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(54) THERAPEUTIC AGENT FOR BREAST CANER COMPRISING BIG3-PHB2 INTERACTION-INHIBITING PEPTIDE DERIVED FROM PHB2

(57) The present invention provides peptides containing the BIG3 polypeptide-binding site in a PHB2 polypeptide, which inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide, and pharmaceutical compositions containing the peptide. The peptides of the present invention have the ability to bind not to PHB2, whose expression is found in organs throughout the human body, but to BIG3, which is a protein highly expressed specifically in particularly estrogen receptor-positive cancer, and have excellent growth suppressive effects on BIG3-positive cancer cells. Accordingly, the peptides of the present invention are useful as therapeutic agents for breast cancer which can avoid expression of side effects.

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Description

[Technical Field]

[0001] The present invention relates to PHB2-derived peptides that inhibit BIG3-PHB2 interaction and therapeutic agents for breast cancer comprising the peptide.

[Background Art]

[0002] Estrogen-receptor α (ERα) plays a key role in the development and progression of breast cancer. The current endocrine therapies for breast cancer mainly target ERα signaling, and use selective ERα modulators (for example, tamoxifen and raloxifene), ERα down-regulators (for example, fulvestrant), and aromatase inhibitors (AI) (NPLs 1 to 3). Among these therapies, a method that uses tamoxifen, which inhibits breast cancer cell proliferation through competitive binding to ERα, is a standard therapy for patients with ERα-positive breast cancer. However, tamoxifen therapy is often ineffective, and the patient may die from recurrent endocrine therapy-resistant tumors (NPLs 4 and 5). Furthermore, compared with tamoxifen, AI, which blocks estrogen synthesis, provides substantial clinical effects such as good efficacy, significant increase in relapse-free survival period, and a prolonged time to disease recurrence in postmenopausal women; however, some patients who have undergone AI treatment still relapse (NPLs 6 and 7). The precise molecular events having effects on the efficacy of these endocrine therapies remain unknown.

[0003] A complex formed between brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3), which is a cancer specific protein, and prohibitin 2 (PHB2), which is a tumor suppressor, plays a key role in estrogen signaling regulation in ERα-positive breast cancer (NPLs 8 and 9). BIG3 binds to PHB2 to inhibit the ability of PHB2, which suppresses the estrogen-dependent transcriptional activation, and thereby causes constitutive ERα activation.

[0004] Based on these findings, strategies of making PHB2 exhibit its tumor suppressive activity by dissociating PHB2 from its complex with BIG3 through inhibition of the BIG3-PHB2 interaction, may become a novel therapy for breast cancer. Based on this strategy, the present inventors have previously developed a dominant negative peptide of BIG3, which specifically inhibits the BIG3-PHB2 interaction (PTL 1). This peptide has been confirmed to suppress breast cancer growth by reactivating the tumor suppressive activity of PHB2 to inhibit ERα-signaling pathways that bring about the growth of breast cancer (PTL 1).

[Citation List]

[Patent Literature]

[0005] [PTL 1] WO 2013/018690

[Non-Patent Literature]

[0006]

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[Summary of Invention]

[Technical Problem]

[0007] As noted above, it has been elucidated that an estrogen receptor (ER) activation regulator, BIG3, interacts with a suppressor, PHB2, to cause constitutive activation of ER and that a BIG3-PHB2 interaction-targeting inhibitory peptide (ERAP; the amino acid sequence at positions 165 to 177 (QMLSDLTLQLRQR; SEQ ID NO: 33) of the BIG3 protein)

has the effect of suppressing estrogen (E2)-dependent breast cancer cell growth (PTL 1; WO2017/126461). However, although ERAP, derived from the BIG3 sequence, achieves the interaction inhibition by binding to PHB2, it cannot be denied that ERAP exerts non-selective effects in organs other than cancer tissue because PHB2 expression is found in organs throughout the human body.

[0008] Accordingly, an objective of the present invention is to provide a therapeutic strategy which targets the BIG3-PHB2 interaction and can be expected to be highly selective for breast cancer.

[Solution to Problem]

[0009] The present inventors designed multiple PHB2-derived peptides (PHB2 peptides) based on the data of candidate protein interaction regions on the PHB2 amino acid sequence predicted through *in silico* analysis, and used these PHB2 peptides for screening to identify interaction regions using the effect of suppressing cell growth as an indicator. As a result, the present inventors succeeded in finding that PHB2 peptide No. 1 (11-RLPAGPRGMGTA-22 (SEQ ID NO: 1)) and PHB2 peptide No. 5 (76-QYPIIYDIRARPRKI-90 (SEQ ID NO: 5)) each have the effect of suppressing growth by about 50%, and in particular that the combination of PHB2 peptides Nos. 1 and 5 exhibits the effects of suppressing growth by 100% and inhibiting the BIG3-PHB2 interaction, as with ERAP. Furthermore, these effects were also observed for peptides consisting of sequences around PHB2 peptides Nos. 1 and 5 and for peptides in which amino acid residues at various positions in PHB2 peptides Nos. 1 and 5 have been substituted.

[0010] On the other hand, the cell growth suppressive effect of PHB2 peptides Nos. 1 and 5 was also observed on triple-negative breast cancer cells which do not express estrogen receptors and such but express BIG3. Moreover, the use of these peptides in combination showed enhancement of the effect. It is considered that in triple-negative breast cancer, which proliferates in a manner independent of proliferative signals such as hormones, its growth is activated by the binding between PHB2 and BIG3 without receipt of these signals in cells. It was suggested that the PHB2-derived peptides may suppress cell growth by inhibiting the binding between BIG3 and PHB2 in breast cancer expressing at least BIG3.

[0011] The present inventors thus found the PHB2-derived peptides which inhibit the BIG3-PHB2 interaction and exert an antitumor effect on E2-dependent breast cancer and triple-negative breast cancer, and completed the present invention. That is, the present invention provides the following peptides and uses thereof.

[1] A peptide, comprising a site binding to a BIG3 polypeptide in a PHB2 polypeptide, wherein the peptide inhibits the binding between the PHB2 polypeptide and the BIG3 polypeptide.

[2] The peptide of [1], wherein the peptide comprises any one or a combination of all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21; all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88; and all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57, in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide).

[3] A peptide, comprising an amino acid sequence selected from the group consisting of (a) to (f) below, wherein the peptide inhibits the binding between a PHB2 polypeptide and a BIG3 polypeptide:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41);
(b) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41);
(c) an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53);
(d) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53);
(e) an amino acid sequence selected from the group consisting of SEQ ID NOs: 82 and 83 (PHB2 sequence-derived peptides Nos. 82 and 83); and
(f) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 82 and 83 (PHB2 sequence-derived peptides Nos. 82 and 83).

[4] The peptide of [3], wherein the peptide comprises an amino acid sequence selected from the group consisting of (a') and (b') below:

(a') an amino acid sequence in which one, two, or several amino acid residues located at positions other than

those corresponding to glycine (Gly/G) at positions 15 and 18 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) are substituted with other amino acid residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41); and

(b') an amino acid sequence in which one, two, or several amino acid residues located at positions other than that corresponding to aspartic acid (Asp/D) at position 82 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) are substituted with other amino acid residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53).

[5] The peptide of any one of [1] to [4], wherein the peptide consists of 80 amino acid residues or less.
[6] The peptide of any one of [1] to [5], wherein the peptide consists of 25 amino acid residues or less.
[7] The peptide of any one of [1] to [6], wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83).
[8] The peptide of any one of [1] to [7], wherein the peptide has been modified with a cell membrane-permeable substance.
[9] The peptide of any one of [1] to [8], wherein the peptide is cyclic.
[10] The peptide of any one of [1] to [9], wherein the peptide is cross-linked.
[11] The peptide of any one of [1] to [10], wherein the peptide has either or both of the following properties (i) and (ii):

(i) suppressing growth of BIG3-positive cells; and
(ii) promoting phosphorylation of a serine residue in the PHB2 polypeptide in BIG3-positive cells.

[12] A polynucleotide encoding the peptide of any one of [1] to [11].
[13] A pharmaceutical composition comprising: at least one ingredient selected from the group consisting of one or more of the peptides of any one of [1] to [11], a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s); and a pharmaceutically acceptable carrier.
[14] The pharmaceutical composition of [13], wherein the composition comprises any one or a combination of: a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide); a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide); and a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide).
[15] The pharmaceutical composition of [13] or [14], which is for suppressing growth of cancer cells or for treatment and/or prophylaxis (prevention) of cancer.
[16] The pharmaceutical composition of [15], wherein the cancer is BIG3-positive cancer.
[17] The pharmaceutical composition of [15] or [16], wherein the cancer is breast cancer.
[18] The pharmaceutical composition of any one of [15] to [17], wherein the cancer is estrogen receptor-positive cancer.
[19] A method for either or both of treatment and prophylaxis (prevention) of cancer, wherein the method comprises administering to a subject at least one selected from the group consisting of one or more of the peptides of any one of [1] to [11]; a polynucleotide(s) encoding the peptide(s); and a pharmaceutically acceptable salt(s) of the peptide(s).
[20] The method of [19], wherein the method comprises administering any one or a combination of: a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide); a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide); and a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide).
[21] A method for either or both of treatment and prophylaxis (prevention) of pharmacotherapy-resistant breast cancer (for example, triple-negative breast cancer), wherein the method comprises: selecting a patient with pharmacotherapy-resistant breast cancer (for example, a patient with triple-negative breast cancer); and administering to a subject at least one selected from the group consisting of one or more of the peptides of any one of [1] to [11], a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s).

[0012] Alternatively, the present invention provides use of at least one selected from the group consisting of one or more of the peptides of any one of [1] to [11] mentioned above, a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s), in the manufacture of a pharmaceutical composition for either or both of treatment and prophylaxis (prevention) of cancer. Furthermore, the present invention relates to at least one

selected from the group consisting of one or more of the peptides of any one of [1] to [11] mentioned above, a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s), for use in either or both of treatment and prophylaxis (prevention) of cancer. Moreover, the present invention relates to methods of manufacturing a pharmaceutical composition for either or both of treatment and prophylaxis (prevention) of cancer, the method comprising mixing or compounding with a carrier at least one selected from the group consisting of one or more of the peptides of any one of [1] to [11] mentioned above, a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s).

[Advantageous Effects of Invention]

[0013] The peptides of the present invention have the ability to bind to BIG3, a protein highly expressed specifically in, among others, estrogen receptor-positive cancer, and not to PHB2, of which expression is observed in organs throughout the human body, and can inhibit the BIG3-PHB2 interaction. Thus, the peptides of the present invention can be expected to have high selectivity for estrogen receptor-positive cancer.

[0014] Furthermore, the peptides of the present invention exhibit a growth suppressive effect not only on estrogen-dependent breast cancer cells but also on triple-negative breast cancer cells. For triple-negative breast cancer, there has so far been no effective molecular target drug, and treatment with existing anticancer agents having strong side effects has been the only option. On the other hand, the cell growth suppressive effect of the peptides of the present invention was not observed in normal mammary gland epithelial cells which did not express BIG3. These suggest that the peptides of the present invention are useful as therapeutic drugs for BIG3-positive cancer, regardless of whether the cancer is hormone-dependent or not.

[Brief Description of Drawings]

[0015]

Fig. 1-1: screening for PHB2 sequence-derived peptides which suppress estrogen-dependent cell growth is shown. (A): Human breast cancer cell line MCF-7 was treated with 10 μM each of PHB2 sequence-derived peptides, and then immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. The full-length sequence of human PHB2 protein is shown below the graph, and synthetic peptides in which eight arginine residues were added to the C terminus of the underlined sequences were used in the assay. Moreover, the bold letters represent amino acids which are suggested to be involved in the interaction with BIG3 by *in silico* analysis, boxed "S" represents the phosphorylation site of PHB2, and the broken line represents a region whose binding to PHB2 has been demonstrated.

Fig. 1-2 (B, C): MCF-7 was treated with 10 μM each of PHB2 sequence-derived peptides surrounding 11-22aa (B) and 76-90aa (C) (SEQ ID NOs: 1, 34 to 43, 5, and 44 to 55), and then immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. The positions of the PHB2-derived sequences contained in the synthetic peptides used in the assay are shown on the left side of the graph.

Fig. 1-3 (D): MCF-7 was treated with the combinations of 10 μM PHB2 sequence-derived peptide 11-22aa with 10 μM each of PHB2 sequence-derived peptides surrounding 76-90aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. (E): MCF-7 was treated with the combinations of 10 μM PHB2 sequence-derived peptide 76-90aa with 10 μM each of PHB2 sequence-derived peptides surrounding 11-22aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 2 shows the effect by combination of PHB2 peptides 11-22aa and 76-90aa on the suppression of estrogen-dependent growth. (A): Human breast cancer cell line MCF-7 was treated with 10 μM each of ERAP (positive control), PHB2 peptides 11-22aa, 76-90aa, and 86-100aa (negative control), a combination of 11-22aa and 76-90aa, and a combination of 76-90aa and 86-100aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. (B): The suppressive effects of PHB2 peptides 11-22aa and 76-90aa and their combination on the interaction between BIG3 and PHB2 in MCF-7 were evaluated by Western blotting. MCF-7 was treated with 1 μM and 10 μM each of PHB2 peptides for 24 hours, and the cells were then lysed and immunoprecipitated with an anti-BIG3 antibody to perform immunoblot analysis using the antibodies shown in the figure. The percent binding inhibition is represented as the ratio when taking the PHB2 band area in untreated cells as 100. (C): Human breast cancer cell line MCF-7 was treated with 10 μM each of ERAP, PHB2 peptides 11-22aa, 76-90aa, and 86-100aa, a

combination of 11-22aa and 76-90aa, and a combination of 76-90aa and 86-100, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the phosphorylation of PHB2 (Ser39) after 24 hours by Western blotting. The strength of phosphorylation is represented as the ratio when taking the phosphorylated band area of ERAP treatment in the presence of estrogen as 1.0.

Fig. 3 shows the effect of PHB2 peptide 11-90aa on estrogen-dependent growth and on the interaction between BIG3 and PHB2. (A): Human breast cancer cell line MCF-7 was treated with PHB2 peptides 11-90aa (20, 50, and 100 μM), 11-22aa (50 μM), and 76-90aa (50 μM), and a combination of 11-22aa and 76-90aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. (B): The inhibition effects of PHB2 peptides 11-90aa and 10 μM ERAP (positive control) on the interaction between BIG3 and PHB2 in MCF-7 were evaluated by Western blotting. (C): Human breast cancer cell line MCF-7 was treated with PHB2 peptide 11-90aa (20, 50, and 100 μM) and 10 μM ERAP, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the phosphorylation of PHB2 (Ser39) after 24 hours by Western blotting. The strength of phosphorylation is represented as the ratio when taking the phosphorylated band area of the untreated cells as 1.0.

Fig. 4 shows schemes of branched and cyclic PHB2 peptides. (A): Linear bound PHB2 peptide. (B): Branched PHB2 peptide. (C): Cyclic PHB2 peptides.

Fig. 5 shows the suppressive effects of branched and cyclic PHB2 peptides on estrogen-dependent growth. (A): Human breast cancer cell line MCF-7 was treated with 10 μM each of linear peptides 11-22aa and 76-90aa, a combination of linear 11-22aa and 76-90aa, the linear bound peptide, the branched bound peptide, cyclic 11-21aa and cyclic 76-88aa, and a combination of the cyclic peptides, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. (B) shows the results of MTT assay showing that linear 11-22aa and linear 76-90aa, the linear bound peptide, the branched bound peptide, and cyclic 11-21aa and cyclic 76-88aa peptides have no effect on growth of normal mammary gland epithelial cells, MCF-10A. (C): Human breast cancer cell line MCF-7 was treated with 10 μM each of linear peptides 11-22aa and 76-90aa, a combination of linear 11-22aa and 76-90aa, the linear bound peptide, the branched bound peptide, cyclic 11-21aa and cyclic 76-88aa, and a combination of the cyclic peptides, and their suppressive effects on the interaction between BIG3 and PHB2 were evaluated by Western blotting.

Fig. 6 shows the effects of suppressing estrogen-dependent growth by a combination of cyclic PHB2 peptides. (A): Human breast cancer cell line MCF-7 was treated with linear 11-22aa and cyclic 11-21aa (left) or with linear 76-90aa and cyclic 76-88aa (right), and then the cells were immediately stimulated with 10 nM estrogen to evaluate the suppressive effect by MTT assay every 24 hours up to 96 hours. (B) represents the results of MTT assay showing that cyclic 11-21aa and cyclic 76-88aa peptides have no effect on the growth of normal mammary gland epithelial cells, MCF-10A. The data represents mean ± standard deviation of three independent experiments.

Fig. 7 shows the concentration-dependent suppressive effects of cyclic PHB2 peptides on estrogen-dependent growth. (A): Human breast cancer cell line MCF-7 was treated with 0.1, 0.5, 1, 2.5, 5, 10, and 20 μM each of cyclic 11-21aa and 76-88aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments. (B): Human breast cancer cell line MCF-7 was treated with cyclic 11-21aa (4 μM), cyclic 76-88aa (2 μM), and their combination, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the suppressive effect by MTT assay every 24 hours up to 96 hours. The data represents mean ± standard deviation of three independent experiments. (C) represents the results of MTT assay showing that cyclic 11-21aa (4 μM), cyclic 76-88aa (2 μM), and their combination have no effect on growth of normal mammary gland epithelial cells, MCF-10A.

Fig. 8-1: PHB2 amino acids important for suppressing estrogen-dependent growth are shown. (A) depicts alanine-mutated PHB2 peptides of No. 1 (11-22aa) and No. 5 (76-90aa). (B, C): Human breast cancer cell line MCF-7 was treated with 10 μM each of the alanine-mutated peptides derived from PHB2 sequence, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 8-2 (D) depicts PHB2 peptides in which 51-57 aa has been added to PHB2 peptides No. 2 (42-50aa) and No. 3 (38-50aa). (E): The PHB2 sequence-derived peptides of (D) were each added at 10 μM, and the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number for 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 9 shows the cell growth suppressive effects of PHB2 peptides 11-22aa (A) and 76-90aa (B) on triple-negative breast cancer cells. Each peptide was diluted in a 3-fold dilution series starting from 20 mM with total 11 concentrations and added to breast cancer cell line MDA-MB-231. The numbers of viable cells were measured 96 hours after the peptide addition, the relative values were calculated based on negative control cells to which no peptide was added, and the values were plotted on the graph. The data represents mean ± standard deviation of three independent experiments. (C) shows the results of examining the combined effect of PHB2 peptides 11-22aa and 76-90aa on

cell growth of MDA-MB-231. The peptides were added to the cells alone or in combination at IC50 value, the numbers of viable cells were measured after 96 hours, the relative values were calculated based on negative control cells to which phosphate buffered saline (PBS) was added, and the values were plotted on the graph. The data represents mean ± standard deviation of three independent experiments.

Fig. 10-1: the suppressive effects of cross-linked PHB2 peptides on estrogen-dependent growth are shown. (A) shows schemes of cross-linking forms. (B) shows PHB2 peptides prepared by adding cysteine to both ends of PHB2 peptides 11-21aa and 76-88aa and cross-linking them (SEQ ID NOs: 106 to 108, 110 to 112, 115 to 117, and 119 to 121). The PHB2 peptides of SEQ ID NOs: 109, 113, 114, 118, and 122 were prepared as non-cross-linked peptides by adding alanine to both ends. The PHB2 peptides of SEQ ID NOs: 106 to 114 were prepared by adding polyarginine to the C terminus. Furthermore, in the PHB2 peptides of 11-21aa, methionine at position 19 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) was substituted with norleucine (Nle) to avoid oxidation during synthesis.

Fig. 10-2 (C): Human breast cancer cell line MCF-7 was treated with cross-linked PHB2 peptides of 11-21aa (left panel) or with cross-linked PHB2 peptides of 76-88aa (right panel), and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 11 shows the suppressive effects of cyclic PHB2 peptides on estrogen-dependent growth. (A) depicts cross-linking types of PHB2 peptides of cyclic 11-21aa (SEQ ID NO: 25) and cyclic 76-88aa (SEQ ID NO: 26). The cyclic PHB2 peptides of SEQ ID NOs: 125 and 128 were prepared as non-cross-linked cyclic peptides by adding alanine to both ends of PHB peptides 11-21aa and 76-88aa. All cyclic PHB2 peptides were prepared by adding an unnatural amino acid and consecutive multiple arginine residues to the C terminus. Furthermore, in the cyclic PHB2 peptides of 11-21aa, methionine at position 19 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) was substituted with norleucine (Nle) to avoid oxidation during synthesis. (B): Human breast cancer cell line MCF-7 was treated with cyclic PHB2 peptides, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 12 shows the effects of modifications of PHB2 peptide 11-22aa on estrogen-dependent growth. (A) depicts PHB2 peptides prepared from the PHB2 peptide of SEQ ID NO: 1 (11-22aa) by substituting glycine at positions 15 and 18 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) with D-alanine and D-leucine. (B): Human breast cancer cell line MCF-7 was treated with the modified PHB2 peptides of 11-22aa, and then the cells were immediately stimulated with 10 nM estrogen to evaluate the cell number after 24 hours by MTT assay. The data represents mean ± standard deviation of three independent experiments.

Fig. 13 shows the effects of cross-linked PHB2 peptides on normal mammary gland epithelial cells, MCF-10A. The figure represents the results of MTT assay showing that cross-linked PHB2 peptides and cyclic PHB2 peptides have no effect on growth of normal mammary gland epithelial cells, MCF-10A. The data represents mean ± standard deviation of three independent experiments.

[Description of Embodiments]

[0016] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that the present invention is not limited to the particular sizes, shapes, dimensions, materials, methodologies, protocols, *etc.* described herein, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

Definitions

[0017] The words "a", "an", and "the" used herein mean "at least one" unless otherwise specifically indicated.

[0018] Herein, unless otherwise specifically indicated, amino acids represented by capital letters indicate L-amino acids. Amino acids represented by lower-case letters indicate D-amino acids. Furthermore, L-amino acids and D-amino acids represented herein may include amino acids in which any of amino group, carboxyl group, and side chains has been modified. Examples of preferred modifications include acetylation of the amino group, amidation of the carboxyl group, tag peptide addition such as FLAG-tagging and HA-tagging, and such.

[0019] Herein, numbers indicating the positions of amino acid residues in amino acid sequences have been given with the N-terminal amino acid residue as number 1 and in order toward the C terminus, unless otherwise specifically indicated.

[0020] The term "BIG3" used herein refers to brefeldin A-inhibited guanine nucleotide-exchange protein 3. BIG3 forms

a complex with PHB2 to inhibit the estrogen-dependent transcriptional activation-suppressing function of PHB2. BIG3 is also referred to as "ARFGEF family member 3 (ARFGEF3)" or "A7322". An example of a representative nucleotide sequence of the human BIG3 gene is shown in SEQ ID NO: 31 (GenBank Accession No. NM_020340.4), and the amino acid sequence encoded by the gene is shown in SEQ ID NO: 32. In the present invention, BIG3 is not limited to that encoded by the aforementioned nucleotide sequence and also encompasses their isoforms and mutants.

[0021] The term "PHB2" used herein refers to prohibitin 2. PHB2 binds to estrogen receptors to inhibit estrogen receptor signaling pathways and suppresses estrogen-dependent cell growth. PHB2 is also referred to as "Repressor of Estrogen Activity (REA)". Examples of representative nucleotide sequences of the human PHB2 gene are shown in SEQ ID NO: 27 (GenBank Accession No. NM_001144831.1) and SEQ ID NO: 29 (GenBank Accession No. NM_001267700.1), and the amino acid sequences encoded by the genes are shown in SEQ ID NO: 28 and SEQ ID NO: 30, respectively. In the present invention, PHB2s are not limited to those encoded by the aforementioned nucleotide sequences and also encompass their isoforms and mutants.

[0022] The term "estrogen receptor" used herein encompasses both estrogen receptor α (ERα) and estrogen receptor β (ERβ). ERα and ERβ are encoded by the ESR1 gene and ESR2 gene, respectively. The nucleotide sequence of a representative human ESR1 gene and the amino acid sequence of a representative human ERα are shown in SEQ ID NO: 86 (GenBank Accession No. NM_000125.3) and SEQ ID NO: 87 (GenBank Accession No. NP_000116.2), respectively. Furthermore, the nucleotide sequence of a representative human ESR2 gene and the amino acid sequence of a representative human ERβ are shown in SEQ ID NO: 88 (GenBank Accession No. NM_001437.2) and SEQ ID NO: 89 (GenBank Accession No. NP_001428.1), respectively. In the present invention, however, the nucleotide sequences and amino acid sequences of estrogen receptor are not limited thereto and also encompass their isoforms and mutants. In a preferred embodiment, the estrogen receptor is ERα. It has been reported that transcriptional activation of ERα and ERβ is both regulated by a PHB2 polypeptide (Montano MM, et al., Proc Natl Acad Sci USA. 96(12): 6947-52 (1999)).

[0023] Herein, the term "estrogen receptor-positive" used in the context of a cell or cancer means that a cell or a cancer cell constituting cancer expresses an estrogen receptor. Whether a cell or cancer is estrogen receptor-positive or not can be confirmed by a known method such as ELISA and immunohistochemical staining. Furthermore, herein, the term "estrogen receptor-negative" used in the context of a cell or cancer means that a cell or a cancer cell constituting cancer does not express an estrogen receptor.

[0024] The term "ERAP" used herein refers to a peptide consisting of the amino acid sequence of SEQ ID NO: 33. The amino acid sequence of SEQ ID NO: 33 is a sequence consisting of the 165th to 177th amino acid residues in the amino acid sequence of BIG3 (SEQ ID NO: 32), and contains amino acid residues important for binding with PHB2 (glutamine (Q) at position 165, aspartic acid (D) at position 169, and glutamine (Q) at position 173 in the amino acid sequence of SEQ ID NO: 32). ERAP has an ability to bind to PHB2 and inhibits BIG3 from forming the complex with PHB2 by binding competitively to PHB2.

[0025] The term "treatment" used herein encompasses alleviation/improvement of at least one symptom caused by a target disease, suppression of progression of the disease, suppression of enlargement of the disease site, and such. For example, "cancer treatment (treatment of cancer)" includes cancer cell growth suppression, suppression of cancer progression, induction of regression/remission of cancer, alleviation/improvement of symptoms accompanying cancer, suppression of cancer metastasis, suppression of postoperative recurrence, and induction of prolonged survival time.

1. PHB2 peptides

[0026] The present invention provides peptides comprising a site binding to a BIG3 polypeptide (a BIG3 polypeptide-binding site) in a PHB2 polypeptide, which inhibit the binding between the PHB2 polypeptide and the BIG3 polypeptide. The peptides of the present invention are also herein referred to as "PHB2 peptides", "PHB2-derived peptides", or "PHB2 sequence-derived peptides".

[0027] The peptides of the present invention have the ability to bind to a BIG3 polypeptide by comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide. Consequently, the peptides competitively inhibit the binding of the PHB2 polypeptide to the BIG3 polypeptide. The PHB2 peptides of the present invention can be salts as long as they have the effect of inhibiting the binding between a PHB2 polypeptide and a BIG3 polypeptide. For example, the PHB2 peptides can be salts with acids (such as inorganic acids and organic acids) or with bases (such as alkaline metals, alkaline earth metals, and amines). The salts with acids include, for example, salts with inorganic acids (for example, hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, and acetic acid) and those with organic acids (for example, acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, and meglumine acid). The salts with bases include, for example, salts with sodium, potassium, calcium, and ammonium. Preferred examples of salts of the peptides of the present invention include acetates, hydrochlorides, meglumine salts, and ammonium salts.

[0028] The "site binding to a BIG3 polypeptide in a PHB2 polypeptide (BIG3 polypeptide-binding site in a PHB2 polypeptide)" means an amino acid residue(s) involved in binding to a BIG3 polypeptide in the amino acid sequence

constituting a PHB2 polypeptide. Such an amino acid residue(s) includes, for example, glycine at positions 15 and 18 and aspartic acid at position 82 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide). Thus, in a preferred embodiment, the peptides of the present invention are peptides which comprise glycine at positions 15 and 18 and aspartic acid at position 82 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) and inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide. Herein, the number of a particular amino acid residue in an amino acid sequence indicates the number of the amino acid residue counted from the N terminus.

[0029] Examples of amino acid sequences comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide include (a) all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21 (SEQ ID NO: 84), (b) all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88 (SEQ ID NO: 85), and (c) all or part of the amino acid sequence (SEQ ID NO: 82) consisting of the amino acids at positions 44 to 57, in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide). Accordingly, preferred examples of the peptides of the present invention include a peptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41);
(b) an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53); and
(c) an amino acid sequence selected from the group consisting of SEQ ID NOs: 82 and 83 (PHB2 sequence-derived peptides Nos. 82 and 83).

[0030] However, the peptides of the present invention are not limited thereto, and the amino acid sequences constituting the peptides are not particularly limited as long as the peptides comprise the BIG3 polypeptide-binding site in a PHB2 polypeptide and have the activity to inhibit the binding between the PHB2 polypeptide and a BIG3 polypeptide.

[0031] In general, it is known that one or more amino acid modifications in a peptide have no effect on the function of the peptide. Indeed, it is known that a peptide having an amino acid sequence in which one or more amino acid residues are modified by substitution, deletion, insertion, and/or addition retains the biological activity of the original peptide (Mark et al., Proc Natl Acad Sci USA 81(18): 5662-6 (1984); Zoller and Smith, Nucleic Acids Res 10(20): 6487-500 (1982); and Dalbadie-McFarland et al., Proc Natl Acad Sci USA 79(21): 6409-13 (1982)). The peptides of the present invention may comprise a substitution or deletion of amino acid residues, for example, at positions other than the BIG3 polypeptide-binding site, in a PHB2-derived amino acid sequence and may have an insertion or addition of amino acid residues at positions which have no effect on the binding to a BIG3 polypeptide, as long as they comprise the BIG3 polypeptide-binding site in a PHB2 polypeptide and have the activity to inhibit the binding between the PHB2 polypeptide and the BIG3 polypeptide. Actually, it is shown in Examples of the present specification that peptides having an amino acid sequence in which an amino acid residue(s) other than the BIG3 polypeptide-binding site in a PHB2 polypeptide has/have been substituted with other amino acid residue(s) also retain the biological activity equal to that of peptides which do not have such substitutions. Accordingly, the peptides of the present invention encompass peptides which comprise an amino acid sequence selected from the group consisting of (a') and (b') below and have an activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide:

(a') an amino acid sequence in which one, two, or several amino acid residues located at positions other than those corresponding to glycine at positions 15 and 18 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) are substituted with other amino acid residues, in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41); and
(b') an amino acid sequence in which one, two, or several amino acid residues located at positions other than that corresponding to aspartic acid at position 82 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) are substituted with other amino acid residues, in an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53).

[0032] In the above (a') and (b'), amino acid residues substituted can be any amino acid residues as long as the resulting peptide maintains the ability to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide. Furthermore, which amino acid residue is substituted can be determined by predicting amino acid residues not involved in the binding to a BIG3 polypeptide by using, for example, a calculation method such as PSIVER. The number of amino acid residues substituted are also not particularly limited as long as the resulting peptide maintains the ability to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide, and one, two, or several amino acid residues can be substituted. "Several" preferably refers to six, five, four, or three.

[0033] In general, it is recognized that a substitution with another amino acid residue which conserves the amino acid side chain characteristics of the original amino acid residue tends to have no effect on the function of the original peptide. Such a substitution is often called a "conservative substitution" or "conservative modification". Accordingly, the substi-

tutions in the above (a') and (b') are preferably performed by conservative substitutions.

[0034] Tables of conservative substitutions presenting functionally similar amino acids are well known in the art. Examples of amino acid side chain characteristics that are desirable to conserve include, for example, hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). In addition, the following eight groups each contain amino acids that are accepted in the art as conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M) (see, *e.g.*, Creighton, Proteins 1984).

[0035] However, the substitutions in the above-mentioned (a') and (b') are not limited thereto, and they may be non-conservative substitutions as long as the peptides maintain the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide.

[0036] The peptides of the present invention can comprise amino acid residues other than the BIG3 polypeptide-binding site in a PHB2 polypeptide as long as they maintain the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide. For example, a fragment of PHB2 polypeptide comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide is preferred as a peptide of the present invention. Accordingly, preferred examples of the peptides of the present invention include PHB2 polypeptides (SEQ ID NOs: 1 and 36 to 41 (PHB2 sequence-derived peptides Nos. 1 and 36 to 41)) comprising glycine at positions 15 and 18 and their surrounding sequence in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) and PHB2 polypeptides (SEQ ID NOs: 5 and 47 to 53 (PHB2 sequence-derived peptides Nos. 5 and 47 to 53)) comprising aspartic acid at position 82 and its surrounding sequence in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide). Other preferred examples of the peptides of the present invention include PHB2 polypeptides (SEQ ID NOs: 82 and 83 (PHB2 sequence-derived peptides Nos. 82 and 83)) comprising amino acids at positions 44 to 57 and their surrounding sequence in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide).

[0037] The following peptides can be exemplified as the PHB2 peptides of the present invention: peptides comprising amino acid sequences of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83), which peptides are composed of, for example, 30 amino acid residues or 20 amino acid residues, typically 19 amino acid residues, preferably 18 amino acid residues, and more preferably 17 amino acid residues or less. The following peptides can be shown as such peptides: peptides comprising an amino acid sequence selected from the amino acid sequences (9 residues) of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83) and an amino acid sequence selected from the full-length amino acid sequence constituting a PHB2 polypeptide, which peptides are composed of 30 amino acid residues or 20 amino acid residues, typically 19 amino acid residues, preferably 18 amino acid residues, and more preferably 17 amino acid residues or less.

[0038] In a preferred embodiment of the present invention, an amino acid to be added to the amino acid sequences of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83) can be zero (that is, the amino acid sequences consisting of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83)) or can be one, two or more consecutive amino acid sequences selected from the full-length amino acid sequence constituting a PHB2 polypeptide (SEQ ID NO: 28 (full-length PHB2 polypeptide)). The amino acid sequences of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83) are those comprising glycine at position 15, glycine at position 18, aspartic acid at position 82, or the amino acid sequence consisting of the amino acids at positions 44 to 57 in the full-length amino acid sequence constituting a PHB2 polypeptide (SEQ ID NO: 28 (full-length PHB2 polypeptide)). Accordingly, in a preferred embodiment of the present invention, an amino acid residue or amino acid sequence to be added to SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83) can be selected from amino acid sequences neighboring glycine at position 15, glycine at position 18, aspartic acid at position 82, or the amino acid sequence consisting of the amino acids at positions 44 to 57 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide)).

[0039] It is desirable that the peptides of the present invention have either or both of the following properties (i) and (ii), in addition to the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide:

(i) promoting the nuclear import of a PHB2 polypeptide in an estrogen receptor-positive cell which expresses a BIG3 polypeptide; and
(ii) promoting the binding between an estrogen receptor present in the nucleus and/or on cell membrane to a PHB2 polypeptide in an estrogen receptor-positive cell which expresses an ERAP1 polypeptide.

[0040] By having either or both of the above properties (i) and (ii), the peptides of the present invention suppress activation of estrogen receptors in BIG3-expressing cells, thereby leading to suppression of growth of estrogen receptor-positive cells. Both the above properties (i) and (ii) of PHB2 peptides can be evaluated according to methods known to one skilled in the art.

[0041] APHB2 polypeptide is known as an estrogen receptor-selective coregulator and suppresses transcriptional activation of estrogen receptors by interaction with them (Kasashima K, J Biol Chem 281(47): 36401-10 (2006)). On the other hand, a BIG3 polypeptide binds to a PHB2 polypeptide to block the nuclear import of a PHB2 polypeptide, thereby inhibiting the interaction between the PHB2 polypeptide and an estrogen receptor in the nucleus. Furthermore, a BIG3 polypeptide blocks the binding between an estrogen receptor present on the cell membrane and a PHB2 polypeptide. As a result of these functions, in cells overexpressing a BIG3 polypeptide, suppression of estrogen receptor activation by the PHB2 polypeptide does not sufficiently work, and enhanced cell growth is induced.

[0042] The peptides of the present invention have the feature of restoring the PHB2 polypeptide's function of suppressing activation of estrogen receptors, the function having been inhibited by the binding to a BIG3 polypeptide, by competitively inhibiting the binding between the BIG3 polypeptide and the PHB2 polypeptide. On the other hand, a PHB2 polypeptide suppresses activation of estrogen receptors through the binding to them. Accordingly, it is desirable that the peptides of the present invention suppress the binding between a BIG3 polypeptide and a PHB2 polypeptide but do not block the binding between an estrogen receptor and the PHB2 polypeptide, and thus do not block the suppression of estrogen receptor activation by the PHB2 polypeptide. As described above, a fragment of PHB2 polypeptide comprising the BIG3 polypeptide-binding site is suitable as a peptide of the present invention; however, a peptide close to the full-length of a PHB2 polypeptide is likely to block the binding between an endogenous PHB2 polypeptide and an estrogen receptor, thereby blocking the suppression of estrogen receptor activation by the endogenous PHB2 polypeptide. Thus, a partial amino acid sequence of PHB2 polypeptide comprised in the peptides of the present invention is preferably 100 residues or less, more preferably 80 residues or less, and even more preferably 70 residues or less. In a more preferred embodiment, a partial amino acid sequence of PHB2 polypeptide comprised in the peptides of the present invention is 50 residues or less, 40 residues or less, 30 residues or less, 25 residues or less, or 20 residues or less. Since the estrogen receptor-binding site in PHB2 is a site consisting of the amino acids at positions 175 to 198 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide), the peptides of the present invention preferably do not comprise this site and in this case, a partial amino acid sequence of PHB2 polypeptide comprised in the peptides of the present invention is desirably 100 residues or less, more preferably 80 residues or less, and even more preferably 70 residues or less, excluding the sequence of amino acids at positions 175 to 198. In a more preferred embodiment, a partial amino acid sequence of PHB2 polypeptide comprised in the peptides of the present invention is 50 residues or less, 40 residues or less, 30 residues or less, 25 residues or less, or 20 residues or less, excluding the sequence of amino acids at positions 175 to 198.

[0043] Furthermore, the peptides of the present invention may comprise additional amino acid sequences other than the amino acid sequence derived from a PHB2 polypeptide as long as they maintain the activity to inhibit the binding between a BIG3 polypeptide and a PHB2 polypeptide and do not block the suppression of estrogen receptor activation by the PHB2 polypeptide. Also in this case, it is desirable that the additional amino acid sequences do not block the binding between an endogenous PHB2 polypeptide and an estrogen receptor. Thus, the peptides of the present invention are preferably a peptide of 100 residues or less, 80 residues or less, or 70 residues or less. In a more preferred embodiment, the peptides of the present invention are peptides of 50 residues or less, 40 residues or less, or 30 residues or less. Preferred examples of amino acid sequences comprised in the peptides of the present invention include, but are not limited to, amino acid sequences constituting cell-permeable peptides described later and linker sequences for coupling other substances.

[0044] Moreover, the peptides of the present invention may be modified with other substances. Herein, the term "modified" used in the context of a peptide means that another substance(s) is/are directly or indirectly coupled to a peptide. Other substances that modify the peptides of the present invention include, but are not limited to, for example, peptides, lipids, saccharides, and natural or synthetic polymers. The peptides of the present invention can have any modifications as long as they maintain the activity to inhibit the binding between a BIG3 polypeptide and a PHB2 polypeptide. Furthermore, the peptides of the present invention may be conferred additional functions by modifications. Examples of the additional functions include, but are not limited to, targeting property, stability, and cell membrane

permeability.

[0045] Preferred examples of modifications in the present invention include introduction of a cell membrane permeable substance. Intracellular structure is usually cut off from the outside world by the cell membrane. Thus, it is difficult to efficiently introduce an extracellular substance into a cell. However, a certain type of substance has cell membrane permeability and can be introduced into a cell without being cut off by the cell membrane. It is possible to confer cell membrane permeability to a substance with no cell membrane permeability by modifying the substance with such a substance having cell membrane permeability (cell membrane permeable substance). Accordingly, the peptides of the present invention can be efficiently introduced into cells by modifying the peptide of the present invention with a cell membrane permeable substance(s). Furthermore, as used herein, "cell membrane permeability" refers to the property of being able to permeate the cell membrane of mammals and enter the cytoplasm. Moreover, a "cell membrane permeable substance" refers to a substance having "cell membrane permeability".

[0046] Examples of the cell membrane permeable substance include, but are not limited to, membrane fusogenic liposomes and cell membrane permeable peptides. For example, membrane fusogenic liposomes are fused to cell membrane to release their contents into a cell. Membrane fusogenic liposomes can be adjusted, for example, by modifying the surface of liposomes with a substance having membrane fusogenicity. Examples of membrane fusogenic liposomes include pH-sensitive liposomes (Yuba E, et al., J. Control. Release, 149, 72-80 (2011)), Sendai virus membrane fusogenic liposomes (WO 97/016171), and liposomes modified with cell membrane permeable peptides. The peptides of the present invention may be encapsulated into a membrane fusogenic liposome to efficiently introduce the peptides into a cell. In the present invention, encapsulation of a peptide into a membrane fusogenic liposome is also encompassed in "modification" of a peptide.

[0047] Various natural or artificially synthesized peptides have so far been reported as cell membrane permeable peptides (Joliot A. & Prochiantz A., Nat Cell Biol. 2004; 6: 189-96). Examples of cell membrane permeable peptides include the following peptides, but are not limited thereto.

[0048]

Polyarginine (Matsushita et al., J. Neurosci.; 21(16), 6000-7 (2003));
Tat/RKKRRQRRR (SEQ ID NO: 90) (Frankel et al., Cell 55(6), 1189-93 (1988)., Green & Loewenstein Cell 55, 1179-88 (1988));
Penetratin/RQIKIWFQNRRMKWKK (SEQ ID NO: 103) (Derossi et al., J. Biol. Chem. 269(14), 10444-50 (1994));
Buforin II/TRSSRAGLQFPVGRVHRLLRK (SEQ ID NO: 91) (Park et al., Proc. Natl Acad. Sci. USA 97(15), 8245-50 (2000));
Transportan/GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO: 92) (Pooga et al., FASEB J. 12(1), 67-77 (1998));
MAP (Model Amphipathic Peptide)/KLALKLALKALKAALKLA (SEQ ID NO: 93) (Oehlke et al., Biochim. Biophys. Acta. 1414(1-2), 127-39 (1998));
K-FGF/AAVALLPAVLLALLAP (SEQ ID NO: 94) (Lin et al., J. Biol. Chem. 270(24), 14255-8 (1995));
Ku70/VPMLK (SEQ ID NO: 95) (Sawada et al., Nature Cell Biol. 5(4), 352-7 (2003));
Ku70/PMLKE (SEQ ID NO: 96) (Sawada et al., Nature Cell Biol. 5(4), 352-7 (2003));
Prion/MANLGYWLLALFVTMWTDVGLCKKRPKP (SEQ ID NO: 97) (Lundberg et al., Biochem. Biophys. Res. Commun. 299(1), 85-90 (2002));
pVEC/LLIILRRRIRKQAHAHSK (SEQ ID NO: 98) (Elmquist et al., Exp. Cell Res. 269(2), 237-44 (2001));
Pep-1/KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 99) (Morris et al., Nature Biotechnol. 19(2), 1173-6 (2001));
SynB1/RGGRLSYSRRRFSTSTGR (SEQ ID NO: 100) (Rousselle et al., Mol. Pharmacol. 57(4), 679-86 (2000));
Pep-7/SDLWEMMMVSLACQY (SEQ ID NO: 101) (Gao et al., Bioorg. Med. Chem. 10(12), 4057-65 (2002)); and
HN-1/TSPLNIHNGQKL (SEQ ID NO: 102); (Hong & Clayman Cancer Res. 60(23), 6551-6 (2000)).

[0049] The above-mentioned polyarginine may be composed of any number of arginine residues. For example, polyarginine may be composed of 5 to 20 arginine residues. The number of arginine residues constituting polyarginine is not particularly limited as long as it does not block the activity of the peptide to inhibit the binding between a BIG3 polypeptide and a PHB2 polypeptide.

[0050] Furthermore, it is known in the art to introduce various particularly useful amino acid mimetics or unnatural amino acids (for example, by substitution, addition, or insertion) in order to increase *in vivo* stability of peptides. Examples of amino acid mimetics or unnatural amino acids to be introduced include, but are not limited to, β-amino acids, D-amino acids, and N-methyl amino acids. Accordingly, such amino acid mimetics or unnatural amino acids can be introduced into the peptides of the present invention to increase *in vivo* stability. Moreover, azapeptides in which α-carbons of amino acids have been substituted with an amino group and techniques for substituting amide bonds in peptides with their equivalents (such as esters, sulfonamides, and alkene isosteres) are also known in the art. Stability of peptides can be confirmed using, for example, peptidases and various biological media such as human plasma and serum (see, for example, Coos Verhoef et al. Eur. J. Drug Metab. Pharmacokin. 11(4): 291-302 (1986)).

[0051] Accordingly, the present invention provides peptides comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide, which inhibit the binding between the PHB2 polypeptide and a BIG3 polypeptide and comprise at least one amino acid mimetic or unnatural amino acid (for example, β-amino acid, D-amino acid, and N-methyl amino acid). In a particular embodiment, the peptides of the present invention comprise an amino acid sequence in which one, two, or several amino acids are substituted with corresponding amino acid mimetics or unnatural amino acids (for example, β-amino acids, D-amino acids, and N-methyl amino acids) in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83 (PHB2 sequence-derived peptides Nos. 1, 5, 36 to 41, 47 to 53, 82, and 83).

[0052] Moreover, the present invention also provides peptides comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide, which inhibit the binding between the PHB2 polypeptide and a BIG3 polypeptide and in which at least one amide bond has been replaced with its equivalent (for example, an ester, sulfonamide, and alkene isostere).

Cyclic and cross-linked peptides

[0053] In a particular embodiment, the peptides of the present invention may be cyclized and their stability can be improved by cyclization. Methods of introducing a cyclic structure into a peptide of the present invention are well known, and for example, a peptide can be cyclized by adding cysteine to the N- and C-termini of a linear peptide and allowing to form a disulfide bond between these cysteines. Herein, such a structure in which side chains of two (a pair of) amino acid residues in an amino acid sequence constituting a peptide are cross-linked (stapled) can be called a "stapling structure", and a cross-linked peptide into which one or more stapling structures have been introduced is also referred to as a "stapled peptide". Positions of amino acid residues forming such an intramolecular crosslink are not limited to the N- and C-termini of the original linear peptide, and amino acid residues present in the original linear peptide may form an intramolecular crosslink, or amino acid residues introduced into the original linear peptide (by, for example, substitution, addition, or insertion) may form an intramolecular crosslink. Amino acid residues forming an intramolecular crosslink are not limited to natural amino acids and may be amino acid mimetics or unnatural amino acids as described above. Moreover, methods of cross-linking a peptide are not limited to disulfide bond formation, and also include cross-linking of cysteine residues through fluorobenzene (for example, by using hexafluorobenzene or decafluorobiphenyl), thioether bond formation, ester bond formation, and a technique for stapling hydrocarbons such as ring-closing olefin metathesis (described in, for example, WO 2017/126461).

[0054] Furthermore, methods of cyclizing a peptide are not limited to methods of forming an intramolecular crosslink as mentioned above, and also include formation of an amide bond between the C-terminal and N-terminal amino acid residues of a peptide. The peptides thus cyclized by various methods are herein referred to as cyclic peptides, and include both cyclic peptides comprising an intramolecular crosslink (*i.e.*, cross-linked peptides; for example, SEQ ID NOs: 25, 26, 123, 124, 126, and 127 depicted in Figs. 4 and 11) and those not comprising an intramolecular crosslink (for example, SEQ ID NOs: 125 and 128 depicted in Fig. 11).

[0055] Accordingly, the present invention provides cyclic peptides comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide, which inhibit the binding between the PHB2 polypeptide and a BIG3 polypeptide and have been cyclized by at least one intramolecular bond. Examples of the intramolecular bond include, but are not limited to, a disulfide bond, crosslink between cysteine residues through fluorobenzene (for example, by using hexafluorobenzene or decafluorobiphenyl), thioether bond, ester bond, thioester bond, bond by a hydrocarbon chain (for example, olefin and aryl), bond by a heterocycle (for example, triazole, oxazole, and thiazole) and amido bond, and combinations thereof.

[0056] Such an intramolecular bond may be formed by amino acid residues at both ends of the original linear peptide of a cyclic peptide, or may be formed by amino acid residues in the linear peptide. Moreover, the intramolecular bond may be formed by amino acid residues in an amino acid sequence derived from a PHB2 polypeptide, or may be formed by amino acid residues introduced into the amino acid sequence (by, for example, substitution, addition, or insertion). Preferred examples of such cyclic peptides of the present invention include cyclic PHB2 peptides of 11-21aa and 76-88aa (cyclic peptides respectively consisting of SEQ ID NO: 25, 106 to 108, 115 to 117, 123, or 124; and SEQ ID NO: 26, 110 to 112, 119 to 121, 126, or 127). These exemplary cyclic peptides are prepared by adding two cysteine residues that form an intramolecular bond (intramolecular crosslink) (SEQ ID NOs: 25, 26, 106 to 108, 110 to 112, 115 to 117, 119 to 121, 123, 124, 126, and 127); adding or substituting unnatural amino acids (SEQ ID NOs: 25, 26, 106 to 108, 115 to 117, 123, 124, 126, and 127); and adding consecutive multiple arginine residues (SEQ ID NOs: 25, 26, 106 to 108, 110 to 112, 123, 124, 126, and 127), in the linear peptides of PHB2 sequence-derived 11-21aa and 76-88aa with the objective of stabilization of the structure (and increase in the activity and improvement of protease resistance accompanied thereby) and improvement of membrane permeability. Such peptides have been cyclized by formation of an intramolecular crosslink between the two cysteine residues introduced (SEQ ID NOs: 25, 26, 106 to 108, 110 to 112, 115 to 117, 119 to 121, 123, 124, 126, and 127) and by formation of an amido bond between the C-terminal and N-terminal amino acid residues added (SEQ ID NOs: 25, 26, 123, 124, 126, and 127) (Figs. 4C, 10A, and 11A). These exemplary cyclic peptides (in particular, cyclic and cross-linked peptides) showed an enhanced growth suppressive

effect as compared to the original linear peptides (Figs. 5A, 10C, and 11B) and the suppressive effect was shown to last for a long time (Fig. 6A).

[0057] The present invention also relates to methods of producing a cyclic peptide, the method comprising:

(a) providing a linear peptide comprising the BIG3 polypeptide-binding site in a PHB2 polypeptide, which inhibits the binding between the PHB2 polypeptide and a BIG3 polypeptide; and
(b) allowing to form at least one intramolecular bond in the linear peptide, thereby cyclizing the linear peptide.

[0058] The intramolecular bond is optionally selected from the group consisting of a disulfide bond, crosslink between cysteine residues through fluorobenzene (for example, by using hexafluorobenzene or decafluorobiphenyl), thioether bond, ester bond, thioester bond, bond by a hydrocarbon chain (for example, olefin and aryl), bond by a heterocycle (for example, triazole, oxazole, and thiazole) and amido bond, and combinations thereof.

[0059] The above methods optionally comprise introducing at least one selected from the group consisting of cysteine residues, an amino acid mimetic(s) or unnatural amino acid(s), and consecutive multiple arginine residues into the linear peptide (by, for example, substitution, addition, or insertion).

[0060] The peptides of the present invention have the feature of having the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide. Whether a peptide produced has the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide or not can be confirmed by comparing the binding level between the PHB2 polypeptide and the BIG3 polypeptide in the presence and absence of the peptide. That is, when the binding level in the presence of a peptide is lower than that in the absence of the peptide, the peptide can be judged to have "the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide".

[0061] The binding level between a PHB2 polypeptide and a BIG3 polypeptide can be measured using various known methods. For example, immunoprecipitation using an anti-PHB2 antibody or an anti-BIG3 antibody, affinity chromatography, a biosensor using surface plasmon resonance phenomenon, and such can be used.

[0062] As a specific method, for example, a PHB2 polypeptide and a BIG3 polypeptide are incubated in the presence and absence of a test peptide. The reaction solution is then immunoprecipitated with an anti-PHB2 antibody or an anti-BIG3 antibody, and the immunoprecipitate is subjected to Western blot analysis. The binding level between the PHB2 polypeptide and the BIG3 polypeptide can be confirmed by detecting at least either one of the BIG3 polypeptide level immunoprecipitated with the anti-PHB2 antibody or the PHB2 polypeptide level immunoprecipitated with the anti-BIG3 antibody. The PHB2 polypeptide and BIG3 polypeptide used here can be adjusted by a known genetic engineering technique. Furthermore, lysates of cells producing these polypeptides can be used. The cell lines as described in Examples of the present specification can be utilized as cells producing these polypeptides.

[0063] Alternatively, the methods as described in Examples of the present specification can also be used. Specifically, estrogen receptor-positive cells are cultured in the presence and absence of a test peptide. The cells are then lysed with an appropriate lysis buffer, and the cell lysate may be used to perform immunoprecipitation and Western blot analysis in the same manner as above.

[0064] A peptide for which "the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide" has been confirmed by any of the above methods is judged to be a peptide having "the activity to inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide".

[0065] Moreover, the peptides of the present invention may have either or both of the following (i) and (ii) as preferred properties:

(i) promoting the nuclear import of a PHB2 polypeptide in an estrogen receptor-positive cell which expresses a BIG3 polypeptide; and
(ii) promoting the binding between an estrogen receptor present in the nucleus and/or on cell membrane and a PHB2 polypeptide in an estrogen receptor-positive cell which expresses a BIG3 polypeptide.

[0066] Whether a peptide of the present invention has the above properties or not can be confirmed by comparing (i) the nuclear import level of a PHB2 polypeptide; and/or (ii) the binding level between an estrogen receptor and a PHB2 polypeptide, in the presence and absence of the peptide of the present invention. That is, when the level in the presence of the peptide of the present invention is higher as compared to that in the absence of the peptide, the peptide can be judged to have the above-mentioned properties (i) and/or (ii).

[0067] Methods well known to one skilled in the art can be used as examples of specific methods for judging the presence or absence of the above-mentioned properties (i) and/or (ii). Specifically, when examining the above property (i), estrogen receptor-positive cells are stimulated with estradiol for 24 hours with or without the addition of a peptide of the present invention. The cells are then fractionated by specific gravity centrifugation, and PHB2 polypeptides present in the nuclear fraction are detected by Western blot analysis and such. When the level of PHB2 polypeptide detected in the nuclear fraction increases in the case where the peptide of the present invention is added as compared to the

case where the peptide is not added, the peptide of the present invention is judged to have the above-mentioned property (i). Moreover, the level of PHB2 polypeptide present in the nucleus can be detected by immunocytochemical staining.

[0068] When examining the above-mentioned property (ii), estrogen receptor-positive cells are stimulated with estradiol for 24 hours with or without the addition of a peptide of the present invention. The cells are then fractionated by specific gravity centrifugation, the cytosolic fraction and the nuclear fraction are immunoprecipitated with an anti-estrogen receptor antibody or an anti-PHB2 antibody, and the immunoprecipitate is subjected to Western blot analysis. As a result, when the binding level between an estrogen receptor and a PHB2 polypeptide in the cytosolic fraction and/or the nuclear fraction increases in the case where the peptide of the present invention is added as compared to the case where the peptide is not added, the peptide of the present invention is judged to have the above-mentioned property (ii).

[0069] The peptides of the present invention can be produced using methods well known to one skilled in the art. For example, the peptides of the present invention can be obtained by chemical synthesis based on their amino acid sequences. Methods for chemical synthesis of a peptide are known and one skilled in the art can chemically synthesize the peptide of the present invention based on amino acid sequence selected as the peptide of the present invention. Chemical synthesis methods of peptide are described, for example, in the documents below:

(i) Peptide Synthesis, Interscience, New York, 1966;
(ii) The Proteins, Vol. 2, Academic Press, New York, 1976;
(iii) "Peptide Synthesis" (in Japanese), Maruzen Co., 1975;
(iv) "Basics and Experiment of Peptide Synthesis" (in Japanese), Maruzen Co., 1985;
(v) "Development of Pharmaceuticals" (in Japanese), Continued Vol. 14 (peptide synthesis), Hirokawa, 1991;
(vi) WO99/67288; and
(vii) Barany G. & Merrifield R.B., Peptides Vol. 2, Solid Phase Peptide Synthesis, Academic Press, New York, 1980, 100-118.

[0070] Alternatively, the peptides of the present invention can be obtained by genetic engineering methods (*e.g.*, Morrison J, J Bacteriology, 132(1): 349-51 (1977); Clark-Curtiss & Curtiss, Methods in Enzymology (eds. Wu et al.), 101: 347-62 (1983)). For example, a polynucleotide encoding a peptide of the present invention is inserted into an appropriate expression vector, and the vector is introduced into appropriate host cells to prepare transformed cells. The transformed cells are then cultured to produce the peptide of the present invention, and the cell extract is adjusted. Standard techniques for purifying proteins can be used to purify the peptide of the present invention from the cell extract. The peptide of the present invention can be purified by, for example, appropriately selecting and combining column chromatography, filter filtration, ultrafiltration, salting-out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, and recrystallization. Furthermore, the peptides of the present invention can be synthesized by *in vitro* translation system in which elements necessary to synthesize protein have been reconstructed *in vitro.*

[0071] When using genetic engineering techniques, the peptides of the present invention can also be expressed as a fusion protein with other peptide. A polynucleotide encoding a peptide of the present invention is ligated in frame with a polynucleotide encoding other peptide and inserted into an appropriate expression vector, and the vector is introduced into appropriate host cells to adjust transformed cells. The host cells are then cultured to allow production of a fusion protein of the peptide of the present invention and the other peptide, and its cell extract is adjusted. The purification of fusion protein from the cell extract can be performed by, for example, capturing the fusion protein by affinity chromatography using a column to which a substance having affinity to the fusion protein has been coupled. Moreover, if the peptide of the present invention has been coupled to another peptide through a linker sequence which can be cleaved by an enzyme such as peptidase, protease, and proteasome, the peptide of the present invention can be separated from the column by treating the fusion protein captured by the column with such an enzyme. Examples of other peptides which can be used to form fusion proteins include the following peptides, but are not limited thereto:

FLAG (Hopp et al., Bio/Technology 6, 1204-10 (1988));
6xHis or 1OxHis consisting of histidine (His) residues;
Influenza Hemagglutinin (HA);
Human c-myc fragment, VSV-GP fragment; p18 HIV fragment;
T7 tag; HSV tag;
E tag; SV40T antigen fragment;
lck tag;
α-Tubulin fragment;
B tag;
Protein C fragment;
GST (Glutathione-S-transferase);

HA (Influenza Hemagglutinin);
Immunoglobulin constant region;
β-Galactosidase; and
MBP (Maltose-binding protein).

2. Polynucleotides encoding the peptides of the present invention, vectors, and host cells

[0072] The present invention also provides polynucleotides encoding the peptide of the present invention. Furthermore, the present invention provides vectors comprising the polynucleotide and host cells comprising the vector. Such polynucleotides, vectors, and host cells can be used to produce the peptides of the present invention.

[0073] The polynucleotides of the present invention can be produced by methods known to one skilled in the art. For example, the polynucleotides of the present invention can be synthesized using solid-phase techniques as described in Beaucage SL & Iyer RP, Tetrahedron, 48: 2223-311 (1992); Matthes et al., EMBO J, 3(4): 801-5 (1984). Moreover, the polynucleotides of the present invention can be adjusted using genetic engineering techniques. For example, primers are produced based on a partial nucleotide sequence of a PHB2 gene (SEQ ID NO: 27) encoding an amino acid sequence selected as a peptide of the present invention, and reverse transcription-PCR is performed by using mRNAs extracted from cells expressing a PHB2 polypeptide as a template. Thus, the polynucleotides of the present invention can be amplified.

[0074] The polynucleotides of the present invention can be inserted into an appropriate expression vector and the vector is introduced into appropriate host cells to produce the peptide of the present invention in the host cells.

[0075] For example, when *E. coli* is selected as a host cell and a vector is amplified in a large amount in *E. coli* (for example, JM109, DH5-alpha, HB101 or XL1 Blue), the vector needs to have an "ori" for amplification in *E. coli* and a marker gene for selection of transformed *E. coli* (for example, a drug resistance gene selected by a drug such as ampicillin, tetracycline, kanamycin, chloramphenicol). For example, the M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script and such can be used. When a vector is used for producing a peptide of the present invention, an expression vector is particularly useful. For example, an expression vector for expression in *E. coli* needs to have the above features for amplification in *E. coli.* When *E. coli* such as JM109, DH5-alpha, HB 101 or XL1 Blue are used as a host cell, the vector needs to have a promoter, for example, lacZ promoter (Ward et al., Nature, 341(6242): 544-6 (1989); FASEB J, 6(7): 2422-7 (1992)), araB promoter (Better et al., Science, 240(4855): 1041-3 (1988)), T7 promoter or the like, that can efficiently express the desired gene in *E. coli.* Additionally, the vector may contain a signal sequence for polypeptide secretion. An exemplary signal sequence that directs the polypeptide to be secreted to the periplasm of the *E. coli* is the pelB signal sequence (Lei et al., J Bacteriol, 169(9): 4379-83 (1987)). Means for introducing the vectors into the target host cells include, for example, the calcium chloride method and the electroporation method.

[0076] In addition to *E. coli*, for example, expression vectors derived from mammalian cells (for example, pcDNA3 (Invitrogen) and pEGF-BOS (Mizushima S., Nucleic Acids Res, 18(17): 5322 (1990)), pEF, pCDM8), expression vectors derived from insect cells (for example, "Bac-to-BAC baculovirus expression system" (GIBCO BRL), pBacPAK8), expression vectors derived from plants (*e.g.*, pMH1, pMH2), expression vectors derived from animal viruses (*e.g.*, pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (*e.g.*, pZIpneo), expression vectors derived from yeast (*e.g.*, "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01) and expression vectors derived from Bacillus subtilis (*e.g.*, pPL608, pKTH50) can be used.

[0077] In order to express the vector in animal cells such as CHO cells, COS cells or NIH3T3 cells, the vector needs to carry a promoter necessary for expression in such cells, for example, the SV40 promoter (Mulligan et al., Nature, 277(5692): 108-14 (1979)), the MMLV-LTR promoter, the EF1-alpha promoter (Mizushima et al., Nucleic Acids Res, 18(17): 5322 (1990)), the CMV promoter and the like, and preferably a marker gene for selecting transformants (for example, a drug resistance gene selected by a drug (*e.g.*, neomycin, G418)). Examples of known vectors with these characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV and pOP13.

[0078] Furthermore, the polynucleotide of the present invention may be inserted into an appropriate vector and the vector is introduced into target cells to produce the peptide of the present invention within the target cells. A peptide of the present invention produced in a target cell inhibits the binding between a PHB2 polypeptide and a BIG3 polypeptide and induces suppression of growth of the target cell. In this case, the vector into which a polynucleotide of the present invention is inserted may be a vector for stably inserting the polynucleotide of the present invention into the genome of the target cell (for example, see Thomas KR & Capecchi MR, Cell, 51(3): 503-12 (1987) for description of cassette vectors for homologous recombination). For example, see Wolff et al., Science, 247: 1465-8 (1990); U.S. Patent No. 5,580,895; U.S. Patent No. 5,589,466; U.S. Patent No. 5,804,566; U.S. Patent No. 5,739,118; U.S. Patent No. 5,736,524; U.S. Patent No. 5,679,647; and WO 98/04720.

[0079] Moreover, the polynucleotide of the present invention can be inserted into, for example, an expression vector such as a viral vector and a bacterial vector. Examples of the expression vector include a host for an attenuated virus of cowpox, fowlpox, and the like (see, for example, U.S. Patent No. 4,722,858). Other examples of vectors that can be

used include Bacille Calmette Guerin (BCG) (Stover et al., Nature, 351(6326): 456-60 (1991)). Other examples include adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, *Salmonella typhi* vectors, and attenuated anthrax toxin vectors (Shata et al., Mol Med Today, 6(2): 66-71 (2000); Shedlock et al., J Leukoc Biol, 68(6): 793-806 (2000); and Hipp et al., In Vivo, 14(5): 571-85 (2000)).

[0080] Peptides of the present invention encompass peptides in which either or both of the N-terminal and C-terminal amino acid residues have been modified. The types of modifications are not particularly limited, but those that do not affect the affinity for BIG3 are preferred. Examples of preferred modifications include acetylation of the N-terminal amino acid residue, amidation of the C-terminal amino acid residue, addition of tag peptides such as HA-tag and FLAG-tag, and such.

[0081] The peptides of the present invention are not limited to those composed of L-amino acids and may be peptides including one or more D-amino acids. The composition ratio of L-amino acids to D-amino acids in the peptides is not particularly limited, and there may be any of the following cases: all amino acid residues may be in L-form (hereinafter referred to as "L-form peptide"); all amino acid residues may be in D-form (hereinafter referred to as "D-form peptide"); or only amino acid residues at a particular position(s) may be in D-form. One preferred embodiment of the peptides of the present invention includes a peptide in which all the amino acid residues have been substituted with D-form amino acid residues in any of the above-mentioned peptides of the present invention. Another preferred embodiment of the peptides of the present invention includes a peptide in which an amino acid residue(s) at a particular position(s) important for the binding to BIG3 has/have been substituted with the corresponding D-form amino acid residue(s). Examples of such a position include positions corresponding to glycine at position 15, glycine at position 18, and aspartic acid at position 82 in the amino acid sequence of SEQ ID NO: 28.

[0082] Furthermore, the peptides of the present invention may be retro-inverso forms of any of the above-mentioned peptides of the present invention. A retro-inverso form has an amino acid sequence that is reversed from that of the original peptide, and all amino acid residues are substituted with D-form amino acid residues. More specifically, a retro-inverso form is a D-form peptide having an amino acid sequence that is reversed from that of the original peptide. Therefore, peptides which are retro-inverso forms of any one of the above-mentioned peptides of the present invention are included as preferred embodiments of the peptides of the present invention.

[0083] Peptides of the present invention may also be in the form of salts. The form of salts is not particularly limited, but pharmaceutically acceptable salts are preferred. Herein, the "pharmaceutically acceptable salt" refers to a salt that retains the pharmacological and pharmaceutical efficacy and characteristics of a peptide. Preferred examples of salts include salts with alkali metals (lithium, potassium, sodium and such), salts with alkaline-earth metals (calcium, magnesium and such), salts with other metals (copper, iron, zinc, manganese and such), salts with organic bases, salts with amines, salts with organic acids (acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, and such), salts with inorganic acids (hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, nitric acid and such), and such. These salts can be prepared according to known methods.

3. Pharmaceutical compositions comprising the peptide or polynucleotide of the present invention and uses thereof

[0084] The present invention also provides pharmaceutical compositions comprising a peptide or a salt thereof of the present invention or a polynucleotide encoding a peptide of the present invention, along with a pharmaceutically acceptable carrier.

[0085] The peptides of the present invention inhibit the binding between a PHB2 polypeptide and a BIG3 polypeptide to induce the suppression of estrogen receptor activation by the PHB2 polypeptide, thereby leading to suppression of growth of estrogen receptor-positive cells. Accordingly, the pharmaceutical compositions of the present invention are useful in either or both of treatment and prophylaxis (prevention) of cell proliferative diseases caused by activation of estrogen receptors. Such cell proliferative diseases include, for example, cancer.

[0086] It is known that among cancers, particularly breast cancer is deeply related to activation of estrogen receptors. A BIG3 polypeptide is a novel estrogen receptor activation regulator and is frequently expressed in many breast cancer specimens and breast cancer cells; meanwhile, it has been confirmed that expression of a BIG3 polypeptide is hardly found in normal tissues (Kim JW, Akiyama M, Park JH, et al. Cancer Sci.; 100(8): 1468-78 (2009)). Thus, it is considered that in breast cancer, expression of a BIG3 polypeptide inhibits the function of a PHB2 polypeptide of suppressing activation of estrogen receptors, thereby growth of breast cancer cells is promoted. Accordingly, the pharmaceutical compositions of the present invention are particularly suitable for either or both of treatment and prophylaxis (prevention) of breast cancer. Furthermore, among breast cancers, the pharmaceutical compositions of the present invention are particularly useful in breast cancers that are estrogen receptor-positive and express BIG3 polypeptides. However, the pharmaceutical compositions of the present invention are not limited to use for breast cancer, and they can be used for any cancer as long as it is estrogen receptor-positive and expresses a BIG3 polypeptide. Examples of estrogen receptor-positive cancer besides breast cancer include endometrial cancer, ovarian cancer, prostate cancer (Nelles JL, et al.,

Expert Rev Endocrinol Metab., 6(3): 437-51 (2011)), and lung cancer (particularly non-small-cell lung cancer) (Stabile LP, et al., Cancer Res., 65(4): 1459-70 (2005); Marquez-Garban DC, et al., Steroids. 72(2): 135-43 (2007)), but are not limited thereto. Cancers to which pharmaceutical compositions of the present invention are applied preferably express BIG3 and PHB2, and estrogen receptor-positive cancers generally express BIG3 and PHB2. Whether a cancer is estrogen receptor-positive can be confirmed by known methods such as ELISA or immunohistochemical staining.

[0087] Furthermore, the peptides of the present invention showed excellent cell growth inhibitory effects in triple-negative breast cancer cells, which are estrogen receptor-negative breast cancer cells (Fig. 9). Generally, triple-negative refers to breast cancer cells lacking expression of HER2, estrogen receptors, and progesterone receptors, which are targeted factors in major drug treatments. Therefore, triple-negative breast cancers are normally resistant to drug treatment. Therefore, the pharmaceutical compositions of the present invention can be applied to either or both of treatment and prophylaxis (prevention) of estrogen receptor-negative breast cancer, and are also useful as pharmaceutical compositions for administration to patients with such treatment-resistant breast cancers.

[0088] More specifically, the present invention provides pharmaceutical compositions comprising a peptide of the present invention, which are for administration to drug therapy-resistant breast cancer patients. The present invention also relates to peptides of the present invention for use in either or both of treatment and prophylaxis (prevention) of drug therapy-resistant breast cancer patients. Furthermore, the present invention relates to use of the peptides of the present invention in the production of pharmaceutical compositions for either or both of treatment and prophylaxis (prevention) of drug therapy-resistant breast cancer patients. The present invention also provides methods for either or both of treatment and prophylaxis (prevention) of breast cancer which comprise the steps of selecting patients having drug therapy-resistant breast cancer, and administering a peptide of the present invention to the selected patients.

[0089] Patients with drug therapy-resistant breast cancer can be identified by observing the therapeutic outcome after common drug therapy. Specifically, when degeneration of the disease focus is not clearly observed by the treatment, one can know that this cancer is treatment-resistant. A condition where enlargement of the disease focus is prevented is included in the degeneration of the disease focus. The above-mentioned triple-negative breast cancer patients are said to have resistance to drug therapies. Triple-negative refers to breast cancers having the features of lacking expression of estrogen receptors and progesterone receptors in addition to HER2. These markers for drug therapy resistance can be evaluated quantitatively by immunostaining and gene expression profiling. For example, the marker status is determined to be negative when the expression level is approximately the same as that of a negative control. For the negative control, treatment-resistant cancer cell lines lacking expression of these markers can be used.

[0090] Pharmaceutical compositions of the present invention can be produced using known drug formulation techniques by mixing a peptide or a salt thereof of the present invention with a pharmaceutically acceptable carrier. Herein, "pharmaceutically acceptable carrier" refers to an inactive substance to be used as diluents or solvents for drugs. For the pharmaceutically acceptable carriers to be used in pharmaceutical compositions of the present invention, carriers generally used for pharmaceutical products can be appropriately selected according to the dosage form of the pharmaceutical compositions to be prepared.

[0091] The dosage forms of the pharmaceutical compositions of the present invention are not particularly limited, and dosage forms generally used for pharmaceutical products such as liquids, tablets, elixirs, capsules, granules, and powders can be selected appropriately. Furthermore, depending on the selected dosage form, additives such as excipients, stabilizers, suspensions, preservatives, surfactants, solubilizing agents, pH adjusters, and aggregation inhibitors can be added appropriately.

[0092] The pharmaceutical compositions of the present invention comprise as an active ingredient a pharmaceutically effective amount of a peptide or a salt thereof of the present invention or a polynucleotide encoding the peptide. A "pharmaceutically effective amount" is an amount sufficient for a pharmaceutical composition of the present invention to accomplish its objective. For example, when a pharmaceutical composition of the present invention is for a pharmaceutical composition for either or both of the treatment and prophylaxis (prevention) of cancer, an example of a pharmaceutically effective amount can be an amount that induces suppression of cancer growth rate, suppression of metastatic potential, prolonged survival time, suppression or delay of cancer development, or alleviation of various clinical symptoms associated with cancer, when administered to a patient. Suppression of cancer growth rate can be, for example, suppression of about 5% or more compared to when the pharmaceutical composition of the present invention is not administered. Preferably, suppression of cancer growth rate can be about 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 75 % or more, 80% or more, 90% or more, or 100% or more.

[0093] The pharmaceutically effective amount can be selected appropriately according to the dosage form of the pharmaceutical compositions, dosage interval, age, gender, body weight, and body surface area of subjects for administration, type of disease, and such. Examples of the content of peptides or salts thereof of the present invention in pharmaceutical compositions of the present invention include 0.001 mg to 1000 mg, 0.01 mg to 100 mg, 0.1 mg to 30 mg, or 0.1 mg to 10 mg, but are not limited thereto.

[0094] Pharmaceutical compositions of the present invention may optionally include other pharmaceutical agents. Examples of other pharmaceutical agents include anti-inflammatory agents, analgesic agents, antipyretics, other ther-

apeutic agents for cancer, and such. Other therapeutic agents for cancer that may be used for pharmaceutical compositions of the present invention are not particularly limited, but when the pharmaceutical compositions are used for estrogen-positive cancers, examples may include hormone therapy agents such as selective ERα modulators (*e.g.*, tamoxifen and raloxifene), ERα down-regulators (*e.g.*, fulvestrant), aromatase inhibitors, LH-RH agonist formulations, and progesterone formulations. These pharmaceutical agents may also be mixed in the form of prodrugs and pharmaceutically acceptable salts.

[0095] Pharmaceutical compositions of the present invention can be administered to a subject by appropriately selecting a suitable administration route depending on the dosage form. The administration route is not particularly limited, but examples include oral administration, intradermal, subcutaneous, intramuscular, intraosseous, peritoneal and intravenous injection, and such. Furthermore, while either systemic administration or local administration near the diseased site is possible, local administration is preferred. More specifically, pharmaceutical compositions of the present invention can be administered by means of injection and such to the cancer tissue or to its vicinity. Alternatively, pharmaceutical compositions of the present invention can be administered surgically into the cancer tissue or to its vicinity. Pharmaceutical compositions of the present invention can also be prepared as a controlled-release preparation by combining them with appropriate carriers.

[0096] Dosage interval of pharmaceutical compositions of the present invention may also be appropriately selected according to the age, gender, body weight, and body surface area of subjects for administration, the disease type and such, as well as the dosage form, administration route, and such of the pharmaceutical compositions of the present invention. Examples of the dosage interval include every day, every four days, and every seven days, but are not limited thereto.

[0097] Dosage of pharmaceutical compositions of the present invention may also be appropriately selected according to the age, gender, body weight, and body surface area of subjects for administration, the disease type and such, as well as the dosage form, administration route, and such of the pharmaceutical compositions of the present invention.

[0098] Examples of the dosage of peptides or salts thereof of the present invention include, for example, 0.001 mg/kg/day to 1000 mg/kg/day, 0.005 mg/kg/day to 500 mg/kg/day, 0.01 mg/kg/day to 250 mg/kg/day, but are not limited thereto.

[0099] Pharmaceutical compositions of the present invention may be used in combination with other pharmaceuticals depending on the condition of the administration subjects. The pharmaceuticals used in combination are not particularly limited, but when the pharmaceutical compositions are used for estrogen receptor-positive cancers, examples may include hormone therapy agents such as selective ERα modulators (*e.g.*, tamoxifen and raloxifene), ERα down-regulators (*e.g.*, fulvestrant), aromatase inhibitors, LH-RH agonist formulations, and progesterone formulations. Among these hormone therapy agents, particularly preferred examples include tamoxifen and fulvestrant.

[0100] When pharmaceutical compositions of the present invention are used for cancer treatment, one may examine whether the cancer to be treated is accompanied by expression of BIG3 and PHB2 before administering the pharmaceutical compositions. Whether BIG3 and PHB2 are expressed in the cancer to be treated can be confirmed by detecting transcription products or translation products of these genes in the samples collected from the subjects. Known methods can be used for detection methods, and for example, methods of detecting transcription products using probes or PCR methods (for example, cDNA microarray method, Northern blotting, and RT-PCR) and methods of detecting translation products using antibodies and such (for example, Western blotting and immunostaining) may be used.

[0101] The present invention also provides articles of manufacture or kits that comprise a pharmaceutical composition of the present invention. The articles of manufacture or kits of the present invention can include a container that houses the pharmaceutical composition of the present invention. An example of an appropriate container includes a bottle, a vial or a test tube, but is not limited thereto. The container may be formed of various materials such as glass or plastic. A label may be attached to the container, and the disease or disease state to which the pharmaceutical composition of the present invention should be used may be described in the label. The label may also indicate directions for administration and such.

[0102] The articles of manufacture or kits of the present invention may further comprise a second container that houses pharmaceutically acceptable diluents optionally, in addition to the container that houses the pharmaceutical composition of the present invention. The articles of manufacture or kits of the present invention may further comprise the other materials desirable from a commercial standpoint and the user's perspective, such as the other buffers, diluents, filters, injection needles, syringes, and package inserts with instructions for use.

[0103] As needed, the pharmaceutical composition of the present invention can be provided in a pack or dispenser device that can contain one or more units of dosage forms containing active ingredients. The pack can include, for example, a metallic foil or a plastic foil such as a blister pack. Instructions for administration can be attached to the pack or dispenser device.

[0104] In another embodiment, the present invention provides the following uses, methods, and such:

(a) uses of the peptide or salt thereof, or polynucleotide encoding the peptide of the present invention in the man-

ufacture of a pharmaceutical composition for either or both of treatment and prophylaxis (prevention) of cancer;
(b) the peptides or salts thereof, or polynucleotides encoding the peptides of the present invention for use in either or both of treatment and prophylaxis (prevention) of cancer;
(c) methods or processes for manufacturing a pharmaceutical composition for either or both of treatment and prophylaxis (prevention) of cancer, the method or process comprising formulating the peptide or salt thereof, or polynucleotide encoding the peptide of the present invention and a pharmaceutically acceptable carrier(s);
(d) methods or processes for manufacturing a pharmaceutical composition for either or both of treatment and prophylaxis (prevention) of cancer, the method or process comprising mixing the peptide or salt thereof, or polynucleotide encoding the peptide of the present invention with a pharmaceutically acceptable carrier(s); and
(e) methods for either or both of treatment and prophylaxis (prevention) of cancer, the method comprising administering the peptide or salt thereof, or polynucleotide encoding the peptide of the present invention to a subject.

[0105] In the above uses, methods and such, cancer is preferably BIG3-positive cancer and may be estrogen receptor-positive cancer or estrogen receptor-negative cancer (for example, triple-negative breast cancer). A preferred example of such cancer includes breast cancer.

[0106] Hereinbelow, the present invention is described in more detail with reference to the Examples. Nevertheless, while the following materials, method and Examples may serve to assist one of ordinary skill in making and using certain embodiments of the present invention, there are only intended to illustrate aspects of the present invention and thus in no way to limit the scope of the present invention. One of ordinary skill in the art can use methods and materials similar or equivalent to those described herein in the practice or testing of the present invention.

[0107] All prior art documents cited herein are incorporated by reference in the present specification.

[Example]

[Example 1] Effects on Estrogen-dependent Breast Cancer

1. Materials and Methods

Cell lines and Culturing Conditions

[0108] Human breast cancer cell line MCF-7 was purchased from JCRB Cell Bank (Osaka, Japan) and maintained in MEM (Thermo Fisher Scientific) supplemented with 10% FBS (Nichirei Biosciences Inc., Tokyo, Japan), 1% Antibiotic/Antimycotic solution (Thermo Fisher Scientific, Waltham, MA, USA), 0.1 mM NEAA (Thermo Fisher Scientific), 1 mM sodium pyruvate (Thermo Fisher Scientific), and 10 μg/mL insulin (Sigma, St. Louis, MO, USA) under 5% CO_2 at 37°C.

[0109] Normal mammary gland epithelial cell line MCF-10A was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and maintained in MEBM (Lonza) supplemented with a Single Quots kit (BPE, hydrocortisone, hEGF, insulin, gentamycin/amphoterin-B) (Lonza, Walkersville, MD, USA) and 100 ng/mL cholera toxin under 5% CO_2 at 37°C.

Cell Growth Assay

[0110] Growth assay on MCF-7 was carried out by seeding cells into 48-well plates (2 x 10^4 cells/200 μL). First, on the next day after seeding, the medium was changed to phenol red-free DMEM/F12 (Thermo Fisher Scientific) supplemented with 10% FBS, 1% Antibiotic/Antimycotic solution, 0.1 mM NEAA, 1 mM sodium pyruvate, and 10 μg/mL insulin. 24 hours later, the cells were treated with 10 nM 17β-estradiol (estrogen, Sigma) alone or with 10 nM estrogen and a PHB2 sequence-derived peptide. Growth assay on MCF-10A was carried out by seeding cells into 48-well plates (2 x 10^4 cells/200 μL). 24 hours after seeding, PHB2 sequence-derived peptide was added. Growth assays were carried out using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). The data are shown by mean ± standard deviation of three independent experiments.

Antibodies and Immunoblot Analyses

[0111] For immunoblot analyses, after performing SDS-PAGE, the membranes blotted with proteins were blocked with 4% BlockAce solution (Dainippon Pharmaceutical, Osaka, Japan) for 3 hours and then incubated to react for 12 hours with antibodies against BIG3 (1:1,000), PHB2 (1:1,000) (Abcam, Cambridge, UK) and phosphorylated PHB2 (Ser39, Scrum, Tokyo, Japan). After allowing interaction with HRP-labeled secondary antibodies (anti-rat IgG-HRP for BIG3, 1:5,000; and anti-rabbit IgG-HRP for PHB2 and phosphorylated PHB2, 1:1,000) (Santa Cruz Biotechnology, Dallas, TX, USA) for 1 hour, the blots were developed with the Enhanced Chemiluminescence (ECL) system (GE

Healthcare, Buckinghamshire, UK) and scanned using the Image Reader LAS-3000 mini (Fujifilm, Tokyo, Japan).

Immunoprecipitation

[0112] For immunoprecipitation, MCF-7 was seeded into 10 cm dishes (2 x 10^6 cells/10 mL), and like cell growth assay, MCF-7 was treated with 10 nM estrogen alone or with 10 nM estrogen and PHB2 sequence-derived peptide. For immunoprecipitation analysis, cell lysates lysed in a cell lysis buffer (50 mM Tris-HCl; pH 8.0, 150 mM NaCl, 0.1% NP-40, and 0.5% CHAPS; 0.1% protease inhibitor cocktail III) were pre-cleared with a rat IgG antibody and rec-Protein G Sepharose 4B (Thermo Fisher Scientific) at 4°C for 3 hours. Then, the supernatants were incubated for reaction with 5 μg of an antibody against BIG3 at 4°C for 12 hours. Next, the antigen-antibody complexes were precipitated using rec-Protein G Sepharose 4B at 4°C for 1 hour. The immunoprecipitated protein complexes were washed four times with the cell lysis buffer. Then, SDS-PAGE and immunoblot analyses were carried out.

Peptide Synthesis

[0113] All peptides were synthesized by the Fmoc solid-phase synthesis method. NovaSyn TGR resin (0.25 mmol amine/g) or Rink Amide AM resin (0.62 mmol amine/g) was used as resin and a manual Fmoc solid-phase synthesis method was utilized. The Fmoc group was removed by the following procedures: reacting with 20% (v/v) piperidine/DMF solution at room temperature for 10 minutes, the resin was washed five to ten times with DMF, and then three equivalents of an Fmoc amino acid was subjected to coupling in a DMF solvent at room temperature for 90 minutes using N,N-diisopropylcarbodiimide (DIPCDI; 3.0 equivalents) and 1-hydroxy benzotriazole hydrate (HOBt·H2O; 3.3 equivalents) or using N,N-diisopropylethylamine (DIPEA; 3.0 equivalents) and N,N,N,N-tetramethyl-O-(benzotriazole-1-yl)uronium hexafluorophosphate (HBTU; 2.9 equivalents). After washing with DMF, methanol, and ethanol and drying, the protected peptide resin was reacted with a cocktail of TFA:thioanisole:m-cresol:1,2-ethanedithiol:water (80:5:5:5:5) in the proportion of 100 mg of the protected peptide resin to 5 mL of the cocktail at room temperature for 90 minutes. After TFA was concentrated by nitrogen stream, the residual was precipitated by adding ether, and the precipitate was washed with ether and then dissolved in an appropriate aqueous solvent to perform preparative purification by HPLC.

2. Results

Screening of PHB2 sequence-derived peptides

[0114] The 20 types of PHB2 protein sequence-derived peptides depicted in Fig. 1A were used to examine the suppressive effects on estrogen (E2)-dependent growth of MCF-7 (treated with 10 μM each peptide for 24 hours). As a result, growth of MCF-7 was significantly promoted by E2 stimulation, while treatments with PHB2-derived peptides No. 1 (11-22aa; SEQ ID NO: 1) and No. 5 (76-90aa; SEQ ID NO: 5) respectively showed the effect of significantly suppressing the E2-dependent growth by about 50% (No: 1: suppression ratio of 58%; No. 5: suppression ratio of 49%). Both peptides almost coincided with the BIG3-binding region predicted by *in silico* analysis (amino acids in bold letters). Furthermore, peptides No. 2 (42-50aa; SEQ ID NO: 2) and No. 3 (38-50aa; SEQ ID NO: 3) also showed the effect of suppressing the E2-dependent growth by 22% and 23%, respectively. However, each PHB2 sequence-derived peptide had a lower suppressive effect on the E2-dependent growth compared to that of ERAP, suggesting that PHB2 may have multiple BIG3-binding regions.

[0115] Next, additional PHB2 sequence-derived peptides surrounding No. 1 (11-22aa) and No. 5 (76-90aa) were synthesized (Figs. 1B and 1C), and the effect of treatment with 10 μM each peptide for 24 hours on the E2-dependent growth was examined. As a result, among the peptides around No. 1 (11-22aa), the PHB2 (11-22aa) peptide showed the highest suppression ratio (63%), while the suppressive effect attenuated with distance from there (Fig. 1B). On the other hand, among the peptides around No. 5 (76-90aa), the PHB2 (76-90aa) peptide showed the highest suppression ratio (51%), and No. 50 (75-89aa) also had almost the same suppressive effect; however, similarly to the above, the suppression ratio decreased with distance from these peptides (Fig. 1C). What is common to these data is that they comprise each amino acid of 11-21aa and 76-88aa, which showed a high score among the BIG3-binding sites predicted by *in silico* analysis, and the data suggested that there are two BIG3-binding sites in PHB2.

[0116] Thus, the combinations of No. 1 (11-22aa) with peptides surrounding No. 5 (76-90aa) and the combinations of No. 5 (76-90aa) with peptides surrounding No. 1 (11-22aa) were examined for suppressive effect on the E2-depentent growth of MCF-7. As a result, treatment with peptide No. 1 (11-22aa) alone almost reproduced the result with a suppression ratio of 65% for the E2-dependent growth, and when this peptide was used in combination with PHB2 peptides surrounding No. 5 (76-90aa), the combinations with No. 5 (76-90aa) and with No. 50 (75-89aa) showed almost complete suppressive effects of 100% and 97%, respectively (Fig. ID). Similarly, treatment with No. 5 (76-90aa) alone showed a suppression ratio of 55%, and when this peptide was used in combination with PHB2 peptides surrounding No. 1 (11-22aa), the

combination with No. 1 (11-22aa) almost completely suppressed the growth, and the combinations with any of the peptides consisting of a region of 5 to 26 aa (Nos. 36, 37, 38, 39, and 40) showed a suppression ratio of 90% or higher (Fig. IE). This suggested the importance of the PHB2 regions of No. 1 (11-22aa) and No. 5 (76-90aa) for the binding to BIG3 and the necessity to develop dominant-negative peptides considering these regions.

Suppressive effects of PHB2 sequence-derived peptides on E2-dependent growth

[0117] Next, the suppressive effect on E2-depentent growth of MCF-7 was examined when the two types of PHB2 sequence-derived peptides (No. 1 and No. 5), which can suppress E2-depentent growth of MCF-7, were used in combination. As a result, compared to each treatment with No. 1 or No. 5 alone, the use of both peptides in combination enhanced the effect and showed the growth suppressive effect of 88% (Fig. 2A). Moreover, enhanced suppressive effects were not observed when No. 5 was treated in combination with No. 6 (86-100aa), which did not show suppressive effect (suppression ratio of about 10%) in treatment with No. 6 alone. This suggested that there may be a BIG3-binding region in each of 11-22 aa and 76-90 aa of PHB2.

[0118] Next, the inhibition of the binding between BIG3 and PHB2 by treatment with 20 μM and 50 μM of No. 1 or No. 5 was examined by immunoprecipitation with a BIG3 antibody. As a result, No. 1 and No. 5 both inhibited the binding between BIG3 and PHB2 in a concentration-dependent manner, and No. 1 and No. 5 showed inhibition ratios of 64% and 80%, respectively, at 50 μM (Fig. 2B). Furthermore, the use of both peptides at 50 μM in combination achieved the inhibition ratio of 87% (Fig. 2B). Next, the effect of each peptide (Nos. 1, 5, and 6) on Ser39 phosphorylation of PHB2 was examined. Compared to Ser39 phosphorylation of PHB2 by treatment with ERAP, a positive control, each treatment with No. 1 or No. 5 alone showed only 40% or 20% band intensity of phosphorylation (Fig. 2C), and even the use of both peptides in combination showed 70% intensity of phosphorylation (Fig. 2C). On the other hand, No. 6 showed 10% band intensity of PHB2 phosphorylation and could hardly induce the phosphorylation (Fig. 2C). Moreover, since even the use of No. 5 and No. 6 in combination showed 20% band intensity, it was suggested that the binding between PHB2 and BIG3 extends over multiple regions of No. 1 and No. 5.

Suppressive effect of a novel peptide (11-90aa) covering the BIG3-binding regions of PHB2 peptides No. 1 and No. 5 on E2-dependent growth

[0119] Since the PHB2 sequence-derived peptides (No. 1 and No. 5) can suppress E2-dependent growth and can induce Ser39 phosphorylation of PHB2 by only 50%, a PHB2 peptide of 11-90aa comprising these two regions was newly synthesized and its effect on E2-dependent growth of MCF-7 was examined. As a result, the PHB2 peptide 11-90aa suppressed MCF-7 which proliferated twofold by 24 hour-E2 stimulation in a concentration-dependent manner, but had a suppression ratio of only 57% even at 50 μM (Fig. 3A), which ratio was almost the same as that of No. 1 and No. 5 peptides.

[0120] It was then evaluated whether the peptide 11-90aa can inhibit the binding between BIG3 and PHB2 and can induce PHB2 phosphorylation. As a result, the PHB2 peptide 11-90aa inhibited the binding between BIG3 and PHB2 greater than an untreated sample, but did not provide a sufficient effect of inhibiting the binding (Fig. 3B). Furthermore, Ser39 phosphorylation of PHB2 was induced in a manner dependent on the concentration of PHB2 peptide 11-90aa, but it was 30% of the phosphorylation obtained by ERAP treatment, and PHB2 peptide 11-90aa could not induce sufficient phosphorylation (Fig. 3C). This may be because the number of amino acids was large, *i.e.* 80, and thus the binding to the α-helical structure of BIG3 was insufficient.

Suppressive effects of linear, branched, and cyclic PHB2 on E2-dependent growth

[0121] Since the combination of PHB2 sequence-derived peptides 11-22aa and 76-90aa enhanced suppression of estrogen-dependent growth to achieve a suppression ratio of 88% (Fig. 2A), a peptide in which both sequences were synthesized linearly (linear bound type; Fig. 4A), a peptide in which both sequences were synthesized with branching (branched bound type; Fig. 4B), and peptides in which each sequence was synthesized in a cyclic form for stabilizing structure and improving membrane permeability (cyclic type; Fig. 4C) were additionally prepared, and the enhancement of the growth suppressive effect of each peptide was examined (treated with 10 μM each peptide for 24 hours). As a result, the linear bound and branched bound PHB2 peptides, in which both sequences were linked *via* a PEG (polyethylene glycol) sequence, showed suppression ratios of 71% and 57%, respectively. These suppression ratios were enhanced compared to that of administration of a single linear peptide, but were lower than that of the use of the peptides in combination (Fig. 5A). Furthermore, cyclic PHB2 peptides of 11-21aa and 76-88aa showed enhanced suppression ratios compared to non-cyclic peptides (Fig. 5A). Moreover, the use of the cyclic peptides in combination achieved an almost complete suppressive effect (suppression ratio of 96%; Fig. 5A), but an apoptosis-like phenomenon in which cells floated could not be confirmed.

[0122] Next, the effects of the cyclic peptides on growth of normal mammary gland epithelial cells, MCF-10A, which do not express ERα and BIG3, were examined (treated with 10 μM each peptide for 24 hours). As a result, although little suppressive effects were observed in the linear bound and cyclic bound PHB2 peptides (Fig. 5B; linear bound type: inhibition ratio of 10%, cyclic 11-21aa: inhibition ratio of 14%, cyclic 76-88aa: inhibition ratio of 15%), it was suggested that most PHB2 peptides specifically suppress E2-dependent growth with little effect on MCF-10A growth.

[0123] Subsequently, it was examined whether these PHB2 peptides can inhibit the interaction between BIG3 and PHB2. As a result, BIG3 strongly bound to PHB2 when the cells were untreated or stimulated with E2 (Fig. 5C) and treatment with each PHB2 peptide alone could hardly inhibit the interaction between BIG3 and PHB2 (Fig. 5C); however, the respective combination of the linear PHB2 peptides and the cyclic PHB2 peptides could markedly inhibit the interaction (Fig. 5C; combination of linear type: inhibition ratio of 67%, combination of cyclic type: inhibition ratio of 81%), and these results suggested that there are two BIG3-binding regions on PHB2. On the other hand, it was judged that the linear bound and branched bound PHB2 peptides cannot cover two BIG3-binding regions on PHB2.

Long-term stability of the suppressive effects of cyclic PHB2 peptides on E2-dependent growth

[0124] Since it was suggested that cyclic PHB2 peptides may acquire low concentration and long-term stability due to enhanced membrane permeability and fixation of structure, the long-term stability for up to 96 hours was examined when cells were treated with 10 μM each cyclic PHB2 peptide alone. As a result, the linear PHB2 peptides 11-22aa and 76-90aa showed suppression ratios of 40% and 61%, respectively, in a 24-hour treatment, but exhibited significantly decreased suppression ratios of 31% and 24%, respectively, after 96 hours (Fig. 6A). Meanwhile, the cyclic peptide 11-21aa and cyclic peptide 76-88aa showed suppression ratios of 67% (53% in a 24-hour treatment) and 72% (58% in a 24-hour treatment), respectively, even after 96 hours (Fig. 6A), and their suppressive effects stably lasted for up to 96 hours. It was considered from these data that the suppressive effects of the cyclic peptides could last for a long time due to stable fixation of their tertiary structure similar to the cross-linked peptides.

[0125] Next, since the cyclic PHB2 peptides could sustain their suppressive effects for up to 96 hours, the effect on growth of MCF-10A, which does not express ERα and BIG3, was examined by treating with 1 μM or 10 μM each cyclic PHB2 peptide for up to 96 hours. As a result, the cyclic peptides of PHB2 sequences 11-21 aa and 76-88 aa each had little effect at 1 μM (both of them had suppression ratios of 5% to 7%), but showed suppression ratios of 10% to 15% at 10 μM (Fig. 6B), suggesting that they have a small non-specific suppressive effect. However, it was considered that the suppression of E2-dependent growth of MCF-7 by the cyclic PHB2 peptides was mainly due to the inhibition of the binding between BIG3 and PHB2.

Concentration-dependent suppressive effect of cyclic PHB2 peptides on E2-dependent growth

[0126] The 50% inhibition concentrations (IC_{50}) of the cyclic PHB2 peptides for E2-dependent growth of MCF-7 were calculated and a synergistic suppressive effect of the peptides at the IC_{50} was examined. As a result, each cyclic PHB2 peptide suppressed E2-dependent growth in a concentration-dependent manner, and the cyclic 11-21aa and cyclic 76-88aa showed IC_{50} values of 4.06 μM and 2.11 μM, respectively (Fig. 7A). Then, 4 μM cyclic 11-21aa and 2 μM cyclic 76-88aa were used to examine the effect of long-term combination, and the use of peptides in combination for 24 hours showed a synergistic suppressive effect of 82%, which effect lasted for up to 96 hours (Fig. 7B; combination: suppression ratio of 88%, cyclic 11-21aa: suppression ratio of 41%, cyclic 76-88aa: suppression ratio of 59%). Furthermore, the peptides at these concentrations had little effect on growth of MCF-10A (Fig. 7C).

Identification of amino acids in PHB2 peptide sequences that are important for the binding to BIG3

[0127] Since PHB2-derived peptides No. 1 (11-22aa: SEQ ID NO: 1) and No. 5 (76-90aa: SEQ ID NO: 5) had a suppressive effect by about 50% on E2-dependent growth, peptides were made in which each amino acid in the peptide sequences of No. 1 and No. 5 was mutated to alanine (Fig. 8A) to identify amino acids important for growth suppression. In the experiment, MCF-7 was seeded, 10 μM each PHB2 peptide and 10 nM estrogen were added 48 hours after seeding, and the cell number was monitored after another 24 hours. First, the amino acids of PHB2 sequence 11-22 aa were evaluated and No. 1 (11-22aa) suppressed estrogen-dependent cell growth by up to 65%, while only alanine-mutated peptides No. 59 and No. 62 (SEQ ID NOs: 59 and 62) attenuated suppression ratios of 19% and 8%, respectively (Fig. 8B). On the other hand, since other alanine-mutated peptides showed almost the same suppression ratio as No. 1 (Fig. 8B), glycines at positions 15 and 18 were considered to be important for the binding to BIG3, suggesting that the suppressive activity may be improved by converting these positions into an isomeric form, a D-amino acid.

[0128] Next, the amino acids of PHB2 sequence 76-90 aa were evaluated. Although the suppression ratio of No. 5 (76-90aa) on estrogen-dependent growth was 54% and nearly reproduced (Fig. 8C), the suppression ratios of alanine-mutated peptides Nos. 71 to 73 (SEQ ID NOs: 71 to 73) attenuated below No. 5 (76-90aa) and were 38%, 37%, and

13%, respectively (Fig. 8C), and particularly aspartic acid at position 82 was considered to be necessary for the binding between BIG3 and PHB2.

[0129] Furthermore, peptides (Fig. 8D; Nos. 82 and 83 (SEQ ID NOs: 82 and 83)) were prepared by adding 51-57aa comprising 53-57 aa, which was predicted to be involved in the interaction between BIG3 and PHB2 by *in silico* analysis (Fig. 1A), to No. 2 (42-50aa: SEQ ID NO: 2) and to No. 3 (38-50aa: SEQ ID NO: 3), which showed a suppressive effect other than peptides No. 1 (11-22aa) and No. 5 (76-90aa), and these peptides were used to examine the effects on estrogen-dependent cell growth. As a result, while the suppression ratios of No. 2 and No. 3 were 20% and 17%, respectively, peptides No. 82 and No. 83, to which the amino acids at positions up to 57 were added, showed improved suppression ratios of 59% and 61%, respectively (Fig. 8E). Thus, it was suggested that by having the amino acids from glutamic acid at position 44 to glycine at position 57, the PHB2 peptides can be comparable in suppression ratio to No. 1 and No. 5.

[Example 2] Effects on Triple-negative Breast Cancer

1. Materials and Methods

Cell lines and Culture Conditions

[0130] Human breast cancer cell line MDA-MB-231 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured using Leibovitz's L-15 medium (Thermo Fisher Scientific) supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) and a 1% antibiotic/antimycotic solution (Wako Pure Chemical, Osaka, Japan) at 37°C without CO_2 concentration control.

Cell Growth Assay

[0131] MDA-MB-231 cells were seeded into 48-well plates at a cell density of 1 x 10^4 cells/200 mL in well. 48 hours later, the medium in each well was exchanged to a medium supplemented with PHB2 peptides 11-22aa or 76-90aa (three-fold serial dilution from 20 mM), and after culturing for another 96 hours, the level of cell growth was measured using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Data were obtained from three independent experiments, a graph (mean ± standard deviation) was produced using a graphing and data analyzing software SigmaPlot (Systat Software, San Jose, CA, USA), and the 50% inhibition concentration (IC_{50}) of the peptide against cell growth was calculated.

Combination assay

[0132] MDA-MB-231 cells were seeded into 48-well plates at a cell density of 1 x 10^4 cells/200 mL in well. 48 hours later, the medium in each well was exchanged to media supplemented with PHB2 peptide 11-22aa (added concentration: IC_{50} value), PHB2 peptide 76-90aa (added concentration: IC_{50} value) and a mixed solution of both peptides (added concentration: respective IC_{50} value), respectively, or to a medium supplemented with phosphate buffered saline (PBS) as a negative control. After culture for another 96 hours, the cell growth level was measured using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The data obtained was used to calculate relative values based on the growth level when PBS was added, and the graph was produced.

2. Results

Growth suppressive effects of PHB2 peptides on a breast cancer cell line

[0133] To examine the cell growth suppressive effects of PHB2 peptides 11-22aa and 76-90aa on the breast cancer cell line MDA-MB-231, serial dilution series of the peptides were prepared and the growth level was measured 96 hours after addition to the cells. As a result, as shown in Figs. 9A and 9B, concentration-dependent suppressive effects on cell growth were observed in both peptides. The 50% inhibition concentrations (IC_{50}) were 0.462 μM in the peptide 11-22aa and 0.273 μM in the peptide 76-90aa, and the peptide 76-90aa showed a more potent growth suppressive effect.

Effect of use of PHB2 peptides 11-22aa and 76-90aa in combination

[0134] To examine the effect of use of PHB2 peptides 11-22aa and 76-90aa in combination on suppression of cell growth, the breast cancer cell line MDA-MB-231 was used to compare the cell growth levels when both peptides were mixed and added at respective IC_{50} and when each peptide was added alone at respective IC_{50}. As a result, as shown

in Fig. 9C, the addition of each peptide alone showed the suppression of growth by about 50% compared to when phosphate buffered saline (PBS), a negative control, was added; however, the combination enhanced the suppressive effect by about 62%.

[Example 3] Effects of Cross-linking PHB2 Peptide on Estrogen-dependent Breast Cancer

1. Materials and Methods

Cell lines and Culturing Conditions

[0135] Human breast cancer cell line MCF-7 was purchased from JCRB Cell Bank (Osaka, Japan) and maintained in MEM (Thermo Fisher Scientific) supplemented with 10% FBS (Nichirei Biosciences Inc., Tokyo, Japan), a 1% Antibiotic/Antimycotic solution (Thermo Fisher Scientific, Waltham, MA, USA), 0.1 mM NEAA (Thermo Fisher Scientific), 1 mM sodium pyruvate (Thermo Fisher Scientific), and 10 μg/mL insulin (Sigma, St. Louis, MO, USA) under 5% CO_2 at 37°C.

[0136] Normal mammary gland epithelial cell line MCF-10A was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and maintained in MEBM (Lonza) supplemented with a Single Quots kit (BPE, hydrocortisone, hEGF, insulin, gentamycin/amphoterin-B) (Lonza, Walkersville, MD, USA) and 100 ng/mL cholera toxin under 5% CO_2 at 37°C.

Cell Growth Assay

[0137] Growth assay on MCF-7 was carried out by seeding cells into 48-well plates (2 x 10^4 cells/200 μL). First, on the next day after seeding, the medium was changed to phenol red-free DMEM/F12 (Thermo Fisher Scientific) supplemented with 10% FBS, 1% Antibiotic/Antimycotic solution, 0.1 mM NEAA, 1 mM sodium pyruvate, and 10 μg/mL insulin. 24 hours later, the cells were treated with 10 nM 17β-estradiol (estrogen, Sigma) alone or with 10 nM estrogen and a PHB2 sequence-derived peptide. Growth assay on MCF-10A was carried out by seeding cells into 48-well plates (2 x 10^4 cells/200 μL). 24 hours after seeding, PHB2 sequence-derived peptide was added. Growth assays were carried out using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). The data are shown by mean ± standard deviation of three independent experiments.

2. Results

Suppressive effects of cross-linked PHB2 peptides on estrogen-dependent growth

[0138] PHB2 peptides 11-21aa and 76-88aa were cross-linked by three types of cross-linking methods (Fig. 10A: hexafluorobenzene cross-linking, decafluorobiphenyl cross-linking, and disulfide cross-linking) to prepare cross-linked PHB2 peptides (stapled PHB2 peptides) (Fig. 10B), and the suppressive effects of these peptides on estrogen-dependent growth were examined. In the experiment, breast cancer cells MCF-7 were seeded, 10 μM each PHB2 peptide and 10 nM estrogen were added after 48 hours, and the cell number was evaluated after another 24 hours by MTT assay.

[0139] Each cross-linked PHB2 peptide of 11-21aa and 76-88aa improved the suppressive effect on estrogen-dependent growth about 1.5-fold compared to non-cross-linked PHB2 peptides (SEQ ID NOs: 109, 113, 114, 118, and 122) (polyarginine addition: Fig. 10C (left); without polyarginine: Fig. 10C (right)). The improved effects did not vary depending on the cross-linking method (stapling method). Moreover, cross-linked PHB2 peptides which do not have polyarginine at the C terminus (SEQ ID NOs: 115, 116, 117, 119, 120, and 121) showed a slightly higher suppression ratios than peptides to which polyarginine was added (with polyarginine: suppression ratio of about 60%; without polyarginine: suppression ratio of about 70%), suggesting that polyarginine may block the function of cross-linked structure.

Suppressive effects of cyclic PHB2 peptides on estrogen-dependent growth

[0140] For improving the cell membrane permeability and the structural stability of cyclic PHB2 peptides (Fig. 4C, SEQ ID NOs: 25 and 26), their cross-linking forms were changed and the suppressive effects on estrogen-dependent growth were examined. In addition to disulfide cross-linking (SEQ ID NOs: 25 and 26) so far examined, hexafluorobenzene cross-linking (Fig. 11A; SEQ ID NOs: 123 and 126) and decafluorobiphenyl cross-linking (Fig. 11A; SEQ ID NOs: 124 and 127) were evaluated. As a result, cyclic PHB2 peptides cross-linked using fluorobenzene (Fig. 11A; SEQ ID NOs: 123, 124, 126, and 127) slightly improved the growth suppressive effect compared to disulfide cross-linking (Fig. 11B), and cyclic PHB2 peptide 11-21aa and cyclic PHB2 peptide 76-88aa enhanced the suppression ratios to about 70% and about 80%. Furthermore, no difference has been observed for fluorobenzene cross-linking between a single cross-linking (hexafluorobenzene cross-linking) and a double cross-linking (decafluorobiphenyl cross-linking).

Effects of modifications of PHB2 peptide 11-22aa on estrogen-dependent growth

[0141] In the PHB2 peptide 11-22aa, glycines at positions 15 and 18 in the amino acid sequence of SEQ ID NO: 28 (full-length PHB2 polypeptide) were considered to be important for the binding to BIG3 (Fig. 8). Then, it was examined whether substitutions of these positions with D-alanine and D-leucine (Fig. 12A) enhance the suppressive activity or not. As a result, while the PHB2 peptide in which glycines at positions 15 and 18 were substituted with D-leucines (SEQ ID NO: 134) showed a suppression ratio of about 65% (Fig. 12B), the substitutions with D-alanines (SEQ ID NO: 133) had no suppressive effect (Fig. 12B). However, since the suppression ratio of PHB2 peptide 11-22aa (SEQ ID NO: 1; Fig. 8B) was about 65%, the substitutions with D-leucine showed little improvement in suppressive effect.

Effects of cross-linked PHB2 peptides on growth of mammary gland epithelial cells

[0142] The effects of cross-linked and cyclic PHB2 peptides on growth of normal mammary gland epithelial cells MCF-10A, which do not express ERα and BIG3, were examined (treated with 10 μM each PHB2 peptide for 24 hours). As a result, all the PHB2 peptides evaluated had no effect on growth of MCF-10A (Fig. 13).

[Industrial Applicability]

[0143] The present invention provides PHB2 amino acid sequence-derived peptides which exert an inhibitory effect on the BIG3-PHB2 interaction and are useful as therapeutic agents for breast cancer. The peptides provided by the present invention are useful in treating cancer such as breast cancer. More specifically, the peptides of the present invention are useful in treating BIG3-positive and/or estrogen receptor-positive cancer. The peptides of the present invention target not PHB2, whose expression is observed in organs throughout the human body, but BIG3, which is a protein highly expressed specifically in particularly estrogen receptor-positive cancer, and thus these peptides can be expected to have high selectivity for estrogen receptor-positive cancer. Moreover, the peptides of the present invention also exert an antitumor effect against triple-negative breast cancer.

SEQUENCE LISTING

<110> TOKUSHIMA UNIVERSITY
ONCOTHERAPY SCIENCE, INC.

<120> THERAPEUTIC AGENTS FOR BREAST CANCER COMPRISING PHB2-DERIVED PEPTIDE INHIBITING BIG3-PHB2 INTERACTION

<130> ONC-A1802P

<150> JP 2018-225660
<151> 2018-11-30

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Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile Ser Ser Pro Thr Gly Ser
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Val Ala Lys Phe Asn Ala Ser Gln Leu Ile Thr Gln Arg Ala Gln Val
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tcc ctg cga gtg ttg tct cga ccc aat gct cag gag ctt cct agc atg        570
Ser Leu Arg Val Leu Ser Arg Pro Asn Ala Gln Glu Leu Pro Ser Met
105                 110                 115                 120

tac cag cgc cta ggg ctg gac tac gag gaa cga gtg ttg ccg tcc att        618
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Leu Ser Gln Met Leu Ser Asp Leu Thr Leu Gln Leu Arg Gln Arg Gln
        165                 170                 175

gag aat acg ata att gaa aac cca gat gtc cca cag gat ttc ggg aat        753
Glu Asn Thr Ile Ile Glu Asn Pro Asp Val Pro Gln Asp Phe Gly Asn
    180                 185                 190

caa ggg tca aca gta gag tcc ctc tgt gat gat gtt gtc tct gta ctc        801
Gln Gly Ser Thr Val Glu Ser Leu Cys Asp Asp Val Val Ser Val Leu
195                 200                 205                 210

acc gtc ctg tgt gag aag ctg caa gcc gcc ata aat gac agc cag cag        849
Thr Val Leu Cys Glu Lys Leu Gln Ala Ala Ile Asn Asp Ser Gln Gln
                215                 220                 225

ctg cag ctt ctc tac ctg gag tgc atc ctg tct gtg ctc agc agc tcc        897
Leu Gln Leu Leu Tyr Leu Glu Cys Ile Leu Ser Val Leu Ser Ser Ser
            230                 235                 240

tcc tcc tcc atg cac ctg cac agg cgc ttc acg gac ctg atc tgg aaa        945
Ser Ser Ser Met His Leu His Arg Arg Phe Thr Asp Leu Ile Trp Lys
        245                 250                 255

aac ctc tgc cct gct ctc atc gtg atc ttg ggg aat cca att cat gac        993
Asn Leu Cys Pro Ala Leu Ile Val Ile Leu Gly Asn Pro Ile His Asp
    260                 265                 270

aaa acc atc acc tct gct cac acc agc agc acc agt acc agc ctg gag       1041
Lys Thr Ile Thr Ser Ala His Thr Ser Ser Thr Ser Thr Ser Leu Glu
275                 280                 285                 290

tcg gac tct gcg tct ccg gga gtg tct gac cac ggc cga gga tca ggc       1089
Ser Asp Ser Ala Ser Pro Gly Val Ser Asp His Gly Arg Gly Ser Gly
                295                 300                 305

tgc tcc tgc act gcg ccg gcc ctg agc gga cct gtg gct cgg act atc       1137
Cys Ser Cys Thr Ala Pro Ala Leu Ser Gly Pro Val Ala Arg Thr Ile
            310                 315                 320

tat tac atc gca gcc gag ctg gtc cgg ctg gtg ggg tct gtg gac tcc       1185
Tyr Tyr Ile Ala Ala Glu Leu Val Arg Leu Val Gly Ser Val Asp Ser
        325                 330                 335

atg aag ccc gtg ctc cag tcc ctc tac cac cga gtg ctg ctc tac ccc       1233
Met Lys Pro Val Leu Gln Ser Leu Tyr His Arg Val Leu Leu Tyr Pro
    340                 345                 350

cca ccc cag cac cgg gtg gaa gcc atc aaa ata atg aaa gag ata ctt       1281
Pro Pro Gln His Arg Val Glu Ala Ile Lys Ile Met Lys Glu Ile Leu
355                 360                 365                 370

ggg agc cca cag cgt ctc tgt gac ttg gca gga ccc agc tcc act gaa       1329
Gly Ser Pro Gln Arg Leu Cys Asp Leu Ala Gly Pro Ser Ser Thr Glu
                375                 380                 385
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tca gag tcc aga aaa aga tca att tca aaa aga aag tct cat ctg gat      1377
Ser Glu Ser Arg Lys Arg Ser Ile Ser Lys Arg Lys Ser His Leu Asp
            390                 395                 400

ctc ctc aaa ctc atc atg gat ggc atg acc gaa gca tgc atc aag ggt      1425
Leu Leu Lys Leu Ile Met Asp Gly Met Thr Glu Ala Cys Ile Lys Gly
        405                 410                 415

ggc atc gaa gct tgc tat gca gcc gtg tcc tgt gtc tgc acc ttg ctg      1473
Gly Ile Glu Ala Cys Tyr Ala Ala Val Ser Cys Val Cys Thr Leu Leu
    420                 425                 430

ggt gcc ctg gat gag ctc agc cag ggg aag ggc ttg agc gaa ggt cag      1521
Gly Ala Leu Asp Glu Leu Ser Gln Gly Lys Gly Leu Ser Glu Gly Gln
435                 440                 445                 450

gtg caa ctg ctg ctt ctg cgc ctt gag gag ctg aag gat ggg gct gag      1569
Val Gln Leu Leu Leu Leu Arg Leu Glu Glu Leu Lys Asp Gly Ala Glu
                455                 460                 465

tgg agc cga gat tcc atg gag atc aat gag gct gac ttc cgc tgg cag      1617
Trp Ser Arg Asp Ser Met Glu Ile Asn Glu Ala Asp Phe Arg Trp Gln
            470                 475                 480

cgg cga gtg ctg tcc tca gaa cac acg ccg tgg gag tca ggg aac gag      1665
Arg Arg Val Leu Ser Ser Glu His Thr Pro Trp Glu Ser Gly Asn Glu
        485                 490                 495

agg agc ctt gac atc agc atc agt gtc acc aca gac aca ggc cag acc      1713
Arg Ser Leu Asp Ile Ser Ile Ser Val Thr Thr Asp Thr Gly Gln Thr
    500                 505                 510

act ctc gag gga gag ttg ggt cag act aca ccc gag gac cat tcg gga      1761
Thr Leu Glu Gly Glu Leu Gly Gln Thr Thr Pro Glu Asp His Ser Gly
515                 520                 525                 530

aac cac aag aac agt ctc aag tcg cca gcc atc cca gag ggt aag gag      1809
Asn His Lys Asn Ser Leu Lys Ser Pro Ala Ile Pro Glu Gly Lys Glu
                535                 540                 545

acg ctg agc aaa gta ttg gaa aca gag gcg gta gac cag cca gat gtc      1857
Thr Leu Ser Lys Val Leu Glu Thr Glu Ala Val Asp Gln Pro Asp Val
            550                 555                 560

gtg cag aga agc cac acg gtc cct tac cct gac ata act aac ttc ctg      1905
Val Gln Arg Ser His Thr Val Pro Tyr Pro Asp Ile Thr Asn Phe Leu
        565                 570                 575

tca gta gac tgc agg aca agg tcc tat gga tct agg tat agt gag agc      1953
Ser Val Asp Cys Arg Thr Arg Ser Tyr Gly Ser Arg Tyr Ser Glu Ser
    580                 585                 590

aat ttt agc gtt gat gac caa gac ctt tct agg aca gag ttt gat tcc      2001
Asn Phe Ser Val Asp Asp Gln Asp Leu Ser Arg Thr Glu Phe Asp Ser
595                 600                 605                 610

tgt gat cag tac tct atg gca gca gaa aag gac tcg ggc agg tcc gac      2049
Cys Asp Gln Tyr Ser Met Ala Ala Glu Lys Asp Ser Gly Arg Ser Asp
                615                 620                 625

gtg tca gac att ggg tcg gac aac tgt tca cta gcc gat gaa gag cag      2097
Val Ser Asp Ile Gly Ser Asp Asn Cys Ser Leu Ala Asp Glu Glu Gln
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                630                 635                 640

aca ccc cgg gac tgc cta ggc cac cgg tcc ctg cga act gcc gcc ctg       2145
Thr Pro Arg Asp Cys Leu Gly His Arg Ser Leu Arg Thr Ala Ala Leu
        645                 650                 655

tct cta aaa ctg ctg aag aac cag gag gcg gat cag cac agc gcc agg       2193
Ser Leu Lys Leu Leu Lys Asn Gln Glu Ala Asp Gln His Ser Ala Arg
    660                 665                 670

ctg ttc ata cag tcc ctg gaa ggc ctc ctc cct cgg ctc ctg tct ctc       2241
Leu Phe Ile Gln Ser Leu Glu Gly Leu Leu Pro Arg Leu Leu Ser Leu
675                 680                 685                 690

tcc aat gta gag gag gtg gac acc gct ctg cag aac ttt gcc tct act       2289
Ser Asn Val Glu Glu Val Asp Thr Ala Leu Gln Asn Phe Ala Ser Thr
                695                 700                 705

ttc tgc tca ggc atg atg cac tct cct ggc ttt gac ggg aat agc agc       2337
Phe Cys Ser Gly Met Met His Ser Pro Gly Phe Asp Gly Asn Ser Ser
            710                 715                 720

ctc agc ttc cag atg ctg atg aac gca gac agc ctc tac aca gct gca       2385
Leu Ser Phe Gln Met Leu Met Asn Ala Asp Ser Leu Tyr Thr Ala Ala
        725                 730                 735

cac tgc gcc ctg ctc ctc aac ctg aag ctc tcc cac ggt gac tac tac       2433
His Cys Ala Leu Leu Leu Asn Leu Lys Leu Ser His Gly Asp Tyr Tyr
    740                 745                 750

agg aag cgg ccg acc ctg gcg cca ggc gtg atg aag gac ttc atg aag       2481
Arg Lys Arg Pro Thr Leu Ala Pro Gly Val Met Lys Asp Phe Met Lys
755                 760                 765                 770

cag gtg cag acc agc ggc gtg ctg atg gtc ttc tct cag gcc tgg att       2529
Gln Val Gln Thr Ser Gly Val Leu Met Val Phe Ser Gln Ala Trp Ile
                775                 780                 785

gag gag ctc tac cat cag gtg ctc gac agg aac atg ctt gga gag gct       2577
Glu Glu Leu Tyr His Gln Val Leu Asp Arg Asn Met Leu Gly Glu Ala
            790                 795                 800

ggc tat tgg ggc agc cca gaa gat aac agc ctt ccc ctc atc aca atg       2625
Gly Tyr Trp Gly Ser Pro Glu Asp Asn Ser Leu Pro Leu Ile Thr Met
        805                 810                 815

ctg acc gat att gac ggc tta gag agc agt gcc att ggt ggc cag ctg       2673
Leu Thr Asp Ile Asp Gly Leu Glu Ser Ser Ala Ile Gly Gly Gln Leu
    820                 825                 830

atg gcc tcg gct gct aca gag tct cct ttc gcc cag agc agg aga att       2721
Met Ala Ser Ala Ala Thr Glu Ser Pro Phe Ala Gln Ser Arg Arg Ile
835                 840                 845                 850

gat gac tcc aca gtg gca ggc gtg gca ttt gct cgc tat att ctg gtg       2769
Asp Asp Ser Thr Val Ala Gly Val Ala Phe Ala Arg Tyr Ile Leu Val
                855                 860                 865

ggc tgc tgg aag aac ttg atc gat act tta tca acc cca ctg act ggt       2817
Gly Cys Trp Lys Asn Leu Ile Asp Thr Leu Ser Thr Pro Leu Thr Gly
            870                 875                 880

cga atg gcg ggg agc tcc aaa ggg ctg gcc ttc att ctg gga gct gaa       2865
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Arg Met Ala Gly Ser Ser Lys Gly Leu Ala Phe Ile Leu Gly Ala Glu
        885                 890                 895

ggc atc aaa gag cag aac cag aag gag cgg gac gcc atc tgc atg agc      2913
Gly Ile Lys Glu Gln Asn Gln Lys Glu Arg Asp Ala Ile Cys Met Ser
    900                 905                 910

ctc gac ggg ctg cgg aaa gcc gca cgg ctg agc tgc gct cta ggc gtt      2961
Leu Asp Gly Leu Arg Lys Ala Ala Arg Leu Ser Cys Ala Leu Gly Val
915                 920                 925                 930

gct gct aac tgc gcc tca gcc ctt gcc cag atg gca gct gcc tcc tgt      3009
Ala Ala Asn Cys Ala Ser Ala Leu Ala Gln Met Ala Ala Ala Ser Cys
                935                 940                 945

gtc caa gaa gaa aaa gaa gag agg gag gcc caa gaa ccc agt gat gcc      3057
Val Gln Glu Glu Lys Glu Glu Arg Glu Ala Gln Glu Pro Ser Asp Ala
            950                 955                 960

atc aca caa gtg aaa cta aaa gtg gag cag aaa ctg gag cag att ggg      3105
Ile Thr Gln Val Lys Leu Lys Val Glu Gln Lys Leu Glu Gln Ile Gly
        965                 970                 975

aag gtg cag ggg gtg tgg ctg cac act gcc cac gtc ttg tgc atg gag      3153
Lys Val Gln Gly Val Trp Leu His Thr Ala His Val Leu Cys Met Glu
    980                 985                 990

gcc atc ctc agc gta ggc  ctg gag atg gga agc  cac aac ccg gac       3198
Ala Ile Leu Ser Val Gly  Leu Glu Met Gly Ser  His Asn Pro Asp
995                 1000                1005

tgc  tgg cca cac gtg ttc  agg gtg tgt gaa tac  gtg ggc acc ctg      3243
Cys  Trp Pro His Val Phe  Arg Val Cys Glu Tyr  Val Gly Thr Leu
1010                1015                1020

gag  cac aac cac ttc agc  gat ggt gcc tcg cag  ccc cct ctg acc      3288
Glu  His Asn His Phe Ser  Asp Gly Ala Ser Gln  Pro Pro Leu Thr
1025                1030                1035

atc  agc cag ccc cag aag  gcc act gga agc gct  ggc ctc ctt ggg      3333
Ile  Ser Gln Pro Gln Lys  Ala Thr Gly Ser Ala  Gly Leu Leu Gly
1040                1045                1050

gac  ccc gag tgt gag ggc  tcg ccc ccc gag cac  agc ccg gag cag      3378
Asp  Pro Glu Cys Glu Gly  Ser Pro Pro Glu His  Ser Pro Glu Gln
1055                1060                1065

ggg  cgc tcc ctg agc acg  gcc cct gtc gtc cag  ccc ctg tcc atc      3423
Gly  Arg Ser Leu Ser Thr  Ala Pro Val Val Gln  Pro Leu Ser Ile
1070                1075                1080

cag  gac ctc gtc cgg gaa  ggc agc cgg ggt cgg  gcc tcc gac ttc      3468
Gln  Asp Leu Val Arg Glu  Gly Ser Arg Gly Arg  Ala Ser Asp Phe
1085                1090                1095

cgc  ggc ggg agc ctc atg  agc ggg agc agc gcg  gcc aag gtg gtg      3513
Arg  Gly Gly Ser Leu Met  Ser Gly Ser Ser Ala  Ala Lys Val Val
1100                1105                1110

ctc  acc ctc tcc acg caa  gcc gac agg ctc ttt  gaa gat gct acg      3558
Leu  Thr Leu Ser Thr Gln  Ala Asp Arg Leu Phe  Glu Asp Ala Thr
1115                1120                1125
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gat  aag ttg aac ctc atg  gcc ttg gga ggt ttt  ctt tac cag ctg        3603
Asp  Lys Leu Asn Leu Met  Ala Leu Gly Gly Phe  Leu Tyr Gln Leu
1130                1135                1140

aag  aaa gca tcg cag tct  cag ctt ttc cat tct  gtt aca gat aca        3648
Lys  Lys Ala Ser Gln Ser  Gln Leu Phe His Ser  Val Thr Asp Thr
1145                1150                1155

gtt  gat tac tct ctg gca  atg cca gga gaa gtt  aaa tcc act caa        3693
Val  Asp Tyr Ser Leu Ala  Met Pro Gly Glu Val  Lys Ser Thr Gln
1160                1165                1170

gac  cga aaa agc gcc ctc  cac ctg ttc cgc ctg  ggg aat gcc atg        3738
Asp  Arg Lys Ser Ala Leu  His Leu Phe Arg Leu  Gly Asn Ala Met
1175                1180                1185

ctg  agg att gtg cgg agc  aaa gca cgg ccc ctg  ctc cac gtg atg        3783
Leu  Arg Ile Val Arg Ser  Lys Ala Arg Pro Leu  Leu His Val Met
1190                1195                1200

cgc  tgc tgg agc ctt gtg  gcc cca cac ctg gtg  gag gct gct tgc        3828
Arg  Cys Trp Ser Leu Val  Ala Pro His Leu Val  Glu Ala Ala Cys
1205                1210                1215

cat  aag gaa aga cat gtg  tct cag aag gct gtt  tcc ttc atc cat        3873
His  Lys Glu Arg His Val  Ser Gln Lys Ala Val  Ser Phe Ile His
1220                1225                1230

gac  ata ctg aca gaa gtc  ctc act gac tgg aat  gag cca cct cat        3918
Asp  Ile Leu Thr Glu Val  Leu Thr Asp Trp Asn  Glu Pro Pro His
1235                1240                1245

ttt  cac ttc aat gaa gca  ctc ttc cga cct ttc  gag cgc att atg        3963
Phe  His Phe Asn Glu Ala  Leu Phe Arg Pro Phe  Glu Arg Ile Met
1250                1255                1260

cag  ctg gaa ttg tgt gat  gag gac gtc caa gac  cag gtt gtc aca        4008
Gln  Leu Glu Leu Cys Asp  Glu Asp Val Gln Asp  Gln Val Val Thr
1265                1270                1275

tcc  att ggt gag ctg gtt  gaa gtg tgt tcc acg  cag atc cag tcg        4053
Ser  Ile Gly Glu Leu Val  Glu Val Cys Ser Thr  Gln Ile Gln Ser
1280                1285                1290

gga  tgg aga ccc ttg ttc  agt gcc ctg gaa aca  gtg cat ggc ggg        4098
Gly  Trp Arg Pro Leu Phe  Ser Ala Leu Glu Thr  Val His Gly Gly
1295                1300                1305

aac  aag tca gag atg aag  gag tac ctg gtt ggt  gac tac tcc atg        4143
Asn  Lys Ser Glu Met Lys  Glu Tyr Leu Val Gly  Asp Tyr Ser Met
1310                1315                1320

gga  aaa ggc caa gct cca  gtg ttt gat gta ttt  gaa gct ttt ctc        4188
Gly  Lys Gly Gln Ala Pro  Val Phe Asp Val Phe  Glu Ala Phe Leu
1325                1330                1335

aat  act gac aac atc cag  gtc ttt gct aat gca  gcc act agc tac        4233
Asn  Thr Asp Asn Ile Gln  Val Phe Ala Asn Ala  Ala Thr Ser Tyr
1340                1345                1350

atc  atg tgc ctt atg aag  ttt gtc aaa gga ctg  ggg gag gtg gac        4278
Ile  Met Cys Leu Met Lys  Phe Val Lys Gly Leu  Gly Glu Val Asp
1355                1360                1365
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tgt  aaa gag att gga gac  tgt gcc cca gca ccc  gga gcc ccg tcc        4323
Cys  Lys Glu Ile Gly Asp  Cys Ala Pro Ala Pro  Gly Ala Pro Ser
1370                 1375                 1380

aca  gac ctg tgc ctc ccg  gcc ctg gat tac ctc  agg cgc tgc tct        4368
Thr  Asp Leu Cys Leu Pro  Ala Leu Asp Tyr Leu  Arg Arg Cys Ser
1385                 1390                 1395

cag  tta ttg gcc aaa atc  tac aaa atg ccc ttg  aag cca ata ttc        4413
Gln  Leu Leu Ala Lys Ile  Tyr Lys Met Pro Leu  Lys Pro Ile Phe
1400                 1405                 1410

ctt  agt ggg aga ctt gcc  ggc ttg cct cga aga  ctt cag gaa cag        4458
Leu  Ser Gly Arg Leu Ala  Gly Leu Pro Arg Arg  Leu Gln Glu Gln
1415                 1420                 1425

tca  gcc agc agt gag gat  gga att gaa tca gtc  ctg tct gat ttt        4503
Ser  Ala Ser Ser Glu Asp  Gly Ile Glu Ser Val  Leu Ser Asp Phe
1430                 1435                 1440

gat  gat gac acc ggt ctg  ata gaa gtc tgg ata  atc ctg ctg gag        4548
Asp  Asp Asp Thr Gly Leu  Ile Glu Val Trp Ile  Ile Leu Leu Glu
1445                 1450                 1455

cag  ctg aca gcg gct gtg  tcc aat tgt cca cgg  cag cac caa cca        4593
Gln  Leu Thr Ala Ala Val  Ser Asn Cys Pro Arg  Gln His Gln Pro
1460                 1465                 1470

cca  act ctg gat tta ctc  ttt gag ctg ttg aga  gat gtg acg aaa        4638
Pro  Thr Leu Asp Leu Leu  Phe Glu Leu Leu Arg  Asp Val Thr Lys
1475                 1480                 1485

aca  cca gga cca ggg ttt  ggt atc tat gca gtg  gtt cac ctc ctc        4683
Thr  Pro Gly Pro Gly Phe  Gly Ile Tyr Ala Val  Val His Leu Leu
1490                 1495                 1500

ctt  cct gtg atg tcc gtt  tgg ctc cgc cgg agc  cat aaa gac cat        4728
Leu  Pro Val Met Ser Val  Trp Leu Arg Arg Ser  His Lys Asp His
1505                 1510                 1515

tcc  tac tgg gat atg gcc  tct gcc aat ttc aag  cac gct att ggt        4773
Ser  Tyr Trp Asp Met Ala  Ser Ala Asn Phe Lys  His Ala Ile Gly
1520                 1525                 1530

ctg  tcc tgt gag ctg gtg  gtg gag cac att caa  agc ttt cta cat        4818
Leu  Ser Cys Glu Leu Val  Val Glu His Ile Gln  Ser Phe Leu His
1535                 1540                 1545

tca  gat atc agg tac gag  agc atg atc aat acc  atg ctg aag gac        4863
Ser  Asp Ile Arg Tyr Glu  Ser Met Ile Asn Thr  Met Leu Lys Asp
1550                 1555                 1560

ctc  ttt gag ttg ctg gtc  gcc tgt gtg gcc aag  ccc act gaa acc        4908
Leu  Phe Glu Leu Leu Val  Ala Cys Val Ala Lys  Pro Thr Glu Thr
1565                 1570                 1575

atc  tcc aga gtg ggc tgc  tcc tgt att aga tac  gtc ctt gtg aca        4953
Ile  Ser Arg Val Gly Cys  Ser Cys Ile Arg Tyr  Val Leu Val Thr
1580                 1585                 1590

gcg  ggc cct gtg ttc act  gag gag atg tgg agg  ctt gcc tgc tgt        4998
Ala  Gly Pro Val Phe Thr  Glu Glu Met Trp Arg  Leu Ala Cys Cys
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1595                1600                1605

gcc  ctg caa gat gcg ttc  tct gcc aca ctc aag  cca gtg aag gac        5043
Ala  Leu Gln Asp Ala Phe  Ser Ala Thr Leu Lys  Pro Val Lys Asp
1610                1615                1620

ctg  ctg ggc tgc ttc cac  agc ggc acg gag agc  ttc agc ggg gaa        5088
Leu  Leu Gly Cys Phe His  Ser Gly Thr Glu Ser  Phe Ser Gly Glu
1625                1630                1635

ggc  tgc cag gtg cga gtg  gcg gcc ccg tcc tcc  tcc cca agt gcc        5133
Gly  Cys Gln Val Arg Val  Ala Ala Pro Ser Ser  Ser Pro Ser Ala
1640                1645                1650

gag  gcc gag tac tgg cgc  atc cga gcc atg gcc  cag cag gtg ttt        5178
Glu  Ala Glu Tyr Trp Arg  Ile Arg Ala Met Ala  Gln Gln Val Phe
1655                1660                1665

atg  ctg gac acc cag tgc  tca cca aag aca cca  aac aac ttt gac        5223
Met  Leu Asp Thr Gln Cys  Ser Pro Lys Thr Pro  Asn Asn Phe Asp
1670                1675                1680

cac  gct cag tcc tgc cag  ctc att att gag ctg  cct cct gat gaa        5268
His  Ala Gln Ser Cys Gln  Leu Ile Ile Glu Leu  Pro Pro Asp Glu
1685                1690                1695

aaa  cca aat gga cac acc  aag aaa agc gtg tct  ttc agg gaa att        5313
Lys  Pro Asn Gly His Thr  Lys Lys Ser Val Ser  Phe Arg Glu Ile
1700                1705                1710

gtg  gtg agc ctg ctg tct  cat cag gtg tta ctc  cag aac tta tat        5358
Val  Val Ser Leu Leu Ser  His Gln Val Leu Leu  Gln Asn Leu Tyr
1715                1720                1725

gac  atc ttg tta gaa gag  ttt gtc aaa ggc ccc  tct cct gga gag        5403
Asp  Ile Leu Leu Glu Glu  Phe Val Lys Gly Pro  Ser Pro Gly Glu
1730                1735                1740

gaa  aag acg ata caa gtg  cca gaa gcc aag ctg  gct ggc ttc ctc        5448
Glu  Lys Thr Ile Gln Val  Pro Glu Ala Lys Leu  Ala Gly Phe Leu
1745                1750                1755

aga  tac atc tct atg cag  aac ttg gca gtc ata  ttc gac ctg ctg        5493
Arg  Tyr Ile Ser Met Gln  Asn Leu Ala Val Ile  Phe Asp Leu Leu
1760                1765                1770

ctg  gac tct tat agg act  gcc agg gag ttt gac  acc agc ccc ggg        5538
Leu  Asp Ser Tyr Arg Thr  Ala Arg Glu Phe Asp  Thr Ser Pro Gly
1775                1780                1785

ctg  aag tgc ctg ctg aag  aaa gtg tct ggc atc  ggg ggc gcc gcc        5583
Leu  Lys Cys Leu Leu Lys  Lys Val Ser Gly Ile  Gly Gly Ala Ala
1790                1795                1800

aac  ctc tac cgc cag tct  gcg atg agc ttt aac  att tat ttc cac        5628
Asn  Leu Tyr Arg Gln Ser  Ala Met Ser Phe Asn  Ile Tyr Phe His
1805                1810                1815

gcc  ctg gtg tgt gct gtt  ctc acc aat caa gaa  acc atc acg gcc        5673
Ala  Leu Val Cys Ala Val  Leu Thr Asn Gln Glu  Thr Ile Thr Ala
1820                1825                1830

gag  caa gtg aag aag gtc  ctt ttt gag gac gac  gag aga agc acg        5718
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Glu  Gln Val Lys Lys Val  Leu Phe Glu Asp Asp  Glu Arg Ser Thr
1835                 1840                 1845

gat  tct tcc cag cag tgt  tca tct gag gat gaa  gac atc ttt gag      5763
Asp  Ser Ser Gln Gln Cys  Ser Ser Glu Asp Glu  Asp Ile Phe Glu
1850                 1855                 1860

gaa  acc gcc cag gtc agc  ccc ccg aga ggc aag  gag aag aga cag      5808
Glu  Thr Ala Gln Val Ser  Pro Pro Arg Gly Lys  Glu Lys Arg Gln
1865                 1870                 1875

tgg  cgg gca cgg atg ccc  ttg ctc agc gtc cag  cct gtc agc aac      5853
Trp  Arg Ala Arg Met Pro  Leu Leu Ser Val Gln  Pro Val Ser Asn
1880                 1885                 1890

gca  gat tgg gtg tgg ctg  gtc aag agg ctg cac  aag ctg tgc atg      5898
Ala  Asp Trp Val Trp Leu  Val Lys Arg Leu His  Lys Leu Cys Met
1895                 1900                 1905

gaa  ctg tgc aac aac tac  atc cag atg cac ttg  gac ctg gag aac      5943
Glu  Leu Cys Asn Asn Tyr  Ile Gln Met His Leu  Asp Leu Glu Asn
1910                 1915                 1920

tgt  atg gag gag cct ccc  atc ttc aag ggc gac  ccg ttc ttc atc      5988
Cys  Met Glu Glu Pro Pro  Ile Phe Lys Gly Asp  Pro Phe Phe Ile
1925                 1930                 1935

ctg  ccc tcc ttc cag tcc  gag tca tcc acc cca  tcc acc ggg ggc      6033
Leu  Pro Ser Phe Gln Ser  Glu Ser Ser Thr Pro  Ser Thr Gly Gly
1940                 1945                 1950

ttc  tct ggg aaa gaa acc  cct tcc gag gat gac  aga agc cag tcc      6078
Phe  Ser Gly Lys Glu Thr  Pro Ser Glu Asp Asp  Arg Ser Gln Ser
1955                 1960                 1965

cgg  gag cac atg ggc gag  tcc ctg agc ctg aag  gcc ggt ggt ggg      6123
Arg  Glu His Met Gly Glu  Ser Leu Ser Leu Lys  Ala Gly Gly Gly
1970                 1975                 1980

gac  ctg ctg ctg ccc ccc  agc ccc aaa gtg gag  aag aag gat ccc      6168
Asp  Leu Leu Leu Pro Pro  Ser Pro Lys Val Glu  Lys Lys Asp Pro
1985                 1990                 1995

agc  cgg aag aag gag tgg  tgg gag aat gcg ggg  aac aaa atc tac      6213
Ser  Arg Lys Lys Glu Trp  Trp Glu Asn Ala Gly  Asn Lys Ile Tyr
2000                 2005                 2010

acc  atg gca gcc gac aag  acc att tca aag ttg  atg acc gaa tac      6258
Thr  Met Ala Ala Asp Lys  Thr Ile Ser Lys Leu  Met Thr Glu Tyr
2015                 2020                 2025

aaa  aag agg aaa cag cag  cac aac ctg tcc gcg  ttc ccc aaa gag      6303
Lys  Lys Arg Lys Gln Gln  His Asn Leu Ser Ala  Phe Pro Lys Glu
2030                 2035                 2040

gtc  aaa gtg gag aag aaa  gga gag cca ctg ggt  ccc agg ggc cag      6348
Val  Lys Val Glu Lys Lys  Gly Glu Pro Leu Gly  Pro Arg Gly Gln
2045                 2050                 2055

gac  tcc ccg ctg ctt cag  cgt ccc cag cac ttg  atg gac caa ggg      6393
Asp  Ser Pro Leu Leu Gln  Arg Pro Gln His Leu  Met Asp Gln Gly
2060                 2065                 2070
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caa  atg cgg cat tcc ttc  agc gca ggc ccc gag  ctg ctg cga cag        6438
Gln  Met Arg His Ser Phe  Ser Ala Gly Pro Glu  Leu Leu Arg Gln
2075                 2080                 2085

gac  aag agg ccc cgc tca  ggc tcc acc ggg agc  tcc ctc agt gtc        6483
Asp  Lys Arg Pro Arg Ser  Gly Ser Thr Gly Ser  Ser Leu Ser Val
2090                 2095                 2100

tcg  gtg aga gac gca gaa  gca cag atc cag gca  tgg acc aac atg        6528
Ser  Val Arg Asp Ala Glu  Ala Gln Ile Gln Ala  Trp Thr Asn Met
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gtg  cta aca gtt ctc aat  cag att cag att ctc  cca gac cag acc        6573
Val  Leu Thr Val Leu Asn  Gln Ile Gln Ile Leu  Pro Asp Gln Thr
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ttc  acg gcc ctc cag ccc  gca gtg ttc ccg tgc  atc agt cag ctg        6618
Phe  Thr Ala Leu Gln Pro  Ala Val Phe Pro Cys  Ile Ser Gln Leu
2135                 2140                 2145

acc  tgt cac gtg acc gac  atc aga gtt cgc cag  gct gtg agg gag        6663
Thr  Cys His Val Thr Asp  Ile Arg Val Arg Gln  Ala Val Arg Glu
2150                 2155                 2160

tgg  ctg ggc agg gtg ggc  cgt gtc tat gac atc  att gtg tag           6705
Trp  Leu Gly Arg Val Gly  Arg Val Tyr Asp Ile  Ile Val
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Tyr Lys Ala Ile Lys Glu Ser Cys Thr Trp Ala Leu Glu Thr Leu Gly

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Gly Leu Asp Thr Ile Val Lys Ile Pro Pro His Val Leu Arg Glu Lys
        35                  40                  45

Cys Leu Leu Pro Leu Gln Leu Ala Leu Glu Ser Lys Asn Val Lys Leu
    50                  55                  60

Ala Gln His Ala Leu Ala Gly Met Gln Lys Leu Leu Ser Glu Glu Arg
65                  70                  75                  80

Phe Val Ser Met Glu Thr Asp Ser Asp Glu Lys Gln Leu Leu Asn Gln
                85                  90                  95

Ile Leu Asn Ala Val Lys Val Thr Pro Ser Leu Asn Glu Asp Leu Gln
            100                 105                 110

Val Glu Val Met Lys Val Leu Leu Cys Ile Thr Tyr Thr Pro Thr Phe
        115                 120                 125

Asp Leu Asn Gly Ser Ala Val Leu Lys Ile Ala Glu Val Cys Ile Glu
    130                 135                 140

Thr Tyr Ile Ser Ser Cys His Gln Arg Ser Ile Asn Thr Ala Val Arg
145                 150                 155                 160

Ala Thr Leu Ser Gln Met Leu Ser Asp Leu Thr Leu Gln Leu Arg Gln
                165                 170                 175

Arg Gln Glu Asn Thr Ile Ile Glu Asn Pro Asp Val Pro Gln Asp Phe
            180                 185                 190

Gly Asn Gln Gly Ser Thr Val Glu Ser Leu Cys Asp Asp Val Val Ser
        195                 200                 205

Val Leu Thr Val Leu Cys Glu Lys Leu Gln Ala Ala Ile Asn Asp Ser
    210                 215                 220

Gln Gln Leu Gln Leu Leu Tyr Leu Glu Cys Ile Leu Ser Val Leu Ser
225                 230                 235                 240

Ser Ser Ser Ser Ser Met His Leu His Arg Arg Phe Thr Asp Leu Ile
                245                 250                 255

Trp Lys Asn Leu Cys Pro Ala Leu Ile Val Ile Leu Gly Asn Pro Ile
            260                 265                 270
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His Asp Lys Thr Ile Thr Ser Ala His Thr Ser Ser Thr Ser Thr Ser
        275                 280                 285

Leu Glu Ser Asp Ser Ala Ser Pro Gly Val Ser Asp His Gly Arg Gly
    290                 295                 300

Ser Gly Cys Ser Cys Thr Ala Pro Ala Leu Ser Gly Pro Val Ala Arg
305                 310                 315                 320

Thr Ile Tyr Tyr Ile Ala Ala Glu Leu Val Arg Leu Val Gly Ser Val
                325                 330                 335

Asp Ser Met Lys Pro Val Leu Gln Ser Leu Tyr His Arg Val Leu Leu
            340                 345                 350

Tyr Pro Pro Pro Gln His Arg Val Glu Ala Ile Lys Ile Met Lys Glu
        355                 360                 365

Ile Leu Gly Ser Pro Gln Arg Leu Cys Asp Leu Ala Gly Pro Ser Ser
    370                 375                 380

Thr Glu Ser Glu Ser Arg Lys Arg Ser Ile Ser Lys Arg Lys Ser His
385                 390                 395                 400

Leu Asp Leu Leu Lys Leu Ile Met Asp Gly Met Thr Glu Ala Cys Ile
                405                 410                 415

Lys Gly Gly Ile Glu Ala Cys Tyr Ala Ala Val Ser Cys Val Cys Thr
            420                 425                 430

Leu Leu Gly Ala Leu Asp Glu Leu Ser Gln Gly Lys Gly Leu Ser Glu
        435                 440                 445

Gly Gln Val Gln Leu Leu Leu Leu Arg Leu Glu Glu Leu Lys Asp Gly
    450                 455                 460

Ala Glu Trp Ser Arg Asp Ser Met Glu Ile Asn Glu Ala Asp Phe Arg
465                 470                 475                 480

Trp Gln Arg Arg Val Leu Ser Ser Glu His Thr Pro Trp Glu Ser Gly
                485                 490                 495

Asn Glu Arg Ser Leu Asp Ile Ser Ile Ser Val Thr Thr Asp Thr Gly
            500                 505                 510

Gln Thr Thr Leu Glu Gly Glu Leu Gly Gln Thr Thr Pro Glu Asp His
        515                 520                 525
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    530                 535                 540

Lys Glu Thr Leu Ser Lys Val Leu Glu Thr Glu Ala Val Asp Gln Pro
545                 550                 555                 560

Asp Val Val Gln Arg Ser His Thr Val Pro Tyr Pro Asp Ile Thr Asn
                565                 570                 575

Phe Leu Ser Val Asp Cys Arg Thr Arg Ser Tyr Gly Ser Arg Tyr Ser
            580                 585                 590

Glu Ser Asn Phe Ser Val Asp Asp Gln Asp Leu Ser Arg Thr Glu Phe
        595                 600                 605

Asp Ser Cys Asp Gln Tyr Ser Met Ala Ala Glu Lys Asp Ser Gly Arg
    610                 615                 620

Ser Asp Val Ser Asp Ile Gly Ser Asp Asn Cys Ser Leu Ala Asp Glu
625                 630                 635                 640

Glu Gln Thr Pro Arg Asp Cys Leu Gly His Arg Ser Leu Arg Thr Ala
                645                 650                 655

Ala Leu Ser Leu Lys Leu Leu Lys Asn Gln Glu Ala Asp Gln His Ser
            660                 665                 670

Ala Arg Leu Phe Ile Gln Ser Leu Glu Gly Leu Leu Pro Arg Leu Leu
        675                 680                 685

Ser Leu Ser Asn Val Glu Glu Val Asp Thr Ala Leu Gln Asn Phe Ala
    690                 695                 700

Ser Thr Phe Cys Ser Gly Met Met His Ser Pro Gly Phe Asp Gly Asn
705                 710                 715                 720

Ser Ser Leu Ser Phe Gln Met Leu Met Asn Ala Asp Ser Leu Tyr Thr
                725                 730                 735

Ala Ala His Cys Ala Leu Leu Leu Asn Leu Lys Leu Ser His Gly Asp
            740                 745                 750

Tyr Tyr Arg Lys Arg Pro Thr Leu Ala Pro Gly Val Met Lys Asp Phe
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Met Lys Gln Val Gln Thr Ser Gly Val Leu Met Val Phe Ser Gln Ala
    770                 775                 780
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Glu Ala Gly Tyr Trp Gly Ser Pro Glu Asp Asn Ser Leu Pro Leu Ile
                805                 810                 815

Thr Met Leu Thr Asp Ile Asp Gly Leu Glu Ser Ser Ala Ile Gly Gly
            820                 825                 830

Gln Leu Met Ala Ser Ala Ala Thr Glu Ser Pro Phe Ala Gln Ser Arg
        835                 840                 845

Arg Ile Asp Asp Ser Thr Val Ala Gly Val Ala Phe Ala Arg Tyr Ile
    850                 855                 860

Leu Val Gly Cys Trp Lys Asn Leu Ile Asp Thr Leu Ser Thr Pro Leu
865                 870                 875                 880

Thr Gly Arg Met Ala Gly Ser Ser Lys Gly Leu Ala Phe Ile Leu Gly
                885                 890                 895

Ala Glu Gly Ile Lys Glu Gln Asn Gln Lys Glu Arg Asp Ala Ile Cys
            900                 905                 910

Met Ser Leu Asp Gly Leu Arg Lys Ala Ala Arg Leu Ser Cys Ala Leu
        915                 920                 925

Gly Val Ala Ala Asn Cys Ala Ser Ala Leu Ala Gln Met Ala Ala Ala
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Ser Cys Val Gln Glu Glu Lys Glu Glu Arg Glu Ala Gln Glu Pro Ser
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Asp Ala Ile Thr Gln Val Lys Leu Lys Val Glu Gln Lys Leu Glu Gln
                965                 970                 975

Ile Gly Lys Val Gln Gly Val Trp Leu His Thr Ala His Val Leu Cys
            980                 985                 990

Met Glu Ala Ile Leu Ser Val Gly  Leu Glu Met Gly Ser  His Asn Pro
        995                 1000                1005

Asp Cys  Trp Pro His Val Phe  Arg Val Cys Glu Tyr  Val Gly Thr
    1010                1015                1020

Leu Glu  His Asn His Phe Ser  Asp Gly Ala Ser Gln  Pro Pro Leu
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Gln Asp Arg Lys Ser Ala Leu His Leu Phe Arg Leu Gly Asn Ala
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Met Arg Cys Trp Ser Leu Val Ala Pro His Leu Val Glu Ala Ala
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Cys His Lys Glu Arg His Val Ser Gln Lys Ala Val Ser Phe Ile
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His Asp Ile Leu Thr Glu Val Leu Thr Asp Trp Asn Glu Pro Pro
1235 1240 1245

His Phe His Phe Asn Glu Ala Leu Phe Arg Pro Phe Glu Arg Ile
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Thr Ser  Ile Gly Glu Leu Val  Glu Val Cys Ser Thr  Gln Ile Gln
    1280                 1285                 1290

Ser Gly  Trp Arg Pro Leu Phe  Ser Ala Leu Glu Thr  Val His Gly
    1295                 1300                 1305

Gly Asn  Lys Ser Glu Met Lys  Glu Tyr Leu Val Gly  Asp Tyr Ser
    1310                 1315                 1320

Met Gly  Lys Gly Gln Ala Pro  Val Phe Asp Val Phe  Glu Ala Phe
    1325                 1330                 1335

Leu Asn  Thr Asp Asn Ile Gln  Val Phe Ala Asn Ala  Ala Thr Ser
    1340                 1345                 1350

Tyr Ile  Met Cys Leu Met Lys  Phe Val Lys Gly Leu  Gly Glu Val
    1355                 1360                 1365

Asp Cys  Lys Glu Ile Gly Asp  Cys Ala Pro Ala Pro  Gly Ala Pro
    1370                 1375                 1380

Ser Thr  Asp Leu Cys Leu Pro  Ala Leu Asp Tyr Leu  Arg Arg Cys
    1385                 1390                 1395

Ser Gln  Leu Leu Ala Lys Ile  Tyr Lys Met Pro Leu  Lys Pro Ile
    1400                 1405                 1410

Phe Leu  Ser Gly Arg Leu Ala  Gly Leu Pro Arg Arg  Leu Gln Glu
    1415                 1420                 1425

Gln Ser  Ala Ser Ser Glu Asp  Gly Ile Glu Ser Val  Leu Ser Asp
    1430                 1435                 1440

Phe Asp  Asp Asp Thr Gly Leu  Ile Glu Val Trp Ile  Ile Leu Leu
    1445                 1450                 1455

Glu Gln  Leu Thr Ala Ala Val  Ser Asn Cys Pro Arg  Gln His Gln
    1460                 1465                 1470

Pro Pro  Thr Leu Asp Leu Leu  Phe Glu Leu Leu Arg  Asp Val Thr
    1475                 1480                 1485

Lys Thr  Pro Gly Pro Gly Phe  Gly Ile Tyr Ala Val  Val His Leu
    1490                 1495                 1500
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Leu Leu  Pro Val Met Ser Val  Trp Leu Arg Arg Ser  His Lys Asp
    1505                 1510                 1515

His Ser  Tyr Trp Asp Met Ala  Ser Ala Asn Phe Lys  His Ala Ile
    1520                 1525                 1530

Gly Leu  Ser Cys Glu Leu Val  Val Glu His Ile Gln  Ser Phe Leu
    1535                 1540                 1545

His Ser  Asp Ile Arg Tyr Glu  Ser Met Ile Asn Thr  Met Leu Lys
    1550                 1555                 1560

Asp Leu  Phe Glu Leu Leu Val  Ala Cys Val Ala Lys  Pro Thr Glu
    1565                 1570                 1575

Thr Ile  Ser Arg Val Gly Cys  Ser Cys Ile Arg Tyr  Val Leu Val
    1580                 1585                 1590

Thr Ala  Gly Pro Val Phe Thr  Glu Glu Met Trp Arg  Leu Ala Cys
    1595                 1600                 1605

Cys Ala  Leu Gln Asp Ala Phe  Ser Ala Thr Leu Lys  Pro Val Lys
    1610                 1615                 1620

Asp Leu  Leu Gly Cys Phe His  Ser Gly Thr Glu Ser  Phe Ser Gly
    1625                 1630                 1635

Glu Gly  Cys Gln Val Arg Val  Ala Ala Pro Ser Ser  Ser Pro Ser
    1640                 1645                 1650

Ala Glu  Ala Glu Tyr Trp Arg  Ile Arg Ala Met Ala  Gln Gln Val
    1655                 1660                 1665

Phe Met  Leu Asp Thr Gln Cys  Ser Pro Lys Thr Pro  Asn Asn Phe
    1670                 1675                 1680

Asp His  Ala Gln Ser Cys Gln  Leu Ile Ile Glu Leu  Pro Pro Asp
    1685                 1690                 1695

Glu Lys  Pro Asn Gly His Thr  Lys Lys Ser Val Ser  Phe Arg Glu
    1700                 1705                 1710

Ile Val  Val Ser Leu Leu Ser  His Gln Val Leu Leu  Gln Asn Leu
    1715                 1720                 1725

Tyr Asp  Ile Leu Leu Glu Glu  Phe Val Lys Gly Pro  Ser Pro Gly
    1730                 1735                 1740
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Glu Glu  Lys Thr Ile Gln Val  Pro Glu Ala Lys Leu  Ala Gly Phe
    1745                 1750                 1755

Leu Arg  Tyr Ile Ser Met Gln  Asn Leu Ala Val Ile  Phe Asp Leu
    1760                 1765                 1770

Leu Leu  Asp Ser Tyr Arg Thr  Ala Arg Glu Phe Asp  Thr Ser Pro
    1775                 1780                 1785

Gly Leu  Lys Cys Leu Leu Lys  Lys Val Ser Gly Ile  Gly Gly Ala
    1790                 1795                 1800

Ala Asn  Leu Tyr Arg Gln Ser  Ala Met Ser Phe Asn  Ile Tyr Phe
    1805                 1810                 1815

His Ala  Leu Val Cys Ala Val  Leu Thr Asn Gln Glu  Thr Ile Thr
    1820                 1825                 1830

Ala Glu  Gln Val Lys Lys Val  Leu Phe Glu Asp Asp  Glu Arg Ser
    1835                 1840                 1845

Thr Asp  Ser Ser Gln Gln Cys  Ser Ser Glu Asp Glu  Asp Ile Phe
    1850                 1855                 1860

Glu Glu  Thr Ala Gln Val Ser  Pro Pro Arg Gly Lys  Glu Lys Arg
    1865                 1870                 1875

Gln Trp  Arg Ala Arg Met Pro  Leu Leu Ser Val Gln  Pro Val Ser
    1880                 1885                 1890

Asn Ala  Asp Trp Val Trp Leu  Val Lys Arg Leu His  Lys Leu Cys
    1895                 1900                 1905

Met Glu  Leu Cys Asn Asn Tyr  Ile Gln Met His Leu  Asp Leu Glu
    1910                 1915                 1920

Asn Cys  Met Glu Glu Pro Pro  Ile Phe Lys Gly Asp  Pro Phe Phe
    1925                 1930                 1935

Ile Leu  Pro Ser Phe Gln Ser  Glu Ser Ser Thr Pro  Ser Thr Gly
    1940                 1945                 1950

Gly Phe  Ser Gly Lys Glu Thr  Pro Ser Glu Asp Asp  Arg Ser Gln
    1955                 1960                 1965

Ser Arg  Glu His Met Gly Glu  Ser Leu Ser Leu Lys  Ala Gly Gly
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1970 1975 1980

Gly Asp Leu Leu Leu Pro Pro Ser Pro Lys Val Glu Lys Lys Asp
1985 1990 1995

Pro Ser Arg Lys Lys Glu Trp Trp Glu Asn Ala Gly Asn Lys Ile
2000 2005 2010

Tyr Thr Met Ala Ala Asp Lys Thr Ile Ser Lys Leu Met Thr Glu
2015 2020 2025

Tyr Lys Lys Arg Lys Gln Gln His Asn Leu Ser Ala Phe Pro Lys
2030 2035 2040

Glu Val Lys Val Glu Lys Lys Gly Glu Pro Leu Gly Pro Arg Gly
2045 2050 2055

Gln Asp Ser Pro Leu Leu Gln Arg Pro Gln His Leu Met Asp Gln
2060 2065 2070

Gly Gln Met Arg His Ser Phe Ser Ala Gly Pro Glu Leu Leu Arg
2075 2080 2085

Gln Asp Lys Arg Pro Arg Ser Gly Ser Thr Gly Ser Ser Leu Ser
2090 2095 2100

Val Ser Val Arg Asp Ala Glu Ala Gln Ile Gln Ala Trp Thr Asn
2105 2110 2115

Met Val Leu Thr Val Leu Asn Gln Ile Gln Ile Leu Pro Asp Gln
2120 2125 2130

Thr Phe Thr Ala Leu Gln Pro Ala Val Phe Pro Cys Ile Ser Gln
2135 2140 2145

Leu Thr Cys His Val Thr Asp Ile Arg Val Arg Gln Ala Val Arg
2150 2155 2160

Glu Trp Leu Gly Arg Val Gly Arg Val Tyr Asp Ile Ile Val
2165 2170 2175

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Gln Met Leu Ser Asp Leu Thr Leu Gln Leu Arg Gln Arg
1 5 10

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<220>
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<400> 34

Met Ala Gln Asn Leu Lys Asp Leu Ala Gly Arg Leu
1 5 10

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<220>
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<400> 35

Gln Asn Leu Lys Asp Leu Ala Gly Arg Leu Pro Ala
1 5 10

<210> 36
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<220>
<223> PHB2 peptide No. 36

<400> 36

Leu Lys Asp Leu Ala Gly Arg Leu Pro Ala Gly Pro
1 5 10

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<220>
<223> PHB2 peptide No. 37

<400> 37

Asp Leu Ala Gly Arg Leu Pro Ala Gly Pro Arg Gly
1 5 10

<210> 38

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<400> 38

Ala Gly Arg Leu Pro Ala Gly Pro Arg Gly Met Gly
1 5 10

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<400> 39

Pro Ala Gly Pro Arg Gly Met Gly Thr Ala Leu Lys
1 5 10

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<220>
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<400> 40

Gly Pro Arg Gly Met Gly Thr Ala Leu Lys Leu Leu
1 5 10

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<400> 41

Arg Gly Met Gly Thr Ala Leu Lys Leu Leu Leu Gly
1 5 10

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<400> 42

Met Gly Thr Ala Leu Lys Leu Leu Leu Gly Ala Gly
1 5 10

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<400> 43

Thr Ala Leu Lys Leu Leu Leu Gly Ala Gly Ala Val
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<220>
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<400> 44

Ile Leu Ala Glu Gly Leu His Phe Arg Ile Pro Trp Phe Gln Tyr
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<400> 46

Gly Leu His Phe Arg Ile Pro Trp Phe Gln Tyr Pro Ile Ile Tyr
1 5 10 15

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<220>
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<400> 47

His Phe Arg Ile Pro Trp Phe Gln Tyr Pro Ile Ile Tyr Asp
1 5 10

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Arg Ile Pro Trp Phe Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala
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<400> 49

Pro Trp Phe Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro
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Phe Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys
1 5 10 15

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Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile Ser
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Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile Ser Ser Pro
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Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile Ser Ser Pro Thr Gly
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<220>
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Ile Arg Ala Arg Pro Arg Lys Ile Ser Ser Pro Thr Gly Ser Lys
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Ala Arg Pro Arg Lys Ile Ser Ser Pro Thr Gly Ser Lys Asp Leu
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Ala Leu Pro Ala Gly Pro Arg Gly Met Gly Thr Ala
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Arg Ala Pro Ala Gly Pro Arg Gly Met Gly Thr Ala
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Arg Leu Pro Ala Gly Pro Ala Gly Met Gly Thr Ala
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Arg Leu Pro Ala Gly Pro Arg Ala Met Gly Thr Ala
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Arg Leu Pro Ala Gly Pro Arg Gly Ala Gly Thr Ala
1 5 10

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Arg Leu Pro Ala Gly Pro Arg Gly Met Ala Thr Ala
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<400> 65

Arg Leu Pro Ala Gly Pro Arg Gly Met Gly Ala Ala
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<400> 66

Ala Leu Pro Ala Gly Pro Ala Gly Met Gly Thr Ala
1 5 10

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<220>
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<400> 67

Ala Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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<220>
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Gln Ala Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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<220>
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<400> 69

Gln Tyr Ala Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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1 5 10 15

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Gln Tyr Pro Ile Ala Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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<220>
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<400> 72

Gln Tyr Pro Ile Ile Ala Asp Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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Gln Tyr Pro Ile Ile Tyr Ala Ile Arg Ala Arg Pro Arg Lys Ile
1 5 10 15

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Gln Tyr Pro Ile Ile Tyr Asp Ala Arg Ala Arg Pro Arg Lys Ile
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Gln Tyr Pro Ile Ile Tyr Asp Ile Ala Ala Arg Pro Arg Lys Ile
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Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Ala Pro Arg Lys Ile
1 5 10 15

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<400> 77

Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Ala Arg Lys Ile
1 5 10 15

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Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Ala Lys Ile
1 5 10 15

<210> 79
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<220>
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<400> 79

Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Ala Ile
1 5 10 15

<210> 80
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Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ala
1 5 10 15

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<400> 81

Gln Ala Pro Ile Ile Tyr Asp Ile Ala Ala Arg Pro Arg Lys Ile
1 5 10 15

<210> 82
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<220>
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<400> 82

Glu Gly Gly His Arg Ala Ile Phe Phe Asn Arg Ile Gly Gly
1 5 10

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<220>
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<400> 83

Glu Ser Val Phe Thr Val Glu Gly Gly His Arg Ala Ile Phe Phe Asn
1 5 10 15

Arg Ile Gly Gly
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<210> 84
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Arg Leu Pro Ala Gly Pro Arg Gly Met Gly Thr
1 5 10

<210> 85
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Gln Tyr Pro Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg
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<210> 86
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<220>
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<400> 86
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atgcgctgcg tcgcctctaa cctcgggctg tgctcttttt ccaggtggcc cgccggtttc 180

tgagccttct gccctgcggg gacacggtct gcaccctgcc cgcggccacg gacc atg 237

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                                                                  Met
                                                                  1

acc atg acc ctc cac acc aaa gca tct ggg atg gcc cta ctg cat cag   285
Thr Met Thr Leu His Thr Lys Ala Ser Gly Met Ala Leu Leu His Gln
            5                   10                  15

atc caa ggg aac gag ctg gag ccc ctg aac cgt ccg cag ctc aag atc   333
Ile Gln Gly Asn Glu Leu Glu Pro Leu Asn Arg Pro Gln Leu Lys Ile
        20                  25                  30

ccc ctg gag cgg ccc ctg ggc gag gtg tac ctg gac agc agc aag ccc   381
Pro Leu Glu Arg Pro Leu Gly Glu Val Tyr Leu Asp Ser Ser Lys Pro
    35                  40                  45

gcc gtg tac aac tac ccc gag ggc gcc gcc tac gag ttc aac gcc gcg   429
Ala Val Tyr Asn Tyr Pro Glu Gly Ala Ala Tyr Glu Phe Asn Ala Ala
50                  55                  60                  65

gcc gcc gcc aac gcg cag gtc tac ggt cag acc ggc ctc ccc tac ggc   477
Ala Ala Ala Asn Ala Gln Val Tyr Gly Gln Thr Gly Leu Pro Tyr Gly
                70                  75                  80

ccc ggg tct gag gct gcg gcg ttc ggc tcc aac ggc ctg ggg ggt ttc   525
Pro Gly Ser Glu Ala Ala Ala Phe Gly Ser Asn Gly Leu Gly Gly Phe
            85                  90                  95

ccc cca ctc aac agc gtg tct ccg agc ccg ctg atg cta ctg cac ccg   573
Pro Pro Leu Asn Ser Val Ser Pro Ser Pro Leu Met Leu Leu His Pro
        100                 105                 110

ccg ccg cag ctg tcg cct ttc ctg cag ccc cac ggc cag cag gtg ccc   621
Pro Pro Gln Leu Ser Pro Phe Leu Gln Pro His Gly Gln Gln Val Pro
    115                 120                 125

tac tac ctg gag aac gag ccc agc ggc tac acg gtg cgc gag gcc ggc   669
Tyr Tyr Leu Glu Asn Glu Pro Ser Gly Tyr Thr Val Arg Glu Ala Gly
130                 135                 140                 145

ccg ccg gca ttc tac agg cca aat tca gat aat cga cgc cag ggt ggc   717
Pro Pro Ala Phe Tyr Arg Pro Asn Ser Asp Asn Arg Arg Gln Gly Gly
                150                 155                 160

aga gaa aga ttg gcc agt acc aat gac aag gga agt atg gct atg gaa   765
Arg Glu Arg Leu Ala Ser Thr Asn Asp Lys Gly Ser Met Ala Met Glu
            165                 170                 175

tct gcc aag gag act cgc tac tgt gca gtg tgc aat gac tat gct tca   813
Ser Ala Lys Glu Thr Arg Tyr Cys Ala Val Cys Asn Asp Tyr Ala Ser
        180                 185                 190

ggc tac cat tat gga gtc tgg tcc tgt gag ggc tgc aag gcc ttc ttc   861
Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe
    195                 200                 205

aag aga agt att caa gga cat aac gac tat atg tgt cca gcc acc aac   909
Lys Arg Ser Ile Gln Gly His Asn Asp Tyr Met Cys Pro Ala Thr Asn
210                 215                 220                 225

cag tgc acc att gat aaa aac agg agg aag agc tgc cag gcc tgc cgg   957
Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg
                230                 235                 240
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ctc cgc aaa tgc tac gaa gtg gga atg atg aaa ggt ggg ata cga aaa      1005
Leu Arg Lys Cys Tyr Glu Val Gly Met Met Lys Gly Gly Ile Arg Lys
            245                 250                 255

gac cga aga gga ggg aga atg ttg aaa cac aag cgc cag aga gat gat      1053
Asp Arg Arg Gly Gly Arg Met Leu Lys His Lys Arg Gln Arg Asp Asp
        260                 265                 270

ggg gag ggc agg ggt gaa gtg ggg tct gct gga gac atg aga gct gcc      1101
Gly Glu Gly Arg Gly Glu Val Gly Ser Ala Gly Asp Met Arg Ala Ala
    275                 280                 285

aac ctt tgg cca agc ccg ctc atg atc aaa cgc tct aag aag aac agc      1149
Asn Leu Trp Pro Ser Pro Leu Met Ile Lys Arg Ser Lys Lys Asn Ser
290                 295                 300                 305

ctg gcc ttg tcc ctg acg gcc gac cag atg gtc agt gcc ttg ttg gat      1197
Leu Ala Leu Ser Leu Thr Ala Asp Gln Met Val Ser Ala Leu Leu Asp
                310                 315                 320

gct gag ccc ccc ata ctc tat tcc gag tat gat cct acc aga ccc ttc      1245
Ala Glu Pro Pro Ile Leu Tyr Ser Glu Tyr Asp Pro Thr Arg Pro Phe
            325                 330                 335

agt gaa gct tcg atg atg ggc tta ctg acc aac ctg gca gac agg gag      1293
Ser Glu Ala Ser Met Met Gly Leu Leu Thr Asn Leu Ala Asp Arg Glu
        340                 345                 350

ctg gtt cac atg atc aac tgg gcg aag agg gtg cca ggc ttt gtg gat      1341
Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Val Asp
    355                 360                 365

ttg acc ctc cat gat cag gtc cac ctt cta gaa tgt gcc tgg cta gag      1389
Leu Thr Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu Glu
370                 375                 380                 385

atc ctg atg att ggt ctc gtc tgg cgc tcc atg gag cac cca ggg aag      1437
Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Gly Lys
                390                 395                 400

cta ctg ttt gct cct aac ttg ctc ttg gac agg aac cag gga aaa tgt      1485
Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys Cys
            405                 410                 415

gta gag ggc atg gtg gag atc ttc gac atg ctg ctg gct aca tca tct      1533
Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser Ser
        420                 425                 430

cgg ttc cgc atg atg aat ctg cag gga gag gag ttt gtg tgc ctc aaa      1581
Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu Lys
    435                 440                 445

tct att att ttg ctt aat tct gga gtg tac aca ttt ctg tcc agc acc      1629
Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser Thr
450                 455                 460                 465

ctg aag tct ctg gaa gag aag gac cat atc cac cga gtc ctg gac aag      1677
Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp Lys
                470                 475                 480

atc aca gac act ttg atc cac ctg atg gcc aag gca ggc ctg acc ctg      1725
Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr Leu
            485                 490                 495
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cag cag cag cac cag cgg ctg gcc cag ctc ctc ctc atc ctc tcc cac      1773
Gln Gln Gln His Gln Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser His
        500                 505                 510

atc agg cac atg agt aac aaa ggc atg gag cat ctg tac agc atg aag      1821
Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Ser Met Lys
    515                 520                 525

tgc aag aac gtg gtg ccc ctc tat gac ctg ctg ctg gag atg ctg gac      1869
Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu Asp
530                 535                 540                 545

gcc cac cgc cta cat gcg ccc act agc cgt gga ggg gca tcc gtg gag      1917
Ala His Arg Leu His Ala Pro Thr Ser Arg Gly Gly Ala Ser Val Glu
                550                 555                 560

gag acg gac caa agc cac ttg gcc act gcg ggc tct act tca tcg cat      1965
Glu Thr Asp Gln Ser His Leu Ala Thr Ala Gly Ser Thr Ser Ser His
            565                 570                 575

tcc ttg caa aag tat tac atc acg ggg gag gca gag ggt ttc cct gcc      2013
Ser Leu Gln Lys Tyr Tyr Ile Thr Gly Glu Ala Glu Gly Phe Pro Ala
        580                 585                 590

acg gtc tga gagctccctg gctcccacac ggttcagata atccctgctg              2062
Thr Val
    595

cattttaccc tcatcatgca ccactttagc caaattctgt ctcctgcata cactccggca     2122

tgcatccaac accaatggct ttctagatga gtggccattc atttgcttgc tcagttctta     2182

gtggcacatc ttctgtcttc tgttgggaac agccaaaggg attccaaggc taaatctttg     2242

taacagctct ctttcccccc tgctatgtta ctaagcgtga ggattcccgt agctcttcac     2302

agctgaactc agtctatggg ttggggctca gataactctg tgcatttaag ctacttgtag     2362

agacccaggc ctggagagta gacattttgc ctctgataag cacttttaa atggctctaa      2422

gaataagcca cagcaaagaa tttaaagtgg ctcctttaat tggtgacttg gagaaagcta     2482

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tccccctata ggaatacaag gggcacacag ggaaggcaga tcccctagtt ggcaagacta     2662

ttttaacttg atacactgca gattcagatg tgctgaaagc tctgcctctg gctttccggt     2722

catgggttcc agttaattca tgcctcccat ggacctatgg agagcagcaa gttgatctta     2782

gttaagtctc cctatatgag ggataagttc ctgatttttg tttttatttt tgtgttacaa     2842

aagaaagccc tccctccctg aacttgcagt aaggtcagct tcaggacctg ttccagtggg     2902

cactgtactt ggatcttccc ggcgtgtgtg tgccttacac aggggtgaac tgttcactgt     2962

ggtgatgcat gatgagggta aatggtagtt gaaaggagca ggggccctgg tgttgcattt     3022

agccctgggg catggagctg aacagtactt gtgcaggatt gttgtggcta ctagagaaca     3082
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agagggaaag tagggcagaa actggataca gttctgaggc acagccagac ttgctcaggg 3142

tggccctgcc acaggctgca gctacctagg aacattcctt gcagaccccg cattgccctt 3202

tgggggtgcc ctgggatccc tggggtagtc cagctcttct tcatttccca gcgtggccct 3262

ggttggaaga agcagctgtc acagctgctg tagacagctg tgttcctaca attggcccag 3322

caccctgggg cacgggagaa gggtggggac cgttgctgtc actactcagg ctgactgggg 3382

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gctgtttgtt taagaagcac cttagtttgt ttaagaagca ccttatatag tataatatat 3982

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aagtccatgg aatagctagt ggtctgtgtt tcttttcgcc attgcctagc ttgccgtaat 5062

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tatacactga aatggccatt gatttaggcc actggcttag agtactcctt cccctgcatg 5362

acactgatta caaatacttt cctattcata ctttccaatt atgagatgga ctgtgggtac 5422

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caattttgca ctgtcttttg agattcaaga aaaatttcta ttctttttt tgcatccaat 5842

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attaatatgc ccttttgccg atgcatacta ttactgatgt gactcggttt tgtcgcagct 6202

ttgctttgtt taatgaaaca cacttgtaaa cctcttttgc actttgaaaa agaatccagc 6262

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aaactaaa 6330

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<213> Homo sapiens

<400> 87

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Gln Ile Gln Gly Asn Glu Leu Glu Pro Leu Asn Arg Pro Gln Leu Lys
20 25 30

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Ile Pro Leu Glu Arg Pro Leu Gly Glu Val Tyr Leu Asp Ser Ser Lys
        35                  40                  45

Pro Ala Val Tyr Asn Tyr Pro Glu Gly Ala Ala Tyr Glu Phe Asn Ala
    50                  55                  60

Ala Ala Ala Ala Asn Ala Gln Val Tyr Gly Gln Thr Gly Leu Pro Tyr
65                  70                  75                  80

Gly Pro Gly Ser Glu Ala Ala Ala Phe Gly Ser Asn Gly Leu Gly Gly
                85                  90                  95

Phe Pro Pro Leu Asn Ser Val Ser Pro Ser Pro Leu Met Leu Leu His
            100                 105                 110

Pro Pro Pro Gln Leu Ser Pro Phe Leu Gln Pro His Gly Gln Gln Val
        115                 120                 125

Pro Tyr Tyr Leu Glu Asn Glu Pro Ser Gly Tyr Thr Val Arg Glu Ala
    130                 135                 140

Gly Pro Pro Ala Phe Tyr Arg Pro Asn Ser Asp Asn Arg Arg Gln Gly
145                 150                 155                 160

Gly Arg Glu Arg Leu Ala Ser Thr Asn Asp Lys Gly Ser Met Ala Met
                165                 170                 175

Glu Ser Ala Lys Glu Thr Arg Tyr Cys Ala Val Cys Asn Asp Tyr Ala
            180                 185                 190

Ser Gly Tyr His Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe
        195                 200                 205

Phe Lys Arg Ser Ile Gln Gly His Asn Asp Tyr Met Cys Pro Ala Thr
    210                 215                 220

Asn Gln Cys Thr Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys
225                 230                 235                 240

Arg Leu Arg Lys Cys Tyr Glu Val Gly Met Met Lys Gly Gly Ile Arg
                245                 250                 255

Lys Asp Arg Arg Gly Gly Arg Met Leu Lys His Lys Arg Gln Arg Asp
            260                 265                 270

Asp Gly Glu Gly Arg Gly Glu Val Gly Ser Ala Gly Asp Met Arg Ala
        275                 280                 285
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Ala Asn Leu Trp Pro Ser Pro Leu Met Ile Lys Arg Ser Lys Lys Asn
    290                 295                 300

Ser Leu Ala Leu Ser Leu Thr Ala Asp Gln Met Val Ser Ala Leu Leu
305                 310                 315                 320

Asp Ala Glu Pro Pro Ile Leu Tyr Ser Glu Tyr Asp Pro Thr Arg Pro
                325                 330                 335

Phe Ser Glu Ala Ser Met Met Gly Leu Leu Thr Asn Leu Ala Asp Arg
            340                 345                 350

Glu Leu Val His Met Ile Asn Trp Ala Lys Arg Val Pro Gly Phe Val
        355                 360                 365

Asp Leu Thr Leu His Asp Gln Val His Leu Leu Glu Cys Ala Trp Leu
    370                 375                 380

Glu Ile Leu Met Ile Gly Leu Val Trp Arg Ser Met Glu His Pro Gly
385                 390                 395                 400

Lys Leu Leu Phe Ala Pro Asn Leu Leu Leu Asp Arg Asn Gln Gly Lys
                405                 410                 415

Cys Val Glu Gly Met Val Glu Ile Phe Asp Met Leu Leu Ala Thr Ser
            420                 425                 430

Ser Arg Phe Arg Met Met Asn Leu Gln Gly Glu Glu Phe Val Cys Leu
        435                 440                 445

Lys Ser Ile Ile Leu Leu Asn Ser Gly Val Tyr Thr Phe Leu Ser Ser
    450                 455                 460

Thr Leu Lys Ser Leu Glu Glu Lys Asp His Ile His Arg Val Leu Asp
465                 470                 475                 480

Lys Ile Thr Asp Thr Leu Ile His Leu Met Ala Lys Ala Gly Leu Thr
                485                 490                 495

Leu Gln Gln Gln His Gln Arg Leu Ala Gln Leu Leu Leu Ile Leu Ser
            500                 505                 510

His Ile Arg His Met Ser Asn Lys Gly Met Glu His Leu Tyr Ser Met
        515                 520                 525

Lys Cys Lys Asn Val Val Pro Leu Tyr Asp Leu Leu Leu Glu Met Leu
    530                 535                 540
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Asp Ala His Arg Leu His Ala Pro Thr Ser Arg Gly Gly Ala Ser Val
545 550 555 560

Glu Glu Thr Asp Gln Ser His Leu Ala Thr Ala Gly Ser Thr Ser Ser
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His Ser Leu Gln Lys Tyr Tyr Ile Thr Gly Glu Ala Glu Gly Phe Pro
580 585 590

Ala Thr Val
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gctgcgagaa ataactgcct cttgaaactt gcagggcgaa gagcaggcgg cgagcgctgg 180

gccggggagg gaccacccga gctgcgacgg gctctggggc tgcggggcag ggctggcgcc 240

cggagcctga gctgcaggag gtgcgctcgc tttcctcaac aggtggcggc ggggcgcgcg 300

ccgggagacc ccccctaatg cgggaaaagc acgtgtccgc attttagaga aggcaaggcc 360

ggtgtgttta tctgcaagcc attatacttg cccacgaatc tttgagaaca ttataatgac 420

ctttgtgcct cttcttgcaa ggtgttttct cagctgttat ctcaagac atg gat ata 477
Met Asp Ile
1

aaa aac tca cca tct agc ctt aat tct cct tcc tcc tac aac tgc agt 525
Lys Asn Ser Pro Ser Ser Leu Asn Ser Pro Ser Ser Tyr Asn Cys Ser
5 10 15

caa tcc atc tta ccc ctg gag cac ggc tcc ata tac ata cct tcc tcc 573
Gln Ser Ile Leu Pro Leu Glu His Gly Ser Ile Tyr Ile Pro Ser Ser
20 25 30 35

tat gta gac agc cac cat gaa tat cca gcc atg aca ttc tat agc cct 621
Tyr Val Asp Ser His His Glu Tyr Pro Ala Met Thr Phe Tyr Ser Pro
40 45 50

gct gtg atg aat tac agc att ccc agc aat gtc act aac ttg gaa ggt 669
Ala Val Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly
55 60 65

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ggg cct ggt cgg cag acc aca agc cca aat gtg ttg tgg cca aca cct        717
Gly Pro Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro
        70                  75                  80

ggg cac ctt tct cct tta gtg gtc cat cgc cag tta tca cat ctg tat        765
Gly His Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr
    85                  90                  95

gcg gaa cct caa aag agt ccc tgg tgt gaa gca aga tcg cta gaa cac        813
Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His
100                 105                 110                 115

acc tta cct gta aac aga gag aca ctg aaa agg aag gtt agt ggg aac        861
Thr Leu Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn
                120                 125                 130

cgt tgc gcc agc cct gtt act ggt cca ggt tca aag agg gat gct cac        909
Arg Cys Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His
            135                 140                 145

ttc tgc gct gtc tgc agc gat tac gca tcg gga tat cac tat gga gtc        957
Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val
        150                 155                 160

tgg tcg tgt gaa gga tgt aag gcc ttt ttt aaa aga agc att caa gga       1005
Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly
    165                 170                 175

cat aat gat tat att tgt cca gct aca aat cag tgt aca atc gat aaa       1053
His Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys
180                 185                 190                 195

aac cgg cgc aag agc tgc cag gcc tgc cga ctt cgg aag tgt tac gaa       1101
Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu
                200                 205                 210

gtg gga atg gtg aag tgt ggc tcc cgg aga gag aga tgt ggg tac cgc       1149
Val Gly Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg
            215                 220                 225

ctt gtg cgg aga cag aga agt gcc gac gag cag ctg cac tgt gcc ggc       1197
Leu Val Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly
        230                 235                 240

aag gcc aag aga agt ggc ggc cac gcg ccc cga gtg cgg gag ctg ctg       1245
Lys Ala Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu Leu Leu
    245                 250                 255

ctg gac gcc ctg agc ccc gag cag cta gtg ctc acc ctc ctg gag gct       1293
Leu Asp Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu Glu Ala
260                 265                 270                 275

gag ccg ccc cat gtg ctg atc agc cgc ccc agt gcg ccc ttc acc gag       1341
Glu Pro Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro Phe Thr Glu
                280                 285                 290

gcc tcc atg atg atg tcc ctg acc aag ttg gcc gac aag gag ttg gta       1389
Ala Ser Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys Glu Leu Val
            295                 300                 305

cac atg atc agc tgg gcc aag aag att ccc ggc ttt gtg gag ctc agc       1437
His Met Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val Glu Leu Ser
        310                 315                 320
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ctg ttc gac caa gtg cgg ctc ttg gag agc tgt tgg atg gag gtg tta       1485
Leu Phe Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met Glu Val Leu
    325                 330                 335

atg atg ggg ctg atg tgg cgc tca att gac cac ccc ggc aag ctc atc       1533
Met Met Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly Lys Leu Ile
340                 345                 350                 355

ttt gct cca gat ctt gtt ctg gac agg gat gag ggg aaa tgc gta gaa       1581
Phe Ala Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys Cys Val Glu
                360                 365                 370

gga att ctg gaa atc ttt gac atg ctc ctg gca act act tca agg ttt       1629
Gly Ile Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr Ser Arg Phe
            375                 380                 385

cga gag tta aaa ctc caa cac aaa gaa tat ctc tgt gtc aag gcc atg       1677
Arg Glu Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val Lys Ala Met
        390                 395                 400

atc ctg ctc aat tcc agt atg tac cct ctg gtc aca gcg acc cag gat       1725
Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala Thr Gln Asp
    405                 410                 415

gct gac agc agc cgg aag ctg gct cac ttg ctg aac gcc gtg acc gat       1773
Ala Asp Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala Val Thr Asp
420                 425                 430                 435

gct ttg gtt tgg gtg att gcc aag agc ggc atc tcc tcc cag cag caa       1821
Ala Leu Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser Gln Gln Gln
                440                 445                 450

tcc atg cgc ctg gct aac ctc ctg atg ctc ctg tcc cac gtc agg cat       1869
Ser Met Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His
            455                 460                 465

gcg agt aac aag ggc atg gaa cat ctg ctc aac atg aag tgc aaa aat       1917
Ala Ser Asn Lys Gly Met Glu His Leu Leu Asn Met Lys Cys Lys Asn
        470                 475                 480

gtg gtc cca gtg tat gac ctg ctg ctg gag atg ctg aat gcc cac gtg       1965
Val Val Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn Ala His Val
    485                 490                 495

ctt cgc ggg tgc aag tcc tcc atc acg ggg tcc gag tgc agc ccg gca       2013
Leu Arg Gly Cys Lys Ser Ser Ile Thr Gly Ser Glu Cys Ser Pro Ala
500                 505                 510                 515

gag gac agt aaa agc aaa gag ggc tcc cag aac cca cag tct cag tga       2061
Glu Asp Ser Lys Ser Lys Glu Gly Ser Gln Asn Pro Gln Ser Gln
                520                 525                 530

cgcctggccc tgaggtgaac tggcccacag aggtcacagg ctgaagcgtg aactccagtg     2121

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Pro Ser Ser Tyr Val Asp Ser His His Glu Tyr Pro Ala Met Thr Phe
        35                  40                  45

Tyr Ser Pro Ala Val Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn
    50                  55                  60

Leu Glu Gly Gly Pro Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp
65                  70                  75                  80

Pro Thr Pro Gly His Leu Ser Pro Leu Val Val His Arg Gln Leu Ser
                85                  90                  95

His Leu Tyr Ala Glu Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser
            100                 105                 110

Leu Glu His Thr Leu Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val
        115                 120                 125

Ser Gly Asn Arg Cys Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg
    130                 135                 140

Asp Ala His Phe Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His
145                 150                 155                 160

Tyr Gly Val Trp Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser
                165                 170                 175

Ile Gln Gly His Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr
            180                 185                 190

Ile Asp Lys Asn Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys
        195                 200                 205

Cys Tyr Glu Val Gly Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys
    210                 215                 220

Gly Tyr Arg Leu Val Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His
225                 230                 235                 240

Cys Ala Gly Lys Ala Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg
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245 250 255

Glu Leu Leu Leu Asp Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu
260 265 270

Leu Glu Ala Glu Pro Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro
275 280 285

Phe Thr Glu Ala Ser Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys
290 295 300

Glu Leu Val His Met Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val
305 310 315 320

Glu Leu Ser Leu Phe Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met
325 330 335

Glu Val Leu Met Met Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly
340 345 350

Lys Leu Ile Phe Ala Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys
355 360 365

Cys Val Glu Gly Ile Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr
370 375 380

Ser Arg Phe Arg Glu Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val
385 390 395 400

Lys Ala Met Ile Leu Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala
405 410 415

Thr Gln Asp Ala Asp Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala
420 425 430

Val Thr Asp Ala Leu Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser
435 440 445

Gln Gln Gln Ser Met Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His
450 455 460

Val Arg His Ala Ser Asn Lys Gly Met Glu His Leu Leu Asn Met Lys
465 470 475 480

Cys Lys Asn Val Val Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn
485 490 495

Ala His Val Leu Arg Gly Cys Lys Ser Ser Ile Thr Gly Ser Glu Cys
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Ser Pro Ala Glu Asp Ser Lys Ser Lys Glu Gly Ser Gln Asn Pro Gln
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Ser Gln
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Arg Leu Leu Arg Lys
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Lys Ala Leu Ala Ala Leu Ala Lys Lys Ile Leu
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<210> 93

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1 5 10 15

Leu Ala

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<400> 103

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Ile Ile Tyr Asp Ile Arg Ala Arg Pro Arg Lys Ile Ser Ser Pro Thr
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Gly Ser Lys Asp Leu
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Arg Arg Arg Arg Arg
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Arg Arg Arg Arg
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20

Claims

1. A peptide, comprising a site binding to a BIG3 polypeptide in a PHB2 polypeptide, wherein the peptide inhibits the binding between the PHB2 polypeptide and the BIG3 polypeptide.

2. The peptide of claim 1, wherein the peptide comprises any one or a combination of all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21; all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88; and all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57, in the amino acid sequence of SEQ ID NO: 28.

3. A peptide, comprising an amino acid sequence selected from the group consisting of (a) to (f) below, wherein the peptide inhibits the binding between a PHB2 polypeptide and a BIG3 polypeptide:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41;
(b) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41;
(c) an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53;
(d) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53;
(e) an amino acid sequence selected from the group consisting of SEQ ID NOs: 82 and 83; and
(f) an amino acid sequence in which one, two, or several amino acids are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 82 and 83.

4. The peptide of claim 3, wherein the peptide comprises an amino acid sequence selected from the group consisting of (a') and (b') below:

(a') an amino acid sequence in which one, two, or several amino acid residues located at positions other than those corresponding to glycine at positions 15 and 18 in the amino acid sequence of SEQ ID NO: 28 are substituted with other amino acid residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 36 to 41; and
(b') an amino acid sequence in which one, two, or several amino acid residues located at positions other than that corresponding to aspartic acid at position 82 in the amino acid sequence of SEQ ID NO: 28 are substituted with other amino acid residues in an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 47 to 53.

5. The peptide of any one of claims 1 to 4, wherein the peptide consists of 80 amino acid residues or less.

6. The peptide of any one of claims 1 to 5, wherein the peptide consists of 25 amino acid residues or less.

7. The peptide of any one of claims 1 to 6, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 36 to 41, 47 to 53, 82, and 83.

8. The peptide of any one of claims 1 to 7, wherein the peptide has been modified with a cell membrane-permeable substance.

9. The peptide of any one of claims 1 to 8, wherein the peptide is cyclic.

10. The peptide of any one of claims 1 to 9, wherein the peptide is cross-linked.

11. The peptide of any one of claims 1 to 10, wherein the peptide has either or both of the following properties (i) and (ii):

(i) suppressing growth of BIG3-positive cells; and
(ii) promoting phosphorylation of a serine residue in the PHB2 polypeptide in BIG3-positive cells.

12. A polynucleotide encoding the peptide of any one of claims 1 to 11.

13. A pharmaceutical composition comprising: at least one ingredient selected from the group consisting of one or more of the peptides of any one of claims 1 to 11, a polynucleotide(s) encoding the peptide(s), and a pharmaceutically acceptable salt(s) of the peptide(s); and a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, wherein the composition comprises any one or a combination of: a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21 in the amino acid sequence of SEQ ID NO: 28; a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57 in the amino acid sequence of SEQ ID NO: 28; and a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88 in the amino acid sequence of SEQ ID NO: 28.

15. The pharmaceutical composition of claim 13 or 14, which is for suppressing growth of cancer cells or for treatment and/or prophylaxis (prevention) of cancer.

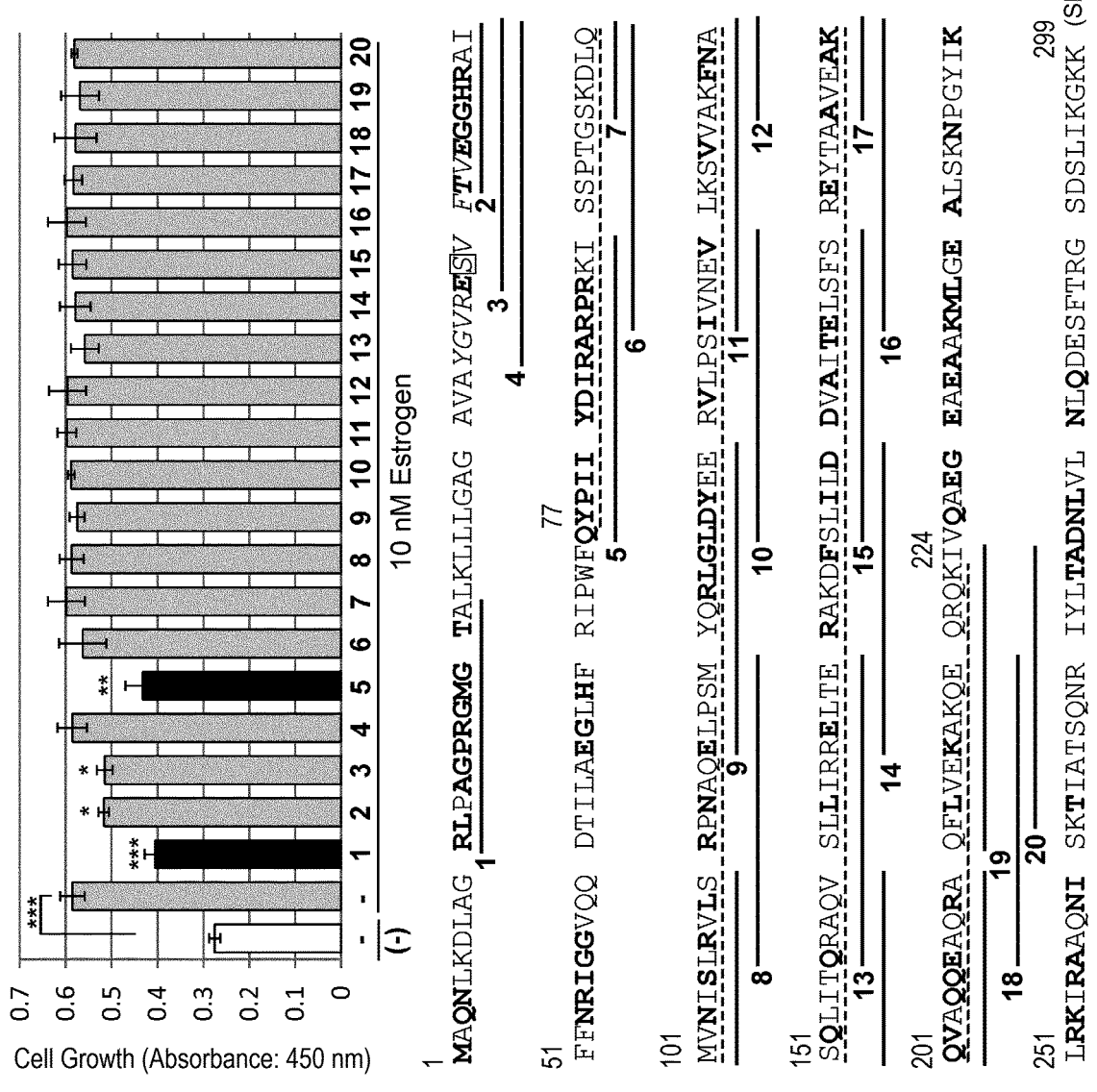
16. The pharmaceutical composition of claim 15, wherein the cancer is BIG3-positive cancer.

17. The pharmaceutical composition of claim 15 or 16, wherein the cancer is breast cancer.

18. The pharmaceutical composition of any one of claims 15 to 17, wherein the cancer is estrogen receptor-positive cancer.

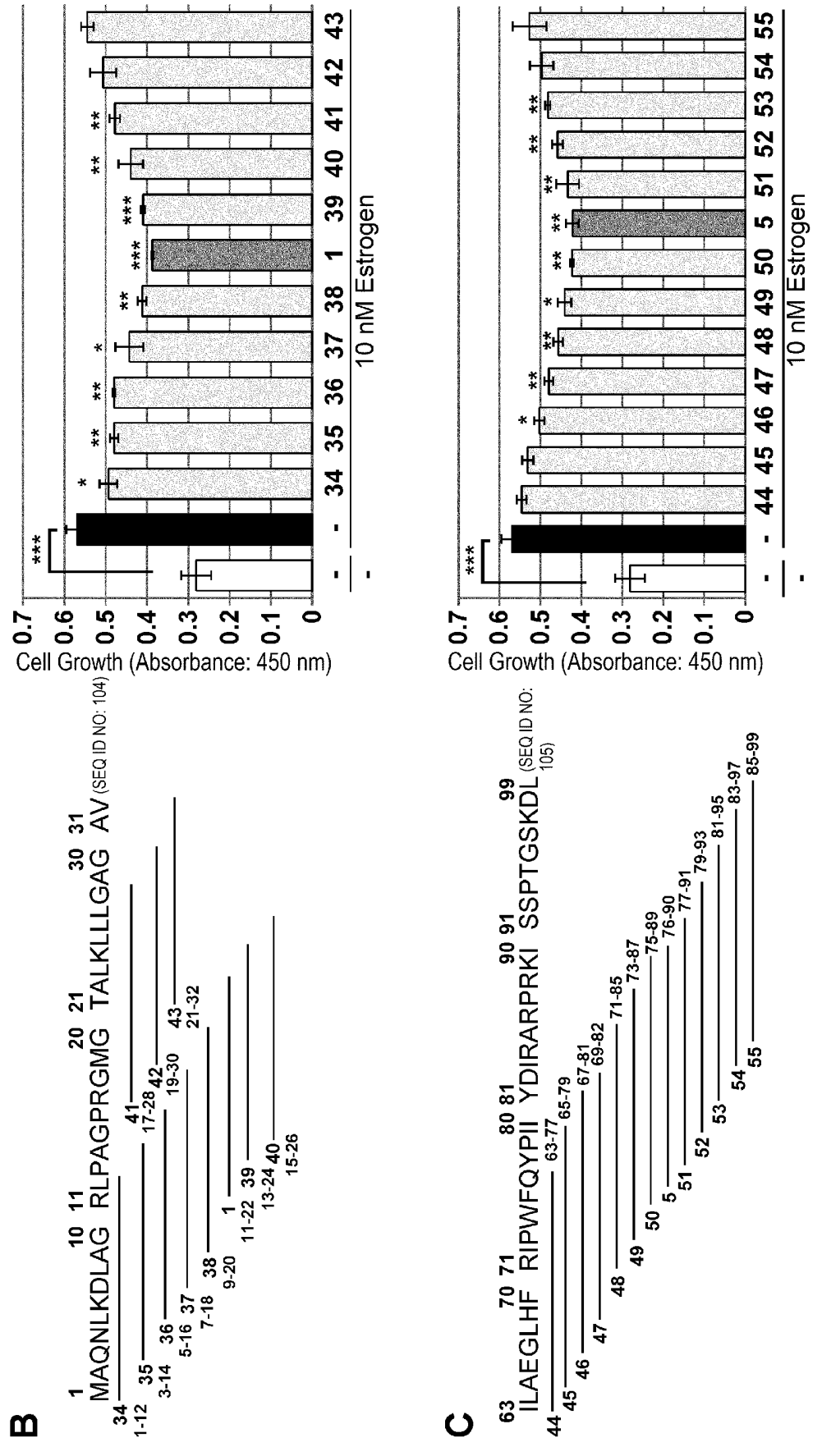
19. A method for either or both of treatment and prophylaxis (prevention) of cancer, wherein the method comprises administering to a subject at least one selected from the group consisting of one or more of the peptides of any one of claims 1 to 11; a polynucleotide(s) encoding the peptide(s); and a pharmaceutically acceptable salt(s) of the peptide(s).

20. The method of claim 19, wherein the method comprises administering any one or a combination of: a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 11 to 21 in the amino acid sequence of SEQ ID NO: 28; a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 44 to 57 in the amino acid sequence of SEQ ID NO: 28; and a peptide comprising all or part of the amino acid sequence consisting of the amino acids at positions 76 to 88 in the amino acid sequence of SEQ ID NO: 28.



(SEQ ID NO: 28)

FIG. 1-1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1 10 11 20 21 30 31
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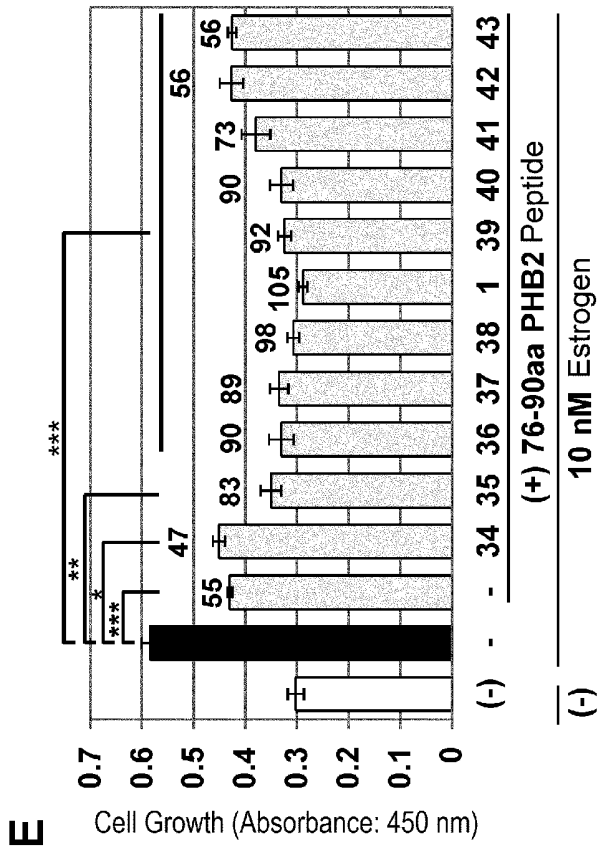
1-12
3-14
5-16
7-18
9-20
11-22
13-24
15-26
17-28
19-30
21-32
Cell Growth (Absorbance: 450 nm)
10 nM Estrogen
C
63 70 71 80 81 90 91 99
ILAEGLHF RIPWFQYPII YDIRARPRKI SSPTGSKDL (SEQ ID NO: 105)
63-77
65-79
67-81
69-82
71-85
73-87
75-89
76-90
77-91
79-93
81-95
83-97
85-99
Cell Growth (Absorbance: 450 nm)
10 nM Estrogen

FIG. 1-2


E
Cell Growth (Absorbance: 450 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
**
*
***
55
47
56
56
(-) - - 34 35 36 37 38 1 39 40 41 42 43
(+) 76-90aa PHB2 Peptide
(-) 10 nM Estrogen

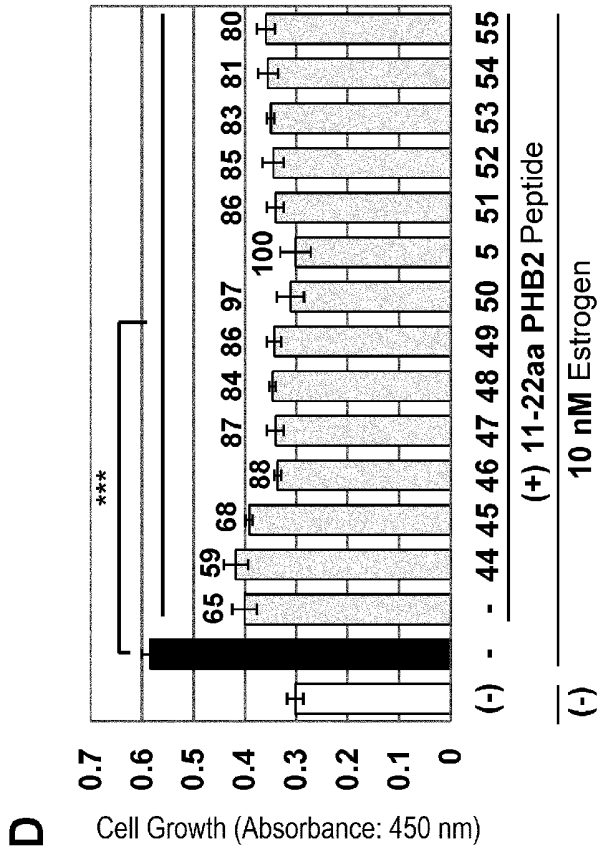

D
Cell Growth (Absorbance: 450 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
65 59 68 88 87 84 86 97 100 86 85 83 81 80
(-) - - 44 45 46 47 48 49 50 5 51 52 53 54 55
(+) 11-22aa PHB2 Peptide
(-) 10 nM Estrogen

FIG. 1-3

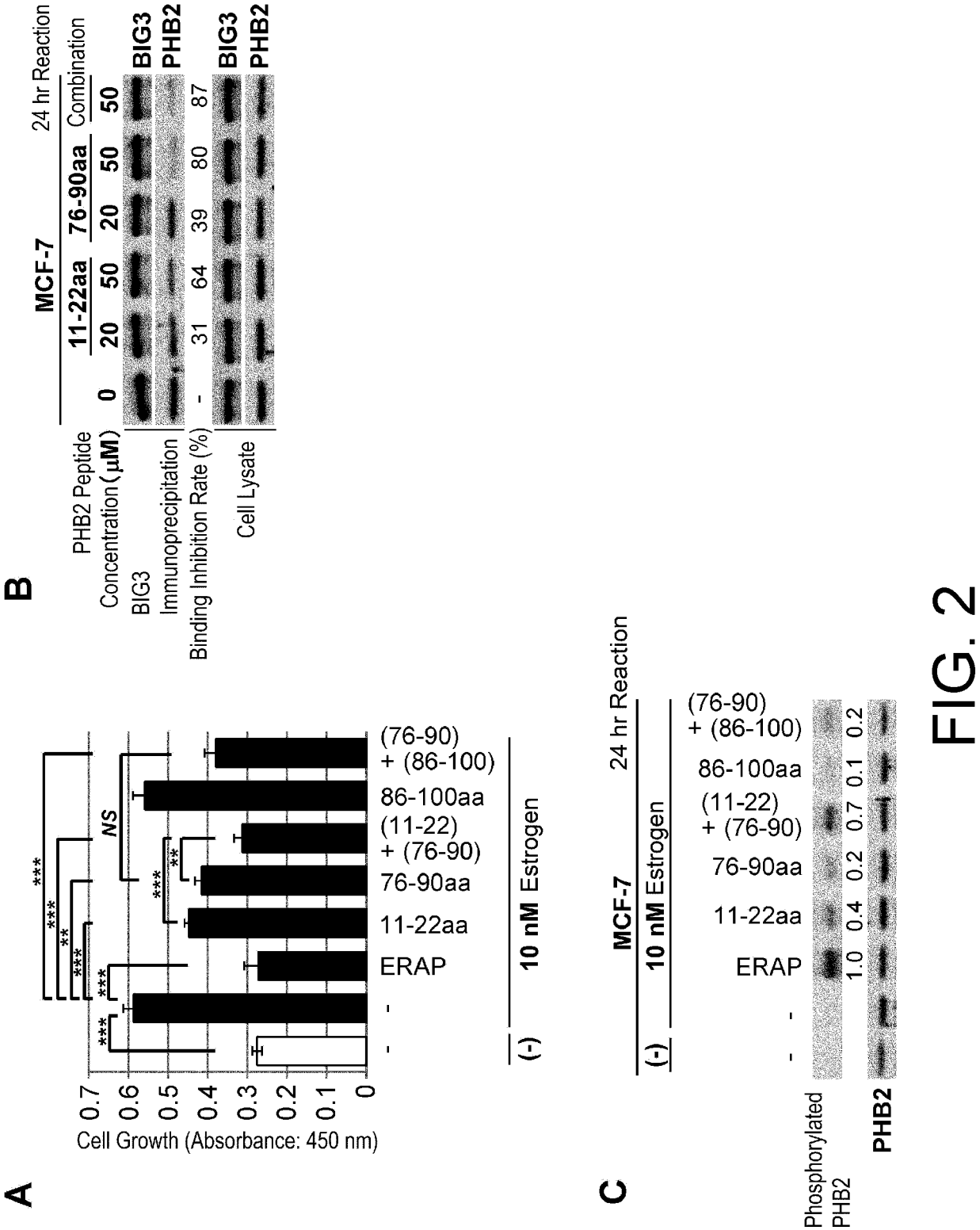

A
Cell Growth (Absorbance: 450 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
***
**
***
***
NS
***
**
- -
ERAP
11-22aa
76-90aa
(11-22)
+ (76-90)
86-100aa
(76-90)
+ (86-100)
(-)
10 nM Estrogen
B
MCF-7
24 hr Reaction
PHB2 Peptide
Concentration (μM)
0
11-22aa
20
50
76-90aa
20
50
Combination
50
BIG3
Immunoprecipitation
PHB2
BIG3
Binding Inhibition Rate (%)
-
31
64
39
80
87
Cell Lysate
BIG3
PHB2
C
MCF-7
24 hr Reaction
(-)
10 nM Estrogen
(76-90)
+ (86-100)
86-100aa
(11-22)
+ (76-90)
76-90aa
11-22aa
ERAP
- -
Phosphorylated
PHB2
1.0
0.4
0.2
0.7
0.1
0.2
PHB2

FIG. 2

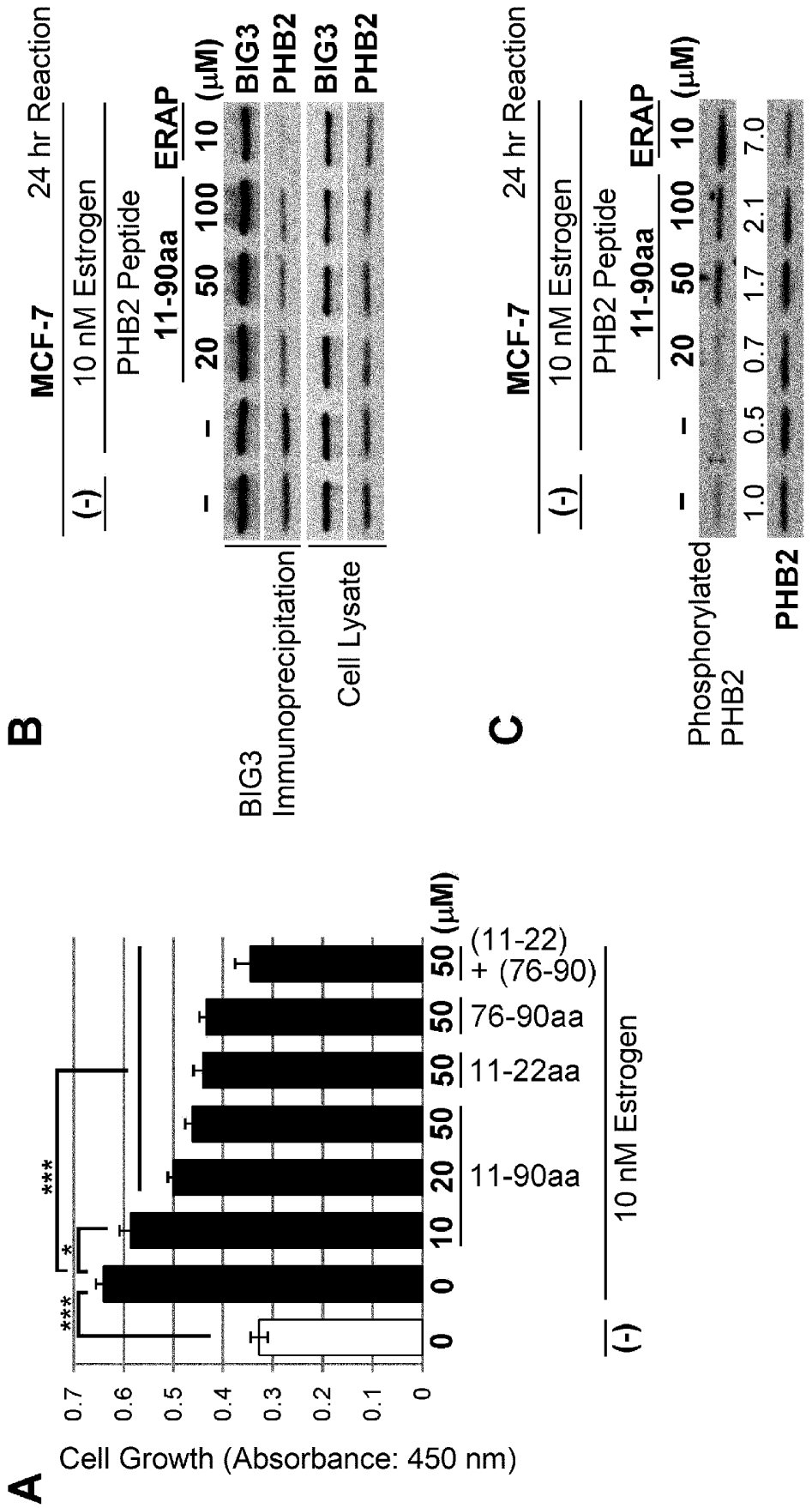

A
Cell Growth (Absorbance: 450 nm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
*
***
0 0 10 20 50 50 50 50 (μM)
(11-22) + (76-90)
76-90aa
11-22aa
11-90aa
(-) 10 nM Estrogen
B
MCF-7 24 hr Reaction
(-) 10 nM Estrogen
PHB2 Peptide
11-90aa ERAP
– – 20 50 100 10 (μM)
BIG3
PHB2
BIG3
PHB2
BIG3 Immunoprecipitation
Cell Lysate
C
MCF-7 24 hr Reaction
(-) 10 nM Estrogen
PHB2 Peptide
11-90aa ERAP
– – 20 50 100 10 (μM)
Phosphorylated PHB2
1.0 0.5 0.7 1.7 2.1 7.0
PHB2

FIG. 3

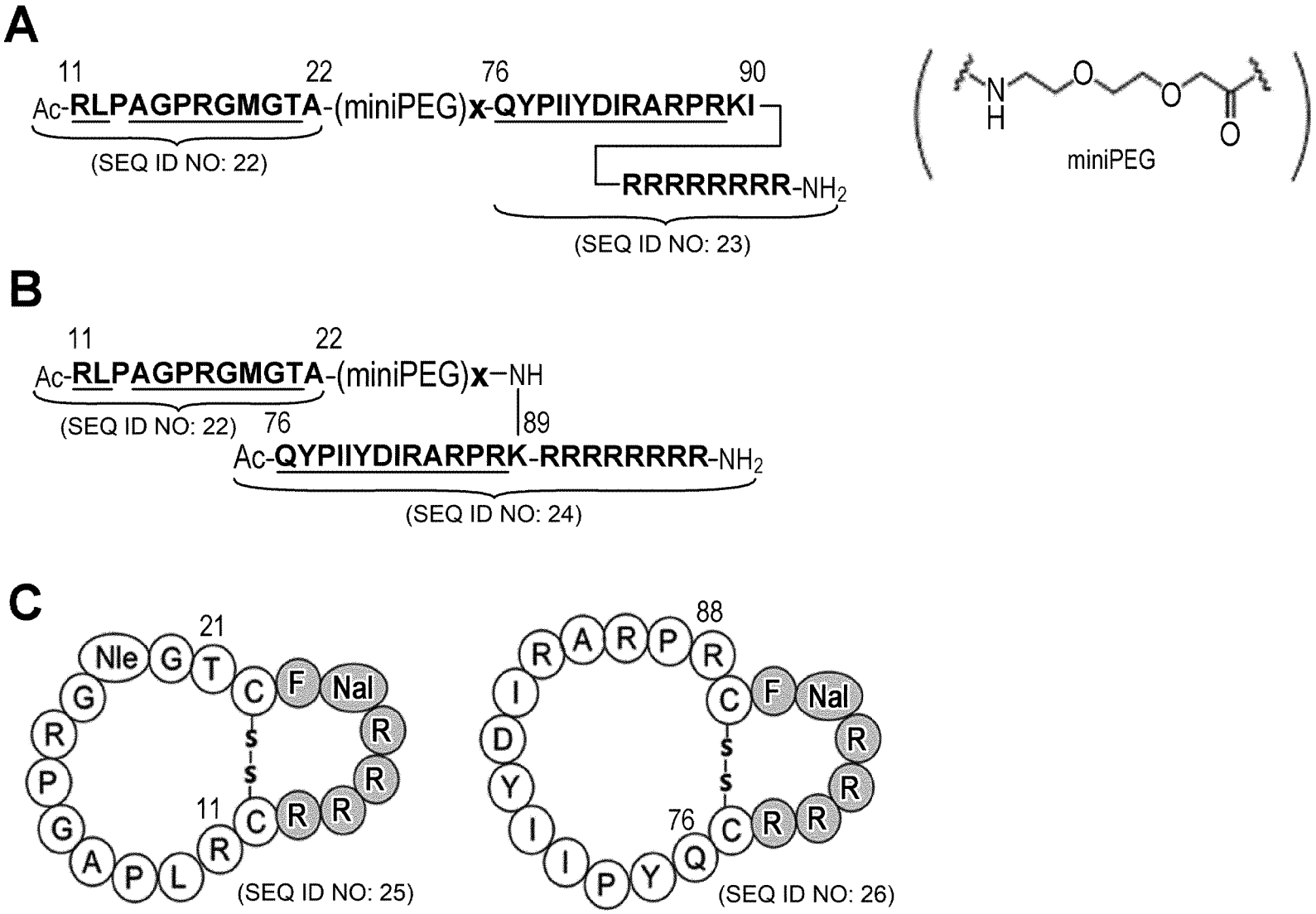

A
11
22
76
90
Ac-RLPAGPRGMGTA-(miniPEG)x-QYPIIYDIRARPRKI
(SEQ ID NO: 22)
RRRRRRRR-NH2
(SEQ ID NO: 23)
miniPEG
B
11
22
Ac-RLPAGPRGMGTA-(miniPEG)x-NH
(SEQ ID NO: 22)
76
89
Ac-QYPIIYDIRARPRK-RRRRRRRR-NH2
(SEQ ID NO: 24)
C
21
11
(SEQ ID NO: 25)
88
76
(SEQ ID NO: 26)

FIG. 4

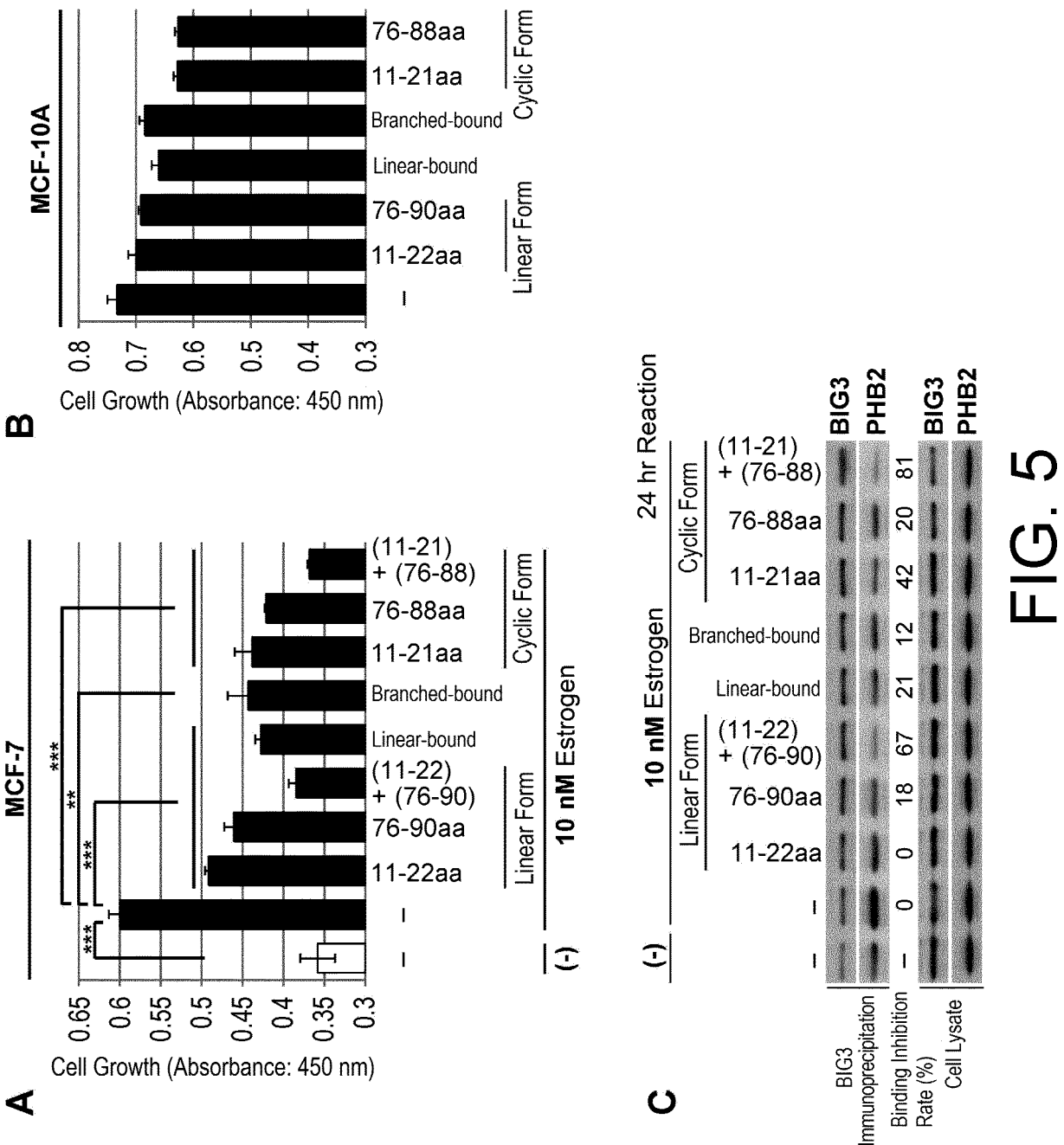

A
MCF-7
Cell Growth (Absorbance: 450 nm)
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
***
**
***
***
(11-21) + (76-88)
76-88aa
11-21aa
Branched-bound
Linear-bound
(11-22) + (76-90)
76-90aa
11-22aa
–
–
Cyclic Form
Linear Form
(-)
10 nM Estrogen
B
MCF-10A
Cell Growth (Absorbance: 450 nm)
0.8
0.7
0.6
0.5
0.4
0.3
76-88aa
11-21aa
Branched-bound
Linear-bound
76-90aa
11-22aa
–
Cyclic Form
Linear Form
C
(-)
10 nM Estrogen
24 hr Reaction
Linear Form
Cyclic Form
(11-21) + (76-88)
76-88aa
11-21aa
Branched-bound
Linear-bound
(11-22) + (76-90)
76-90aa
11-22aa
–
–
BIG3
PHB2
BIG3 Immunoprecipitation
Binding Inhibition Rate (%)
– 0 0 18 67 21 12 42 20 81
BIG3
PHB2
Cell Lysate

FIG. 5

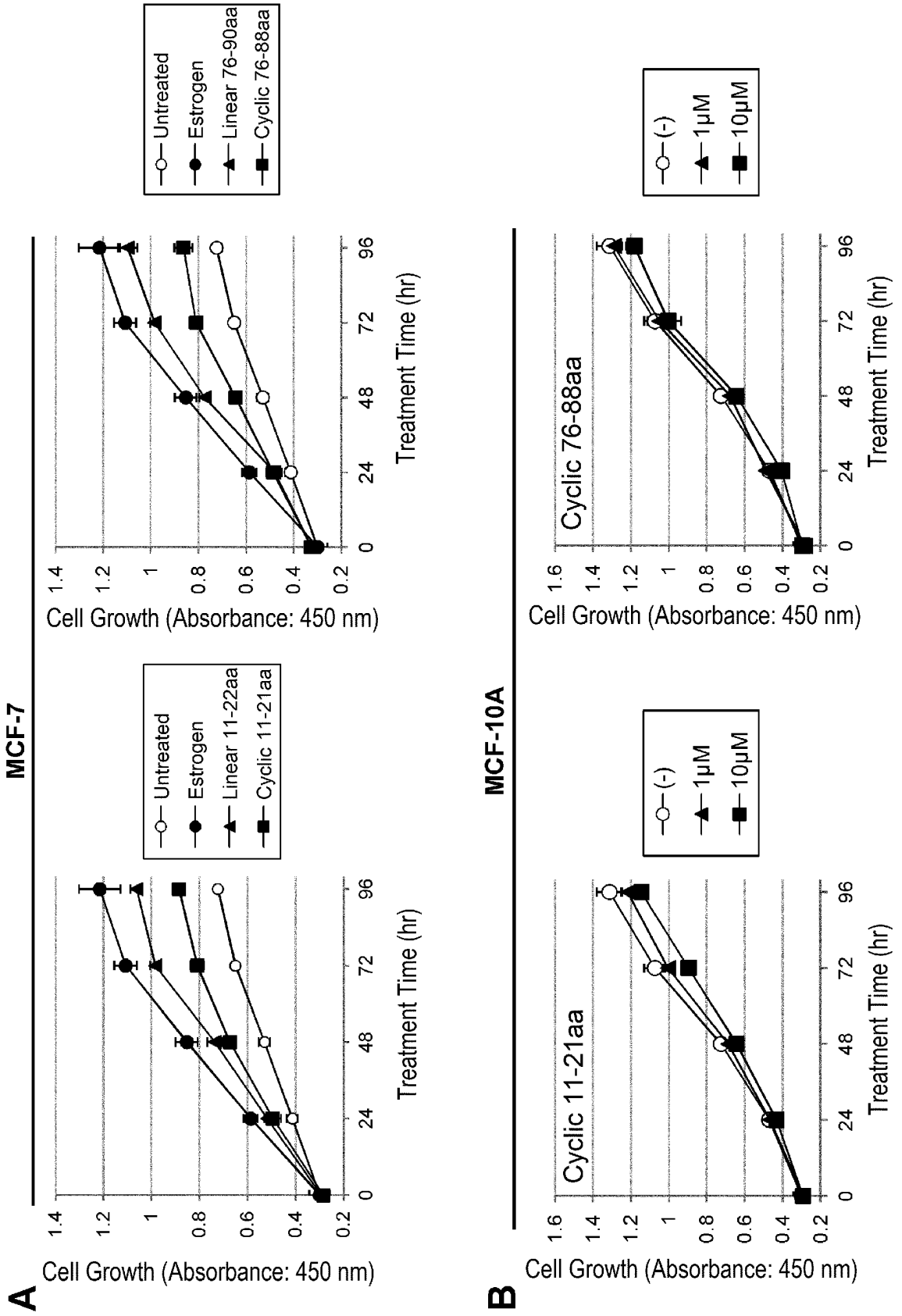

A
MCF-7
Cell Growth (Absorbance: 450 nm)
Treatment Time (hr)
Untreated
Estrogen
Linear 11-22aa
Cyclic 11-21aa
Linear 76-90aa
Cyclic 76-88aa
B
MCF-10A
Cyclic 11-21aa
Cyclic 76-88aa
(-)
1μM
10μM

FIG. 6

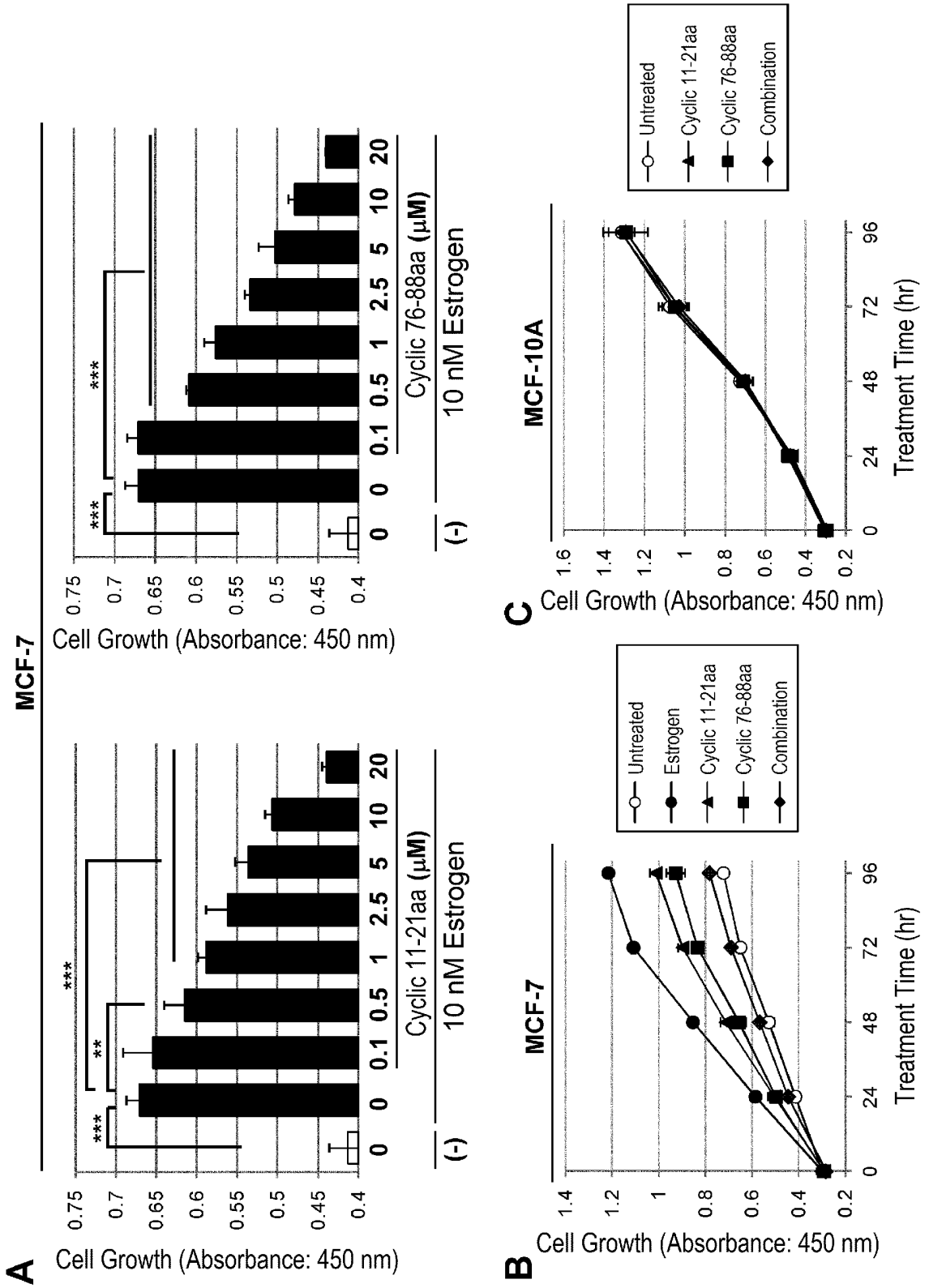

A
MCF-7
Cell Growth (Absorbance: 450 nm)
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
***
**
***
0 0 0.1 0.5 1 2.5 5 10 20
Cyclic 11-21aa (μM)
(-) 10 nM Estrogen
***
***
0 0 0.1 0.5 1 2.5 5 10 20
Cyclic 76-88aa (μM)
(-) 10 nM Estrogen
B
MCF-7
Cell Growth (Absorbance: 450 nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0 24 48 72 96
Treatment Time (hr)
Untreated
Estrogen
Cyclic 11-21aa
Cyclic 76-88aa
Combination
C
MCF-10A
Cell Growth (Absorbance: 450 nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0 24 48 72 96
Treatment Time (hr)
Untreated
Cyclic 11-21aa
Cyclic 76-88aa
Combination

FIG. 7

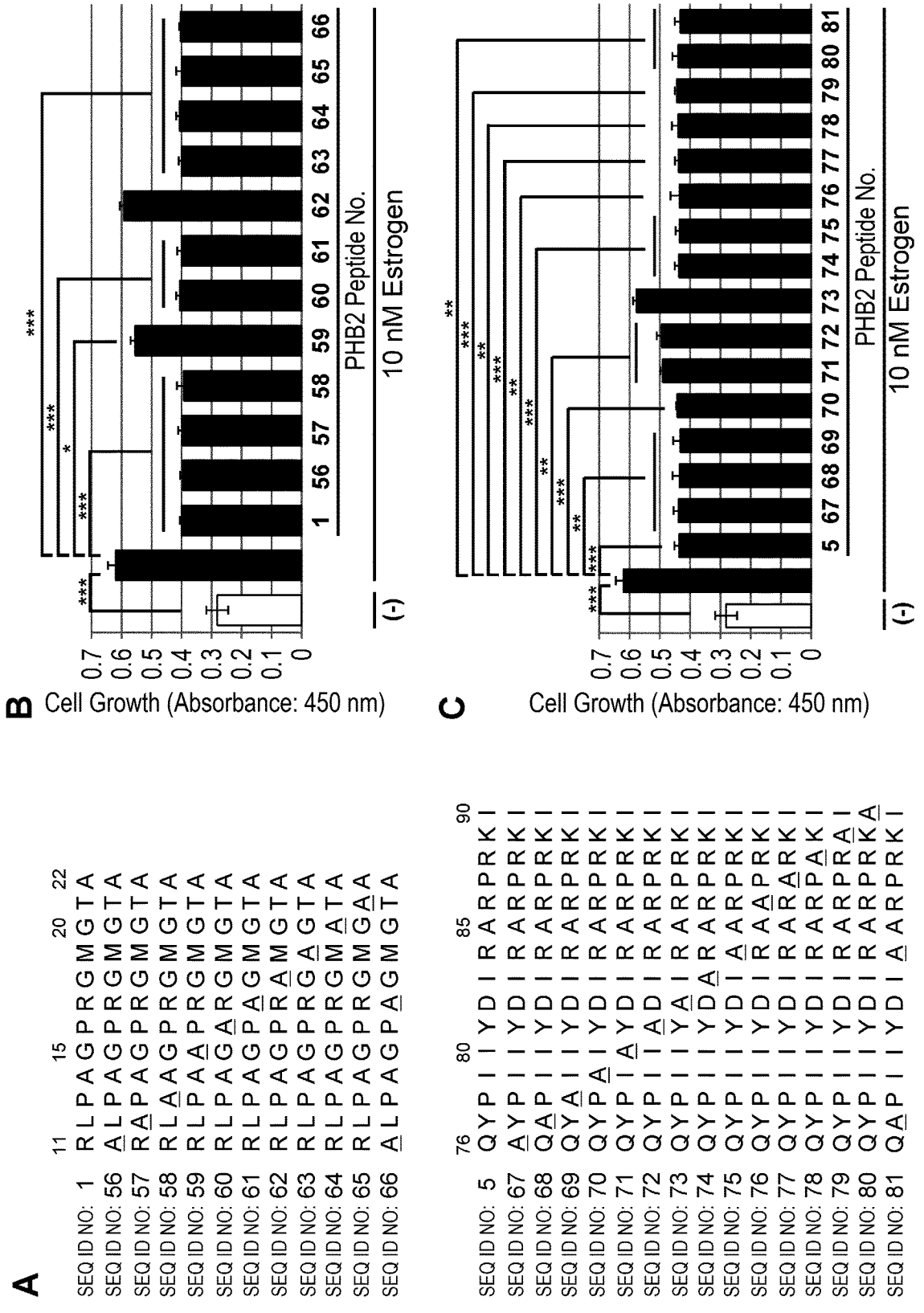
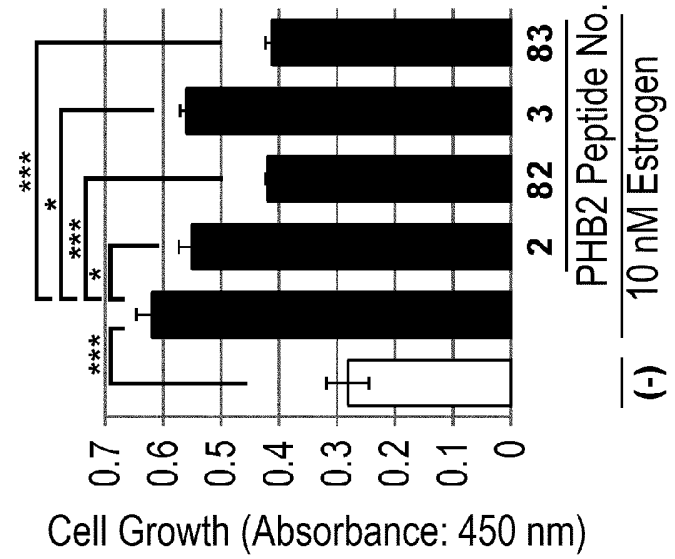

A
SEQ ID NO: 1 RLPAGPRGMGTA
SEQ ID NO: 56 ALPAGPRGMGTA
SEQ ID NO: 57 RAPAGPRGMGTA
SEQ ID NO: 58 RLAAGPRGMGTA
SEQ ID NO: 59 RLPAAPRGMGTA
SEQ ID NO: 60 RLPAGARGMGTA
SEQ ID NO: 61 RLPAGPAGMGTA
SEQ ID NO: 62 RLPAGPRAMGTA
SEQ ID NO: 63 RLPAGPRGAGTA
SEQ ID NO: 64 RLPAGPRGMATA
SEQ ID NO: 65 RLPAGPRGMGAA
SEQ ID NO: 66 ALPAGPAGMGTA
SEQ ID NO: 5 QYPIIYDIRARPRKI
SEQ ID NO: 67 AYPIIYDIRARPRKI
SEQ ID NO: 68 QAPIIYDIRARPRKI
SEQ ID NO: 69 QYAIIYDIRARPRKI
SEQ ID NO: 70 QYPAIYDIRARPRKI
SEQ ID NO: 71 QYPIAYDIRARPRKI
SEQ ID NO: 72 QYPIIADIRARPRKI
SEQ ID NO: 73 QYPIIYAIRARPRKI
SEQ ID NO: 74 QYPIIYDARARPRKI
SEQ ID NO: 75 QYPIIYDIAARPRKI
SEQ ID NO: 76 QYPIIYDIRAARPRKI
SEQ ID NO: 77 QYPIIYDIRARARKI
SEQ ID NO: 78 QYPIIYDIRARPAKI
SEQ ID NO: 79 QYPIIYDIRARPRAI
SEQ ID NO: 80 QYPIIYDIRARPRKA
SEQ ID NO: 81 QAPIIYDIAARPRKI
B
Cell Growth (Absorbance: 450 nm)
PHB2 Peptide No.
1 56 57 58 59 60 61 62 63 64 65 66
(-)
10 nM Estrogen
C
Cell Growth (Absorbance: 450 nm)
PHB2 Peptide No.
5 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81
(-)
10 nM Estrogen

FIG. 8-1



E

D

	38	40				45				50				55	57
SEQ ID NO: 2			T	V	E	G	G	H	R	A	I				
SEQ ID NO: 82					E	G	G	H	R	A	I	F	F	N	R	I	G	G
SEQ ID NO: 3	E	S	V	F	T	V	E	G	G	H	R	A	I		
SEQ ID NO: 83	E	S	V	F	T	V	E	G	G	H	R	A	I	F	F	N	R	I	G	G

FIG. 8-2

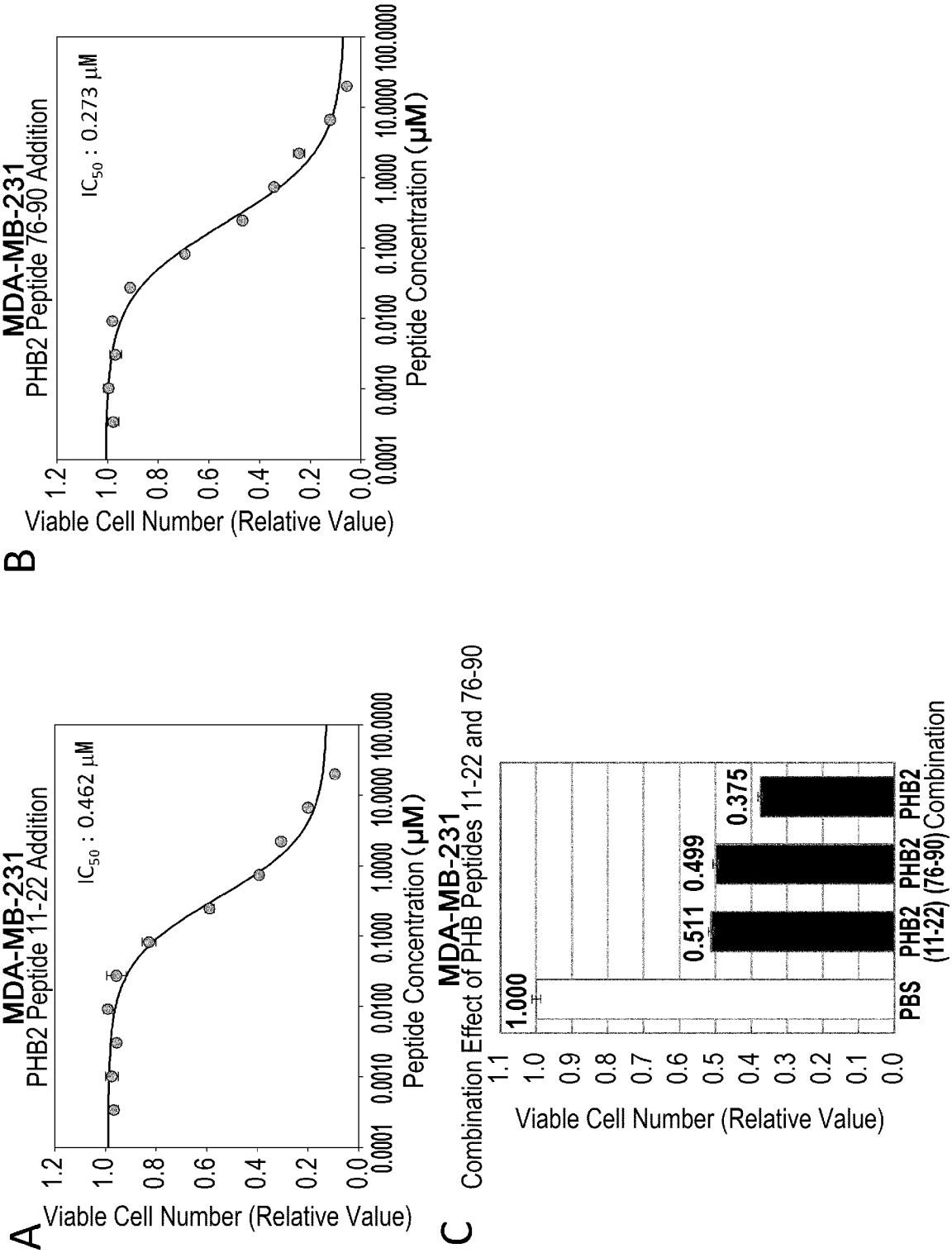
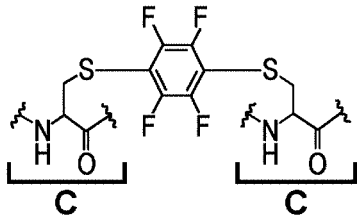

A
MDA-MB-231
PHB2 Peptide 11-22 Addition
IC50 : 0.462 μM
Viable Cell Number (Relative Value)
Peptide Concentration (μM)
B
MDA-MB-231
PHB2 Peptide 76-90 Addition
IC50 : 0.273 μM
Viable Cell Number (Relative Value)
Peptide Concentration (μM)
C
MDA-MB-231
Combination Effect of PHB Peptides 11-22 and 76-90
Viable Cell Number (Relative Value)
1.000
0.511
0.499
0.375
PBS
PHB2 (11-22)
PHB2 (76-90)
PHB2 Combination

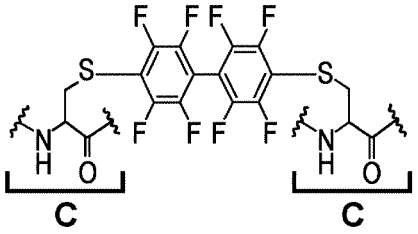
FIG. 9

A

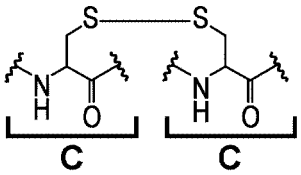
Hexafluorobenzene Crosslink



Decafluorobiphenyl Crosslink



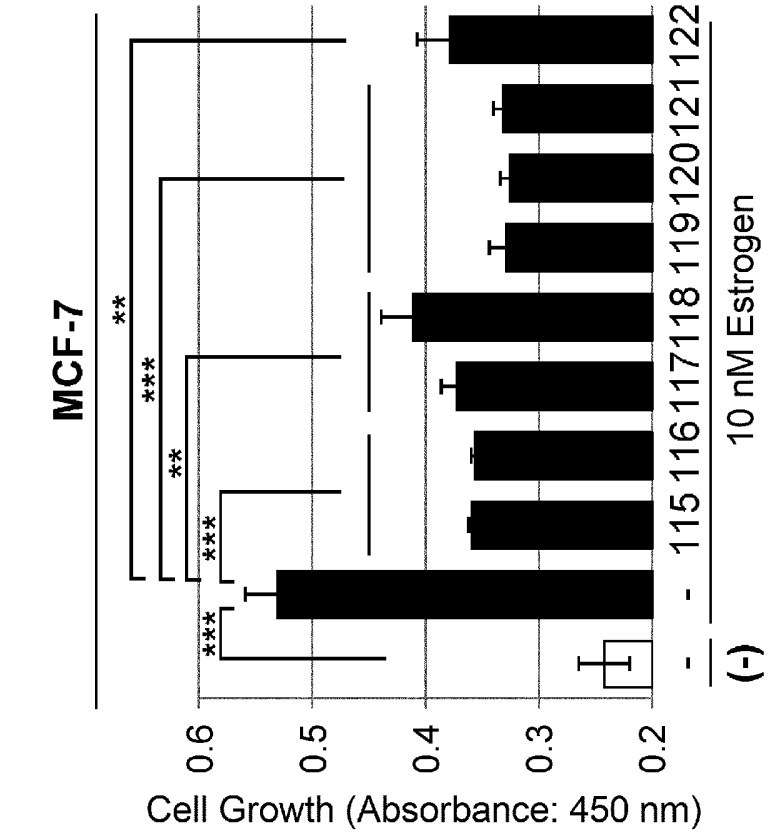
Disulfide Crosslink



B

Sequence	Type of Crosslink	
CRLPAGPRG-Nle-GTC-RRRRRRRR (11, 19, 21)	Hexafluorobenzene Crosslink	SEQ ID NO: 106
	Decafluorobiphenyl Crosslink	SEQ ID NO: 107
	Disulfide Crosslink	SEQ ID NO: 108
ARLPAGPRG-Nle-GTA-RRRRRRRR	Non-crosslink	SEQ ID NO: 109
CQYPIIYDIRARPRC-RRRRRRRR (76, 88)	Hexafluorobenzene Crosslink	SEQ ID NO: 110
	Decafluorobiphenyl Crosslink	SEQ ID NO: 111
	Disulfide Crosslink	SEQ ID NO: 112
AQYPIIYDIRARPRA-RRRRRRRR	Non-crosslink	SEQ ID NO: 113
ARLPAGPRGMGTA-RRRRRRRR	Non-crosslink	SEQ ID NO: 114
CRLPAGPRG-Nle-GTC (11, 19, 21)	Hexafluorobenzene Crosslink	SEQ ID NO: 115
	Decafluorobiphenyl Crosslink	SEQ ID NO: 116
	Disulfide Crosslink	SEQ ID NO: 117
ARLPAGPRG-Nle-GTA	Non-crosslink	SEQ ID NO: 118
CQYPIIYDIRARPRC (76, 88)	Hexafluorobenzene Crosslink	SEQ ID NO: 119
	Decafluorobiphenyl Crosslink	SEQ ID NO: 120
	Disulfide Crosslink	SEQ ID NO: 121
AQYPIIYDIRARPRA	Non-crosslink	SEQ ID NO: 122

FIG. 10-1


MCF-7
Cell Growth (Absorbance: 450 nm)
0.6
0.5
0.4
0.3
0.2
**
***
**
***
***
- - 115 116 117 118 119 120 121 122
(-) 10 nM Estrogen

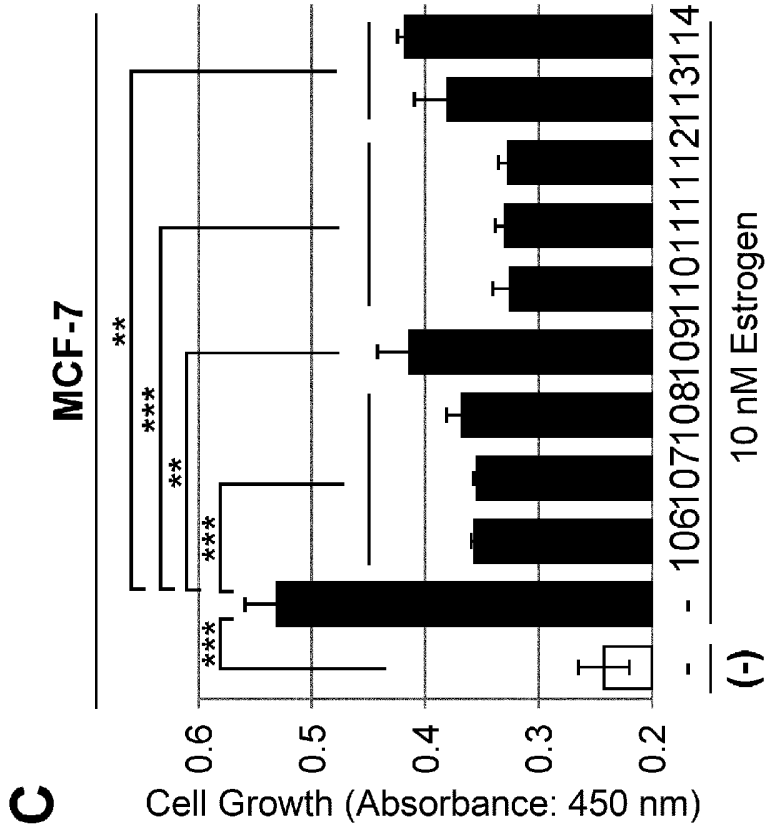

C
MCF-7
Cell Growth (Absorbance: 450 nm)
0.6
0.5
0.4
0.3
0.2
**
***
**
***
***
- - 106 107 108 109 110 111 112 113 114
(-) 10 nM Estrogen

FIG. 10-2

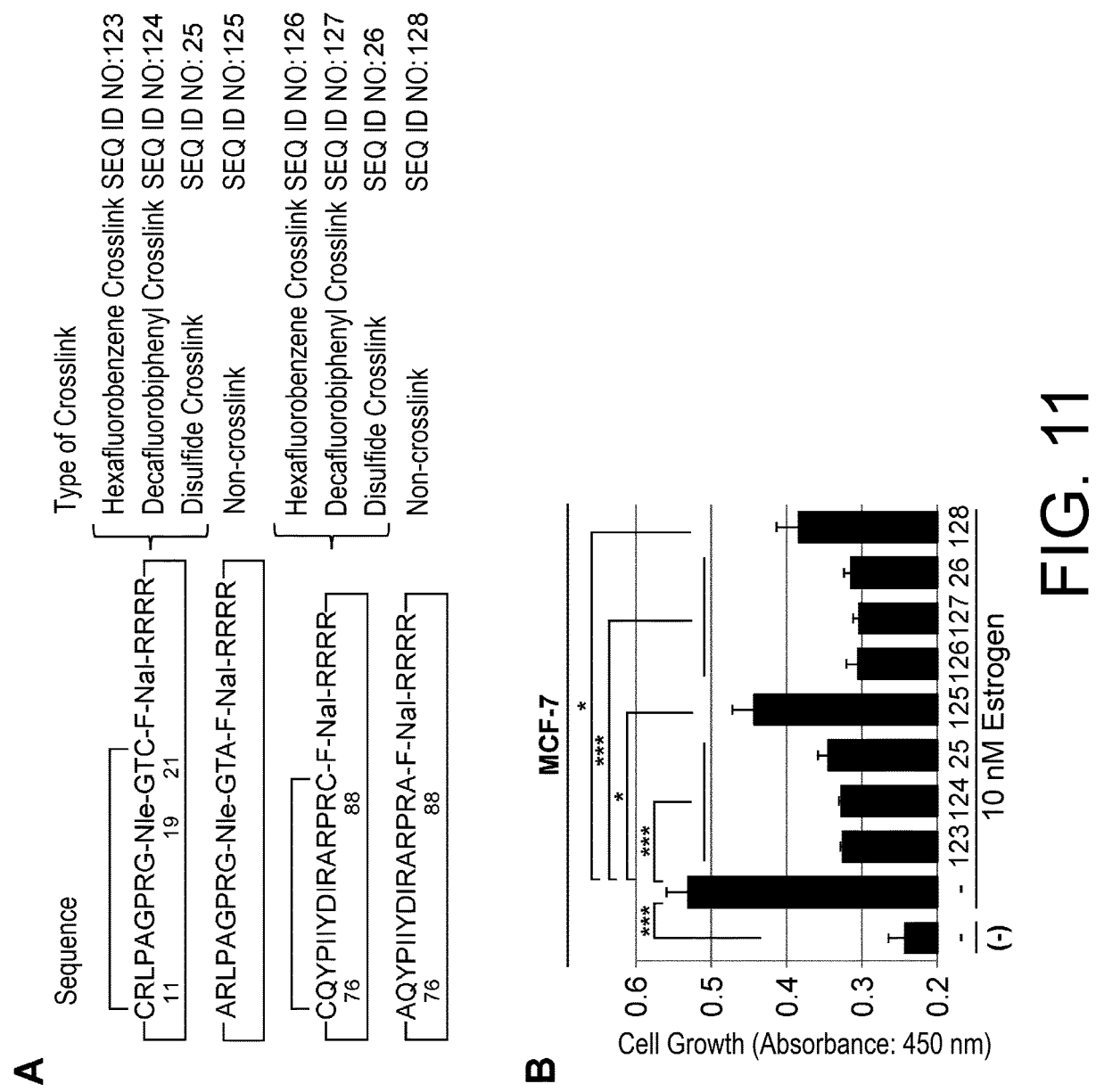

A
Sequence
Type of Crosslink
CRLPAGPRG-Nle-GTC-F-Nal-RRRR
11 19 21
Hexafluorobenzene Crosslink SEQ ID NO:123
Decafluorobiphenyl Crosslink SEQ ID NO:124
Disulfide Crosslink SEQ ID NO: 25
ARLPAGPRG-Nle-GTA-F-Nal-RRRR
Non-crosslink SEQ ID NO:125
CQYPIIYDIRARPRC-F-Nal-RRRR
76 88
Hexafluorobenzene Crosslink SEQ ID NO:126
Decafluorobiphenyl Crosslink SEQ ID NO:127
Disulfide Crosslink SEQ ID NO:26
AQYPIIYDIRARPRA-F-Nal-RRRR
76 88
Non-crosslink SEQ ID NO:128
B
MCF-7
Cell Growth (Absorbance: 450 nm)
0.2
0.3
0.4
0.5
0.6
***
***
*
***
*
*
- - 1 2 3 12 4 25 125 126 127 26 128
(-) 10 nM Estrogen

FIG. 11

A

	Sequence (15 18)	Mutation Site
SEQ ID NO: 129	RLPAGPRAMGTA-RRRRRRRR	G18 → D-Ala
SEQ ID NO: 130	RLPAAPRGMGTA-RRRRRRRR	G15 → D-Ala
SEQ ID NO: 131	RLPAGPRLMGTA-RRRRRRRR	G18 → D-Leu
SEQ ID NO: 132	RLPALPRGMGTA-RRRRRRRR	G15 → D-Leu
SEQ ID NO: 133	RLPAAPRAMGTA-RRRRRRRR	G15 → D-Ala、G18 → D-Ala
SEQ ID NO: 134	RLPALPRLMGTA-RRRRRRRR	G15 → D-Leu、G18 → D-Leu
SEQ ID NO: 135	RLPAAPRLMGTA-RRRRRRRR	G15 → D-Ala、G18 → D-Leu
SEQ ID NO: 136	RLPALPRAMGTA-RRRRRRRR	G15 → D-Leu、G18 → D-Ala

B

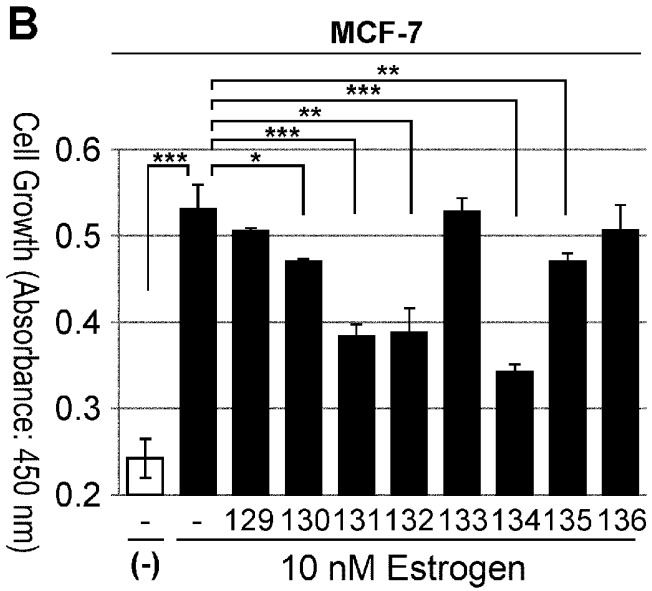


FIG. 12

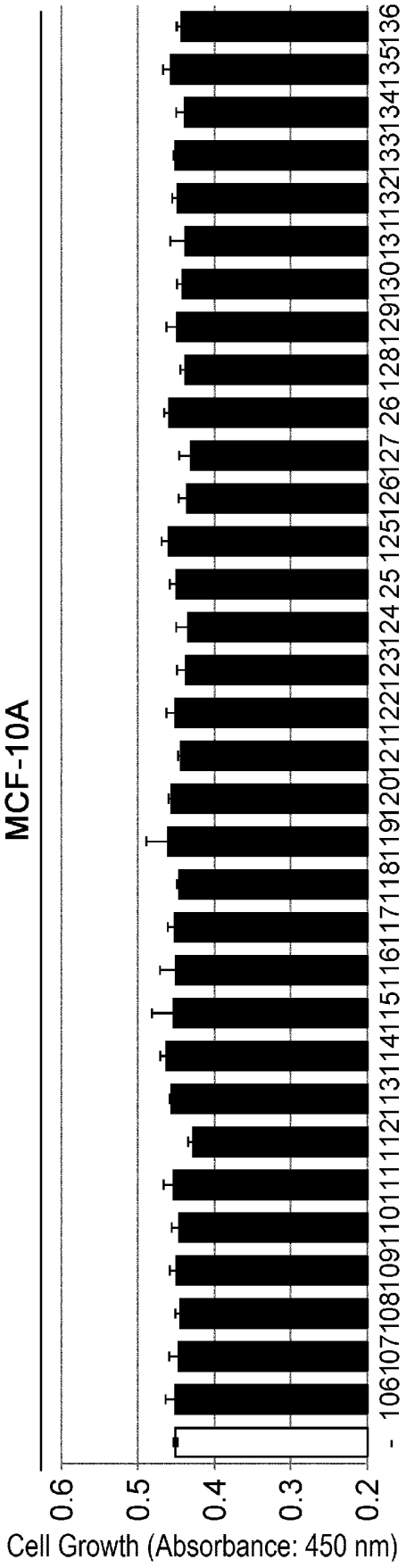

MCF-10A
Cell Growth (Absorbance: 450 nm)
0.6
0.5
0.4
0.3
0.2
-
1
06
10
7
10
8
10
9
11
0
10
11
11
11
2
11
3
11
4
11
5
11
6
11
7
11
8
11
9
12
0
12
1
12
2
12
3
12
4
12
5
12
6
12
7
12
8
12
9
13
0
13
1
13
2
13
3
13
4
13
5
13
6

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2019/046505

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl. C12N15/12(2006.01)i, A61K38/10(2006.01)i, A61K38/12(2006.01)i, A61K48/00(2006.01)i, A61P35/00(2006.01)i, A61P43/00(2006.01)i, C07K7/06(2006.01)i, C07K7/08(2006.01)i, C07K14/47(2006.01)i
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl. C12N15/12, A61K38/10, A61K38/12, A61K48/00, A61P35/00, A61P43/00, C07K7/06, C07K7/08, C07K14/47

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2019
Registered utility model specifications of Japan 1996-2019
Published registered utility model applications of Japan 1994-2019

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JSTPlus/JMEDPlus/JST7580(JDreamIII),
CAplus/REGISTRY/MEDLINE/EMBASE/BIOSIS/REGISTRY(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CHEN, Y. A. et al., Brefeldin A-inhibited guanine nucleotide-exchange protein 3 (BIG3) is predicted to interact with its partner through an ARM-type α-helical structure, BMC Research Notes, 2014, vol. 7, no. 435, pp. 1-8, particularly, abstract, background, fig. 3, 6	1-6, 11-12 1-20
Y	WO 2013/018690 A1 (TOKUSHIMA UNIVERSITY) 07 February 2013, claims, paragraphs [0008], [0022] & US 2014/0162952 A1, claims, paragraphs [0011], [0143] & EP 2738255 A1	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
12.12.2019	24.12.2019
Name and mailing address of the ISA/ Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2019/046505

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	吉丸哲郎 ほか, 新規 A キナーゼアンカータンパク質 BIG3 による抑制因子 PHB2 の制御は HER2 乳がん細胞増殖に必須である, 第 41 回日本分子生物学会年会 要旨集, 09 November 2018, 2P-0632, (YOSHIMARU, Tetsuro et al., A-kinase anchoring protein BIG3-PHB2 axis is required for progression of HER2-rich breast cancer cells), non-official translation (Abstracts of the 41st Annual Meeting of the Molecular Biology Society of Japan)	1-20

Form PCT/ISA/210 (continuation of second sheet) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

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- WO 2013018690 A **[0005]**
- WO 2017126461 A **[0007] [0053]**
- WO 97016171 A **[0046]**
- WO 9967288 A **[0069]**
- US 5580895 A **[0078]**
- US 5589466 A **[0078]**
- US 5804566 A **[0078]**
- US 5739118 A **[0078]**
- US 5736524 A **[0078]**
- US 5679647 A **[0078]**
- WO 9804720 A **[0078]**
- US 4722858 A **[0079]**

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- **FISHER, B. et al.** *J. Natl. Cancer Inst.,* 2005, vol. 97 (22), 1652-62 **[0006]**
- **JORDAN, V. C.** *Nature Rev. Drug Discov.,* 2003, vol. 2 (3), 205-13 **[0006]**
- **CLARKE, R. et al.** *Pharmacol. Rev.,* 2001, vol. 53 (1), 25-71 **[0006]**
- **FISHER, B. et al.** *J. Natl. Cancer Inst.,* 2001, vol. 93 (9), 684-90 **[0006]**
- **CHLEBOWSKI, R. et al.** *Breast,* 2009, vol. 2, 1-11 **[0006]**
- **CHUMSRI, S. et al.** *J. Steroid Biochem. Mol. Biol.,* 2011, vol. 125 (1-2), 13-22 **[0006]**
- **KIM, J. W. et al.** *Cancer Sci.,* 2009, vol. 100 (8), 1468-78 **[0006]**
- **YOSHIMARU, T. et al.** *Nat. Commun.,* 2013, vol. 4, 2443 **[0006]**
- **YOSHIMARU, T. et al.** *Sci Rep.,* 2017, vol. 7 (1), 1821 **[0006]**
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