, Q9Y490, P49841, Q92934, P02452, P21333, P16144, Q13177, P10451, O14974, Q14315, P05412, Q15746, P28482, P31749	213	201	5085	1.9003574	0.9275308	1.71E-01	20.719655
T8/NT8	hsa04920:Adipocytokine signaling pathway	8	1.1251758	**2.06E-02**	Q9Y4H2, P54646, P35568, Q8N5S9, P40763, P31749, O15111, Q96RR4	213	67	5085	2.8505361	0.9363084	1.68E-01	21.619998
T8/NT8	hsa04620:Toll-like receptor signaling pathway	10	1.4064698	**2.42E-02**	O43318, Q13546, P05412, P28482, Q16539, Q9NYJ8, P36507, P10451, P31749, O15111	213	101	5085	2.3636871	0.960387	1.83E-01	24.844586
T8/NT8	hsa04012:ErbB signaling pathway	9	1.2658228	**2.77E-02**	P05412, P49841, Q92934, P28482, Q13177, P36507, P46527, P42229, P31749	213	87	5085	2.4696455	0.9754317	1.96E-01	27.954395
T8/NT8	hsa04330:Notch signaling pathway	6	0.8438819	**4.45E-02**	Q13547, Q9Y6R0, Q9Y618, Q92769, Q13573, Q8TDB6	213	47	5085	3.0476476	0.9975498	2.84E-01	41.245623
T9/NT9	hsa04530:Tight junction	13	2.5742574	**1.24E-03**	Q96A65, Q9H4G0, O95049, P35222, P35579, Q9P2M7, P55196, P35580, Q7Z406, P31749, P35749, Q9Y624, Q07157	166	134	5085	2.9718126	0.1402452	1.40E-01	1.4159514
T9/NT9	hsa04510:Focal adhesion	16	3.1683168	**2.04E-03**	P02751, P18206, Q9Y490, Q92934, P02452, P21333, P16144, P10451, Q15942, P35222, Q14315, P22105, P05412, Q15746, B2ZZ83, P31749	166	201	5085	2.4384104	0.2204942	1.17E-01	2.3234027
T9/NT9	hsa03040:Spliceosome	12	2.3762376	**2.42E-03**	Q9UKV3, P52272, P26368, O75643, Q13247, P49756, Q13573, Q8NI27, O75533, P61978, Q00839, P38159	166	126	5085	2.9173838	0.2556182	9.37E-02	2.7474917
T9/NT9	hsa05221:Acute myeloid leukemia	8	1.5841584	**2.49E-03**	Q92934, Q06455, P10398, P36507, P40763, P31749, O15111, P29590	166	58	5085	4.2251766	0.2620032	7.31E-02	2.8265259
T9/NT9	hsa04910:Insulin signaling pathway	12	2.3762376	**4.14E-03**	Q9Y4H2, Q92934, P54646, Q15642, O95685, P62753, P10398, P36507, P35568, P13861, P46019, P31749	166	135	5085	2.7228916	0.397191	9.63E-02	4.6645079
T9/NT9	hsa04662:B cell receptor signaling pathway	8	1.5841584	**1.04E-02**	P05412, P29350, O95999, Q9BXL7, Q14934, P36507, P31749, O15111	166	75	5085	3.2674699	0.719697	1.91E-01	11.310903
T9/NT9	hsa04370:VEGF signaling pathway	8	1.5841584	**1.04E-02**	Q92934, P29474, Q14934, Q16539, P36507, P04792, P47712, P31749	166	75	5085	3.2674699	0.719697	1.91E-01	11.310903
T9/NT9	hsa04920:Adipocytokine signaling pathway	7	1.3861386	**2.08E-02**	Q9Y4H2, P54646, P35568, Q8N5S9, P40763, P31749, O15111	166	67	5085	3.2004136	0.9227555	3.06E-01	21.468611
T9/NT9	hsa04660:T cell receptor signaling pathway	9	1.7821782	**2.33E-02**	P05412, P29350, O95999, Q9BXL7, Q14934, Q16539, P36507, P31749, O15111	166	108	5085	2.5527108	0.9439676	3.02E-01	23.812309
T9/NT9	hsa04010:MAPK signaling pathway	16	3.1683168	**2.57E-02**	P10636, P21333, P15336, Q9Y4G8, P47712, O15111, O95819, Q14315, P05412, P16949, Q14934, Q16539, P36507, B2ZZ83, P04792, P31749	166	267	5085	1.8356572	0.9582575	2.97E-01	25.900039
T9/NT9	hsa04670:Leukocyte transendothelial migration	9	1.7821782	**3.70E-02**	P55196, P15311, P18206, O60716, P26038, Q96FS4, Q16539, P35222, Q9Y624	166	118	5085	2.3363794	0.9899851	3.69E-01	35.23915
T9/NT9	hsa04520:Adherens junction	7	1.3861386	**3.80E-02**	P55196, P29350, P18206, Q8WWI1, O60716, P35222, Q07157	166	77	5085	2.7847755	0.9910909	3.49E-01	35.950324
T9/NT9	hsa04810:Regulation of actin cytoskeleton	13	2.5742574	**4.56E-02**	P02751, Q9NYB9, P18206, P26038, P16144, P10398, P35579, P35580, Q15746, P15311, Q7Z406, P36507, Q13576	166	215	5085	1.8521995	0.9966421	3.78E-01	41.584981
T10/NT10	hsa04530:Tight junction	18	2.6509573	**5.76E-05**	Q9H4G0, O95049, P35221, P11171, P35222, P35579, Q13813, P55196, Q9P2M7, P35580, Q5JTD0, Q7Z406, Q8NI35, Q9Y624, P31749, P35749, Q07157, Q14247	222	134	5085	3.0768455	0.0072945	7.29E-03	0.0668322
T10/NT	hsa04510:Focal	21	3.0927835	**3.95E-04**	P18206, Q9Y490, P49841, Q92934, P02452, P16144,	222	201	5085	2.3931021	0.0489262	1.25E-02	0.4570234

Table. 13 (continued)

10	adhesion				P21333, Q13177, Q9Y4G6, P10451, Q15942, P35222, O14974, P04049, P22105, P05412, Q13905, Q05397, P49023, P28482, P31749							
T10/NT 10	hsa04370:VEGF signaling pathway	12	1.7673049	**3.42E-04**	P04049, Q92934, Q05397, P49023, P29474, P28482, Q14934, Q08209, Q16539, P36507, P04792, P31749	222	75	5085	3.6648649	0.0424668	1.44E-02	0.3954783
T10/NT 10	hsa04012:ErbB signaling pathway	13	1.9145803	**3.32E-04**	Q92934, P49841, Q13177, P10398, P42229, P04049, P05412, Q05397, P28482, P16333, P36507, P31749, P46527	222	87	5085	3.4226468	0.0412915	2.09E-02	0.3843201
T10/NT 10	hsa03040:Spliceosome	15	2.2091311	**1.03E-03**	P52272, O75643, Q13573, O60231, Q13247, P49756, Q07955, Q9UKV3, Q08170, Q9Y5S9, P08621, P61978, P09651, Q15365, P38159	222	126	5085	2.726834	0.1224251	2.15E-02	1.1854332
T10/NT 10	hsa04810:Regulation of actin cytoskeleton	21	3.0927835	**9.40E-04**	Q9NYB9, P18206, P26038, P16144, P10398, Q13177, Q9NZN5, O14974, P35579, Q14155, P35580, Q9UBI6, P04049, Q9Y2I7, Q05397, Q15052, Q7Z406, P49023, P28482, P36507, Q13576	222	215	5085	2.2372722	0.1126095	2.36E-02	1.0850175
T10/NT 10	hsa05213:Endometrial cancer	9	1.3254786	**1.61E-03**	P04049, P49841, Q92934, P28482, P35221, P10398, P36507, P35222, P31749	222	52	5085	3.9643971	0.1853304	2.89E-02	1.8543085
T10/NT 10	hsa04660:T cell receptor signaling pathway	13	1.9145803	**2.33E-03**	O95999, P49841, Q08209, Q13177, P04049, P05412, Q14934, P28482, P16333, Q16539, P36507, P31749, Q12959	222	108	5085	2.7571321	0.2565939	3.64E-02	2.671292
T10/NT 10	hsa05221:Acute myeloid leukemia	9	1.3254786	**3.28E-03**	P04049, Q92934, P28482, P10398, P36507, P40763, P42229, P31749, P29590	222	58	5085	3.554287	0.3414775	4.09E-02	3.742917
T10/NT 10	hsa04270:Vascular smooth muscle contraction	13	1.9145803	**3.17E-03**	Q14573, P10398, Q9NZN5, O14974, O60237, Q96A00, Q9Y6F6, P04049, Q05682, P28482, P36507, O15085, P35749	222	112	5085	2.6586631	0.3318033	4.38E-02	3.6146426
T10/NT 10	hsa04662:B cell receptor signaling pathway	10	1.4727541	**4.84E-03**	P04049, P05412, O95999, P49841, P28482, Q14934, Q08209, P36507, P31749, P07948	222	75	5085	3.0540541	0.4603011	5.45E-02	5.4761706
T10/NT 10	hsa05220:Chronic myeloid leukemia	9	1.3254786	**1.54E-02**	Q13547, P04049, Q92934, P28482, P10398, P36507, P46527, P42229, P31749	222	75	5085	2.7486486	0.8605715	1.23E-01	16.465458
T10/NT 10	hsa05215:Prostate cancer	10	1.4727541	**1.45E-02**	P04049, P14625, P49841, Q92934, P28482, P10398, P36507, P35222, P46527, P31749	222	89	5085	2.5736411	0.8440867	1.24E-01	15.608679
T10/NT 10	hsa00010:Glycolysis / Gluconeogenesis	8	1.1782032	**1.45E-02**	P04406, P60174, P36871, P18669, Q96G03, P04075, P08559, P17858	222	60	5085	3.0540541	0.8430846	1.33E-01	15.559291
T10/NT 10	hsa04722:Neurotrophin signaling pathway	12	1.7673049	**1.83E-02**	P04049, P05412, Q13905, Q9Y5V3, P49841, Q92934, P28482, Q9ULH0, Q16539, P36507, P35568, P31749	222	124	5085	2.2166521	0.904157	1.36E-01	19.276359
T10/NT 10	hsa04910:Insulin signaling pathway	13	1.9145803	**1.38E-02**	Q92934, P49841, Q15642, O95685, P10398, P35568, P13861, P04049, Q13905, P28482, P36507, P31749, P46019	222	135	5085	2.2057057	0.8289625	1.37E-01	14.892193
T10/NT 10	hsa04666:Fc gamma R-mediated phagocytosis	10	1.4727541	**2.15E-02**	Q92608, P04049, Q9Y2I7, Q9ULH1, P28482, P29966, O43150, P49006, P31749, P07948	222	95	5085	2.4110953	0.936581	1.42E-01	22.263646
T10/NT 10	hsa00030:Pentose phosphate pathway	5	0.736377	**2.14E-02**	P37837, P36871, Q96G03, P04075, P17858	222	25	5085	4.5810811	0.9359705	1.49E-01	22.195613
T10/NT 10	hsa04144:Endocytosis	15	2.2091311	**2.83E-02**	Q8NDX1, Q9ULH1, Q15276, Q8N6H7, O43150, Q9NP61, Q9BXF6, Q99961, Q9Y2I7, Q96PU5, Q9UQN3, Q96B97, Q15075, Q00610, Q7L804	222	184	5085	1.8672885	0.9740198	1.67E-01	28.347314
T10/NT 10	hsa04720:Long-term potentiation	8	1.1782032	**2.72E-02**	P04049, Q14573, Q13522, P28482, Q08209, P10398, P36507, O14974	222	68	5085	2.6947536	0.9697753	1.68E-01	27.350326
T10/NT 10	hsa04670:Leukocyte transendothelial migration	11	1.6200295	**3.16E-02**	P55196, P18206, Q05397, P49023, O60716, P26038, Q96FS4, P35221, Q16539, P35222, Q9Y624	222	118	5085	2.1352497	0.9831067	1.69E-01	31.108923
T10/NT	hsa05211:Renal cell	8	1.1782032	**3.13E-02**	P04049, P05412, Q13905, P28482, P10398, Q13177,	222	70	5085	2.6177606	0.982283	1.75E-01	30.808793

Table. 13 (continued)

10	carcinoma				P36507,P31749							
T10/NT 10	hsa05216:Thyroid cancer	5	0.736377	**3.51E-02**	P12270,P28482,P36507,P35222,Q16204	222	29	5085	3.9492078	0.9893578	1.79E-01	33.95539
T10/NT 10	hsa04010:MAPK signaling pathway	19	2.7982327	**4.10E-02**	P10636,Q08209,P21333,P15336,Q9Y4G8,Q13177, O95819,Q9UBI6,P04049,P05412,P16949,P28482, Q14934,P11831,Q16539,P36507,Q9NYJ8,P04792, P31749	222	267	5085	1.6299727	0.9951076	1.92E-01	38.479787
T10/NT 10	hsa00520:Aminosugar and nucleotidesugar metabolism	6	0.8836524	**4.06E-02**	Q06210,P36871,Q96G03,Q9UJ70,O60701,O95394	222	44	5085	3.1234644	0.9947976	1.97E-01	38.133693
T11/NT 11	hsa04530:Tightjunction	23	3.1420765	**6.44E-08**	O14493,Q9H4G0,P16989,Q3MNF1,Q9NYL2, O95049,Q9UDY2,P35221,Q05655,P35579, Q13813,O43491,P55196,Q9P2M7,P35580, Q16625,Q5JTD0,Q7Z406,Q8NI35,O43707,Q9Y624, P78369,P35749,Q07157	226	134	5085	3.8619403	8.56E-06	8.56E-06	7.53E-05
T11/NT 11	hsa04270:Vascular smoothmuscle contraction	16	2.1857923	**1.04E-04**	Q14573,P63092,Q3MNF1,Q13464,P10398, Q05655,O60237,Q9NZN5,O14974,Q96A00, Q9Y6F6,P04049,Q05682,P28482,P36507,O15085, P35749	226	112	5085	3.2142857	0.0137585	6.90E-03	0.1217248
T11/NT 11	hsa04810:Regulationof actin cytoskeleton	23	3.1420765	**1.74E-04**	P08648,P18206,P26038,Q13464,Q9UQB8,P10398, Q9NZN5,O14974,P35579,Q14155,P35580,P04049, Q15052,Q7Z406,P49023,P25054,P26010,P28482, Q76I76,O43707,Q9NRY4,P36507,Q13576	226	215	5085	2.4069767	0.0229065	7.69E-03	0.2035185
T11/NT 11	hsa04670:Leukocyte transendothelial migration	15	2.0491803	**6.35E-04**	O14493,P18206,P26038,Q96FS4,Q13464,P35221, P55196,Q16625,P49023,Q15080,O60716, Q9NRY4,O43707,Q9Y624,P78369	226	118	5085	2.8601695	0.0809759	2.09E-02	0.7396365
T11/NT 11	hsa03040:Spliceosome	15	2.0491803	**1.22E-03**	P52272,P51991,Q13573,Q13247,Q07955, Q9UKV3,Q16629,Q9Y5S9,P08621,Q5VTL8, Q13242,P61978,P09651,Q15365,P38159	226	126	5085	2.6785714	0.1502805	3.20E-02	1.4214971
T11/NT 11	hsa04510:Focal adhesion	19	2.5956284	**3.15E-03**	P08648,P18206,Q9Y490,Q92934,Q13464,P21333, P10451,Q15942,O14974,Q03135,Q14315,P04049, P05412,P49023,Q9NVD7,P26010,P28482, Q9NRY4,O43707	226	201	5085	2.1268657	0.3425831	6.75E-02	3.6203464
T11/NT 11	hsa04520:Adherens junction	10	1.3661202	**6.48E-03**	P55196,O43318,P18206,Q8WWI1,O60716, P28482,Q9UQB8,P35221,O43707,Q07157	226	77	5085	2.9220779	0.5788665	1.02E-01	7.3211535
T11/NT 11	hsa05213:Endometrial cancer	8	1.0928962	**7.39E-03**	P04049,Q92934,P25054,P28482,P35221,P10398, P36507,O43524	226	52	5085	3.4615385	0.6273717	1.04E-01	8.3128594
T11/NT 11	hsa04722:Neurotrophin signaling pathway	13	1.7759563	**8.30E-03**	Q13233,Q92934,Q9ULH0,Q05655,P35568, O43524,Q99523,Q9Y4H2,P04049,Q13480,P05412, P28482,P36507	226	124	5085	2.358871	0.6699201	1.05E-01	9.2850054
T11/NT 11	hsa05412:Arrhythmoge nic right ventricular cardiomyopathy (ARVC)	10	1.3661202	**5.95E-03**	P02545,P08648,P15924,Q14126,P26010,P35221, O43707,Q99959,P16615,P11532	226	76	5085	2.9605263	0.5476174	1.07E-01	6.7360995
T11/NT 11	hsa03010:Ribosome	10	1.3661202	**1.41E-02**	P15880,P55795,P05387,P05386,P62888,P61247, P62753,P42677,P23396,P26373	226	87	5085	2.5862069	0.8483653	1.45E-01	15.281282
T11/NT 11	hsa05221:Acute myeloidleukemia	8	1.0928962	**1.33E-02**	P04049,Q7Z4J5,Q92934,P28482,P10398,P36507, P42229,P29590	226	58	5085	3.1034483	0.8313104	1.49E-01	14.483689
T11/NT 11	hsa04910:Insulin signaling pathway	13	1.7759563	**1.57E-02**	Q13131,Q93100,Q92934,O95685,P62753,P10398, P35568,P13861,Q9Y4H2,P04049,P28482,Q16822, P36507	226	135	5085	2.1666667	0.8786906	1.50E-01	16.926986
T11/NT 11	hsa04120:Ubiquitin mediatedproteolysis	13	1.7759563	**1.75E-02**	O15344,Q9C0C9,Q13233,Q15751,Q7Z6Z7, Q9Y385,Q14669,Q9UJX2,O00308,Q92466,	226	137	5085	2.1350365	0.9045369	1.54E-01	18.658616

Table. 13 (continued)

					O95071, Q8NHZ8, P29590							
T11/NT 11	hsa04912:GnRH signaling pathway	10	1.3661202	**2.85E-02**	P04049, P05412, Q14573, P63092, Q13233, Q9Y2U5, P52564, P28482, Q05655, P36507	226	98	5085	2.2959184	0.9785959	2.26E-01	28.677823
T11/NT 11	hsa04370:VEGF signaling pathway	8	1.0928962	**4.69E-02**	P04049, Q92934, P49023, P29474, P28482, Q14934, P36507, P04792	226	75	5085	2.4	0.9983097	3.29E-01	42.945012
T11/NT 11	hsa05220:Chronic myeloid leukemia	8	1.0928962	**4.69E-02**	P04049, Q9UQC2, P11274, Q92934, P28482, P10398, P36507, P42229	226	75	5085	2.4	0.9983097	3.29E-01	42.945012
T12/NT 12	hsa04530:Tight junction	4	5.5555556	**1.68E-02**	O14493, Q9H4G0, O95049, P78369	22	134	5085	6.8995929	0.5094701	5.09E-01	14.5256
T12/NT 12	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3	4.1666667	**3.85E-02**	P15924, Q99959, P11532	22	76	5085	9.1238038	0.8080886	5.62E-01	30.493339
T12/NT 12	hsa04510:Focal adhesion	4	5.5555556	**4.79E-02**	Q9Y490, Q9NVD7, Q14185, Q07889	22	201	5085	4.5997286	0.8727504	4.97E-01	36.510042
T13/NT 13	hsa04530:Tight junction	20	3.8314176	**6.65E-09**	O14493, Q9H4G0, P12931, Q3MNF1, Q9NYL2, Q6P1M3, O95049, Q9UDY2, P35221, P35222, P35579, Q13813, P55196, Q9P2M7, Q7Z406, Q8NI35, P31749, P78369, P35749, Q07157, Q14247	149	134	5085	5.0936592	6.91E-07	6.91E-07	7.43E-06
T13/NT 13	hsa03040:Spliceosome	16	3.0651341	**2.86E-06**	Q8WWY3, P51991, O60231, Q9NW64, Q13247, Q07955, P08107, Q9UKV3, P62995, P08621, Q5VTL8, Q13242, P61978, P09651, Q15365, P38159	149	126	5085	4.3336529	2.98E-04	1.49E-04	0.0032018
T13/NT 13	hsa04510:Focal adhesion	17	3.256705	**2.11E-04**	P18206, P12931, Q9Y490, Q92934, P02452, P21333, P35222, O14974, Q14315, P04049, P05412, Q15746, P49023, Q9NVD7, Q14185, P46108, P31749	149	201	5085	2.8864069	0.0217139	7.29E-03	0.2357442
T13/NT 13	hsa04520:Adherens junction	10	1.9157088	**3.50E-04**	P55196, P18206, P12931, Q8WWI1, O60716, Q9UQB8, P35221, P35222, P18031, Q07157	149	77	5085	4.432145	0.0357818	9.07E-03	0.3909831
T13/NT 13	hsa04270:Vascular smooth muscle contraction	11	2.1072797	**1.43E-03**	P04049, Q14573, P63092, Q15746, Q05682, Q3MNF1, P10398, Q14643, O60237, O14974, P35749, Q9Y6F6	149	112	5085	3.3518097	0.1385427	2.94E-02	1.5905425
T13/NT 13	hsa05220:Chronic myeloid leukemia	8	1.532567	**5.82E-03**	Q13547, P04049, Q92934, Q92769, P10398, P46108, P42229, P31749	149	75	5085	3.6402685	0.4551154	9.62E-02	6.3194314
T13/NT 13	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	8	1.532567	**6.26E-03**	P02545, P15924, Q14126, P35221, P17661, Q99959, P35222, P11532	149	76	5085	3.5923702	0.4795188	8.91E-02	6.7797895
T13/NT 13	hsa04012:ErbB signaling pathway	8	1.532567	**1.28E-02**	P04049, P05412, P12931, Q92934, P10398, P46108, P42229, P31749	149	87	5085	3.1381625	0.7392836	1.55E-01	13.457195
T13/NT 13	hsa04540:Gap junction	8	1.532567	**1.44E-02**	P04049, Q14573, P63092, P12931, Q9Y2U5, Q14643, Q07157, P48730	149	89	5085	3.067642	0.7795733	1.55E-01	15.005095
T13/NT 13	hsa04910:Insulin signaling pathway	10	1.9157088	**1.63E-02**	Q13131, P04049, Q92934, Q8N122, O95685, P10398, P46108, P13861, P18031, P31749	149	135	5085	2.5279642	0.818809	1.57E-01	16.777525
T13/NT 13	hsa05213:Endometrial cancer	6	1.1494253	**1.69E-02**	P04049, Q92934, P35221, P10398, P35222, P31749	149	52	5085	3.9377904	0.8300959	1.49E-01	17.351015
T13/NT 13	hsa04670:Leukocyte transendothelial migration	9	1.7241379	**2.10E-02**	P55196, O14493, P18206, Q15080, P49023, O60716, P35221, P35222, P78369	149	118	5085	2.6029462	0.8900443	1.68E-01	21.128666
T13/NT 13	hsa04370:VEGF signaling pathway	7	1.3409962	**2.13E-02**	P04049, P12931, Q92934, P49023, P29474, P04792, P31749	149	75	5085	3.1852349	0.893129	1.58E-01	21.369585
T13/NT 13	hsa05221:Acute myeloid leukemia	6	1.1494253	**2.60E-02**	P04049, Q92934, P10398, P42229, P31749, P29590	149	58	5085	3.5304328	0.9350912	1.77E-01	25.47394
T13/NT 13	hsa04720:Long-term potentiation	6	1.1494253	**4.70E-02**	P04049, Q14573, Q13522, P10398, Q14643, O14974	149	68	5085	3.0112515	0.9932899	2.84E-01	41.608884

Table. 13 (continued)

T13/NT 13	hsa04810:Regulation of actin cytoskeleton	12	2.2988506	**4.77E-02**	P04049, P18206, Q15746, Q7Z406, P49023, Q14185, Q9UQB8, P19634, P10398, P46108, O14974, P35579	149	215	5085	1.9047916	0.9938063	2.72E-01	42.109451
T14/NT 14	hsa04530:Tight junction	20	3.2894737	**3.65E-07**	O14493, Q9H4G0, Q3MNF1, P16989, Q6P1M3, O95049, Q9UDY2, P35221, P35222, P35579, P55196, Q9P2M7, P35580, Q16625, Q7Z406, Q8NI35, Q9Y624, P78369, P35749, Q07157, Q14247	190	134	5085	3.9945012	4.79E-05	4.79E-05	4.26E-04
T14/NT 14	hsa03040:Spliceosome	16	2.6315789	**5.58E-05**	P52272, Q13573, O60231, Q13247, P49756, Q07955, Q9UKV3, Q16629, P62995, Q5VTL8, Q13242, P61978, Q00839, P09651, Q15365, P38159	190	126	5085	3.3984962	0.0072869	3.65E-03	0.0650832
T14/NT 14	hsa04270:Vascular smooth muscle contraction	13	2.1381579	**8.32E-04**	P63092, Q3MNF1, Q13464, P10398, Q9NZN5, O60237, P47712, Q96A00, Q9Y6F6, P04049, Q15746, P36507, O15085, P35749	190	112	5085	3.106438	0.1032587	3.57E-02	0.965499
T14/NT 14	hsa04810:Regulation of actin cytoskeleton	19	3.125	**9.41E-04**	P18206, O60610, Q13464, P19634, P10398, Q9NZN5, P35579, Q14155, P35580, P04049, Q15746, Q15052, Q7Z406, P25054, P26010, Q9Y2X7, Q14185, P36507, Q13576	190	215	5085	2.3651163	0.1160829	3.04E-02	1.0924018
T14/NT 14	hsa04910:Insulin signaling pathway	13	2.1381579	**4.13E-03**	Q13131, Q92934, Q308M2, Q14432, O95685, P49815, P62753, P10398, P13861, Q9Y4H2, P04049, Q16822, P36507	190	135	5085	2.577193	0.4184421	1.03E-01	4.7106193
T14/NT 14	hsa03010:Ribosome	10	1.6447368	**4.69E-03**	P15880, P55795, P05387, P05386, P62888, P61247, P62753, P42677, P23396, P26373	190	87	5085	3.076225	0.4596029	9.75E-02	5.3312569
T14/NT 14	hsa05130:Pathogenic Escherichia coli infection	8	1.3157895	**4.80E-03**	O43639, Q92974, Q16625, Q13464, P05783, P35222, P19338, Q14247	190	57	5085	3.7562327	0.4676489	8.61E-02	5.4575902
T14/NT 14	hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)	9	1.4802632	**6.80E-03**	P02545, P15924, Q14126, P26010, P35221, P17661, Q99959, P35222, P11532	190	76	5085	3.1693213	0.5908833	1.06E-01	7.6477751
T14/NT 14	hsa04520:Adherens junction	9	1.4802632	**7.35E-03**	P55196, P08069, O43318, P18206, Q8WWI1, O60716, P35221, P35222, Q07157	190	77	5085	3.1281613	0.6196813	1.02E-01	8.2458909
T14/NT 14	hsa04510:Focal adhesion	16	2.6315789	**7.38E-03**	P18206, O60610, Q92934, Q308M2, Q13464, P21333, P10451, P35222, Q14315, P04049, P08069, P05412, Q15746, Q9NVD7, P26010, Q14185	190	201	5085	2.1304006	0.6210144	9.25E-02	8.2745655
T14/NT 14	hsa05213:Endometrial cancer	7	1.1513158	**1.20E-02**	P04049, Q92934, P25054, P35221, P10398, P36507, P35222	190	52	5085	3.6027328	0.7931399	1.33E-01	13.087384
T14/NT 14	hsa04670:Leukocyte transendothelial migration	10	1.6447368	**3.06E-02**	P55196, O14493, P18206, Q16625, O60716, Q13464, P35221, P35222, Q9Y624, P78369	190	118	5085	2.2680642	0.983051	2.88E-01	30.439663
T14/NT 14	hsa05210:Colorectal cancer	8	1.3157895	**3.57E-02**	P08069, P04049, P05412, Q92934, Q308M2, P25054, P10398, P35222	190	84	5085	2.5488722	0.9914331	3.07E-01	34.538812
T14/NT 14	hsa04012:ErbB signaling pathway	8	1.3157895	**4.20E-02**	P04049, P05412, O43639, Q92934, Q308M2, P10398, P36507, P42229	190	87	5085	2.46098	0.9963749	3.31E-01	39.363299

Table. 13 (continued)

Accession	Protein	Peptide	Global	1T/1NT	KEGG Path
P55196	Afadin	TQVLsPDSLFTAk	S1721	-1.63	AJ, TJ
Q9H4G0	Band 4.1-like protein 1	hQAsINELk	S510	-1.64	TJ
Q9H4G0	Band 4.1-like protein 1	rLPSsPAsPSPk	S541;S544	-1.11	TJ
Q9H4G0	Band 4.1-like protein 1	SLsPIIGk	S784	-1.53	TJ
Q9H4G0	Band 4.1-like protein 1	gGFsETRIEk	S820	-1.71	TJ
O43491	Band 4.1-like protein 2	qksYTLVVAk	S87	-1.28	TJ
Q9UQB8	Brain-specific angiogenesis inhibitor 1-associated protein 2	SSsmAAGLER	S366	1.41	AJ
Q9UQB8	Brain-specific angiogenesis inhibitor 1-associated protein 2	SSsMAAGLER	S366	1.63	AJ
P35221	Catenin alpha-1	SRTsVQTEDDQLIAGQSAR	S655	1.12	AJ, TJ
P35222	Catenin beta-1	rTsmGGTQQQFVEGVR	S552	-1.03	FA, AJ, TJ
P35222	Catenin beta-1	rtSmGGTQQQFVEGVR	T551	-1.12	FA, AJ, TJ
O60716	Catenin delta-1	gSLAsLDsLRk	S349;S352	-1.12	AJ
O60716	Catenin delta-1	SDFQVNLNNAsR	S857	-1.08	AJ
O60716	Catenin delta-1	SQSSHsYDDSTLPLIDR	S864	-1.99	AJ
Q9P2M7	Cingulin	SHsQASLAGPGPVDPSNR	S131	-1.36	TJ
Q9P2M7	Cingulin	SNsmLELAPk	S149	-1.07	TJ
Q9P2M7	Cingulin	SNsMLELAPk	S149	-1.87	TJ
Q96A65	Exocyst complex component 4	dAsVPLIDVTNLPTPR	S226	-1.45	TJ
P21333	Filamin-A	aFGPGLQGGSAGsPAR	S1084	1.86	FA
P21333	Filamin-A	cSGPGLsPGmVR	S1459	1.94	FA
P21333	Filamin-A	SsFTVDcSk	S2577	1.03	FA
Q14315	Filamin-C	lGsFGSITR	S2233	1.47	FA
P49840	Glycogen synthase kinase-3 alpha	qLVRGEPNVSyIcSR	Y279	1.14	FA
P16144	Integrin beta-4	vLsTSsTLTR	S1483;S1486	1.25	FA
Q9Y624	Junctional adhesion molecule A	kVIYSQPsAR	S284	-1.00	TJ
Q8WWI1	LIM domain only protein 7	SRsTTELDDYSTNk	S1423	-1.06	AJ
Q8WWI1	LIM domain only protein 7	TSTTGVATTQsPTPR	S1586	1.16	AJ
O43318	Mitogen-activated protein kinase kinase kinase 7	rmsADmSEIEAR	S389	1.13	AJ
P35580	Myosin-10	aLsLAR	S1487	1.94	TJ
P35749	Myosin-11	aLsLAR	S1487	1.94	TJ
P35749	Myosin-11	vIENADGsEEETDTR	S1954	1.56	TJ
P35749	Myosin-11	mTESsLPSASk	S638	1.78	TJ
Q7Z406	Myosin-14	aLsLTR	S1504	2.71	TJ
P35579	Myosin-9	aLsLAR	S1480	1.94	TJ
P10451	Osteopontin	fRIsHELDsASSEVN	S303;S308	1.42	FA
P10451	Osteopontin	iSHELDsASSEVN	S308	1.56	FA
Q05655	Protein kinase C delta type	aRLsYSDk	S645	-1.52	TJ

Table. 14

P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEMEVSLAkPk	S124	-1.28	FA, TJ
P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEmEVSLAkPk	S124	-1.16	FA, TJ
P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	S124;S129	-1.04	FA, TJ
Q14247	Src substrate cortactin	akTQtPPVsPAPQPTEER	T401;S405	-2.17	TJ
Q9Y490	Talin-1	aSVPTIQDQASAmQLsQcAk	S1021	1.34	FA
Q9Y490	Talin-1	cVscLPGQR	S1201	2.12	FA
Q9Y490	Talin-1	lLsDSLPPSTGTFQEAQSR	S1225	1.02	FA
Q9Y490	Talin-1	gLAGAVsELLR	S620	2.47	FA
Q9Y490	Talin-1	vVAPTIssPVcQEQLVEAGR	S729	1.37	FA
Q07157	Tight junction protein ZO-1	iDsPGFkPASQQk	S912	-2.96	AJ, TJ
P06241	Tyrosine-protein kinase Fyn	lTEERDGsLNQSSGYR	S21	1.15	FA, AJ
P29350	Tyrosine-protein phosphatase non-receptor type 6	dLsGLDAETLLk	S10	1.79	AJ
P18206	Vinculin	dPSAsPGDAGEQAIR	S290	1.56	FA, AJ
Q15942	Zyxin	fSPVtPk	T270	1.24	FA

Table. 14 (continued)

CASE #	Uniprot	Protein	Peptide	T/NT	Global	Phos Site 1_Function	Phos Site 2_Function	Drug
1	P47712	Cytosolic phospholipase A2	qNPSRcsVSLSNVEAR	1.63	S727	enzymatic activity, induced		
1	P49840	Glycogen synthase kinase-3 alpha	qLVRGEPNVSyIcSR	1.14	Y279	enzymatic activity, induced		
1	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	-1.53	S232	enzymatic activity, inhibited		
1	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-1.67	S232	enzymatic activity, inhibited		
1	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVsYR	-1.10	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
1	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEMEVSLAkPk	-1.28	S124	enzymatic activity, induced		GSK2141795
1	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEmEVSLAkPk	-1.16	S124	enzymatic activity, induced		GSK2141796
1	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	-1.04	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141797
1	P06241	Tyrosine-protein kinase Fyn	lTEERDGsLNQSSGYR	1.15	S21	enzymatic activity, induced		Dasatinib
4	P28482	Mitogen-activated protein kinase 1	vADPDHDHTGFLtEyVATR	-1.93	T185;Y187	enzymatic activity, induced	enzymatic activity, induced	AEZS-131
4	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEMEVSLAkPk	1.69	S124	enzymatic activity, induced		GSK2141795
4	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEmEVSLAkPk	1.59	S124	enzymatic activity, induced		GSK2141796
4	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	1.31	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141797
5	P13569	Cystic fibrosis transmembrane conductance regulator	rLsLVPDSEQGEAILPR	-1.11	S737	enzymatic activity, inhibited		
5	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	gScNLSRVDsTTcLFPVEEk	-1.02	S261	enzymatic activity, induced		
5	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	vDsTTcLFPVEEk	-1.25	S261	enzymatic activity, induced		

Table. 15

5	P28482	Mitogen-activated protein kinase 1	vADPDHDHTGFLtEyVATR	1.05	T185;Y187	enzymatic activity, induced	enzymatic activity, induced	AEZS-131
5	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	-1.41	S232	enzymatic activity, inhibited		
5	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-1.82	S232	enzymatic activity, inhibited		
5	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVsYR	-1.53	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
6	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	gScNLSRVDsTTcLFPVEEk	-1.33	S261	enzymatic activity, induced		
6	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	vDsTTcLFPVEEk	-1.14	S261	enzymatic activity, induced		
6	P49840	Glycogen synthase kinase-3 alpha	gEPNVSyIcSR	1.03	Y279	enzymatic activity, induced		
6	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	-1.10	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141795
7	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	gScNLSRVDsTTcLFPVEEk	1.11	S261	enzymatic activity, induced		
7	P49841	Glycogen synthase kinase-3 beta	TTsFAESckPVQQPSAFGSmk	-1.11	S9	enzymatic activity, inhibited		
7	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	1.12	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141795
7	P06241	Tyrosine-protein kinase Fyn	dGsLNQSSGYR	-1.14	S21	enzymatic activity, induced		Dasatinib
8	P13569	Cystic fibrosis transmembrane conductance regulator	rLsLVPDSEQGEAILPR	-4.50	S737	enzymatic activity, inhibited		
8	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	vDsTTcLFPVEEk	-1.13	S261	enzymatic activity, induced		
8	P49841	Glycogen synthase kinase-3 beta	TTsFAESckPVQQPSAFGSMk	2.05	S9	enzymatic activity, inhibited		

Table. 15 (continued)

8	P49841	Glycogen synthase kinase-3 beta	TTsFAESckPVQQPSAFGSmk	1.23	S9	enzymatic activity, inhibited		
8	P49841	Glycogen synthase kinase-3 beta	gEPNVSyIcSR	2.32	Y216	enzymatic activity, induced		
8	P49841	Glycogen synthase kinase-3 beta	qLVRGEPNVSyIcSR	1.13	Y216	enzymatic activity, induced		
8	Q13547	Histone deacetylase 1	iAcEEEFsDsEEEGEGGRk	-1.04	S421;S423	enzymatic activity, induced	enzymatic activity, induced	Vorinostat
8	Q92769	Histone deacetylase 2	iAcDEEFsDSEDEGEGGRR	1.43	S422	enzymatic activity, inhibited		Vorinostat
8	P28482	Mitogen-activated protein kinase 1	vADPDHDHTGFLtEyVATR	1.44	T185;Y187	enzymatic activity, induced	enzymatic activity, induced	AEZS-131
8	Q16539	Mitogen-activated protein kinase 14	hTDDEMtGyVATR	1.33	T180;Y182	enzymatic activity, induced	enzymatic activity, induced	
8	Q16539	Mitogen-activated protein kinase 14	hTDDEMTGyVATR	-1.34	Y182	enzymatic activity, induced		
8	P29474	Nitric oxide synthase, endothelial	iRtQsFSLQER	-1.23	T1175;S1177		enzymatic activity, induced	
8	P29474	Nitric oxide synthase, endothelial	iRtQSFsLQER	-1.77	T1175;S1179		enzymatic activity, induced	
8	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-2.68	S232	enzymatic activity, inhibited		
8	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVsYR	-1.44	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
8	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsMSDPGVsYR	-1.27	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
8	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEMEVSLAkPk	-1.57	S124	enzymatic activity, induced		GSK2141795
8	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	-2.86	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141795
9	P13569	Cystic fibrosis transmembrane conductance regulator	rLsLVPDSEQGEAILPR	-2.35	S737	enzymatic activity, inhibited		
9	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	gScNLSRVDsTTcLFPVEEk	-1.28	S261	enzymatic activity, induced		
9	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	vDsTTcLFPVEEk	-2.06	S261	enzymatic activity, induced		

Table. 15 (continued)

9	Q16539	Mitogen-activated protein kinase 14	hTDDEMtGyVATR	1.39	T180;Y182	enzymatic activity, induced	enzymatic activity, induced	
9	P29474	Nitric oxide synthase, endothelial	iRtQSFsLQER	-1.22	T1175;S1179		enzymatic activity, induced	
9	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	-1.03	S232	enzymatic activity, inhibited		
9	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-2.07	S232	enzymatic activity, inhibited		
9	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVsYR	-1.14	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
9	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	-1.26	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141795
9	Q15139	Serine/threonine-protein kinase D1	aLGERVsIL	1.28	S910	enzymatic activity, induced		
10	Q9Y2I7	1-phosphatidylinositol-3-phosphate 5-kinase	SAsITNLSLDR	-1.39	S307	enzymatic activity, induced		
10	P13569	Cystic fibrosis transmembrane conductance regulator	rLsLVPDSEQGEAILPR	-4.08	S737	enzymatic activity, inhibited		
10	Q06210	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	vDsTTcLFPVEEk	-2.33	S261	enzymatic activity, induced		
10	P49841	Glycogen synthase kinase-3 beta	TTsFAESckPVQQPSAFGSMk	1.01	S9	enzymatic activity, inhibited		
10	P49841	Glycogen synthase kinase-3 beta	gEPNVSyIcSR	1.80	Y216	enzymatic activity, induced		
10	P49841	Glycogen synthase kinase-3 beta	qLVRGEPNVSyIcSR	1.57	Y216	enzymatic activity, induced		
10	Q13547	Histone deacetylase 1	iAcEEEFsDsEEEGEGGRk	-1.57	S421;S423	enzymatic activity, induced	enzymatic activity, induced	Vorinostat
10	P28482	Mitogen-activated protein kinase 1	vADPDHDHTGFLtEyVATR	1.28	T185;Y187	enzymatic activity, induced	enzymatic activity, induced	AEZS-131
10	Q16539	Mitogen-activated protein kinase 14	hTDDEMtGyVATR	2.20	T180;Y182	enzymatic activity, induced	enzymatic activity, induced	
10	P29474	Nitric oxide synthase, endothelial	iRtQsFSLQER	-1.94	T1175;S1177		enzymatic activity, induced	
10	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-2.81	S232	enzymatic activity, inhibited		

Table. 15 (continued)

10	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVsYR	-1.58	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
10	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsMSDPGVsYR	-1.47	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited	
10	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNsGAEEMEVSLAkPk	1.26	S124;S129	enzymatic activity, induced	enzymatic activity, induced	GSK2141795
10	P04049	RAF proto-oncogene serine/threonine-protein kinase	SAsEPSLHR	1.49	S621	enzymatic activity, inhibited/induced		Sorafenib
10	P10398	Serine/threonine-protein kinase A-Raf	SAsEPSLHR	1.49	S582	enzymatic activity, induced		Sorafenib
10	Q15139	Serine/threonine-protein kinase D1	aLGERVsIL	-1.23	S910	enzymatic activity, induced		
11	P52564	Dual specificity mitogen-activated protein kinase kinase 6	mcDFGISGYLVDsVAk	-2.23	S207	enzymatic activity, inhibited		
11	P49840	Glycogen synthase kinase-3 alpha	qLVRGEPNVSyIcSR	1.38	Y279	enzymatic activity, induced		
11	P28482	Mitogen-activated protein kinase 1	vADPDHDHTGFLtEyVATR	-1.07	T185;Y187	enzymatic activity, induced	enzymatic activity, induced	AEZS-131
11	P29474	Nitric oxide synthase, endothelial	iRtQsFSLQER	1.03	T1175;S1177		enzymatic activity, induced	
11	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	-2.81	S232	enzymatic activity, inhibited		
11	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-3.45	S232	enzymatic activity, inhibited		
11	P04049	RAF proto-oncogene serine/threonine-protein kinase	SAsEPSLHR	-1.71	S621	enzymatic activity, inhibited/induced		Sorafenib
11	P10398	Serine/threonine-protein kinase A-Raf	SAsEPSLHR	-1.71	S582	enzymatic activity, induced		Sorafenib
11	Q9Y385	Ubiquitin-conjugating enzyme E2 J1	qIsFkAEVNSSGk	-1.42	S184	enzymatic activity, induced		
13	P49840	Glycogen synthase kinase-3 alpha	gEPNVSyIcSR	1.15	Y279	enzymatic activity, induced		
13	Q13547	Histone deacetylase 1	iAcEEEFsDsEEEGEGGRk	1.66	S421;S423	enzymatic activity, induced	enzymatic activity, induced	Vorinostat

Table. 15 (continued)

13	Q92769	Histone deacetylase 2	iAcDEEFsDsEDEGEGGRR	1.42	S422;S424	enzymatic activity, inhibited	enzymatic activity, inhibited	Vorinostat
13	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	2.82	S232	enzymatic activity, inhibited		
13	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHsmSDPGVSYR	1.89	S293	enzymatic activity, inhibited		
13	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YHGHSMsDPGVsYR	1.01	S295;S300		enzymatic activity, inhibited	
13	P31749	RAC-alpha serine/threonine-protein kinase	SGsPSDNSGAEEMEVSLAkPk	1.48	S124	enzymatic activity, induced		GSK2141795
13	P04049	RAF proto-oncogene serine/threonine-protein kinase	SAsEPSLHR	1.91	S621	enzymatic activity, inhibited/induced		Sorafenib
13	P10398	Serine/threonine-protein kinase A-Raf	SAsEPSLHR	1.91	S582	enzymatic activity, induced		Sorafenib
13	P18031	Tyrosine-protein phosphatase non-receptor type 1	YRDVsPFDHSR	1.38	S50	enzymatic activity, inhibited/induced		
14	Q14432	cGMP-inhibited 3,5-cyclic phosphodiesterase A	rTsLPcIPR	-1.42	S312	enzymatic activity, induced		
14	P47712	Cytosolic phospholipase A2	qNPSRcsVsLSNVEAR	1.18	S727;S729	enzymatic activity, induced		
14	P52564	Dual specificity mitogen-activated protein kinase kinase 6	mcDFGISGYLVDsVAk	-2.78	S207	enzymatic activity, inhibited		
14	P49840	Glycogen synthase kinase-3 alpha	qLVRGEPNVSyIcSR	1.08	Y279	enzymatic activity, induced		
14	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGMGTsVER	-3.02	S232	enzymatic activity, inhibited		
14	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	YGmGTsVER	-2.82	S232	enzymatic activity, inhibited		
		Pyruvate dehydrogenase E1						

Table. 15 (continued)

Table. 15 (continued)

14	P08559	component subunit alpha, somatic form, mitochondrial Pyruvate dehydrogenase E1	YHGHsmSDPGVsYR	-1.38	S293;S300	enzymatic activity, inhibited	enzymatic activity, inhibited
14	P08559	component subunit alpha, somatic form, mitochondrial	YHGHsMSDPGVsYR	-1.08	S293;S300	enzymatic activity, inhibited	
14	P04049	RAF proto-oncogene serine/threonine-protein kinase	STsTPNVHMVSTTLPVDSR	-1.25	S259	enzymatic activity, inhibited	Sorafenib
14	Q9Y385	Ubiquitin-conjugating enzyme E2 J1	qIsFkAEVNSSGk	-1.12	S184	enzymatic activity, induced	