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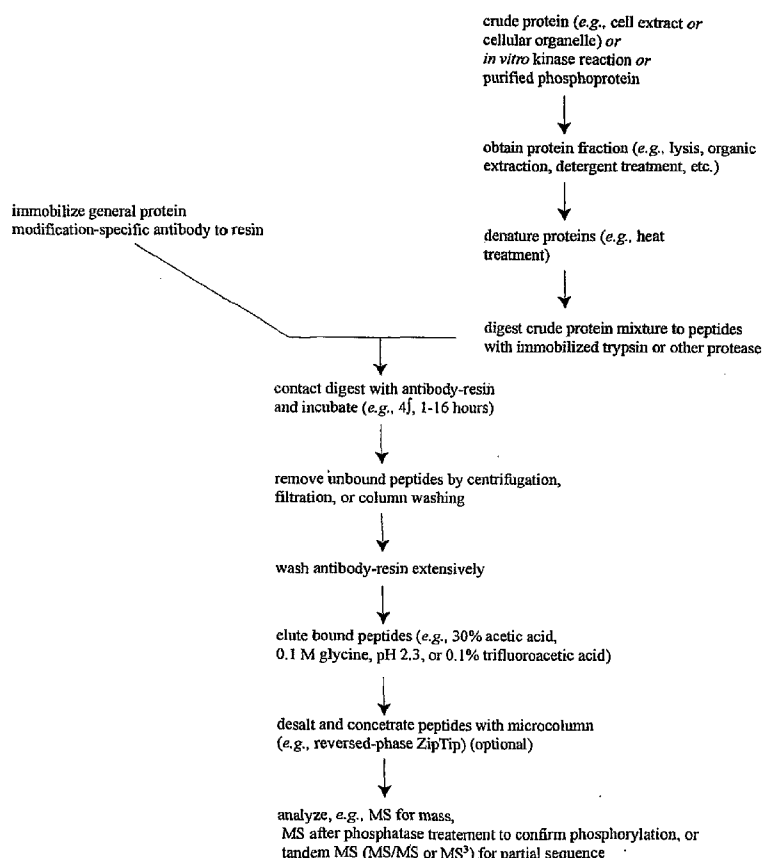
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(54) Title: REAGENTS FOR THE DETECTION OF PROTEIN PHOSPHORYLATION IN ATM AND ATR KINASE SIGNALING PATHWAYS



(57) Abstract: The invention discloses nearly 300 novel phosphorylation sites identified in signal transduction proteins and pathways underlying human ATM/ATR kinase signaling pathways, and provides phosphorylation-site specific antibodies and heavy-isotope labeled peptides (AQUA peptides) for the selective detection, profiling and quantification of these phosphorylated sites/proteins, as well as methods of using the reagents for such purpose. Among the phosphorylation sites identified are sites occurring in the following protein types: DNA repair proteins, Adaptor/Scaffold proteins, Cell cycle regulation proteins, G Protein/GTPase Activating/Guanine Nucleotide Exchange Factor proteins, DNA binding proteins, DNA replication proteins, Kinases, Disease associated proteins proteins, Methyltransferase, Ubiquitin conjugating proteins, Proteases, Phosphatases, and Transcription proteins.

WO 2007/127335 A2



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REAGENTS FOR THE DETECTION OF PROTEIN PHOSPHORYLATION IN ATM AND ATR KINASE SIGNALING PATHWAYS

RELATED APPLICATIONS

This application claims the benefit of, and priority to, U.S.S.N.
5 60/795,440, filed April 27, 2006, presently pending, the disclosure of
which is incorporated herein, in its entirety, by reference

FIELD OF THE INVENTION

The invention relates generally to antibodies and peptide reagents
for the detection of protein phosphorylation, and to protein
10 phosphorylation in cancer.

BACKGROUND OF THE INVENTION

The activation of proteins by post-translational modification is an
important cellular mechanism for regulating most aspects of biological
organization and control, including growth, development, homeostasis,
15 and cellular communication. Protein phosphorylation, for example, plays
a critical role in the etiology of many pathological conditions and diseases,
including cancer, developmental disorders, autoimmune diseases, and
diabetes. Yet, in spite of the importance of protein modification, it is not
yet well understood at the molecular level, due to the extraordinary
20 complexity of signaling pathways, and the slow development of
technology necessary to unravel it.

Protein phosphorylation on a proteome-wide scale is extremely
complex as a result of three factors: the large number of modifying
proteins, e.g. kinases, encoded in the genome, the much larger number of
25 sites on substrate proteins that are modified by these enzymes, and the

- 2 -

dynamic nature of protein expression during growth, development, disease states, and aging. The human genome, for example, encodes over 520 different protein kinases, making them the most abundant class of enzymes known. See Hunter, *Nature* 411: 355-65 (2001). Most
5 kinases phosphorylate many different substrate proteins, at distinct threonine, serine, and/or threonine residues. Indeed, it is estimated that one-third of all proteins encoded by the human genome are phosphorylated, and many are phosphorylated at multiple sites by different kinases. See Graves *et al.*, *Pharmacol. Ther.* 82: 111-21 (1999).

10 Many of these phosphorylation sites regulate critical biological processes and may prove to be important diagnostic or therapeutic targets for molecular medicine. For example, of the more than 100 dominant oncogenes identified to date, 46 are protein kinases. See Hunter, *supra*. Understanding which proteins are modified by these
15 kinases will greatly expand our understanding of the molecular mechanisms underlying oncogenic transformation. Therefore, the identification of, and ability to detect, phosphorylation sites on a wide variety of cellular proteins is crucially important to understanding the key signaling proteins and pathways implicated in the progression of diseases
20 like cancer.

Such pathways include the ability of a non-cancerous cell to stop progression through the cell cycle in response to genomic defects is termed the DNA damage checkpoint. See Hartwell *et al.*, *Science*, 246(4930): 629-34 (1989). It is thought that activation of these checkpoint
25 mechanisms allows time for cells to repair or bypass DNA damage without potentially lethal entry into mitosis.

Central to the proper function of the DNA damage checkpoint are the phosphoinositol 3 phosphate kinase (PI3K) like kinases, ataxia telangiectasia mutated (ATM), ATM and Rad3 related (ATR), DNA
30 dependent protein kinase (DNA-PK), and the more recently discovered

- 3 -

member of the family, SMG1. See Abraham *et al.*, *Genes Dev.* 15:2177-2196 (2001). In response to DNA damage, these kinases are activated (through as of yet undetermined mechanisms), leading to activation of signaling pathways that (1) block progression through the cell cycle, (2)
5 coordinate repair activities, and (3) affect transcription of DNA damage response genes. It has been shown that ATM is activated by the presence of double stranded breaks (DSBs) in the genome, such as those generated by gamma irradiation (IR). See Banin *et al.*, *Science*, 281(5383): 1674-7. (1998). In contrast, ATR is activated by a variety of
10 genotoxic agents, such as UV light, alkylating agents, and perturbation of DNA replication by inhibitors such as aphidicolin and hydroxyurea. See Cliby *et al.*, *EMBO J.*, 17(1): 159-69. (1998). It has also been demonstrated that ATR has a role in normal cell cycle progression, as ATR deletion results in embryonic lethality. See de Klein *et al.*, *Curr Biol.*
15 10(8):479-82. (2000). The role of DNA-PK is less clear, though it is known to be involved in the response to both DSBs and UV damage of DNA. See Park *et al.*, *J Biol Chem.*, 274 (45):32520-7 (1999).

Once activated and/or localized, various adaptor proteins control the activities of ATM/ATR/DNA-PK. In the case of ATR, the adaptor
20 ATRIP has been shown to be important not only for proper localization of ATR in response to damage, but also for signaling to downstream targets. Like ATR, an adaptor also controls ATM localization and activity. In this case the adaptor is the aforementioned heterotrimeric complex of Mre11, NBS1, and Rad50 (M/R/N). This complex has been shown to localize to
25 sites of Double Strand Breaks (DSB's) early in the damage response, and is thought to be important in activating ATM at sites of damage. Proper function of DNA-PK has similarly been shown to depend on another adaptor complex made up of the Ku proteins. See Cortez *et al.*, 2001, Falck *et al.*, 2005, Lee and Paul, 2005).

- 4 -

Many effects of ATM and ATR are mediated by their so-called effector kinases, Chk1 for ATR, and Chk2 for ATM. See Guo *et al.*, *Genes Dev.* 14(21): 2745-56. (2000). ATR or ATM activates these kinases through phosphorylation in response to damage, and they subsequently carry out some of the best-characterized functions of the DNA damage checkpoint. As adaptor proteins regulate ATM/ATR, the functions of Chk1, and possibly of Chk2, also appear to be regulated by adaptors. The protein Claspin, for example, has been shown to be necessary for the ATR-dependent phosphorylation and activation of Chk1. See Kumagai *et al.*, *J. Cell Biol.* 142:1559-69 (2001). There is currently no known adaptor for Chk2, however, some have speculated that BRCA1 may fulfill this role. See Cortez *et al.*, *Science*, 294(5547):1713-6 (1999).

Although the best-characterized pathways of the DNA damage checkpoint rely on the action of the effector kinases, Chk1 or Chk2, it is clear that ATM/ATR/DNA-PK target other DNA damage response proteins independently. Other examples of known ATM/ATR substrates include BRCA1, p95/NBS1, MDM2, CtIP, 4E-BP1, SMC1, H2AX, and 53BP1. See Kastan *et al.*, *Mol Cell Biol.*, 1(3):179-86 (2000). In more general terms, it has been shown that the PI3K-like family of kinases has a substrate preference for serine or threonine with a glutamine at the +1 position (the S/TQ motif). Additionally, positively charged residues surrounding the S/T-Q motif inhibit efficient substrate phosphorylation by ATM/ATR (Kim, 1999, O'Neill *et al.*, 2000). New substrates of the PI3K-like kinases are continually being discovered, although the discovery process has to date been laborious, finding only one or a few new substrates at a time.

Despite the identification of a few key molecules involved in ATM and ATR protein kinase signaling pathways, the vast majority of signaling protein changes underlying these pathways remains unknown. There is,

- 5 -

therefore, relatively scarce information about kinase-driven signaling pathways and phosphorylation sites relevant to ATM and ATR. This has hampered a complete and accurate understanding of how protein activation within signaling pathways may be driving the malfunction of the DNA damage checkpoint and cancer.

Accordingly, there is a continuing and pressing need to unravel the molecular mechanisms of ATM/ATR kinase-driven oncogenesis in cancer by identifying the downstream signaling proteins mediating cellular transformation. Identifying particular phosphorylation sites on such signaling proteins and providing new reagents, such as phospho-specific antibodies and AQUA peptides, to detect and quantify them remains particularly important to advancing our understanding of the biology of this pathway. Moreover, identification of downstream signaling molecules and phosphorylation sites involved in ATM/ATR signaling and development of new reagents to detect and quantify these sites and proteins may lead to improved diagnostic/prognostic markers, as well as novel drug targets, for the detection and treatment of cancer.

SUMMARY OF THE INVENTION

The invention discloses nearly 300 novel phosphorylation sites identified in signal transduction proteins and pathways relevant to ataxia telangiectasia mutated (ATM) and ATM Rad3 related (ATR) kinase signaling and provides new reagents, including phosphorylation-site specific antibodies and AQUA peptides, for the selective detection and quantification of these phosphorylated sites/proteins. Also provided are methods of using the reagents of the invention for the detection, profiling and quantification of the disclosed phosphorylation sites.

BRIEF DESCRIPTION OF THE DRAWINGS

- 6 -

FIG. 1 – Is a diagram broadly depicting the immunoaffinity isolation and mass-spectrometric characterization methodology (IAP) employed to identify the novel phosphorylation sites disclosed herein.

FIG. 2 – Is a table (corresponding to Table 1) enumerating the ATM and/or ATR signaling protein phosphorylation sites disclosed herein: Column A = the name of the parent protein; Column B = the SwissProt accession number for the protein (human sequence); Column C = the protein type/classification; Column D = the threonine or serine residue (in the parent protein amino acid sequence) at which phosphorylation occurs within the phosphorylation site; Column E = the phosphorylation site sequence encompassing the phosphorylatable residue (residue at which phosphorylation occurs (and corresponding to the respective entry in Column D) appears in lowercase; Column F = the type of disease in which the phosphorylation site was discovered; and Column G = the cell type(s) in which the phosphorylation site was discovered.

FIG. 3 – is an exemplary mass spectrograph depicting the detection of the serine 395 phosphorylation site in NuMA-1 (see Row 40 in Figure 2/Table 1), as further described in Example 1 (red and blue indicate ions detected in MS/MS spectrum); S* indicates the phosphorylated serine (shown as lowercase "s" in Figure 2).

FIG. 4 – is an exemplary mass spectrograph depicting the detection of the threonine 286 phosphorylation site in NLK (see Row 173 in Figure 2/Table 1), as further described in Example 1 (red and blue indicate ions detected in MS/MS spectrum); T* indicates the phosphorylated threonine (shown as lowercase "t" in Figure 2).

FIG. 5 – is an exemplary mass spectrograph depicting the detection of the serine 352 phosphorylation site in Bcl-9 (see Row 58 in Figure 2/Table 1), as further described in Example 1 (red and blue indicate ions detected in MS/MS spectrum); S* indicates the phosphorylated serine (shown as lowercase "s" in Figure 2).

- 7 -

FIG. 6 – is an exemplary mass spectrograph depicting the detection of the serine 951 phosphorylation site in Smc1 (see Row 111 in Figure 2/ Table 1), as further described in Example 1 (red and blue indicate ions detected in MS/MS spectrum); S* indicates the phosphorylated serine
5 (shown as lowercase "s" in Figure 2).

FIG. 7 – is an exemplary mass spectrograph depicting the detection of the serine 161, 164 and 172 phosphorylation sites in FOXJ2 (see Rows 224-226 in Figure 2/ Table 1), as further described in Example 1 (red and blue indicate ions detected in MS/MS spectrum); S* indicates the
10 phosphorylated serine (shown as lowercase "s" in Figure 2).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, nearly 300 novel protein phosphorylation sites in signaling proteins and pathways underlying ATM and ATR kinase signaling have now been discovered. These newly
5 described phosphorylation sites were identified by employing the techniques described in "Immunoaffinity Isolation of Modified Peptides From Complex Mixtures," U.S. Patent Publication No. 20030044848, Rush *et al.*, using cellular extracts from a variety of glioblastoma-derived cell lines, *e.g.* MO59K, 293T etc., as further described below. The novel
10 phosphorylation sites (threonine or serine), and their corresponding parent proteins, disclosed herein are listed in Table 1. These phosphorylation sites correspond to numerous different parent proteins (the full sequences of which (human) are all publicly available in SwissProt database and their Accession numbers listed in Column B of
15 Table 1/Fig. 2), each of which fall into discrete protein type groups, for example DNA repair proteins, Protein Kinases, and Vesicle proteins, etc. (see Column C of Table 1), the phosphorylation of which is relevant to signal transduction activity underlying ATM and ATR kinase signaling, as disclosed herein.

20 The discovery of the nearly 300 novel protein phosphorylation sites described herein enables the production, by standard methods, of new reagents, such as phosphorylation site-specific antibodies and AQUA peptides (heavy-isotope labeled peptides), capable of specifically detecting and/or quantifying these phosphorylated sites/proteins. Such
25 reagents are highly useful, *inter alia*, for studying signal transduction events underlying the progression of cancer. Accordingly, the invention provides novel reagents -- phospho-specific antibodies and AQUA peptides -- for the specific detection and/or quantification of as ATM and/or ATR kinase signaling protein/polypeptide only when
30 phosphorylated (or only when not phosphorylated) at a particular

- 9 -

phosphorylation site disclosed herein. The invention also provides methods of detecting and/or quantifying one or more phosphorylated ATM and/or ATR kinase signaling proteins using the phosphorylation-site specific antibodies and AQUA peptides of the invention and methods of
5 obtaining a phosphorylation profile of such proteins (e.g. Kinases).

In part, the invention provides an isolated phosphorylation site-specific antibody that specifically binds a given ATM and/or ATR kinase signaling protein only when phosphorylated (or not phosphorylated, respectively) at a particular threonine or serine enumerated in Column D
10 of Table 1/Figure 2 comprised within the phosphorylatable peptide site sequence enumerated in corresponding Column E. In further part, the invention provides a heavy-isotope labeled peptide (AQUA peptide) for the detection and quantification of a given ATM and/or ATR kinase signaling protein, the labeled peptide comprising a particular
15 phosphorylatable peptide site/sequence enumerated in Column E of Table 1/Figure 2 herein. For example, among the reagents provided by the invention is an isolated phosphorylation site-specific antibody that specifically binds the ILF2 transcription protein only when phosphorylated (or only when not phosphorylated) at threonine 388 (see Row 254 (and
20 Columns D and E) of Table 1/Figure 2). By way of further example, among the group of reagents provided by the invention is an AQUA peptide for the quantification of phosphorylated ILF2 transcription protein, the AQUA peptide comprising the phosphorylatable peptide sequence listed in Column E, Row 254, of Table 1/Figure 2 (which encompasses
25 the phosphorylatable threonine at position 388).

In one embodiment, the invention provides an isolated phosphorylation site-specific antibody that specifically binds a human ATM and/or ATR kinase signaling protein selected from Column A of Table 1 (Rows 2-301) only when phosphorylated at the threonine or
30 serine residue listed in corresponding Column D of Table 1, comprised

- 10 -

within the phosphorylatable peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-300), wherein said antibody does not bind said signaling protein when not phosphorylated at said threonine or serine. In another embodiment, the invention provides an isolated
5 phosphorylation site-specific antibody that specifically binds an ATM and/or ATR kinase signaling protein selected from Column A of Table 1 only when not phosphorylated at the threonine or serine residue listed in corresponding Column D of Table 1, comprised within the peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-
10 300), wherein said antibody does not bind said signaling protein when phosphorylated at said threonine or serine. Such reagents enable the specific detection of phosphorylation (or non-phosphorylation) of a novel phosphorylatable site disclosed herein. The invention further provides immortalized cell lines producing such antibodies. In one preferred
15 embodiment, the immortalized cell line is a rabbit or mouse hybridoma.

In another embodiment, the invention provides a heavy-isotope labeled peptide (AQUA peptide) for the quantification of an ATM and/or ATR kinase signaling protein selected from Column A of Table 1, said labeled peptide comprising the phosphorylatable peptide sequence listed
20 in corresponding Column E of Table 1 (SEQ ID NOs: 1-300), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D of Table 1. In certain preferred embodiments, the phosphorylatable threonine or serine within the labeled peptide is phosphorylated, while in other preferred embodiments, the
25 phosphorylatable residue within the labeled peptide is not phosphorylated.

Reagents (antibodies and AQUA peptides) provided by the invention may conveniently be grouped by the type of ATM and/or ATR kinase signaling protein in which a given phosphorylation site (for which reagents are provided) occurs. The protein types for each respective
30 protein (in which a phosphorylation site has been discovered) are

- 11 -

provided in Column C of Table 1/Figure 2, and include: Adaptor/Scaffold proteins, Apoptosis proteins, Calcium-binding proteins, Cell Cycle Regulation proteins, DNA replication proteins, Channel proteins, Chaperone proteins, Contractile proteins, Cellular Metabolism enzymes, Cytoskeletal proteins, DNA repair proteins, DNA binding proteins, Endoplasmic reticulum protein, enzyme proteins, G protein and GTPase Activating proteins, Guanine Nucleotide Exchange Factors, Helicase proteins, Isomerase proteins, Ligase proteins, Methyltransferase proteins Lipid Kinases, Lipid Binding proteins, Lipid Phosphatases, Phosphatase proteins Mitochondrial proteins, Motor proteins, DNA Repair/ Binding/Transcription proteins, Kinase Proteins, Phosphodiesterases, Proteases, Serine/Threonine Protein Kinase, Protein Phosphatases, RNA binding proteins, Transferase proteins Receptors, Secreted proteins, Translation/Transporter proteins, Ubiquitin Conjugating System proteins, and Vesicle proteins. Each of these distinct protein groups is considered a preferred subset of ATM and/or ATR signal transduction protein phosphorylation sites disclosed herein, and reagents for their detection/quantification may be considered a preferred subset of reagents provided by the invention.

Particularly preferred subsets of the phosphorylation sites (and their corresponding proteins) disclosed herein are those occurring on the following protein types/groups listed in Column C of Table 1/Figure 2, DNA repair proteins, transcription proteins, kinase proteins, Phosphatases, G protein/GTPase Activating proteins/Guanine Nucleotide Exchange Factors, DNA replication proteins, DNA binding proteins, Disease associated proteins, Adaptor/Scaffold proteins, Cell cycle regulation proteins, Proteases, Methyltransferases and Ubiquitin conjugating proteins. Accordingly, among preferred subsets of reagents provided by the invention are isolated antibodies and AQUA peptides

- 12 -

useful for the detection and/or quantification of the foregoing preferred protein/phosphorylation site subsets.

In one subset of preferred embodiments, there is provided:

- 5 (i) An isolated phosphorylation site-specific antibody that specifically binds an DNA repair protein selected from Column A, Rows 90-100, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 90-100, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 90-100, of Table 1 (SEQ ID NOs: 89-99), wherein said antibody
10 does not bind said protein when not phosphorylated at said threonine or serine.
- (ii) An equivalent antibody to (i) above that only binds the DNA repair protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).
- 15 (iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of an DNA repair protein selected from Column A, Rows 90-100, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 90-100, of Table 1 (SEQ ID NOs: 89-99), which sequence comprises the phosphorylatable
20 threonine or serine listed in corresponding Column D, Rows 90-100, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following DNA repair protein phosphorylation sites are particularly preferred: MRE11A (S648),
25 NBS1 (S397), and NBS1 (S58) (see SEQ ID NOs: 91, 95 and 96).

In a second subset of preferred embodiments there is provided:

- (i) An isolated phosphorylation site-specific antibody that specifically binds a Transcription protein selected from Column A, Rows 221-266, of Table 1 only when phosphorylated at the threonine or serine listed in

- 13 -

corresponding Column D, Rows 221-266, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

(ii) An equivalent antibody to (i) above that only binds the Transcription protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Transcription protein selected from Column A, Rows 221-266, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 221-266, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following Transcription protein phosphorylation sites are particularly preferred: FOXJ2 (S161), MYT1L (S991), 53BP1 (S105), MYST2 (S50), NCOA2 (S716) and YAP1 (T973) (see SEQ ID NOs: 224, 230, 251, 257, 259 and 262).

In another subset of preferred embodiments there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a Protein Kinase selected from Column A, Rows 149 and 166 -181, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 149 and 166 -181, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 149 and 166 -181, of Table 1 (SEQ ID NOs: 148 and 165 -180), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

- 14 -

(ii) An equivalent antibody to (i) above that only binds the Protein Kinase when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the
5 quantification of a Protein Kinase selected from Column A, Rows 149 and 166 -181, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 149 and 166 -181, of Table 1 (SEQ ID NOs: 148 and 165 -180), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D,
10 Rows 149 and 166 -181, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following Protein Kinase phosphorylation sites are particularly preferred: NLK (T286) and WNK1 (S167) (see SEQ ID NOs: 172 and 180).

15 In still another subset of preferred embodiments there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a Phosphatase selected from Column A, Rows 157-161 and 182-192, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1,
20 comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

(ii) An equivalent antibody to (i) above that only binds the Phosphatase
25 when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Phosphatase selected from Column A, Rows 157-161 and 182-192, said labeled peptide comprising the phosphorylatable

- 15 -

peptide sequence listed in corresponding Column E, Rows 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1.

5 In still another subset of preferred embodiments there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a G protein/GTPase/Guanine nucleotide exchange factor selected from Column A, Rows 119-135, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 119-135, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 119-135, of Table 1 (SEQ ID NOs: 118-134), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

(ii) An equivalent antibody to (i) above that only binds the G protein/GTPase/Guanine nucleotide exchange factor when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a G protein/GTPase/Guanine nucleotide exchange factor selected from Column A, Rows 119-135, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 119-135, of Table 1 (SEQ ID NOs: 118-134), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 119-135, of Table 1.

25 In still another subset of preferred embodiments there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a DNA replication protein selected from Column A, Rows 101-114, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 101-114, of Table 1, comprised within the

- 16 -

phosphorylatable peptide sequence listed in corresponding Column E, Rows 101-114 of Table 1 (SEQ ID NOs: 100-113), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

- 5 (ii) An equivalent antibody to (i) above that only binds the DNA replication protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

- (iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a DNA replication protein selected from Column A, Rows
10 101-114, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 101-114, of Table 1 (SEQ ID NOs: 100-113), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 101-114, of Table 1.

- 15 Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following DNA replication protein phosphorylation site is particularly preferred: SMC1 (S951) (see SEQ ID NO: 110).

In yet another subset of preferred embodiments, there is provided:

- 20 (i) An isolated phosphorylation site-specific antibody that specifically binds a DNA binding protein selected from Column A, Rows 70-89, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E,
25 Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.
- (ii) An equivalent antibody to (i) above that only binds the DNA binding protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

- 17 -

- (iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a DNA binding protein that is a DNA binding protein selected from Column A, Rows 70-89, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1.

In yet another subset of preferred embodiments, there is provided:

- (i) An isolated phosphorylation site-specific antibody specifically binds a Disease associated protein selected from Column A, Rows 56-69, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.
- (ii) An equivalent antibody to (i) above that only binds the Disease associated protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).
- (iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Disease associated protein selected from Column A, Rows 56-69, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following Disease associated protein phosphorylation site is particularly preferred: Bcl-9 (S352) (see SEQ ID NO: 57).

In yet another subset of preferred embodiments, there is provided:

- 18 -

- (i) An isolated phosphorylation site-specific antibody that specifically binds an Adaptor/Scaffold protein selected from Column A, Rows 2-22, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 2-22, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.
- (ii) An equivalent antibody to (i) above that only binds the Adaptor/Scaffold protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).
- (iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Adaptor/Scaffold protein selected from Column A, Rows 2-22, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), which sequence comprises the phosphorylatable serine and threonine listed in corresponding Column D, Rows 2-22, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following Adaptor/Scaffold protein phosphorylation sites are particularly preferred: DOK-1 (S310) (see SEQ ID NO: 5).

In still another subset of preferred embodiments, there is provided:

- (i) An isolated phosphorylation site-specific antibody that specifically binds a Cell cycle regulation protein selected from Column A, Rows 27-42, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 27-42, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

- 19 -

(ii) An equivalent antibody to (i) above that only binds the Cell cycle regulation protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the
5 quantification of a Cell cycle regulation protein selected from Column A, Rows 27-42, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 27-42, of
10 Table 1.

Among this preferred subset of reagents, antibodies and AQUA peptides for the detection/quantification of the following Cell cycle regulation protein phosphorylation sites are particularly preferred: KI-67 (S2925) and NuMA-1 (S395) (see SEQ ID NOs: 33 and 39).

15 In still another subset of preferred embodiments, there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a Protease selected from Column A, Rows 163-165, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1, comprised within the
20 phosphorylatable peptide sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

(ii) An equivalent antibody to (i) above that only binds the Protease
25 when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Protease selected from Column A, Rows 163-165, said labeled peptide comprising the phosphorylatable peptide

- 20 -

sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1.

5 In still another subset of preferred embodiments, there is provided:

(i) An isolated phosphorylation site-specific antibody that specifically binds a Methyltransferase selected from Column A, Rows 153-156, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 153-156, of Table 1, comprised within the
10 phosphorylatable peptide sequence listed in corresponding Column E, Rows 153-156, of Table 1 (SEQ ID NOs: 152-155), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

(ii) An equivalent antibody to (i) above that only binds the
15 Methyltransferase when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a Methyltransferase selected from Column A, Rows 153-156, said labeled peptide comprising the phosphorylatable peptide
20 sequence listed in corresponding Column E, Rows 153-156, of Table 1 (SEQ ID NOs: 152-155), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 153-156, of Table 1.

In still another subset of preferred embodiments, there is provided:

25 (i) An isolated phosphorylation site-specific antibody that specifically binds a Ubiquitin conjugating protein selected from Column A, Rows 285-298, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 285-298, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E,

- 21 -

Rows 285-298, of Table 1 (SEQ ID NOs: 284-297), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

(ii) An equivalent antibody to (i) above that only binds the Ubiquitin
5 conjugating protein when *not* phosphorylated at the disclosed site (and does not bind the protein when it *is* phosphorylated at the site).

(iii) A heavy-isotope labeled peptide (AQUA peptide) for the
quantification of a Ubiquitin conjugating protein selected from Column A,
Rows 285-298, said labeled peptide comprising the phosphorylatable
10 peptide sequence listed in corresponding Column E, Rows 285-298, of
Table 1 (SEQ ID NOs: 284-297), which sequence comprises the
phosphorylatable threonine or serine listed in corresponding Column D,
Rows 285-298, of Table 1.

Among this preferred subset of reagents, antibodies and AQUA
15 peptides for the detection/quantification of the following Ubiquitin
conjugating protein phosphorylation sites are particularly preferred:
UREB1 (T485) (see SEQ ID NO: 291).

The invention also provides, in part, an immortalized cell line
producing an antibody of the invention, for example, a cell line producing
20 an antibody within any of the foregoing preferred subsets of antibodies. In
one preferred embodiment, the immortalized cell line is a rabbit hybridoma
or a mouse hybridoma.

In certain other preferred embodiments, a heavy-isotope labeled
peptide (AQUA peptide) of the invention (for example, an AQUA peptide
25 within any of the foregoing preferred subsets of AQUA peptides)
comprises a disclosed site sequence wherein the phosphorylatable
threonine or serine is phosphorylated. In certain other preferred
embodiments, a heavy-isotope labeled peptide of the invention comprises
a disclosed site sequence wherein the phosphorylatable threonine or
30 serine is *not* phosphorylated.

- 22 -

The foregoing subsets of preferred reagents of the invention should not be construed as limiting the scope of the invention, which, as noted above, includes reagents for the detection and/or quantification of disclosed phosphorylation sites on any of the other protein type/group subsets (each a preferred subset) listed in Column C of Table 1/Figure 2.

Also provided by the invention are methods for detecting or quantifying a ATM and/or ATR kinase signaling protein that is threonine- or serine- phosphorylated, said method comprising the step of utilizing one or more of the above-described reagents of the invention to detect or quantify one or more ATM and/or ATR kinase signaling protein(s) selected from Column A of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D of Table 1. In certain preferred embodiments of the methods of the invention, the reagents comprise a subset of preferred reagents as described above.

Also provided by the invention is a method for obtaining a phosphorylation profile of protein kinases that are phosphorylated in ATM and/or ATR kinase signaling pathways, said method comprising the step of utilizing one or more isolated antibody that specifically binds a protein kinase selected from Column A, Rows 149 and 166-181, of Table 1 only when phosphorylated at the tyrosine listed in corresponding Column D, Rows 149 and 166-181, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 138-165, of Table 1 (SEQ ID NOs: 148 and 165-180), to detect the phosphorylation of one or more of said protein kinases, thereby obtaining a phosphorylation profile for said kinases.

The identification of the disclosed novel ATM and/or ATR kinase signaling protein phosphorylation sites, and the standard production and use of the reagents provided by the invention are described in further detail below and in the Examples that follow.

- 23 -

All cited references are hereby incorporated herein, in their entirety, by reference. The Examples are provided to further illustrate the invention, and do not in any way limit its scope, except as provided in the claims appended hereto.

5 Table 1. Newly Discovered ATM and/or ATR Phosphorylation Sites.

1	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	SEQ ID NO
2	AKAP12	NP_005091.2	Adaptor/scaffold	T1264	TEGIQEADQYADEK	SEQ ID NO: 1
3	AKAP13	NP_006729.4	Adaptor/scaffold	T1112	RENISHNIQDILIPNVLLSQEKE	SEQ ID NO: 2
4	AKAP13	NP_006729.4	Adaptor/scaffold	S1123	RENISHNTQDILIPNVLLSQEKE	SEQ ID NO: 3
5	ATG16L1	NP_110430.5	Adaptor/scaffold	S269	RLsQPAGGLDSITNIFGR	SEQ ID NO: 4
6	Dok1	NP_001372.1	Adaptor/scaffold	S310	IAPCPsQDSLYSDPLDSTSAQAGEGVQR	SEQ ID NO: 5
7	MACF1	NP_149033.2	Adaptor/scaffold	S5511	SGGRKsLSQPTPPMPILSQSEAKNPR	SEQ ID NO: 6
8	MACF1	NP_149033.2	Adaptor/scaffold	S5513	SGGRKSLsQPTPPMPILSQSEAKNPR	SEQ ID NO: 7
9	MACF1	NP_149033.2	Adaptor/scaffold	S5506	sGGRKSLsQPTPPMPILSQSEAKNPR	SEQ ID NO: 8
10	MACF1	NP_149033.2	Adaptor/scaffold	S5524	SGGRKSLsQPTPPMPILsQSEAKNPR	SEQ ID NO: 9
11	NDFIP2	NP_061953.1	Adaptor/scaffold	S205	AAAMAAAAAETsQR	SEQ ID NO: 10
12	rabenosyn 5	NP_071735.2	Adaptor/scaffold	S217	ESLSTHTSPsQSPNSVHGSR	SEQ ID NO: 11
13	RANBP3	NP_003615.1	Adaptor/scaffold	S186	ALSQTVPSsSGTNGVSLPADCTGAVPAASPD TAAWR	SEQ ID NO: 12
14	RANBP3	NP_003615.1	Adaptor/scaffold	S211	ALSQTVPSsSGTNGVSLPADCTGAVPAAsPD TAAWR	SEQ ID NO: 13
15	RANBP3	NP_003615.1	Adaptor/scaffold	S125	TsSLTQFPPSQSEER	SEQ ID NO: 14
16	RANBP3	NP_003615.1	Adaptor/scaffold	S133	TSSLTQFPPSQSEER	SEQ ID NO: 15
17	RANBP3	NP_003615.1	Adaptor/scaffold	T124	ISSLTQFPPSQSEER	SEQ ID NO: 16
18	RANBP3	NP_003615.1	Adaptor/scaffold	S126	TSSLTQFPPSQSEER	SEQ ID NO: 17
19	RANBP3	NP_003615.1	Adaptor/scaffold	T128	TSSLTQFPPSQSEER	SEQ ID NO: 18
20	RANBP3	NP_003615.1	Adaptor/scaffold	S135	TSSLTQFPPSQsEER	SEQ ID NO: 19
21	SAKS1	NP_056937.2	Adaptor/scaffold	S202	YGGsVGSQPPVAPePGVPSSPsQEPP TKR	SEQ ID NO: 20
22	VANGL1	NP_620409.1	Adaptor/scaffold	S88	TEEVQDDNWGETTTAITGTSEHSLsQEDIAR	SEQ ID NO: 21
23	acinus	NP_055792.1	Apoptosis	S132	HSTPHAAFPNSQIGeEMsQNSFIK	SEQ ID NO: 22
24	requiem	NP_006259.1	Apoptosis	S113	EGLIsQDGSSLEALLR	SEQ ID NO: 23
25	UACA	NP_060473.2	Apoptosis	T181	PIICQLLIDRGADVNSRDK	SEQ ID NO: 24
26	SDF4	NP_057260.2	Calcium-binding protein	T193	LNEELKVDEEIQEVLLENLKDR	SEQ ID NO: 25
27	CENPF	NP_057427.3	Cell cycle regulation	S1255	GDLETsNLQDMQsQEISGLKDCEIDAEK	SEQ ID NO: 26
28	CLASP1	NP_056097.1	Cell cycle regulation	S1196	FSFRsQEDLNEPIKR	SEQ ID NO: 27
29	CNAP1	NP_055680.2	Cell cycle regulation	T577	LLNILGLIFKGAASIQEKNPR	SEQ ID NO: 28
30	cyclin C	NP_005181.2	Cell cycle regulation	S278	MPKPKPPPNSEGEQGPNGSQNsSYSQS	SEQ ID NO: 29
31	cyclin C	NP_005181.2	Cell cycle regulation	S279	MPKPKPPPNSEGEQGPNGSQNsSYSQS	SEQ ID NO: 30
32	cyclin C	NP_005181.2	Cell cycle regulation	S275	MPKPKPPPNSEGEQGPNGsQNSSYSQS	SEQ ID NO: 31
33	cyclin C	NP_005181.2	Cell cycle regulation	S281	MPKPKPPPNSEGEQGPNGSQNSSYSQS	SEQ ID NO: 32

- 24 -

34	KI-67	NP_002408.3	Cell cycle regulation	S2925	AQPLEDLASFQELsQTPGHTEELANGAADS FTSAPK	SEQ ID NO: 33
35	MDC1	NP_055456.1	Cell cycle regulation	T659	ENLTDLVWDTDTLGESIQPQR	SEQ ID NO: 34
36	MDC1	NP_055456.1	Cell cycle regulation	S1086	QDGsQEAPeAPLSSELEPFHKKPK	SEQ ID NO: 35
37	MDC1	NP_055456.1	Cell cycle regulation	S513	sQASTTVQINTQVEK	SEQ ID NO: 36
38	NIPA	NP_057562.3	Cell cycle regulation	S472	QsQPAETDSMSLSEK	SEQ ID NO: 37
39	NuMA-1	NP_006176.2	Cell cycle regulation	T1744	LPRIQPDGTSVPGEPASPIQR	SEQ ID NO: 38
40	NuMA-1	NP_006176.2	Cell cycle regulation	S395	LSQLEEHLSQLQDNPPQEK	SEQ ID NO: 39
41	TPX2	NP_036244.2	Cell cycle regulation	S310	GCTIVKPFNLsQGK	SEQ ID NO: 40
42	VCP	NP_009057.1	Cell cycle regulation	S784	FPSGNQGGAGPsQGGSGGTGGSVYTEDN DDLYG	SEQ ID NO: 41
43	CLIC1	NP_001279.2	Channel, chloride	S163	VLDNYLTSPLEEVDETSAEDEGVsQR	SEQ ID NO: 42
44	SCN2A	NP_066287.2	Channel, sodium	S629	HSNVsQAsRASR	SEQ ID NO: 43
45	SCN2A	NP_066287.2	Channel, sodium	S632	HSNVsQASRASr	SEQ ID NO: 44
46	SCN2A	NP_066287.2	Channel, sodium	S626	HSNVsQASRASR	SEQ ID NO: 45
47	TBCB	NP_001272.2	Chaperone; Cytoskeletal protein	S110	VEKYTIsQEAYDQR	SEQ ID NO: 46
48	ELMO2	NP_573403.1	Cytoskeletal protein	S517	QSERMsQDDFQSPPIVELR	SEQ ID NO: 47
49	ezrin	NP_003370.2	Cytoskeletal protein	S539	QLLTLSSELsQAR	SEQ ID NO: 48
50	lamin A/C	NP_005563.1	Cytoskeletal protein	T394	LRLSPSPISQR	SEQ ID NO: 49
51	lamin A/C	NP_005563.1	Cytoskeletal protein	S395	LRLSPSPtsQR	SEQ ID NO: 50
52	NEXN	NP_653174.2	Cytoskeletal protein	T193	NMVVDDDSPEMYKIsQEFLTPGK	SEQ ID NO: 51
53	NEXN	NP_653174.2	Cytoskeletal protein	S195	TIsQEFLTPGK	SEQ ID NO: 52
54	TMSB1 0	NP_066926.1	Cytoskeletal protein	T23	TEIQEKNTLPKTETIEQEK	SEQ ID NO: 53
55	tubulin, alpha-1	NP_005991.1	Cytoskeletal protein; Motor protein	S232	LIsQIVSSITASLR	SEQ ID NO: 54
56	ataxin-3	NP_004984.2	Disease associated	S265	NIsQDMTQTSGTNLTSEELR	SEQ ID NO: 55
57	ataxin-3	NP_004984.2	Disease associated	T269	NISQDMTQTSGTNLTSEELR	SEQ ID NO: 56
58	Bcl-9	NP_004317.2	Disease associated	S352	APPPPPVSSGEPPTLGENPDGLsQEQLHR	SEQ ID NO: 57
59	BCL9L	NP_872363.1	Disease associated	S942	SPTLSQVHsPLVTSPSANLK	SEQ ID NO: 58
60	BCL9L	NP_872363.1	Disease associated	S938	SPTLSQVHSPLVTSPSANLK	SEQ ID NO: 59
61	BCL9L	NP_872363.1	Disease associated	T946	SPTLSQVHSPLVtSPSANLK	SEQ ID NO: 60
62	BCL9L	NP_872363.1	Disease associated	S947	SPTLSQVHSPLVTsPSANLK	SEQ ID NO: 61
63	MAGE- D2	NP_055414.2	Disease associated	S162	AQETEAAAPSOAPADEPEPEsAAAsQENQ DTRPK	SEQ ID NO: 62
64	NBEAL1	XP_00113443 2.1	Disease associated	S1299	RLsQISAGETeYNTQDSK	SEQ ID NO: 63
65	PAGE1	NP_003776.2	Disease associated	S144	GDGPDVQELGLPNPEEVKTPEDEGGsQP	SEQ ID NO: 64
66	PRCC	NP_005984.3	Disease associated	S267	QITQEEDDsDEEVAPENFFSLPEK	SEQ ID NO: 65
67	PRCC	NP_005984.3	Disease associated	T261	QIIQEEDDSDEEVAPENFFSLPEK	SEQ ID NO: 66
68	SART2	NP_037484.1	Disease associated	S797	IFAlsQQQQQSK	SEQ ID NO: 67
69	STEAP1	NP_036581.1	Disease associated	S337	INKTEICsQL	SEQ ID NO: 68
70	ARID1A	NP_006006.3	DNA binding protein	S604	FPPPQELsQDSFGSQASSAPSMSSK	SEQ ID NO: 69
71	ARID1B	NP_059989.1	DNA binding protein	S574	YQPQQDMsQEGYGTR	SEQ ID NO: 70
72	CBX3	NP_009207.2	DNA binding protein	S79	GFTDADNTWEPEENLDCPELIEAFNLsQK	SEQ ID NO: 71
73	NUCB1	NP_006175.2	DNA binding protein	S369	AQRLsQETEALGR	SEQ ID NO: 72
74	RPA1	NP_002936.1	DNA binding protein	T180	AAGPSLSHTSGGIQSK	SEQ ID NO: 73
75	UKp68	NP_079100.2	DNA binding protein	S450	LQIDPVMAETLQMsQDYDMESMVHADTR	SEQ ID NO: 74
76	UKp68	NP_079100.2	DNA binding protein	S390	TRTsQEELLAeVVQGQSR	SEQ ID NO: 75

- 25 -

77	WDHD1	NP_009017.1	DNA binding protein	T826	AAELTAIQVEEEEEEDFRK	SEQ ID NO: 76
78	ZAP	NP_064504.2	DNA binding protein	S327	ATDLGGTSQAGTsQR	SEQ ID NO: 77
79	ZAP	NP_064504.2	DNA binding protein	S335	FLENGsQEDLLHGNPGSTYLASNSTsAPNW K	SEQ ID NO: 78
80	ZMYM4	NP_005086.2	DNA binding protein	S122	RVTQHEsDNENEIQINQK	SEQ ID NO: 79
81	ZMYM4	NP_005086.2	DNA binding protein	T118	RVIQHESDNENEIQINQK	SEQ ID NO: 80
82	ZNF262	NP_005086.2	DNA binding protein	T1070	KTLSQGESQISEHELFDTK	SEQ ID NO: 81
83	ZNF262	NP_005086.2	DNA binding protein	S1064	KTLSQGESQTSEHELFDTK	SEQ ID NO: 82
84	ZNF262	NP_005086.2	DNA binding protein	S1068	KTLSQGESQTSEHELFDTK	SEQ ID NO: 83
85	ZNF262	NP_005086.2	DNA binding protein	S1071	KTLSQGESQTsEHELFDTK	SEQ ID NO: 84
86	ZNF262	NP_005086.2	DNA binding protein	S1249	SDPLGsTQDHALsQESSEPGCR	SEQ ID NO: 85
87	ZNF262	NP_005086.2	DNA binding protein	T1250	SDPLGSIQDHALsQESSEPGCR	SEQ ID NO: 86
88	ZNF262	NP_005086.2	DNA binding protein	S1256	SDPLGSTQDHALsQESSEPGCR	SEQ ID NO: 87
89	Nucleoli n	NP_005372.2	DNA binding protein; Helicase; RNA binding protein	T405	NLPYKVIQDELKEVFEDAAEIR	SEQ ID NO: 88
90	FANCA	NP_000126.2	DNA repair	S1449	QQAAPDADLsQEPHLF	SEQ ID NO: 89
91	FANCD 2	NP_149075.2	DNA repair	T596	SESPSLIQR	SEQ ID NO: 90
92	MRE11 A	NP_005581.2	DNA repair	S648	IMsQSQVSK	SEQ ID NO: 91
93	MRE11 A	NP_005581.2	DNA repair	S650	IMSQsQVSK	SEQ ID NO: 92
94	NBS1	NP_002476.2	DNA repair	S615	MDIETNDTFSDEAVPESSKIsQENEIGK	SEQ ID NO: 93
95	NBS1	NP_002476.2	DNA repair	S397	MLsQDAPTVK	SEQ ID NO: 94
96	NBS1	NP_002476.2	DNA repair	S58	NHAVLTANFSVTNLsQTDEIPVLTk	SEQ ID NO: 95
97	PMS2	NP_000526.1	DNA repair	T597	LVNtQDMSASQVDVAVK	SEQ ID NO: 96
98	RAD23 A	NP_005044.1	DNA repair	S357	NENLAANFLsQNFDDDE	SEQ ID NO: 97
99	Rif1	NP_060621.3	DNA repair	T1518	KADPENIKSEGDIQDIVDKSSEK	SEQ ID NO: 98
100	TDP1	NP_060789.2	DNA repair	S563	FFAGsQEPMATFPVPYDLPPELYGSK	SEQ ID NO: 99
101	BAZ1A	NP_038476.2	DNA replication	S286	IHIsQEDNVANK	SEQ ID NO: 100
102	CAF-1B	NP_005432.1	DNA replication	S409	GsSPGPRPVEGTPASRTQDPSSPGTTPPQ AR	SEQ ID NO: 101
103	CAF-1B	NP_005432.1	DNA replication	S410	GSsPGPRPVEGTPASRTQDPSSPGTTPPQ AR	SEQ ID NO: 102
104	CAF-1B	NP_005432.1	DNA replication	S428	GSSPGPRPVEGTPASRTQDPsSPGTTPPQ AR	SEQ ID NO: 103
105	CAF-1B	NP_005432.1	DNA replication	T424	GSSPGPRPVEGTPASRIQDPSSPGTTPPQA R	SEQ ID NO: 104
106	PSF2	NP_057179.1	DNA replication	T180	LRTNLQPLESIQSQDF	SEQ ID NO: 105
107	PSF2	NP_057179.1	DNA replication	S179	TNLQPLEsTQSQDF	SEQ ID NO: 106
108	PSF2	NP_057179.1	DNA replication	S182	TNLQPLESTQsQDF	SEQ ID NO: 107
109	RFC1	NP_002904.3	DNA replication	T193	RKELSQNIDESGLNDEAIAK	SEQ ID NO: 108
110	RFC1	NP_002904.3	DNA replication	S196	RKELSQNTDEsGLNDEAIAK	SEQ ID NO: 109
111	Smc1	NP_006297.2	DNA replication	S951	GTMDLIsQEEGSSQGEDSVSGSQR	SEQ ID NO: 110
112	TERF2I P	NP_061848.2	DNA replication	S206	YLLGDAPVSPSsQK	SEQ ID NO: 111
113	TOPBP 1	NP_008958.1	DNA replication	S405	DFAPSEKHEQADEDLLsQYENGSSSTVVEAK	SEQ ID NO: 112
114	TOPBP 1	NP_008958.1	DNA replication	T975	GVLIQTLEMR	SEQ ID NO: 113
115	TOR1AI P2	NP_659471.1	Endoplasmic reticulum	S150	EASDGTGAsQEPPTTDSQEAQSPGHSSAG QEGEDTLR	SEQ ID NO: 114
116	TOR1AI P2	NP_659471.1	Endoplasmic reticulum	S158	EASDGTGAsQEPPTTDSQEAQSPGHSSAG QEGEDTLR	SEQ ID NO: 115
117	GAPDH	NP_002037.2	Enzyme, cellular	T184	VIHDFNGIVEGLMTTVHAIATQK	SEQ ID NO: 116

- 26 -

			metabolism			
118	deoxyhy pusine synthas e	NP_001921.1	Enzyme, misc.	S78	KLEPLsQDEDQHADLTQSR	SEQ ID NO: 117
119	CSN1	NP_004118.3	G protein regulator, misc.	T479	SPPREGSQGELIPANSQSR	SEQ ID NO: 118
120	centauri n-delta 1	NP_056045.2	GTPase activating protein, ARF	S580	NDWISILLNALKSQSLTsQSQAVVTPEK	SEQ ID NO: 119
121	centauri n-delta 1	NP_056045.2	GTPase activating protein, ARF	S582	NDWISILLNALKSQSLTsQsQAVVTPEK	SEQ ID NO: 120
122	SIPA1L 1	NP_056371.1	GTPase activating protein, misc.	T1190	SPASIDRQNIQSDIGGSGK	SEQ ID NO: 121
123	ARHGA P18	NP_277050.2	GTPase activating protein, Rac/Rho	S610	FLsQESGVAQTLK	SEQ ID NO: 122
124	ARHGA P18	NP_277050.2	GTPase activating protein, Rac/Rho	S69	SISQDsLDELSMEDYWIELENIKK	SEQ ID NO: 123
125	ARHGA P18	NP_277050.2	GTPase activating protein, Rac/Rho	S66	SlsQDSLDELSMEDYWIELENIKK	SEQ ID NO: 124
126	ARHGA P6	NP_038267.1	GTPase activating protein, Rac/Rho	S569	AGPCSLsQGNLSPNWPR	SEQ ID NO: 125
127	Cdc42E P1	NP_008992.3	GTPase activating protein, Rac/Rho	T34	RLIADMISHPLGDFR	SEQ ID NO: 126
128	SIARD1 3	NP_443083.1	GTPase activating protein, Rac/Rho	S271	MHEFHsQENLVVHIPK	SEQ ID NO: 127
129	ARHGE F2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	S147	ILSQsTDSLNMNR	SEQ ID NO: 128
130	ARHGE F2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	S145	ILsQSTDsLNMNR	SEQ ID NO: 129
131	ARHGE F2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	T148	RILSQSIDSLNMNR	SEQ ID NO: 130
132	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	T1627	DGVEDIDSQGDGSSQPDtISIAsR	SEQ ID NO: 131
133	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	S1629	DGVEDIDSQGDGSSQPDtIsIASR	SEQ ID NO: 132
134	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	S1632	DGVEDIDSQGDGSSQPDtISIAsR	SEQ ID NO: 133
135	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	S1623	DGVEDIDSQGDGSSQPDtISIAsR	SEQ ID NO: 134
136	CHD-6	NP_115597.3	Helicase	S1714	KVLsQEPSSFQESpSTNTESR	SEQ ID NO: 135
137	DDX11	NP_004390.3	Helicase	T465	QNPNIQSLsQTGTTELK	SEQ ID NO: 136
138	DDX11	NP_004390.3	Helicase	S469	QNPNTQSLsQTGTTELK	SEQ ID NO: 137
139	DDX18	NP_006764.3	Helicase	S74	QKPMNVGLSETQNGGmsQEAVGNIK	SEQ ID NO: 138
140	DDX9	NP_001348.2	Helicase	S321	LAQFEPsQR	SEQ ID NO: 139
141	DFFA	NP_998731.1	Hydrolase, esterase	S257	QAPELSLsQDLVGGNQGH	SEQ ID NO: 140
142	DFFA	NP_004392.1	Hydrolase, esterase	S107	WAYNNSDGGTAWIsQESFDVDETDSGAGL K	SEQ ID NO: 141
143	AMPD2	NP_004028.3	Hydrolase, non- esterase	S168	QIsQDVKLEPDILLR	SEQ ID NO: 142

144	RENT1	NP_002902.2	Hydrolase, non-esterase	S1116	AYQHGGVTGLsQY	SEQ ID NO: 143
145	RENT1	NP_002902.2	Hydrolase, non-esterase	S1089	sQIDVALsQDSTYQGER	SEQ ID NO: 144
146	RENT1	NP_002902.2	Hydrolase, non-esterase	S1096	SQIDVALsQDSTYQGER	SEQ ID NO: 145
147	PPIE	NP_006103.1	Isomerase	T126	TLEENKEEEGSEPPKAEIQEGEPIAK	SEQ ID NO: 146
148	PPIL1	NP_057143.1	Isomerase	S149	VGMVETNsQDRPVDDVK	SEQ ID NO: 147
149	deoxycytidine kinase	NP_000779.1	Kinase (non-protein)	T64	WCNVQSiQDEFEELTMSQK	SEQ ID NO: 148
150	ACSS2	NP_061147.1	Ligase	T264	AELGMGDSISQSPPIKR	SEQ ID NO: 149
151	ACSS2	NP_061147.1	Ligase	S267	AELGMGDSTsQsPPIKR	SEQ ID NO: 150
152	ATP11C	NP_775965.2	Lipid binding protein	S445	GVTQEVdGLsQTDGTLTYFDKVDK	SEQ ID NO: 151
153	BAT8	NP_006700.2	Methyltransferase	S569	GDGVTTPAGTAAPAPPLsQDVPGR	SEQ ID NO: 152
154	COQ3	NP_059117.3	Methyltransferase	S86	LYsTSQTTVDsGEVK	SEQ ID NO: 153
155	COQ3	NP_059117.3	Methyltransferase	T90	LYSTSQITVDsGEVK	SEQ ID NO: 154
156	COQ3	NP_059117.3	Methyltransferase	S88	LYTsQTTVDsGEVK	SEQ ID NO: 155
157	Pnk1	NP_009185.2	Phosphatase (non-protein)	T118	TPESQPDIPPGTPLVsQDEKR	SEQ ID NO: 156
158	Pnk1	NP_009185.2	Phosphatase (non-protein)	S114	TPEsQPDTPPGTPLVsQDEKR	SEQ ID NO: 157
159	Pnk1	NP_009185.2	Phosphatase (non-protein)	S126	TPESQPDTPPGTPLVsQDEKR	SEQ ID NO: 158
160	PTP1B	NP_002818.1	PHOSPHATASE; Phosphatase; Protein phosphatase, tyrosine (non-receptor)	S363	GSPLNAAPYGIesMSQDTEVR	SEQ ID NO: 159
161	PTP1B	NP_002818.1	PHOSPHATASE; Phosphatase; Protein phosphatase, tyrosine (non-receptor)	S365	GSPLNAAPYGIesMsQDTEVR	SEQ ID NO: 160
162	PLCG1	NP_002651.2	Phospholipase	S1264	YQQPFEDFRIsQEHLADHFDSR	SEQ ID NO: 161
163	ST14	NP_068813.1	Protease (non-proteasomal)	S203	VVMLPPRARSLKSFVVTsVAFPTDsK	SEQ ID NO: 162
164	PSMC1	NP_002793.2	Protease (proteasomal subunit)	S4	MGQsQSGGHGPGGGKK	SEQ ID NO: 163
165	PSMD4	NP_002801.1	Protease (proteasomal subunit)	S266	MTIsQQEFGR	SEQ ID NO: 164
166	TIF1-beta	NP_005753.1	Protein kinase	S824	FSAVLVEPPMSLPAGLsQELSGGPGD GP	SEQ ID NO: 165
167	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S196	TSISPLNVVQGAsVNSSSQTAQVTK	SEQ ID NO: 166
168	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S199	TSISPLNVVQGASVNsSSQTAQVTK	SEQ ID NO: 167
169	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S201	TSISPLNVVQGASVNSSSQTAQVTK	SEQ ID NO: 168
170	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S200	TSISPLNVVQGASVNsSQTAQVTK	SEQ ID NO: 169
171	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-	T203	TSISPLNVVQGASVNSSSQIAAQVTK	SEQ ID NO: 170

			receptor)			
172	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non- receptor)	T208	TSISPLNVVQGASVNSSSQTAQVIK	SEQ ID NO: 171
173	NLK	NP_057315.2	Protein kinase, Ser/Thr (non- receptor)	T286	HMIQEVVTQYYR	SEQ ID NO: 172
174	RIPK2	NP_003812.1	Protein kinase, Ser/Thr (non- receptor)	S176	MMSLSQSR	SEQ ID NO: 173
175	SMG1	NP_055907.3	Protein kinase, Ser/Thr (non- receptor)	T3550	SNTGQKIQPDVMSQNAR	SEQ ID NO: 174
176	SMG1	NP_055907.3	Protein kinase, Ser/Thr (non- receptor)	S3556	SNTGQKTQPDVMSQNAR	SEQ ID NO: 175
177	STK33	NP_112168.1	Protein kinase, Ser/Thr (non- receptor)	T491	TPViPSQGTATKYPK	SEQ ID NO: 176
178	STK33	NP_112168.1	Protein kinase, Ser/Thr (non- receptor)	T496	TPVTPSQGIATKYPK	SEQ ID NO: 177
179	STK33	NP_112168.1	Protein kinase, Ser/Thr (non- receptor)	S493	TPVTPsQGTATKYPK	SEQ ID NO: 178
180	VRK1	NP_003375.1	Protein kinase, Ser/Thr (non- receptor)	T378	EIEESKEPGVEDTEWSNIQTTEEAIQTR	SEQ ID NO: 179
181	WNK1	NP_061852.1	Protein kinase, Ser/Thr (non- receptor)	S167	PVsQP SLVGSK	SEQ ID NO: 180
182	PPP1R1 2C	NP_060077.1	Protein phosphatase, regulatory subunit	T560	RSIQGVTLTDLK	SEQ ID NO: 181
183	PPP1R7	NP_002703.1	Protein phosphatase, regulatory subunit	S12	GAGQQQsQEMMEVDR	SEQ ID NO: 182
184	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non- receptor)	S183	SGGGTGEEPGsQGLNGEAGPEDSTRETPs QENGPTAK	SEQ ID NO: 183
185	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non- receptor)	T199	SGGGTGEEPGsQGLNGEAGPEDSTREiPS QENGPTAK	SEQ ID NO: 184
186	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non- receptor)	S201	SGGGTGEEPGsQGLNGEAGPEDSTRETPs QENGPTAK	SEQ ID NO: 185
187	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non- receptor)	S262	LSSGDPSTsPSLSQTTPSKDQDQSR	SEQ ID NO: 186
188	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non- receptor)	S264	LSSGDPSTSPsLSQTTPSKDQDQSRK	SEQ ID NO: 187
189	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non- receptor)	T268	LSSGDPSTSPSLsQITPSKDQDQSRK	SEQ ID NO: 188
190	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non- receptor)	S266	LSSGDPSTSPSLsQTTPSKDQDQSRK	SEQ ID NO: 189

191	PTPN14	NP_005392.2	Protein phosphatase, tyrosine (non-receptor)	S486	NLNIINTHAYNQPEDLVYsQPEMR	SEQ ID NO: 190
192	PTPN21	NP_008970.1	Protein phosphatase, tyrosine (non-receptor)	S492	NLNIGSSYAYSRPAALVYsQPEIR	SEQ ID NO: 191
193	VEGFR-2	NP_002244.1	Receptor tyrosine kinase	T1217	FHYDNIAGISQYLQNSK	SEQ ID NO: 192
194	OGFR	NP_031372.2	Receptor, misc.	S420	IALNLEGCALsQGSRLR	SEQ ID NO: 193
195	OGFR	NP_031372.2	Receptor, misc.	T325	KVEEEGSPGDPDHEASIQGR	SEQ ID NO: 194
196	OGFR	NP_031372.2	Receptor, misc.	S315	KVEEEGSPGDPDHEASTQGR	SEQ ID NO: 195
197	OGFR	NP_031372.2	Receptor, misc.	T426	IGTQEVGGQDPGEAVQPCR	SEQ ID NO: 196
198	OGFR	NP_031372.2	Receptor, misc.	T428	TGQEVGGQDPGEAVQPCR	SEQ ID NO: 197
199	PDGFR L	NP_006198.1	Receptor, misc.	S254	AEAGGRsQISVK	SEQ ID NO: 198
200	HEAB	NP_006822.1	RNA binding protein	S344	VGAPTIPDSCLPLGMSQEDNQLK	SEQ ID NO: 199
201	POP4	NP_006618.1	RNA binding protein	S10	SVIYHALsQK	SEQ ID NO: 200
202	RALY	NP_031393.2	RNA binding protein	S279	TRDDGDEEGLLTHSEEELEHsQDTDADDG ALQ	SEQ ID NO: 201
203	RBM16	NP_055707.3	RNA binding protein	T619	SSEPVKETVQTTQSPVPVEK	SEQ ID NO: 202
204	RBM16	NP_055707.3	RNA binding protein	T615	SSEPVKETVQTIQSPTPVEK	SEQ ID NO: 203
205	RBM34	NP_055829.1	RNA binding protein	S94	NEEEESTSQIERPLsQEPK	SEQ ID NO: 204
206	SFRS14	NP_055699.1	RNA binding protein	T7	RRIQETFDVAVLQEK	SEQ ID NO: 205
207	SFRS7	BAC03661.1	RNA binding protein	S136	FLRLSQsP	SEQ ID NO: 206
208	SFRS7	BAC03661.1	RNA binding protein	S134	FLRLsQSP	SEQ ID NO: 207
209	SFRS8	NP_004583.2	RNA binding protein	T642	KPQLIQEELEAK	SEQ ID NO: 208
210	SRm300	NP_057417.3	RNA binding protein	T383	HGGSPQPLAITPLSQEPVNPPSEASPTR	SEQ ID NO: 209
211	SRm300	NP_057417.3	RNA binding protein	S395	HGGSPQPLATTPLSQEPVNPPsEASPTR	SEQ ID NO: 210
212	SSA2	NP_004591.2	RNA binding protein	S19	QIANsQDGYVWQVTDMMNR	SEQ ID NO: 211
213	UTP14A	NP_006640.2	RNA binding protein	S434	KRsELSQDAEPAGSQETK	SEQ ID NO: 212
214	UTP14A	NP_006640.2	RNA binding protein	S453	KRSELSQDAEPAGSQETKDSGsQEVLSCLR	SEQ ID NO: 213
215	UTP14A	NP_006640.2	RNA binding protein	S14	LAESLLALsQQEELADLPK	SEQ ID NO: 214
216	UTP14A	NP_006640.2	RNA binding protein	T448	RSELSQDAEPAGSQEIK	SEQ ID NO: 215
217	UTP14A	NP_006640.2	RNA binding protein	S437	SELSQDAEPAGSQETK	SEQ ID NO: 216
218	UTP14A	NP_006640.2	RNA binding protein	S445	SELSQDAEPAGsQETK	SEQ ID NO: 217
219	TAF15	NP_003478.1	RNA binding protein; Translation initiation complex	S94	QSSYSQQPYNNQGGQQNNMESSGsQGGR	SEQ ID NO: 218
220	MFGE8	NP_005919.1	Secreted protein	S42	NPCHNGGLCEEIsQEVNR	SEQ ID NO: 219
221	CUTL1	NP_853530.1	Transcription factor	S734	QAPLsQSDITILTPK	SEQ ID NO: 220

222	ELYS	NP_056261.3	Transcription factor	S1797	GLsQNQQIPQNSVTPR	SEQ ID NO: 221
223	FBP3	NP_003925.1	Transcription factor	S569	QQVAFYGGTLGQAQAHsQEQ	SEQ ID NO: 222
224	FOXJ2	NP_060886.1	Transcription factor	S164	RHPPDDDLsQDsPEQEASKSPR	SEQ ID NO: 223
225	FOXJ2	NP_060886.1	Transcription factor	S161	RHPPDDDLsQDSPEQEASKSPR	SEQ ID NO: 224
226	FOXJ2	NP_060886.1	Transcription factor	S172	RHPPDDDLsQDSPEQEASKsPR	SEQ ID NO: 225
227	MBD1	NP_056669.1	Transcription factor	T449	QEKADtQDEWTPGTAVLTSPVLVPGCPSK	SEQ ID NO: 226
228	MCEF	NP_055238.1	Transcription factor	S836	TIsQSSSLK	SEQ ID NO: 227
229	MYT1L	NP_055840.2	Transcription factor	S994	QKDGyLNGSQFsWKSvK	SEQ ID NO: 228
230	MYT1L	NP_055840.2	Transcription factor	S997	QKDGyLNGSQFSWksvK	SEQ ID NO: 229
231	MYT1L	NP_055840.2	Transcription factor	S991	QKDGyLNGsQFSWKSvK	SEQ ID NO: 230
232	RFX5	NP_000440.1	Transcription factor	S605	EHVLQSSLSQEhKDPKATPP	SEQ ID NO: 231
233	TCEAL3	NP_116315.1	Transcription factor	S65	REDEGEpGDEGQLEDEGsQEKGGR	SEQ ID NO: 232
234	ZNF264	NP_003408.1	Transcription factor	S80	KEDLSQDTCpGDKGKPK	SEQ ID NO: 233
235	ZNF281	NP_036614.1	Transcription factor	S395	GATSAEPGSSNHTNMGNLAVLSQGNTSSSR	SEQ ID NO: 234
236	ZNF281	NP_036614.1	Transcription factor	S807	NLESSTGFQIPsQELASQIDPQKDIEPR	SEQ ID NO: 235
237	TAF1C	NP_005670.2	Transcription initiation complex	S856	ATRSQQHTPVLsSSQPLRKKPR	SEQ ID NO: 236
238	TAF1C	NP_005670.2	Transcription initiation complex	S857	ATRSQQHTPVLsSSQPLRKKPR	SEQ ID NO: 237
239	TAF1C	NP_005670.2	Transcription initiation complex	S848	ATRSQQHTPVLSSSQPLRKKPR	SEQ ID NO: 238
240	TAF1C	NP_005670.2	Transcription initiation complex	S858	ATRSQQHTPVLSSsQPLRKKPR	SEQ ID NO: 239
241	TFIIF	NP_004119.1	Transcription initiation complex	S142	LQIESSKPVRLsQQLDK	SEQ ID NO: 240
242	SSRP1	NP_003137.1	Transcription initiation complex; Transcription, coactivator/corepressor	T170	FYVPPiQEDGVDpVEAFaQNVLSK	SEQ ID NO: 241
243	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T855	ADDPLRLDQELQQPQtQEK	SEQ ID NO: 242
244	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S580	FVPAENDsILMNP AQDGEVQLsQNDDKTK	SEQ ID NO: 243
245	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S566	FVPAENDsILMNP AQDGEVQLsQNDDKTK	SEQ ID NO: 244
246	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T543	IDEDGENIQIEDTEPMSPVLNSK	SEQ ID NO: 245
247	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T859	LDQELQQPQTQEKISNSLTEDSK	SEQ ID NO: 246
248	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S860	LDQELQQPQTQEKtsNSLTEDSK	SEQ ID NO: 247
249	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S862	LDQELQQPQTQEKTSNsLTEDSK	SEQ ID NO: 248
250	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S1104	QSQQPMKPISPVKDPVSPAsQK	SEQ ID NO: 249
251	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T302	SPEPEVLSiQEDLFDQSNK	SEQ ID NO: 250

- 31 -

252	53BP1	NP_005648.1	Transcription, coactivator/corepres sor	S105	VADPVDSSNLDTCGSIsQVIEQLPQPNR	SEQ ID NO: 251
253	HBXAP	NP_057662.3	Transcription, coactivator/corepres sor	S524	DADSSISVLEIHsQK	SEQ ID NO: 252
254	ILF2	NP_004506.2	Transcription, coactivator/corepres sor	T388	AYEKPPEKKEGEEEEENTEEPPQGEES MEIQE	SEQ ID NO: 253
255	MYST2	NP_008998.1	Transcription, coactivator/corepres sor	S52	SSARLSQsSQDSSPVR	SEQ ID NO: 254
256	MYST2	NP_008998.1	Transcription, coactivator/corepres sor	S56	SSARLSQSSQDsSPVR	SEQ ID NO: 255
257	MYST2	NP_008998.1	Transcription, coactivator/corepres sor	S57	SSARLSQSSQDsPVR	SEQ ID NO: 256
258	MYST2	NP_008998.1	Transcription, coactivator/corepres sor	S50	SSARLSQSSQDSSPVR	SEQ ID NO: 257
259	MYST2	NP_008998.1	Transcription, coactivator/corepres sor	S53	SSARLSQsSQDSSPVR	SEQ ID NO: 258
260	NCOA2	NP_006531.1	Transcription, coactivator/corepres sor	S716	LTAEATGKDLsQESSSTAPGSEVTIKQEPVS PK	SEQ ID NO: 259
261	RBBP5	NP_005048.2	Transcription, coactivator/corepres sor	S525	VQAEIsQPLTAGGAISELL	SEQ ID NO: 260
262	SLTM	NP_00101386 5.1	Transcription, coactivator/corepres sor	S955	EWHGPPsQGPPSYHDTRR	SEQ ID NO: 261
263	YAP1	NP_079031.2	Transcription, coactivator/corepres sor	T973	SQLPTLEQDGGtQNPVSPGMSQELR	SEQ ID NO: 262
264	YAP1	NP_006097.1	Transcription, coactivator/corepres sor	S321	SQLPTLEQDGGTQNPVSSPGMsQELR	SEQ ID NO: 263
265	ZNF318	NP_055160.2	Transcription, coactivator/corepres sor	S214	YISQEEGPLsPFLGQLDEDYR	SEQ ID NO: 264
266	ZNF318	NP_055160.2	Transcription, coactivator/corepres sor	S207	YIsQEEGPLSPFLGQLDEDYR	SEQ ID NO: 265
267	AASDH PPT	NP_056238.2	Transferase	S258	HQDVPsQDDSKPTQR	SEQ ID NO: 266
268	ATE1	NP_008972.2	Transferase	S169	KEEPQELLQsQDFVGEK	SEQ ID NO: 267
269	TGM7	NP_443187.1	Transferase	T25	EHHiQEMGVKRLTVR	SEQ ID NO: 268
270	UGP2	NP_006750.3	Transferase	S13	AMsQDGASQFQEVIR	SEQ ID NO: 269
271	eIF2B	NP_003899.2	Translation initiation complex	T31	KPFMLDEEGDIQTEETQPSETK	SEQ ID NO: 270
272	ATP13A 1	NP_065143.1	Transporter, active	S817	DRLsQVLRDLEDESTPIVK	SEQ ID NO: 271
273	NUP93	NP_055484.2	Transporter, active	T159	ILHTLLASGEDALDFIQESEPSYISDVGPPG R	SEQ ID NO: 272
274	NUP93	NP_055484.2	Transporter, active	S52	TLTRTsQETADVK	SEQ ID NO: 273
275	SLC22A 7	NP_696961.2	Transporter, active	T409	LTQAGILLGTALAFGTRLLVSSDMKSWSTVL AVMGK	SEQ ID NO: 274
276	SLC22A 7	NP_696961.2	Transporter, active	T405	LIQAGTLLGTALAFGTRLLVSSDMKSWSTVL AVMGK	SEQ ID NO: 275
277	SLC22A 7	NP_696961.2	Transporter, active	T419	LTQAGTLLGTALAFGIRLLVSSDMKSWSTVL AVMGK	SEQ ID NO: 276

- 32 -

278	SLC25A4	NP_001142.2	Transporter, active; Transporter, facilitator	T84	YFPIQALNFAFK	SEQ ID NO: 277
279	NHE-1	NP_003038.2	Transporter, facilitator	S787	SKETSSPGTDDVFTAPSDSPsSQR	SEQ ID NO: 278
280	NHE-1	NP_003038.2	Transporter, facilitator	S788	SKETSSPGTDDVFTAPSDSPsSQR	SEQ ID NO: 279
281	NUP107	NP_065134.1	Transporter, facilitator	S37	VLLQAsQDENFGNTTPR	SEQ ID NO: 280
282	TPR	NP_003283.2	Transporter, facilitator	S2034	AADsQNSGEGNTGAAESSFSQEVSR	SEQ ID NO: 281
283	TPR	NP_003283.2	Transporter, facilitator	S2050	AADSQNSGEGNTGAAESSFsQEVSR	SEQ ID NO: 282
284	SASH1	NP_056093.3	Tumor suppressor	S90	RVsQDLEVEKPDASPTSLQLR	SEQ ID NO: 283
285	BRAP	NP_006759.2	Ubiquitin conjugating system	T474	CIQLNTKVAKLTNELK	SEQ ID NO: 284
286	BRAP	NP_006759.2	Ubiquitin conjugating system	T484	CTQLNTKVAKLTNELK	SEQ ID NO: 285
287	DCUN1D1	NP_065691.2	Ubiquitin conjugating system	S31	TAVSCLsQNDWK	SEQ ID NO: 286
288	HYD	NP_056986.2	Ubiquitin conjugating system	S1549	RI sQSQPVR	SEQ ID NO: 287
289	MUF1	NP_006360.3	Ubiquitin conjugating system	T327	RSIQESL TAGGTDLKR	SEQ ID NO: 288
290	NOT4	NP_037448.2	Ubiquitin conjugating system	S71	KPYPEDPAVYKPLsQEELQR	SEQ ID NO: 289
291	UBLE1A	NP_005491.1	Ubiquitin conjugating system	S185	VAKVsQGVEDGPDTKR	SEQ ID NO: 290
292	UREB1	NP_113584.3	Ubiquitin conjugating system	T485	IQRPNtIQEGEEMETdMDGVQCIPQR	SEQ ID NO: 291
293	UREB1	NP_113584.3	Ubiquitin conjugating system	S2372	sGEDESQEDVLMDEAPSNLSQASTLQANR	SEQ ID NO: 292
294	UREB1	NP_113584.3	Ubiquitin conjugating system	S2394	SGEDESQEDVLMDEAPSNLSQAsTLQANR	SEQ ID NO: 293
295	UREB1	NP_113584.3	Ubiquitin conjugating system	S2377	SGEDESQEDVLMDEAPSNLSQASTLQANR	SEQ ID NO: 294
296	UREB1	NP_113584.3	Ubiquitin conjugating system	S2391	SGEDESQEDVLMDEAPSNLSQASTLQANR	SEQ ID NO: 295
297	USP29	NP_065954.1	Ubiquitin conjugating system	T17	tGMTKLKEALiETVQRQK	SEQ ID NO: 296
298	USP36	NP_079366.3	Ubiquitin conjugating system	S1053	MSAVsQDAIEDSR	SEQ ID NO: 297
299	COG1	NP_061184.1	Vesicle protein	T657	VKIQEIPTQAK	SEQ ID NO: 298
300	ICA1	NP_004959.2	Vesicle protein	S100	FLRsQGfQDK	SEQ ID NO: 299
301	SNX25	NP_114159.2	Vesicle protein	S410	EEEKHAsQMISNK	SEQ ID NO: 300

The short name for each protein in which a phosphorylation site has presently been identified is provided in Column A, and its SwissProt accession number (human) is provided Column B. The protein type/group into which each protein falls is provided in Column C. The

5 identified threonine or serine residue at which phosphorylation occurs in a

- 33 -

given protein is identified in Column D, and the amino acid sequence of the phosphorylation site encompassing the serine or threonine residue is provided in Column E (lower case t = the threonine, or lower case s = the serine (identified in Column D)) at which phosphorylation occurs. Table 1
5 above is identical to Figure 2, except that the latter includes the disease and cell type(s) in which the particular phosphorylation site was identified (Columns F and G).

The identification of these 300 phosphorylation sites is described in more detail in Part A below and in Example 1.

10 **Definitions.**

As used herein, the following terms have the meanings indicated:

"Antibody" or "antibodies" refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE, including F_{ab} or antigen-recognition fragments thereof, including chimeric, polyclonal, and monoclonal
15 antibodies. The term "does not bind" with respect to an antibody's binding to one phospho-form of a sequence means does not substantially react with as compared to the antibody's binding to the other phospho-form of the sequence for which the antibody is specific.

"ATM and/or ATR kinase signaling protein" means any protein (or
20 poly-peptide derived therefrom) enumerated in Column A of Table 1/Figure 2, which is disclosed herein as being phosphorylated in one or more of the disclosed cell line(s). ATM and/or ATR kinase signaling proteins may be serine/threonine kinases, or direct substrates of such kinases, or may be indirect substrates downstream of such kinases in
25 signaling pathways. An ATM and/or ATR kinase signaling protein may also be phosphorylated in other cell lines harboring activated kinase activity.

- 34 -

“Heavy-isotope labeled peptide” (used interchangeably with AQUA peptide) means a peptide comprising at least one heavy-isotope label, which is suitable for absolute quantification or detection of a protein as described in WO/03016861, “Absolute Quantification of Proteins and
5 Modified Forms Thereof by Multistage Mass Spectrometry” (Gygi *et al.*), further discussed below.

“Protein” is used interchangeably with polypeptide, and includes protein fragments and domains as well as whole protein.

“Phosphorylatable amino acid” means any amino acid that is
10 capable of being modified by addition of a phosphate group, and includes both forms of such amino acid.

“Phosphorylatable peptide sequence” means a peptide sequence comprising a phosphorylatable amino acid.

“Phosphorylation site-specific antibody” means an antibody that
15 specifically binds a phosphorylatable peptide sequence/epitope only when phosphorylated, or only when not phosphorylated, respectively. The term is used interchangeably with “phospho-specific” antibody.

A. Identification of Novel ATM and/or ATR Protein Phosphorylation Sites.

20 The nearly 300 novel ATM and/or ATR kinase signaling protein phosphorylation sites disclosed herein and listed in Table 1/Figure 2 were discovered by employing the modified peptide isolation and characterization techniques described in “Immunoaffinity Isolation of Modified Peptides From Complex Mixtures,” U.S. Patent Publication No.
25 20030044848, Rush *et al.* (the teaching of which is hereby incorporated herein by reference, in its entirety) using cellular extracts from the following human glioblastoma-derived cell lines and patient samples: M059K, 293 T, M059J, M059K+J and human embryonic kidney cells. The isolation and identification of phosphopeptides from these cell lines, using

- 35 -

an ATM/ATR substrate antibody and a phospho-Chk2(T26/S28)/VCP(S784) antibody, which recognize the phosphorylated motifs LS*Q and S*Q, respectively, is described in detail in Example 1 below. See *Cell Signaling Technology Inc. 2006-2006 catalogue #'s 2851 and 2664, respectively*. In addition to the nearly 300 previously unknown protein phosphorylation sites (threonine and serine) discovered, many known phosphorylation sites were also identified (not described herein). The immunoaffinity/mass spectrometric technique described in the '848 Patent Publication (the "IAP" method) -- and employed as described in detail in the Examples -- is briefly summarized below.

The IAP method employed generally comprises the following steps: (a) a proteinaceous preparation (e.g. a digested cell extract) comprising phosphopeptides from two or more different proteins is obtained from an organism; (b) the preparation is contacted with at least one immobilized ATM/ATR substrate antibody or phospho-chk2(T26/S28)/VCP(S784) antibody; (c) at least one phosphopeptide specifically bound by the immobilized antibody in step (b) is isolated; and (d) the modified peptide isolated in step (c) is characterized by mass spectrometry (MS) and/or tandem mass spectrometry (MS-MS). Subsequently, (e) a search program (e.g. Sequest) may be utilized to substantially match the spectra obtained for the isolated, modified peptide during the characterization of step (d) with the spectra for a known peptide sequence. A quantification step employing, e.g. SILAC or AQUA, may also be employed to quantify isolated peptides in order to compare peptide levels in a sample to a baseline.

In the IAP method as employed herein, at least one immobilized ATM/ATR substrate antibody or phospho-chk2(T26/S28)/VCP(S784) antibody (commercially available from Cell Signaling Technology, Inc., Beverly, MA, Cat #'s 2851 and 2664, respectively, recognizing the phosphorylated motifs LS*Q and S*Q) were used in the immunoaffinity

- 36 -

step to isolate the widest possible number of phospho-threonine and phospho-serine containing peptides from the cell extracts.

Extracts from the following cell lines were employed: M059K, 293 T, M059J, M059K+J and human embryonic kidney cells. These cells
5 were treated with 50 mJ/m² UV and allowed to rest for two (2) hours.

As described in more detail in the Examples, lysates were prepared from these cells line and digested with trypsin after treatment with DTT and iodoacetamide to alkylate cysteine residues. Before the immunoaffinity step, peptides were pre-fractionated by reversed-phase
10 solid phase extraction using Sep-Pak C₁₈ columns to separate peptides from other cellular components. The solid phase extraction cartridges were eluted with varying steps of acetonitrile. Each lyophilized peptide fraction was redissolved in MOP IP buffer and treated with ATM/ATR substrate antibody or phospho-chk2(T26/S28)/VCP(S784) antibody
15 immobilized on protein A-Sepharose or Protein A-Sepharose. Immunoaffinity-purified peptides were eluted with 0.15% TFA and a portion of this fraction was concentrated with Stage or Zip tips and analyzed by LC-MS/MS, using a ThermoFinnigan LCQ Deca XP Plus as well as LTQ ion trap mass spectrometer. Peptides were eluted from a 10
20 cm x 75 µm reversed-phase column with a 45-min linear gradient of acetonitrile. MS/MS spectra were evaluated using the program Sequest with the NCBI human protein database.

This revealed a total of nearly 300 novel threonine or serine phosphorylation sites in signaling pathways affected by kinase activation
25 or active in ATM/ATR cells. The identified phosphorylation sites and their parent proteins are enumerated in Table 1/Figure 2. The threonine or serine (human sequence) at which phosphorylation occurs is provided in Column D, and the peptide sequence encompassing the phosphorylatable threonine or serine residue at the site is provided in
30 Column E. Figure 2 also shows the particular type of ATM/ATR

- 37 -

associated disease (see Column G) and cell line(s) (see Column F) in which a particular phosphorylation site was discovered.

As a result of the discovery of these phosphorylation sites, phospho-specific antibodies and AQUA peptides for the detection of and quantification of these sites and their parent proteins may now be produced by standard methods, described below. These new reagents will prove highly useful in, e.g., studying the signaling pathways and events underlying the progression of ATM/ATR associated diseases and the identification of new biomarkers and targets for diagnosis and treatment of such diseases.

B. Antibodies and Cell Lines

Isolated phosphorylation site-specific antibodies that specifically bind a ATM and/or ATR kinase signaling protein disclosed in Column A of Table 1 only when phosphorylated (or only when not phosphorylated) at the corresponding amino acid and phosphorylation site listed in Columns D and E of Table 1/Figure 2 may now be produced by standard antibody production methods, such as anti-peptide antibody methods, using the phosphorylation site sequence information provided in Column E of Table 1. For example, two previously unknown NBS DNA repair protein phosphorylation sites (serine 397 and 58) (see Rows 95 and 96 of Table 1/Fig. 2) are presently disclosed. Thus, antibodies that specifically bind either of these novel NBS DNA repair protein sites can now be produced, e.g. by immunizing an animal with a peptide antigen comprising all or part of the amino acid sequence encompassing the respective phosphorylated residue (e.g. a peptide antigen comprising the sequence set forth in Rows 95 and 96 respectively, Column E, of Table 1 (SEQ ID NOs: 94 and 95, respectively) (which encompasses the phosphorylated serine at positions 397 and 58 in NBS), to produce an antibody that only binds NBS kinase when phosphorylated at that site.

- 38 -

Polyclonal antibodies of the invention may be produced according to standard techniques by immunizing a suitable animal (e.g., rabbit, goat, etc.) with a peptide antigen corresponding to the ATM and/or ATR phosphorylation site of interest (i.e. a phosphorylation site enumerated in Column E of Table 1, which comprises the corresponding phosphorylatable amino acid listed in Column D of Table 1), collecting immune serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures. For example, a peptide antigen corresponding to all or part of the novel Dok1 Adaptor/Scaffold phosphorylation site disclosed herein (SEQ ID NO: 5 = IAPCPsQDSL~~S~~YSDPLDSTSAQAGEGVQR, encompassing phosphorylated serine 310 (see Row 6 of Table 1)) may be used to produce antibodies that only bind Dok1 when phosphorylated at Ser310. Similarly, a peptide comprising all or part of any one of the phosphorylation site sequences provided in Column E of Table 1 may employed as an antigen to produce an antibody that only binds the corresponding protein listed in Column A of Table 1 when phosphorylated (or when *not* phosphorylated) at the corresponding residue listed in Column D. If an antibody that only binds the protein when phosphorylated at the disclosed site is desired, the peptide antigen includes the phosphorylated form of the amino acid. Conversely, if an antibody that only binds the protein when not phosphorylated at the disclosed site is desired, the peptide antigen includes the non-phosphorylated form of the amino acid.

Peptide antigens suitable for producing antibodies of the invention may be designed, constructed and employed in accordance with well-known techniques. See, e.g., ANTIBODIES: A LABORATORY MANUAL, Chapter 5, p. 75-76, Harlow & Lane Eds., Cold Spring Harbor Laboratory (1988); Czernik, *Methods In Enzymology*, 201: 264-283 (1991); Merrifield, *J. Am. Chem. Soc.* 85: 21-49 (1962)).

- 39 -

It will be appreciated by those of skill in the art that longer or shorter phosphopeptide antigens may be employed. See *Id.* For example, a peptide antigen may comprise the full sequence disclosed in Column E of Table 1/Figure 2, or it may comprise additional amino acids
5 flanking such disclosed sequence, or may comprise of only a portion of the disclosed sequence immediately flanking the phosphorylatable amino acid (indicated in Column E by lowercase "t" or "s"). Typically, a desirable peptide antigen will comprise four or more amino acids flanking each side of the phosphorylatable amino acid and encompassing it. Polyclonal
10 antibodies produced as described herein may be screened as further described below.

Monoclonal antibodies of the invention may be produced in a hybridoma cell line according to the well-known technique of Kohler and Milstein. See *Nature* 265: 495-97 (1975); Kohler and Milstein, *Eur. J.*
15 *Immunol.* 6: 511 (1976); see also, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel *et al.* Eds. (1989). Monoclonal antibodies so produced are highly specific, and improve the selectivity and specificity of diagnostic assay methods provided by the invention. For example, a solution
20 containing the appropriate antigen may be injected into a mouse or other species and, after a sufficient time (in keeping with conventional techniques), the animal is sacrificed and spleen cells obtained. The spleen cells are then immortalized by fusing them with myeloma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. Rabbit fusion hybridomas, for example, may be produced as
25 described in U.S Patent No. 5,675,063, C. Knight, Issued October 7, 1997. The hybridoma cells are then grown in a suitable selection media, such as hypoxanthine-aminopterin-thymidine (HAT), and the supernatant screened for monoclonal antibodies having the desired specificity, as described below. The secreted antibody may be recovered from tissue

- 40 -

culture supernatant by conventional methods such as precipitation, ion exchange or affinity chromatography, or the like.

Monoclonal Fab fragments may also be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. See, 5 e.g., W. Huse, *Science* 246: 1275-81 (1989); Mullinax *et al.*, *Proc. Nat'l Acad. Sci.* 87: 8095 (1990). If monoclonal antibodies of one isotype are preferred for a particular application, particular isotypes can be prepared directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype 10 by using the sib selection technique to isolate class-switch variants (Steplewski, *et al.*, *Proc. Nat'l. Acad. Sci.*, 82: 8653 (1985); Spira *et al.*, *J. Immunol. Methods*, 74: 307 (1984)).

The preferred epitope of a phosphorylation-site specific antibody of the invention is a peptide fragment consisting essentially of about 8 to 17 15 amino acids including the phosphorylatable threonine or serine, wherein about 3 to 8 amino acids are positioned on each side of the phosphorylatable threonine (for example, the requiem serine 113 phosphorylation site sequence disclosed in Row 24, Column E of Table 1), and antibodies of the invention thus specifically bind a target ATM 20 and/or ATR kinase signaling polypeptide comprising such epitopic sequence. Particularly preferred epitopes bound by the antibodies of the invention comprise all or part of a phosphorylatable site sequence listed in Column E of Table 1, including the phosphorylatable amino acid.

Included in the scope of the invention are equivalent non-antibody 25 molecules, such as protein binding domains or nucleic acid aptamers, which bind, in a phospho-specific manner, to essentially the same phosphorylatable epitope to which the phospho-specific antibodies of the invention bind. See, e.g., Neuberger *et al.*, *Nature* 312: 604 (1984). Such equivalent non-antibody reagents may be suitably employed in the 30 methods of the invention further described below.

- 41 -

Antibodies provided by the invention may be any type of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE, including F_{ab} or antigen-recognition fragments thereof. The antibodies may be monoclonal or polyclonal and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or human, or may be chimeric antibodies. See, e.g., M. Walker *et al.*, *Molec. Immunol.* 26: 403-11 (1989); Morrision *et al.*, *Proc. Nat'l. Acad. Sci.* 81: 6851 (1984); Neuberger *et al.*, *Nature* 312: 604 (1984)). The antibodies may be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Pat. No. 4,474,893 (Reading) or U.S. Pat. No. 4,816,567 (Cabilly *et al.*) The antibodies may also be chemically constructed by specific antibodies made according to the method disclosed in U.S. Pat. No. 4,676,980 (Segel *et al.*)

The invention also provides immortalized cell lines that produce an antibody of the invention. For example, hybridoma clones, constructed as described above, that produce monoclonal antibodies to the ATM and/or ATR kinase signaling protein phosphorylation sties disclosed herein are also provided. Similarly, the invention includes recombinant cells producing an antibody of the invention, which cells may be constructed by well known techniques; for example the antigen combining site of the monoclonal antibody can be cloned by PCR and single-chain antibodies produced as phage-displayed recombinant antibodies or soluble antibodies in *E. coli* (see, e.g., ANTIBODY ENGINEERING PROTOCOLS, 1995, Humana Press, Sudhir Paul editor.)

Phosphorylation site-specific antibodies of the invention, whether polyclonal or monoclonal, may be screened for epitope and phospho-specificity according to standard techniques. See, e.g. Czernik *et al.*, *Methods in Enzymology*, 201: 264-283 (1991). For example, the antibodies may be screened against the phospho and non-phospho peptide library by ELISA to ensure specificity for both the desired antigen

- 42 -

(i.e. that epitope including a phosphorylation site sequence enumerated in Column E of Table 1) and for reactivity only with the phosphorylated (or non-phosphorylated) form of the antigen. Peptide competition assays may be carried out to confirm lack of reactivity with other phospho-
5 epitopes on the given ATM and/or ATR kinase signaling protein. The antibodies may also be tested by Western blotting against cell preparations containing the signaling protein, e.g. cell lines over-expressing the target protein, to confirm reactivity with the desired phosphorylated epitope/target.

10 Specificity against the desired phosphorylated epitope may also be examined by constructing mutants lacking phosphorylatable residues at positions outside the desired epitope that are known to be phosphorylated, or by mutating the desired phospho-epitope and confirming lack of reactivity. Phosphorylation-site specific antibodies of
15 the invention may exhibit some limited cross-reactivity to related epitopes in non-target proteins. This is not unexpected as most antibodies exhibit some degree of cross-reactivity, and anti-peptide antibodies will often cross-react with epitopes having high homology to the immunizing peptide. See, e.g., Czernik, *supra*. Cross-reactivity with non-target
20 proteins is readily characterized by Western blotting alongside markers of known molecular weight. Amino acid sequences of cross-reacting proteins may be examined to identify sites highly homologous to the ATM and/or ATR kinase signaling protein epitope for which the antibody of the invention is specific.

25 In certain cases, polyclonal antisera may exhibit some undesirable general cross-reactivity to phosphothreonine or phosphoserine itself, which may be removed by further purification of antisera, e.g. over a phosphotyramine column. Antibodies of the invention specifically bind their target protein (i.e. a protein listed in Column A of Table 1) only when
30 phosphorylated (or only when not phosphorylated, as the case may be) at

- 43 -

the site disclosed in corresponding Columns D/E, and do not (substantially) bind to the other form (as compared to the form for which the antibody is specific).

Antibodies may be further characterized via immunohistochemical (IHC) staining using normal and diseased tissues to examine ATM and/or ATR phosphorylation and activation status in diseased tissue. IHC may be carried out according to well-known techniques. See, e.g., **ANTIBODIES: A LABORATORY MANUAL**, Chapter 10, Harlow & Lane Eds., Cold Spring Harbor Laboratory (1988). Briefly, paraffin-embedded tissue (e.g. tumor tissue) is prepared for immunohistochemical staining by deparaffinizing tissue sections with xylene followed by ethanol; hydrating in water then PBS; unmasking antigen by heating slide in sodium citrate buffer; incubating sections in hydrogen peroxide; blocking in blocking solution; incubating slide in primary antibody and secondary antibody; and finally detecting using ABC avidin/biotin method according to manufacturer's instructions.

Antibodies may be further characterized by flow cytometry carried out according to standard methods. See Chow *et al.*, *Cytometry (Communications in Clinical Cytometry)* 46: 790-100 (2001). Briefly and by way of example, the following protocol for cytometric analysis may be employed: samples may be centrifuged on Ficoll gradients to remove erythrocytes, and cells may then be fixed with 2% paraformaldehyde for 10 minutes at 37 °C followed by permeabilization in 90% methanol for 30 minutes on ice. Cells may then be stained with the primary phosphorylation-site specific antibody of the invention (which detects a ATM and/or ATR signal transduction protein enumerated in Table 1), washed and labeled with a fluorescent-labeled secondary antibody. Additional fluorochrome-conjugated marker antibodies (e.g. CD45, CD34) may also be added at this time to aid in the subsequent identification of specific hematopoietic cell types. The cells would then be analyzed on a

- 44 -

flow cytometer (e.g. a Beckman Coulter FC500) according to the specific protocols of the instrument used.

Antibodies of the invention may also be advantageously conjugated to fluorescent dyes (e.g. Alexa488, PE) for use in multi-parametric analyses along with other signal transduction (phospho-CrkL, phospho-Erk 1/2) and/or cell marker (CD34) antibodies.

Phosphorylation-site specific antibodies of the invention specifically bind to a human ATM and/or ATR signal transduction protein or polypeptide only when phosphorylated at a disclosed site, but are not limited only to binding the human species, *per se*. The invention includes antibodies that also bind conserved and highly homologous or identical phosphorylation sites in respective ATM and/or ATR proteins from other species (e.g. mouse, rat, monkey, yeast), in addition to binding the human phosphorylation site. Highly homologous or identical sites conserved in other species can readily be identified by standard sequence comparisons, such as using BLAST, with the human ATM and/or ATR signal transduction protein phosphorylation sites disclosed herein.

C. Heavy-Isotope Labeled Peptides (AQUA Peptides).

The novel ATM and/or ATR kinase signaling protein phosphorylation sites disclosed herein now enable the production of corresponding heavy-isotope labeled peptides for the absolute quantification of such signaling proteins (both phosphorylated and not phosphorylated at a disclosed site) in biological samples. The production and use of AQUA peptides for the absolute quantification of proteins (AQUA) in complex mixtures has been described. See WO/03016861, "Absolute Quantification of Proteins and Modified Forms Thereof by Multistage Mass Spectrometry," Gygi *et al.* and also Gerber *et al. Proc.*

- 45 -

Natl. Acad. Sci. U.S.A. 100: 6940-5 (2003) (the teachings of which are hereby incorporated herein by reference, in their entirety).

The AQUA methodology employs the introduction of a known quantity of at least one heavy-isotope labeled peptide standard (which
5 has a unique signature detectable by LC-SRM chromatography) into a digested biological sample in order to determine, by comparison to the peptide standard, the absolute quantity of a peptide with the same sequence and protein modification in the biological sample. Briefly, the AQUA methodology has two stages: peptide internal standard selection
10 and validation and method development; and implementation using validated peptide internal standards to detect and quantify a target protein in sample. The method is a powerful technique for detecting and quantifying a given peptide/protein within a complex biological mixture, such as a cell lysate, and may be employed, *e.g.*, to quantify change in
15 protein phosphorylation as a result of drug treatment, or to quantify differences in the level of a protein in different biological states.

Generally, to develop a suitable internal standard, a particular peptide (or modified peptide) within a target protein sequence is chosen based on its amino acid sequence and the particular protease to be used
20 to digest. The peptide is then generated by solid-phase peptide synthesis such that one residue is replaced with that same residue containing stable isotopes (^{13}C , ^{15}N). The result is a peptide that is chemically identical to its native counterpart formed by proteolysis, but is easily distinguishable by MS via a 7-Da mass shift. A newly synthesized AQUA internal standard
25 peptide is then evaluated by LC-MS/MS. This process provides qualitative information about peptide retention by reverse-phase chromatography, ionization efficiency, and fragmentation via collision-induced dissociation. Informative and abundant fragment ions for sets of native and internal standard peptides are chosen and then specifically
30 monitored in rapid succession as a function of chromatographic retention

- 46 -

to form a selected reaction monitoring (LC–SRM) method based on the unique profile of the peptide standard.

The second stage of the AQUA strategy is its implementation to measure the amount of a protein or modified protein from complex mixtures. Whole cell lysates are typically fractionated by SDS-PAGE gel electrophoresis, and regions of the gel consistent with protein migration are excised. This process is followed by in-gel proteolysis in the presence of the AQUA peptides and LC–SRM analysis. (See Gerber *et al. supra.*) AQUA peptides are spiked in to the complex peptide mixture obtained by digestion of the whole cell lysate with a proteolytic enzyme and subjected to immunoaffinity purification as described above. The retention time and fragmentation pattern of the native peptide formed by digestion (e.g. trypsinization) is identical to that of the AQUA internal standard peptide determined previously; thus, LC–MS/MS analysis using an SRM experiment results in the highly specific and sensitive measurement of both internal standard and analyte directly from extremely complex peptide mixtures. Because an absolute amount of the AQUA peptide is added (e.g. 250 fmol), the ratio of the areas under the curve can be used to determine the precise expression levels of a protein or phosphorylated form of a protein in the original cell lysate. In addition, the internal standard is present during in-gel digestion as native peptides are formed, such that peptide extraction efficiency from gel pieces, absolute losses during sample handling (including vacuum centrifugation), and variability during introduction into the LC–MS system do not affect the determined ratio of native and AQUA peptide abundances.

An AQUA peptide standard is developed for a known phosphorylation site sequence previously identified by the IAP-LC-MS/MS method within a target protein. One AQUA peptide incorporating the phosphorylated form of the particular residue within the site may be developed, and a second AQUA peptide incorporating the non-

- 47 -

phosphorylated form of the residue developed. In this way, the two standards may be used to detect and quantify both the phosphorylated and non-phosphorylated forms of the site in a biological sample.

Peptide internal standards may also be generated by examining
5 the primary amino acid sequence of a protein and determining the boundaries of peptides produced by protease cleavage. Alternatively, a protein may actually be digested with a protease and a particular peptide fragment produced can then be sequenced. Suitable proteases include, but are not limited to, serine proteases (e.g. trypsin, chymotrypsin), metallo
10 proteases (e.g. PUMP1), chymotrypsin, cathepsin, pepsin, thermolysin, carboxypeptidases, etc.

A peptide sequence within a target protein is selected according to one or more criteria to optimize the use of the peptide as an internal standard. Preferably, the size of the peptide is selected to minimize the
15 chances that the peptide sequence will be repeated elsewhere in other non-target proteins. Thus, a peptide is preferably at least about 6 amino acids. The size of the peptide is also optimized to maximize ionization frequency. Thus, peptides longer than about 20 amino acids are not preferred. The preferred range is about 7 to 15 amino acids. A peptide
20 sequence is also selected that is not likely to be chemically reactive during mass spectrometry, thus sequences comprising cysteine, tryptophan, or methionine are avoided.

A peptide sequence that does not include a modified region of the target region may be selected so that the peptide internal standard can be
25 used to determine the quantity of all forms of the protein. Alternatively, a peptide internal standard encompassing a modified amino acid may be desirable to detect and quantify only the modified form of the target protein. Peptide standards for both modified and unmodified regions can be used together, to determine the extent of a modification in a particular
30 sample (*i.e.* to determine what fraction of the total amount of protein is

- 48 -

represented by the modified form). For example, peptide standards for both the phosphorylated and unphosphorylated form of a protein known to be phosphorylated at a particular site can be used to quantify the amount of phosphorylated form in a sample.

- 5 The peptide is labeled using one or more labeled amino acids (*i.e.* the label is an actual part of the peptide) or less preferably, labels may be attached after synthesis according to standard methods. Preferably, the label is a mass-altering label selected based on the following considerations: The mass should be unique to shift fragment masses
10 produced by MS analysis to regions of the spectrum with low background; the ion mass signature component is the portion of the labeling moiety that preferably exhibits a unique ion mass signature in MS analysis; the sum of the masses of the constituent atoms of the label is preferably uniquely different than the fragments of all the possible amino acids. As a
15 result, the labeled amino acids and peptides are readily distinguished from unlabeled ones by the ion/mass pattern in the resulting mass spectrum. Preferably, the ion mass signature component imparts a mass to a protein fragment that does not match the residue mass for any of the 20 natural amino acids.
- 20 The label should be robust under the fragmentation conditions of MS and not undergo unfavorable fragmentation. Labeling chemistry should be efficient under a range of conditions, particularly denaturing conditions, and the labeled tag preferably remains soluble in the MS buffer system of choice. The label preferably does not suppress the
25 ionization efficiency of the protein and is not chemically reactive. The label may contain a mixture of two or more isotopically distinct species to generate a unique mass spectrometric pattern at each labeled fragment position. Stable isotopes, such as ^2H , ^{13}C , ^{15}N , ^{17}O , ^{18}O , or ^{34}S , are among preferred labels. Pairs of peptide internal standards that
30 incorporate a different isotope label may also be prepared. Preferred

- 49 -

amino acid residues into which a heavy isotope label may be incorporated include leucine, proline, valine, and phenylalanine.

Peptide internal standards are characterized according to their mass-to-charge (m/z) ratio, and preferably, also according to their retention time on a chromatographic column (e.g. an HPLC column). Internal standards that co-elute with unlabeled peptides of identical sequence are selected as optimal internal standards. The internal standard is then analyzed by fragmenting the peptide by any suitable means, for example by collision-induced dissociation (CID) using, e.g., argon or helium as a collision gas. The fragments are then analyzed, for example by multi-stage mass spectrometry (MS^n) to obtain a fragment ion spectrum, to obtain a peptide fragmentation signature. Preferably, peptide fragments have significant differences in m/z ratios to enable peaks corresponding to each fragment to be well separated, and a signature that is unique for the target peptide is obtained. If a suitable fragment signature is not obtained at the first stage, additional stages of MS are performed until a unique signature is obtained.

Fragment ions in the MS/MS and MS^3 spectra are typically highly specific for the peptide of interest, and, in conjunction with LC methods, allow a highly selective means of detecting and quantifying a target peptide/protein in a complex protein mixture, such as a cell lysate, containing many thousands or tens of thousands of proteins. Any biological sample potentially containing a target protein/peptide of interest may be assayed. Crude or partially purified cell extracts are preferably employed. Generally, the sample has at least 0.01 mg of protein, typically a concentration of 0.1-10 mg/mL, and may be adjusted to a desired buffer concentration and pH.

A known amount of a labeled peptide internal standard, preferably about 10 femtomoles, corresponding to a target protein to be detected/quantified is then added to a biological sample, such as a cell

- 50 -

lysate. The spiked sample is then digested with one or more protease(s) for a suitable time period to allow digestion. A separation is then performed (e.g. by HPLC, reverse-phase HPLC, capillary electrophoresis, ion exchange chromatography, etc.) to isolate the labeled internal
5 standard and its corresponding target peptide from other peptides in the sample. Microcapillary LC is a preferred method.

Each isolated peptide is then examined by monitoring of a selected reaction in the MS. This involves using the prior knowledge gained by the characterization of the peptide internal standard and then requiring the
10 MS to continuously monitor a specific ion in the MS/MS or MSⁿ spectrum for both the peptide of interest and the internal standard. After elution, the area under the curve (AUC) for both peptide standard and target peptide peaks are calculated. The ratio of the two areas provides the absolute quantification that can be normalized for the number of cells used in the
15 analysis and the protein's molecular weight, to provide the precise number of copies of the protein per cell. Further details of the AQUA methodology are described in Gygi *et al.*, and Gerber *et al. supra*.

In accordance with the present invention, AQUA internal peptide standards (heavy-isotope labeled peptides) may now be produced, as
20 described above, for any of the nearly 300 novel ATM and/or ATR kinase signaling protein phosphorylation sites disclosed herein (see Table 1/Figure 2). Peptide standards for a given phosphorylation site (e.g. the serine 395 in NuMA-1 – see Row 40 of Table 1) may be produced for both the phosphorylated and non-phosphorylated forms of the site (e.g.
25 see NuMA-1 site sequence in Column E, Row 41 of Table 1 (SEQ ID NO: 39) and such standards employed in the AQUA methodology to detect and quantify both forms of such phosphorylation site in a biological sample.

AQUA peptides of the invention may comprise all, or part of, a
30 phosphorylation site peptide sequence disclosed herein (see Column E of

- 51 -

Table 1/Figure 2). In a preferred embodiment, an AQUA peptide of the invention comprises a phosphorylation site sequence disclosed herein in Table 1/Figure 2. For example, an AQUA peptide of the invention for detection/quantification of MRE11A DNA repair protein when phosphorylated at serine S648 may comprise the sequence IMsQSQVSK (s=phosphoserine), which comprises phosphorylatable serine 648 (see Row 92, Column E; (SEQ ID NO: 91)). Heavy-isotope labeled equivalents of the peptides enumerated in Table 1/Figure 2 (both in phosphorylated and unphosphorylated form) can be readily synthesized and their unique MS and LC-SRM signature determined, so that the peptides are validated as AQUA peptides and ready for use in quantification experiments.

The phosphorylation site peptide sequences disclosed herein (see Column E of Table 1/Figure 2) are particularly well suited for development of corresponding AQUA peptides, since the IAP method by which they were identified (see Part A above and Example 1) inherently confirmed that such peptides are in fact produced by enzymatic digestion (trypsinization) and are in fact suitably fractionated/ionized in MS/MS. Thus, heavy-isotope labeled equivalents of these peptides (both in phosphorylated and unphosphorylated form) can be readily synthesized and their unique MS and LC-SRM signature determined, so that the peptides are validated as AQUA peptides and ready for use in quantification experiments.

Accordingly, the invention provides heavy-isotope labeled peptides (AQUA peptides) for the detection and/or quantification of any of the ATM and/or ATR phosphorylation sites disclosed in Table 1/Figure 2 (see Column E) and/or their corresponding parent proteins/polypeptides (see Column A). A phosphopeptide sequence comprising any of the phosphorylation sequences listed in Table 1 may be considered a preferred AQUA peptide of the invention. For example, an AQUA peptide

- 52 -

comprising the sequence PVsQPSLVGSK (SEQ ID NO: 180) (where s may be either phosphoserine or serine, and where V = labeled valine (e.g. ^{14}C)) is provided for the quantification of phosphorylated (or non-phosphorylated) WNK1 kinase (Ser167) in a biological sample (see Row 5 181 of Table 1, serine 167 being the phosphorylatable residue within the site). However, it will be appreciated that a larger AQUA peptide comprising a disclosed phosphorylation site sequence (and additional residues downstream or upstream of it) may also be constructed. Similarly, a smaller AQUA peptide comprising less than all of the residues 10 of a disclosed phosphorylation site sequence (but still comprising the phosphorylatable residue enumerated in Column D of Table 1/Figure 2) may alternatively be constructed. Such larger or shorter AQUA peptides are within the scope of the present invention, and the selection and production of preferred AQUA peptides may be carried out as described 15 above (see Gygi *et al.*, Gerber *et al. supra.*).

Certain particularly preferred subsets of AQUA peptides provided by the invention are described above (corresponding to particular protein types/groups in Table 1, for example, Protein Kinases or Phosphatases). Example 4 is provided to further illustrate the construction and use, by 20 standard methods described above, of exemplary AQUA peptides provided by the invention. For example, the above-described AQUA peptides corresponding to both the phosphorylated and non-phosphorylated forms of the disclosed WNK1 kinase serine, 167 phosphorylation site (see Row 181 of Table 1/Figure 2) may be used to 25 quantify the amount of phosphorylated WNK1 kinase (Ser167) in a biological sample, e.g. a tumor cell sample (or a sample before or after treatment with a test drug).

AQUA peptides of the invention may also be employed within a kit that comprises one or multiple AQUA peptide(s) provided herein (for the 30 quantification of a ATM and/or ATR signal transduction protein disclosed

- 53 -

in Table 1/Figure 2), and, optionally, a second detecting reagent conjugated to a detectable group. For example, a kit may include AQUA peptides for both the phosphorylated and non-phosphorylated form of a phosphorylation site disclosed herein. The reagents may also include
5 ancillary agents such as buffering agents and protein stabilizing agents, e.g., polysaccharides and the like. The kit may further include, where necessary, other members of the signal-producing system of which system the detectable group is a member (e.g., enzyme substrates), agents for reducing background interference in a test, control reagents,
10 apparatus for conducting a test, and the like. The test kit may be packaged in any suitable manner, typically with all elements in a single container along with a sheet of printed instructions for carrying out the test.

AQUA peptides provided by the invention will be highly useful in
15 the further study of signal transduction anomalies underlying cancer, including both solid and blood borne cancers, and in identifying diagnostic/bio-markers of these diseases, new potential drug targets, and/or in monitoring the effects of test compounds on ATM and/or ATR signal transduction proteins and pathways.

20 D. Immunoassay Formats

Antibodies provided by the invention may be advantageously employed in a variety of standard immunological assays (the use of AQUA peptides provided by the invention is described separately above). Assays may be homogeneous assays or heterogeneous assays. In a
25 homogeneous assay the immunological reaction usually involves a phosphorylation-site specific antibody of the invention), a labeled analyte, and the sample of interest. The signal arising from the label is modified, directly or indirectly, upon the binding of the antibody to the labeled analyte. Both the immunological reaction and detection of the extent

- 54 -

thereof are carried out in a homogeneous solution. Immunochemical labels that may be employed include free radicals, radioisotopes, fluorescent dyes, enzymes, bacteriophages, coenzymes, and so forth.

In a heterogeneous assay approach, the reagents are usually the specimen, a phosphorylation-site specific antibody of the invention, and suitable means for producing a detectable signal. Similar specimens as described above may be used. The antibody is generally immobilized on a support, such as a bead, plate or slide, and contacted with the specimen suspected of containing the antigen in a liquid phase. The support is then separated from the liquid phase and either the support phase or the liquid phase is examined for a detectable signal employing means for producing such signal. The signal is related to the presence of the analyte in the specimen. Means for producing a detectable signal include the use of radioactive labels, fluorescent labels, enzyme labels, and so forth. For example, if the antigen to be detected contains a second binding site, an antibody which binds to that site can be conjugated to a detectable group and added to the liquid phase reaction solution before the separation step. The presence of the detectable group on the solid support indicates the presence of the antigen in the test sample. Examples of suitable immunoassays are the radioimmunoassay, immunofluorescence methods, enzyme-linked immunoassays, and the like.

Immunoassay formats and variations thereof that may be useful for carrying out the methods disclosed herein are well known in the art. See generally E. Maggio, *Enzyme-Immunoassay*, (1980) (CRC Press, Inc., Boca Raton, Fla.); see also, e.g., U.S. Pat. No. 4,727,022 (Skold *et al.*, "Methods for Modulating Ligand-Receptor Interactions and their Application"); U.S. Pat. No. 4,659,678 (Forrest *et al.*, "Immunoassay of Antigens"); U.S. Pat. No. 4,376,110 (David *et al.*, "Immunometric Assays Using Monoclonal Antibodies"). Conditions suitable for the formation of

- 55 -

reagent-antibody complexes are well described. *See id.* Monoclonal antibodies of the invention may be used in a "two-site" or "sandwich" assay, with a single cell line serving as a source for both the labeled monoclonal antibody and the bound monoclonal antibody. Such assays
5 are described in U.S. Pat. No. 4,376,110. The concentration of detectable reagent should be sufficient such that the binding of a target ATM and/or ATR signal transduction protein is detectable compared to background.

Phosphorylation site-specific antibodies disclosed herein may be conjugated to a solid support suitable for a diagnostic assay (*e.g.*, beads,
10 plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as precipitation. Antibodies, or other target protein or target site-binding reagents, may likewise be conjugated to detectable groups such as radiolabels (*e.g.*, ^{35}S , ^{125}I , ^{131}I), enzyme labels (*e.g.*, horseradish peroxidase, alkaline phosphatase), and
15 fluorescent labels (*e.g.*, fluorescein) in accordance with known techniques.

Antibodies of the invention may also be optimized for use in a flow cytometry (FC) assay to determine the activation/phosphorylation status of a target ATM and/or ATR signal transduction protein in patients before,
20 during, and after treatment with a drug targeted at inhibiting phosphorylation at such a protein at the phosphorylation site disclosed herein. For example, bone marrow cells or peripheral blood cells from patients may be analyzed by flow cytometry for target ATM and/or ATR signal transduction protein phosphorylation, as well as for markers
25 identifying various hematopoietic cell types. In this manner, activation status of the malignant cells may be specifically characterized. Flow cytometry may be carried out according to standard methods. *See, e.g. Chow et al., Cytometry (Communications in Clinical Cytometry) 46: 72–78 (2001).* Briefly and by way of example, the following protocol for
30 cytometric analysis may be employed: fixation of the cells with 1% para-

- 56 -

formaldehyde for 10 minutes at 37 °C followed by permeabilization in 90% methanol for 30 minutes on ice. Cells may then be stained with the primary antibody (a phospho-specific antibody of the invention), washed and labeled with a fluorescent-labeled secondary antibody. Alternatively, the cells may be stained with a fluorescent-labeled primary antibody. The cells would then be analyzed on a flow cytometer (e.g. a Beckman Coulter EPICS-XL) according to the specific protocols of the instrument used. Such an analysis would identify the presence of activated ATM and/or ATR signal transduction protein(s) in the malignant cells and reveal the drug response on the targeted protein.

Alternatively, antibodies of the invention may be employed in immunohistochemical (IHC) staining to detect differences in signal transduction or protein activity using normal and diseased tissues. IHC may be carried out according to well-known techniques. See, e.g., ANTIBODIES: A LABORATORY MANUAL, supra. Briefly, paraffin-embedded tissue (e.g. tumor tissue) is prepared for immunohistochemical staining by deparaffinizing tissue sections with xylene followed by ethanol; hydrating in water then PBS; unmasking antigen by heating slide in sodium citrate buffer; incubating sections in hydrogen peroxide; blocking in blocking solution; incubating slide in primary antibody and secondary antibody; and finally detecting using ABC avidin/biotin method according to manufacturer's instructions.

Antibodies of the invention may be also be optimized for use in other clinically-suitable applications, for example bead-based multiplex-type assays, such as IGEN, Luminex™ and/or Bioplex™ assay formats, or otherwise optimized for antibody arrays formats, such as reversed-phase array applications (see, e.g. Paweletz *et al.*, *Oncogene* 20(16): 1981-89 (2001)). Accordingly, in another embodiment, the invention provides a method for the multiplex detection of ATM and/or ATR protein phosphorylation in a biological sample, the method comprising utilizing

- 57 -

two or more antibodies or AQUA peptides of the invention to detect the presence of two or more phosphorylated ATM and/or ATR kinase signaling proteins enumerated in Column A of Table 1/Figure 2. In one preferred embodiment, two to five antibodies or AQUA peptides of the invention are employed in the method. In another preferred embodiment, 5 six to ten antibodies or AQUA peptides of the invention are employed, while in another preferred embodiment eleven to twenty such reagents are employed.

Antibodies and/or AQUA peptides of the invention may also be employed within a kit that comprises at least one phosphorylation site-specific antibody or AQUA peptide of the invention (which binds to or detects a ATM and/or ATR signal transduction protein disclosed in Table 1/Figure 2), and, optionally, a second antibody conjugated to a detectable group. In some embodiments, the kit is suitable for multiplex assays and 10 comprises two or more antibodies or AQUA peptides of the invention, and in some embodiments, comprises two to five, six to ten, or eleven to twenty reagents of the invention. The kit may also include ancillary agents such as buffering agents and protein stabilizing agents, *e.g.*, polysaccharides and the like. The kit may further include, where 20 necessary, other members of the signal-producing system of which system the detectable group is a member (*e.g.*, enzyme substrates), agents for reducing background interference in a test, control reagents, apparatus for conducting a test, and the like. The test kit may be packaged in any suitable manner, typically with all elements in a single 25 container along with a sheet of printed instructions for carrying out the test.

The following Examples are provided only to further illustrate the invention, and are not intended to limit its scope, except as provided in the claims appended hereto. The present invention encompasses

- 58 -

modifications and variations of the methods taught herein which would be obvious to one of ordinary skill in the art.

EXAMPLE 1

5 **Isolation of Phosphoserine and/or Phosphothreonine-Containing
 Peptides from Extracts of Human Cancer Cell Lines and
 Identification of Novel Phosphorylation Sites.**

 In order to discover previously unknown ATM and/or ATR signal
transduction protein phosphorylation sites, IAP isolation techniques were
employed to identify phosphothreonine- and/or phosphoserine- containing
10 peptides in cell extracts from the following cell lines: M059K, 293 T,
M059J, M059K+J and human embryonic kidney cells.

 Tryptic phosphothreonine- and phosphoserine- containing peptides
were purified and analyzed from extracts of each of the 3 cell lines
mentioned above, as follows. Cells were cultured in DMEM medium
15 (M059K and J) or RPMI 1640 medium (293 cells) supplemented with
10% fetal bovine serum and penicillin/streptomycin. Cells were harvested
by scraping plates. After complete aspiration of medium, cells were
harvested in 10mL lysis buffer per 2×10^8 cells (20 mM HEPES pH 8.0, 9 M
urea, 1 mM sodium vanadate, supplemented with 2.5 mM sodium pyro-
20 phosphate, 1 mM β -glycerol-phosphate) and sonicated.

 Sonicated cell lysates were cleared by centrifugation at 20,000 x g,
and proteins were reduced with DTT at a final concentration of 4.1 mM
and alkylated with iodoacetamide at 8.3 mM. For digestion with trypsin,
protein extracts were diluted in 20 mM HEPES pH 8.0 to a final
25 concentration of 2 M urea and soluble TLCK-trypsin (Worthington) was
added at 10-20 μ g/mL. Digestion was performed for overnight days at
room temperature.

 Trifluoroacetic acid (TFA) was added to protein digests to a final
concentration of 1%, precipitate was removed by centrifugation, and

- 59 -

digests were loaded onto Sep-Pak C₁₈ columns (Waters) equilibrated with 0.1% TFA. A column volume of 0.7-1.0 ml was used per 2×10^8 cells. Columns were washed with 15 volumes of 0.1% TFA, followed by 4 volumes of 5% acetonitrile (MeCN) in 0.1% TFA. Peptide fraction I was
5 obtained by eluting columns with 2 volumes each of 8, 12, and 15% MeCN in 0.1% TFA and combining the eluates. Fractions II and III were a combination of eluates after eluting columns with 18, 22, 25% MeCN in 0.1% TFA and with 30, 35, 40% MeCN in 0.1% TFA, respectively. All peptide fractions were pooled and lyophilized.

10 Peptides from each fraction corresponding to 2×10^8 cells were dissolved in 1 ml of IAP buffer (20 mM Tris/HCl or 50 mM MOPS pH 7.2, 10 mM sodium phosphate, 50 mM NaCl) and insoluble matter (mainly in peptide fractions III) was removed by centrifugation. IAP was performed on each peptide fraction separately. The ATM/ATR substrate antibody or
15 phospho-chk2(T26/S28)/VCP(S784) antibody (commercially available from Cell Signaling Technology, Inc., Beverly, MA, Cat #'s 2851 and 2664, respectively, recognizing the phosphorylated motifs LS*Q and S*Q) were coupled at 4 mg/ml beads to protein G or protein A agarose (Roche), respectively. Immobilized antibody (15 μ l, 60 μ g) was added as
20 1:1 slurry in IAP buffer to 1 ml of each peptide fraction, and the mixture was incubated overnight at 4° C with gentle rotation. The immobilized antibody beads were washed three times with 1 ml IAP buffer and twice with 1 ml water, all at 4° C. Peptides were eluted from beads by incubation with 75 μ l of 0.1% TFA at room temperature for 10 minutes.

25 Alternatively, one single peptide fraction was obtained from Sep-Pak C₁₈ columns by elution with 2 volumes each of 10%, 15%, 20 %, 25 %, 30 %, 35 % and 40 % acetonitrile in 0.1% TFA and combination of all eluates. IAP on this peptide fraction was performed as follows: After lyophilization, peptide was dissolved in 1.4 ml IAP buffer (MOPS pH 7.2,
30 10 mM sodium phosphate, 50 mM NaCl) and insoluble matter was

- 60 -

removed by centrifugation. Immobilized antibody (40 μ l, 160 μ g) was added as 1:1 slurry in IAP buffer, and the mixture was incubated overnight at 4° C with gentle shaking. The immobilized antibody beads were washed three times with 1 ml IAP buffer and twice with 1 ml water, all at 4° C. Peptides were eluted from beads by incubation with 55 μ l of 0.15% TFA at room temperature for 10 min (eluate 1), followed by a second elution of the beads (eluate 2) with 45 μ l of 0.15% TFA. Both eluates were combined.

Analysis by LC-MS/MS Mass Spectrometry.

40 μ l or more of IAP eluate were purified by 0.2 μ l StageTips or ZipTips. Peptides were eluted from the microcolumns with 1 μ l of 40% MeCN, 0.1% TFA (fractions I and II) or 1 μ l of 60% MeCN, 0.1% TFA (fraction III) into 7.6 μ l of 0.4% acetic acid/0.005% heptafluorobutyric acid. This sample was loaded onto a 10 cm x 75 μ m PicoFrit capillary column (New Objective) packed with Magic C18 AQ reversed-phase resin (Michrom Bioresources) using a Famos autosampler with an inert sample injection valve (Dionex). The column was then developed with a 45-min linear gradient of acetonitrile delivered at 200 nl/min (Ultimate, Dionex), and tandem mass spectra were collected in a data-dependent manner with an LCQ Deca XP Plus ion trap mass spectrometer essentially as described by Gygi *et al.*, *supra*.

Database Analysis & Assignments.

MS/MS spectra were evaluated using TurboSequest in the Sequest Browser package (v. 27, rev. 12) supplied as part of BioWorks 3.0 (ThermoFinnigan). Individual MS/MS spectra were extracted from the raw data file using the Sequest Browser program CreateDta, with the following settings: bottom MW, 700; top MW, 4,500; minimum number of ions, 20; minimum TIC, 4×10^5 ; and precursor charge state, unspecified. Spectra were extracted from the beginning of the raw data file before

- 61 -

sample injection to the end of the eluting gradient. The IonQuest and VuDta programs were not used to further select MS/MS spectra for Sequest analysis. MS/MS spectra were evaluated with the following TurboSequest parameters: peptide mass tolerance, 2.5; fragment ion
5 tolerance, 0.0; maximum number of differential amino acids per modification, 4; mass type parent, average; mass type fragment, average; maximum number of internal cleavage sites, 10; neutral losses of water and ammonia from b and y ions were considered in the correlation analysis. Proteolytic enzyme was specified except for spectra collected
10 from elastase digests.

Searches were performed against the NCBI human protein database (as released on August 24, 2004 and containing 27, 960 protein sequences). Cysteine carboxamidomethylation was specified as a static modification, and phosphorylation was allowed as a variable modification
15 on serine and/or threonine. Furthermore, it should be noted that certain peptides were originally isolated in mouse and later normalized to human sequences as shown by Table1/Figure2.

In proteomics research, it is desirable to validate protein identifications based solely on the observation of a single peptide in one
20 experimental result, in order to indicate that the protein is, in fact, present in a sample. This has led to the development of statistical methods for validating peptide assignments, which are not yet universally accepted, and guidelines for the publication of protein and peptide identification results (see Carr *et al.*, *Mol. Cell Proteomics* 3: 531-533 (2004)), which
25 were followed in this Example. However, because the immunoaffinity strategy separates phosphorylated peptides from unphosphorylated peptides, observing just one phosphopeptide from a protein is a common result, since many phosphorylated proteins have only one threonine or serine phosphorylated site. For this reason, it is appropriate to use
30 additional criteria to validate phosphopeptide assignments. Assignments

- 62 -

are likely to be correct if any of these additional criteria are met: (i) the same sequence is assigned to co-eluting ions with different charge states, since the MS/MS spectrum changes markedly with charge state; (ii) the site is found in more than one peptide sequence context due to sequence overlaps from incomplete proteolysis or use of proteases other than trypsin; (iii) the site is found in more than one peptide sequence context due to homologous but not identical protein isoforms; (iv) the site is found in more than one peptide sequence context due to homologous but not identical proteins among species; and (v) sites validated by MS/MS analysis of synthetic phosphopeptides corresponding to assigned sequences, since the ion trap mass spectrometer produces highly reproducible MS/MS spectra. The last criterion is routinely employed to confirm novel site assignments of particular interest.

All spectra and all sequence assignments made by Sequest were imported into a relational database. The following Sequest scoring thresholds were used to select phosphopeptide assignments that are likely to be correct: $RSp < 6$, $XCorr \geq 2.2$, and $DeltaCN > 0.099$. Further, the assigned sequences could be accepted or rejected with respect to accuracy by using the following conservative, two-step process.

In the first step, a subset of high-scoring sequence assignments should be selected by filtering for $XCorr$ values of at least 1.5 for a charge state of +1, 2.2 for +2, and 3.3 for +3, allowing a maximum RSp value of 10. Assignments in this subset should be rejected if any of the following criteria were satisfied: (i) the spectrum contains at least one major peak (at least 10% as intense as the most intense ion in the spectrum) that can not be mapped to the assigned sequence as an *a*, *b*, or *y* ion, as an ion arising from neutral-loss of water or ammonia from a *b* or *y* ion, or as a multiply protonated ion; (ii) the spectrum does not contain a series of *b* or *y* ions equivalent to at least six uninterrupted residues; or (iii) the sequence is not observed at least five times in all the studies conducted

- 63 -

(except for overlapping sequences due to incomplete proteolysis or use of proteases other than trypsin).

In the second step, assignments with below-threshold scores should be accepted if the low-scoring spectrum shows a high degree of similarity to a high-scoring spectrum collected in another study, which simulates a true reference library-searching strategy.

EXAMPLE 2

Production of Phospho-specific Polyclonal Antibodies for the Detection of ATM and/or ATR kinase signaling Protein Phosphorylation

Polyclonal antibodies that specifically bind a ATM and/or ATR signal transduction protein only when phosphorylated at the respective phosphorylation site disclosed herein (see Table 1/Figure 2) are produced according to standard methods by first constructing a synthetic peptide antigen comprising the phosphorylation site sequence and then immunizing an animal to raise antibodies against the antigen, as further described below. Production of exemplary polyclonal antibodies is provided below.

A. MYT1L (serine 991).

A 17 amino acid phospho-peptide antigen, QKDGYLNGs*QFSWKSVK (where s*= phosphoserine) that corresponds to the sequence encompassing the serine 991 phosphorylation site in human MYT1L transcription factor (see Row 231 of Table 1; SEQ ID NO: 230), plus cysteine on the C-terminal for coupling, is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to immunize animals to produce (and

- 64 -

subsequently screen) phospho-specific MYT1L (ser 991) polyclonal antibodies as described in Immunization/ Screening below.

B. MYST2 (serine 50).

A 16 amino acid phospho-peptide antigen,

5 SSARLs*QSSQDSSPVR (where s*= phosphoserine) that corresponds to the sequence encompassing the serine 50 phosphorylation site in human MYST2 transcription protein (see Row 258 of Table 1 (SEQ ID NO: 257)), plus cysteine on the C-terminal for coupling, is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies,
10 Inc., Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to immunize animals to produce (and subsequently screen) phospho-specific MYST2 (ser 50) polyclonal antibodies as described in Immunization/Screening below.

15 **C. MRE11A (serine 648).**

A 9 amino acid phospho-peptide antigen, IMs*QSQVSK (where s*= phosphoserine) that corresponds to the sequence encompassing the serine 648 phosphorylation site in human MRE11A DNA repair protein (see Row 92 of Table 1 (SEQ ID NO: 91), plus cysteine on the C-terminal
20 for coupling, is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to immunize animals to produce (and subsequently screen) phospho-specific MRE11A (ser
25 648) antibodies as described in Immunization/Screening below.

- 65 -

Immunization/Screening.

A synthetic phospho-peptide antigen as described in A-C above is coupled to KLH, and rabbits are injected intradermally (ID) on the back with antigen in complete Freund's adjuvant (500 µg antigen per rabbit).

- 5 The rabbits are boosted with same antigen in incomplete Freund's adjuvant (250 µg antigen per rabbit) every three weeks. After the fifth boost, bleeds are collected. The sera are purified by Protein A-affinity chromatography by standard methods (see ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor, *supra.*). The eluted immunoglobulins are
- 10 further loaded onto a non-phosphorylated synthetic peptide antigen-resin Knetes column to pull out antibodies that bind the non-phosphorylated form of the phosphorylation site. The flow through fraction is collected and applied onto a phospho-synthetic peptide antigen-resin column to isolate antibodies that bind the phosphorylated form of the site. After
- 15 washing the column extensively, the bound antibodies (*i.e.* antibodies that bind a phosphorylated peptide described in A-C above, but do not bind the non-phosphorylated form of the peptide) are eluted and kept in antibody storage buffer.

- The isolated antibody is then tested for phospho-specificity using
- 20 Western blot assay using an appropriate cell line that expresses (or overexpresses) target phospho-protein (*i.e.* phosphorylated MYTL1, MYST2 and MRE11A), for example, M059J, M059K M059K+J respectively. Cells are cultured in DMEM or RPMI supplemented with 10% FBS. Cells are collected, washed with PBS and directly lysed in cell
- 25 lysis buffer. The protein concentration of cell lysates is then measured. The loading buffer is added into cell lysate and the mixture is boiled at 100 °C for 5 minutes. 20 µl (10 µg protein) of sample is then added onto 7.5% SDS-PAGE gel.

- 66 -

A standard Western blot may be performed according to the Immunoblotting Protocol set out in the CELL SIGNALING TECHNOLOGY, INC. 2003-04 Catalogue, p. 390. The isolated phospho-specific antibody is used at dilution 1:1000. Phosphorylation-site specificity of the antibody will be shown by binding of only the phosphorylated form of the target protein. Isolated phospho-specific polyclonal antibody does not (substantially) recognize the target protein when not phosphorylated at the appropriate phosphorylation site in the non-stimulated cells (*e.g.* MYTL1 is not bound when not phosphorylated at serine 74).

In order to confirm the specificity of the isolated antibody, different cell lysates containing various phosphorylated signal transduction proteins other than the target protein are prepared. The Western blot assay is performed again using these cell lysates. The phospho-specific polyclonal antibody isolated as described above is used (1:1000 dilution) to test reactivity with the different phosphorylated non-target proteins on Western blot membrane. The phospho-specific antibody does not significantly cross-react with other phosphorylated signal transduction proteins, although occasionally slight binding with a highly homologous phosphorylation-site on another protein may be observed. In such case the antibody may be further purified using affinity chromatography, or the specific immunoreactivity cloned by rabbit hybridoma technology.

EXAMPLE 3

Production of Phospho-specific Monoclonal Antibodies for the Detection of ATM and/or ATR kinase signaling Protein Phosphorylation

Monoclonal antibodies that specifically bind a ATM and/or ATR signal transduction protein only when phosphorylated at the respective phosphorylation site disclosed herein (see Table 1/Figure 2) are produced according to standard methods by first constructing a synthetic peptide

- 67 -

antigen comprising the phosphorylation site sequence and then immunizing an animal to raise antibodies against the antigen, and harvesting spleen cells from such animals to produce fusion hybridomas, as further described below. Production of exemplary monoclonal
5 antibodies is provided below.

A. requiem (serine 113):

A 16 amino acid phospho-peptide antigen, EGLIs*QDGSSLEALLR (where s*= phosphoserine) that corresponds to the sequence encompassing the serine 113 phosphorylation site in human requiem
10 apoptosis protein (see Row 24 of Table 1 (SEQ ID NO: 23)), plus cysteine on the C-terminal for coupling, is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to
15 immunize animals and harvest spleen cells for generation (and subsequent screening) of phospho-specific monoclonal requiem (ser 113) antibodies as described in Immunization/Fusion/Screening below.

B. NuMA-1 (serine 395).

A 19 amino acid phospho-peptide antigen
20 LSQLEEHLs*QLQDNPPQEK (where s*= phosphoserine) that corresponds to the sequence encompassing the serine 395 phosphorylation site in human NuMA-1 cell cycle regulation protein (see Row 40 of Table 1 (SEQ ID NO: 39)), plus cysteine on the C-terminal for coupling, is constructed according to standard synthesis techniques
25 using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to immunize animals and harvest spleen cells for generation (and subsequent

- 68 -

screening) of phospho-specific monoclonal NuMA-1 (ser395) antibodies as described in Immunization/Fusion/Screening below.

C. NLK (threonine 286).

5 A 12 amino acid phospho-peptide antigen, HMt*QEVTQYYR (where t*= phosphothreonine) that corresponds to the sequence encompassing the threonine 286 phosphorylation site in human NLK protein kinase (see Row 173 of Table 1 (SEQ ID NO: 172)), plus cysteine on the C-terminal for coupling, is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc.,
10 Symphony peptide synthesizer. See ANTIBODIES: A LABORATORY MANUAL, *supra.*; Merrifield, *supra.* This peptide is then coupled to KLH and used to immunize animals and harvest spleen cells for generation (and subsequent screening) of phospho-specific monoclonal NLK (thr286) antibodies as described in Immunization/Fusion/Screening below.

15 **Immunization/Fusion/Screening.**

A synthetic phospho-peptide antigen as described in A-C above is coupled to KLH, and BALB/C mice are injected intradermally (ID) on the back with antigen in complete Freund's adjuvant (e.g. 50 µg antigen per mouse). The mice are boosted with same antigen in incomplete Freund's
20 adjuvant (e.g. 25 µg antigen per mouse) every three weeks. After the fifth boost, the animals are sacrificed and spleens are harvested.

Harvested spleen cells are fused to SP2/0 mouse myeloma fusion partner cells according to the standard protocol of Kohler and Milstein (1975). Colonies originating from the fusion are screened by ELISA for
25 reactivity to the phospho-peptide and non-phospho-peptide forms of the antigen and by Western blot analysis (as described in Example 1 above). Colonies found to be positive by ELISA to the phospho-peptide while negative to the non-phospho-peptide are further characterized by Western blot analysis. Colonies found to be positive by Western blot

- 69 -

analysis are subcloned by limited dilution. Mouse ascites are produced from a single clone obtained from subcloning, and tested for phospho-specificity (against the requiem, NuMA-1, or NLK phospho-peptide antigen, as the case may be) on ELISA. Clones identified as positive on
5 Western blot analysis using cell culture supernatant as having phospho-specificity, as indicated by a strong band in the induced lane and a weak band in the uninduced lane of the blot, are isolated and subcloned as clones producing monoclonal antibodies with the desired specificity.

Ascites fluid from isolated clones may be further tested by Western
10 blot analysis. The ascites fluid should produce similar results on Western blot analysis as observed previously with the cell culture supernatant, indicating phospho-specificity against the phosphorylated target (e.g. NLK phosphorylated at threonine 286).

EXAMPLE 4

15 **Production and Use of AQUA Peptides for the Quantification of ATM and/or ATR kinase signaling Protein Phosphorylation**

Heavy-isotope labeled peptides (AQUA peptides (internal standards)) for the detection and quantification of a ATM and/or ATR signal transduction protein only when phosphorylated at the respective
20 phosphorylation site disclosed herein (see Table 1/Figure 2) are produced according to the standard AQUA methodology (see Gygi *et al.*, Gerber *et al.*, *supra.*) methods by first constructing a synthetic peptide standard corresponding to the phosphorylation site sequence and incorporating a heavy-isotope label. Subsequently, the MSⁿ and LC-SRM signature of
25 the peptide standard is validated, and the AQUA peptide is used to quantify native peptide in a biological sample, such as a digested cell extract. Production and use of exemplary AQUA peptides is provided below.

- 70 -

A. WNK1 (serine 167).

An AQUA peptide comprising the sequence, PVs*QPSLVGSK (s*= phosphoserine; sequence incorporating $^{14}\text{C}/^{15}\text{N}$ -labeled leucine (indicated by bold L), which corresponds to the serine 167 phosphorylation site in human WNK1 kinase (see Row 181 in Table 1 (SEQ ID NO: 180)), is constructed according to standard synthesis techniques using, *e.g.*, a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (see Merrifield, *supra.*) as further described below in Synthesis & MS/MS Signature. The WNK1 (ser167) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated WNK1 (ser167) in the sample, as further described below in Analysis & Quantification.

B. 53BP1 (serine 105).

An AQUA peptide comprising the sequence VADPVDSSNLDTCSIs*QVIEQLPQPNR (s*= phosphoserine; sequence incorporating $^{14}\text{C}/^{15}\text{N}$ -labeled leucine (indicated by bold L), which corresponds to the serine 105 phosphorylation site in human 53BP1 kinase (see Row 252 in Table 1 (SEQ ID NO: 251)), is constructed according to standard synthesis techniques using, *e.g.*, a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (see Merrifield, *supra.*) as further described below in Synthesis & MS/MS Signature. The 53BP1 (ser105) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated 53BP1 (ser105) in the sample, as further described below in Analysis & Quantification.

C. PSF2 (serine 182)

An AQUA peptide comprising the sequence, TNLQPLESTQs*QDF (y*= phosphoserine; sequence incorporating $^{14}\text{C}/^{15}\text{N}$ -labeled phenylalanine (indicated by bold F), which corresponds to the serine 182

- 71 -

phosphorylation site in human PSF2 DNA replication protein (see Row 108 in Table 1 (SEQ ID NO: 107)), is constructed according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (see Merrifield, *supra.*) as further
5 described below in Synthesis & MS/MS Signature. The PSF2 (ser182) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated PSF2 (ser182) in the sample, as further described below in Analysis & Quantification.

D. FOXJ2 (serine 161).

10 An AQUA peptide comprising the sequence, RHPPDDDLs*QDSPEQEASKSPR (s*= phosphoserine; sequence incorporating ¹⁴C/¹⁵N-labeled proline (indicated by bold P), which corresponds to the serine 161 phosphorylation site in human FOXJ2 protein (see Row 225 in Table 1 (SEQ ID NO: 224)), is constructed
15 according to standard synthesis techniques using, e.g., a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (see Merrifield, *supra.*) as further described below in Synthesis & MS/MS Signature. The FOXJ2 (ser161) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated FOXJ2 (ser161) in the sample, as further
20 described below in Analysis & Quantification.

Synthesis & MS/MS Spectra.

Fluorenylmethoxycarbonyl (Fmoc)-derivatized amino acid monomers may be obtained from AnaSpec (San Jose, CA). Fmoc-derivatized stable-isotope monomers containing one ¹⁵N and five to nine ¹³C atoms may be
25 obtained from Cambridge Isotope Laboratories (Andover, MA). Preloaded Wang resins may be obtained from Applied Biosystems. Synthesis scales may vary from 5 to 25 μmol. Amino acids are activated *in situ* with 1-H-benzotriazolium, 1-bis(dimethylamino) methylene]-hexafluorophosphate (1-),3-oxide:1-hydroxybenzotriazole hydrate and coupled at a 5-fold molar

- 72 -

excess over peptide. Each coupling cycle is followed by capping with acetic anhydride to avoid accumulation of one-residue deletion peptide by-products. After synthesis peptide-resins are treated with a standard scavenger-containing trifluoroacetic acid (TFA)-water cleavage solution, and the peptides are precipitated by addition to cold ether. Peptides (*i.e.* a desired AQUA peptide described in A-D above) are purified by reversed-phase C18 HPLC using standard TFA/acetonitrile gradients and characterized by matrix-assisted laser desorption ionization-time of flight (Biflex III, Bruker Daltonics, Billerica, MA) and ion-trap (ThermoFinnigan, LCQ DecaXP) MS.

MS/MS spectra for each AQUA peptide should exhibit a strong y -type ion peak as the most intense fragment ion that is suitable for use in an SRM monitoring/analysis. Reverse-phase microcapillary columns (0.1 Å~ 150–220 mm) are prepared according to standard methods. An Agilent 1100 liquid chromatograph may be used to develop and deliver a solvent gradient [0.4% acetic acid/0.005% heptafluorobutyric acid (HFBA)/7% methanol and 0.4% acetic acid/0.005% HFBA/65% methanol/35% acetonitrile] to the microcapillary column by means of a flow splitter. Samples are then directly loaded onto the microcapillary column by using a FAMOS inert capillary autosampler (LC Packings, San Francisco) after the flow split. Peptides are reconstituted in 6% acetic acid/0.01% TFA before injection.

Analysis & Quantification.

Target protein (*e.g.* a phosphorylated protein of A-D above) in a biological sample is quantified using a validated AQUA peptide (as described above). The IAP method is then applied to the complex mixture of peptides derived from proteolytic cleavage of crude cell extracts to which the AQUA peptides have been spiked in.

- 73 -

LC-SRM of the entire sample is then carried out. MS/MS may be performed by using a ThermoFinnigan (San Jose, CA) mass spectrometer (LCQ DecaXP ion trap or TSQ Quantum triple quadrupole). On the DecaXP, parent ions are isolated at 1.6 m/z width, the ion injection
5 time being limited to 150 ms per microscan, with two microscans per peptide averaged, and with an AGC setting of 1×10^8 ; on the Quantum, Q1 is kept at 0.4 and Q3 at 0.8 m/z with a scan time of 200 ms per peptide. On both instruments, analyte and internal standard are analyzed in alternation within a previously known reverse-phase retention window;
10 well-resolved pairs of internal standard and analyte are analyzed in separate retention segments to improve duty cycle. Data are processed by integrating the appropriate peaks in an extracted ion chromatogram (60.15 m/z from the fragment monitored) for the native and internal standard, followed by calculation of the ratio of peak areas multiplied by
15 the absolute amount of internal standard (*e.g.*, 500 fmol).

- 74 -

WHAT IS CLAIMED IS:

1. A method for obtaining a phosphorylation profile of protein Kinases that are phosphorylated in human leukemia signaling pathways, said method comprising the step of utilizing one or more isolated antibody that
5 specifically binds a protein Kinase selected from Column A, Rows 58-74, of Table 1 only when phosphorylated at the tyrosine listed in corresponding Column D, Rows 58-74, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 58-74, of Table 1 (SEQ ID NOs: 57-73), to detect the phosphorylation of
10 one or more of said protein Kinases, thereby obtaining a phosphorylation profile for said Kinases.
2. A method for detecting or quantifying a signaling protein that is threonine- or serine- phosphorylated in ATM and/or ATR kinase signaling pathways, said method comprising the step of utilizing one or more of the
15 following reagents to detect or quantify one or more ATM and/or ATR kinase signaling protein(s) selected from Column A of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D of Table 1:
 - (i) an isolated phosphorylation site-specific antibody that
20 specifically binds said protein only when phosphorylated at the threonine or serine listed in corresponding Column D of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-300), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine; and/or
 - 25 (ii) a heavy-isotope labeled peptide (AQUA peptide) for the quantification of said protein, said labeled peptide comprising the phosphorylation site peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-300).

- 75 -

3. The method of claim 2, wherein said protein is an DNA repair protein selected from Column A, Rows 90-100 of Table 1, and wherein

(i) said antibody specifically binds said DNA repair protein only when phosphorylated at the threonine or serine listed in corresponding

5 Column D, Rows 90-100, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 90-100, of Table 1 (SEQ ID NOs: 89-99), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 90-100, of Table 1

10 (SEQ ID NOs: 89-99), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 90-100, of Table 1.

4. The method of claim 2, wherein said protein is a Transcription protein selected from Column A, Rows 221-266, of Table 1, and wherein

(i) said antibody specifically binds said Transcription protein only when phosphorylated at the threonine or serine listed in corresponding

15 Column D, Rows 221-266, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), and

(ii) said labeled peptide comprises the phosphorylation site peptide

20 sequence listed in corresponding Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 221-266, of Table 1.

5. The method of claim 2, wherein said protein is a Kinase selected from Column A, Rows 149 and 166 -181, of Table 1, and wherein

25 (i) said antibody specifically binds said Kinase only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 149 and 166 -181, of Table 1, comprised within the

- 76 -

phosphorylation site sequence listed in corresponding Column E, Rows 149 and 166 -181 of Table 1 (SEQ ID NOs: 148 and 165 -180), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 149 and 166 -181, of
5 Table 1 (SEQ ID NOs: 148 and 165 -180), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 149 and 166 -181, of Table 1.

6. The method of claim 2, wherein said protein is a Phosphatase selected from Column A, Rows 157-161 and 182-192 of Table 1, and
10 wherein

(i) said antibody specifically binds said Phosphatase only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows
15 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), comprising the
20 phosphorylated threonine or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1.

7. The method of claim 2, wherein said protein is a G Protein/GTPase/Guanine Nucleotide Exchange Factor selected from Column A, Rows 119-135, of Table 1, and wherein

25 (i) said antibody specifically binds said G Protein/GTPase/Guanine Nucleotide Exchange Factor only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 119-135, of Table 1, comprised within the phosphorylation site sequence listed in

- 77 -

corresponding Column E, Rows 119-135, of Table 1 (SEQ ID NOs: 118-134), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 119-135, of Table 1
5 (SEQ ID NOs: 118-134), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 119-135, of Table 1.

8. The method of claim 2, wherein said protein is a DNA replication protein selected from Column A, Rows 101-114, of Table 1, and wherein

(i) said antibody specifically binds said DNA replication protein only
10 when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 101-114, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 101-114, of Table 1 (SEQ ID NOs: 100-113), and

(ii) said labeled peptide comprises the phosphorylation site peptide
15 sequence listed in corresponding Column E, Rows 101-114, of Table 1 (SEQ ID NOs: 100-113), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 101-114, of Table 1.

9. The method of claim 2, wherein said protein is a DNA binding protein selected from Column A, Rows 70-89, of Table 1, and wherein

(i) said antibody specifically binds said DNA binding protein only
20 when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), and

(ii) said labeled peptide comprises the phosphorylation site peptide
25 sequence listed in corresponding Column E, Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1.

- 78 -

10. The method of claim 2, wherein said protein is a Disease associated protein selected from Column A, Rows 56-69, of Table 1, and wherein

5 (i) said antibody specifically binds said Disease associated protein only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), and

10 (ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), comprising the phosphorylated serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1.

11. The method of claim 2, wherein said protein is an Adaptor/Scaffold protein selected from Column A, Rows 2-22, of Table 1, and wherein

15 (i) said antibody specifically binds said Adaptor/Scaffold protein only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 2-22, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), and

20 (ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), comprising the phosphorylated serine or threonine listed in corresponding Column D, Rows 2-22, of Table 1.

12. The method of claim 2, wherein said protein is a Cell cycle regulation protein selected from Column A, Rows 27-42, of Table 1, and wherein

(i) said antibody specifically binds said Cell cycle regulation protein only when phosphorylated at the threonine or serine listed in

- 79 -

corresponding Column D, Rows 27-42, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), and

- (ii) said labeled peptide comprises the phosphorylation site
5 sequence listed in corresponding Column E, Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), comprising the phosphorylated threonine or serine listed in corresponding Column D, Rows 27-42, of Table 1.

13. The method of claim 2, wherein said protein is a Protease selected from Column A, Rows 163-165, of Table 1, and wherein

- 10 (i) said antibody specifically binds said Protease only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), and

- 15 (ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), comprising the phosphorylated serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1.

14. The method of claim 2, wherein said protein is a Methyltransferase
20 selected from Column A, Rows 153-156, of Table 1, and wherein

- (i) said antibody specifically binds a Methyltransferase only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 153-156, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows 153-156, of Table 1
25 (SEQ ID NOs: 152-155), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 153-156, of Table 1

- 80 -

(SEQ ID NOs: 152-155), comprising the phosphorylated serine or threonine listed in corresponding Column D, Rows 153-156 of Table 1.

15. The method of claim 2, wherein said protein is a Ubiquitin conjugating protein selected from Column A, Rows 285-298, of Table 1,
5 and wherein

(i) said antibody specifically binds a Ubiquitin conjugating protein only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 285-298, of Table 1, comprised within the phosphorylation site sequence listed in corresponding Column E, Rows
10 285-298, of Table 1 (SEQ ID NOs: 284-297), and

(ii) said labeled peptide comprises the phosphorylation site peptide sequence listed in corresponding Column E, Rows 285-298, of Table 1 (SEQ ID NOs: 284-297), comprising the phosphorylated serine or threonine listed in corresponding Column D, Rows 285-298 of Table 1.

15 16. An isolated phosphorylation site-specific antibody that specifically binds a human ATM and/or ATR kinase signaling protein selected from Column A of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E of
20 Table 1 (SEQ ID NOs: 1-300), wherein said antibody does not bind said signaling protein when not phosphorylated at said threonine or serine.

17. An isolated phosphorylation site-specific antibody that specifically binds a human ATM and/or ATR kinase signaling protein selected from Column A of Table 1 only when not phosphorylated at the threonine or
25 serine listed in corresponding Column D of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E of Table 1 (SEQ ID NOs: 1-300), wherein said antibody does not bind said signaling protein when phosphorylated at said threonine or serine.

- 81 -

18. A heavy-isotope labeled peptide (AQUA peptide) for the quantification of a human ATM and/or ATR kinase signaling protein selected from Column A of Table 1, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E of
5 Table 1 (SEQ ID NOs: 1-300), comprising the phosphorylatable threonine or serine listed in corresponding Column D, Rows 2-301, of Table 1.

19. The labeled peptide of claim 18, wherein said phosphorylatable threonine or serine is phosphorylated.

20. The labeled peptide of claim 18, wherein said phosphorylatable
10 threonine or serine is not phosphorylated.

21. An immortalized cell line producing the antibody of claim 16 or 17.

22. The cell line of claim 21, wherein said immortalized cell line is a rabbit hybridoma or a mouse hybridoma.

23. The antibody of claim 16, wherein said antibody specifically binds a
15 DNA repair protein selected from Column A, Rows 90-100, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 90-100, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 90-100, of Table 1 (SEQ ID NOs: 89-99), wherein said antibody
20 does not bind said protein when not phosphorylated at said threonine or serine.

24. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a DNA repair protein selected from Column A, Rows 90-100, said labeled peptide
25 comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 90-100, of Table 1 (SEQ ID NOs: 89-99), which

- 82 -

sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 90-100, of Table 1.

25. The antibody of claim 16, wherein said antibody specifically binds a Transcription protein selected from Column A, Rows 221-266, of Table 1
5 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 221-266, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), wherein said antibody does not bind said protein when not phosphorylated at said threonine or
10 serine.

26. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Transcription protein selected from Column A, Rows 221-266, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding
15 Column E, Rows 221-266, of Table 1 (SEQ ID NOs: 220-265), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 221-266, of Table 1.

27. The antibody of claim 16, wherein said antibody specifically binds a Kinase selected from Column A, Rows 149 and 166 -181, of Table 1 only
20 when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 149 and 166 -181, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 149 and 166 -181, of Table 1 (SEQ ID NOs: 148 and 165 -180), wherein said antibody does not bind said protein when not phosphorylated
25 at said threonine or serine.

28. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Kinase selected

- 83 -

from Column A, Rows 149 and 166 -181, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 149 and 166 -181, of Table 1 (SEQ ID NOs: 148 and 165 -180), which sequence comprises the phosphorylatable threonine or serine listed
5 in corresponding Column D, Rows 149 and 166 -181, of Table 1.

29. The antibody of claim 16, wherein said antibody specifically binds a Phosphatase selected from Column A, Rows 157-161 and 182-192, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1,
10 comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

30. The heavy-isotope labeled peptide (AQUA peptide) of claim 18,
15 wherein said labeled peptide is for the quantification of a Phosphatase selected from Column A, Rows 157-161 and 182-192, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 157-161 and 182-192, of Table 1 (SEQ ID NOs: 156-160 and 181-191), which sequence comprises the phosphorylatable threonine
20 or serine listed in corresponding Column D, Rows 157-161 and 182-192, of Table 1.

31. The antibody of claim 16, wherein said antibody specifically binds a G Protein/GTPase/Guanine Nucleotide Exchange Factor from Column A, Rows 119-135, of Table 1 only when phosphorylated at the threonine or
25 serine listed in corresponding Column D, Rows 119-135, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 119-135 of Table 1 (SEQ ID NOs: 118-

- 84 -

134), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

32. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a G
5 Protein/GTPase/Guanine Nucleotide Exchange Factor selected from Column A, Rows 119-135, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 119-135, of Table 1 (SEQ ID NOs: 118-134), which sequence comprises the phosphorylatable threonine or serine listed in corresponding
10 Column D, Rows 119-135, of Table 1.

33. The antibody of claim 16, wherein said antibody specifically binds a DNA replication protein selected from Column A, Rows 101-114, of Table 1 only when phosphorylated at the threonine or serine listed in
15 corresponding Column D, Rows 101-114, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 101-114, of Table 1 (SEQ ID NOs: 100-113), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

34. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a ATM and/or ATR
20 kinase signaling protein that is a DNA replication protein selected from Column A, Rows 101-114, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 101-114, of Table 1 (SEQ ID NOs: 100-113), which sequence
25 comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 101-114, of Table 1.

- 85 -

35. The antibody of claim 16, wherein said antibody specifically binds a DNA binding protein selected from Column A, Rows 70-89, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

36. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a DNA binding protein selected from Column A, Rows 70-89, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 70-89, of Table 1 (SEQ ID NOs: 69-88), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 70-89, of Table 1.

37. The antibody of claim 16, wherein said antibody specifically binds a Disease associated protein selected from Column A, Rows 56-69, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

38. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Disease associated protein selected from Column A, Rows 56-69, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 56-69, of Table 1 (SEQ ID NOs: 55-68), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 56-69, of Table 1.

- 86 -

39. The antibody of claim 16, wherein said antibody specifically binds an Adaptor/Scaffold Protein selected from Column A, Rows 2-22, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 2-22, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E,
5 Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

40. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of an Adaptor/Scaffold
10 Protein selected from Column A, Rows 2-22, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 2-22, of Table 1 (SEQ ID NOs: 1-21), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 2-22, of Table 1.

15 41. The antibody of claim 16, wherein said antibody specifically binds a Cell cycle regulation protein selected from Column A, Rows 27-42, of Table 1 only when phosphorylated at the threonine or serine listed in corresponding Column D, Rows 27-42, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E,
20 Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), wherein said antibody does not bind said protein when not phosphorylated at said threonine or serine.

42. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Cell cycle regulation protein selected from Column A, Rows 27-42, said labeled
25 peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 27-42, of Table 1 (SEQ ID NOs: 26-41), which sequence comprises the phosphorylatable threonine or serine listed in corresponding Column D, Rows 27-42, of Table 1.

- 87 -

43. The antibody of claim 16, wherein said antibody specifically binds a Protease selected from Column A, Rows 163-165, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

44. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Protease selected from Column A, Rows 163-165, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 163-165, of Table 1 (SEQ ID NOs: 162-164), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 163-165, of Table 1.

45. The antibody of claim 16, wherein said antibody specifically binds a Methyltransferase selected from Column A, Rows 153-156, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 153-156, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 153-156, of Table 1 (SEQ ID NOs: 152-155), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

46. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Methyltransferase selected from Column A, Rows 153-156, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 153-156, of Table 1 (SEQ ID NOs: 152-155), which sequence

- 88 -

comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 153-156, of Table 1.

47. The antibody of claim 16, wherein said antibody specifically binds a Ubiquitin conjugating system protein selected from Column A, Rows 285-298, of Table 1 only when phosphorylated at the serine or threonine listed in corresponding Column D, Rows 285-298, of Table 1, comprised within the phosphorylatable peptide sequence listed in corresponding Column E, Rows 285-298, of Table 1 (SEQ ID NOs: 284-297), wherein said antibody does not bind said protein when not phosphorylated at said serine or threonine.

48. The heavy-isotope labeled peptide (AQUA peptide) of claim 18, wherein said labeled peptide is for the quantification of a Ubiquitin conjugating system protein selected from Column A, Rows 285-298, said labeled peptide comprising the phosphorylatable peptide sequence listed in corresponding Column E, Rows 285-298, of Table 1 (SEQ ID NOs: 284-297), which sequence comprises the phosphorylatable serine or threonine listed in corresponding Column D, Rows 285-298, of Table 1.

49. An immortalized cell line producing the antibody of any one of claims 23, 25, 27, 29, 32, 33, 35, 37, 39, 41, 43, 45 and 47.

50. The cell line of claim 49, wherein said immortalized cell line is a rabbit hybridoma or a mouse hybridoma.

51. The heavy-isotope labeled peptide of any one of claims 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46 and 48 wherein said phosphorylatable threonine or serine is phosphorylated.

- 89 -

52. The heavy-isotope labeled peptide of any one of claims 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44 and 46 and 48, wherein said phosphorylatable threonine or serine is not phosphorylated.

FIGURE 1

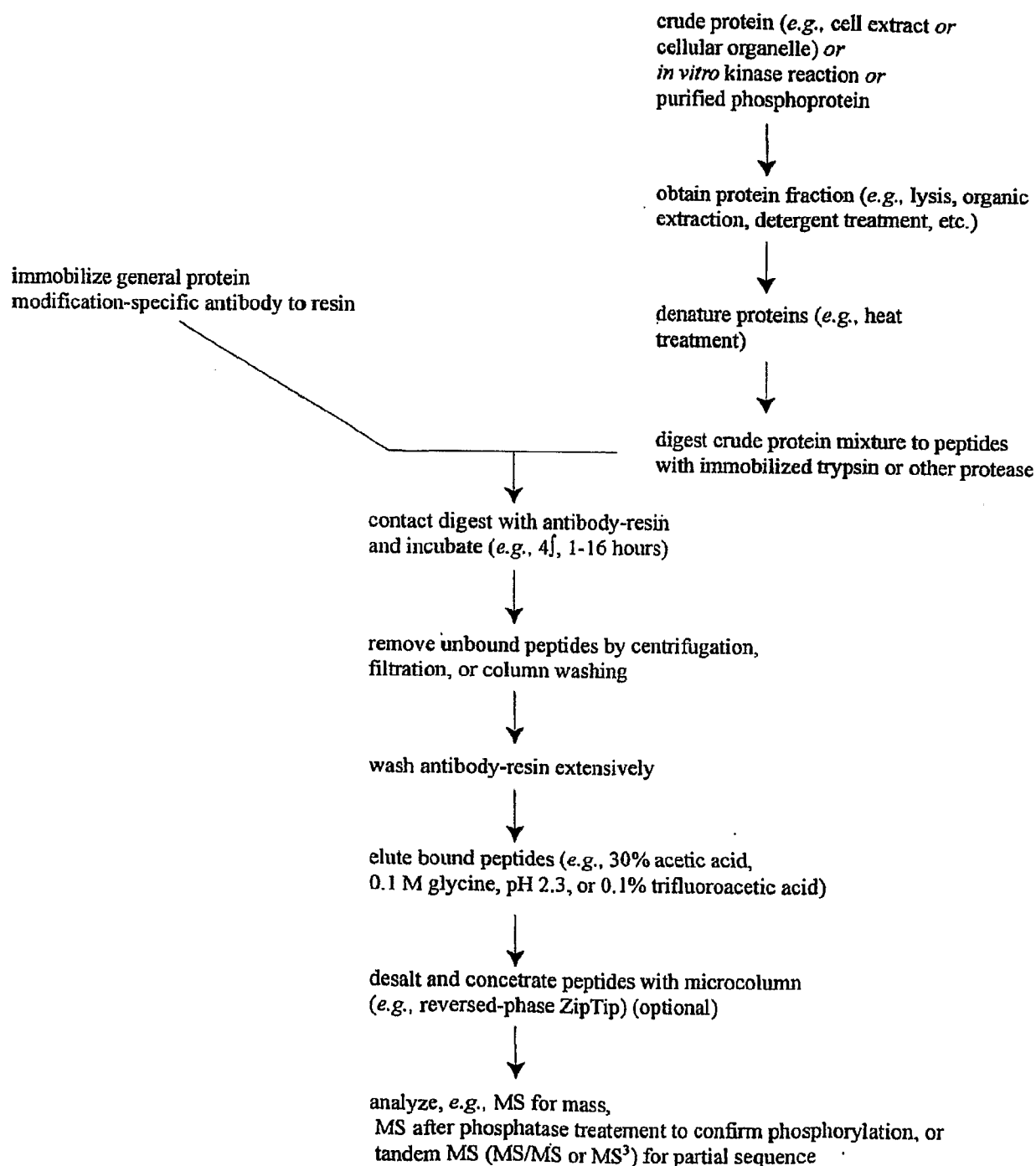


Figure 2

1

	A	B	C	D	E	F	G	H
1	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	Disease	Cell Line / Tissue / Patient	SEQ ID NO
2	AKAP12	NP_005891.2	Adaptor/scaffold	T1264	TEGIDEADQYADEK	glioblastoma	M059K	SEQ ID NO: 1
3	AKAP13	NP_006729.4	Adaptor/scaffold	T1112	RENISHNIQDILIPNVLSSQEK	transformed kidney	293T	SEQ ID NO: 2
4	AKAP13	NP_006729.4	Adaptor/scaffold	S1123	RENISHNTQDILIPNVLSSQEK	transformed kidney	293T	SEQ ID NO: 3
5	ATG16L1	NP_110430.5	Adaptor/scaffold	S269	RLSQAGGLDLSITMFGK	glioblastoma	M059K	SEQ ID NO: 4
6	Dok1	NP_001372.1	Adaptor/scaffold	S310	IAPCPsCDSLYSDPLDSTSQAQAGEGVQR	glioblastoma	M059K	SEQ ID NO: 5
7	MACF1	NP_149033.2	Adaptor/scaffold	S5511	SGGRKSLSQPTPPMPILSQSEAKNPR	glioblastoma	M059K+J	SEQ ID NO: 6
8	MACF1	NP_149033.2	Adaptor/scaffold	S5513	SGGRKSLSQPTPPMPILSQSEAKNPR	glioblastoma	M059K+J	SEQ ID NO: 7
9	MACF1	NP_149033.2	Adaptor/scaffold	S5506	SGGRKSLSQPTPPMPILSQSEAKNPR	glioblastoma	M059K+J	SEQ ID NO: 8
10	MACF1	NP_149033.2	Adaptor/scaffold	S5524	SGGRKSLSQPTPPMPILSQSEAKNPR	glioblastoma	M059K+J	SEQ ID NO: 9
11	NDIFP2	NP_061953.1	Adaptor/scaffold	S205	AAAMAAAETSSQR	glioblastoma	M059K	SEQ ID NO: 10
12	rabenosyn 5	NP_071735.2	Adaptor/scaffold	S217	ESLSTHTSPsQSPNSVHGSR	glioblastoma	M059K+J	SEQ ID NO: 11
13	RANBP3	NP_003615.1	Adaptor/scaffold	S188	ALsQTVPSSTNGVSLPADCTGAVPAASPTAAWR	glioblastoma	M059K	SEQ ID NO: 12
14	RANBP3	NP_003615.1	Adaptor/scaffold	S211	ALSQTVPSSTNGVSLPADCTGAVPAASPTAAWR	glioblastoma	M059K	SEQ ID NO: 13
15	RANBP3	NP_003615.1	Adaptor/scaffold	S125	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 14
16	RANBP3	NP_003615.1	Adaptor/scaffold	S139	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 15
17	RANBP3	NP_003615.1	Adaptor/scaffold	T124	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 16
18	RANBP3	NP_003615.1	Adaptor/scaffold	S126	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 17
19	RANBP3	NP_003615.1	Adaptor/scaffold	T128	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 18
20	RANBP3	NP_003615.1	Adaptor/scaffold	S135	TsSLTQFPSSQSEER	glioblastoma	M059K	SEQ ID NO: 19
21	SAKS1	NP_056937.2	Adaptor/scaffold	S202	YGGSVGSQPPVPAEPGPVPSPsQEPPTKR	glioblastoma	M059J, M059K	SEQ ID NO: 20
22	VANGL1	NP_020409.1	Adaptor/scaffold	S88	TEEVODDNWGETTATGTSEHSIQEDDIAR	glioblastoma	M059K	SEQ ID NO: 21
23	acinus	NP_055792.1	Apoptosis	S132	HSTPAHFQPNSSQIGEMSQNSFIK	glioblastoma	M059K	SEQ ID NO: 22
24	regulem	NP_006259.1	Apoptosis	S113	EGLeQDGSLEALLR	glioblastoma	M059K	SEQ ID NO: 23
25	UAGA	NP_060473.2	Apoptosis	T181	PIGQLLIDRAGDVNSRDK	glioblastoma	M059K	SEQ ID NO: 24
26	SDF4	NP_057260.2	Calcium-binding protein	T193	LNEELKVDDEQEVLENLKKR	glioblastoma	M059J, M059K+J	SEQ ID NO: 25
27	CENPF	NP_057427.3	Cell cycle regulation	S1255	GDLETSLNQDMQsQESIGLDCDEDAEEK	glioblastoma	M059K	SEQ ID NO: 26
28	CLASP1	NP_056097.1	Cell cycle regulation	S1196	FSFRsQEDLNPEIKR	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 27
29	CHAP1	NP_055680.2	Cell cycle regulation	T577	LLNLGLIFKGPAAISQENKPR	glioblastoma	M059K	SEQ ID NO: 28
30	cyclin C	NP_005181.2	Cell cycle regulation	S278	MPKPKPPPNSEGEQGPNGSQNSYSQS	glioblastoma, transformed kidney	293T, M059K	SEQ ID NO: 29
31	cyclin C	NP_005181.2	Cell cycle regulation	S279	MPKPKPPPNSEGEQGPNGSQNSYSQS	glioblastoma, transformed kidney	293T, M059K	SEQ ID NO: 30
32	cyclin C	NP_005181.2	Cell cycle regulation	S275	MPKPKPPPNSEGEQGPNGSQNSYSQS	glioblastoma	M059J, M059K	SEQ ID NO: 31
33	cyclin C	NP_005181.2	Cell cycle regulation	S281	MPKPKPPPNSEGEQGPNGSQNSYSQS	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 32
34	KI-67	NP_002408.3	Cell cycle regulation	S2925	AQPLDELASFQELSQTPGHTEELANGAADSFTSAPK	transformed kidney	293T	SEQ ID NO: 33
35	MDC1	NP_055456.1	Cell cycle regulation	T659	ENLTLVDVTDITLGEISQAPQR	glioblastoma	M059K+J	SEQ ID NO: 34
36	MDC1	NP_055456.1	Cell cycle regulation	S1086	QDGSQEAPEARLSSELEFPHKPKK	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 35
37	MDC1	NP_055456.1	Cell cycle regulation	S613	sQASTTVDINTQVEK	glioblastoma	M059K+J	SEQ ID NO: 36
38	NIPA	NP_057562.3	Cell cycle regulation	S472	QsSQPAETDSMSLSSEK	glioblastoma	M059K+J	SEQ ID NO: 37
39	NuMA-1	NP_006176.2	Cell cycle regulation	T1744	LPRIQPDGTSVPGEPAFSPISQR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 38
40	NuMA-1	NP_006176.2	Cell cycle regulation	S395	LSQLEELHsQLQDNPPQEK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 39
41	TPX2	NP_036244.2	Cell cycle regulation	S310	GCTIVKPFNLsQYK	glioblastoma	M059J	SEQ ID NO: 40
42	VCP	NP_009057.1	Cell cycle regulation	S784	FPSGNGGAGPssQSGGGGTGGSVYTEDNDDLYG	glioblastoma	M059K, M059K+J	SEQ ID NO: 41
43	CLIC1	NP_001279.2	Channel, chloride	S163	VLDNYLTSPLEPVEDTSQADEGVsQR	glioblastoma	M059K	SEQ ID NO: 42
44	SCN2A	NP_068287.2	Channel, sodium	S629	HSNVsQASRASR	glioblastoma	M059K+J	SEQ ID NO: 43
45	SCN2A	NP_068287.2	Channel, sodium	S632	HSNVsQASRASR	glioblastoma	M059K+J	SEQ ID NO: 44
46	SCN2A	NP_068287.2	Channel, sodium	S626	HSNVsQASRASR	glioblastoma	M059K+J	SEQ ID NO: 45
47	TBCB	NP_001272.2	Chaperone; Cytoskeletal protein	S110	VEKYTIsQEAVDQR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 46
48	ELMO2	NP_073403.1	Cytoskeletal protein	S517	QSERMsQDQFQSPPIVELR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 47
49	ezrin	NP_003370.2	Cytoskeletal protein	S639	QLTLTSELSQAR	glioblastoma	M059K	SEQ ID NO: 48
50	lamin A/C	NP_005563.1	Cytoskeletal protein	T394	LRLSFSPISQR	glioblastoma	M059J	SEQ ID NO: 49
51	lamin A/C	NP_005563.1	Cytoskeletal protein	S395	LRLSFSPISQR	glioblastoma	M059J, M059K	SEQ ID NO: 50
52	NEXN	NP_053174.2	Cytoskeletal protein	T193	NMVVDDDSPEMYKUSQELFTPGK	glioblastoma	M059K	SEQ ID NO: 51
53	NEXN	NP_053174.2	Cytoskeletal protein	S195	TIsQEFLLTPGK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 52
54	TMSB10	NP_066926.1	Cytoskeletal protein	T23	TEIQEKNTLPTKETIEQEK	glioblastoma	M059K+J	SEQ ID NO: 53
55	tubulin, alpha-1	NP_005991.1	Cytoskeletal protein; Motor protein	S232	LIsQVSSITASLR	glioblastoma	M059K	SEQ ID NO: 54
56	ataxin-3	NP_004984.2	Disease associated	S265	NIsQDMTQTSNTLTSEELR	glioblastoma	M059K	SEQ ID NO: 55
57	ataxin-3	NP_004984.2	Disease associated	T269	NIsQDMTQTSNTLTSEELR	glioblastoma	M059K	SEQ ID NO: 56
58	Bcl-9	NP_004317.2	Disease associated	S362	APPPPPVSSGEPPTLTGENPDGLsQDEQLEHR	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 57
59	BCL9L	NP_072363.1	Disease associated	S942	SPTLSQVHSPLVTSANLK	glioblastoma	M059K+J	SEQ ID NO: 58
60	BCL9L	NP_072363.1	Disease associated	S938	SPTLSQVHSPLVTSANLK	glioblastoma	M059K+J	SEQ ID NO: 59
61	BCL9L	NP_072363.1	Disease associated	T946	SPTLSQVHSPLVTSANLK	glioblastoma	M059K+J	SEQ ID NO: 60
62	BCL9L	NP_072363.1	Disease associated	S947	SPTLSQVHSPLVTSANLK	glioblastoma	M059K+J	SEQ ID NO: 61
63	MAGE-D2	NP_055414.2	Disease associated	S162	AQETEAAPsQAPADEPEPESAAQsQENQDTRPK	glioblastoma	M059K	SEQ ID NO: 62
64	NBEAL1	XP_001134432.1	Disease associated	S1299	RLsQISAGETTYNTQSK	glioblastoma	M059K	SEQ ID NO: 63
65	PAGE1	NP_003776.2	Disease associated	S144	GDGPDVQELGLPNPEEVKTPEDEEGQsQP	glioblastoma	M059K	SEQ ID NO: 64
66	PRCC	NP_005984.3	Disease associated	S287	QIQTEEDDsDEVAPENFSLPEK	glioblastoma	M059K	SEQ ID NO: 65
67	PRCC	NP_005984.3	Disease associated	T261	QIQTEEDDsDEVAPENFSLPEK	glioblastoma	M059K	SEQ ID NO: 66
68	SART2	NP_037484.1	Disease associated	S797	IFAsQQQQsQSK	glioblastoma	M059J	SEQ ID NO: 67
69	STEAP1	NP_036581.1	Disease associated	S337	INKTIsQsQL	glioblastoma	M059K	SEQ ID NO: 68
70	ARID1A	NP_006006.3	DNA binding protein	S604	FPPPELsQDSFGSQAASAPMTSSK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 69
71	ARID1B	NP_059989.1	DNA binding protein	S574	YQFQQDMsQEGYTR	glioblastoma	M059K	SEQ ID NO: 70
72	CBX3	NP_009207.2	DNA binding protein	S79	GFTDADNTWPEENLDCPELIEFLNSCK	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 71
73	NUCB1	NP_006175.2	DNA binding protein	S369	AQRIsQETEAQR	glioblastoma	M059J, M059K	SEQ ID NO: 72
74	RPA1	NP_002938.1	DNA binding protein	T180	AAGPSLSHTSGGQSK	glioblastoma	M059K, M059K+J	SEQ ID NO: 73

Figure 2

2

	A	B	C	D	E	F	G	H
1	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	Disease	Cell Line / Tissue / Patient	SEQ ID NO
75	UKP58	NP_079100.2	DNA binding protein	S450	LOIDPVMAETLQMeQDYDDMESVHADTR	transformed kidney	293T	SEQ ID NO: 74
76	UKP58	NP_079100.2	DNA binding protein	S390	TRISQELLAEVVGQQR	glioblastoma	M059K	SEQ ID NO: 75
77	WDH1	NP_009017.1	DNA binding protein	T826	AAELTAIQVEEVEEEDFRK	glioblastoma	M059K+J	SEQ ID NO: 76
78	ZAP	NP_064504.2	DNA binding protein	S327	ATDLGGTSAQGTQR	transformed kidney	293T	SEQ ID NO: 77
79	ZAP	NP_064504.2	DNA binding protein	S335	FLENGsQEDLLHGNGPGSTYLSNLTGAPNWK	glioblastoma	M059J, M059K	SEQ ID NO: 78
80	ZMYM4	NP_005086.2	DNA binding protein	S122	RVTQHSQDNENEIQNK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 79
81	ZMYM4	NP_005086.2	DNA binding protein	T118	RVIQHESDNENEIQNK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 80
82	ZNF262	NP_005086.2	DNA binding protein	T1070	KTLSQGESQISEHEFLDTK	glioblastoma	M059K	SEQ ID NO: 81
83	ZNF262	NP_005086.2	DNA binding protein	S1064	KTLSQGESQISEHEFLDTK	glioblastoma	M059K	SEQ ID NO: 82
84	ZNF262	NP_005086.2	DNA binding protein	S1068	KTLSQGESQISEHEFLDTK	glioblastoma	M059K	SEQ ID NO: 83
85	ZNF262	NP_005086.2	DNA binding protein	S1071	KTLSQGESQISEHEFLDTK	glioblastoma	M059K	SEQ ID NO: 84
86	ZNF262	NP_005086.2	DNA binding protein	S1249	SDPLGstQDHALSSESSEPGCR	glioblastoma, transformed kidney	293T, M059K	SEQ ID NO: 85
87	ZNF262	NP_005086.2	DNA binding protein	T1250	SDPLGstQDHALSSESSEPGCR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 86
88	ZNF262	NP_005086.2	DNA binding protein	S1256	SDPLGstQDHALSSESSEPGCR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 87
89	Nucleolin	NP_005372.2	DNA binding protein; Helicase; RNA binding	T405	NLPYKVKQDELKEVFEDAAEIR	glioblastoma	M059K	SEQ ID NO: 88
90	FANCA	NP_000126.2	DNA repair	S1449	QQAAPDADLSQEPHLF	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 89
91	FANCD2	NP_149075.2	DNA repair	T696	SESPSLQER	glioblastoma	M059J, M059K	SEQ ID NO: 90
92	MRE11A	NP_005581.2	DNA repair	S648	IMSQSQVSK	glioblastoma	M059J, M059K	SEQ ID NO: 91
93	MRE11A	NP_005581.2	DNA repair	S650	IMSQSQVSK	glioblastoma	M059K	SEQ ID NO: 92
94	NBS1	NP_002476.2	DNA repair	S615	MDIETNDTFSDEAVPESSKISQENEIGK	glioblastoma	M059J, M059K	SEQ ID NO: 93
95	NBS1	NP_002476.2	DNA repair	S397	MLsQDAPTVK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 94
96	NBS1	NP_002476.2	DNA repair	S68	NHAVLTANFVSNTLSQTDEIPVLTk	glioblastoma	M059K	SEQ ID NO: 95
97	PMS2	NP_003526.1	DNA repair	T597	LVNQDMSAQGVDAVK	glioblastoma	M059J, M059K	SEQ ID NO: 96
98	RAD23A	NP_005044.1	DNA repair	S357	NENLAANFLSsQNFDE	glioblastoma	M059J, M059K	SEQ ID NO: 97
99	R81	NP_060821.3	DNA repair	T1518	KADPENIKSEGQGGQIDVKSSEK	glioblastoma	M059J	SEQ ID NO: 98
100	TDP1	NP_060789.2	DNA repair	S553	FFAGSQEPMAFPVPYDLPPELYGSK	glioblastoma	M059J, M059K	SEQ ID NO: 99
101	BAZ1A	NP_038476.2	DNA replication	S286	IIHsQEDMANK	glioblastoma	M059J, M059K	SEQ ID NO: 100
102	CAF-1B	NP_005432.1	DNA replication	S409	GsSPGPRPVEGTASRTQDPSSPGTTPQAR	glioblastoma	M059K	SEQ ID NO: 101
103	CAF-1B	NP_005432.1	DNA replication	S410	GsSPGPRPVEGTASRTQDPSSPGTTPQAR	glioblastoma	M059K	SEQ ID NO: 102
104	CAF-1B	NP_005432.1	DNA replication	S428	GSSPGPRPVEGTASRTQDPsSPGTTTPQAR	glioblastoma	M059K	SEQ ID NO: 103
105	CAF-1B	NP_005432.1	DNA replication	T424	GSSPGPRPVEGTASRTQDPSSPGTTPQAR	glioblastoma	M059K	SEQ ID NO: 104
106	PSF2	NP_057179.1	DNA replication	T180	LRTNLQPLESsQSQDF	glioblastoma	M059K	SEQ ID NO: 105
107	PSF2	NP_057179.1	DNA replication	S179	TNLQPLEStQSQDF	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 106
108	PSF2	NP_057179.1	DNA replication	S182	TNLQPLEStQSQDF	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 107
109	RFC1	NP_002804.3	DNA replication	T193	RKELSQNIDESGLNDEIAK	glioblastoma	M059K	SEQ ID NO: 108
110	RFC1	NP_002804.3	DNA replication	S196	RKELSQNIDESGLNDEIAK	glioblastoma	M059K	SEQ ID NO: 109
111	Smc1	NP_006297.2	DNA replication	S951	GTMDISQEEGSSQGESVSsGSGQR	glioblastoma	M059K	SEQ ID NO: 110
112	TERF2IP	NP_061848.2	DNA replication	S206	YLLGDAPVSPSsQK	glioblastoma	M059K	SEQ ID NO: 111
113	TOPBP1	NP_008358.1	DNA replication	S405	DFAPSEKHEQADELLsQYENGSSSTVVEAK	glioblastoma	M059J	SEQ ID NO: 112
114	TOPBP1	NP_008358.1	DNA replication	T975	GVLTQTLsMR	glioblastoma	M059J	SEQ ID NO: 113
115	TOR1AIP2	NP_659471.1	Endoplasmic reticulum	S150	EASDGTGAsQEPPTTDSQEAQSPGHSSAGQEGEDTLR	transformed kidney	293T	SEQ ID NO: 114
116	TOR1AIP2	NP_659471.1	Endoplasmic reticulum	S158	EASDGTGAsQEPPTTDSQEAQSPGHSSAGQEGEDTLR	transformed kidney	293T	SEQ ID NO: 115
117	GAPDH	NP_002037.2	Enzyme, cellular metabolism	T184	VIHDFNGIVEGLMTTYVATAICK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 116
118	deoxyhypusine synthase	NP_001921.1	Enzyme, misc.	S78	KLEPLsQDEQDQADLTQSR	glioblastoma, transformed kidney	293T, M059K, M059K+J	SEQ ID NO: 117
119	CSN1	NP_004118.3	G protein regulator, misc.	T479	SPPREGSQGELIPANSQSR	glioblastoma	M059K	SEQ ID NO: 118
120	centaurin-delta-1	NP_058045.2	GTPase activating protein, ARF	S580	NDWISILNALKSQSLTsQDAVTPKEK	glioblastoma	M059K+J	SEQ ID NO: 119
121	centaurin-delta-1	NP_058045.2	GTPase activating protein, ARF	S582	NDWISILNALKSQSLTsQDAVTPKEK	glioblastoma	M059K+J	SEQ ID NO: 120
122	SIPA1L1	NP_056371.1	GTPase activating protein, misc.	T1199	SPASIBRONIQSDIGSGSK	glioblastoma	M059K+J	SEQ ID NO: 121
123	ARHGAP18	NP_277050.2	GTPase activating protein, Rac/Rho	S610	FLsQESGVAQTLK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 122
124	ARHGAP18	NP_277050.2	GTPase activating protein, Rac/Rho	S69	SISQSLsDELsMEDYWIENIKK	glioblastoma	M059K	SEQ ID NO: 123
125	ARHGAP18	NP_277050.2	GTPase activating protein, Rac/Rho	S66	SISQSLsDELsMEDYWIENIKK	glioblastoma	M059K	SEQ ID NO: 124
126	ARHGAP8	NP_038267.1	GTPase activating protein, Rac/Rho	S569	AGPCSLsQGNLSPNWR	glioblastoma	M059K	SEQ ID NO: 125
127	Cdc42EP1	NP_008992.3	GTPase activating protein, Rac/Rho	T34	RLADIMSHPLGDFR	glioblastoma	M059K	SEQ ID NO: 126
128	SIARD13	NP_443083.1	GTPase activating protein, Rac/Rho	S271	MHEFHSQENLVVHsPK	glioblastoma	M059K	SEQ ID NO: 127
129	ARHGEF2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	S147	ILSQsTDSLNMNR	glioblastoma	M059K	SEQ ID NO: 128
130	ARHGEF2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	S145	ILsQSTDSLNMNR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 129
131	ARHGEF2	NP_004714.2	Guanine nucleotide exchange factor, Rac/Rho	T148	RILSQSDSLNMNR	glioblastoma	M059K	SEQ ID NO: 130
132	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	T1627	DGVEDIDSQGGSSQPDTSIASR	glioblastoma	M059K	SEQ ID NO: 131
133	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	S1629	DGVEDIDSQGGSSQPDTSIASR	glioblastoma	M059K	SEQ ID NO: 132
134	HAPIP	NP_003938.1	Guanine nucleotide exchange factor, Rac/Rho	S1632	DGVEDIDSQGGSSQPDTSIASR	glioblastoma	M059K	SEQ ID NO: 133

Figure 2

3

1	A	B	C	D	E	F	G	H
	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	Disease	Cell Line / Tissue / Patient	SEQ ID NO
135	HAPIP	NP_003638.1	Guanine nucleotide exchange factor, Rac/Rho	S1623	DGVEDIDSGQDGSQPDTISIASR	glioblastoma	M059K	SEQ ID NO: 134
136	CHD-6	NP_115597.3	Helicase	S1714	KVLsQEPSSFQESPTNTESR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K-J	SEQ ID NO: 135
137	DDX11	NP_004390.3	Helicase	T465	QNPNIQSLSQGTGTELK	glioblastoma	M059J	SEQ ID NO: 136
138	DDX11	NP_004390.3	Helicase	S469	QNPNTQSLsQTGTTELK	glioblastoma	M059J	SEQ ID NO: 137
139	DDX18	NP_005764.3	Helicase	S74	QKPMNVGLSETQNGMSEAVGNIK	glioblastoma	M059K	SEQ ID NO: 138
140	DOX9	NP_001348.2	Helicase	S321	LAQFEPsQR	glioblastoma	M059J, M059K, M059K-J	SEQ ID NO: 139
141	DFFA	NP_998731.1	Hydrolase, esterase	S257	QAPELSLSsQDLEVGGNGQH	glioblastoma	M059K	SEQ ID NO: 140
142	DFFA	NP_004392.1	Hydrolase, esterase	S107	WAYNNSDGGTAWsQESFDVDETDSGAGLK	glioblastoma	M059J, M059K	SEQ ID NO: 141
143	AMPD2	NP_004028.3	Hydrolase, non-esterase	S168	QIsQDVKLEPDILLR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K-J	SEQ ID NO: 142
144	RENT1	NP_002902.2	Hydrolase, non-esterase	S1116	AYQHGGTGLsQY	glioblastoma	M059J, M059K-J	SEQ ID NO: 143
145	RENT1	NP_002902.2	Hydrolase, non-esterase	S1089	sQDVALSQDSTYQGER	glioblastoma	M059K	SEQ ID NO: 144
146	RENT1	NP_002902.2	Hydrolase, non-esterase	S1096	SQDVALsQDSTYQGER	glioblastoma, transformed kidney	293T, M059J, M059K, M059K-J	SEQ ID NO: 145
147	PPIE	NP_006103.1	Isomerase	T126	TLEENKEEGSEPPKAEIGEGEPIAK	glioblastoma	M059K	SEQ ID NO: 146
148	PPI1	NP_057143.1	Isomerase	S149	VGMVETNsQDRPVDDVK	glioblastoma	M059K	SEQ ID NO: 147
149	deoxycytidine kinase	NP_000779.1	Kinase (non-protein)	T64	WCNVQSIQDEFEELTMSQK	glioblastoma	M059K	SEQ ID NO: 148
150	ACSS2	NP_061147.1	Ligase	T264	AELGMGDSISQSPPIKR	glioblastoma	M059K	SEQ ID NO: 149
151	ACSS2	NP_061147.1	Ligase	S267	AELGMGDSISQSPPIKR	glioblastoma	M059K	SEQ ID NO: 150
152	ATP11C	NP_775965.2	Lipid binding protein	S445	GVTQEVQGLsQTDGILTYFDKVK	glioblastoma	M059K	SEQ ID NO: 151
153	BAI8	NP_006700.2	Methyltransferase	S569	GDGVTPAGTAAPPLsQDVPGR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K-J	SEQ ID NO: 152
154	COQ3	NP_058117.3	Methyltransferase	S88	LYTSQTTVDSGEVK	glioblastoma	M059K	SEQ ID NO: 153
155	COQ3	NP_058117.3	Methyltransferase	T90	LYTSQTTVDSGEVK	glioblastoma	M059K	SEQ ID NO: 154
156	COQ3	NP_058117.3	Methyltransferase	S88	LYTSQTTVDSGEVK	glioblastoma	M059K	SEQ ID NO: 155
157	Pnk1	NP_009185.2	Phosphatase (non-protein)	T118	TPESQDIPPGTPLVSDQDEKR	glioblastoma	M059J, M059K	SEQ ID NO: 156
158	Pnk1	NP_009185.2	Phosphatase (non-protein)	S114	TPESQDTPPGTPLVSDQDEKR	glioblastoma	M059J, M059K	SEQ ID NO: 157
159	Pnk1	NP_009185.2	Phosphatase (non-protein)	S126	TPESQDTPPGTPLVSDQDEKR	glioblastoma	M059J, M059K	SEQ ID NO: 158
160	PTP1B	NP_002818.1	PHOSPHATASE; Phosphatase; Protein phosphatase, tyrosine (non-receptor)	S363	GSPLNAPYGIESMSQDTEVR	transformed kidney	293T	SEQ ID NO: 159
161	PTP1B	NP_002818.1	PHOSPHATASE; Phosphatase; Protein phosphatase, tyrosine (non-receptor)	S365	GSPLNAPYGIESMSQDTEVR	transformed kidney	293T	SEQ ID NO: 160
162	PLCG1	NP_002651.2	Phospholipase	S1234	YQQPFEDFRIsQEHLDHFDSR	glioblastoma	M059J, M059K	SEQ ID NO: 161
163	ST14	NP_068813.1	Protease (non-)	S203	VVMLPPRARSLSKFVVTsVAFPTDSK	glioblastoma	M059K	SEQ ID NO: 162
164	PSMC1	NP_002793.2	Protease (proteasomal subunit)	S4	MGQsQSGGHGPGGGKK	glioblastoma	M059K, M059K-J	SEQ ID NO: 163
165	PSMD4	NP_002801.1	Protease (proteasomal subunit)	S266	MTIsQEFGR	glioblastoma	M059J, M059K, M059K-J	SEQ ID NO: 164
166	TIF1-beta	NP_005753.1	Protein kinase	S824	FSAVLVEPPPMsLPGAGLSsQELSGGPGDGP	glioblastoma	M059K	SEQ ID NO: 165
167	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S196	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 166
168	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S199	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 167
169	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S201	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 168
170	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	S200	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 169
171	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	T203	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 170
172	BRDT	NP_001717.2	Protein kinase, Ser/Thr (non-receptor)	T208	TSISPLNVQGVASVNSSQTAAQVTX	glioblastoma	M059K-J	SEQ ID NO: 171
173	NLK	NP_057315.2	Protein kinase, Ser/Thr (non-receptor)	T286	HMIQEVVTQYR	glioblastoma	M059J, M059K-J	SEQ ID NO: 172
174	RIPK2	NP_003812.1	Protein kinase, Ser/Thr (non-receptor)	S176	MMSLSQSR	glioblastoma	M059J, M059K-J	SEQ ID NO: 173
175	SMG1	NP_055907.3	Protein kinase, Ser/Thr (non-receptor)	T3550	SNTGQKIQDVMsQNAR	glioblastoma	M059J, M059K, M059K-J	SEQ ID NO: 174
176	SMG1	NP_055907.3	Protein kinase, Ser/Thr (non-receptor)	S3556	SNTGQKTQDVMsQNAR	glioblastoma	M059J, M059K, M059K-J	SEQ ID NO: 175
177	STK33	NP_112168.1	Protein kinase, Ser/Thr (non-receptor)	T491	TPVPSQGTATKYPAK	glioblastoma	M059K, M059K-J	SEQ ID NO: 176
178	STK33	NP_112168.1	Protein kinase, Ser/Thr (non-receptor)	T496	TPVTPSQGTATKYPAK	glioblastoma	M059K, M059K-J	SEQ ID NO: 177
179	STK33	NP_112168.1	Protein kinase, Ser/Thr (non-receptor)	S493	TPVTPsQGTATKYPAK	glioblastoma	M059K, M059K-J	SEQ ID NO: 178
180	VRK1	NP_003375.1	Protein kinase, Ser/Thr (non-receptor)	T378	EIEESKEPGVEDTEWSNIQTTEEAIQTR	glioblastoma	M059K, M059K-J	SEQ ID NO: 179
181	WNK1	NP_061852.1	Protein kinase, Ser/Thr (non-receptor)	S167	PVsQPSLVGSX	glioblastoma	M059K-J	SEQ ID NO: 180
182	PPP1R12C	NP_060077.1	Protein phosphatase, regulatory subunit	T560	RSIQVTLTDLK	glioblastoma	M059K, M059K-J	SEQ ID NO: 181
183	PPP1R7	NP_002703.1	Protein phosphatase, regulatory subunit	S12	GAGQQQsQEMMEVDR	glioblastoma	M059J, M059K, M059K-J	SEQ ID NO: 182
184	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non-receptor)	S183	SGGGTGEEPGsQGLNGEAGPEDSTRETPSQENGPTAK	glioblastoma	M059K	SEQ ID NO: 183
185	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non-receptor)	T199	SGGGTGEEPGsQGLNGEAGPEDSTRETPSQENGPTAK	glioblastoma	M059K	SEQ ID NO: 184
186	PPM1G	NP_002698.1	Protein phosphatase, Ser/Thr (non-receptor)	S201	SGGGTGEEPGsQGLNGEAGPEDSTRETPSQENGPTAK	glioblastoma	M059K	SEQ ID NO: 185

Figure 2

	A	B	C	D	E	F	G	H
1	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	Disease	Cell Line / Tissue / Patient	SEQ ID NO
187	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non-receptor)	S262	LSSGDPSTsPSLSQTTPSKDQDQSR	glioblastoma	M059K	SEQ ID NO: 188
188	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non-receptor)	S264	LSSGDPSTSPsLSQTTPSKDQDQSRK	glioblastoma	M059K	SEQ ID NO: 187
189	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non-receptor)	T268	LSSGDPSTSPSLsQTTPSKDQDQSRK	glioblastoma	M059J	SEQ ID NO: 188
190	EYA3	NP_001981.2	Protein phosphatase, tyrosine (non-receptor)	S266	LSSGDPSTSPSLsQTTPSKDQDQSRK	glioblastoma	M059K	SEQ ID NO: 189
191	PTPN14	NP_005392.2	Protein phosphatase, tyrosine (non-receptor)	S488	NLNINTHAYNQPEDLVYsQPEMR	glioblastoma	M059K, M059K+J	SEQ ID NO: 190
192	PTPN21	NP_006970.1	Protein phosphatase, tyrosine (non-receptor)	S492	NLNIGSSYAYSRAALVYsQPEIR	glioblastoma	M059K	SEQ ID NO: 191
193	VEGFR-2	NP_002244.1	Receptor tyrosine kinase	T1217	FHYDNAGISQYLQNSK	glioblastoma	M059K	SEQ ID NO: 192
194	OGFR	NP_031372.2	Receptor, misc.	S420	IALNLEGCLsQGSILR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 193
195	OGFR	NP_031372.2	Receptor, misc.	T325	KVEEEGSPGDPDHEASIQGR	glioblastoma	M059J	SEQ ID NO: 194
196	OGFR	NP_031372.2	Receptor, misc.	S315	KVEEEGSPGDPDHEASTQGR	glioblastoma	M059J	SEQ ID NO: 195
197	OGFR	NP_031372.2	Receptor, misc.	T426	IGTQEVGGQDPGEAVQPCR	glioblastoma	M059K	SEQ ID NO: 196
198	OGFR	NP_031372.2	Receptor, misc.	T428	TGQEVGGQDPGEAVQPCR	glioblastoma	M059K+J	SEQ ID NO: 197
199	PDGFR1	NP_006198.1	Receptor, misc.	S254	AEAGGRsQISVK	glioblastoma	M059J	SEQ ID NO: 198
200	HEAB	NP_006822.1	RNA binding protein	S344	VGAPITPDSCPLGMsQEDNQLK	glioblastoma	M059J, M059K	SEQ ID NO: 199
201	POP4	NP_006618.1	RNA binding protein	S10	SVIYHALsQK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 200
202	RALY	NP_031393.2	RNA binding protein	S279	TRDDGDEELGTHSEEEHsQDQDADGALQ	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 201
203	RBM16	NP_055707.3	RNA binding protein	T619	SSEPVKETVQTQSPSPVEK	glioblastoma	M059K+J	SEQ ID NO: 202
204	RBM16	NP_055707.3	RNA binding protein	T615	SSEPVKETVQTQSPSPVEK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 203
205	RBM34	NP_055829.1	RNA binding protein	S94	NEEEESTSQIERLsQEPK	glioblastoma	M059J, M059K	SEQ ID NO: 204
206	SFRS14	NP_055699.1	RNA binding protein	T7	RIQETFDVQLQEK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 205
207	SFRS7	BAC03661.1	RNA binding protein	S136	FRLSQsP	glioblastoma	M059J	SEQ ID NO: 206
208	SFRS7	BAC03661.1	RNA binding protein	S134	FRLsQSP	glioblastoma	M059J	SEQ ID NO: 207
209	SFRS8	NP_004583.2	RNA binding protein	T642	KPLIQEELQEK	glioblastoma	M059K	SEQ ID NO: 208
210	SRm300	NP_057417.3	RNA binding protein	T383	HGGSPQPLATPLSQEPVNPSPASPTR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 209
211	SRm300	NP_057417.3	RNA binding protein	S395	HGGSPQPLATPLSQEPVNPSPASPTR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 210
212	SSA2	NP_004591.2	RNA binding protein	S19	QIANsQDGYVWQVTDMNR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 211
213	UTP14A	NP_006640.2	RNA binding protein	S434	KRSELSDAEPAGSQETK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 212
214	UTP14A	NP_006640.2	RNA binding protein	S453	KRSELSDAEPAGSQETKDSQsQEVLSLR	glioblastoma	M059J, M059K	SEQ ID NO: 213
215	UTP14A	NP_006640.2	RNA binding protein	S14	LAESLLAsQEEELADLPK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 214
216	UTP14A	NP_006640.2	RNA binding protein	T448	RSELSDAEPAGSQEK	glioblastoma	M059K, M059K+J	SEQ ID NO: 215
217	UTP14A	NP_006640.2	RNA binding protein	S437	SELsQDAEPAGSQETK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 216
218	UTP14A	NP_006640.2	RNA binding protein	S445	SELSDAEPAGSQETK	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 217
219	TAF15	NP_003476.1	RNA binding protein; Translation initiation	S94	QSSYSQQPYNNQGGQNNMESSGsQGGK	glioblastoma	M059K, M059K+J	SEQ ID NO: 218
220	MFGE8	NP_005919.1	Secreted protein	S42	NPCHNGGLCEEIsQEVK	glioblastoma	M059J	SEQ ID NO: 219
221	CUTL1	NP_053530.1	Transcription factor	S734	QAPLsQSDITLTPK	glioblastoma	M059K	SEQ ID NO: 220
222	ELYS	NP_056281.3	Transcription factor	S1797	GLsQNGQIPQNSVTPR	glioblastoma	M059K	SEQ ID NO: 221
223	FBP3	NP_003925.1	Transcription factor	S559	QQVAFYQGTLLGQAQAHsQEQ	glioblastoma, transformed kidney	293T, M059K, M059K+J	SEQ ID NO: 222
224	FOXJ2	NP_060886.1	Transcription factor	S154	RHPPDDLLSQDsPEQEASKSPR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 223
225	FOXJ2	NP_060886.1	Transcription factor	S151	RHPPDDLLSQDsPEQEASKSPR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 224
226	FOXJ2	NP_060886.1	Transcription factor	S172	RHPPDDLLSQDsPEQEASKSPR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 225
227	MED1	NP_056689.1	Transcription factor	T449	QEKADIQDEWTPGTAVLTSPLVPGCPsK	glioblastoma	M059K	SEQ ID NO: 226
228	MCEP	NP_055238.1	Transcription factor	S836	TIsQSSSLK	glioblastoma	M059K	SEQ ID NO: 227
229	MYT1L	NP_055840.2	Transcription factor	S994	QKDGYNLNGSsQFWSKsVK	glioblastoma	M059K	SEQ ID NO: 228
230	MYT1L	NP_055840.2	Transcription factor	S997	QKDGYNLNGSsQFWSKsVK	glioblastoma	M059K	SEQ ID NO: 229
231	MYT1L	NP_055840.2	Transcription factor	S991	QKDGYNLNGSsQFWSKsVK	glioblastoma	M059K	SEQ ID NO: 230
232	RFX5	NP_000440.1	Transcription factor	S605	EHVLQSSLSsQEHKDPKATPP	glioblastoma	M059J	SEQ ID NO: 231
233	TCEAL3	NP_116315.1	Transcription factor	S65	REDEGEQGEQLEDEGsQEKQGR	glioblastoma	M059K	SEQ ID NO: 232
234	ZNF264	NP_003408.1	Transcription factor	S80	KEDLSQDTCPQDKGPKP	glioblastoma	M059K	SEQ ID NO: 233
235	ZNF281	NP_036614.1	Transcription factor	S395	GATSAEPGSSNHTNMGNLAVLSQNGTSSSR	glioblastoma	M059K	SEQ ID NO: 234
236	ZNF281	NP_036614.1	Transcription factor	S807	NLESSTGFGQIPsQELASQIDPQKDIEPR	glioblastoma	M059K	SEQ ID NO: 235
237	TAF1C	NP_005670.2	Transcription initiation	S856	ATRSQQHTPVLsSSQPLRKKPR	glioblastoma	M059K	SEQ ID NO: 236
238	TAF1C	NP_005670.2	Transcription initiation	S857	ATRSQQHTPVLsSSQPLRKKPR	glioblastoma	M059K	SEQ ID NO: 237
239	TAF1C	NP_005670.2	Transcription initiation	S848	ATRSQQHTPVLsSSQPLRKKPR	glioblastoma	M059K	SEQ ID NO: 238
240	TAF1C	NP_005670.2	Transcription initiation	S858	ATRSQQHTPVLsSSQPLRKKPR	glioblastoma	M059K	SEQ ID NO: 239
241	TFIIF	NP_004119.1	Transcription initiation complex	S142	LQIESSKPYRLsCQLDK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 240
242	SSRP1	NP_003137.1	Transcription initiation complex; Transcription, coactivator/corepressor	T170	FVYPPIQEDGVDPVEAFANQLVSK	glioblastoma	M059J, M059K	SEQ ID NO: 241
243	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T855	ADDPLRLDQELQQPQIEK	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 242
244	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S580	FVPAENDSILNPAQDGEVQLsQNDKDKTK	glioblastoma	M059J, M059K	SEQ ID NO: 243

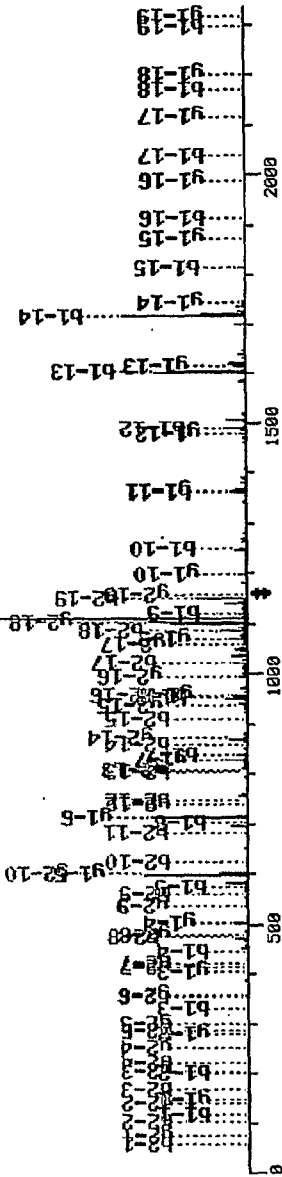
Figure 2

1	A	B	C	D	E	F	G	H
	Protein Name	Stable Accession No.	Protein Type	Stable Phospho-Residue	Phosphorylation Site Sequence	Disease	Cell Line / Tissue / Patient	SEQ ID NO
245	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S560	FVPAENDSILMNPADQGEVLSQNDKTK	glioblastoma	M059J	SEQ ID NO: 244
246	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T543	IDEDGEMQIEDTEPMSPVLNSK	glioblastoma	M059K+J	SEQ ID NO: 245
247	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T859	LDQELQPPQTQEKISNSLTEDSK	glioblastoma	M059J, M059K	SEQ ID NO: 246
248	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S860	LDQELQPPQTQEKISNSLTEDSK	glioblastoma	M059K	SEQ ID NO: 247
249	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S862	LDQELQPPQTQEKISNSLTEDSK	glioblastoma	M059J, M059K	SEQ ID NO: 248
250	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S1104	QSQQPMKPISPVKDPVPSAsQK	glioblastoma, transformed kidney	293T, M059J, M059K	SEQ ID NO: 249
251	53BP1	NP_005648.1	Transcription, coactivator/corepressor	T302	SPEPEVLSIQEDLFDQSNK	glioblastoma	M059J	SEQ ID NO: 250
252	53BP1	NP_005648.1	Transcription, coactivator/corepressor	S105	VADPVDSSNLDTCGSLQVIEQLCPNR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 251
253	HBXAP	NP_057042.3	Transcription, coactivator/corepressor	S524	DADSSISVLEHsQK	glioblastoma	M059K	SEQ ID NO: 252
254	ILF2	NP_004506.2	Transcription, coactivator/corepressor	T388	AYEKPEKKEGEEEEETEPPQEEEESEMEQE	glioblastoma	M059K	SEQ ID NO: 253
255	MYST2	NP_008998.1	Transcription, coactivator/corepressor	S52	SSARLSQSsQDSSPVR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 254
256	MYST2	NP_008998.1	Transcription, coactivator/corepressor	S66	SSARLSQSSQDsSPVR	glioblastoma	M059K	SEQ ID NO: 255
257	MYST2	NP_008998.1	Transcription, coactivator/corepressor	S57	SSARLSQSSQDsSPVR	glioblastoma	M059K, M059K+J	SEQ ID NO: 256
258	MYST2	NP_008998.1	Transcription, coactivator/corepressor	S50	SSARLSQSSQDSSPVR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 257
259	MYST2	NP_008998.1	Transcription, coactivator/corepressor	S53	SSARLSQSsQDSSPVR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 258
260	NCOA2	NP_008531.1	Transcription, coactivator/corepressor	S716	LTAETGKDLsQESSSTAPGSEVTKQEPVSPK	glioblastoma	M059K	SEQ ID NO: 259
261	RBBP5	NP_005048.2	Transcription, coactivator/corepressor	S525	VOAELsQPLTAGGASSELL	glioblastoma	M059K	SEQ ID NO: 260
262	SLTM	NP_001013865.1	Transcription, coactivator/corepressor	S955	EWHGPPsQGPSYHDTTR	glioblastoma	M059K+J	SEQ ID NO: 261
263	YAP1	NP_079031.2	Transcription, coactivator/corepressor	T973	SQLPTLEQDGGITNPVSPGMSQELR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 262
264	YAP1	NP_008097.1	Transcription, coactivator/corepressor	S321	SQLPTLEQDGGITNPVSPGMSQELR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 263
265	ZNF318	NP_055160.2	Transcription, coactivator/corepressor	S214	YISQEEGPLsPFLGQLDEYR	glioblastoma	M059J, M059K	SEQ ID NO: 264
266	ZNF318	NP_055160.2	Transcription, coactivator/corepressor	S207	YIsQEEGPLsPFLGQLDEYR	glioblastoma	M059J, M059K	SEQ ID NO: 265
267	AASDHPPT	NP_056238.2	Transferase	S258	HQDVPSQDDSKPTQR	glioblastoma	M059K	SEQ ID NO: 266
268	ATE1	NP_008972.2	Transferase	S169	KEEPCELLQsQDFVGEK	glioblastoma	M059J, M059K	SEQ ID NO: 267
269	TGM7	NP_443187.1	Transferase	T25	EHHQEMGVKRLTVR	glioblastoma	M059J	SEQ ID NO: 268
270	UGP2	NP_008750.3	Transferase	S13	AMsQDGAQsQFQEVIR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 269
271	atf2B	NP_003899.2	Translation initiation	T31	KPFMLDEEGDQTEETQPSSTK	glioblastoma	M059K+J	SEQ ID NO: 270
272	ATP13A1	NP_065143.1	Transporter, active	S817	DRLsQVLRLDEESTPVR	glioblastoma	M059K	SEQ ID NO: 271
273	NUP93	NP_055484.2	Transporter, active	T159	ILHTLLASGEDALDQSEPSYISDVGPGR	glioblastoma	M059J	SEQ ID NO: 272
274	NUP93	NP_055484.2	Transporter, active	S52	TLTRTsQETADVR	glioblastoma	M059K	SEQ ID NO: 273
275	SLC22A7	NP_666961.2	Transporter, active	T409	LQAGTLGLTALAFGTRLLVSSDMKsWSVTLAVMGK	glioblastoma	M059K	SEQ ID NO: 274
276	SLC22A7	NP_666961.2	Transporter, active	T405	LQAGTLGLTALAFGTRLLVSSDMKsWSVTLAVMGK	glioblastoma	M059K	SEQ ID NO: 275
277	SLC22A7	NP_666961.2	Transporter, active	T419	LQAGTLGLTALAFGTRLLVSSDMKsWSVTLAVMGK	glioblastoma	M059K	SEQ ID NO: 276
278	SLC25A4	NP_001142.2	Transporter, active; Transporter, facilitator	T84	YFPQALNFAFK	glioblastoma	M059J	SEQ ID NO: 277
279	NHE-1	NP_003038.2	Transporter, facilitator	S787	SKETSSPGTDVFTAPSDSPsQSR	glioblastoma	M059K	SEQ ID NO: 278
280	NHE-1	NP_003038.2	Transporter, facilitator	S788	SKETSSPGTDVFTAPSDSPsQSR	glioblastoma	M059K	SEQ ID NO: 279
281	NUP107	NP_085134.1	Transporter, facilitator	S37	VLLQAsQDENFGNTTPR	glioblastoma	M059K	SEQ ID NO: 280
282	TPR	NP_003283.2	Transporter, facilitator	S2034	AADsQNSGEGNTGAESSFSQEVSR	glioblastoma	M059K, M059K+J	SEQ ID NO: 281
283	TPR	NP_003283.2	Transporter, facilitator	S2050	AADsQNSGEGNTGAESSFSQEVSR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 282
284	SASH1	NP_056093.3	Tumor suppressor	S90	RVsQDLEVEKPDASPTSLQLR	glioblastoma	M059K	SEQ ID NO: 283
285	BRAP	NP_006759.2	Ubiquitin conjugating	T474	CIQLNTKVKLTINELK	glioblastoma	M059K	SEQ ID NO: 284
286	BRAP	NP_006759.2	Ubiquitin conjugating	T484	CTQLNTKVKLTINELK	glioblastoma	M059J	SEQ ID NO: 285
287	DCUN1D1	NP_085891.2	Ubiquitin conjugating	S31	TAVSCLsQNDWK	glioblastoma	M059K	SEQ ID NO: 286
288	HYD	NP_055986.2	Ubiquitin conjugating	S1549	RIsQSQPVR	glioblastoma	M059K	SEQ ID NO: 287
289	MUF1	NP_005360.3	Ubiquitin conjugating system	T327	RSIQESLTAGTDLKR	glioblastoma	M059J, M059K, M059K+J	SEQ ID NO: 288
290	NOT4	NP_037448.2	Ubiquitin conjugating system	S71	KYPEDPAVYKPLsQEELQR	glioblastoma, transformed kidney	293T, M059J, M059K, M059K+J	SEQ ID NO: 289
291	UBE1A	NP_005491.1	Ubiquitin conjugating	S185	VAKVsQGVEDGPDTRR	glioblastoma	M059K	SEQ ID NO: 290
292	UBE1	NP_11384.3	Ubiquitin conjugating	T485	IQRPNTQEGEEMETDMDGVCIPQR	glioblastoma	M059K	SEQ ID NO: 291
293	UBE1	NP_11384.3	Ubiquitin conjugating	S2372	sGEDESQEDVLMDEAPSNLSOASTLOANR	glioblastoma	M059K	SEQ ID NO: 292
294	UBE1	NP_11384.3	Ubiquitin conjugating	S2394	SGEDESQEDVLMDEAPSNLSOASTLOANR	glioblastoma	M059K	SEQ ID NO: 293
295	UBE1	NP_11384.3	Ubiquitin conjugating	S2377	SGEDESQEDVLMDEAPSNLSOASTLOANR	glioblastoma	M059K	SEQ ID NO: 294
296	UBE1	NP_11384.3	Ubiquitin conjugating	S2391	SGEDESQEDVLMDEAPSNLSOASTLOANR	glioblastoma	M059K	SEQ ID NO: 295
297	USP29	NP_055954.1	Ubiquitin conjugating	T17	IGMTKLKEALIEYVQRDK	glioblastoma	M059J	SEQ ID NO: 296
298	USP36	NP_079366.3	Ubiquitin conjugating	S1053	MSAVsQDAIEDSR	glioblastoma	M059K	SEQ ID NO: 297
299	COG1	NP_061184.1	Vesicle protein	T657	VKIQEIPQAK	glioblastoma	M059K	SEQ ID NO: 298
300	ICA1	NP_004959.2	Vesicle protein	S100	FLRsQGFQDK	glioblastoma	M059J, M059K+J	SEQ ID NO: 299
301	SNX25	NP_114159.2	Vesicle protein	S410	EEKHAsQNIJSNK	glioblastoma	M059K	SEQ ID NO: 300

FIGRUE 3.

LSQLEEHLpSQLQDNPPQEK

Seq #	b	y	(+1)
L 1	114.2	2314.4	19
S 2	201.2	2201.3	18
Q 3	329.4	2114.2	17
L 4	442.5	1986.1	16
E 5	571.7	1872.9	15
E 6	700.8	1743.8	14
H 7	837.9	1614.7	13
L 8	951.1	1477.5	12
S* 9	1118.1	1364.4	11
Q 10	1246.3	1197.3	10
L 11	1359.4	1069.2	9
Q 12	1487.6	956.0	8
D 13	1602.7	827.9	7
N 14	1716.8	712.8	6
P 15	1813.9	598.7	5
P 16	1911.0	501.6	4
Q 17	2029.1	404.4	3
E 18	2168.2	276.2	2
K 19	2296.4	147.2	1



FIGRUE 4.

HMpTQEVVTQYYR

Seq #	b	y	(+1)
H 1	138.1	1635.7	12
N 2	269.3	1498.6	11
T* 3	450.4	1367.4	10
Q 4	578.6	1186.3	9
E 5	707.7	1058.2	8
V 6	806.8	929.1	7
V 7	906.0	829.9	6
T 8	1007.1	730.8	5
Q 9	1135.2	629.7	4
Y 10	1298.4	501.6	3
Y 11	1461.5	338.4	2
R 12	1617.7	175.2	1

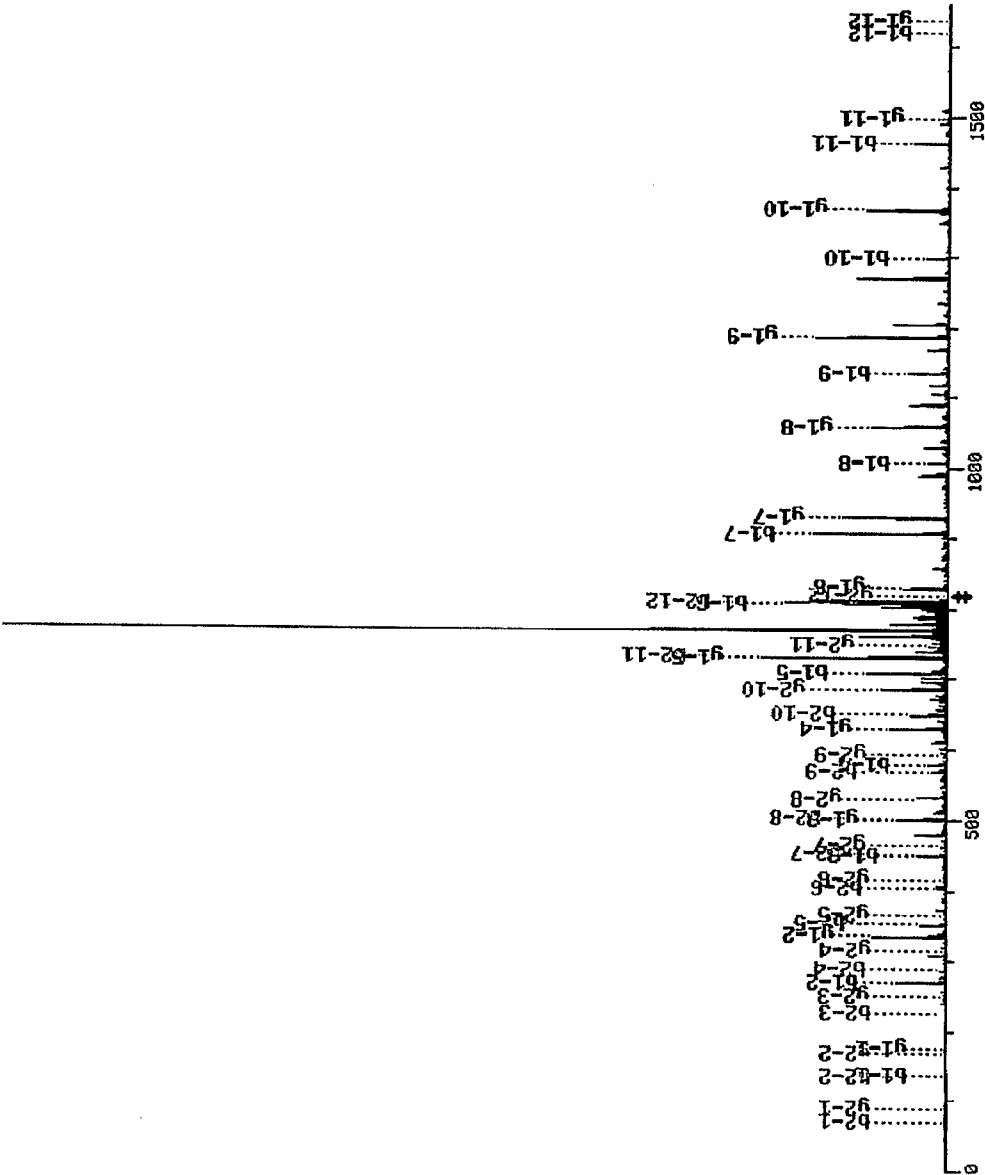
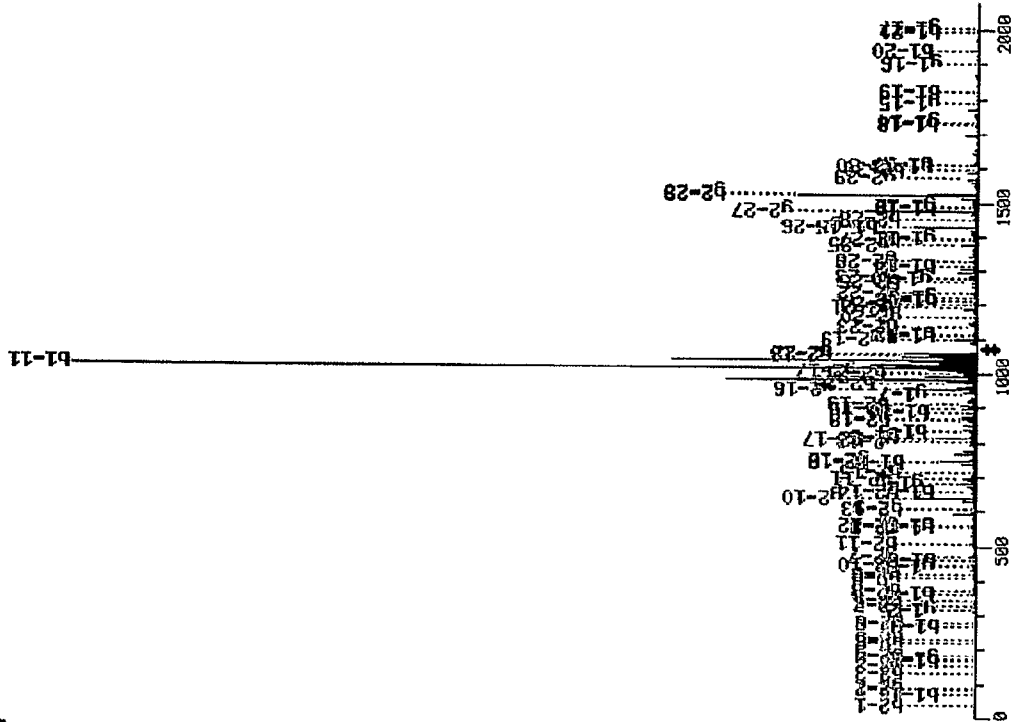


FIGURE 5.

APPPPPVSSGEPPTLGENPDGLpSQEQLEHR

Seq #	b	y	(+1)Seq #	b	y	(+2)
A 1	72.1	3214.4	30 A 1	36.5	1607.7	30
P 2	169.2	3143.3	29 P 2	85.1	1572.2	29
P 3	266.3	3046.2	28 P 3	133.7	1523.6	28
P 4	363.4	2949.1	27 P 4	182.2	1475.0	27
P 5	460.6	2852.0	26 P 5	230.8	1426.5	26
P 6	557.7	2754.8	25 P 6	279.3	1377.9	25
V 7	656.8	2657.7	24 V 7	328.9	1329.4	24
S 8	743.9	2558.6	23 S 8	372.4	1279.8	23
S 9	831.0	2471.5	22 S 9	416.0	1236.3	22
G 10	888.0	2384.4	21 G 10	444.5	1192.7	21
E 11	1017.1	2327.4	20 E 11	509.1	1164.2	20
P 12	1114.2	2198.3	19 P 12	557.6	1099.6	19
P 13	1211.4	2101.2	18 P 13	606.2	1051.1	18
T 14	1312.5	2004.0	17 T 14	656.7	1002.5	17
L 15	1425.6	1902.9	16 L 15	713.3	952.0	16
G 16	1482.7	1789.8	15 G 16	741.8	895.4	15
E 17	1611.8	1732.7	14 E 17	806.4	866.9	14
N 18	1725.9	1603.6	13 N 18	863.5	802.3	13
P 19	1823.0	1489.5	12 P 19	912.0	745.3	12
D 20	1938.1	1392.4	11 D 20	969.6	696.7	11
G 21	1995.2	1277.3	10 G 21	998.1	639.2	10
L 22	2108.3	1220.2	9 L 22	1054.7	610.6	9
S* 23	2275.4	1107.1	8 S* 23	1138.2	554.0	8
Q 24	2403.5	940.0	7 Q 24	1202.3	470.5	7
E 25	2532.6	811.9	6 E 25	1266.8	406.4	6
Q 26	2660.8	682.8	5 Q 26	1330.9	341.9	5
L 27	2773.9	554.6	4 L 27	1387.5	277.8	4
E 28	2903.0	441.5	3 E 28	1452.0	221.2	3
H 29	3040.2	312.4	2 H 29	1520.6	156.7	2
R 30	3196.4	175.2	1 R 30	1598.7	88.1	1



GTMDDDIpSQEEGSSQGEDSVSGSQR

FIGURE 6.

Seq #	b	y	(+1)Seq #	b	y	(+2)
G 1	58.1	2647.5	24 G 1	29.5	1224.2	24
T 2	159.2	2590.4	23 T 2	80.1	1295.7	22
M 3	290.4	2483.3	22 M 3	145.7	1245.2	22
D 4	405.4	2358.1	21 D 4	203.2	1179.6	21
D 5	520.5	2243.0	20 D 5	260.8	1122.0	20
I 6	633.7	2128.0	19 I 6	317.4	1064.5	19
S 7	720.8	2014.8	18 S 7	360.9	1007.9	18
Q 8	848.9	1927.7	17 Q 8	425.0	964.4	17
E 9	978.0	1799.6	16 E 9	489.5	900.3	16
E 10	1107.1	1670.5	15 E 10	554.1	835.7	15
G 11	1164.2	1541.4	14 G 11	582.6	771.2	14
S 12	1251.3	1484.3	13 S 12	626.1	742.7	13
S* 13	1418.3	1397.2	12 S* 13	709.7	699.1	12
Q 14	1546.5	1230.1	11 Q 14	773.7	615.6	11
G 15	1603.5	1102.0	10 G 15	802.3	551.5	10
E 16	1732.6	1045.0	9 E 16	866.8	523.0	9
D 17	1847.7	915.8	8 D 17	924.4	458.4	8
S 18	1934.8	800.8	7 S 18	967.9	400.9	7
V 19	2033.9	713.7	6 V 19	1017.5	357.3	6
S 20	2121.0	614.5	5 S 20	1061.0	307.8	5
G 21	2178.1	527.5	4 G 21	1089.5	264.2	4
S* 22	2345.1	470.4	3 S* 22	1173.1	225.7	3
Q 23	2473.3	303.3	2 Q 23	1237.1	152.2	2
R 24	2629.5	175.2	1 R 24	1315.2	88.1	1

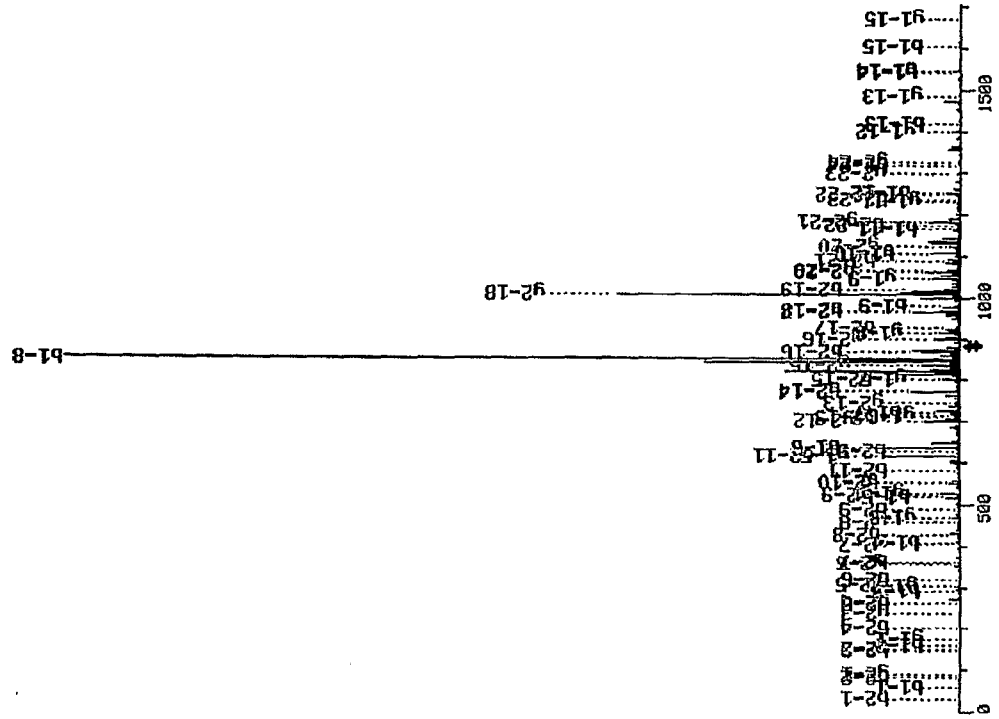


FIGURE 7.
RHPPDDDLpSQDpSPEQEASKpSPR

Seq #	b	y	(+1)seq #	b	y	(+2)
R 1	157.2	2732.6	22 R 1	79.1	1366.8	22
R 2	294.3	2576.4	21 R 2	147.7	1288.7	21
P 3	391.5	2429.2	20 P 3	196.2	1220.1	20
P 4	488.6	2342.1	19 P 4	244.8	1171.6	19
D 5	603.7	2245.0	18 D 5	302.3	1123.0	18
D 6	718.7	2129.9	17 D 6	359.9	1065.5	17
D 7	833.8	2014.8	16 D 7	417.4	1007.9	16
L 8	947.0	1899.7	15 L 8	474.0	950.4	15
S* 9	1114.1	1786.6	14 S* 9	557.5	893.8	14
Q 10	1242.2	1619.5	13 Q 10	621.6	810.3	13
D 11	1357.3	1491.4	12 D 11	679.2	746.2	12
S* 12	1524.4	1376.3	11 S* 12	762.7	688.7	11
P 13	1621.5	1209.2	10 P 13	811.2	605.1	10
E 14	1750.6	1112.1	9 E 14	875.8	556.6	9
Q 15	1878.7	983.0	8 Q 15	939.9	492.0	8
E 16	2007.8	854.9	7 E 16	1004.4	427.9	7
A 17	2078.9	725.7	6 A 17	1040.0	363.4	6
S 18	2166.0	654.7	5 S 18	1083.5	327.8	5
K 19	2254.2	567.6	4 K 19	1147.6	284.3	4
S* 20	2461.3	439.4	3 S* 20	1231.1	220.2	3
P 21	2558.4	272.3	2 P 21	1279.7	126.7	2
R 22	2714.6	175.2	1 R 22	1357.8	88.1	1

