



(86) **Date de dépôt PCT/PCT Filing Date:** 2014/11/12
(87) **Date publication PCT/PCT Publication Date:** 2015/05/21
(85) **Entrée phase nationale/National Entry:** 2016/04/21
(86) **N° demande PCT/PCT Application No.:** US 2014/065173
(87) **N° publication PCT/PCT Publication No.:** 2015/073509
(30) **Priorités/Priorities:** 2013/11/12 (US61/902,877);
2014/03/17 (US61/954,046)

(51) **Cl.Int./Int.Cl.** **C40B 30/08** (2006.01),
C12N 9/12 (2006.01), **C40B 20/00** (2006.01)
(71) **Demandeur/Applicant:**
DANA-FARBER CANCER INSTITUTE, INC., US
(72) **Inventeurs/Inventors:**
ZHAO, JEAN, US;
WANG, YUBAO, US
(74) **Agent:** RICHES, MCKENZIE & HERBERT LLP

(54) **Titre : BIOMARQUEUR DE L'ACTIVITE DE MELK ET SES PROCEDES D'UTILISATION**
(54) **Title: BIOMARKER FOR MELK ACTIVITY AND METHODS OF USING SAME**

(57) **Abrégé/Abstract:**

The methods of the present invention, relate to the surprising determination that the level of phosphorylation of position 406 (e.g., a serine residue.) of human eukaryotic initiation factor 4B (eIF4B), or a corresponding phosphorylatable amino acid of an ortholog thereof, serves as a biomarker for MELK enzymatic (e.g., kinase) and/or oncogenic activity. The methods of the present invention further relate to the surprising determination that the level of phosphorylation of position 3 (e.g., a threonine residue) and/or position 10 (e.g., a serine residue) and/or position 11 (e.g., a threonine residue) of human Histone H3, or a corresponding phosphorylatable amino acid of an ortholog thereof, also serves as a biomarker for MELK enzymatic (e.g., kinase) and/or oncogenic activity.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 May 2015 (21.05.2015)

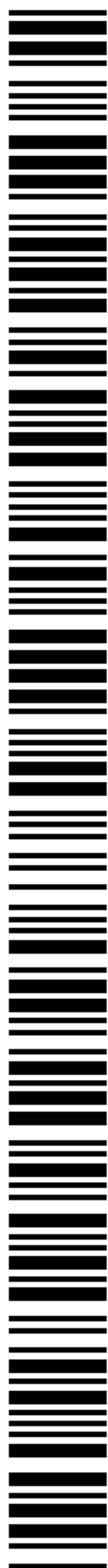
(10) International Publication Number
WO 2015/073509 A3

- (51) **International Patent Classification:**
C40B 30/08 (2006.01) *C12N 9/12* (2006.01)
C40B 20/00 (2006.01)
- (21) **International Application Number:**
PCT/US2014/065173
- (22) **International Filing Date:**
12 November 2014 (12.11.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/902,877 12 November 2013 (12.11.2013) US
61/954,048 17 March 2014 (17.03.2014) US
- (71) **Applicant:** DANA-FARBER CANCER INSTITUTE, INC. [US/US]; 450 Brookline Avenue, Boston, MA 02215-5450 (US).
- (72) **Inventors:** ZHAO, Jean; 9 Bradford Terrace, #6, Brookline, MA 02446 (US). WANG, Yubao; 268 Grove Street, Apt. 4, Newton, MA 02466 (US).
- (74) **Agents:** SMITH, DeAnn F. et al.; Foley Hoag LLP, Seaport West, 155 Seaport Blvd, Boston, MA 02210-2600 (US).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM,

- AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
- Published:**
- *with international search report (Art. 21(3))*
 - *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- (88) **Date of publication of the international search report:**
14 January 2016

(54) **Title:** BIOMARKER FOR MELK ACTIVITY AND METHODS OF USING SAME

(57) **Abstract:** The methods of the present invention, relate to the surprising determination that the level of phosphorylation of position 406 (e.g., a serine residue.) of human eukaryotic initiation factor 4B (cIF4B), or a corresponding phosphorylatable amino acid of an ortholog thereof, serves as a biomarker for MELK enzymatic (e.g., kinase) and/or oncogenic activity. The methods of the present invention further relate to the surprising determination that the level of phosphorylation of position 3 (e.g., a threonine residue) and/or position 10 (e.g., a serine residue) and/or position 11 (e.g., a threonine residue) of human Histone H3, or a corresponding phosphorylatable amino acid of an ortholog thereof, also serves as a biomarker for MELK enzymatic (e.g., kinase) and/or oncogenic activity.



WO 2015/073509 A3

BIOMARKER FOR MELK ACTIVITY AND METHODS OF USING SAME

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Application Nos. 61/954,046,
5 filed on 17 March 2014, and 61/902,877, filed on 12 November 2013; the entire contents of
each of said applications is incorporated herein in its entirety by this reference.

Background of the Invention

The protein kinase, maternal embryonic leucine zipper kinase (MELK), is known to
10 be involved in regulating cell cycle progression, cellular proliferation, apoptosis, and
mRNA splicing (Badouel *et al.* (2006) *Cell Cycle* 5:883-889 and Badouel *et al.* (2010) *Exp.*
Cell Res. 316:2166-2173). MELK has also been identified using gene expression profile
analyses to be associated with a number of cancers, including breast, lung, bladder,
lymphoma, and cervical cancer cells and mammary tumor formation in animal models
15 (Komatsu *et al.* (2013) *Int. J. Oncol.* 42:478-506; Pickard *et al.* (2009) *Breast Cancer Res.*
11:R60; Hebbard *et al.* (2010) *Cancer Res.* 70:8863-8873; Lin *et al.* (2007) *Breast Cancer*
Res. 9:R17; WO 2004/031413; WO 2007/7013665; and WO 2006/085684). Despite this
association, however, functional analyses of MELK-mediated oncogenesis have not been
performed to date and the mechanisms of MELK-mediated oncogenesis and, by extension,
20 assays for determining agents useful in regulating such oncogenesis, are not known. This
lack of understanding has prevented the identification of biomarkers that reliably report
MELK enzymatic and/or oncogenic activity. While certain MELK-targeting inhibitors of
kinase activity are known (see, for example, Chung *et al.* (2012) *Oncotarget* 3:1629-1640),
there is a clear need in the art to identify biomarkers of MELK-mediated ontogenesis in
25 order to provide rapid and effective means for evaluating MELK-targeted anti-cancer
therapies.

Summary of the Invention

The present invention is based, at least in part, on the discovery that the level of
30 phosphorylation of position 406 (*e.g.*, a serine residue) of human eukaryotic initiation factor
4B (eIF4B) is a reliable biomarker for maternal embryonic leucine zipper kinase (MELK)
activity suitable for use in measuring MELK enzymatic activity for preclinical and clinical
applications. Similarly, the present invention is based, at least in part, on the discovery that

the level of phosphorylation of position 3 (e.g., a threonine residue) and/or position 10 (e.g., a serine residue) of human Histone H3 and/or position 11 (e.g., a threonine residue) is also a reliable biomarker for MELK activity suitable for use in measuring MELK enzymatic activity or preclinical and clinical applications.

5 In one aspect, a method of identifying an agent which inhibits kinase or oncogenic activity of human maternal embryonic leucine zipper kinase (MELK) or an ortholog thereof, comprising a) contacting a sample comprising i) human MELK or an ortholog thereof and ii) human eukaryotic initiation factor 4B (eIF4B) or an ortholog thereof, with the agent; and b) determining the ability of the agent to inhibit Ser-406 phosphorylation of
10 human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic activity of human MELK or the ortholog thereof, is provided. In one embodiment, the inhibition of said Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B is determined
15 by comparing the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, in the sample relative to a control. In another embodiment, the control is the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B in the sample relative to said amount in the absence of the agent or at an earlier
20 timepoint after contact of the sample with the agent. In still another embodiment, the inhibition of said Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B is determined by comparing the ratio of the amount of Ser-406 phosphorylated human eIF4B, or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, in the sample relative to the
25 total amount of human eIF4B or ortholog thereof, to a control. In yet another embodiment, the control is the ratio of the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B in the sample relative to said ratio in the absence of the agent or at an earlier timepoint after contact of the sample with the agent. In another embodiment, the method further comprises determining
30 the amount of a protein translated from an RNA with highly structured 5'UTR, optionally wherein the protein is selected from the group consisting of cellular myelocytomatosis oncogene (c-Myc), X-linked inhibitor of apoptosis protein (XIAP), and ornithine decarboxylase (ODC1). In still another embodiment, the method further comprises a step

of determining whether the agent directly binds said human eIF4B or said ortholog thereof, or said human MELK or said ortholog thereof. In yet another embodiment, the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples. In another embodiment, the sample comprises cells (e.g., cancer cells, such as a cancer selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases). In still another embodiment, the cells are obtained from a subject. In yet another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In another embodiment, the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B (e.g., a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay). In still another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B. In yet another embodiment, said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In still another embodiment, the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%.

In another aspect, a method for assessing the efficacy of an agent for inhibiting kinase activity of human MELK or an ortholog thereof in a subject, comprising a) detecting in a subject sample at a first point in time, the amount of Ser-406 phosphorylated human eIF4B or the amount of a human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B; b) repeating step a) during at one or more subsequent points in time after administration of the agent; and c) comparing the amount of phosphorylated human

eIF4B or ortholog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Ser-406 phosphorylated human eIF4B or the amount of the human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B in the first point in time relative to at least one subsequent point in time, indicates that the agent

5 inhibits kinase activity of human MELK or the ortholog thereof, is provided. In one embodiment, the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B (*e.g.*, a

10 radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay). In another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding

15 ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B. In still another embodiment, the sample is selected from the group consisting of *ex vivo* and *in vivo* samples. In yet another embodiment, the sample comprises cancer cells (*e.g.*, cancer cells selected from the group consisting of any cancer

20 in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases). In another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In still another embodiment, the sample in step a) and/or step b) is a

25 portion of a single sample obtained from the subject. In yet another embodiment, the sample in step a) and/or step b) is a portion of pooled samples obtained from the subject. In another embodiment, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer between the first point in time and the subsequent point in time. In still another embodiment, said human eIF4B or ortholog

30 thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In yet another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In another embodiment, the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a

corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%.

In still another aspect, a method of treating a subject afflicted with cancer comprising administering to the subject an agent that inhibits Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B, thereby treating the subject afflicted with the cancer, is provided. In one embodiment, the agent is administered in a pharmaceutically acceptable formulation. In another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In still another embodiment, the agent directly binds said human eIF4B or the ortholog thereof, or said human MELK or the ortholog thereof. In yet another embodiment, the cancer is selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases. In another embodiment, said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In still another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In yet another embodiment, the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%. In another embodiment, the method further comprises administering one or more additional anti-cancer agents.

In yet another aspect, a method of determining the function or activity of human MELK or an ortholog, comprising a) detecting in a sample the amount of Ser-406 phosphorylated human eIF4B or the amount of a human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B; b) repeating step a) in the same sample or a test sample at one or more subsequent points in time after manipulation of the sample and/or manipulation of the same sample or test sample; and c) comparing the amount of phosphorylated human eIF4B or ortholog thereof detected in step a) with said amount detected in step b), wherein a modulated of Ser-406 phosphorylated human eIF4B or the amount of the human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B in the first point in time relative to at least one subsequent point in time and/or at least one subsequent manipulation of the same sample or test sample, indicates that the function or activity of human MEL or an ortholog thereof is modulated, is provided. In one embodiment, the amount of Ser-406 phosphorylated human eIF4B or a

corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B (*e.g.*, a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay). In another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B. In still another embodiment, the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples. In yet another embodiment, the sample comprises cells or the method uses a cell-based assay. In another embodiment, the cells are cancer cells selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases. In still another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In yet another embodiment, the same sample or test sample in step a) and/or step b) is a portion of a single sample obtained from a subject. In another embodiment, the same sample or test sample in step a) and/or step b) is a portion of pooled samples obtained from a subject. In still another embodiment, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer between the first point in time and the subsequent point in time. In yet another embodiment, said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In another embodiment, the manipulation of the sample is selected from the group consisting of contacting the sample with a test agent, contacting the sample with an upstream signal of the MELK signaling pathway, and contacting the sample with a MELK inhibitor. In still another embodiment, the test agent is a small molecule, or an antibody or antigen-binding fragment thereof. In yet another embodiment, the test agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%.

In another aspect, a method of identifying an agent which inhibits kinase or oncogenic activity of human MELK or an ortholog thereof, comprising: a) contacting a sample comprising i) human MELK or an ortholog thereof and ii) human Histone H3 or an ortholog thereof, with the agent; and b) determining the ability of the agent to inhibit Thr-3 phosphorylation of human Histone H3 or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3; and/or Ser-10 phosphorylation of human Histone H3 or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3; and/or Thr-11 phosphorylation of human Histone H3 or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic activity of human MELK or the ortholog thereof is provided. In one embodiment, the inhibition of said Thr-3 phosphorylation and/or Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, is determined by comparing the amount of Thr-3 phosphorylated human Histone H3 and/or Ser-10 phosphorylated human Histone H3 and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, in the sample relative to a control. In another embodiment, the control is the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, in the sample relative to said amount in the absence of the agent or at an earlier timepoint after contact of the sample with the agent. In still another embodiment, the inhibition of said Thr-3 phosphorylation and/or Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, is determined by comparing the ratio of the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, in the sample relative to the total amount of human Histone H3 or ortholog thereof, to a control. In yet another embodiment, the control is the ratio of the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3 in the sample relative to said ratio in the absence of the agent or at an earlier timepoint after contact of the sample with the agent. In another embodiment, the method further comprises determining the amount of a mitosis-specific protein. In still another embodiment, the

method further comprises a step of determining whether the agent directly binds said human Histone H3 or said ortholog thereof, or said human MELK or said ortholog thereof. In yet another embodiment, the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples. In another embodiment, the sample comprises cells, such as

5 cancer cells (*e.g.*, cells from a cancer selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases). In still another embodiment, the cells are obtained from a subject. In yet another embodiment, the sample is selected from the group consisting of

10 tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In another embodiment, the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent which specifically binds with Thr-3

15 phosphorylated or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3. In still another embodiment, the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot

20 assay. In yet another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding

25 phosphorylated ortholog of human Histone H3. In another embodiment, the human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In still another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In yet another embodiment, the agent decreases the amount of Thr-3 phosphorylated and/or Ser-

30 10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

In still another aspect, a method for assessing the efficacy of an agent for inhibiting kinase activity of human MELK or an ortholog thereof in a subject, comprising: a)

detecting in a subject sample at a first point in time, the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of a human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3; b) repeating step a) during at one or more subsequent points in time
5 after administration of the agent; and c) comparing the amount of phosphorylated human Histone H3 or ortholog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of the human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3, in the first
10 point in time relative to at least one subsequent point in time, indicates that the agent inhibits kinase activity of human MELK or the ortholog thereof is provided. In one embodiment, the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent
15 which specifically binds with Thr-3 phosphorylated human Histone H3 or Ser-10 phosphorylated human Histone H3 or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3. In another embodiment, the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a
20 chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. In still another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated or Ser-10
25 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylated ortholog of human Histone H3. In yet another embodiment, the sample is selected from the group consisting of *ex vivo* and *in vivo* samples. In another embodiment, the sample comprises cancer cells (*e.g.*, cancer cells selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having
30 an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases). In still another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In yet another embodiment, the sample

in step a) and/or step b) is a portion of a single sample obtained from the subject. In another embodiment, the sample in step a) and/or step b) is a portion of pooled samples obtained from the subject. In still another embodiment, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer
5 between the first point in time and the subsequent point in time. In yet another embodiment, the human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In still another embodiment, the agent decreases the amount of
10 Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

In yet another aspect, a method of treating a subject afflicted with cancer comprising administering to the subject an agent that inhibits Thr-3 phosphorylation and/or Ser-10
15 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, thereby treating the subject afflicted with the cancer is provided. In one embodiment, the agent is administered in a pharmaceutically acceptable formulation. In another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In still another embodiment,
20 the agent directly binds said human Histone H3 or the ortholog thereof, or said human MELK or the ortholog thereof. In yet another embodiment, the cancer is selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases. In another
25 embodiment, the human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In still another embodiment, the agent is a small molecule, or an antibody or antigen-binding fragment thereof. In yet another embodiment, the agent decreases the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11
30 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%. In another embodiment, the method further comprises administering one or more additional anti-cancer agents.

In another aspect, a method of determining the function or activity of human MELK or an ortholog, comprising: a) detecting in a sample the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human histone H3 or the amount of a human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3; b) repeating step a) in the same sample or a test sample at one or more subsequent points in time after manipulation of the sample and/or manipulation of the same sample or test sample; and c) comparing the amount of phosphorylated human Histone H3 or ortholog thereof detected in step a) with said amount detected in step b), wherein a modulated amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of the human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3, in the first point in time relative to at least one subsequent point in time and/or at least one subsequent manipulation of the same sample or test sample, indicates that the function or activity of human MELK or an ortholog thereof is modulated is provided. In one embodiment, the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent which specifically binds with Thr-3 phosphorylated or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3. In another embodiment, the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. In still another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylated ortholog of human Histone H3. In yet another embodiment, the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples. In another embodiment, the sample comprises cells or the method uses a cell-based assay. In still another embodiment, the cells are cancer cells selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or

Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases. In yet another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In another embodiment, the same sample or test sample in step a) and/or step

5 b) is a portion of a single sample obtained from a subject. In still another embodiment, the same sample or test sample in step a) and/or step b) is a portion of pooled samples obtained from a subject. In yet another embodiment, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer between the first point in time and the subsequent point in time. In another embodiment, the human Histone

10 H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1. In still another embodiment, the manipulation of the sample is selected from the group consisting of contacting the sample with a test agent, contacting the sample with an upstream signal of the MELK signaling pathway, and contacting the sample with a MELK inhibitor. In yet another embodiment,

15 the test agent is a small molecule, or an antibody or antigen-binding fragment thereof. In another embodiment, the test agent decreases the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

20 It will also be understood that certain embodiments of the present invention can be used with more than one method described herein, according to knowledge available to the skilled artisan.

Brief Description of Figures

25 **Figure 1** shows that MELK interacts with eIF4B. Flag-MELK was conditionally expressed in MDA-MB-468 cells. Mitotic lysates were subjected to anti-Flag immunoprecipitation followed by tandem mass spectrometric analysis. The left panel shows the number of peptides recovered from the immunoprecipitates. The right panel shows validation of the interaction between MELK and eIF4B during mitosis. Note that

30 Flag-MELK is doxycycline-inducible.

Figure 2 shows the results of peptide library screening to identify an optimal substrate motif for MELK. The top panel shows the results of a spatially arrayed peptide library subjected to *in vitro* phosphorylation using recombinant full-length MELK. Each

peptide contains one residue fixed at one of nine positions relative to the centrally fixed phosphoacceptor (*i.e.*, serine or threonine). Reactions were spotted onto the membrane and the spots were exposed to a phosphor storage screen. The bottom panel shows a sequence logo generated using quantified and normalized data from the screen. Note that MELK has
 5 a strong selection for arginine at the -3 position relative to the phosphoacceptor site.

Figure 3 shows that MELK phosphorylates eIF4B at S406 *in vitro*. Recombinant full-length MELK or the kinase domain of MELK was subjected to *in vitro* kinase assays using immunoprecipitated Flag-eIF4B (wild type) or Flag-eIF4B (S406A). Robust phosphorylation of eIF4B at S406 was observed in the presence of MELK. This
 10 phosphorylation was abolished when wild type (wt) eIF4B was replaced with a mutant eIF4B (S406A).

Figure 4 shows that MELK does not phosphorylates eIF4B at S422 *in vitro*. Recombinant full-length or kinase domain of MELK was subjected to *in vitro* kinase assay using immunoprecipitated Flag-eIF4B (wild type) or Flag-eIF4B (S422A). Reactions were
 15 analyzed by immunoblotting.

Figure 5 shows that MELK regulates phosphorylation of eIF4B at S406 *in vivo*. Left panels show MELK knockdown impairs the phosphorylation of eIF4B at S406. MDA-MB-468 cells stably expressing tetracycline-inducible (tet-on) small hairpin MELK (shMELK) in the presence or absence of doxycycline were harvested through treatment of
 20 nocodazole, and subjected to immunoblotting. Right panels show that a MELK inhibitor impairs the phosphorylation of eIF4B at S406. Mitotic MDA-MB-468 cells were treated for 30 min with vehicle or 200 nM OTSSP167, a MELK inhibitor (Chung *et al.* (2012) *Oncotarget* 3:1629-1640). Lysates were used for immunoblotting.

Figure 6 shows the results of treating mitotic cells for 30 min with mTOR inhibitors
 25 (*e.g.*, Rapamycin and Torin 1) versus treating such cells with MELK inhibitors (*e.g.*, OTSSP67). The results indicate that MELK inhibition, but not mTOR inhibition, suppressed the phosphorylation of eIF4B at S406.

Figure 7 shows that knocking down MELK or eIF4B decreases the protein abundance of XIAP, c-Myc, and ODC1 during mitosis. MDA-MB-468 and MDA-MB-231
 30 cells stably expressing tet-on shMELK or sh-eIF4B were treated with doxycycline or vehicle control. Mitotic cells were harvested by nocodazole-induced arrest at prometaphase. Note that the mRNA of XIAP, c-Myc, and ODC1 have been shown to contain structured 5'UTR and their total levels remain unchanged.

Figure 8 shows that knocking down MELK decreases the translation of luciferase driven by the 5' UTR of c-Myc or ODC1 during mitosis. MDA-MB-231 cells stably expressing tet-on shMELK were transfected with the indicated bicistronic vector in the presence or absence of doxycycline. Nocodazole-arrested mitotic cells were harvested two
5 days after transfection and subjected to a luciferase assay. The ratio of Renilla luciferase to firefly luciferase (RL/FL) was normalized to the value of control vector. Note that the left bar corresponds to Dox (-) and the right bar corresponds to Dox (+) for each pair of bars shown in the graph reporting relative RL/FL ratios.

Figure 9 shows that MELK phosphorylates Histone H3 at Thr-3, Ser-10 and Thr-11
10 *in vitro*. Recombinant Histone H3 was incubated with or without recombinant MELK (kinase domain) for 30 min. at 30 °C in the presence of ATP. Reactions were terminated by adding SDS sample buffer. Samples were then subjected to immunoblotting using the indicated antibodies.

Figure 10 shows that knocking down MELK decreases the mitotic phosphorylation
15 of Histone H3 at Thr-3, Ser-10 and Thr-11, but not Ser-28. MDA-MB-468 cells stably transduced with tet-on shMELK were untreated or treated with doxycycline (200 ng/ml) in order to induce gene silencing. Cells were then treated with nocodazole (200 ng/ml) for 20 hours. Mitotic cells were harvested by shake-off and cell lysates were subjected to immunoblotting using the indicated antibodies.

Figure 11 shows that knocking down MELK does not affect the phosphorylation of
20 Aurora kinases, which are known kinases for Histone H3 at Ser 10. MDA-MB-468 cells stably transduced with tet-on shMELK were untreated or treated with doxycycline (200 ng/ml) to induce gene silencing. Cells were treated with nocodazole (200 ng/ml) for 20 hours. Mitotic cells were harvested by shake-off and cell lysates were subjected to
25 immunoblotting using the indicated antibodies.

Figure 12 shows that a MELK inhibitor suppresses MELK-induced
phosphorylation of Histone H3 at Ser 10 *in vitro*. Recombinant Histone H3 was incubated without or with recombinant MELK (kinase domain) for 30 min. at 30 °C in the absence or presence of OTSSP167 (200 ng/ml final). Reactions were terminated by adding SDS
30 sample buffer. Samples were subjected to immunoblotting using the indicated antibodies.

Figure 13 shows that a MELK inhibition suppresses the mitotic phosphorylation of Histone H3 at Thr-3, Ser-10 and Thr-11, but not Ser-28, in a concentration-dependent manner. Mitotic cells were harvested through nocodazole-induced cell cycle arrest at

prometaphase (200 ng/ml nocodazole, 20 hours). Cells were treated with the small chemical inhibitor of MELK, OTSSP167, for 30 min, at the indicated concentrations. Cell lysates were then prepared for immunoblotting.

5 **Detailed Description of the Invention**

The methods of the present invention relate to the surprising determination that the level of phosphorylation of position 406 (*e.g.*, a serine residue) of human eukaryotic initiation factor 4B (eIF4B), or a corresponding phosphorylatable amino acid of an ortholog thereof, serves as a biomarker for MELK enzymatic (*e.g.*, kinase) and/or oncogenic
10 activity. Specifically, decreased phosphorylation of, for example, Ser-406 of human eIF4B (*e.g.*, by directly or indirectly inhibiting MELK-mediated phosphorylation of Ser-406) corresponds with a reduction in MELK enzymatic activity (*e.g.*, kinase activity) and MELK-mediated oncogenic effects. Such a biomarker is particularly advantageous for preclinical and clinical applications because the phosphorylation state of eIF4B is directly
15 associated with the MELK oncogene itself. Similarly, the methods of the present invention also relate to the surprising determination that the level of phosphorylation of position 10 (*e.g.*, a serine residue) of human Histone H3, or a corresponding phosphorylatable amino acid of an ortholog thereof, and/or the level of phosphorylation of position 11 (*e.g.*, a threonine residue) of human Histone H3, or a corresponding phosphorylatable amino acid
20 of an ortholog thereof, serves as a biomarker for MELK enzymatic (*e.g.*, kinase) and/or oncogenic activity. Specifically, decreased phosphorylation of, for example, Thr-3 of human Histone H3 (*e.g.*, by directly or indirectly inhibiting MELK-mediated phosphorylation of Thr-3) and/or Ser-10 of human Histone H3 (*e.g.*, by directly or indirectly inhibiting MELK-mediated phosphorylation of Ser-10) and/or Thr-11 of human
25 Histone H3 (*e.g.*, by directly or indirectly inhibiting MELK-mediated phosphorylation of Thr-11) corresponds with a reduction in MELK enzymatic activity (*e.g.*, kinase activity) and MELK-mediated oncogenic effects. Such a biomarker is particularly advantageous for preclinical and clinical applications because the phosphorylation state of Histone H3 is directly associated with the MELK oncogene itself. In some embodiments, Ser-406 of
30 human eIF4B, Thr-3 of human Histone H3, Ser-10 of human Histone H3, and/or Thr-11 of human Histone H3, as well as any corresponding phosphorylatable amino acid of an ortholog thereof, including in any combination thereof, are contemplated for use according to the present invention. In other embodiments, Ser-28 of human eIF4B or a corresponding

phosphorylatable amino acid of an ortholog thereof is not regulated by MELK and is not used according to the present invention.

A. MELK, eIF4B, and Histone H3 Molecules

5 As used herein, "MELK" refers to the MELK member of the protein kinase superfamily and is alternatively known as "pEG3 kinase," "protein kinase Eg3," "protein kinase," and "serine/threonine-protein kinase MELK." At least nine splice variants encoding nine distinct human MELK isoforms exist. Human MELK transcript variant 1 (NM_014791.3) encodes the long human MELK isoform 1 (NP_55606.1). Human MELK
10 transcript variant 2 (NM_001256685.1) lacks an exon in the 3' coding region compared to transcript variant 1, but maintains the reading frame and results in an isoform (NP_001243614.1) that is shorter than isoform 1. Human MELK transcript variant 3 (NM_001256687.1) lacks an exon in the 5' coding region compared to transcript variant 1, but maintains the reading frame and results in an isoform (NP_001243616.1) that is shorter
15 than isoform 1. Human MELK transcript variant 4 (NM_001256688.1) lacks two consecutive exons in the 5' coding region compared to transcript variant 1, but maintains the reading frame and results in an isoform (NP_001243617.1) that is shorter than isoform 1. Human MELK transcript variant 5 (NM_001256689.1) initiates translation at an alternate start codon and lacks an exon in the 5' coding region compared to transcript
20 variant 1 and thus results in an isoform (NP_001243618.1) that is shorter than and has a distinct N-terminus from isoform 1. Human MELK transcript variant 6 (NM_001256690.1) initiates translation at an alternate start codon and lacks two consecutive exons in the 5' coding region compared to transcript variant 1, but maintains the reading frame and results in an isoform (NP_001243619.1) that is shorter than and has a
25 distinct N-terminus from isoform 1. Human MELK transcript variant 7 (NM_001256691.1) initiates translation at an alternate start codon and lacks two exons in the 5' coding region compared to transcript variant 1, but maintains the reading frame and results in an isoform (NP_001243620.1) that is shorter than and has a distinct N-terminus from isoform 1. Human MELK transcript variant 8 (NM_001256692.1) lacks three exons
30 in the 5' coding region and initiates translation at a downstream, in-frame start codon compared to transcript variant 1 and results in an isoform (NP_001243621.1) that has a shorter N-terminus than isoform 1. Finally, human MELK transcript variant 9 (NM_001256693.1) lacks two consecutive exons in the 5' coding region and initiates translation at a downstream, in-frame start codon compared to transcript variant 1 and

results in an isoform (NP_001243622.1) that has a shorter N-terminus than isoform 1. The protein domains and structural basis for the regulation of MELK autophosphorylation and activation of kinase activities on target proteins is known (see, at least Cao *et al.* (2013) *PLoS One* 8:e70031 and Canevari *et al.* (2013) *Biochemistry* 52:6380-6387).

5 Mouse MELK nucleic acid (NM_010790.2) and amino acid (NP_034920.2) sequences are publicly available on the GenBank database maintained by the U.S. National Center for Biotechnology Information. Nucleic acid and polypeptide sequences of MELK orthologs in species other than mice and humans are also well known and include, for example, chimpanzee MELK (XM_001169038.3, XP_001169038.1, XM_001168991.3,
10 XP_001168991.1, XM_001168745.3, XP_001168745.1, XM_001168775.3, XP_001168775.1, XM_003951427.1, XP_003951476.1, XM_520578.4, XP_520578.3, XM_001168822.3, XP_001168822.2, XM_003312085.2, XP_003312133.1, XM_003951428.1, XP_003951477.1, XM_003312086.2, and XP_003312134.1), monkey MELK (XM_001115076.2 and XP_001115076.2), dog MELK (XM_003431578.1,
15 XP_003431626.1, XM_538730.3, XP_538730.2, XM_003431579.1, and XP_003431627.1), cow MELK (NM_001111260.1 and NP_001104730.1), rat MELK (NM_001108662.1 and NP_001102132.1), chicken MELK (NM_001031509.1 and NP_001026680.1), and zebrafish MELK (NM_206888.2 and NP_996771.2).

 As used herein, “eIF4B” refers to the eukaryotic translation initiation factor 4B
20 member of the eukaryotic translation initiation factor family and is alternatively known as “EIF-4B” and “PRO1843.” Human eIF4B nucleic acid (NM_001417.4) and amino acid (NP_001408.2) sequences are publicly available on the GenBank database maintained by the U.S. National Center for Biotechnology Information. Nucleic acid and polypeptide sequences of eIF4B orthologs in species other than humans are also well known and
25 include, for example, mouse eIF4B (NM_145625.3 and NP_663600.2), chimpanzee eIF4B (XM_003313676.1, XP_003313724.1, XM_001142097.3, and XP_001142097.3), monkey eIF4B (NM_001195808.1 and NP_001182737.1), dog eIF4B (XM_853888.2, XP_858981.2, XM_853812.2, and XP_858905.2), cow eIF4B (NM_001035028.2 and NP_001030200.1), rat eIF4B (NM_001008324.1 and NP_001008325.1), and chicken
30 eIF4B (XM_003643408.2 and XP_003643456.2). In addition, “Ser-406” of eIF4B refers to the amino acid numbering of the human eIF4B. Accordingly, a skilled artisan will readily understand that Ser-406 of the human eIF4B polypeptide is conserved across numerous species and that although those specific residues may be referenced herein, the methods of

the present invention apply equally well to the corresponding residues (*e.g.*, phosphorylatable amino acids) of isoforms, homologs, and orthologs in other species corresponding to said Ser-406 of human eIF4B.

As used herein, the term "Histone H3" refers to the H3 member of the Histone family, which comprises proteins used to form the structure of nucleosomes in eukaryotic cells. Eukaryotes have chromatin arranged around proteins in the form of nucleosomes, which are the smallest subunits of chromatin and include approximately 146-147 base pairs of DNA wrapped around an octamer of core histone proteins (two each of H2A, H2B, H3, and H4). Mammalian cells have three known sequence variants of Histone H3 proteins, denoted H3.1, H3.2 and H3.3, that are highly conserved differing in sequence by only a few amino acids. Post-translational modification of Histone H3 residues are important in many cellular processes and phosphorylation of serine 10 and/or serine 28 are important for cell division and proliferation regulation. Phosphorylated Histone H3 at serine 10 is a specific biomarker for mitotic cells, similar to other well-known mitosis-specific markers, such as phosphorylated MPM-2, phosphorylated retinoblastoma protein 1 (Rb), phosphorylated cdc2, BubR1, cyclin B1, cdc25c, cdk1, cdc27, and the like. Any serine, threonine, or tyrosine residue can be phosphorylated. In some embodiments, other possible phosphorylation sites include threonine 3, threonine 6, threonine 11, serine 31, tyrosine 41, serine 57, threonine 80, and threonine 107.

As used herein, the term "Histone H3" can refer to H3.1, H3.2, or H3.3 individually or collectively. These amino acid sequences include a methionine as residue number 1 that is cleaved off when the protein is processed. Thus, for example, serine 11 in the Histone H3 amino acid sequences shown in Table 1 below corresponds to serine (Ser) 10 of the present invention. These three protein variants are encoded by at least fifteen different genes/transcripts. Sequences encoding the Histone H3.1 variant are publicly available as HIST1H3A (NM_003529.2; NP_003520.1), HIST1H3B (NM_003537.3; NP_003528.1), HIST1H3C (NM_003531.2; NP_003522.1), HIST1H3D (NM_003530.3; NP_003521.2), HIST1H3E (NM_003532.2; NP_003523.1), HIST1H3F (NM_021018.2; NP_066298.1), HIST1H3G (NM_003534.2; NP_003525.1), HIST1H3H (NM_003536.2; NP_003527.1), HIST1H3I (NM_003533.2; NP_003524.1), and HIST1H3J (NM_003535.2; NP_003526.1). Sequences encoding the Histone H3.2 variant are publicly available as HIST2H3A (NM_001005464.2; NP_001005464.1), HIST2H3C (NM_021059.2; NP_066403.2), and HIST2H3D (NM_001123375.1; NP_001116847.1). Sequences encoding the Histone H3.3

variant are publicly available as H3F3A (NM_002107.3; NP_002098.1) and H3F3B (NM_005324.3; NP_005315.1). See U.S. Pat. Publ. 2012/0202843 for additional details. Moreover, polypeptide sequences for Histone H3 orthologs, as well as nucleic acid sequences that encode such polypeptides, are well-known in many species, and include, for example, Histone H3.1 orthologs in mice (NM_013550.4; NP_038578.2), chimpanzee (XM_527253.4; XP_527253.2), monkey (XM_001088298.2; XP_001088298.1), dog (XM_003434195.1; XP_003434243.1), cow (XM_002697460.1; XP_002697506.1), rat (XM_001055231.2; XP_001055231.1), and zebrafish (NM_001100173.1; NP_001093643.1). Histone H3.2 orthologs in mice (NM_178215.1; NP_835587.1), chimpanzee (XM_524859.4; XP_524859.2), monkey (XM_001084245.2; XP_001084245.1), dog (XM_003640147.1; XP_003640195.1), cow (XM_002685500.1; XP_002685546.1), rat (NM_001107698.1; NP_001101168.1), chicken (XM_001233027.2; XP_001233028.1), and zebrafish (XM_002662732.1; XP_002662778.1). Similarly, Histone H3.3 orthologs in mice (XM_892026.4; XP_897119.3), monkey (XM_001085836.2; XP_001085836.1), cow (NM_001099370.1; NP_001092840.1), rat (NM_053985.2; NP_446437.1), chicken (NM_205296.1; NP_990627.1), and zebrafish (NM_200003.1; NP_956297.1), are well-known. Antibodies for the detection of phosphorylated H3 histone, such as phosphorylated Histone H3 at Thr-3, Ser-10, Thr-11, and other phosphorylatable residues of Histone H3, as well as methods for making such antibodies are known in the art. In addition, for example, "Ser-10" of Histone H3 refers to the amino acid numbering of the human Histone H3. Accordingly, a skilled artisan will readily understand that Ser-10 of the human Histone H3 polypeptide is conserved across numerous species and that although those specific residues may be referenced herein, the methods of the present invention apply equally well to the corresponding residues (*e.g.*, phosphorylatable amino acids) of isoforms, homologs, and orthologs in other species corresponding to said Ser-10 of human Histone H3. The same applies to Thr-3 and Thr-11.

Representative MELK, eIF4B, and Histone H3 orthologs are provided herein (*e.g.*, at least at Table 1 and the Examples) as follows:

30 Table 1

Human MELK (isoform 1) cDNA Sequence (NM_014791.3)

```

1 atgaaagatt atgatgaact totcaaatat tatgaattac atgaaactat tgggacaggt
61 ggctttgcaa aggtcaaact tgcctgocat atccttactg gagagatggt agctataaaa
35 121 atcatggata aaaacacact agggagtgat ttgccccgga tcaaaacgga gattgaggcc

```

181 ttgaagaacc tgagacatca gcataatatgt caactctacc atgtgctaga gacagccaac
 241 aaaatattca tggttottga gtactgccct ggaggagagc tgtttgacta tataatttcc
 301 caggatcgcc tgtcagaaga ggagaccggt gttgtcttcc gtcagatagt atctgctggt
 361 gcttatgtgc acagccaggg ctatgctcac agggacctca agccagaaaa tttgctgttt
 5 421 gatgaatata ataaattaaa gctgattgac tttgggtctct gtgcataaac caagggtaac
 481 aaggattacc atctacagac atgctgtggg agtctggctt atgcagcacc tgagttaata
 541 caaggcaaat catatcttgg atcagaggca gatgtttgga gcatgggcat actgttatat
 601 gttcttatgt gtggattttct accatttgat gatgataatg taatggcttt atacaagaag
 661 attatgagag gaaaatatga tgttcccaag tggctctctc ccagtagcat tctgcttctt
 10 721 caacaaatgc tgcagggtga cccaaagaaa cggattttct tgaaaaatct attgaaccat
 781 ccctggatca tgcagatta caactatctt gttgagtggc aaagcaagaa tctttttatt
 841 caoctcgatg atgattgcgt aacagaactt tctgtacatc acagaaacaa caggcaaaaa
 901 atggaggatt taatttcaat gtggcagtat gatcacctca cggctacctt tcttctgctt
 961 ctagccaaga aggtctgggg aaaaccagtt cgtttaaggt tttcttcttt ctctgtgga
 15 1021 caagccagtg ctaccccatc cacagacatc aagtcaaata attggagtct ggaagatgug
 1081 accgcaagtg ataaaaatta tgtggcggga ttaatagact atgattgggt tgaagatgat
 1141 ttatcaacag gtgctgctac tccccgaaca tcacagtta ccaagtaact gacagaatca
 1201 aatgggggtg aatctaaatc attaaactca gcttatgca gaacacctgc aaataaatta
 1261 aagaacaaag aaaatgtata tactcttaag tctgctgtaa agaatagaaga gtactttatg
 20 1321 tttcttgagc caagactcc agttaataag aaccagcata agagagaaat actcactaag
 1381 ccaaatcgtt acactacacc ctcaaaagct agaaaccagt gctgaaaga aactccaatt
 1441 aaaataccag taaattcaac aggaacagac aagttaatga cagggtgcat tagccctgag
 1501 agggggtgcc gctcagtgga attggatctc aaccaagcac atatggagga gactccaaaa
 1561 agaaagggag ccaaagtgtt tgggagcctt gaaggggggt tggataaggt tatcactgtg
 25 1621 ctcaccagga gcaaaaggaa gggttctgcc agagacgggc ccagaagact aaagcttcac
 1681 tataacgtga ctacaactag attagtgaat ccagatcaac tgttgaatga aataatgtct
 1741 attcttccaa agaagcatgt tgactttgta caaaagggtt atacactgaa gtgtcaaaaa
 1801 cagtcagatt ttgggaaagt gacaatgcaa tttgaattag aagtgtgcaa gcttcaaaaa
 1861 cccgatgtgg tgggtatcag gaggcagcgg cttaagggcg atgctgggtt ttacaaaaga
 30 1921 ttagtggaag acatccatc tagctgcaag gtataa

Human MELK (isoform 1) Amino Acid Sequence (NP_55606.1)

1 mkdydellky yelhetigtg gfakvklach iltgemvaik imdkntlgsc lprikteiea
 61 lknlrhghic qlyhvletan kifmvleycp ggelfdyiis qdrlseeetr vvfriqvsva
 35 121 ayvhsqgyah rdlkpenilf deyhklklid fgicakpkga kdyhlqtccg slayaapeli
 181 qgksylgsea dwsmgilly vlmogflpfd ddhvmalykk imrgkydvpk wlspsalil
 241 qqmlqvdpkk rismknlhnh pwimqdynyp vewqsknpfi hldddcvtel svhhrnnrqt
 301 medlislwqy dhltatylil lakkargkpvr rrlssfsccg qasatpftdi kennwsledv
 361 tasdknyvag lidydscedd lctgaatprt sqfthywtes ngvesksltp alcrtpankl
 40 421 knkenvytpk savkneeyfm fpepktpvnr nqhkrelttn pnrytppska nqciketpi
 481 kipvnstgtd klmtgvispe rrcrsveldl nqahmeetpk rkgakvfqsl ergldkvitv
 541 ltrskrkgsa rdgprrikth ynvtttrilv pdqilneims ilpkkhvdfv qkgytikoqt
 601 qsdfigkvtmq felevcqlqk pdvvgirrrr lkgdawvykr lvedilssck v

Human MELK (isoform 2) cDNA Sequence (NM_001256685.1)

1 atgaaagatt atgatgaact totcaaatat tatgaattac atgaaactat tgggacaggt
 61 ggctttgcaa aggtcaaac tgcctgccat atccttactg gagagatggg agctataaaa
 121 atcatggata aaaacacact agggagtgat ttgccccgga tcaaaacgga gattgagggc
 5 181 ttgaagaacc tgagacatca gcatatatgt caactctacc atgtgctaga gacagccaac
 241 aaaatattca tggttcttga gtactgccct ggaggagagc tgtttgacta tataatttcc
 301 caggatcgcc tgtcagaaga ggagaccggt gttgtcttcc gtcagatagt atctgctggt
 361 gcttatgtgc acagccaggg ctatgctcac agggacctca agccagaaaa ttgctgtgtt
 421 gatgaatata ataaattaaa gctgattgac tttggctctc gtgcaaaacc caagggtaac
 10 481 aaggattacc atctacagac atgctgtggg agtctgggtt atgcagcacc tgagttaata
 541 caaggcaaat catatcttgg atcagaggca gatgttttga gcatgggcat actgttatat
 601 gttcttatgt gtggatttct accatttgat gatgataatg taatggcttt atacaagaag
 661 attatgagag gaaaatatga tgttcccaag tggctctctc ccagtagcat totgcttctt
 721 caacaaatgc tgcagggtga cccaaagaaa cggatttcta tgaaaaatct attgaaccat
 15 781 ccctggatca tgcaagatta caactatcct gttgagtggc aaagcaagaa tccctttatt
 841 cacctcgatg atgattgogt aacagaactt tctgtacatc acagaaacaa caggcaaaaa
 901 atggaggatt taatttcaat gtggcagtat gatcacctca cggctaccta tcttctggtt
 961 ctagccaaga aggtcggggg aaaaccagtt cgtttaaggt tttcttcttt ctctgttga
 1021 caagccagtg ctaccccatc cacagacatc aagtttacca agtactggac agaatcaaat
 20 1081 ggggtggaat ctaaatcatt aactccagcc ttatgcagaa cacctgcasa taaatttaag
 1141 aacaaagaaa atgtatatac tccaaagtct gctgtaaaag atgaagagta ctttatgttt
 1201 cctgagccaa agactccagt taataagaac cagcataaga gagaaatact cactacgcca
 1261 aatcgttaca ctacaccctc aaaagctaga aaccagtgcc tgaagaaaac tccaattaaa
 1321 ataccagtaa attcaacagg aacagacaag ttaatgacag gtgtcattag ccctgagagg
 25 1381 cgggtccggt cagtgggaatt ggatctcaac caagcacata tggaggagac tccaaaaaga
 1441 aaggagagcca aagtgttttg gagccttgaa aggggggttg ataaggttat cactgtgctc
 1501 accaggagca aaaggaaggg ttctgccaga gacgggcccc gaagactaaa gcttcaactat
 1561 aacgtgacta caactagatt agtgaatcca gatcaactgt tgaatgaaat aatgtctatt
 1621 cttccaaaga agcatgttga ctttgtacaa aagggttata cactgaagtg tcaaacacag
 30 1681 tcagattttg gaaagtgaac aatgcaattt gaattagaag tgtgccagct tcaaaaaccc
 1741 gatgtggtgg gtatcaggag gcagcggctt aaggggcagc cctgggttta caaaagatta
 1801 gtggaagaca tctatctag ctgcaaggta taa

Human MELK (isoform 2) Amino Acid Sequence (NP_001243614.1)

35 1 mkdydeliky yelhetigtg gfakvklach iltgemvaik imdkntlgss lprikteiea
 61 lknlrhghic qlyhvletan kifmvleyp ggelfdyiis qdriseeetr vvfirgivsav
 121 ayyhsqgyah rdlkpenilf deyhklklid fgicakpkgn kdyhlgtccg slayaapeli
 181 qgksylgsea dwsmgilly vlmogflpfd ddnvmalykk imrgkydvpk wlspsailll
 241 qqmlqvdpkk risaknlilnh pwimqdynyp vewqsknpfi hldddcvtei svhhrnnrqt
 40 301 medlislwqy dhltatylil lakkargkpvr rlrllsfsag qasatpftdi kftkywtesn
 361 gvesksitpa lcrtpanklk nkenvytpks avkneeyfmf pepktpvnks qhkreibltp
 421 nryttpskar nqclketpik ipvnstgtdk lmtgvisper rcrsveldin qahmeetpkr
 481 kgakvfgele rgldkvitvi trskrkgsar dgprlrkihy svtttrlnvp dqllineimsi
 541 lpkxhvdvfg kgytlkcqtg sdfgkvtnqf elevcqlqkp dvvgirrqri kgdawvykri

601 vedilssckv

Human MELK (isoform 3) cDNA Sequence (NM_001256687.1)

1 atgaaagatt atgatgaact tctcaaatat tatgaattac atgaaactat tgggacaggt
 5 61 ggcttttgcaa aggtcaaaact tgcctgocat atccttactg gagagatggg agctataaaa
 121 atcatggata aaaacacact agggagtgat ttgccccgga tcaaaacgga gattgaggcc
 181 ttgaagaacc tgagacatca gcataatatgt caactctacc atgtgctaga gacagccaac
 241 aaaatattca tggttcttga ggaaaatttg ctgtttgatg aatatcataa attaaagctg
 301 attgactttg gtctctgtgc aaaacccaag ggtaacaagg attaccatct acagacatgc
 10 361 tgtgggagtc tggcttatgc agcacctgag ttaatacaag gcaaatcata tcttggatca
 421 gaggcagatg tttggagcat gggcatactg ttatatgttc ttatgtgtgg atttctacca
 481 tttgatgatg ataattgaat ggctttatac aagaagatta tgagaggaaa atatgatgtt
 541 cccaagtggc tctctcccag tagcattctg cttcttcaac aatgtctgca ggtggacca
 601 aagaaacgga tttctatgaa aaatctattg aaccatccct ggatcatgca agattacaac
 15 661 tatcctgttg agtggcaasg caagaatcct tttattcacc tcgatgatga ttgcgtaca
 721 gaactttctg tacatcacag aaacaacagg caaacaatgg aggatttaat ttcactgttg
 781 cagtatgatc acctcacggc tacctatcct ctgcttctag ccaagaaggc tcggggaaaa
 841 ccagttcgtt taaggcttct tctttctctc tgtggacaag ccagtgtcac cccattcaca
 901 gacatcaagt caaataattg gagtcggaa gatgtgacgg caagtataa aaattatgtg
 20 961 gcgggattaa tagactatga ttgggtgtga gatgattht caacaggtgc tgcactccc
 1021 cgaacatcac agtttaccac gtactggaca gaatcaaatt ggggtggaatc taaatcatta
 1081 actccagcct tatgcagaac acctgcaaat aaattaaaga acaaagaaaa tgtatatact
 1141 cctaagtctg ctgtaaagaa tgaagagtac tttatgtttc ctgagccaaa gactccagtt
 1201 aataagaacc agcataagag agaaatactc actacgccaa atcgttacac tacaccctca
 25 1261 aaagctagaa accagtgcct gaaagaaact ccaattaaaa taccagtaaa ttcaacagga
 1321 acagacaagt taatgacagg tgtcattagc cctgagaggg ggtgcgcctc agtggaaatg
 1381 gatctcaacc aagcacatat ggaggagact ccaaaaagaa agggagccaa agtgtttggg
 1441 agccttgaaa ggggggttga taaggttatc actgtgtctc ccaggagcaa aaggaagggt
 1501 tctgccagag acggggcccag aagactaaag cttcactata acgtgactac aactagatta
 30 1561 gtgaatccag atcaactgtt gaatgaaata atgtctatcc tccaaagaa gcattgtgac
 1621 tttgtacaaa agggttatac actgaagtgt caaacacagt cagatttttg gaaagtgaca
 1681 atgcaatttg aattagaagt gtgccagctt caaaaacccg atgtggtggg tatcaggagg
 1741 cagcggotta agggcgatgc ctgggtttac aaaagattag tggaagacat cctatctagc
 1801 tgcaaggtat aa

35

Human MELK (isoform 3) Amino Acid Sequence (NP_001243616.1)

1 mkdydeliky yelhetigtg gfakvklach iltgemvaik imdknclgsd lprikteiea
 61 lknlrhghic qlyhvletan kifmvleenl lfdeybkkl idfglcakpk gnkdyblqtc
 121 cgsiayaape liqgksylgs eadvwamgil lyvlmcgflp fdddnvmaly kkimrgkydv
 40 181 pkwlspseil liqqmlqvdp kkrismknll nhpwimqdyn ypvewqsknp fihldddcvt
 241 elsvhhrnnr qtmedlislw qydhltatyl lllakkarqk pvrllrlsfs cqqasatpft
 301 diksnwsle dvtsdknyv aglidydwee ddltsgaatp rtsqftkywt esngvesksl
 361 tpalortpan klknkenvyt phsavkneey fmfpepktpv nknqhkrel ttprnyttps
 421 karnqclket pikipvnstg tdklmqgis perrcrsvel dingahmeet pkrkgakvfg

481 slergldkvi tvltskrkg sardgprrik lhynvttrrl vnpdqlinei mailpkkhvd
 541 fvgkgytiko qtgsdfgkvt mqfelevcql qkpdvvgirr grlkgdawvy krivedilss
 601 ckv

5 Human MELK (isoform 4) cDNA Sequence (NM_001256688.1)

1 atgaaagatt atgatgaact totcaaatat tatgaattac atgaaactat tgggacaggt
 61 ggctttgcaa aggtcaaaact tgcctgccat atccttactg gagagatggg agctataaaa
 121 atcatggata aaaacacact agggagtgat ttgccccgga tcaaaacgga gattgaggcc
 181 ttgaagaacc tgagacatca gcataatatgt caactctacc atgtgctaga gacagccaac
 10 241 aaaatattca tggttcttga gggtaacaag gattaccatc tacagacatg ctgtgggagt
 301 ctggcttatg cagcaactga gtaatacaaa ggcaaatcat accttggatc agaggcagat
 361 gtttggagca tgggcatact gttatatgtt ctatgtgtg gatttctacc atttgatgat
 421 gataatgtaa tggctttata caagaagatt atgagaggaa aatatgatgt tcccaagtgg
 481 ctctctccca gtagcattct gcttcttcaa caaatgctgc aggtggaccc aaagaaacgg
 15 541 atttctatga aaaatctatt gaaccatccc tggatcatgc aagattacaa ctatcctggt
 601 gagtggcaaa gcaagaatcc ttttattcac ctgatgatg attgcgtaac agaactttct
 661 gtacatcaca gaaacaacag gcaacaatg gaggatttaa tttcactgtg gcagtatgat
 721 caccctcagg ctacctatct totgcttcta gccaaagaag ctccggggaaa accagttcgt
 781 ttaaggcttt ctcttttctc ctgtggacaa gccagtgcta ccccatcac agacatcaag
 20 841 tcaasteatt ggagtctgga agatgtgacc gcaagtgata aaattatgt ggcgggatta
 901 atagactatg attggtgtga agatgattta tcaacagggt ctgctactcc ccgaacatca
 961 cagtttacca agtactggac agaatacaat ggggtggaat ctaaatcatt aactccagcc
 1021 ttatgcagaa cactgcaaa taaattaaag aacaaagaaa atgtatatac tctaagtct
 1081 gctgtaaaga atgaagagta ctttatgttt cctgagccaa agactccagt taataagaac
 25 1141 cagcataaga gagaaatact cactacgcca aatcggtaca ctacaccctc aaaagctaga
 1201 aaccagtgcc tgaagaaaac tccaattaaa ataccagtaa attcaacagg aacagacaag
 1261 ttaatgacag gtgtcattag cctgagaggg cggtgccgct cagtgggaatt ggatctcaac
 1321 caagcacata tggaggagac tccaaaaaga aaggagacca aagtgtttgg gagccttgaa
 1381 aggggggttg ataaggttat cactgtgctc accaggagca aaaggaaagg ttctgccaga
 30 1441 gaogggccca gaagaactaaa gcttcaatat aacgtgacta caactagatt agtgaatcca
 1501 gatcaactgt tgaatgaaat aatgtctatt ctccaaaga agcatgttga ctttgtacaa
 1561 aagggttata cactgaagtg tcaaacacag tcagattttg ggaaagtga aatgcaattt
 1621 gaattagaag tgtgccagct tcaaaaaccc gatgtggtgg gtatcaggag gcagcggctt
 1681 aaggggcatg cctgggttta caaagatta gtggaagaca tctatctag ctgcaaggta
 35 1741 taa

Human MELK (isoform 4) Amino Acid Sequence (NP_001243617.1)

1 mkdydeliky yelhetigtg gfakvklach iltgemvaik imdkntlgad lpriktelea
 61 lknirbqhic qlybvletan kifmvlegnk dyhlqtccgs layaapeliq gksyigsead
 40 121 vwamgillyv lmcgflpidd dnmalykki mrgkydvpkw lspssilliq qmlqvdpkkr
 181 ismknllnbp wimqdynypv ewqsknpfi hlddcvtels vhhrrnrgtm edllslwqyd
 241 hltatyllll akkargkpvr lrlssfscgg asatpftdik snnwsledvt asdknyvagl
 301 idydwceddl stgaatprts qftkywtesn gveskaltpa lortpankik nkenvytpks
 361 avkneeyfmf pepktpvnkn qhkreltthp nryttskar nqclketpik ipvnstgudk

421 lmtgvisper rcraveidln qahmeetpkr kgakvfgsle rgldkvitvi trskrkgsar
 481 dgprrlkily nvttrrlvnp dqllneimsi lpkxhvdvq kgytlkcgqg sdfgkvtnqf
 541 elevcqlqkp dyvgirrrgl kgdawvykri vedilssckv

5 Human MELK (isoform 5) cDNA Sequence (NM_001256689.1)

1 atgatgaact tctcaaatac tatgaattac atgaaactat tgggacagag tgatttgccc
 61 cggatcaaaa cggagattga ggctttgaag aacctgagac atcagcatat atgtcaactc
 121 taccatgtgc tagagacagc caacaaaata ttcattgttc ttgagtactg ccttggagga
 181 gagctgtttg actatataat ttcccaggat cgcctgtcag aagaggagac ccgggttgtc
 10 241 ttccgtcaga tagtatctgc tgttgcttat gtgcacagcc agggctatgc tcacagggac
 301 ctcaagccag aaaatttgcg gtttgatgaa tatcataaat taaagctgat tgaatttggg
 361 ctctgtgcaa aacccaaggg taacaaggat taccatctac agacatgctg tgggagtctg
 421 gcttatgcag cactgaggtt aatacaagga aaatcatatc ttggatcaga ggcagatgtt
 481 tggagcatgg gcatactgtt atatgttctt atgtgtggat ttctaccatt tgatgatgat
 15 541 aatgtaattg ctttatacaa gaagattatg agaggaaaat atgatgttcc caagtggctc
 601 tctcccagta gcattctgct tcttcaacaa atgctgcagg tggacccaaa gaaacggatt
 661 tctatgaaaa atctattgaa ccatccctgg atcatgcaag attacaacta tctgtttgag
 721 tggcaaagca agaattcctt tatccacctc gatgatgatt gcgtaacaga actttctgta
 781 catcacagaa acaacaggca aacaatggag gatttaattt cactgtggca gtatgatcac
 20 841 ctcaaggcta cctatctctt gcttctagcc aagaaggctc ggggaaaaac agttcgttta
 901 aggtcttctt ctttctctct tggacaagcc agtgcctacc cattcacaga catcaagtca
 961 aataattgga gcttggaaga tctgaccgca agtgataaaa attatgtggc gggattaata
 1021 gaactatgatt ggtgtgaaga tgatttatca acaggtgctg ctactccccg aacatcacag
 1081 tttaaccaag actggacaga atcaaatggg gtggaatcta aatcattaac tccagcctta
 25 1141 tgcagaacac ctgcaataaa attaaagaac aaagaaaatg tatatactcc taagtctgct
 1201 gtaagaatg aagagtactt tatgtttctt gagccaaaga ctccagttaa taagaaccag
 1261 cataagagag aaatactcac taagccaaat cgttacacta caccctcaaa agctagaaac
 1321 cagtgcctga aagaaactcc aattaaaata ccagtaaatc caacaggaac agacaagtta
 1381 atgacaggtg tcattagccc tgagaggcgg tgcgctcag tgggaattgga tctcaaccac
 30 1441 gcacatatgg aggagactcc aaaaagaaag ggagccaaag tgtttgggag ctttgaaagg
 1501 gggttggata aggttatcac tctgctcacc aggagcaaaa ggaagggttc tgccagagac
 1561 gggccacaga gactaaagct tcaactatac gtgactacaa ctgattagat gaatccagat
 1621 caactgttga atgaaataat gtctattctt ccaagagaag atgttgactt tgtacaaaag
 1681 ggttatcac tgaagtgtca aacacagtcg gattttggga aagtgacaat gcaatttgaa
 35 1741 ttagaagtgt gccagcttca aaaaccogag gtgggtgggt tcaggaggca ggggtttaag
 1801 ggcatgctt gggtttacaa aagattagtg gaagacatcc tatctagctg caaggtataa

Human MELK (isoform 5) Amino Acid Sequence (NP_001243618.1)

1 mnfnfnimny mkligqsdip rikteleak nlrhghicql yhvletanki fmvleycpqg
 40 61 elfdyllsqd rlseeetrvv frqivsavay vhsqgyahrd lkpenilfde yhkiklidfg
 121 lcakpkgnkd yhlqtccqsl ayaapeligg ksylgseadv wsmgillyvi mcgflpfddd
 181 nvmalykkim rgkydvpkwl spssilllqq mlqvdpkkri smknllnhpw imqdynypve
 241 wqsknpfihl dddcvtelsv hhrnnrgtme dlislwqydh ltatylllla kkargkpvrll
 301 rlssfsogqa sarpftdiks nnwsledvta sdknyvagli dydwceddis tgaatprtsq

361 ftkywtesng vesksitpal crtpanklkn kenvytpksa vkneeyfmfp epktpvnknq
 421 hkreiltttn ryttpskarn golketpiki pvnstgtdkl mtgvisperr crsvelldng
 481 ahmeetpkrk gkvfgsler gldkvitvit rskrkgsard gprxlklhyn vtttrlvnpd
 541 glilneimsil pkkhvdfvgk gytllkqgtqs dfgkvtnqfe levclqkpd vvgirrgrik
 5 601 gdawvykrlv edilssckv

Human MELK (isoform 6) cDNA Sequence (NM_001256690.1)

1 atgatgaact tctcaaatat tatgaattac atgaaactat tgggacagta ctgccctgga
 61 ggagagctgt ttgactatat aatttcccag gatgcctgt cagaagagga gaccocgggtt
 10 121 gtcttccgtc agatagtatc tgetgttgct tatgtgcaca gccagggcta tgcacacagg
 181 gaactcaagc cagaaaattt gctgtttgat gaatatcata aattaaagct gatagacttt
 241 ggtctctgtg caaaacccaa gggtaacaag gattaccatc tacagacatg ctgtgggagt
 301 ctggccttatg cagcacctga gtttaataca ggcaaatcat atcttggatc agaggcagat
 361 gtttggagca tgggcatact gttatatgtt cttatgtgtg gatttctacc atttgatgat
 15 421 gataatgtaa tggctttata caagaagatt atgagaggaa aatatgatgt tcccaagtgg
 481 ctctctccca gtagcattct gcttcttcaa caaatgctgc aggtgggccc aaagaaacgg
 541 atttctatga aaaatctatt gaaccatccc tggatcatgc aagattacaa ctatcctgtt
 601 gagtggcaaa gcaagaatcc ttttattcac ctgatgatg attgcgtaac agaactttct
 661 gtacatcaca gaaacaacag gcaacaatg gaggatttaa ttctactgtg gcagtatgat
 20 721 cactcaccgg ctacctatct tctgcttcta gccagaagg ctgggggaaa accagttcgt
 781 ttaaggcttt cttctttctc ctgtggacaa gccagtgcct cccattcac agacatcaag
 841 tcaataatt ggagtctgga agatgtgacc gcaagtgata aaaattatgt ggcgggatta
 901 atagactatg attgggtgtg agatgattta tcaacagggt ctgctactcc ccgaacatca
 961 cagtttacca agtactggac agaatacaat ggggtggaat ctaaatcatt aactccagcc
 25 1021 ttatgcagaa cactgcgaaa taaattaaag aacaaagaaa atgtatatac tctaagtct
 1081 gctgtaaaga atgaagagta ctttatgttt cctgagccaa agactccagt taataagaac
 1141 cagcataaga gagaaatact cactacgcca aatcgttaca ctacaccctc aaaagctaga
 1201 aaccagtgcc tgaagagaaac tccaattaaa ataccagtaa attcaacagg aacagacaag
 1261 ttaatgacag gtgtcattag ccttgagagg cgggtgcgct cagtgggaatt ggatctcaac
 30 1321 caagcacata tggaggagac tccaaaaaga aaggggagcca aagtgtttgg gagccttgaa
 1381 aggggggttg ataaggttat cactgtgctc accaggagca aaaggaaggg ttctgccaga
 1441 gaggggccc gaagactaaa gcttcaatat aacgtgacta caactagatt agtgaatcca
 1501 gatcaactgt tgaatgaaat aatgtctatt ctcccaaaga agcatgttga ctttgtacaa
 1561 aagggttata cactgaagtg tcaaacacag tcagattttg ggaaagtgac aatgcaattt
 35 1621 gaattagaag tctgccagct tcaaaaaccc gatgtggtgg gtatcaggag gcagcggctt
 1681 aagggcgatg cctgggttta caaaagatta gtggaagaca tctatctag ctgcaaggta
 1741 taa

Human MELK (isoform 6) Amino Acid Sequence (NP_001243619.1)

40 1 mnnfsnimny mkilggycpq gelldyilsq driseeetrv vfrqivsava yvhsqgyahr
 61 dlkpenllfd eyhklklidf glcakpkgnk dyhlgtccgs layaapelilg gksylgsead
 121 vwsngillyv lmcgflpfdd dnmalykki mrgkydvpkw lspssillilg qmlqvdpkkr
 181 ismknllnlp wimgdynypv ewqsknpfi hlddovtels vhhnnrqtgm edllslwqyd
 241 hitatyllil akkargkpvr lrissfscgq asatpftdik snnwaledvt asdknyvagi

301 idydwoeddl stgaatprts qftkywtesn gvesksitpa lcrtpankik nkenvytpks
 361 avkneeyfmf pepktpvnkn qhkrellttp nryttpskar ngolketpik ipvnstgtdk
 421 lmtgvisper rcrsveidl n qahmeetpkr kgakvfgsle rgldkvitvi trskrkqsar
 481 dgprrlklyhy nvttrrlvnp dqllineimsi lpkkhvdvfg kgytlkcqtg sdfgkvtnqf
 5 541 elevcqlqkp dvvgirrql kgdawvykrl vedilssckv

Human MELK (isoform 7) cDNA Sequence (NM_001256691.1)

1 atgatgaact tctcaaatat tatgaattac atgaaactat tgggacagag tgatttgccc
 61 cggatcassaa cggagattga ggccttgaag aacctgagac atcagcatat atgtcaactc
 10 121 taccatgtgc tagagacagc caacaaaata ttcattggtc ttgaggaaaa tttgctgttt
 181 gatgaatata ataaattaaa gctgattgac tttgggtctc gtgcataaac caagggtaac
 241 aaggattacc atctacagac atgctgtggg agctgtggtt atgcagcacc tgagttaata
 301 caaggcaaat catatcttgg atcagaggca gatgttttga gcatgggcat actgttatat
 361 gttcttatgt gtggatttct accatttgat gatgataatg taatggcttt atacaagaag
 15 421 attatgagag gaaaatatga tgttcccaag tggctctctc ccagtagcat tctgcttctt
 481 caacaaatgc tgcaggtgga cccaaagaaa cggatttcta tgaaaaatct attgaaccat
 541 ccttgatca tgcagatta caactatcct gttgagtggc aaagcaagaa tctttttact
 601 caactcgatg atgattgcgt aacagaactt tctgtacatc acagaaacaa caggcaacaa
 661 atggaggatt taatttcact gtggcagtat gatcacctca cggctaccta tcttctgctt
 20 721 ctagccaaga aggtcggggg aaaaccagtt cgtttaaggg tttcttcttt ctctgttga
 781 caagccagtg ctaccccatc cacagacatc aagtcaata attggagtct ggaagatgtg
 841 accgcaagtg ataaaaatta tgtggcggga ttaatagact atgattgggtg tgaagatgat
 901 ttatcaacag gtgctgctac tccccgaaca tcacagttaa ccaagtactg gacagaatca
 961 aatgggggtg aactataatc attaactcca gcttatgca gaacacctgc aaataaatta
 25 1021 aagaacaaag aaaatgtata tactcctaag tctgctgtta agaatgaaga gtaacttatg
 1081 tttcttgagc caaagactcc agttaataag aaccagcata agagagaaat actcactaag
 1141 ccaaatcgtt acactacacc ctcaaaagct agaaaccagt gcttgaagaa aactccaatt
 1201 aaaataccag taaattcaac aggaacagac aagttaatga caggtgtcat tagccctgag
 1261 aggcgggtgc gctcagtgga attggatctc aaccaagcac atatggagga gactccaaaa
 30 1321 agaaagggag ccaaagtgtt tgggagcctt gaaagggggg tggataaggt tatcactgtg
 1381 ctacaccagga gcaaaaggaa gggttctgcc agagacgggc ccagaagact aaagcttcac
 1441 tataacgtga ctacaactag attagtgaat ccagatcaac tgttgaatga aataatgtct
 1501 attcttccaa agaagcatgt tgactttgta caaaaggggt atacactgaa gtgtcaaaaa
 1561 cagtcagatt ttgggaaagt gacaatgcaa tttgaattag aagtgtgcca gcttcaaaaa
 35 1621 cccgatgtgg tgggtatcag gaggcagcgg ctttaagggc atgcctgggt ttacaaaaga
 1681 ttagtggaag acatcctatc tagctgcaag gtataa

Human MELK (isoform 7) Amino Acid Sequence (NP_001243620.1)

1 mnfnfnimny mkligqsdip rikteleaik nlrhghicql yhvletanki fmvleenlif
 40 61 deyhkiklid fgicakpkgn kdyhigtccg slayaapeli qgksyigsea dvwsmgilly
 121 vlmcgflpfd ddhvmalykk imrgkydvpk wlpssellll qgmllqvdpkk rismknlilh
 181 pwimqdynyp vewqsknpfi hldddovtel svhhrrnnrqt medllslwgy dhltatylil
 241 lakkarqkpvl rlrllsfscg qasatpftdi ksnwaledv tasdknyvag lidydwoedd
 301 lctgaapprt sqftkywtes ngvesksitp alcrtpanki knkenvytpk savkneeyfm

361 fpepktopvkn nqhkrelitt pnryttpska nqclketpi kipvnstgtd klmtgvispe
 421 rrcrsveldl ngahmeetpk rkgakvfgsl ergldkvitv ltrskrkgsa rdgprrlkih
 481 ynvtttrlvn pdqlilneims ilpkkhvdfv qkgytlikcgt qsdgkvtmq felevcqlqk
 541 pdvvgirrqr lkgdawvykr lvedilssck v

5

Human MELK (isoform 8) cDNA Sequence (NM_001256692.1)

1 atgggttcttg aggaaaattt gctgtttgat gaatatcata aattaaagct gattgacttt
 61 ggtctctgtg caaaacccaa gggtaacaag gattaccatc tacagacatg ctgtgggagt
 121 ctggccttatg cagcacctga gttatataca ggcaaatcat atcttggtac agaggcagat
 10 181 gtttggagca tgggcatact gttatatgtt cttatgtgtg gatttctacc atttgatgat
 241 gataatgtaa tggctttata caagaagatt atgagaggaa aatatgatgt tcccaagtgg
 301 ctctctccca gtagcattct gcttcttcaa caaatgctgc aggtggaccc aaagaaacgg
 361 atttctatga aaaatctatt gaaccatccc tggatcatgc aagattacaa ctatcctgtt
 421 gagtggcaaa gcaagaatcc ttttattcac ctgatgatg attgcgtaac agaactttct
 15 481 gtacatcaca gaaacaacag gcaacaatg gaggatttaa tttcactgtg gcagtatgat
 541 caoctcaagg ctacctatct tctgcttcta gccagaagg ctgggggaaa accagttcgt
 601 ttaaggcttt ctcttttctc ctgtggacaa gccagtgcta ccccatcac agacatcaag
 661 tcaataaatt ggagtctgga agatgtgacc gcaagtgata aaaattatgt ggcgggatta
 721 atagactatg atgggtgtga agatgattta tcaacagggt ctgctactcc ccgaacatca
 20 781 cagtttacca agtactggac agaatacaat ggggtggaat cttaatcatt aactccagcc
 841 ttatgcagaa cactgcacaa taaattaaag aacaaagaaa atgtatatac tcttaagtct
 901 gctgtaaaaga atgaagagta ctttatgttt cctgagccaa agactccagt taataagaac
 961 cagcataaga gagaaatact cactacgcca aatcgttaca ctacaccctc aaaagctaga
 1021 aaccagtgcc tgaaagaaac tccaattaaa ataccagtaa attcaacagg aacagacaag
 25 1081 ttaatgacag gtgtcattag ccttgagagg cgggtgocgt cagtggaaat ggatctcaac
 1141 caagcacata tggaggagac tccaaaaaga aagggagcca aagtgtttgg gagccttgaa
 1201 aggggggttg ataagggtat cactgtgctc accaggagca aaagggaagg ttctgccaag
 1261 gacgggcccc gaagaactaaa gottcactat aacgtgacta caactagatt agtgaatcca
 1321 gatcaactgt tgaatgaaat aatgtctatt cttccaaaga agcatgttga ctttgtacaa
 30 1381 aagggttata cactgaagtg tcaaacacag tcagattttg ggaaagtgac aatgcaattt
 1441 gaattagaag tgtgccagct tcaaaaaccc gatgtggtgg gstatcaggag gcagcgggct
 1501 aagggcgatg cctgggttta caaaagatta gtggaagaca tctatctag ctgcaaggta
 1561 taa

35 Human MELK (isoform 8) Amino Acid Sequence (NP_001243621.1)

1 mvleenllfd eyhklklidf glcakpkgnk dyhlgtccgs layaapeliq gksylgsead
 61 vwsngillyv lmogflpfdd dnvmalykki mrgkydvpkw lspssilliq qmlqvdpkkr
 121 isaknlinhp wimgdynypv ewqsknpfih ldddcvtels vbhrrnrgtm edllslwqyd
 181 hitatyllil akkargkpvr lrissfscgq asatpftdik snwsledvt asdknyvagi
 40 241 idydwceddl stgaatprts qftkywtesn gveskaltpa lcrtpanklk nkenvytpks
 301 avkneeyfmf pepktopvkn qhkrelitt pnryttpskar nqclketpik ipvnstgtdk
 361 lmtgvisper rrcrsveldln qahmeetpk rkgakvfgsl ergldkvitvl trskrkgsar
 421 dgprrlkih ynvtttrlvn pdqlilneims ilpkkhvdfv qkgytlikcgt qsdgkvtmqf
 481 elevcqlqk pdvvgirrqr lkgdawvykr lvedilssckv

45

Human MELK (isoform 9) cDNA Sequence (NM_001256693.1)

1 atggggcatac tgttatatgt tobtatgtgt ggattttotac catttgatga tgataaatgta
 61 atggcctttat acaagaagat tatgagagga aaatatgatg ttcccaagtg gctctctccc
 121 agtagcattc tgctttctca acasatgctg cagggtggacc caaagaaaag gattttotatg
 5 181 aaaaatctat tgaaccatcc ctggatcatg caagattaca actatcctgt tgagtggcaa
 241 agcaagaatc cttttattca cctcgatgat gattgctgaa cagaactttc tgtacatcac
 301 agaaacaaca ggcaaacat ggaggattta atttcaactgt ggcagtatga tcacctcag
 361 gctacctatc ttctgcttct agccaagaag gctcggggaa aaccagttcg tttaaggctt
 421 tobtctttct cctgtggaca agccagtgct acccattca cagacatcaa gtcaataaat
 10 481 tggagtcagg aagatgtgac cgcaagtgat aaaaattatg tggcgggatt aatagactat
 541 gattgggtgtg aagatgattt atcaacaggt gctgctactc ccgaacatc acagtttacc
 601 aagtactgga cagaatcaaa tgggggtgaa tctaaatcat taactccagc cttatgcaga
 661 acacctgcaa ataaattaaa gaacaaagaa aatgtatatc ctcttaagtc tgctgttaag
 721 aatgaagagt actttatgtt toctgagcca aagactccag ttaataagaa ccagcataag
 15 781 agagaaatac tcaactagcc aaatcgttac actacacct caaaagctag aaaccagtgc
 841 ctgaaagaaa ctccaattaa aataccagta aattoaacag gaacagacaa gttaatgaca
 901 ggtgtcatta gccctgagag gcgggtgcgc tcagtggaat tggatctcaa ccaagcacat
 961 atggaggaga ctccaaaaag aaaggggagc aaagtgtttg ggagccttga aaggggggtg
 1021 gataagggtt tcaactgtgt caccaggagc aaaaggaagg gttctgccag agacggggcc
 20 1081 agaagactaa agcttcaact taactgtact acaactagat tagtgaatcc agatcaactg
 1141 ttgaatgaaa taatgtctat tobtccaaag aagcatgttg actttgtaca aaagggttat
 1201 acactgaagt gtcaaacaca gtcagatttt gggaaagtga caatgcaatt tgaattagaa
 1261 gtgtgccagc ttcaaaaacc cgatgtgtgt ggtatcagga ggcagcggct taaggcgcat
 1321 gcctggggtt acaaaagatt agtggagagc atctatctc gctgcaaggt ataa
 25

Human MELK (isoform 9) Amino Acid Sequence (NP_001243622.1)

1 mgillyvlmc gflpfdddnv malykkimrg kydvpkwlsp ssllllqgmI qvdppkkrism
 61 knlinhpwim qdynypvewq sknpfihidd dovtelshhh rnnrqtmedi islwqydhlt
 121 atylliliakk argkpvrllri ssfscggasa tpftdiksm wledvtasd knyvaglidy
 30 181 dwceddlstg aatprtsqft kywtesngve skaltpalor tpanklknke nvytpksavk
 241 neeyfmfpep ktpvknqhk reilttpnry ttpakarnqc lketpikipv netgtdkimt
 301 gvisperrcr sveldingah meetpkrkga kvfgslergl dkvitvltrs krkgsardgp
 361 rrlklhynvt ttrlvnpdql lneimsilpk khvdfvqkgy tlkqqtqsdg gkvtnqfele
 421 vcqlqkpdvv girrgrlkgd awvykrlved ilssckv
 35

Mouse MELK cDNA Sequence (NM_010790.2)

1 atgaaagatt atgacgaact cctcaaatac tatgaactat atgaaacgat tgggacaggt
 61 ggctttgcaa aggtcaaaact ggcttgccat gtctcactg gagagatagt agctataaaa
 121 atcatggata agaatgcgtt agggagtgat ttgccccgag tcaaaactga gatcgatgag
 40 181 ctgaagagtc tgagacatca gcacatatgt cagctctacc atgtgctgga gacaaagaac
 241 aaaatattca tggttctgga gtactgtcca ggaggagagc tgtttgacta cataatctcc
 301 caggatcgcc tgtcggaaga ggagaccggg gtcgtcttcc gtcagatact gtctgcagtt
 361 gcgtatgtcc acagccaggg ctatgccac agggacotca aaccagaaaa ttattatttt
 421 gatgaaaatc ataagctaaa gctgattgac tttggtcttt gtgcaaaacc caaggggcaac
 45 481 aaggactacc atctgcagac gtgctgtggg agccttgctt atgcagctcc tgaactaata

541 caaggggaagt cgtaccttgg atcagaggca gatgttttga gcatgggcat cctcctgtat
 601 gtgctcatgt gtggatttct accatttgat gatgataatg tcatggcttt gtacaagaag
 661 ataatgagag ggaaatacga agttcctaag tggctctctc ccagtagcat tctgtctctc
 721 cagcagatgt tgcagggtga cccaaagaaa cggatttcta tgagaaatct cctgaaccat
 5 781 cccgtgggtca tgcagatta cagctgtccc gtggagtggc aaagcaagac tcccttgact
 841 cactcogatg aggattgcgt gacagagctt tctgtacatc accgcagcag caggcagaca
 901 atggaggatt taatttcgtc gtggcagtao gatcacctca cagccaccta ccttctgctt
 961 ctagccaaga agggccgggg gaagccggct cgtctacaga tctgtcctt ctcttggtga
 1021 accgccagca ccactccaaa gtcaagaat ctgagcctgg aagatatgag cacaagtgat
 10 1081 gataactgtg tggctggatt gatagactat gaattgtgtg aagataaatt attagctccc
 1141 aagacgccac aggttaccac acacttggca gaatcaatc accgcagcatc taaatcccca
 1201 gcgccagggg tacgcagagc agtggcaaat aaattaatgg acaaagaaaa tgtgtgcact
 1261 cccaagtctt ctgtgaagaa tgaagagcag tttgtatctt ctgagccgaa gattccagtt
 1321 agtaagaacc agtataagag agaaataccc gcctcaccac cccgtttccc aacacotgca
 15 1381 aaagctagag cccagtgcct gagagaagcc cccgttagaa caccagggaa ttccgcagga
 1441 gcagacacac taacgacagg tctcattagc cccgagagga ggtgccgttc aatggacgtg
 1501 gatctcaacc aggcacacat ggaggatacc ccgaaaaaga aaggaaccaa tgtgtttggg
 1561 agccttgaga gaggactgga taaggttctc actgcgctca caaggaacaa gaagaagggc
 1621 tctgccagag atggaccaag aaagcgaaag ctgcactaca atgtgactac aactcgcctg
 20 1681 gtgaaccccg accagctccc gagcgaaatc atggctattc ttccaaagaa gaacgtggac
 1741 ttgttacaga aaggttacac tctaaagtgt caaacgcagt ctgattttgg caaagtgaca
 1801 atgcagtttg aactggaagt gtgccagctg cagagacctg acgtggtagg catccggaga
 1861 cagcggctga aggtgatgc ctgggtttac aagagattag tgaagatat cttgtctgga
 1921 tgcaagatgt ga

25

Mouse MELK Amino Acid Sequence (NP_034920.2)

1 mkdydeliky yelyetigtg gfakvklach vltgemvaik imdknalgsd lprvkteida
 61 lkslrhghic glyhvletkn kifmwleycp ggelfdyils qdriseeetr vvfrqilsav
 121 ayyvhsqgyah rdlkpenilf denhklklid fgicakpkgn kdyhlqtccg slayaapeli
 30 181 ggksylgsea dvwsngilly vlmcgflpfd ddnvmalykk imrgkyevpk wlspsilili
 241 qgmigvdpkk rismrnilnb pwvmgdyscp vewgsktplt hidedcvtel svhhrsrqg
 301 medlisswgy dhltatylili lakkargkpa siqlilafscg taattpkakn lsledmsted
 361 dncvagliidy elcedkilap ktpqvtkhia eanhaaskap apgvrravan klmdkenvct
 421 pkssvkneeq fvfsepkipv skngykreip asptriptpa karaqclrea pvrtpgnsag
 35 481 adtlittgvis perrcrsmdv dlnqahmedt pkkkgtnvfg slergldkvi taltrnkckg
 541 sardgprkrk lhynvttrri vnpdqlisei mailpkknvd fvqkgytiko gtqsdfigkvt
 601 mgfelevcql qrpdvvgirr qrlkgdawvy krlvedilsg ckm

Human eIF4B cDNA Sequence (NM_001417.4)

40 1 atggcggcct cagcaaaaaa gaagaataag aaggggaaga ctatctccct aacagacttt
 61 ctggctgagg atgggggtac tgggtggagga agcacctatg ttccaaaacc agtcagctgg
 121 gctgatgaaa cggatgacct ggaaggagat gtttcgacca cttggcacag taacgatgac
 181 gatgtgtata gggcgcctcc aattgacctc tccatccttc ccactgctcc acgggctgct
 241 cgggaaccca atatcgaccg gagccgtctt cccaaatcgc caccctacac tgcttttcta
 45 301 ggaacacctac cctatgatgt tacagaagag tcaattaagg aattctttcg aggattaaat

361 atcagtgacg tgcgtttacc acgtgaaccc agcaatccag agagggttgaa aggttttggg
 421 tatgctgaat ttgaggacct ggattccctg ctcagtgccc tgagttctca tgaagagtct
 481 ctaggtaaca ggagaattcg agtggacggt gctgatcaag cacaggataa agacagggat
 541 gatcgttctt ttggccgtga tagaaatcgg gattctgaca aaacagatac agactggagg
 5 601 gctcgtcctg ctacagacag ctttgatgac taccaccta gaagagggtga tgatagcttt
 661 ggagacaagt atcgagatcg ttatgattca gaccggtatc gggatgggta tcgggatggg
 721 tatcgggatg gccacgccc ggatatggat cgatatgggt gccgggatcg ctatgatgac
 781 cgaggcagca gagactatga tagaggctat gattcccgga taggcagtgg cagaagagca
 841 tttggcagtg ggtatcgacg ggatgatgac tacagaggag ggggggaccg ctatgaagac
 10 901 cgatatgaca gacgggatga tcggtcgttg agctccagag atgattactc tcgggatgat
 961 tataggcgtg atgatagagg tcccccccaa agaccacaac tgaatctaaa gctcggagt
 1021 actcctaagg aagatgattc ctctgctagt acctcccagt ccactcgagc tgcttctatc
 1081 tttggagggg caaagcctgt tgacacagct gctagagaaa gagaagtaga agaacggcta
 1141 cagaaggaac aagagaagtt gcagcgtcag ctggatgagc caaaactaga acgacggcct
 15 1201 cgggagagac acccaagctg gcgaagtga gaaactcagg aacgggaacg gtcgaggaca
 1261 ggaagtgagt catcacaaac tgggacctcc accacatcta gcagaaatgc acgaaggaga
 1321 gagagtgaga agtctctaga aaatgaaaca ctcaataagg aggaagattg ccactctcca
 1381 acttctaaac ctcccaaac tgaatcagcc cttaaaggtaa tgcagcccc ccaccaaaag
 1441 gagaatgctt ggggtgaagcg aagttctaac cctcctgctc gatctcagag ctacagacac
 20 1501 gagcagcagt cccctacaag tgggtggggg aaagtagctc cagctcaacc atctgaggaa
 1561 ggaccaggaa ggaaagatga aaataaagta gatgggatga atgccccaaa agggccaaact
 1621 gggaactcta gccgtggtcc aggagacgga gggaacagag accacgggaa ggagtcagat
 1681 aggaagatg gcaaaaagga tcaagactcc agatctgcac ctgagccaaa gaaacctgag
 1741 gaaaatccag cttccaagtt cagttctgca agcaagtatg ctgctctctc tgttgatggg
 25 1801 gaagatgaaa atgagggaga agattatgcc gaatag

Human eIF4B Amino Acid Sequence (NP_001408.2)

1 maasakkknk kgktisltdf laedgggtggg styvskpvsu adetddlegd vsttwhsndd
 61 dvyrappidr silptapraa repnidrsrl pkeppytatf gnlpydvtee sikeffirgin
 30 121 isavrlprep snperlkgfg yaefedldsl lsalslnees lgnrrirvdy adqagdkdrd
 181 drsfgrdrnr dsdkttdtwr arpatdsfdd ypprrgddsf gdkyrdryds dryrdgyrdg
 241 yrdgprrdmd ryggrdrydd rgendydrgy dsrigegrra fgsgyrrddd yrgggdryed
 301 rydrddrsw srrddysrdd yrrddrgppq rpklnikprs tpkeddssas tsqstraasi
 361 fggakpydta arereveeri qkeqeklgrg ldepklerrp rerhpswre etqerersrt
 35 421 gsessqtgts ttssrnarrr esekslenet lnkeedchsp tskppkpdqp lkvmppppk
 481 enawvkrrsn pparsqasdt eqqsptsggg kvapaqpsee gpgkdenkv dgmnapkgqt
 541 gnssrgpgdg gnrhwhkesd rhdgkkdqd ssaepkpkpe enpaskfssa skyaaalsvdg
 601 edenegedy a

40 Mouse eIF4B cDNA Sequence (NM_145625.3)

1 atggcgggct cagcaaaaaa gaagaataag aaggggaaga ccattctcct aacggacttt
 61 ctagctgagg atggagggaac tgggtggagga agcacctatg tccccaaacc agtcagctgg
 121 gctgatgaaa cagacgatct ggaaggagat gtgtcaacaa cttggcatag taacgatgat
 181 gacgtgtaca gggcgctcc aattgacctc tccattcttc ccactgctcc acgggctgct

241 egggaaccca atattgacog gagecgtott cccaagtcgc caccctacac tgcttttcta
 301 gggaatctgc cctatgatgt gacagaagac tccattaagg atttcttttag aggattaaat
 361 atcagcgttg tacgcttacc acgggaaccc agaatccag acaggttgaa aggtttcggo
 421 tacgcagaat ttgaggacct ggattctctg ctcaagtgcg tgagtctcaa tgaagagtct
 5 481 ctaggttaaca ggagaattcg tgtggatgtt gctgatcaag cacaggataa agacagggat
 541 gaccgttctt ttggtcgaga tagaaatcgg gattctgaca aaacagacac agactggagg
 601 gcccgctcca ccacagacag ttttgatgac taccaccta gaagaggcga tgatagcttt
 661 ggagacaagt atcgagatcg ttaagattca gaccggtatc gggatgggta tagggacgga
 721 tatcgggacg gccacgcag agacatggac cgtatgggg gccgggatcg ctatgatgac
 10 781 cgaggcagca gagactatga ccgaggctat gactccagga taggcagtgg cagaagggca
 841 tttggaagtg ggtaccggag agatgatgac tacagaggag gtggggaccg ctatgaagac
 901 cgtatgaca gacgggatga tcggctcgtg agctccaggg atgactactc tcgggatgat
 961 tataggcgtg atgacagagg tcccccccag agaccagac tgaacctcaa gctcgaagc
 1021 gctcctaagg aggatgaogc ctccgccagc acctcccagt ccagccgggc agctccatc
 15 1081 tttggagggg cgaagcctgt tgacacagct gctagggaaa gagaagtaga ggagcggcta
 1141 cagaaggagc aggagaagct gcagcgtcag ctgggatgagc caaaactaga ccgcccggcc
 1201 cgggagagac acccaagctg gcgaagtga gaaactcagg aaagagaacg gtcaaggaca
 1261 ggaagtgagt catcgacagc tggggcctca gccacatctg gcagaaatac acgaaggaga
 1321 gagagtgaga agtctctaga aaatgaaaac ctcaataaag aagaagactg tcaactctca
 20 1381 acctctaagc ctctaaacc tgaccagcct ctaaaggtaa tgccagcccc tccaccaag
 1441 gagaatgctt gggatgaagc aagctctaac cctcccgccc gatctcagag ctacacaca
 1501 gagcagcgtt cccctacaag tggatggagg aaagtactg cagtccagcc cctgaggaa
 1561 ggaccatcaa gaaaagatgg aaataaagtg gatgtggtg gggccacaca aggcacagct
 1621 ggaagctgca gccgtggctc cggggatgga gggagcagag accactggaa ggaactggat
 25 1681 aggaaggatg gcaaaaaaga tcaagactcc agatctgcgc ctgagccaaa gaaacctgag
 1741 gagaacccag cctctaagtt cagctctgca agcaagtacg ctgctctgtc tgtggatggc
 1801 gaggatgagg atgagggcga cgactgcact gaggtag

Mouse eIF4B Amino Acid Sequence (NP_663600.2)

30 1 maasakkkknk kgktisltdf laedgggtggg styvpkpvsw adetddlegd vattwhsndd
 61 dvyrappidr silptapraa repnidrsrl pkspyytafl gnlpdyvted sikdffrgln
 121 isavnlprep snpdrkkgfg yaefedldsl lsalslnees lgnrrirvdy adgaqdkdrd
 181 drsfgrdrnr dsdktdtdwr arpttdsfdd ypprrgddsf gdkyrdryds dryrdgyrdg
 241 yrdgprrdmd ryggrdrydd rgsrdyargy dsrigsgrra fgsgyrrdd yrgggdryed
 35 301 rydrrddrsw srrddysrdd yrrddrgppq xprlnikprs apkeddasa tsqssraasi
 361 fggakpvdta arereveeri qkeqeklqrq ldepklrrp rerhpswrse etqererert
 421 gsessqtgas atsgrrnrrr esekalenet lnkeedchsp tspppkpdp lkvmppppk
 481 enawvkrsen pparsqssdt eqspstsggg kvaavqppee gparkdgnkv dvvgatggga
 541 gsasrgpgdg gsrhskdld rhdgkkgdgs rsapepkpe enpaskfssa skyaaalvdg
 40 601 ededegddct e

Monkey eIF4B cDNA Sequence (NM_001195808.1)

1 ctctcccaac atggcggcct cagcaaaaaa gaagaataag aaggggaaga ctatctccct
 61 aacagacttt ctggctgagg atgggggtac tggatggagg agcaactatg tttccaaacc

121 agtcagctgg gctgatgaaa cggatgacct ggaaggagat gtttcaacaa cgtggcacag
 181 taatgacgac gatgtgtaca gggcgctcc aattgacctt tccatcttcc ccaatgctcc
 241 acgggctgct cgggaaccca atatcgaccg gagcctgtctt cccaaatcgc caccctacac
 301 tgcttttcta ggggaacctac cctatgatgt gacagaagaa tcaattaagg aattcttttag
 5 361 aggattaaat atcagtgcag tgcgtttacc acgtgaaccc agcaatccag agagggttga
 421 aggttttggg ttagctgaat ttgaggacct ggattccctg ctcagtgcc ctagtctcaa
 481 tgaagagctc ctaggtaaca ggagaattcg agtggacgtt gctgatcaag cacaggataa
 541 agacagggat gatcgttctt ttggcctgta tagaaatcgg gattctgaca aaacagatac
 601 agactggagg gctcgtctct ctacagacag ctttgatgac taaccacctc gaagaggtga
 10 661 tgatagcttt ggagacaagt atcgagatcg ttatgattca gaccggtatc gggatgggta
 721 tcgggatggc ccaocccggg atatggatcg atatgggtggc cgggatcgtt atgatgaccg
 781 aggcagcaga gactatgata gaggctatga ttcccgata ggcagtggca gaagagcatt
 841 tggcagtggg taccgcaggg atgatgacta cagaggaggc ggggaccgat atgaagaccg
 901 atacgacaga cgggatgatc ggctcgtggg ctccagagat gattactctc gggatgatta
 15 961 taggcgogat gacagaggto cccctcaaag acccaactg aatctaaagc ctccgagtac
 1021 tccaaaggaa gatgattcct ctgctagtag ctcccagtc agtcagagctg cttctatctt
 1081 tggaggggca aagcctgttg acacagctgc tagagaaga gaagtagaag aacggctaca
 1141 gaaggaacaa gagaagtggc agcgtcagct ggatgagcca aaactagaac gacggcctcg
 1201 ggagagacac ccaagctggc gaagtgaaga aactcaggaa cgggaacggc cgaggacagg
 20 1261 aagtgagtca tcacagactg ggacctccgc cacatctggc agaaatgcac gaaggagaga
 1321 gagtgagaag tctctagaaa atgaaacact caataaggag gaagattgac actctccaac
 1381 ttctaaacct cccaaacctg atcagccctt aaaggtaatg ccagcccttc caccaaagga
 1441 gaatgcttgg gtgaagcgaa gtcttaaccc tccagctcga tctcagagct cagacacaga
 1501 gcagcaatcc cctacaagtg gtgggggaaa agtagctcca gctcaacct ctgagggaag
 25 1561 accagcaagg aaagatgaaa ataaagtaga tgggatgsat gtcccaaaag gccaaactgg
 1621 gaacctctagc cgtggaccag gagacggagg gaacaaagac cactgggaag agtcagatag
 1681 gaaagatggc aaaaaggatc aagactccag atctgcacct gagccaaaga aacctgagga
 1741 aaatccagct tcgaagtcca gtcttgcaag caagtatgct gctctctctg ttgatgggtg
 1801 agatgaaaac gagggagaag attatgcoga atagacctct acatcctgtg ctttctccta
 30 1861 gttctctctc acctggaac attcagagag aatcaaaac cctatccag acaagacaaa
 1921 ataaaactca ccatctcttg aagaccttct ttaccttttt ttaaaaacaa aaatgaaat
 1981 tatcttgcac gctgctgcag cctttaaagt attaaagtaa ctggagaatc gccaatatag
 2041 ccagagagaa agggactaca gcttttttag ggaagagttg tgggtgtgta

35 Monkey eIF4B Amino Acid Sequence (NP_001182737.1)

1 maasakkknk kgktisltdf laedgggtgg styvskpvs adetddlegd vsttwhsdd
 61 dvyrappidr silptapnaa repnidrrrl pkpppytafl gnlpydvtee sikeffrgln
 121 isavrlprep snperlkqfg yaefedldsl lsalslnees lgnrrirvdr adqagdkdrd
 181 drsfgrdrnr dsdktdtdwr arpatdsfdd ypprrgddsf gdkyrdryds dryrdgyrdg
 40 241 prrdmdrygg rdryddrgsr dydrgydsri gsgrrafgsg yrrdddyrgg gdryedrydr
 301 rddrawssrd dysrddyrrd drgppqrpkl nikprstpkc ddssastsgs sraasifgga
 361 kpvdtarar eveerlqkeq eklqrqldep klerrprerh pwrseetge rersrtges
 421 sqtgtsatsg rnarrresek slenetlnke edchsptskp pkpdqplkvm pappphenaw
 481 vkrssnppar sqssdteqqs ptsgggkvap aqpseegpar kdenkvdgmn vpkqgtgtss

541 rgpgdggnkd hwkesdrkdg kkdqdersap epkkpeenpa skfssaskya alsvdgeden
601 egedyae

Cow eIF4B cDNA Sequence (NM_001035028.2)

5 1 atggcggcct cagcgasaaa gaagaataag aaggggaaga ctatctccct aacagacttt
61 ctggctgagg atggagggac tggtaggagg agcacctatg tccccaaacc agtcagctgg
121 gctgatgaaa cagacgatct ggaaggggat gtttcaacca ctgggcatag taatgatgat
181 gatgtgtatc gggcacctcc aattgacctg tccatcctgc ccactgctcc acgggctgct
241 cgggaaccca atatcgaccg gaggcgtctt cccaaatctc caccctacac tgcctttcta
10 301 gggaacctgc cctatgatgt gacagaagac tccattaagg aattctttag aggattaaat
361 atcagtgcag tgcgtttacc gcgtgaaccc agcaatcctg agaggttaaa aggttttggt
421 tatgcagagt ttgaggacct ggattccttg ctacgtgctt tgagcctcaa cgaagagtct
481 ctaggtaaca ggagaattcg agtggacctt gctgatcaag cacaggataa agacagggat
541 gatcgttctt ttggccgaga tagaaatcgt gattctgaca aaacagatac agactggagg
15 601 gcccgctcctg ctgcagacag ctttgatgac taccgcgcca gaaggggtga tgatagcttt
661 ggagacaagt atcgagatcg ttacgattca gacagatata gtgatgggta tggggacagt
721 taccgtgatg gccacgcccg ggacatggat cgatacgggg gccgagatcg ctatgatgac
781 cgaggtggca gagactatga cagaggctac gattccagga taggcagtgg cagaagagca
841 ttoggtagcg ggtaccggag ggatgatgac tacagaggag gcggggaccg ctatgaagac
20 901 agatacgaca gacgagatga ccggtcctgg agttccagag atgattactc tggggatgat
961 tacaggcggg atgatagagg tccccctcaa agacccaaac tgaacctaaa gcctcggagt
1021 actcctaagg aagatgatto ctccgctagc acctcccagt ccagtctgct agcctctatc
1081 tttaggagggg caaagcctgt tgacacagct gctagagaac gagaagtaga agagcggcta
1141 cagaaggaac aggagaaact gcagcgtcag ctggatgagc caaaactaga acgacggcct
25 1201 cgggagagac acccaagctg gcgaagtga gaaactcagg aacgggaacg atcgaggaca
1261 ggaagtgagt catcacagac tgggacctca gccacatctg gcagaaatgc aagaagaaga
1321 gagagtgaga agtctttaga aaatgaaacc cccaataaag aggaagactg tcagtctcca
1381 acttctaagg ctccccaaac tgaacagcct ctaaaggtaa tgccagcccc tccaccaaa
1441 gagaatgctt gggtagaagg aagttctaac cctcctgctc gatctcagag ctccagacaca
30 1501 gacgagcagt cccctacaag tggtaggagg aaagtagttc cagctcaact atctgaggaa
1561 ggatcagcaa ggaagatga aaataaagta gatggggtga gtgccccaaa aggccaaagt
1621 gggagctcca gccgtggctc gggagatggg gggaacaaag accactggaa ggaggcagac
1681 aggaagatg gcaaaaagga tcacgactcc agatctgcac ctgagccaaa gaaagctgaa
1741 gaaaatccag cctcgaagtt cagatctgca agcaagtagc ctactctcgc cattgacggt
35 1801 gaagatgaaa atgagggaga ttacaccgaa tag

Cow eIF4B Amino Acid Sequence (NP_001030200.1)

1 maasakkknk kgktisltdf laedgggtgg styvpkpvsw adetddlegd vettwhsddd
61 dvyrappidr silptapnaa repnidrsrl pkspyytafl gnlpydvted sikeffrgln
40 121 isavrlprep snperlkqfg yaefedlds1 lsalsinees lgnrrirvdy adgaqdkdrd
181 drsfgrdrnr dsdkttdtwd arpaadsfdd ypprrgdsf gdkyrdryds dryrdgyrds
241 yrdgprxrdm ryggrrdrydd rggrrdydgy dsrlsgsrra fgsgyrrddd yrgggdryed
301 rydrxrdrsw serddydrdd yxrddrgppq rpklnlkprs tpkeddsas tsqsaraasi
361 fggakpvdaa arereveerl qkeqeklgrq ldepklerrp rerhpswrse etqerersrt

421 gsessqtgts atagrnarr esekslenet pnkeedcosp tskppkpeqp lkvmpppppk
 481 enawvkresn pparsqssdt eggspteggg kvvpaglsee gsarkdenkv dgvsapkggs
 541 gsssrqpgdg gnkdhwead rkdqkkdhds rsapepkkae enpaskfrsa skyatlaidg
 601 edenegdyte

5

Rat eIF4B cDNA Sequence (NM_001008324.1)

1 atggcggcct cagcaaaaaa gaagaataag aaggggaaga ccctctccct aacagacttt
 61 ctagctgagg atgggggaac tggtaggagg agcacctatg tccccaaacc agtcagctgg
 121 gctgatgaaa cagacgatct ggaaggagat gtgtcaacaa cttggcatag taacgatgac
 10 181 gatgtgtaca gggcacctcc tattgaccgt tccatccttc ccaactgctcc acgggctgct
 241 cgggaaccca atattgatcg gagccgtctt cccaagtcac caccctaac tgccttctca
 301 gggaatctgc cctatgatgt gacagaagac tctattaagg atttctttag aggattaaat
 361 atcagcgttg tacgcttgcc gcgtgagccc agcaatccag acaggttgaa aggttttggc
 421 tatgccgaat ttgaggatct ggattctctg ctacgtgctc tgagtctcaa tgaagagtct
 15 481 ctaggttaaca ggagaattcg ggtggatgtt gctgatcaag cacaggataa agacagggat
 541 gaccgttctt ttggtcgaga tagaaatcgg gattctgaca agacagacac agactggagg
 601 gcccgctcctg ccacagacag ctttgatgac taccaccta gacgaggtga tgacagcttc
 661 ggagacaagt atcgagatcg ttaagagtcg gacgggtatc gggatgggta tagggacgga
 721 tatcgggacg gccacgcag agacatggac cgttatgggg gccgggatcg ctatgatgac
 20 781 cgaggcagca gagactatga ccgaggttat gactccagga taggcagtgg cagaagagca
 841 tttggaagtg ggtaccggag ggatgacgac tacagaggag gtggggaccg ctatgaagat
 901 cgctatgaca gacgggacga tcgggtcatg agtccaggg acgattactc tggggacgat
 961 tacaggcgtg atgacagagg tcccccccaa agacccaaac tgaatctaaa gctcggagt
 1021 actcctaaag aagatgattc ctctgctagc acctcccagt ccagccgagc ggcttctatc
 25 1081 tttggagggg cgaagcctgt tgacacagct gctagagaaa gagaagtaga ggagcggcta
 1141 cagaaggagc aggagaagct gcagcgtcag ctggatgagc caaaaactaga ccgccggccc
 1201 cgggagagac acccaagttg gcgaagtga gaaactcagg aaagagaacg gtcgaggaca
 1261 ggaagtgagt catcgacagc tgggacctca gccacatctg gcagaaatac accaaggaga
 1321 gagagtgaga agtctctaga aaatgaaacc ctcaataaag aagaagactg tcaactctca
 30 1381 acctctaagc ctctaaacc tgaccagcct ctaaaggtaa tgccagcccc tccaccaaag
 1441 gagaatgctt gggatgaagc aagctctaac cctcctgctc gatctcagag ctacagacac
 1501 gagcagcctt cccctacaag tggtaggagg aaagtctgct cagctcagcc ctctgaggaa
 1561 ggaccatcaa ggaagatga aactaaagtg gatggggtga gccaccacaa aggcagact
 1621 ggacactcca gccgtggctc tggggatgga gggagcagag accactggaa ggagtctgat
 35 1681 aggaaggacg gcaaaaaaga tcaagactcc agatctgcac ctgagccaaa gaaatctgag
 1741 gagaaccgag cctctaagtt cagttctgca agcaagtacg ctgctctgtc tgtggacggt
 1801 gaggatgagg atgagggaga cgaactgcact gactag

Rat eIF4B Amino Acid Sequence (NP_001008325.1)

40 1 maasakkknk kgktialtdf laedgggtgg styvpkpvs adetddlegd vettwhsdd
 61 dvyrappidr silptapraa repnidrsrl pkspptaf1 gnlpdyvted sikdfrgln
 121 isavrlprep snpdrlkgfg yaefedlds1 lsalslnees lgnrrirvdv adqagdkdrd
 181 drsfgrdrnr dsdktdtdwr arpatdsfdd ypprrgdsf gdkyrdryes dryrdgyrdg
 241 yrdgprxdmd ryggdrdrydd rgsrdydrgy dsrigsgrra fgsgyrddd yrgggdryed

301 rydrrddrsw ssrddysrdd yrrddrgppq rpklnikpra tpkeddsas taqssraasi
 361 fggakpydta arereveeri qkeqeklgrq ldepklrrp rerhpswrse etqererert
 421 gsessqtgts atsgntrrrr esekslenet lnkeedchsp tskppkpdqp lkvmppppk
 481 enawvkrssn pparsqsadt eqpsptsggg kvapaqpsee gprkdetkv dgvttkggt
 5 541 ghssrgpgdg qsrhskeld rkdgkkdgdz nsapepkke enraskfssa skyaalsvdg
 601 ededegddct e

Human Histone H3.1 Amino Acid Sequence (NP_003520.1)

1 marckqtark stggkaprkq latkaarksa patggvkkph ryrpgtvalr eirrygkste
 10 61 llirklpfqr lvreiaqdfk tdlrfqssav malqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Mouse Histone H3.1 Amino Acid Sequence (NP_038578.2):

1 marckqtark stggkaprkq latkaarksa patggvkkph ryrpgtvalr eirrygkste
 15 61 llirklpfqr lvreiaqdfk tdlrfqssav malqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Human Histone H3.2 Amino Acid Sequence (NP_001005464.1):

1 marckqtark stggkaprkq latkaarksa patggvkkph ryrpgtvalr eirrygkste
 20 61 llirklpfqr lvreiaqdfk tdlrfqssav malqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Mouse Histone H3.2 Amino Acid Sequence (NP_835587.1):

1 marckqtark stggkaprkq latkaarksa patggvkkph ryrpgtvalr eirrygkste
 25 61 llirklpfqr lvreiaqdfk tdlrfqssav malqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Human Histone H3.3 Amino Acid Sequence (NP_002098.1):

1 marckqtark stggkaprkq latkaarksa pstggvkkph ryrpgtvalr eirrygkste
 30 61 llirklpfqr lvreiaqdfk tdlrfqsaai galqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Mouse Histone H3.3 Amino Acid Sequence (NP_032237.1):

1 marckqtark stggkaprkq latkaarksa pstggvkkph ryrpgtvalr eirrygkste
 35 61 llirklpfqr lvreiaqdfk tdlrfqsaai galqeaceay lvglfedtnl caihakrvti
 121 mpkdiqlarr irgera

Nucleic acid and protein molecules (*e.g.*, those of MELK, eIF4B, and orthologs thereof across species) that differ due to degeneracy of the genetic code or due to encoding
 40 or having “non-essential”, “conservative”, “stereoisomers”, or “unconventional” amino acids that do not appreciably alter the enzymatic (*e.g.*, kinase) and/or eIF4B Ser-406-regulatory ability of MELK are included within the scope of the invention. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Stereoisomers (*e.g.*, D-amino acids) of the twenty

conventional amino acids, unnatural amino acids such as alpha,alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides described herein. There is a known and definite correspondence between the amino acid sequence of a particular protein and the nucleotide sequences that can code for the protein, as defined by the genetic code (shown below). Likewise, there is a known and definite correspondence between the nucleotide sequence of a particular nucleic acid and the amino acid sequence encoded by that nucleic acid, as defined by the genetic code.

10 GENETIC CODE

Alanine (Ala, A)	GCA, GCC, GCG, GCT
Arginine (Arg, R)	AGA, ACG, CGA, CGC, CGG, CGT
Asparagine (Asn, N)	AAC, AAT
Aspartic acid (Asp, D)	GAC, GAT
15 Cysteine (Cys, C)	TGC, TGT
Glutamic acid (Glu, E)	GAA, GAG
Glutamine (Gln, Q)	CAA, CAG
Glycine (Gly, G)	GGA, GGC, GGG, GGT
Histidine (His, H)	CAC, CAT
20 Isoleucine (Ile, I)	ATA, ATC, ATT
Leucine (Leu, L)	CTA, CTC, CTG, CTT, TTA, TTG
Lysine (Lys, K)	AAA, AAG
Methionine (Met, M)	ATG
Phenylalanine (Phe, F)	TTC, TTT
25 Proline (Pro, P)	CCA, CCC, CCG, CCT
Serine (Ser, S)	AGC, AGT, TCA, TCC, TCG, TCT
Threonine (Thr, T)	ACA, ACC, ACG, ACT
Tryptophan (Trp, W)	TGG
Tyrosine (Tyr, Y)	TAC, TAT
30 Valine (Val, V)	GTA, GTC, GTG, GTT
Termination signal (end)	TAA, TAG, TGA

An important and well known feature of the genetic code is its redundancy, whereby, for most of the amino acids used to make proteins, more than one coding nucleotide triplet may be employed (for example, illustrated above). Therefore, a number

of different nucleotide sequences may code for a given amino acid sequence. Such nucleotide sequences are considered functionally equivalent since they result in the production of the same amino acid sequence in all organisms (although certain organisms may translate some sequences more efficiently than they do others). Moreover, 5 occasionally, a methylated variant of a purine or pyrimidine may be found in a given nucleotide sequence. Such methylations do not affect the coding relationship between the trinucleotide codon and the corresponding amino acid. In addition, a skilled artisan will understand how to mutate nucleotides of a specific codon so as to specifically alter an encoded amino acid based on the relevant codon chart. Additional desired nucleic acid 10 and/or amino acid modifications can be engineered using site-directed mutagenesis and PCR-mediated mutagenesis techniques.

The “nucleic acid” can take any of a number of forms (*e.g.*, DNA, mRNA, cDNA) that encode a biomarker described herein. For example, such biomarker nucleic acid molecules include DNA (*e.g.*, genomic DNA and cDNA) comprising the entire or a partial 15 sequence of a desired gene or the complement or hybridizing fragment of such a sequence. The biomarker nucleic acid molecules also include RNA comprising the entire or a partial sequence of a desired gene or the complement of such a sequence, wherein all thymidine residues are replaced with uridine residues. A “transcribed polynucleotide” is a polynucleotide (*e.g.*, an RNA, a cDNA, or an analog of one of an RNA or cDNA) which is 20 complementary to or homologous with all or a portion of a mature RNA made by transcription of a biomarker of the present invention, at least in part, and normal post-transcriptional processing (*e.g.*, splicing), if any, of the transcript, and reverse transcription of the transcript.

The terms “homology” or “identity,” as used interchangeably herein, refer to 25 sequence similarity between two polynucleotide sequences or between two polypeptide sequences, with identity being a more strict comparison. The phrases “percent identity or homology” and “% identity or homology” refer to the percentage of sequence similarity found in a comparison of two or more polynucleotide sequences or two or more polypeptide sequences. Two or more sequences can be anywhere from 0-100% similar, or any integer 30 value there between. Identity or similarity can be determined by comparing a position in each sequence that may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same nucleotide base or amino acid, then the molecules are identical at that position. A degree of similarity or identity between

polynucleotide sequences is a function of the number of identical or matching nucleotides at positions shared by the polynucleotide sequences. A degree of identity of polypeptide sequences is a function of the number of identical amino acids at positions shared by the polypeptide sequences. A degree of homology or similarity of polypeptide sequences is a function of the number of amino acids at positions shared by the polypeptide sequences. The term "substantial homology" refers to homology of at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more (e.g., about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more). In one embodiment, biomarker nucleic acid molecules encode a protein or portion thereof which includes an amino acid sequence which is sufficiently homologous to an amino acid sequence described herein such that the protein or portion thereof maintains, for example, the ability to phosphorylate eIF4B, to phosphorylate Histone H3, and/or the ability to be phosphorylated by MELK.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. The alignment can be performed using the Clustal Method. Multiple alignment parameters include GAP Penalty =10, Gap Length Penalty = 10. For DNA alignments, the pairwise alignment parameters can be Htuple=2, Gap penalty=5, Window=4, and Diagonal saved=4. For protein alignments, the pairwise alignment parameters can be Ktuple=1, Gap penalty=3, Window=5, and Diagonals Saved=5. Similarly, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0) (available online), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Methods for the production of nucleic acids (*e.g.*, MELK, eIF4B, and/or mRNAs translated from nucleic acids having structured 5' regions are known in the art and include standard hybridization, PCR, and/or synthetic nucleic acid techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.

A "biomarker protein" is a protein encoded by or corresponding to a biomarker of the present invention. The terms "protein" and "polypeptide" are used interchangeably herein. In one embodiment, the protein is at least 50%, 60%, 70%, 80%, 90%, and 95% or more (*e.g.*, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 10 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more) homologous to the entire amino acid sequence of a MELK and/or eIF4B and/or Histone H3 protein described herein. In addition, biologically active portions of MELK and/or eIF4B and/or Histone H3 proteins described herein are included which have at least 50%, 60%, 70%, 80%, 90%, and 95% or more (*e.g.*, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 15 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more) homology to a fragment of a MELK and/or eIF4B and/or Histone H3 protein described herein, *e.g.*, a domain or motif, and that is capable of phosphorylating eIF4B, phosphorylating Histone H3, or being phosphorylated by MELK. Typically, biologically active portions (peptides, *e.g.*, peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100, 150, 200, 250, 300, 350, 400, 20 450, or more amino acids in length) comprise a domain or motif, *e.g.*, a MELK kinase domain, eIF4B domain encompassing an amino acid residue phosphorylatable by MELK such as a MELK-mediated phosphorylation substrate motif having an arginine at -3 amino acid residue positions relative to serine/threonine (*e.g.*, Ser406 or Ser422 of human eIF4B), or Histone H3 domain encompassing an amino acid residue phosphorylatable by MELK 25 such as a human Histone H3 region comprising Ser10. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein.

Methods for the production of proteins (*e.g.*, MELK and/or eIF4B and/or Histone H3) are known in the art and include *e.g.*, the expression of the protein in appropriate cells 30 starting from a cDNA or the production by subsequent addition of amino acids to a starting amino acid (see Current Protocols, John Wiley & Sons, Inc., New York). Furthermore, methods for the production of protein fragments are known in the art and include the cleavage of the protein with appropriate proteases or the generation of nucleic acid

fragments encoding the protein fragments and subsequent expression of the fragments in appropriate cells. Methods for the production of mutated proteins, *e.g.*, by exchanging and/or deleting one or more amino acids, are known in the art.

5 **B. Diagnostic Methods**

Methods are provided for identifying agents, such as small molecules and antibodies, which inhibit oncogenic and/or kinase activity of human MELK or an ortholog thereof, comprising: a) contacting a sample comprising i) human MELK or an ortholog thereof and ii) human eukaryotic initiation factor 4B (eIF4B) or an ortholog thereof, with
10 the agent; and b) determining the ability of the agent to inhibit Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic activity of human MELK or the ortholog thereof. Similarly, methods are provided for identifying agents, such as small molecules and antibodies, which inhibit
15 oncogenic and/or kinase activity of human MELK or an ortholog thereof, comprising: a) contacting a sample comprising i) human MELK or an ortholog thereof and ii) human Histone H3 or an ortholog thereof, with the agent; and b) determining the ability of the agent to inhibit Thr-3 phosphorylation and/or Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in
20 the ortholog of human Histone H3, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic activity of human MELK or the ortholog thereof. These methods are also referred to herein as drug screening assays and typically include the step of screening a candidate/test compound or agent for the ability to interact with (*e.g.*, bind to) a MELK and/or eIF4B and/or Histone H3 protein, to modulate the phosphorylation of
25 eIF4B by MELK, to modulate the interaction of a phosphorylatable residue of eIF4B with a MELK-mediated intracellular signaling target, to modulate the phosphorylation of Histone H3 by MELK, and/or to modulate the interaction of a phosphorylatable residue of Histone H3 with a MELK-mediated intracellular signaling target. Test compounds or agents which
30 have one or more of these abilities can be used as drugs to treat disorders characterized by aberrant, abnormal, and/or unwanted MELK and/or eIF4B and/or Histone H3 nucleic acid expression and/or protein activity, such as cancer. Candidate/test compounds include, for example, small organic and inorganic molecules (*e.g.*, molecules obtained from combinatorial and natural product libraries). Similarly, antibody agents that, for example

bind to MELK in a manner that modulates phosphorylation of a residue by MELK, modulates eIF4B activity normally driven by MELK-mediated phosphorylation, and/or modulates Histone H3 activity normally driven by MELK-mediated phosphorylation, can be useful agents. The skilled artisan can also readily make other modulatory agents, such as aptamers, antisense RNA, siRNA, that are capable of interacting with MELK nucleic acids and/or proteins to affect MELK-mediated phosphorylation of eIF4B or Histone H3 (see, at least Chung et al. (2012) 3:1629-1640; WO 2013/109388; WO 2012/016082; WO 2013/045539; each of which is incorporated herein in its entirety by this reference.

The term "sample," "tissue sample," "subject sample," "subject cell or tissue sample" or "specimen" each refer to a collection of similar cells obtained from a tissue of a subject or subject either as *in vitro* (e.g., cultured), *ex vivo*, or *in vivo* (e.g., isolated primary cells) samples. The source of the tissue sample may be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, or aspirate; blood or any blood constituents; bodily fluids such as whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow, amniotic fluid, peritoneal fluid or interstitial fluid; or cells from any time in gestation or development of the subject. The tissue sample may contain compounds that are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics or the like. The sample may further comprise cancer cells, such as ovarian, lung, breast, and multiple myeloma cancer cells or any cancer in which MELK and/or eIF4B and/or Histone H3 is amplified or overexpressed, has an activating mutation, or is activated by other kinases.

The terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, e.g., a mammal including a non-primate (e.g., a cow, pig, horse, donkey, goat, camel, cat, dog, guinea pig, rat, mouse, sheep) and a primate (e.g., a monkey, such as a cynomolgous monkey, gorilla, chimpanzee and a human).

The term "inhibit" refers to a statistically significant decrease in a metric of interest, such as the reduction of Thr-3 phosphorylated and/or Ser-10-phosphorylated and/or Thr-11-phosphorylated Histone H3, Ser-406-phosphorylated eIF4B, MELK enzymatic activity (e.g., kinase activity), cancer progression, and the like. Such statistically significant decrease can be at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more relative to a control. For example, a test compound administered and analyzed according to the methods described herein can comprise a *bona fide* inhibitor of MELK enzymatic

activity (e.g., kinase activity) by decreasing Ser-406-phosphorylated eIF4B amounts, Thr-3 phosphorylated Histone H3 amounts, Ser-10-phosphorylated Histone H3 amounts, and/or Thr-11-phosphorylated Histone H3 amounts by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to that of no MELK ligand administration or over a given amount of time. In one embodiment, the term "MELK inhibitor" is a substance, such as a small molecule, antibody, antisense nucleic acid, small interfering nucleic acid, which interferes with the phosphorylation of human eIF4B at Ser-406 or at a corresponding phosphorylation site in an eIF4B ortholog thereof, the phosphorylation of human Histone H3 at Thr-3 or at a corresponding phosphorylation site in a Histone H3 ortholog thereof, the phosphorylation of human Histone H3 at Ser-10 or at a corresponding phosphorylation site in a Histone H3 ortholog thereof, and/or the phosphorylation of human Histone H3 at Thr-11 or at a corresponding phosphorylation site in a Histone H3 ortholog thereof. Exemplary MELK inhibitors are well known in the art, such as OTSSP167, siomycin A, thiostrepton, and anti-MELK antibodies are disclosed, for example, in Chung *et al.* (2012) *Oncotarget* 3:1629-1640; WO 2013/045539; WO 2013/109388; and WO 2012/016082; each of which is incorporated in its entirety herein by this reference.

The term "altered amount" of a biomarker or "altered level" of a biomarker refers to increased or decreased expression, modification, and/or activity of a biomarker of the present invention, at least in part in a sample as compared to that in a control sample.

The amount of a biomarker in a subject is "significantly" higher or lower than the normal amount of a biomarker, if the amount of the biomarker is greater or less, respectively, than the normal level by an amount greater than the standard error of the assay employed to assess amount, or at least two, three, four, five, ten or more times that amount. Alternatively, the amount of the biomarker in the subject can be considered "significantly" higher or lower than the normal amount if the amount is at least about two, at least about three, at least about four, or at least about five times, higher or lower, respectively, than the normal amount of the biomarker (e.g., in a control sample or the average expression level of the biomarkers of the present invention in several control samples).

"Likely to," as used herein, refers to an increased probability, that an item, object, thing or person will occur such as at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or more (or any range inclusive). Thus, in one embodiment, an agent that is likely to inhibit MELK-mediated phosphorylation of

eIF4B has an increased probability of inhibiting Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B. In another embodiment, an agent that is likely to inhibit MELK-mediated phosphorylation of Histone H3 has an increased probability of inhibiting Thr-3 phosphorylation, Ser-10

5 phosphorylation, and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3.

Test compounds of the present invention, at least in part, can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; 10 synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

15 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and 25 Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, the inhibition of Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B is determined 30 by comparing the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, in the sample relative to a control. The control can be the amount of amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B in the

sample relative to said amount in the absence of the agent or at an earlier timepoint after contact of the sample with the agent. The phosphorylation level of eIF4B is generally determined by measuring the amount of phosphorylated eIF4B protein and, optionally, of unphosphorylated eIF4B, and normalizing the amount of phosphorylated protein to the total protein in the sample being analyzed. The calculated response phosphorylation level in the presence of the test compound and the basal or background phosphorylation levels (e.g., in the absence of the test compound or at a earlier timepoint after test compound administration) are thus not affected by differences in the absolute quantity of the indicator protein at a given time.

The discriminatory time point, or predetermined time after administering the test compound to cells, can be selected to achieve a calibrated statistically significant difference between Ser-406 phosphorylation levels in the sample relative to controls. The difference may be maximal at the predetermined time but that is not required and depends on other parameters of the test. In addition, whereas the calculation of ratios as described herein is beneficial in providing useful comparative numbers, calculation of absolute differences between phosphorylated eIF4B levels upon administration of test compounds relative to controls, and between test subjects and control subjects, could also be employed and would be effective.

In some embodiments, the methods described above can further comprise determining the amount of determining the amount of a protein translated from an mRNA with highly structured 5' untranslated region (5'UTR), optionally wherein the protein is selected from the group consisting of cellular myelocytomatosis oncogene (c-Myc), X-linked inhibitor of apoptosis protein (XIAP), and ornithine decarboxylase (ODC1). It is known that eIF4B stimulates the helicase activity of eIF4A for unwinding the secondary structure of 5'UTR of mRNA and that eIF4B is important for the translation of mRNA with structured 5'UTR (Dmitriev *et al.* (2003) *Mol. Cell Biol.* 23:8925-8933 and Shahbazian *et al.* (2010) *Mol. Cell Biol.* 30 1478-1485). The skilled artisan is well aware of mRNA with structured 5'UTR encoding oncogenic proteins, such as c-Myc, XIAP (X-linked inhibitor of apoptosis protein), ODC (ornithine decarboxylase), VEGF, HIF-1alpha, and the like (see, at least Bert *et al.* (2006) *RNA* 12:1074-1083).

Phosphorylation is a biochemical reaction in which a phosphate group is added to Ser, Thr or Tyr residues of a protein and is catalyzed by protein kinase enzymes. Phosphorylation normally modifies the functions of target proteins, often causing

activation. As part of the cell's homeostatic mechanisms, phosphorylation is only a transient process that is reversed by other enzyme called phosphatases. Therefore, protein phosphorylation levels change over time and can be evaluated in a number of well-known manners, including, for example, by immunological approaches. For example, the amount
5 of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B. Such an immunoassay comprise a number of well known forms, including, without limitation, a radioimmunoassay, a Western blot
10 assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. General techniques to be used in performing the various immunoassays noted above and other variations of the techniques, such as *in situ* proximity ligation assay (PLA), fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA),
15 enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA), ELISA, etc. alone or in combination or alternatively with NMR, MALDI-TOF, LC-MS/MS, are known to those of ordinary skill in the art.

In one embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay
20 using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B. Such an enzyme immunoassay is particularly advantageous because identifying differences in protein levels between related kinase
25 family members or isoforms given the relatively high homology between kinases among themselves and also among their phosphorylated forms.

Immunological reagents for identifying eIF4B in both phosphorylated and non-phosphorylated forms, as well as for detecting MELK, are well known in the art and can be generated using standard techniques, such as by inoculating host animals with appropriate
30 eIF4B phosphor-peptides. Such anti-MELK, anti-eIF4B, and/or anti-phospho-eIF4B antibody reagents (e.g., monoclonal antibody) can be used to isolate and/or determine the amount of the respective proteins such as in a cellular lysate. Such reagents can also be used to monitor protein levels in a cell or tissue, e.g., white blood cells or lymphocytes, as

part of a clinical testing procedure, *e.g.*, in order to monitor an optimal dosage of an inhibitory agent. Detection can be facilitated by coupling (*e.g.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

The screening assays described above can further be adapted to identify candidate/test compounds which modulate (*e.g.*, stimulate or inhibit) the interaction (and most likely MELK and eIF4B oncogenic activity as well) between an eIF4B protein and a target eIF4B protein with which the eIF4B protein normally interacts or modulates to verify that MELK-mediated enzymatic activity has been reduced in accordance with the reduced amounts of phosphorylated eIF4B levels. Examples of such target molecules or substrates include certain protein encoded by mRNA with structured 5'UTR, such as those described further herein.

As described above for the identification of eIF4B phosphorylation, Thr-3 phosphorylation, Ser-10 phosphorylation, and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, and modulation (*e.g.*, inhibition) thereof, can similarly be determined.

In another embodiment, the invention provides assays for screening candidate/test compounds which interact with (*e.g.*, bind to) MELK and/or eIF4B and/or Histone H3 protein. "Binding compound" shall refer to a binding composition, such as a small molecule, an antibody, a peptide, a peptide or non-peptide ligand, a protein, an oligonucleotide, an oligonucleotide analog, such as a peptide nucleic acid, a lectin, or any other molecular entity that is capable of specifically binding to a target protein or molecule or stable complex formation with an analyte of interest, such as a complex of proteins. "Binding moiety" means any molecule to which molecular tags can be directly or indirectly attached that is capable of specifically binding to an analyte. Binding moieties include, but

are not limited to, antibodies, antibody binding compositions, peptides, proteins, nucleic acids and organic molecules having a molecular weight of up to about 1000 daltons and containing atoms selected from the group consisting of hydrogen, fluoride, carbon, oxygen, nitrogen, sulfur and phosphorus. Typically, the assays are cell-based assays. The cell, for
5 example, can be of mammalian origin expressing MELK and/or eIF4B and/or Histone H3, *e.g.*, a cancer cell.

In other embodiments, the assays are cell-free assays which include the steps of combining a MELK and/or eIF4B and/or Histone H3 protein or a biologically active portion thereof, and a candidate/test compound, *e.g.*, under conditions which allow for
10 interaction of (*e.g.*, binding of) the candidate/test compound to the MELK and/or eIF4B and/or Histone H3 protein or biologically active portion thereof to form a complex, and detecting the formation of a complex, in which the ability of the candidate compound to interact with (*e.g.*, bind to) the MELK and/or eIF4B and/or Histone H3 polypeptide or biologically active fragment thereof is indicated by the presence of the candidate compound
15 in the complex. Formation of complexes between the MELK and/or eIF4B and/or Histone H3 protein and the candidate compound can be quantitated, for example, using standard immunoassays. Such analyses would identify test compounds as MELK and/or eIF4B and/or Histone H3 ligands.

To perform the above drug screening assays, it can be desirable to immobilize either
20 MELK and/or eIF4B and/or Histone H3 or its target molecule to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Interaction (*e.g.*, binding of) of MELK and/or eIF4B and/or Histone H3 to a target molecule, in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants.
25 Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion polypeptide can be provided which adds a domain that allows the polypeptide to be bound to a matrix. For example, glutathione-S-transferase-MELK, -Histone H3, and/or -eIF4B fusion polypeptides can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtitre plates, which
30 are then combined with the cell lysates (*e.g.*, ³⁵S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly,

or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of MELK-, Histone H3-, and/or eIF4B-binding polypeptide found in the bead fraction quantitated from the gel using standard electrophoretic techniques.

5 Other techniques for immobilizing polypeptides on matrices can also be used in the exemplary drug screening assays of the invention. For example, MELK and/or eIF4B and/or Histone H3 or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated MELK and/or eIF4B and/or Histone H3 molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art
10 (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with MELK and/or eIF4B and/or Histone H3, but which do not interfere with binding of the polypeptide to its target molecule can be derivatized to the wells of the plate, and MELK and/or eIF4B and/or Histone H3 trapped in the wells by antibody conjugation. As
15 described above, preparations of a MELK- and/or eIF4B-binding polypeptide and a candidate compound are incubated in the MELK- and/or eIF4B- and/or Histone H3-presenting wells of the plate, and the amount of complex trapped in the well can be quantitated. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using
20 antibodies reactive with the MELK and/or eIF4B and/or Histone H3 target molecule, or which are reactive with MELK and/or eIF4B and/or Histone H3 polypeptide and compete with the target molecule; as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

In another aspect, a method for assessing the efficacy of an agent for inhibiting
25 kinase activity of human MELK or an ortholog thereof in a subject, comprising: a) detecting in a subject sample at a first point in time, the amount of Ser-406 phosphorylated human eIF4B or the amount of a human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B; b) repeating step a) during at one or more subsequent points in time after administration of the agent; and c) comparing the amount of phosphorylated
30 human eIF4B or ortholog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Ser-406 phosphorylated human eIF4B or the amount of the human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B in the first point in time relative to at least one subsequent point in time, indicates that the

agent inhibits kinase activity of MELK or the ortholog thereof, is provided. Similarly, a method for assessing the efficacy of an agent for inhibiting kinase activity of human MELK or an ortholog thereof in a subject, comprising: a) detecting in a subject sample at a first point in time, the amount of Thr-3 phosphorylated, Ser-10 phosphorylated, and/or Thr-11 phosphorylated human Histone H3, or the amount of a human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3; b) repeating step a) during at one or more subsequent points in time after administration of the agent; and c) comparing the amount of phosphorylated human Histone H3 or ortholog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Thr-3 phosphorylated, Ser-10 phosphorylated, and/or Thr-11 phosphorylated human Histone H3, or the amount of the human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3 in the first point in time relative to at least one subsequent point in time, indicates that the agent inhibits kinase activity of MELK or the ortholog thereof, is provided.

As used herein, "time course" shall refer to the amount of time between an initial event and a subsequent event. For example, with respect to a subject's cancer progression, time course may relate to a subject's disease and may be measured by gauging significant events in the course of the disease, wherein the first event may be diagnosis and the subsequent event may be proliferation, metastasis, etc.

Once binding is confirmed, additional assays, such as kinase assays to determine inhibition of phosphorylation effects, can be performed according to well-known methods in the art. For example, assays for determining MELK kinase activity are well known in the art (see, for example, the publications described herein and incorporated by reference in their entirety). Briefly, MELK can be incubated with a suitable substrate in a buffer allowing phosphorylation of eIF4B or Histone H3. Phosphorylation of the substrate can be detected using a labeled phosphate group, such as the use of the radioactive label ^{32}P present as the ATP source in the buffer. Alternatively, antibodies specific for the phosphorylated products of eIF4B catalytic activity can be used to detect activity. As will be apparent to those of ordinary skill in the art, the assays are easily amenable to high through-put technologies using robotics and automated processes. Alternatively, the MELK kinase activity can be assayed using a synthetic substrate, such as a peptide library. MELK activity can also be assayed by detecting downstream targets of the kinase such as those described herein.

Ser-406-phosphorylated eIF4B can be analyzed according to any of the methods and using any of the samples described herein (*e.g.*, single subject samples or pooled subject samples). Candidate compounds which produce a statistically significant change in phosphorylated-eIF4B-dependent responses (*e.g.*, inhibition of human eIF4B phosphorylation at Ser-406 or a corresponding phosphorylatable amino acid residue in an eIF4B ortholog thereof) can be identified. Such statistically significant changes can be measured according to a number of criteria and/or relative to a number of controls. For example, significant modulation of phosphorylation of Ser-406 can be assessed if the output under analysis is inhibited by 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 3.1-, 3.2-, 3.3-, 3.4-, 3.5-, 3.6-, 3.7-, 3.8-, 3.9-, 4.0-, 4.1-, 4.2-, 4.3-, 4.4-, 4.5-, 4.6-, 4.7-, 4.8-, 4.9-, 5.0-, 5.5-, 6.0-, 6.5-, 7.0-, 7.5-, 8.0-, 8.5-, 9.0-, 9.5-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-fold or more different (including any range inclusive), relative to a control. In one embodiment, between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

As described above for the identification and/or analysis of eIF4B phosphorylation, Thr-3 phosphorylated, Ser-10 phosphorylated, and/or Thr-11 phosphorylated Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, and modulation (*e.g.*, inhibition) thereof, can similarly be identified and/or analyzed.

The term "cancer" refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells may exist alone within an animal, or may be a non-tumorigenic cancer cell, such as a leukemia cell. As used herein, the term "cancer" includes premalignant as well as malignant cancers. Cancers include, but are not limited to, B cell cancer, *e.g.*, multiple myeloma, Waldenström's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland

cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Also included are any cancers in which the gene encoding MELK and/or eIF4B and/or Histone H3 is amplified or overexpressed, or has an activating mutation, or the MELK and/or eIF4B and/or Histone H3 is hyper-activated by other kinases. In some embodiments, ovarian cancers, including serous cystadenocarcinoma, head and neck cancers, including non-small cell lung cancer (NSCLC), squamous cell carcinoma, pancreatic cancer, colon cancer, prostate cancer, and/or gliomas can be preferred.

“Treat,” “treatment,” and other forms of this word refer to the administration of an agent that inhibits the ability of 1) MELK to phosphorylate eIF4B and/or Histone H3 and/or 2) the ability of eIF4B or Histone H3 to be phosphorylated by MELK, to cause a cancer to be ameliorated, to extend the expected survival time of the subject and/or time to progression of a cancer or the like.

“Responsiveness,” to “respond” to treatment, and other forms of this verb, as used herein, refer to the reaction of a subject to treatment with an agent capable of inhibiting the ability of 1) MELK to phosphorylate eIF4B or Histone H3 and/or 2) the ability of eIF4B or Histone H3 to be phosphorylated by MELK. As an example, a subject responds to treatment of the subject or cell thereof with an agent if the assayed condition is modulated by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to that of no administration of the agent or over a given amount of time.

C. Treatment Methods

MELK and/or eIF4B and/or Histone H3 inhibitors described herein can be used to treat cancer. In one embodiment, a method of treating a subject afflicted with cancer comprising administering to the subject an agent that inhibits Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B, for example an agent that specifically modulates Ser-406 phosphorylation, thereby treating the subject afflicted with the cancer. In another embodiment, such MELK and/or eIF4B and/or Histone H3 inhibitors can also be used to determine the efficacy, toxicity, or side effects of treatment with such an agent. These methods of treatment generally include the steps of administering modulators in a pharmaceutical composition, as described further below, to a subject in need of such treatment, *e.g.*, a subject with cancer or at risk for developing cancer.

The term "administering" is intended to include routes of administration which allow the agent to perform its intended function of inhibiting the ability of MELK to phosphorylate eIF4B, the ability of eIF4B to be phosphorylated by MELK, the ability of MELK to phosphorylate Histone H3, and/or the ability of Histone H3 to be phosphorylated
5 by MELK. Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, intrathecal, etc.), oral, inhalation, and transdermal. The injection can be bolus injections or can be continuous infusion. Depending on the route of administration, the agent can be coated with or disposed in a selected material to protect it from natural conditions which may
10 detrimentally affect its ability to perform its intended function. The agent may be administered alone, or in conjunction with a pharmaceutically acceptable carrier. The agent also may be administered as a prodrug, which is converted to its active form *in vivo*.

The term "effective amount" of an agent inhibiting the ability of MELK to phosphorylate eIF4B and/or the ability of eIF4B to be phosphorylated by MELK is that
15 amount necessary or sufficient to inhibit the ability of MELK to phosphorylate eIF4B and/or the ability of eIF4B to be phosphorylated by MELK in the subject or population of subjects as measured, for example, by the levels of Ser-406-phosphorylated human eIF4B or a corresponding phosphorylatable residue in an eIF4B ortholog thereof according to the methods described above. The same analysis applies to inhibiting the ability of MELK to
20 phosphorylate Histone H3 and/or the ability of Histone H3 to be phosphorylated by MELK. The effective amount can vary depending on such factors as the type of therapeutic agent(s) employed, the size of the subject, or the severity of the disorder.

It will be appreciated that individual dosages may be varied depending upon the requirements of the subject in the judgment of the attending clinician, the severity of the
25 condition being treated and the particular compound being employed. In determining the therapeutically effective amount or dose, a number of additional factors may be considered by the attending clinician, including, but not limited to: the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the desired time course of treatment; the species of mammal; its size, age, and general health; the
30 specific disease involved; the degree of or involvement or the severity of the disease; the response of the individual subject; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the kind of concurrent treatment; and other relevant circumstances.

Treatment can be initiated with smaller dosages that are less than the effective dose of the compound. Thereafter, in one embodiment, the dosage should be increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

5 The effectiveness of any particular agent to treat cancers can be monitored by comparing two or more samples obtained from subjects undergoing cancer treatment. In general, a first sample is obtained from the subject prior to beginning therapy and one or more samples during treatment. In such a use, a baseline of expression of cells from subjects with cancer prior to therapy is determined and then changes in the baseline state of
10 expression of cells from subjects with cancer is monitored during the course of therapy. Alternatively, two or more successive samples obtained during treatment can be used without the need of a pre-treatment baseline sample. In such a use, the first sample obtained from the subject is used as a baseline for determining whether the expression of cells from subjects with metabolic disorders is increasing or decreasing.

15 MELK and/or eIF4B and/or Histone H3 inhibitors can be administered in pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of the inhibitor formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. For example, formulations can be adapted for (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions),
20 tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, buccal, or sublingual surfaces; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) nasal/aerosol, for example, as an aqueous aerosol, liposomal
25 preparation or solid particles containing the compound, based on well-known methods in the pharmaceutical arts.

 The phrase "pharmaceutically acceptable" is employed herein to refer to those agents, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals
30 without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

 The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid

filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some

5 examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil,

10 cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20)

15 phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "pharmaceutically-acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the agents that reduce the phosphorylation levels of PKC- ι and/or activity encompassed by the invention. These salts can be

20 prepared in situ during the final isolation and purification of the agents, or by separately reacting a purified agents agent in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate,

25 tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like (See, for example, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In addition, the methods described herein can further comprise treating subjects with MELK and/or eIF4B and/or Histone H3 inhibitors in addition to administering one or more

30 additional anti-cancer agents and/or use samples from subjects exposed to such anti-cancer agents. Anti-cancer agents are well known to the skilled artisan and include, without limitation, chemotherapy and radiation, as well as immunotherapy, hormone therapy, and

gene therapy using nucleic acid molecules and/or proteins that are linked to the initiation, progression, and/or pathology of a tumor or cancer.

Chemotherapy includes the administration of a chemotherapeutic agent. Such a chemotherapeutic agent may be, but is not limited to, those selected from among the following groups of compounds: platinum compounds, cytotoxic antibiotics, antimetabolites, anti-mitotic agents, alkylating agents, arsenic compounds, DNA topoisomerase inhibitors, taxanes, nucleoside analogues, plant alkaloids, and toxins; and synthetic derivatives thereof. Exemplary compounds include, but are not limited to, alkylating agents: cisplatin, treosulfan, and trofosfamide; plant alkaloids: vinblastine, paclitaxel, docetaxol; DNA topoisomerase inhibitors: teniposide, crisnatol, and mitomycin; anti-folates: methotrexate, mycophenolic acid, and hydroxyurea; pyrimidine analogs: 5-fluorouracil, doxifluridine, and cytosine arabinoside; purine analogs: mercaptopurine and thioguanine; DNA antimetabolites: 2'-deoxy-5-fluorouridine, aphidicolin glycinate, and pyrazoloimidazole; and antimitotic agents: halichondrin, colchicine, and rhizoxin.

Compositions comprising one or more chemotherapeutic agents (*e.g.*, FLAG, CHOP) may also be used. FLAG comprises fludarabine, cytosine arabinoside (Ara-C) and G-CSF. CHOP comprises cyclophosphamide, vincristine, doxorubicin, and prednisone. In another embodiment, PARP (*e.g.*, PARP-1 and/or PARP-2) inhibitors are used and such inhibitors are well known in the art (*e.g.*, Olaparib, ABT-888, BSI-201, BGP-15 (N-Gene Research Laboratories, Inc.); INO-1001 (Inotek Pharmaceuticals Inc.); PJ34 (Soriano *et al.*, 2001; Pacher *et al.*, 2002b); 3-aminobenzamide (Trevigen); 4-amino-1,8-naphthalimide; (Trevigen); 6(5H)-phenanthridinone (Trevigen); benzamide (U.S. Pat. Re. 36,397); and NU1025 (Bowman *et al.*). In still another embodiment, the chemotherapeutic agents are platinum compounds, such as cisplatin, carboplatin, oxaliplatin, nedaplatin, and iproplatin. Other antineoplastic platinum coordination compounds are well known in the art, can be modified according to well-known methods in the art, and include the compounds disclosed in U.S. Pat. Nos. 4,996,337, 4,946,954, 5,091,521, 5,434,256, 5,527,905, and 5,633,243, all of which are incorporated herein by reference. The foregoing examples of chemotherapeutic agents are illustrative, and are not intended to be limiting.

Radiation therapy can also comprise an additional anti-cancer agent. The radiation used in radiation therapy can be ionizing radiation. Radiation therapy can also be gamma rays, X-rays, or proton beams. Examples of radiation therapy include, but are not limited to, external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, Pd-

103, Ir-192), intravenous administration of radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal ³²P radiation therapy, and/or total abdominal and pelvic radiation therapy. For a general overview of radiation therapy, see Hellman, Chapter 16: Principles of Cancer Management: Radiation Therapy, 6th edition, 2001, DeVita *et al.*,
5 eds., J. B. Lippencott Company, Philadelphia. The radiation therapy can be administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. The radiation treatment can also be administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass. Also encompassed is the use of photodynamic therapy comprising the
10 administration of photosensitizers, such as hematoporphyrin and its derivatives, Vertoporphin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A; and 2BA-2-DMHA.

Additional anti-cancer agents include immunotherapy, hormone therapy, and gene therapy. Such therapies include, but are not limited to, the use of antisense polynucleotides,
15 ribozymes, RNA interference molecules, triple helix polynucleotides and the like, where the nucleotide sequence of such compounds are related to the nucleotide sequences of DNA and/or RNA of genes that are linked to the initiation, progression, and/or pathology of a tumor or cancer. For example, oncogenes, growth factor genes, growth factor receptor genes, cell cycle genes, DNA repair genes, and others, may be targeted in such therapies.

20 Immunotherapy may comprise, for example, use of cancer vaccines and/or sensitized antigen presenting cells. Immunotherapy can also involve derepression of immunoinhibitory pathways, such as by targeting PD-L1, PD-L2, PD-1, CTLA-4, and the like. The immunotherapy can involve passive immunity for short-term protection of a host, achieved by the administration of an antibody directed against a cancer antigen or disease
25 antigen (*e.g.*, administration of a monoclonal antibody, optionally linked to a chemotherapeutic agent or toxin, to a tumor antigen). Immunotherapy can also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines.

Hormonal therapeutic treatments can comprise, for example, hormonal agonists, hormonal antagonists (*e.g.*, flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide
30 acetate (LUPRON), LH-RH antagonists), inhibitors of hormone biosynthesis and processing, and steroids (*e.g.*, dexamethasone, retinoids, deltoids, betamethasone, cortisol, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), vitamin A derivatives (*e.g.*, all-trans retinoic acid (ATRA));

vitamin D3 analogs; antigestagens (e.g., mifepristone, onapristone), or antiandrogens (e.g., cyproterone acetate).

In one embodiment, anti-cancer therapy used for cancers whose phenotype is determined by the methods of the invention can comprise one or more types of therapies described herein including, but not limited to, chemotherapeutic agents, immunotherapeutics, anti-angiogenic agents, cytokines, hormones, antibodies, polynucleotides, radiation and photodynamic therapeutic agents. For example, combination therapies can comprise one or more chemotherapeutic agents and radiation, one or more chemotherapeutic agents and immunotherapy, or one or more chemotherapeutic agents, radiation and chemotherapy.

Exemplification

This invention is further illustrated by the following examples, which should not be construed as limiting.

Example 1: Materials and Methods For Examples 2-3

a. Plasmids

Human eIF4B was cloned from the reverse transcription products of total RNA extracted from human mammary epithelial cells (HMECs), using the primers (forward: ATGGCGGCCTCAGCAAAAAG; reverse: CTATTCGGCATAATCTTCTC). The 1.8 kb PCR product was then used as template for amplifying Flag-tagged or HA-tagged eIF4B with restriction sites. The constructs (pWzl-Flag-eIF4B, pTrex-eIF4B-HA) were verified by sequencing. Site-directed mutagenesis of eIF4B was performed using QuickChange XL (Stratagene), and all mutant constructs were confirmed by sequencing.

To generate pLKO-tet-on shRNA targeting human eIF4B, synthesized oligonucleotides were annealed and ligated with digested pLKO vector. The sequences for scramble, sh-eIF4B-1, sh-eIF4B-2 are GTGGACTCTTGAAAGTACTAT, GGACCAGGAAGGAAAGATGAA, and GCGGAGAAACACCTTGATCTT, respectively.

b. Retroviral and lentiviral gene delivery

Retroviruses were generated by transfecting HEK293T cells with retroviral plasmids and packaging DNA. Generally, 1.6 µg pWzl DNA, 1.2 µg pCG-VSVG and 1.2 µg pCG-gap/pol, 12 ul lipid of Metafectene Pro (Biontex) were used. DNA and lipid were

diluted in 300 μ l PBS respectively and mixed. After 15 minutes (min.) of incubation, they were added to one 6-cm dish that was seeded with 3 million HEK293T cells one day earlier. Viral supernatant was collected 48 hours (h) and 72 hours post-transfection. The supernatant was filtered through 0.45 μ m membrane, and was added to target cells in the presence of 8 μ g/ml polybrene (Millipore). Lentiviruses were generated with a similar approach with the exception of HEK293T cells that were transfected with 2 μ g pLKO DNA, 1.5 μ g pCMV-dR8.91, and 0.5 μ g pMD2-VSVG. Cells were selected with antibiotics starting 72 h after initial infection. Puromycin and blasticidin were used at the final concentrations of 1.5 μ g/ml and 4 μ g/ml respectively.

c. Immunoblotting

For treatment with nocodazole, cells were refreshed with medium containing nocodazole (200 ng/ml). Twenty hours after treatment, floating mitotic cells were harvested by gentle shake-off. For drug treatment, cells were seeded in multi-well plate, in the presence of OTSSP167 (ChemExpress, HY15512; 10 mM stock made in DMSO).

Cells were harvested and lysed with RIPA buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) supplemented with protease inhibitors cocktail (Roche) and phosphatase inhibitors cocktail (Thermo Scientific). Cleared lysates were analyzed for protein concentration using a BCA kit (Thermo Scientific). Equal amount of protein (10-20 μ g) was resolved on SDS-PAGE, and was subsequently transferred onto a nitrocellulose or polyvinylidene difluoride membrane (Amersham). The membrane was blocked with 5% non-fat milk and was then incubated with primary antibodies overnight at 4°C. After washing, the membrane was incubated with fluorophore-conjugated secondary antibodies for 1 h at room temperature. The membrane was then washed and scanned with an Odyssey® Infrared scanner (Li-Cor Biosciences).

The following antibodies were used for immunoblotting or immunoprecipitation. MELK (Epitomics, 2916), p-eIF4B (S406) (Cell Signaling, 8151), p-eIF4B (S422) (Cell Signaling, 3591), eIF4B (Cell Signaling, 3592), c-Myc (Cell Signaling, 5605), XIAP (Cell Signaling, 2045), p-Akt (S473) (Cell Signaling, 4060), p-MAPK (T202/Y204) (Cell Signaling, 4370), cleaved PARP (Asp214) (Cell Signaling, 9541), Aurora A (Cell Signaling, 4718), Aurora B (Cell Signaling, 3094), p-Aurora A (T288)/Aurora B (T232)/Aurora C(T198) (Cell Signaling, 2914), p-Histone H3 (T3) (Cell Signaling, 13576), p-Histone H3 (S10) (Cell Signaling, 3377), p-Histone H3 (T11) (Cell Signaling, 9767), p-

Histone H3 (S28) (Cell Signaling, 9713), ODC (Sigma, O1136), Vinculin (Sigma, V9131, alpha-tubulin (Sigma, T9016), anti-HA magnetic beads (Pierce, 88836), anti-Flag magnetic beads (Sigma, M8823). Secondary antibodies used were Alexa Fluor 680 goat anti-rabbit IgG (Invitrogen, A-21109) and IRDye800-conjugated anti-mouse IgG (Rockland).

5

d. *In vitro* kinase assay

Flag-tagged eIF4B or Flag-eIF4B (S406A) was transfected into HEK293T cells (4 µg DNA for cells in one 60 mm dish). Thirty-six hours after transfection, cells were lysed with IP buffer (100 mM NaCl, 50 mM Tris, pH 7.5, 0.5% NP-40, 0.5% Sodium deoxycholate, supplemented with protease/phosphatase inhibitor cocktail). Lysates were cleared via incubating with anti-mouse IgG conjugated to magnetic beads (4°C, 30 min), and then immunoprecipitated with anti-Flag M2 magnetic beads (Sigma) (4°C, 120 min). The beads with bound antigens were washed 5 times with IP buffer. Beads during the last wash were aliquoted into 1.5 ml microcentrifuge tubes. After removal of IP buffer, the beads were washed once with 1x kinase buffer without ATP (5 mM Tris, pH 7.5, 5 mM - β glycerophosphate, 2 mM dithiothreitol, 0.1 mM Na₃VO₄, 10 mM MgCl₂; Cell Signaling). After the wash, 40 µl 1x kinase buffer with 200 mM ATP was added to each tube, followed by 5 µl buffer without or with 500 ng recombinant MELK. The reaction was incubated at 30°C for 30 min, and terminated by adding 40 µl 2x SDS sample buffer. The samples were then boiled and subjected to immunoblotting. *In vitro* kinase assays with Histone H3 were performed as above, except that recombinant Histone H3.1 (New England BioLabs, M2503S) was used (50 ng *per* reaction).

15

20

e. Positional scanning peptide library screen

25

30

Active full-length human MELK was purified from insect cells. The positional scanning peptide library screen was performed as described in Turk *et al.* (2006) *Nat. Protocol*, 1:375. Briefly, a set of 180 (or 198) biotin-conjugated peptides with the following sequence, Y-A-X-X-X-X-X-S/T-X-X-X-X-A-G-K-K-biotin, was used. In the sequence, S/T means an equimolar mixture of Ser and Thr. For each peptide, one of the nine X positions represents one of the twenty total amino acids. Peptides were arrayed in a 384-well plates in buffer containing 50 mM HEPES, pH 7.5, 20 mM MgCl₂, 0.02 mg/ml BSA, 0.01% Brij 35, 5 mM DTT, 0.5 mM EGTA, and active MELK and γ-[³²P]-ATP was added to wells (final [peptide] = 50 µM, and [ATP] = 100 µM, 0.025 µCi/µl in each well). After incubating for 2 h at 30°C, aliquots of the reactions were spotted onto a streptavidin

membrane. The membrane was quenched, washed extensively, dried, and exposed to a phosphor storage screen.

Example 2: Phosphorylation Status of eIF4B is a Biomarker of MELK Enzymatic and Oncogenic Activity

To seek a potential molecular mechanism underlying the importance of MELK for cancer (such as basal-like breast cancer (BBC)), multiple experimental approaches, including immunoprecipitation-tandem mass spectrometry, and phospho-peptide mapping, were explored. When Flag-tagged MELK was immunoprecipitated in mitotic cell lysates and subsequently subject to mass spectrometry analysis, it was found that a translation initiation factor, eIF4B, had a strong association with MELK during mitosis (Figure 1). Using positional phospho-peptide mapping, an optimal substrate motif for MELK was identified having a strong selection for arginine at the -3 position relative to serine/threonine (Figure 2).

There are two regions flanking the residues of human eIF4B (*i.e.*, at Ser406 and Ser422), which contain the MELK phosphorylation motif (Figures 3 and 4). To test whether MELK is capable of phosphorylating human eIF4B at these two sites, *in vitro* kinase assays were performed using purified recombinant MELK with immunoprecipitated eIF4B. It was found that Ser406, but not Ser422, of human eIF4B was readily phosphorylated by full-length MELK or the kinase domain of MELK, and that the observed phosphorylation was abolished when the serine at 406 of human eIF4B was mutated to alanine or other manipulations of MELK (Figures 3-5). These results are specifically dependent upon MELK, as inhibition of mitotic cells with mTOR inhibitors, such as rapamycin and torins, do not produce the same results (Figure 6; van Gorp *et al.* (2009) *Oncogene* 28:95-106). These data indicate that MELK is a kinase that phosphorylates human eIF4B at S406 and that eIF4B orthologs in other species are similarly phosphorylated due to the highly conserved sequence and structural composition of the eIF4B polypeptide region harboring the phosphorylation site (Table 2).

Table 2**eIF 4B S406**

Human	S406	RERHPSWRSE
Chimpanzee	S406	RERHPSWRSE
Monkey	S402	RERHPSWRSE
Cattle	S406	RERHPSWRSE
Dog	S406	RERHPSWRSE
Mouse	S406	RERHPSWRSE
Rat	S406	RERHPSWRSE
Zebrafish	S403	RERHPSWRSE
Fission yeast	S315	RERSTSRKPS

It is known that eIF4B stimulates the helicase activity of eIF4A for unwinding the secondary structure of 5'UTR of mRNA (Dmitriev *et al.* (2003) *Mol. Cell Biol.* 23:8925-8933 and Shahbazian *et al.* (2010) *Mol. Cell Biol.* 30 1478-1485). Many of these mRNAs encode oncogenic proteins, such as c-Myc, XIAP (X-linked inhibitor of apoptosis protein) and ODC (ornithine decarboxylase). It was determined herein that down-regulation of MELK reduced phosphorylation of eIF4B (p-eIF4B) at S406 in MDA-MB-468 cells during mitosis, which also resulted in markedly reduced levels of c-Myc, XIAP and ODC1 (Figures 7 and 8). Thus, MELK-mediated S406 phosphorylation of eIF4B during mitosis, is functionally important for the optimal translation of mRNAs with highly structured 5'UTR, many of which are known to be oncogenic, such as c-Myc, XIAP, and ODC1. Together, these data indicate that MELK is a novel kinase that regulates eIF4B during mitosis and thereby mediates the translation of mRNAs that harbor structured 5'-UTR and are important for the survival and proliferation of cancer cells. Thus, the level of eIF4B phosphorylation mediated by MELK is a target engagement biomarker for MELK oncogenic activity useful for preclinical and clinical applications.

Example 3: Phosphorylation Status of Histone H3 is a Biomarker of MELK Enzymatic and Oncogenic Activity

Since MELK protein abundance is highest during mitosis, a cell cycle phase when Histone H3 is heavily phosphorylated, a link between MELK and Histone H3 phosphorylation was suggested. In fact, the region flanking the residues of human Histone H3 at Thr-11 contains the optimal MELK phosphorylation motif described in Example 2 above.

To test whether MELK is capable of phosphorylating human Histone H3 at threonine 3 (Thr3), serine 10 (Ser10) and threonine 11 (Thr11), *in vitro* kinase assays were performed using a purified recombinant kinase domain of human MELK and human Histone H3. It was found that Thr3, Ser10, and Thr11 of human Histone H3 were readily phosphorylated by the kinase domain of MELK (Figure 9).

Down-regulation of MELK reduced phosphorylation of Histone H3 at Thr3, Ser10 and Thr11 in MDA-MB-468 cells during mitosis, but did not affect the phosphorylation of Aurora kinases A, B, or C, which are known kinases that phosphorylate Histone H3 at Ser10 (Figures 10 and 11). Similarly, inhibition of MELK using the small chemical MELK inhibitor, OTSSP167, in MDA-MB-468 cells reduced phosphorylation of Histone H3 at Thr3/Ser10/Thr11 (Figure 12). An OTSSP167 concentration-dependent reduction in phosphorylation of Histone H3 at Thr3, Ser10 and Thr11, but not Ser28, was also observed (Figure 13).

These data indicate that MELK is a kinase that phosphorylates human Histone H3 at least at Thr3, Ser10 and Thr11, but not Ser28, and that Histone H3 orthologs in other species are similarly phosphorylated due to the highly conserved sequence and structural composition of the Histone H3 polypeptide region harboring the phosphorylation site (Table 3).

Table 3

Histone H3

Human	T3/S10/T11	MARTKQTARKSTGGKA
Chimpanzee	T3/S10/T11	MARTKQTARKSTGGKA
Monkey	T3/S10/T11	MARTKQTARKSTGGKA
Cattle	T3/S10/T11	MARTKQTARKSTGGKA
Dog	T3/S10/T11	MARTKQTARKSTGGKA
Mouse	T3/S10/T11	MARTKQTARKSTGGKA
Rat	T3/S10/T11	MARTKQTARKSTGGKA
Zebrafish	T3/S10/T11	MARTKQTARKSTGGKA
Fission yeast	T3/S10/T11	MARTKQTARKSTGGKA

Together, these data indicate that MELK is a novel kinase that regulates Histone H3 phosphorylation during mitosis and is therefore potentially important for the proliferation of cells (e.g., cancer cells). Thus, the level of Histone H3 phosphorylation mediated by MELK is a target engagement biomarker for MELK oncogenic activity useful for preclinical and clinical applications.

Incorporation by Reference

The contents of all references, patent applications, patents, and published patent applications, as well as the Figures and the Sequence Listing, cited throughout this application are hereby incorporated by reference.

5

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

10 claims.

What is claimed:

1. A method of identifying an agent which inhibits kinase or oncogenic activity of human maternal embryonic leucine zipper kinase (MELK) or an ortholog thereof, comprising:
 - 5 a) contacting a sample comprising i) human MELK or an ortholog thereof and ii) human eukaryotic initiation factor 4B (eIF4B) or an ortholog thereof, with the agent; and
 - b) determining the ability of the agent to inhibit Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic
 - 10 activity of human MELK or the ortholog thereof.
2. The method of claim 1, wherein the inhibition of said Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B is determined by comparing the amount of Ser-406 phosphorylated human eIF4B or
- 15 a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, in the sample relative to a control.
3. The method of claim 2, wherein the control is the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the
- 20 ortholog of human eIF4B in the sample relative to said amount in the absence of the agent or at an earlier timepoint after contact of the sample with the agent.
4. The method of claim 1, wherein the inhibition of said Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human
- 25 eIF4B is determined by comparing the ratio of the amount of Ser-406 phosphorylated human eIF4B, or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B, in the sample relative to the total amount of human EIF4B or ortholog thereof, to a control.
- 30 5. The method of claim 4, wherein the control is the ratio of the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B in the sample relative to said ratio in the absence of the agent or at an earlier timepoint after contact of the sample with the agent.

6. The method of any one of claims 1-5, further comprising determining the amount of a protein translated from an RNA with highly structured 5'UTR, optionally wherein the protein is selected from the group consisting of cellular myelocytomatosis oncogene (c-Myc), X-linked inhibitor of apoptosis protein (XIAP), and ornithine decarboxylase (ODC1).
7. The method of claim 1, further comprising a step of determining whether the agent directly binds said human eIF4B or said ortholog thereof, or said human MELK or said ortholog thereof.
8. The method of claim 1, wherein the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples.
9. The method of claim 1, wherein the sample comprises cells.
10. The method of claim 9, wherein the cells are cancer cells.
11. The method of claim 10, wherein the cancer is selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases.
12. The method of claim 9, wherein the cells are obtained from a subject.
13. The method of claim 1, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.
14. The method of any one of claims 1-5, wherein the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds to Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B.
15. The method of claim 14, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme

immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

16. The method of claim 15, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B.

17. The method of any one of claims 1-5, wherein said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

18. The method of any one of claims 1-5, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

19. The method of any one of claims 1-5, wherein the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%.

20. A method for assessing the efficacy of an agent for inhibiting kinase activity of human MELK or an ortholog thereof in a subject, comprising:

a) detecting in a subject sample at a first point in time, the amount of Ser-406 phosphorylated human eIF4B or the amount of a human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B;

b) repeating step a) during at one or more subsequent points in time after administration of the agent; and

c) comparing the amount of phosphorylated human eIF4B or ortholog thereof detected in step a) with said amount detected in step b),

wherein a higher amount of Ser-406 phosphorylated human eIF4B or the amount of the human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B in the first point in time relative to at least one subsequent point in time, indicates that the agent inhibits kinase activity of human MELK or the ortholog thereof.

21. The method of claim 20, wherein the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B.

22. The method of claim 21, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

23. The method of claim 22, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a corresponding phosphorylated ortholog of human eIF4B.

24. The method of claim 20, wherein the sample is selected from the group consisting of *ex vivo* and *in vivo* samples.

25. The method of claim 20, wherein the sample comprises cancer cells.

26. The method of claim 25, wherein the cancer cells are cells selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases.

27. The method of claim 20, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.

28. The method of claim 20, wherein the sample in step a) and/or step b) is a portion of a single sample obtained from the subject.

29. The method of claim 20, wherein the sample in step a) and/or step b) is a portion of pooled samples obtained from the subject.

30. The method of claim 20, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

5 31. The method of claim 20, wherein said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

10 32. The method of claim 20, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

33. The method of claim 20, wherein the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B by at least 50%.

15 34. A method of treating a subject afflicted with cancer comprising administering to the subject an agent that inhibits Ser-406 phosphorylation of human eIF4B or a corresponding phosphorylatable amino acid in an ortholog of human eIF4B, thereby treating the subject afflicted with the cancer.

20 35. The method of claim 34, wherein the agent is administered in a pharmaceutically acceptable formulation.

25 36. The method of claim 34, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

37. The method of claim 34, wherein the agent directly binds said human eIF4B or the ortholog thereof, or said human MELK or the ortholog thereof.

30 38. The method of claim 34, wherein the cancer is selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases.

39. The method of claim 34, wherein said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

5 40. The method of claim 34, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

41. The method of claim 34, wherein the agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the
10 ortholog of human eIF4B by at least 50%.

42. The method of claim 34, further comprising administering one or more additional anti-cancer agents.

15 43. A method of determining the function or activity of human MELK or an ortholog, comprising:

a) detecting in a sample the amount of Ser-406 phosphorylated human eIF4B or the amount of a human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B;

20 b) repeating step a) in the same sample or a test sample at one or more subsequent points in time after manipulation of the sample and/or manipulation of the same sample or test sample; and

c) comparing the amount of phosphorylated human eIF4B or ortholog thereof detected in step a) with said amount detected in step b),

25 wherein a modulated of Ser-406 phosphorylated human eIF4B or the amount of the human eIF4B ortholog phosphorylated at a corresponding amino acid of human eIF4B in the first point in time relative to at least one subsequent point in time and/or at least one subsequent manipulation of the same sample or test sample, indicates that the function or activity of human MEL or an ortholog thereof is modulated.

30 44. The method of claim 43, wherein the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the ortholog of human eIF4B is determined by an immunoassay using a reagent which specifically binds with Ser-406 phosphorylated human eIF4B or corresponding phosphorylated ortholog of human eIF4B.

35

45. The method of claim 44, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

5

46. The method of claim 45, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human eIF4B or corresponding ortholog of human eIF4B and a detection antibody or fragment thereof which specifically binds with Ser-406 phosphorylated human eIF4B or a
10 corresponding phosphorylated ortholog of human eIF4B.

47. The method of claim 43, wherein the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples.

15

48. The method of claim 43, wherein the sample comprises cells or the method uses a cell-based assay.

49. The method of claim 48, wherein the cells are cancer cells selected from the group consisting of any cancer in which MELK or eIF4B is amplified or overexpressed, any
20 cancer having an activating mutation of MELK or eIF4B, and any cancer in which MELK or eIF4B is activated by other kinases.

50. The method of claim 43, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine,
25 stool, and bone marrow.

51. The method of claim 43, wherein the same sample or test sample in step a) and/or step b) is a portion of a single sample obtained from a subject.

30

52. The method of claim 43, wherein the same sample or test sample in step a) and/or step b) is a portion of pooled samples obtained from a subject.

53. The method of claim 43, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for
35 cancer, and/or is in remission from cancer.

54. The method of claim 43, wherein said human eIF4B or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

5 55. The method of claim 43, wherein the manipulation of the sample is selected from the group consisting of contacting the sample with a test agent, contacting the sample with an upstream signal of the MELK signaling pathway, and contacting the sample with a MELK inhibitor.

10 56. The method of claim 55, wherein the test agent is a small molecule, or an antibody or antigen-binding fragment thereof.

57. The method of claim 55 or 56, wherein the test agent decreases the amount of Ser-406 phosphorylated human eIF4B or a corresponding phosphorylatable amino acid in the
15 ortholog of human eIF4B by at least 50%.

58. A method of identifying an agent which inhibits kinase or oncogenic activity of human MELK or an ortholog thereof, comprising:

- a) contacting a sample comprising i) human MELK or an ortholog thereof and ii)
20 human Histone H3 or an ortholog thereof, with the agent; and
- b) determining the ability of the agent to inhibit Thr-3 phosphorylation of human Histone H3 or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3; and/or Ser-10 phosphorylation of human Histone H3 or a corresponding
25 phosphorylatable amino acid in the ortholog of human Histone H3; and/or Thr-11 phosphorylation of human Histone H3 or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, wherein decreased phosphorylation identifies an agent which inhibits kinase or oncogenic activity of human MELK or the ortholog thereof.

59. The method of claim 58, wherein the inhibition of said Thr-3 phosphorylated and/or
30 Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, is determined by comparing the amount of Thr-3 phosphorylated human Histone H3 and/or Ser-10 phosphorylated human Histone H3 and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3,
35 in the sample relative to a control.

60. The method of claim 59, wherein the control is the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, in the sample relative to said amount in the absence of the agent or at an earlier timepoint after contact of the sample with the agent.

61. The method of claim 58, wherein the inhibition of said Thr-3 phosphorylation and/or Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3 is determined by comparing the ratio of the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, in the sample relative to the total amount of human Histone H3 or ortholog thereof, to a control.

62. The method of claim 61, wherein the control is the ratio of the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3 in the sample relative to said ratio in the absence of the agent or at an earlier timepoint after contact of the sample with the agent.

63. The method of any one of claims 58-62, further comprising determining the amount of a mitosis-specific protein.

64. The method of claim 58, further comprising a step of determining whether the agent directly binds said human Histone H3 or said ortholog thereof, or said human MELK or said ortholog thereof.

65. The method of claim 58, wherein the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples.

66. The method of claim 58, wherein the sample comprises cells.

67. The method of claim 66, wherein the cells are cancer cells.

68. The method of claim 67, wherein the cancer is selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases.

5

69. The method of claim 66, wherein the cells are obtained from a subject.

70. The method of claim 58, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.

10

71. The method of any one of claims 58-62, wherein the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3.

15

72. The method of claim 71, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

20

73. The method of claim 72, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylated ortholog of human Histone H3.

25

74. The method of any one of claims 58-62, wherein said human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

30

75. The method of any one of claims 58-62, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

35

76. The method of any one of claims 58-62, wherein the agent decreases the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human
5 Histone H3, by at least 50%.

77. A method for assessing the efficacy of an agent for inhibiting kinase activity of human MELK or an ortholog thereof in a subject, comprising:

a) detecting in a subject sample at a first point in time, the amount of Thr-3
10 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of a human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3;

b) repeating step a) during at one or more subsequent points in time after administration of the agent; and

15 c) comparing the amount of phosphorylated human Histone H3 or ortholog thereof detected in step a) with said amount detected in step b),

wherein a higher amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of the human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3, in the first
20 point in time relative to at least one subsequent point in time, indicates that the agent inhibits kinase activity of human MELK or the ortholog thereof.

78. The method of claim 77, wherein the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding
25 phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated human Histone H3 or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3.

30 79. The method of claim 78, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

80. The method of claim 79, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylated ortholog of human Histone H3.

81. The method of claim 77, wherein the sample is selected from the group consisting of *ex vivo* and *in vivo* samples.

82. The method of claim 77, wherein the sample comprises cancer cells.

83. The method of claim 82, wherein the cancer cells are cells selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases.

84. The method of claim 77, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.

85. The method of claim 77, wherein the sample in step a) and/or step b) is a portion of a single sample obtained from the subject.

86. The method of claim 77, wherein the sample in step a) and/or step b) is a portion of pooled samples obtained from the subject.

87. The method of claim 77, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

88. The method of claim 77, wherein said human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

89. The method of claim 77, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

90. The method of claim 77, wherein the agent decreases the amount of Thr-3
5 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

91. A method of treating a subject afflicted with cancer comprising administering to the
10 subject an agent that inhibits Thr-3 phosphorylation and/or Ser-10 phosphorylation and/or Thr-11 phosphorylation of human Histone H3, or a corresponding phosphorylatable amino acid in an ortholog of human Histone H3, thereby treating the subject afflicted with the cancer.

15 92. The method of claim 91, wherein the agent is administered in a pharmaceutically acceptable formulation.

93. The method of claim 91, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

20

94. The method of claim 91, wherein the agent directly binds said human Histone H3 or the ortholog thereof, or said human MELK or the ortholog thereof.

95. The method of claim 91, wherein the cancer is selected from the group consisting of
25 any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which MELK or Histone H3 is activated by other kinases.

96. The method of claim 91, wherein said human Histone H3 or ortholog thereof, and/or
30 said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.

97. The method of claim 91, wherein the agent is a small molecule, or an antibody or antigen-binding fragment thereof.

35

98. The method of claim 91, wherein the agent decreases the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

5

99. The method of claim 91, further comprising administering one or more additional anti-cancer agents.

100. A method of determining the function or activity of human MELK or an ortholog, comprising:

10

a) detecting in a sample the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human histone H3 or the amount of a human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3;

b) repeating step a) in the same sample or a test sample at one or more subsequent points in time after manipulation of the sample and/or manipulation of the same sample or test sample; and

15

c) comparing the amount of phosphorylated human Histone H3 or ortholog thereof detected in step a) with said amount detected in step b),

wherein a modulated amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or the amount of the human Histone H3 ortholog phosphorylated at a corresponding amino acid of human Histone H3, in the first point in time relative to at least one subsequent point in time and/or at least one subsequent manipulation of the same sample or test sample, indicates that the function or activity of human MELK or an ortholog thereof is modulated.

25

101. The method of claim 100, wherein the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, is determined by an immunoassay using a reagent which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or corresponding phosphorylated ortholog of human Histone H3.

30

102. The method of claim 101, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme

immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.

103. The method of claim 102, wherein the enzyme immunoassay is a sandwich enzyme
5 immunoassay using a capture antibody or fragment thereof which specifically binds with human Histone H3 or corresponding ortholog of human Histone H3 and a detection antibody or fragment thereof which specifically binds with Thr-3 phosphorylated and/or Ser-10 phosphorylated or Thr-11 phosphorylated human Histone H3, or a corresponding phosphorylated ortholog of human Histone H3.

104. The method of claim 100, wherein the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples.

105. The method of claim 100, wherein the sample comprises cells or the method uses a
15 cell-based assay.

106. The method of claim 105, wherein the cells are cancer cells selected from the group consisting of any cancer in which MELK or Histone H3 is amplified or overexpressed, any cancer having an activating mutation of MELK or Histone H3, and any cancer in which
20 MELK or Histone H3 is activated by other kinases.

107. The method of claim 100, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.

25 108. The method of claim 100, wherein the same sample or test sample in step a) and/or step b) is a portion of a single sample obtained from a subject.

109. The method of claim 100, wherein the same sample or test sample in step a) and/or
30 step b) is a portion of pooled samples obtained from a subject.

110. The method of claim 100, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

35

111. The method of claim 100, wherein said human Histone H3 or ortholog thereof, and/or said human MELK or ortholog thereof, comprises a nucleic acid sequence or amino acid sequence set forth in Table 1.
- 5 112. The method of claim 100, wherein the manipulation of the sample is selected from the group consisting of contacting the sample with a test agent, contacting the sample with an upstream signal of the MELK signaling pathway, and contacting the sample with a MELK inhibitor.
- 10 113. The method of claim 112, wherein the test agent is a small molecule, or an antibody or antigen-binding fragment thereof.
114. The method of claim 112 or 113, wherein the test agent decreases the amount of Thr-3 phosphorylated and/or Ser-10 phosphorylated and/or Thr-11 phosphorylated human
15 Histone H3, or a corresponding phosphorylatable amino acid in the ortholog of human Histone H3, by at least 50%.

Figure 1

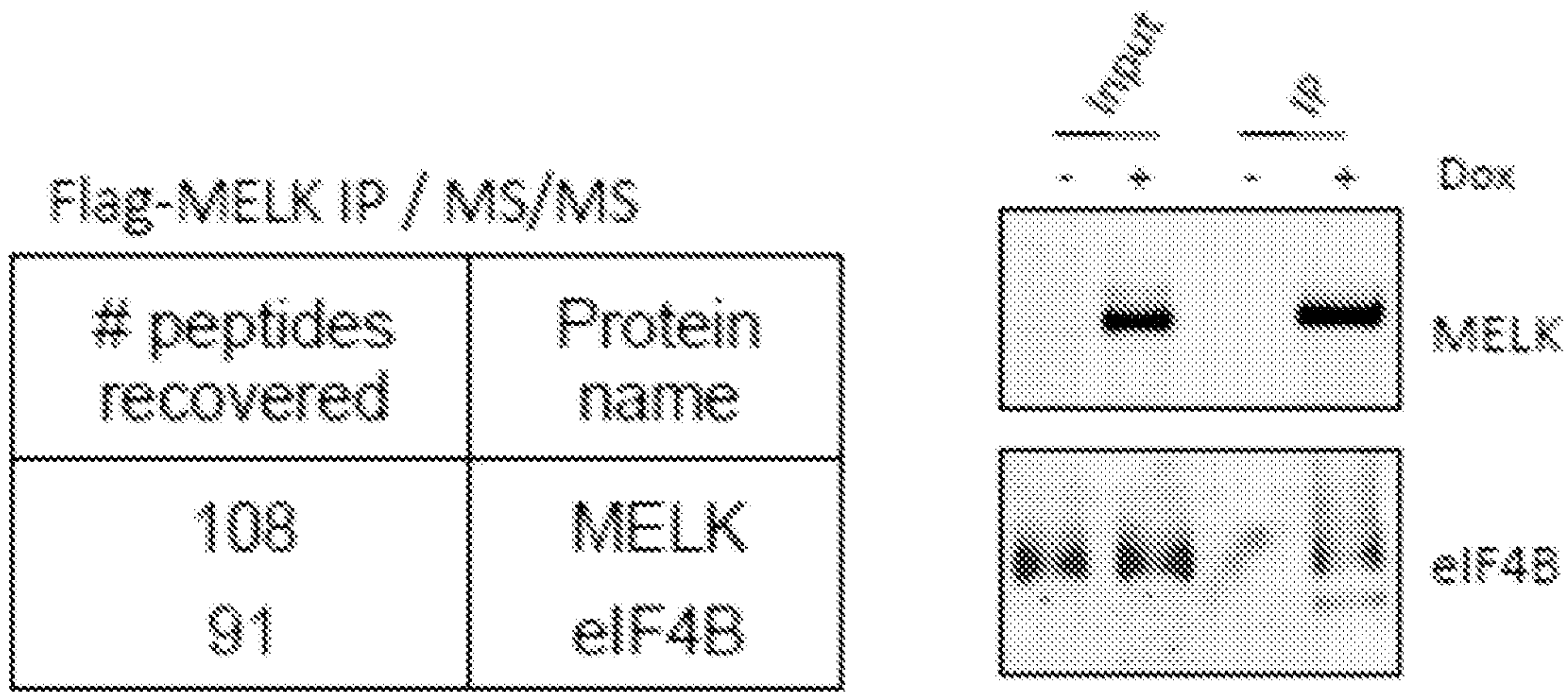


Figure 2

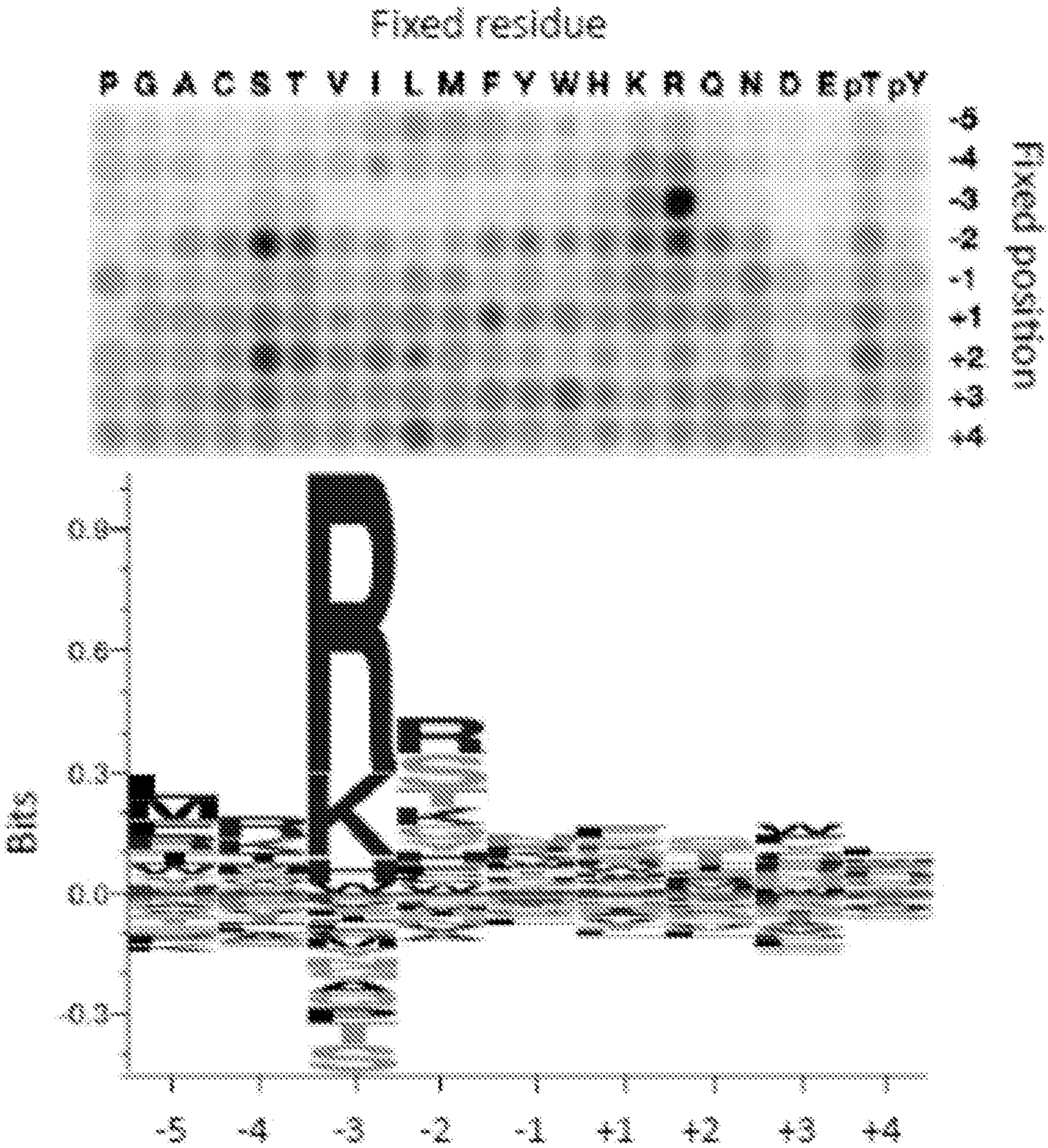


Figure 3

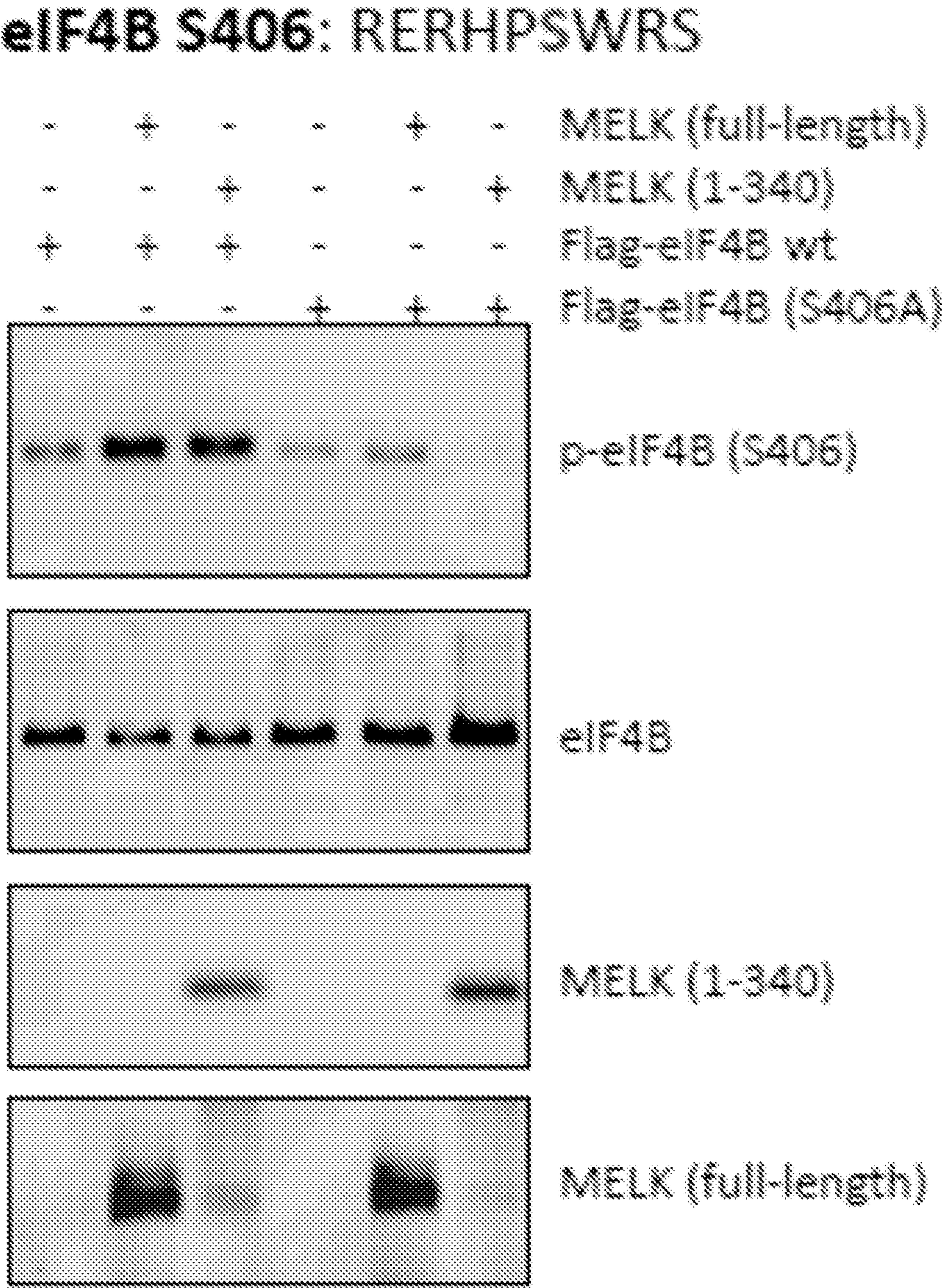


Figure 4

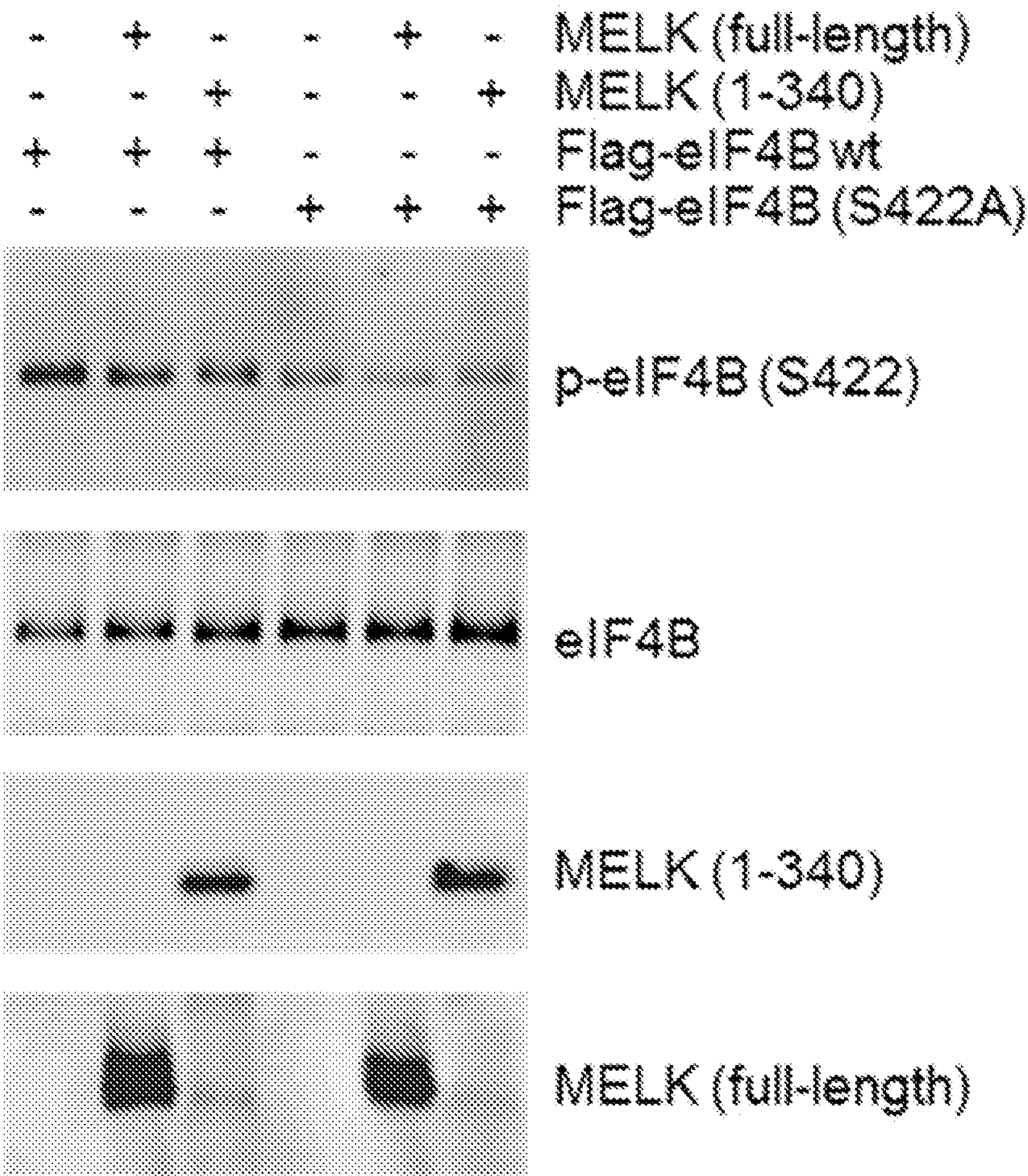


Figure 5

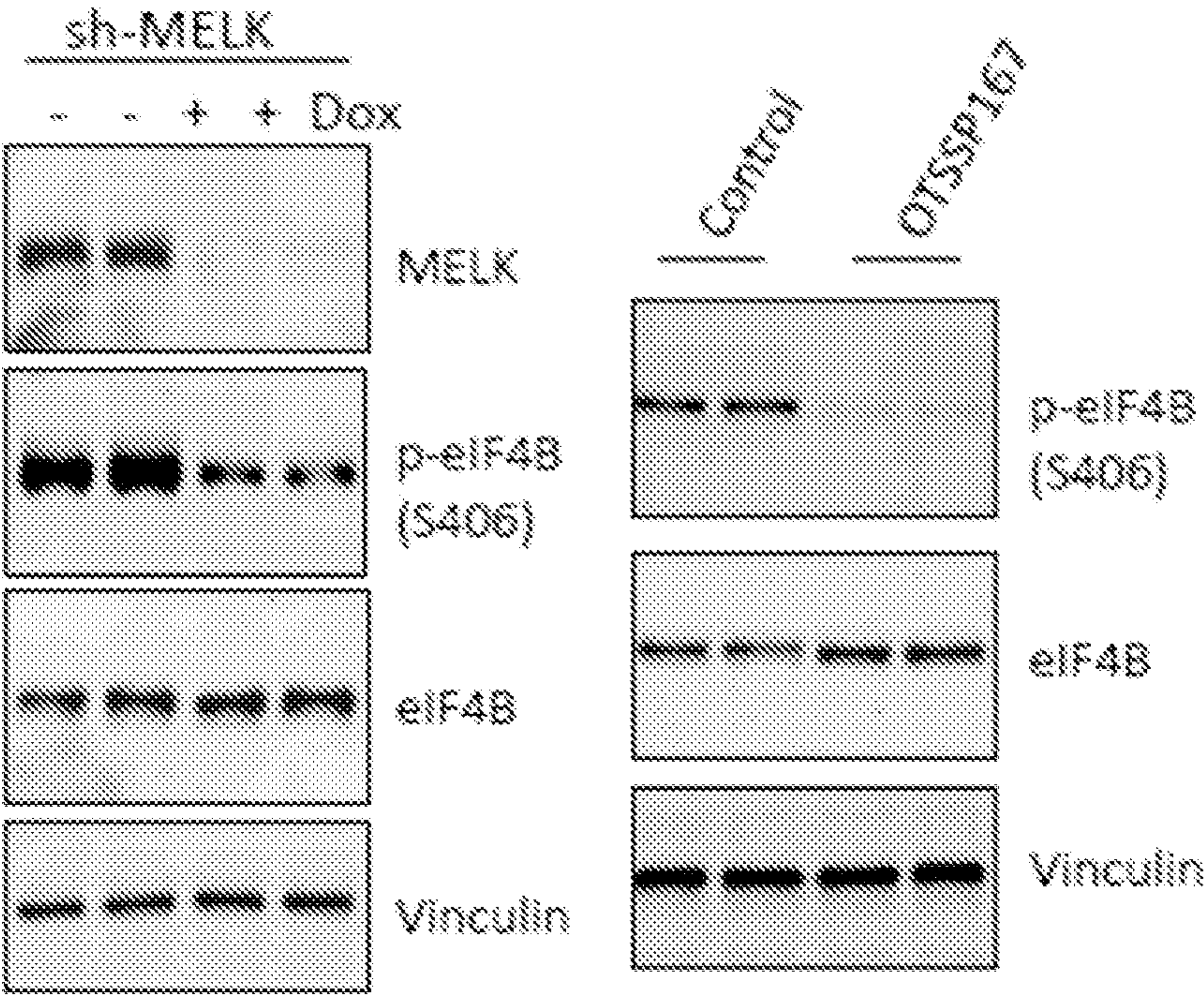


Figure 6

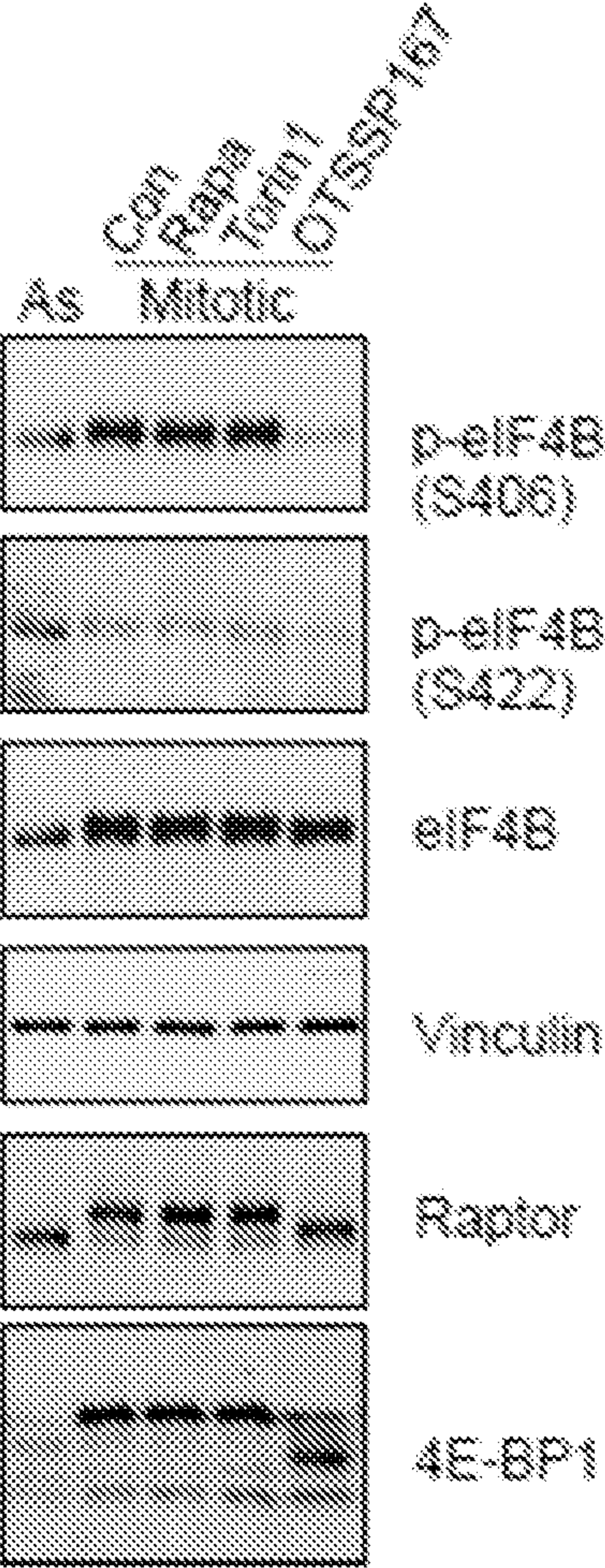


Figure 7

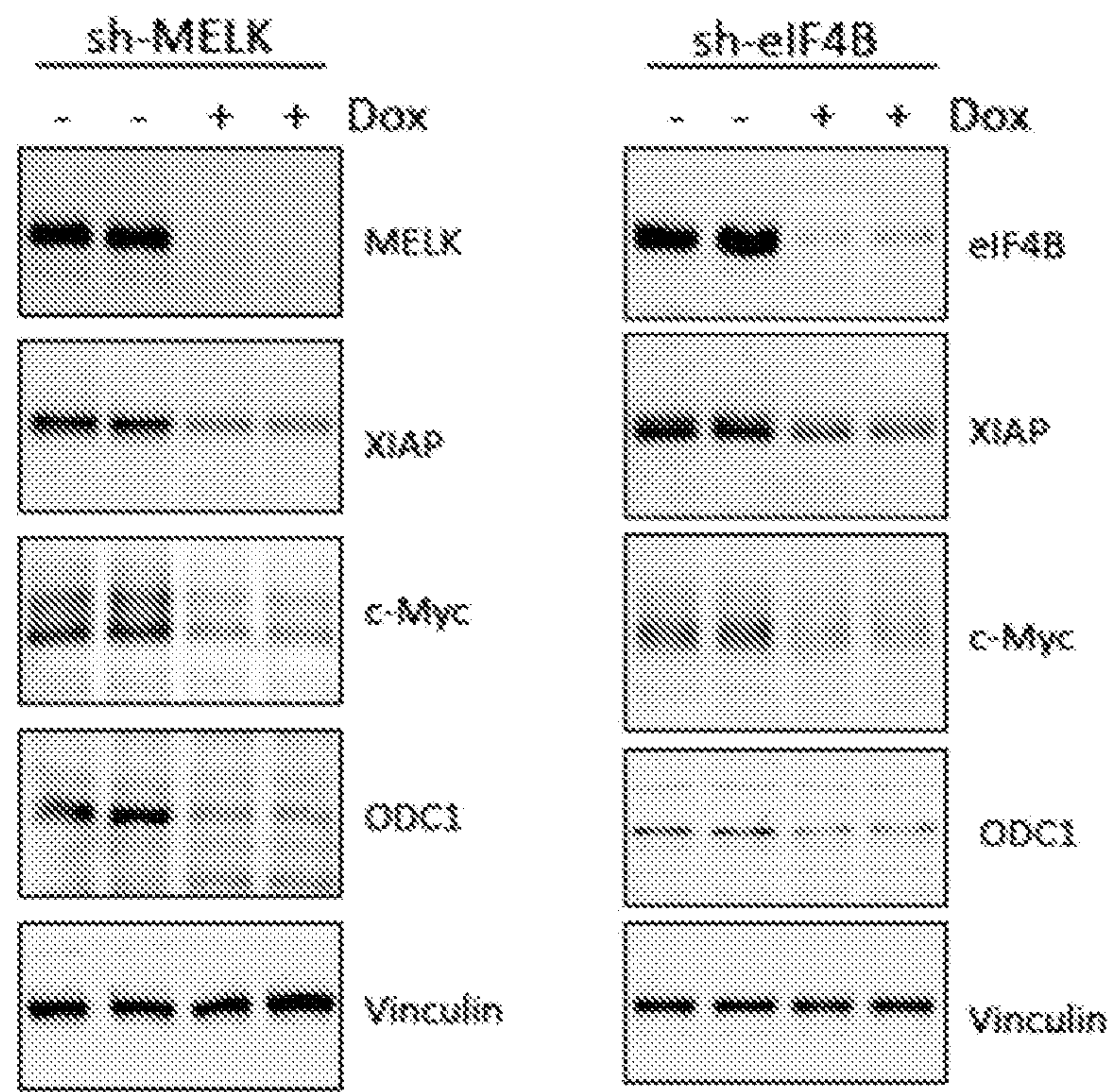


Figure 8

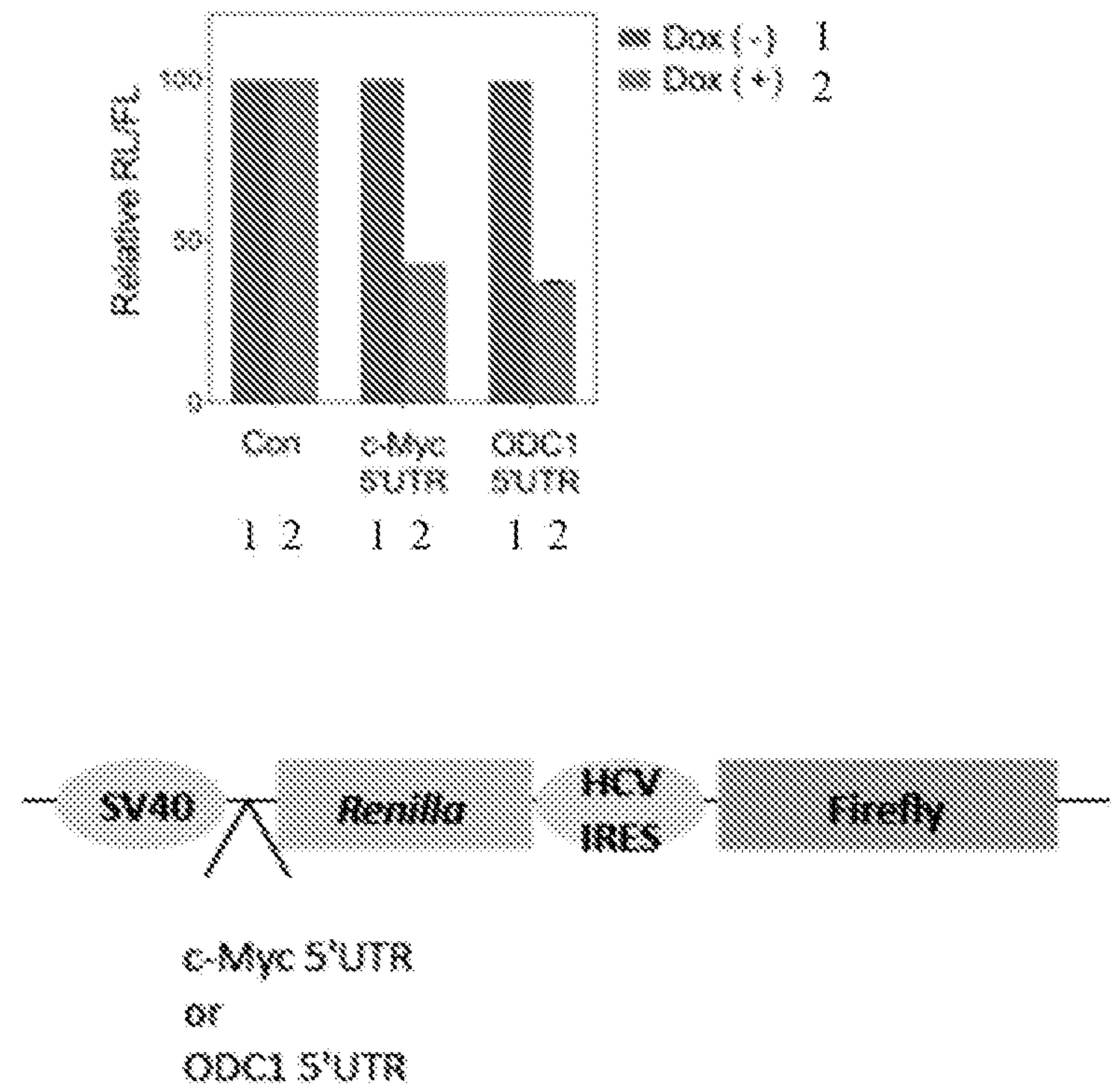


Figure 9

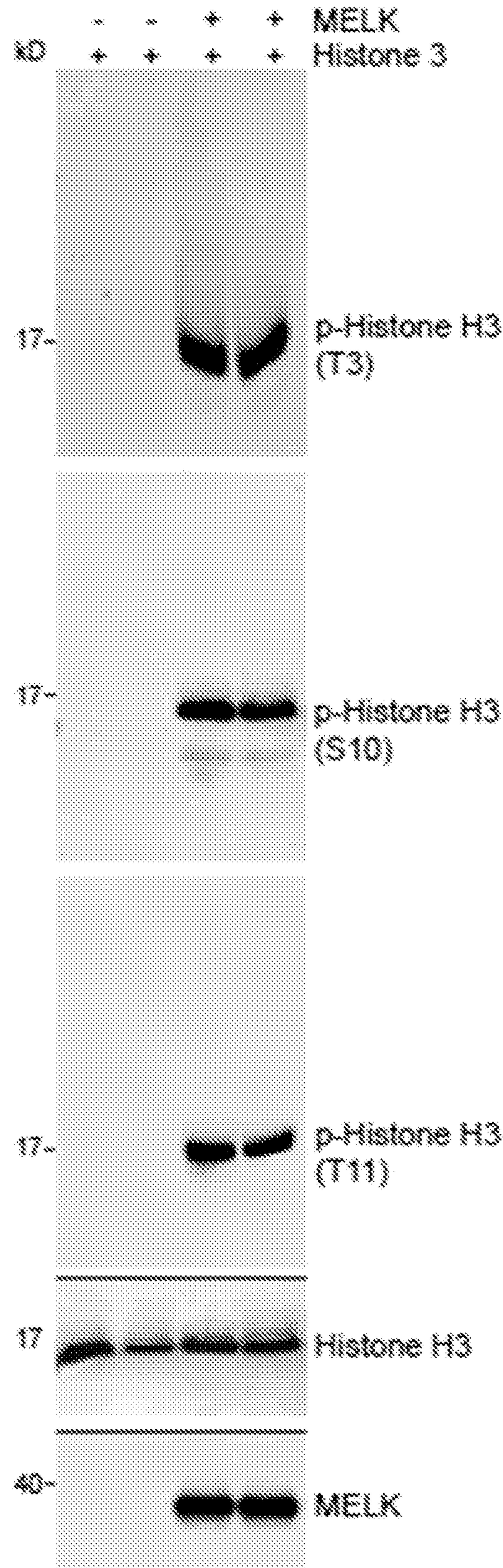


Figure 10

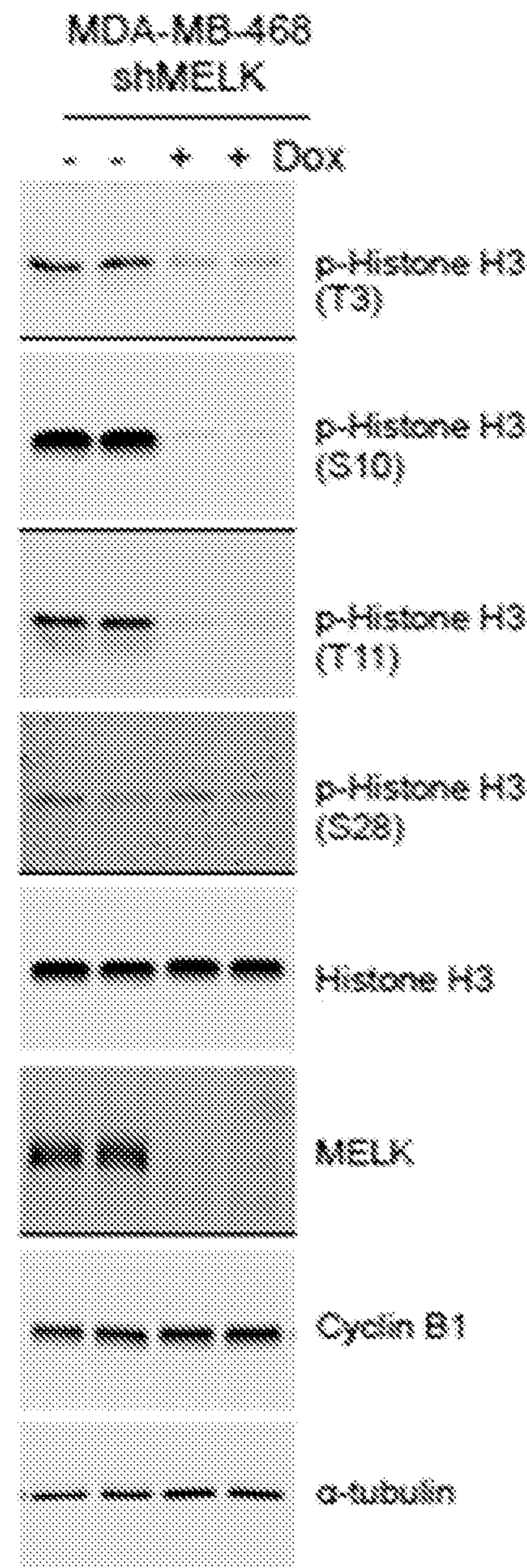


Figure 11

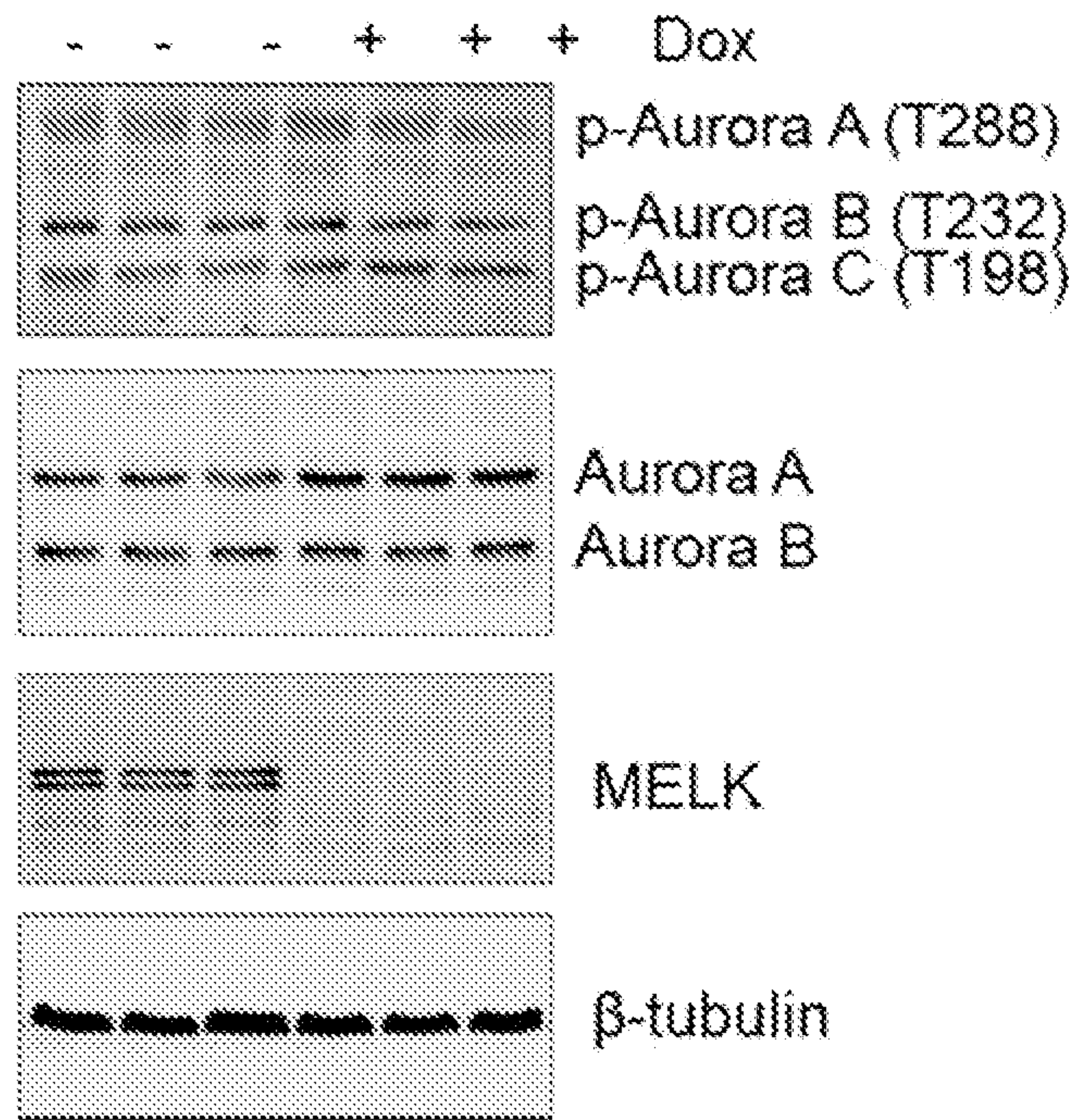


Figure 12

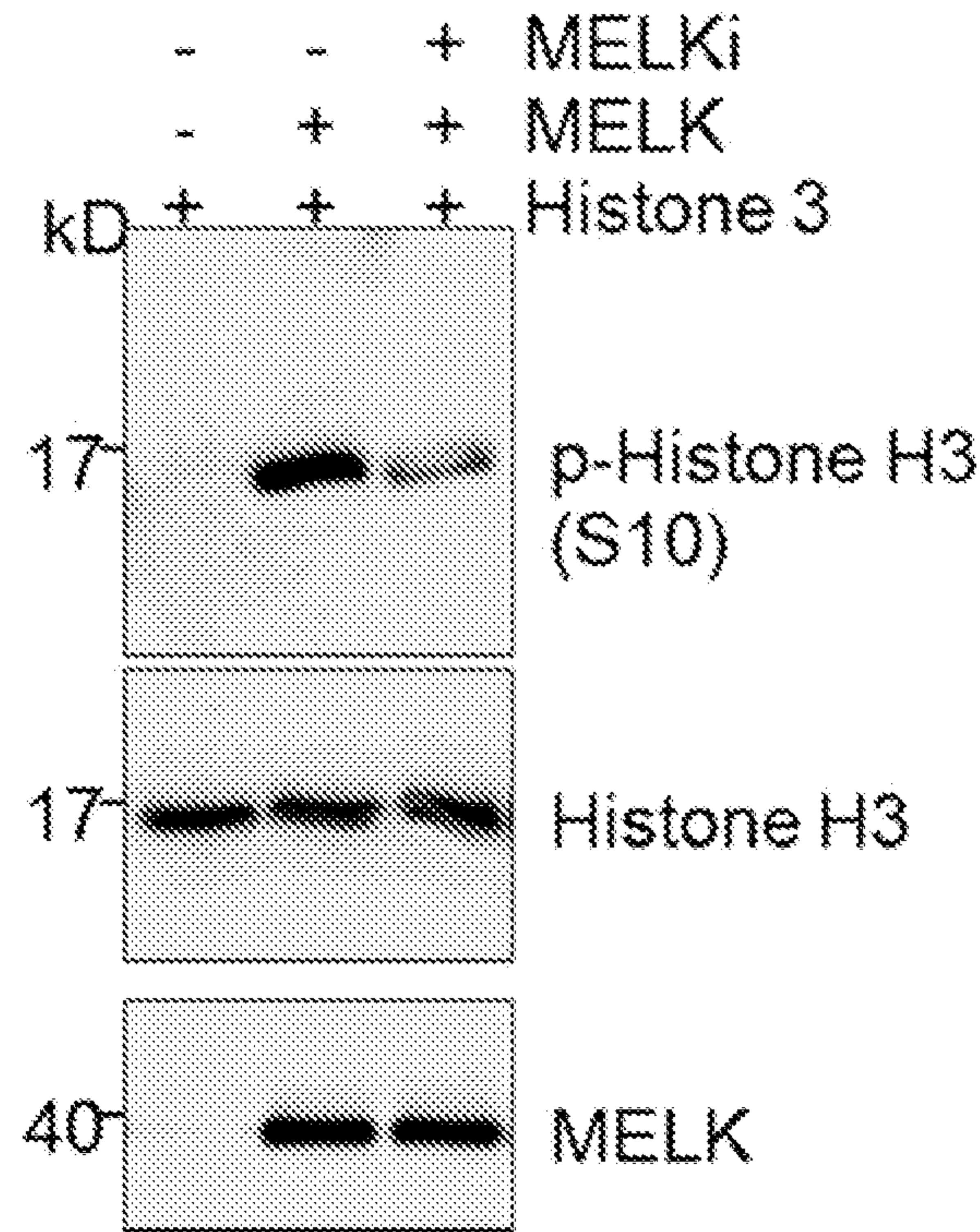


Figure 13

