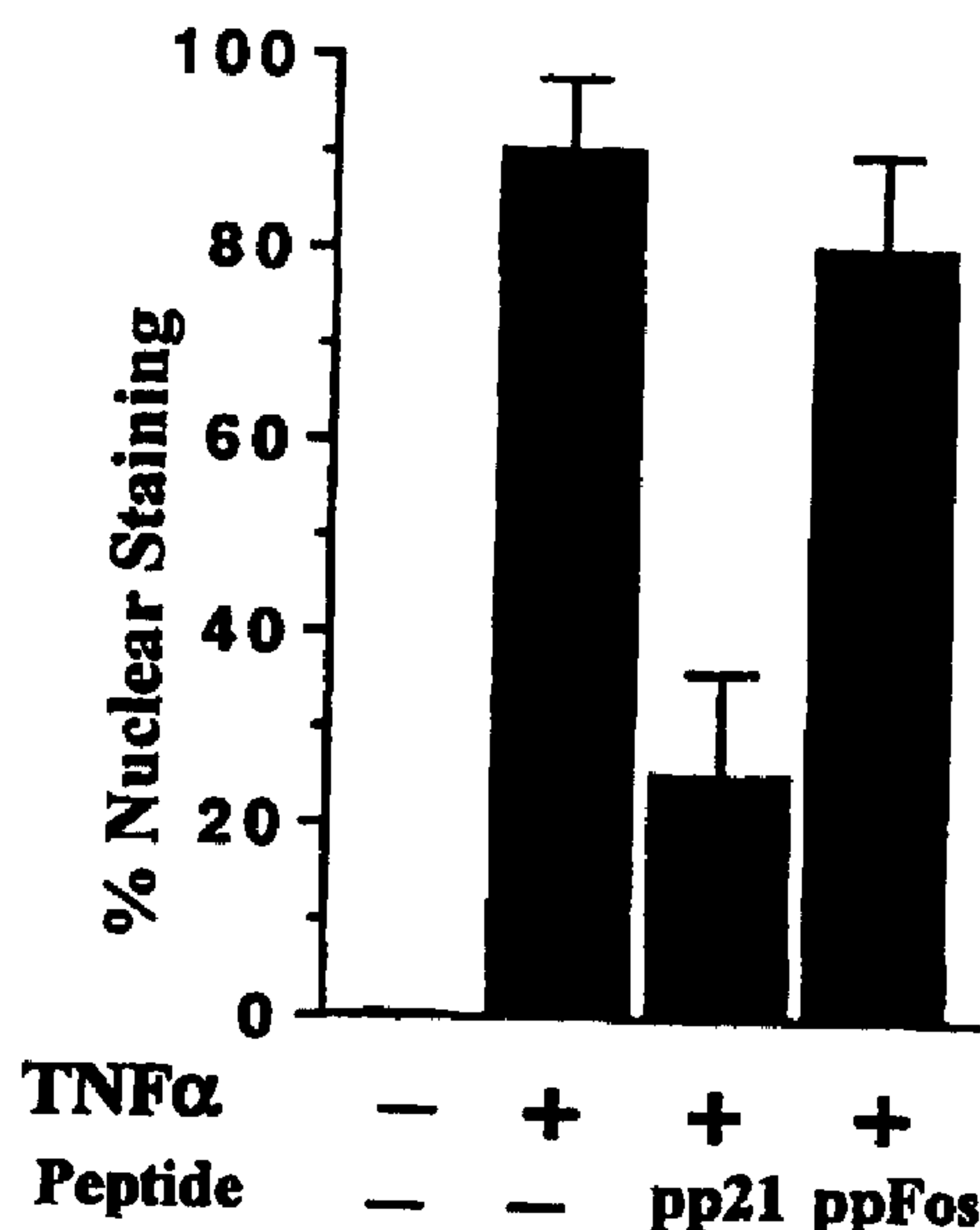




- (72) MANNING, ANTHONY, US
(72) MERCURIO, FRANK, US
(72) ALKALAY, IRIT, IL
(72) BEN-NERIAH, YINON, IL
(72) CIECHANOVER, AARON, IL
(72) YARON, AVRAHAM, IL
(71) SIGNAL PHARMACEUTICALS, INC., US
(71) YISSUM RESEARCH DEVELOPMENT COMPANY OF THE
HEBREW UNIVERSITY OF JERUSALEM, IL
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(54) **COMPOSITIONS ET PROCEDES SERVANT A MODULER
L'ACTIVATION DE κ B-NF CELLULAIRE**
(54) **COMPOSITIONS AND METHODS FOR MODULATING
CELLULAR NF- κ B ACTIVATION**



(57) Compositions et procédés servant à moduler l'activation du facteur nucléaire κ B (NF- κ B). Ces compositions contiennent un ou plusieurs agents modulant l'ubiquitination, soit de I κ B α , soit de I κ B β . On peut utiliser ces compositions afin de traiter des maladies associées à l'activation de NF- κ B. Les agents modulants incluent des peptides présentant un domaine de reconnaissance d'ubiquitine ligase de E3.

(57) Compositions and methods for modulating the activation of nuclear factor κ B (NF- κ B) are provided. The compositions comprise one or more agents that modulate ubiquitination of phosphorylated I κ B α and/or I κ B β . Such compositions may be used for treating diseases associated with NF- κ B activation. Modulating agents include peptides that comprise a recognition domain for E3 ubiquitin ligase.

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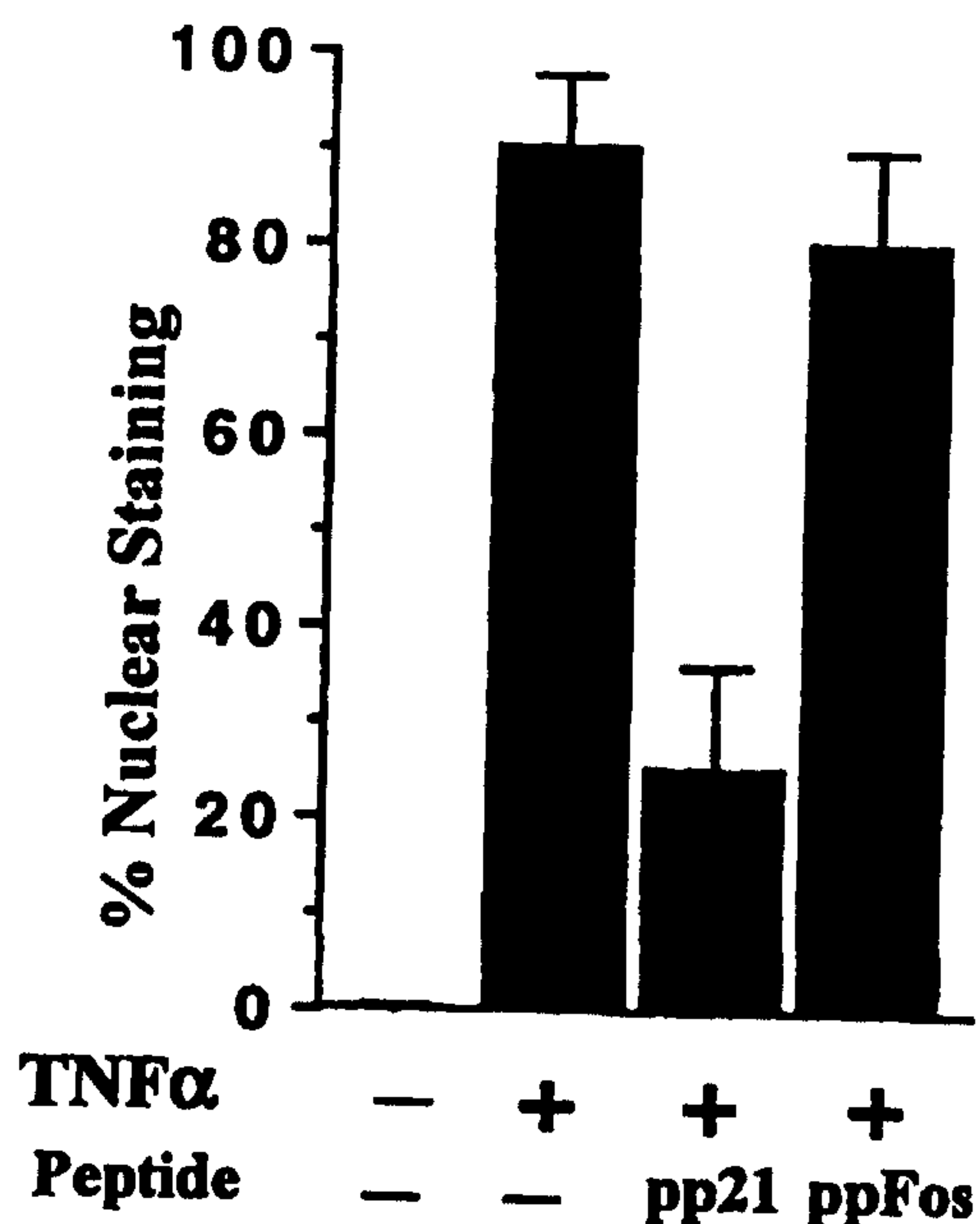
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(30) Priority Data:
08/802,322 18 February 1997 (18.02.97) US(71) Applicants: SIGNAL PHARMACEUTICALS, INC. [US/US];
5555 Oberlin Drive, San Diego, CA 92121 (US). YISSUM
RESEARCH DEVELOPMENT COMPANY OF THE HE-
BREW UNIVERSITY OF JERUSALEM [IL/IL]; P.O. Box
4279, 91042 Jerusalem (IL).(72) Inventors: MANNING, Anthony; 12333 Goldfish Court,
San Diego, CA 92129 (US). MERCURIO, Frank; 3674
Carmel View Road, San Diego, CA 92130 (US). AL-
KALAY, Irit; 70 Sderot Herzl, 96142 Jerusalem (IL).
BEN-NERIAH, Yinon; 5 Mevo Duvdevan, 80805 Zion (IL).
CIECHANOVER, Aaron; 21 Peretz Bernstein, 34981 Haifa
(IL). YARON, Avraham; 1 Emil Zola, 93107 Jerusalem
(IL).(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP,
6300 Columbia Center, 701 Fifth Avenue, Seattle, WA
98104-7092 (US).(81) Designated States: AU, CA, JP, European patent (AT, BE,
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(57) Abstract

Compositions and methods for modulating the activation of nuclear factor κ B (NF- κ B) are provided. The compositions comprise one or more agents that modulate ubiquitination of phosphorylated I κ B α and/or I κ B β . Such compositions may be used for treating diseases associated with NF- κ B activation. Modulating agents include peptides that comprise a recognition domain for E3 ubiquitin ligase.



COMPOSITIONS AND METHODS FOR MODULATING CELLULAR NF- κ B ACTIVATION

TECHNICAL FIELD

The present invention relates generally to compositions and methods for
5 modulating the activation of nuclear factor κ B (NF- κ B). The invention is more particularly related to agents that modulate ubiquitination of phosphorylated I κ B α and/or I κ B β , to methods for identifying such agents and to methods for treating diseases associated with NF- κ B activation. Modulating agents encompassed by the present invention include peptides that comprise a recognition domain for E3 ubiquitin ligase.

10 BACKGROUND OF THE INVENTION

NF- κ B is a transcription factor that plays a pivotal role in the highly specific pattern of gene expression observed for immune, inflammatory and acute phase response genes, including interleukin 1, interleukin 8, tumor necrosis factor and certain cell adhesion molecules. Like other members of the Rel family of transcriptional
15 activators, NF- κ B is sequestered in an inactive form in the cytoplasm of most cell types. A variety of extracellular stimuli including mitogens, cytokines, antigens, stress inducing agents, UV light and viral proteins initiate a signal transduction pathway that ultimately leads to NF- κ B release and activation.

Important modulators of NF- κ B activation are the inhibitor proteins
20 I κ B α and I κ B β (referred to herein as I κ B), which associate with (and thereby inactivate) NF- κ B *in vivo*. Activation and nuclear translocation of NF- κ B occurs following signal-induced phosphorylation of I κ B, which leads to proteolysis via the ubiquitin pathway. For I κ B α , the stimulus-induced phosphorylation at serines 32 and 36 renders the inhibitor a target for ubiquitination at lysines 21 and 22, resulting in degradation.
25 Similarly, phosphorylation of I κ B β at serines 19 and 23 renders the inhibitor a target for ubiquitination at lysine 9. However, neither the site at which I κ Bs are recognized by the ubiquitin system, nor the component(s) of the ubiquitin system mediating I κ B recognition have been identified.

Degradation of a protein via the ubiquitin pathway proceeds by two discrete and successive steps: (a) covalent attachment of multiple ubiquitin molecules to the protein substrate, and (b) degradation of the targeted protein by the 26S proteasome complex. The ubiquitin pathway consists of several components that act in concert and in a hierarchical manner (for reviews, *see* Ciechanover, *Cell* 79:13, 1994; Hochstrasser, *Curr. Op. Cell. Biol.* 7:215, 1995; Jentsch and Schlenker, *Cell* 82:881, 1995; Deshaies, *Trends Cell Biol.* 5:428, 1995). One such component, a single E1 enzyme, carries out activation of ubiquitin. Several major species of E2 enzymes have been characterized in mammalian cells, plants, and yeast. E2 enzymes probably bind to the ligase E3 (Reiss and Hersko, *J. Biol. Chem.* 265:3685, 1990; Dohmen et al., *Proc. Natl. Acad. Sci. USA* 88:7351, 1991) and it appears that each E2 enzyme can act with one or more E3 proteins (Nuber et al., *J. Biol. Chem.* 271:2795, 1996; Orian et al., *J. Biol. Chem.* 270:21707, 1995; Stancovski et al., *Mol. Cell. Biol.* 15:7106, 1995; Gonen et al., *J. Biol. Chem.* 271:302, 1996).

Only few E3 enzymes (ubiquitin ligases) have been described. Mammalian E3 α (UBR1 in yeast) and E3 β recognize protein substrates via their free N-terminal amino acid residues ("N-end rule"; Varshavsky, *Cell* 69:725, 1992; Hershko and Ciechanover, *Ann. Rev. Biochem.* 61:761, 1992). Cdc53 is probably an E3 involved in targeting phosphorylated G1 cyclins (Willems et al., *Cell* 86:453, 1996). E6-AP is involved in recognition of p53 (Scheffner et al., *Cell* 75:495, 1993), and a series of unique E6-AP homologous proteins have been identified (Huibregtse et al., *Proc. Natl. Acad. Sci. USA* 92:2563, 1995): Nedd4 is involved the degradation of the epithelial Na⁺ channel (Staub et al., *Embo J.* 15:2371, 1996) and RSP5 (NIP1) is involved in tagging the permeases Gap1 and Fur1 (Hein et al., *Mol. Microbiol.* 18:77, 1995), whereas Pub1 targets Cdc25 (Nefsky and Beach, *EMBO J.* 15:1301, 1996). Several other E3 enzymes that have been recently isolated appear to be involved in the degradation of c-Fos, a subset of muscle proteins, and in the processing of p105, the NF- κ B precursor (Orian et al., *J. Biol. Chem.* 270:21707, 1995; Stancovski et al., *Mol. Cell. Biol.* 15:7106, 1995; Gonen et al., *J. Biol. Chem.* 271:302, 1996). Thus, it appears that the ligases represent a large, mostly unraveled family of enzymes and, except for the mode of recognition of the

“N-end rule” ligases (E3 α and E3 β), the recognition motifs of all other known substrates of the ubiquitin system have not been identified.

Accordingly, there is a need in the art for an improved understanding of I κ B degradation via the ubiquitin pathway, and for the identification of modulators of this degradation process for use in treating diseases associated with activation of NF- κ B. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for modulating the activation of nuclear factor κ B (NF- κ B) by modulating ubiquitination of phosphorylated I κ B α and/or I κ B β . Within one aspect, the present invention provides a method for assaying the ability of an I κ B polypeptide to undergo ubiquitination *in vivo*, comprising: (a) incubating an I κ B polypeptide with a cellular extract, wherein the step of incubating is carried out under conditions and for a time sufficient to allow phosphorylation of the I κ B polypeptide and formation of a complex comprising phosphorylated I κ B polypeptide and NF- κ B; (b) subsequently subjecting the complex to *in vitro* ubiquitination; and (c) subsequently evaluating the extent of ubiquitination of the complex, and thereby assaying the ability of the I κ B polypeptide to undergo ubiquitination *in vivo*.

Within another aspect, a method for identifying an agent that modulates ubiquitination of I κ B α and/or I κ B β is provided, comprising: (a) incubating a candidate agent with an I κ B polypeptide and a cellular extract, wherein the step of incubating is carried out under conditions and for a time sufficient to allow phosphorylation of the I κ B polypeptide and formation of a complex comprising phosphorylated I κ B polypeptide and NF- κ B; and (b) subsequently measuring the ability of the candidate agent to modulate ubiquitination of the complex, and therefrom identifying an agent that modulates ubiquitination of I κ B α and/or I κ B β .

The present invention also provides agents that modulate ubiquitination of I κ B α and/or I κ B β . Such agents include peptides that comprise a recognition domain for E3 ubiquitin ligase including, for example, the peptides recited in SEQ ID NO:5 -

SEQ ID NO:9. Isolated DNA molecules and recombinant expression vectors encoding peptide agents, as well as host cells transformed or transfected with such an expression vector, are also provided.

Within another aspect, the present invention provides pharmaceutical
5 compositions comprising one or more agents that modulate ubiquitination of I κ B α and/or I κ B β in combination with a pharmaceutically acceptable carrier.

The present invention also provides, within further aspects, methods for modulating NF- κ B activity in a patient and for treating a patient afflicted with a disorder associated with NF- κ B activation, comprising administering to a patient a
10 pharmaceutical composition as described above. Disorders associated with NF- κ B activation include inflammatory diseases, autoimmune diseases, cancer and viral infection.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All
15 references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D are autoradiograms depicting the results of SDS-PAGE analysis of ubiquitination assays performed in the presence and absence of representative
20 modulating agents. Unless otherwise indicated, the substrate was an ^{35} S-labelled, HA-tagged I κ B polypeptide that was phosphorylated and NF- κ B complex-associated.

In Figure 1A, lane 1 shows the ubiquitination of an I κ B α polypeptide that contains alanine residues at positions 32 and 36 (S32/36A; SEQ ID NO:13) and lane 2 shows the ubiquitination of a non-phosphorylated wild-type I κ B α polypeptide (SEQ ID
25 NO:12). In lanes 3-14, the ubiquitination substrate was wild-type I κ B α (SEQ ID NO:12). In lane 3, ubiquitination was performed in the absence of ATP; and in lanes 4-14 the reaction was performed in the presence of ATP γ S with (lanes 5-14) or without (lane 4) a candidate peptide modulating agent. The candidate agents shown are: 400 μ M c-Fos phosphopeptide (ppFos (SEQ ID NO:10), lane 5); 400 μ M serine 32, 36 to alanine

substituted I κ B α peptide (pp21S/A (SEQ ID NO:11), lane 6); 40 μ M doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lane 7); 400 μ M non-phosphorylated I κ B α peptide (p21 (SEQ ID NO:9), lane 8); 100 μ M singly phosphorylated I κ B α peptides (ppS32 (SEQ ID NO:9), lane 9; ppS36 (SEQ ID NO:9), lane 10); and 40 μ M shorter, doubly phosphorylated I κ B α peptides (pp19 (SEQ ID NO:8), lane 11); pp15 (SEQ ID NO:7), lane 12; pp11 (SEQ ID NO:6), lane 13; pp7 (SEQ ID NO:5), lane 14).

In Figure 1B, the ubiquitination substrate was free wild type I κ B α (SEQ ID NO:12, lanes 1-3) or free S32/36A substituted I κ B α (SEQ ID NO:13, lanes 4-6). The reaction was performed in the absence (lanes 1 and 4) or presence (lanes 2, 3, 5 and 6) of ATP γ S. 40 μ M doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9) was added to the conjugation reaction mixture in the samples shown in lanes 3 and 6.

In Figure 1C, the ubiquitination of bulk cellular proteins in HeLa extract is shown. Lane 1 shows the ubiquitination in the absence of ATP, and lane 5 shows the ubiquitination in the presence of ATP. In lanes 3-5, candidate modulating agents were added: 40 μ M doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lane 2); 400 μ M c-Fos phosphopeptide (ppFos (SEQ ID NO:10), lane 3); and 400 μ M non-phosphorylated I κ B α peptide (p21 (SEQ ID NO:9), lane 4).

In Figure 1D, the ubiquitination substrate was phosphorylated (lanes 2-7) or non-phosphorylated (lane 1) wild type I κ B β (SEQ ID NO:14). Reactions were performed in the absence (lane 2) or presence (lanes 1, 3-7) of ATP γ S, and with (lanes 4-7) or without (lanes 1-3) a candidate peptide modulating agent. The candidate agents shown are: 40 μ M doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lane 4); 400 μ M c-Fos phosphopeptide (ppFos (SEQ ID NO:10), lane 5); 40 μ M doubly phosphorylated I κ B α peptide (pp19 (SEQ ID NO:8), lane 6); and 400 μ M non-phosphorylated I κ B α peptide (p21 (SEQ ID NO:9), lane 7).

Figure 2 is an autoradiogram depicting the results of an *in vitro* ubiquitin-dependent degradation assay performed using extracts from stimulated HeLa cells. In each lane of the SDS-PAGE, the level of phosphorylated (upper band) and non-phosphorylated (lower band) HA-tagged I κ B α polypeptide (SEQ ID NO:12) following

the degradation assay is shown. Lane 1 shows the level of these polypeptides following a degradation assay performed without ATP. In lanes 2-6, ATP was included in the reaction mixture. 40 μ M candidate modulating agents were added to the reactions shown in lanes 3-6: doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lane 3);
5 doubly phosphorylated I κ B α peptide (pp19 (SEQ ID NO:8), lane 4); c-Fos phosphopeptide (ppFos (SEQ ID NO:10), lane 5); and non-phosphorylated I κ B α peptide (p21 (SEQ ID NO:9), lane 6).

Figure 3A is an autoradiogram depicting the results of SDS-PAGE analysis of ubiquitination assays performed using flow-through fractions of HeLa cell
10 lysate fractionated over modulating agent columns. In each case, the substrate was a ³⁵S-labelled, HA-tagged I κ B α polypeptide (SEQ ID NO:12) that was phosphorylated and NF- κ B complex-associated. Lane 1 shows the level of ubiquitination using a non-fractionated extract. In lanes 2-9, the extract was fractionated over a peptide-Sepharose[®] column. The peptides used were: c-Fos phosphopeptide (ppFos (SEQ ID
15 NO:10), lane 2); serine 32, 36 to alanine substituted I κ B α peptide (pp21S/A (SEQ ID NO:11), lane 3); doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lanes 4-6); and doubly phosphorylated I κ B α peptide (pp19 (SEQ ID NO:8), lanes 7-9). In addition, reticulocyte Fraction II (160 μ g) was added to the ubiquitination reactions shown in lanes 5 and 8, and Fraction I (160 μ g) was added to the reactions in lanes 6
20 and 9.

Figure 3B is an autoradiogram showing the ubiquitination of bulk cellular proteins in HeLa extract. Lane 1 shows the ubiquitination in the absence of ATP, and lane 2 shows the ubiquitination in the presence of ATP, but without candidate modulating agent. In lanes 3-6, candidate modulating agents were added: 40 μ M doubly
25 phosphorylated I κ B α peptide (pp19 (SEQ ID NO:8), lane 3); 400 μ M c-Fos phosphopeptide (ppFos (SEQ ID NO:10), lane 4); 400 μ M serine 32, 36 to alanine substituted I κ B α peptide (pp21S/A (SEQ ID NO:11), lane 5); and 40 μ M doubly phosphorylated I κ B α peptide (pp21 (SEQ ID NO:9), lane 6).

Figures 4A-4F are micrographs showing the effect of candidate
30 modulating agents on nuclear NF- κ B translocation. In Figures 4A-C, pp21 (Figures 4A

and 4B) or ppFos (Figure 4C) was microinjected into the cytoplasm of HeLa cells. Cells were then activated immediately with $\text{TNF}\alpha$ and immunostained with anti-p65 antibodies. In Figures 4D-F, pp21 (Figure 4D) or ppFos (Figure 4F) was injected into the cytoplasm of human vascular endothelial cells (HUVEC). Cells were then activated immediately with $\text{TNF}\alpha$ and immunostained with anti-E-selectin antibodies. Figure 4E is a phase contrast photograph of Figure 4D. In each micrograph, the injected cells are marked by large arrows. A non-injected, E-selectin negative cell is marked by a small arrow in Figures 4D and 4E.

Figures 4G and 4H are graphs presenting a summary of the microinjection experiments shown in Figures 4A - 4F. In Figure 4G, the percent of HeLa cells displaying nuclear p65 staining is shown. 90 and 42 cells were microinjected with pp21 and ppFos, respectively. Figure 4H shows the percent of HUVEC displaying E-selectin staining. 160 and 36 cells were microinjected with pp21 and ppFos, respectively. For each graph, column 1 shows the level in the absence of candidate modulating agent and $\text{TNF}\alpha$ activation. Columns 2-4 show the level following $\text{TNF}\alpha$ activation in the absence of candidate modulating agent (column 2) or in the presence of pp21 (column 3) or ppFos (column 4).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods useful for modulating the activation of nuclear factor κB (NF- κB) and for treating diseases associated with such activation. In particular, the invention is directed to agents that modulate ubiquitination of phosphorylated I κB (*i.e.*, I $\kappa\text{B}\alpha$ and/or I $\kappa\text{B}\beta$), and to methods for identifying such agents.

In response to a stimulus, I κB associated with NF- κB is activated (*i.e.*, phosphorylated), rendering I κB a target for degradation and thereby releasing and activating NF- κB . It has been found, within the context of the present invention, that phosphorylated and NF- κB -associated I κB is recognized by a specific ubiquitin ligase, E3. The N-terminal signal-induced phosphorylation site that is functionally conserved between I $\kappa\text{B}\alpha$ and I $\kappa\text{B}\beta$ constitutes the E3 recognition motif and is distinct from the

nearby ubiquitination site. Peptides corresponding to this motif, and variants thereof, inhibit the ubiquitination of I κ B and its subsequent degradation, and such peptides are modulating agents within the scope of the present invention.

Within one aspect, the present invention provides an *in vitro* ubiquitination assay that reproduces the *in vivo* ubiquitination of I κ B α with high fidelity. *In vivo*, I κ B is targeted for degradation by phosphorylation at serines 32 and 36, while altered forms of I κ B α that contain alanine residues at positions 32 and 36 are not subject to ubiquitin conjugation. Similarly, phosphorylation at serines 19 and 23 is required for ubiquitination of I κ B β . However, free I κ B is recognized by the ubiquitin system in a non-discriminatory manner (*i.e.*, phosphorylation is not required). The ubiquitination assay provided herein allows regulation of I κ B ubiquitination that corresponds to the regulation observed *in vivo*.

I κ B polypeptides for use in a ubiquitination assay as described herein may be native human I κ B α (SEQ ID NO:1) or I κ B β (SEQ ID NO:3), or may be a variant of a native protein. As used herein, a variant is a polypeptide that contains one or more substitutions and/or modifications. Variants include truncated polypeptides and polypeptides containing additional amino acid sequences that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the polypeptide. Polypeptide variants of I κ B are modified such that the ability of the variant to be phosphorylated and ubiquitinated within a ubiquitination assay as described herein is not substantially diminished. Preferably, the I κ B polypeptide is labeled. For example, ^{35}S may be incorporated into a I κ B polypeptide by *in vitro* translation of the polypeptide in the presence of ^{35}S -methionine, using standard techniques.

An I κ B polypeptide may generally be prepared from DNA encoding the polypeptide by expression of the DNA in cultured host cells or by translation using an *in vitro* system such as wheat germ extract. If host cells are employed, such cells are preferably are bacteria, yeast, baculovirus-infected insect cells or mammalian cells. The recombinant DNA may be cloned into any expression vector suitable for use within the

host cell, using techniques well known to those of ordinary skill in the art. *In vitro* translation of polypeptide may generally be performed according to the manufacturer's instructions.

The DNA sequences expressed in this manner may encode native I κ B α or I κ B β , or may encode portions or variants of a native I κ B. DNA molecules encoding variants may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the DNA sequence may also, or alternatively, be removed to permit preparation of truncated polypeptides and DNA encoding additional sequences such as "tags" may be added to the 5' or 3' end of the DNA molecule. For example, DNA encoding an I κ B polypeptide may also encode an epitope, such that the recombinant protein contains the epitope at the N- or C-terminus. Epitopes such as glutathione-S transferase protein (GST), HA (hemagglutinin)-tag, FLAG and Histidine-tag may be added using techniques well known to those of ordinary skill in the art.

Expressed I κ B polypeptides may be used without purification following *in vitro* translation. Alternatively, a polypeptide may be isolated in substantially pure form. An I κ B polypeptide may be isolated to a purity of at least 80% by weight, preferably to a purity of at least 95% by weight, and more preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the representative purification method described herein or the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography.

Within a ubiquitination assay as provided herein, cellular extracts from stimulated or non-stimulated Jurkat, HeLa, THP-1 or endothelial cells are incubated *in vitro* with an I κ B polypeptide in the presence of ATP and the phosphatase inhibitor okadaic acid. Cellular extracts may generally be prepared according to the method of Alkalay et al., *Proc. Natl. Acad. Sci. USA* 92:10599, 1995. The incubation is performed under conditions sufficient to result in phosphorylation of the I κ B polypeptide (at serines 32 and 36 for I κ B α and variants thereof) and association of the phosphorylated polypeptide (pI κ B) with the cellular-derived NF- κ B complex. For example, I κ B

polypeptide may be incubated with HeLa or Jurkat cell extract, ATP and okadaic acid. Incubation for 90 minutes at 30°C is generally sufficient to allow phosphorylation of the IκB polypeptide. Following this incubation, the pIκB/NF-κB complex may be immunopurified with, for example, anti-p65 antibodies and subjected to *in vitro* ubiquitination in a cell free system, as described by Alkalay et al., *Proc. Natl. Acad. Sci. USA* 92:10599, 1995. The level of ubiquitination may then be evaluated using the well known techniques of SDS-PAGE, followed by autoradiography.

Under these conditions, a wild type ³⁵S-pIκBα polypeptide generates multiply ubiquitinated species in the presence of ATPγS (see Figure 1A, lane 4). Neither ³⁵S-labeled S32/36A mutant of IκBα (lane 1), nor the non-phosphorylated wild type ³⁵S-IκBα (lane 2) are ubiquitinated. However, free forms of either mutant or wild type IκBα are readily conjugated (Figure 1B). Similarly, a free (but not a complex-associated) lysine 21, 22 mutant of IκBα can be ubiquitinated *in vitro*. Thus, unlike ubiquitination assays performed using free IκB polypeptides, the ubiquitination assay provided herein targets only IκB polypeptides that are complex-associated and appropriately phosphorylated.

In another aspect of the present invention, a ubiquitination assay as described above may be used to identify agents that modulate ubiquitination of IκB. Modulating agents may include antibodies (*e.g.*, monoclonal), peptides and other drugs that stimulate or, preferably, inhibit ubiquitination of an IκBα and/or IκBβ polypeptide. In general, such agents may be identified by including a candidate modulating agent in the ubiquitination reaction, which may otherwise be performed as described above, and evaluating the effect of the agent on the level of ubiquitination. A suitable concentration of candidate agent for use in such an assay generally ranges from about 0.1 μM to about 1 mM. For peptide candidate agents, a peptidase inhibitor such as Bestatin (40 μg/mL) may also be added, and the amount of peptide preferably ranges from about 10 μM to about 1 mM. A candidate agent that results in a statistically significant effect on the level of ubiquitination is a modulating agent encompassed by the present invention.

As noted above, it has been found, within the context of the present invention, that complex-associated IκB is recognized by a specific ubiquitin ligase, E3.

Accordingly, modulating agents within the scope of the present invention include, but are not limited to, peptides that comprise a recognition domain for E3 ubiquitin ligase. Such peptides may be derived from the N-terminal signaling domain (residues 1 to 54 of native I κ B α or I κ B β) and, at minimum, should contain the signaling phosphorylation site
5 (residues 32 to 36 of native I κ B α or residues 19 to 23 of native I κ B β). Peptide modulating agents may generally be prepared using standard automated synthesis techniques or by expression of recombinant DNA encoding the desired peptide. Such agents may differ in sequence from native I κ B α and I κ B β due to one or more substitutions and/or modifications, as described above, provided that the peptide variant
10 inhibits ubiquitination of an I κ B polypeptide.

For maximal inhibition, peptide modulating agents should be phosphorylated; preferably at both of the native phosphorylation sites (*e.g.*, serines 32 and 36 of I κ B α), although singly phosphorylated peptides may be employed. Phosphorylated peptides may be prepared using well known techniques. For example
15 phosphoserine residues may be incorporated into a peptide during synthesis. Alternatively, a peptide may be phosphorylated using standard techniques following synthesis.

In general, peptide modulating agents may be prepared using standard techniques, incorporating amino acids and/or amino acid analogs. During synthesis,
20 active groups of amino acids and/or amino acid analogs may be protected as necessary using, for example, a t-butyldicarbonate (t-BOC) group or a fluorenylmethoxy carbonyl (Fmoc) group. Amino acids and amino acid analogs may be purchased commercially (*e.g.*, Sigma Chemical Co.; Advanced Chemtec) or synthesized using methods known in the art. Peptides may be synthesized using a solid phase method, in which the peptides
25 are attached to a resin such as 4-methylbenzhydrylamine (MBHA), 4-(oxymethyl)-phenylacetamido methyl- and 4-(hydroxymethyl)phenoxy methyl-copoly(styrene-1% divinylbenzene) (Wang resin), all of which are commercially available, or to p-nitrobenzophenone oxime polymer (oxime resin) which can be synthesized as described by De Grado and Kaiser, *J. Org. Chem.* 47:3258, 1982. Those skilled in the art will
30 realize that the choice of amino acids and/or amino acid analogs will depend, in part, on

the specific physical, chemical or biological characteristics desired. Such characteristics are determined, in part, by the method of administration and the target location within a patient.

Selective modification of the reactive groups in a peptide can also impart
5 desirable characteristics. Peptides can be manipulated while still attached to the resin to obtain N-terminal modified compounds such as an acetylated peptide or can be removed from the resin using hydrogen fluoride or an equivalent cleaving agent and then modified. Compounds synthesized containing the C-terminal carboxy group (Wang resin) can be modified after cleavage from the resin or, in some cases, prior to solution
10 phase synthesis. Methods for modifying the N-terminus or C-terminus of a peptide are well known in the art and include, for example, methods for acetylation of the N-terminus or amidation of the C-terminus. Similarly, methods for modifying side chains of the amino acids or amino acid analogs are well known to those skilled in the art of peptide synthesis. The choice of modifications made to reactive groups present on the
15 peptide will be determined by the desired characteristics.

A modulating agent may also be a cyclic peptide. A cyclic peptide can be obtained by inducing the formation of a covalent bond between, for example, the amino group at the N-terminus of the peptide and the carboxyl group at the C-terminus. Alternatively, a cyclic peptide can be obtained by forming a covalent bond between a
20 terminal reactive group and a reactive amino acid side chain or between two reactive side chains. It will be apparent to those of skill in the art that a cyclic peptide is selected based on the desired properties. For example, a cyclic peptide may provide increased stability, increased solubility, decreased immunogenicity or decreased clearance *in vivo*.

A newly synthesized peptide can be purified using a method such as
25 reverse phase high performance liquid chromatography (RP-HPLC) or other methods of separation based on size or charge. Furthermore, a purified peptide can be characterized using these and other well known methods such as amino acid analysis and mass spectrometry.

Some representative examples of peptide modulating agents are provided
30 in Table I.

Table I
Representative Peptide Modulating Agents

Peptide	Sequence
pp7	CDS*GLDS*M
pp11	CDDRHDS*GLDS*M
pp15	CDDRHDS*GLDS*MKDEE
pp19	CERLLDDRHDS*GLDS*MKDEE
pp21	CKKERLLDDRHDS*GLDS*MKDEE

5 * indicates phosphorylated residue

Further characterization of modulating agents may be achieved using a ubiquitin-dependent *in vitro* degradation assay. Such an assay may generally be performed as described by Alkalay et al., *Proc. Natl. Acad. Sci. USA* 92:10599, 1995.

10 Within this assay, pI κ B α from stimulated cells is degraded *in vitro* in a ubiquitin-dependent manner, whereas non-phosphorylated I κ B α from the same cell extract is not subject to degradation. Modulating agents that inhibit ubiquitination of I κ B α should also result in stabilization of pI κ B α within such an *in vitro* degradation assay.

Modulating agents as described herein may generally be used to

15 specifically inhibit cellular NF- κ B functions. Such inhibition may generally be demonstrated by microinjection of the agent (e.g., about 5 mg/mL of a peptide agent) into a suitable cell (e.g., HeLa cell or primary human vascular endothelial cell). Following microinjection, cells are stimulated (e.g., with TNF α) and incubated to allow NF- κ B activation. In HeLa cells, TNF α induces rapid nuclear translocation of NF- κ B

20 into the nucleus, which may be detected by staining with p65-specific antibodies. Modulating agents induce a statistically significant decrease in NF- κ B translocation, and may reduce such translocation to undetectable levels.

Primary human vascular endothelial cells (HUVEC) respond to TNF α stimulation by surface expression of NF- κ B regulated adhesion proteins such as ICAM-

1, V-CAM-1 and E-selectin (Read et al., *Immunity* 2:493,1995; Chen et al., *J. Immunol* 155:3538, 1995). E-selectin expression is particularly NF- κ B dependent and is the major inducible endothelial adhesion molecule for initial neutrophil attachment and rolling on activated endothelium. Stimulated cells may be fixed and stained to detect
5 expression of one or more NF- κ B regulated adhesion proteins. Microinjection of a modulating agent results in a statistically significant inhibition of such expression, but does not affect the expression of NF- κ B independent adhesion proteins, such as ICAM2.

Modulating agents may also be used to modulate ubiquitination of I κ B α and/or I κ B β in a patient, thereby modulating NF- κ B cellular function *in vivo*. As used
10 herein, a "patient" may be any mammal, including a human, and may be afflicted with a disease associated with NF- κ B activation, or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Diseases associated with NF- κ B activation include inflammatory diseases, autoimmune diseases, cancer and viral infection.

15 Treatment refers to administration of a modulating agent as described herein. For administration to a patient, one or more such compounds are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not
20 interfere with the activity of the active ingredient). Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Representative carriers include physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Optionally, a
25 pharmaceutical composition may additionally contain preservatives and/or other additives such as, for example, antimicrobial agents, anti-oxidants, chelating agents and/or inert gases, and/or other active ingredients.

Alternatively, a pharmaceutical composition may comprise a polynucleotide encoding a modulating agent (such that the modulating agent is generated
30 *in situ*) in combination with a physiologically acceptable carrier. In such pharmaceutical

compositions, the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, as well as colloidal dispersion systems, including liposomes. Appropriate nucleic acid expression systems contain the necessary polynucleotide
5 sequences for expression in the patient (such as a suitable promoter and terminating signal). DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other
10 pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV).
15 A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that encodes the ligand for a receptor on a specific target cell (to render the vector target specific). For example, retroviral vectors can be made target specific by inserting a nucleotide sequence encoding a sugar, a glycolipid, or a protein. Targeting may also be
20 accomplished using an antibody, by methods known to those of ordinary skill in the art.

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the
25 control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ 2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a

tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.* 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., *Biotechniques* 6:882, 1988).

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity and may be, for example, organ-specific, cell-specific, and/or organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticuloendothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

Routes and frequency of administration, as well doses, will vary from patient to patient. In general, the pharmaceutical compositions may be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity or transdermally. Between 1 and 6 doses may be administered daily. A suitable dose is an amount that is sufficient to show improvement in the symptoms of a patient afflicted with a disease associated with NF- κ B activation. Such improvement may be detected by monitoring inflammatory responses (*e.g.*, edema, transplant rejection, hypersensitivity) or through an improvement in clinical symptoms associated with the disease. In general, the amount of modulating agent present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 1 μ g to about 100 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1Identification of Modulating Agents Using Ubiquitination Assay

5

This Example illustrates a representative ubiquitination assay, and the use of such an assay to evaluate candidate modulating agents.

A. *In vitro* Ubiquitination Assay

HA-tagged I κ B α or HA-tagged I κ B β cDNAs (Haskill et al., *Cell* 10 65:1281-1289, 1991) were translated *in vitro* in wheat germ extract in the presence of ³⁵S-methionine according to the manufacturer's instructions (Promega, Madison, WI). To phosphorylate I κ B α or I κ B β , 1 μ l of the extract containing the labeled protein was incubated for 90 minutes at 30°C in a reaction mixture having a final volume of 30 μ l: 100 μ g HeLa or Jurkat cell extract (prepared as described by Alkalay et al., *Proc. Natl.* 15 *Acad. Sci. USA* 92:10599, 1995), 2mM ATP and 1 μ M okadaic acid. During this incubation, the labeled I κ B polypeptide was phosphorylated at serines 32 and 36, and associated with the endogenous NF- κ B complex (data not shown).

Following incubation, 1 μ l of anti-p65 serum was added, and the NF- κ B immune complex was immobilized to Protein A-Sepharose[®] and subjected to *in vitro* 20 ubiquitination in HeLa cell extract as described by Alkalay et al.. Ubiquitinated proteins were separated by SDS-PAGE and visualized by autoradiography.

As shown in Figure 1A, only wild type ³⁵S-pI κ B α generated multiply ubiquitinated species (lane 4). Neither ³⁵S-labeled S32/36A mutant of I κ B α (lane 1) nor the non-phosphorylated wild type ³⁵S-I κ B α (lane 2) were ubiquitinated, and no 25 ubiquitination of pI κ B α was seen in the absence of ATP (lane 3).

The physiological relevance of this assay was further documented by comparison of *in vitro* ubiquitination of free ³⁵S-I κ B to that of a complex-associated, phosphorylated substrate. Whereas a complex-associated S32/36A mutant was not subject to ubiquitin conjugation in accordance with its *in vivo* fate, free forms of either

mutant or wild type I κ B α were readily conjugated (Figure 1B). Similarly, only free, but not a complex-associated lysine 21, 22 mutant of I κ B α could be ubiquitinated *in vitro* (data not shown). Thus, while the free I κ B α is recognized by the ubiquitin system in a non-discriminatory manner, the complex-associated inhibitor is masked unless it is
5 appropriately phosphorylated.

B. Identification of Peptide Modulating Agents

To identify the I κ B α -ubiquitin ligase recognition motif, various peptides were added at varying concentrations to the reaction mixtures in the presence of the peptidase inhibitor Bestatin (40 μ g/ml). The peptides spanned the N-terminal signaling
10 domain of the protein, and were phosphorylated at one or both serine residues (32 and 36), or were unmodified or serine-substituted. These peptides were included in the ubiquitination reaction at different concentrations and tested for inhibition of pI κ B α specific ubiquitination. When conjugation of free I κ B α was monitored, the translated protein was added directly to the conjugation reaction mixture.

15 Only peptides that were phosphorylated at both serine 32 and 36 (pI κ B α peptides) effectively inhibited pI κ B α ubiquitination (Figure 1A, lanes 7, 11-14). A c-Fos phosphopeptide (ppFos, lane 5), a serine 32, 36 to alanine substituted I κ B α peptide (p21 S/A, lane 6) and a non-phosphorylated peptide (p21, lane 8) had no detectable effect on the ubiquitination of pI κ B at a concentration of 400 μ M. The IC₅₀ of the
20 phosphorylated I κ B α peptides were calculated and representative inhibitory concentrations are shown in Figure 1A. Doubly phosphorylated I κ B α peptides inhibited the pI κ B conjugation reaction (lanes 7, 11-14) at an IC₅₀ of 5 μ M. The sequences of these peptides are provided in Table I, above, and in SEQ ID NOs:5-9. In contrast, singly phosphorylated peptides (lanes 9, 10) inhibited the pI κ B α conjugation at an IC₅₀
25 of 400 μ M. The minimal size peptide tested (pp7, lane 14), merely spanning the signaling phosphorylation site, was sufficient to effectively inhibit the ubiquitination, although at somewhat higher IC₅₀ (10 μ M). Thus, a peptide comprising residues 21 to 41 of SEQ ID NO:1 comprises a recognition domain for E3 ubiquitin ligase.

Interestingly, lysine residues 21 and 22 are not essential for inhibition, implying that the ubiquitin-system recognition site is distinct from the actual conjugation site.

The specificity of the peptide modulating agents was tested in two other ubiquitin-conjugation reactions: the conjugation of free wild type (Figure 1B lanes 1-3) or S32/36A mutant I κ B α (Figure 1B, lanes 4-6) and the ubiquitin conjugation to the bulk of cellular proteins in HeLa extract (detected by ^{125} I-labeled ubiquitin according to Alkalay et al., Figure 1C). Neither reaction was affected by the addition of a peptide modulating agent or a control peptide.

Peptide modulating agents were found to abolish the ubiquitination of the pI κ B α related substrate pI κ B β (Figure 1D). Similar to the conjugation of pI κ B α , the specific conjugation of the I κ B β also required an associated NF- κ B complex (not shown) and prior phosphorylation at the I κ B α -homologous residues Ser 19 and 23. An I κ B β substrate prepared in the absence of phosphatase inhibitors was not subject to ubiquitination (Figure 1D, lane 1). Peptide modulating agents affected pI κ B β ubiquitination at an IC₅₀ that was similar to that observed for pI κ B α (Figure 1D, lanes 4-7). Hence, it appears that the same enzyme(s) target both I κ Bs for ubiquitin-dependent degradation.

The inhibitory pI κ B α peptides were tested in a complementary ubiquitin-dependent *in vitro* degradation assay (Orian et al., *J. Biol. Chem.* 270:21707, 1995; Stancovski et al., *Mol. Cell. Biol.* 15:7106, 1995). Using this assay, only pI κ B α derived from stimulated cells is degraded *in vitro* in a ubiquitin-dependent manner, whereas the non-phosphorylated I κ B α from the same cell extract is not subject to degradation. Incorporation of the conjugation-inhibitory phosphopeptide modulating agents into the degradation assay resulted in stabilization of the pI κ B α substrate (Figure 2, lanes 3, 4) whereas the non-phosphorylated peptide agent or a control phospho-Fos peptide had no effect on the specific pI κ B α degradation (lanes 5, 6). Trimming the peptides at Lys 21/22 did not diminish the degradation inhibitory effect (lane 4), indicating that the peptides do not abolish pI κ B α degradation by exhausting the ubiquitin-proteasome system as conjugatable substrates.

Example 2

Identification of Ubiquitin System Component Involved in Substrate Recognition

This Example illustrates the identification of a specific E3 that is
5 responsible for recognition of pIkB polypeptides.

pIkB α -ubiquitin conjugation and degradation requires a full complement of the ubiquitin system enzymes: E1, a specific E2 derived from the ubiquitin system fraction I, E2F1 (Alkalay et al., *Proc. Natl. Acad. Sci. USA* 92:10599, 1995; Chen et al., *Cell* 84:853, 1996) and a Fraction II-component E3. To identify the ubiquitin system
10 component involved in the substrate recognition, HeLa lysate was fractionated over IkB α phosphopeptide modulating agent columns, and the flow-through fractions were assayed for pIkB α conjugation. Peptides were coupled to NHS-Sepharose[®] (Pharmacia) according to the manufacturer's instructions at a concentration of 2 mg/ml. 100 μ g of HeLa extract were incubated with 2.5 μ l coupled resin in the presence of 0.1% NP40
15 and 3% ovalbumin for 1 hour at 4°C. The resin was discarded and the unbound material tested in the ubiquitination assay described above.

Whereas a flow-through fraction from a control phosphopeptide column and an S32/36A peptide column retained full IkB α conjugation capacity (Figure 3A, lanes 2, 3) flow-through fractions from two different pIkB α peptides lost their IkB α
20 specific conjugation capacity (lanes 4, 7). The depleted conjugating activity could be complemented by reticulocyte Fraction II (lanes 5, 8) that contains all the known species of E3 enzymes (Ciechanover, *Cell* 79:13, 1994). Complementation could not be obtained by the addition of Fraction I or Fraction I and E1 (lanes 6 and 9, respectively), indicating that the peptide columns depleted an E3 rather than E2 or E1. Again, IkB α
25 lysine residues 21 and 22 were dispensable for retaining the E3 (compare Figure 3A, lane 7 to lane 4), emphasizing the distinction between the substrate recognition and conjugation site. The peptide column depletion was found to be specific for the IkB E3, as all flow-through fractions maintained full activity in random HeLa protein conjugation (as detected by measuring the conjugation of ¹²⁵I ubiquitin, Figure 3B). This indicates
30 that a specific E3 is responsible for recognition of the pIkBs at the identified motif.

Example 3

Effect of Representative Peptide Modulating Agents on Cellular NF- κ B Activation

5 This Example illustrates the inhibition of cellular NF- κ B activation by microinjection of peptide modulating agents.

 HeLa cells were plated on a grid coverslips (Cellocate, Eppendorf) 18 hours before microinjection. Microinjection was performed with a 22 amino acid pI κ B α peptide (pp21; Table I and SEQ ID NO:9) or a control phospho-Fos peptide (SEQ ID
10 NO:10) using a semi-automated apparatus (Eppendorf). Peptides were injected into the cell cytoplasm at a concentration of 5 mg/ml in 100 mM KCl, 5mM Na₂HPO₄ (pH 7.2), and immediately activated with TNF α (200 units/mL) for either 20 minutes (for NF- κ B translocation) or 3 hours (for E-selectin expression). Following activation, the cells were fixed and stained with p65 specific antibodies (Mercurio et al., *Genes & Dev.*
15 7:705, 1993; Santa Cruz) or monoclonal anti-E-selectin antibodies (R&D Systems).

 In the absence of peptide modulating agent, TNF α induces rapid nuclear translocation of NF- κ B into the nucleus, as shown by the p65 nuclear staining of 90% of the cells (*see* Figure 4G, column 2). The pp21 peptide abolished TNF α -stimulated NF- κ B activation in 50%-70% of the microinjected cells in several experiments (*see*
20 representative fields in Figures 4A and 4B; and Figure 4G, column 3). In contrast, the control pp-Fos peptide had no effect on the rate of NF- κ B induced nuclear translocation, as compared to non-microinjected cells (Figures 4C and 4G, column 4).

 To further assess the functional consequences of NF- κ B inhibition, the I κ B-E3 inhibitory peptide was microinjected into primary human vascular endothelial
25 cells (HUVEC; Chen et al, *J. Immunol* 155:3538, 1995). These cells respond to TNF α stimulation by surface expression of NF- κ B regulated adhesion proteins, such as E-selectin. HUVEC cells were plated, microinjected and stimulated as described above. Three hours post stimulation the cells were fixed and stained for expression of the NF- κ B dependent E-selectin. 75%-85% of the HUVEC cells were intensely stained for
30 E-selectin following TNF α stimulation in several experiments. Microinjection of the

pp21 peptide resulted in the inhibition of E-selectin expression in 70%-80% of the microinjected cells (Figure 4D; and Figure 4H, column 3). In contrast, the control pp-Fos peptide had no effect on E-selectin expression, as compared to non-microinjected cells (Figures 4F and 4H, column 4). Microinjection of a control, S32/36A substituted
 5 IkB α peptide had no effect on the rate of E-selectin expression (data not shown).

These results demonstrate that the subunit-specific degradation of the signal-induced phosphorylated IkB α and IkB β is mediated by a specific E3. The recognition domain for E3 ubiquitin ligase is a short sequence, centered around the two
 10 signal-acquired phosphoserines conserved in both IkBs, representing the first biologically relevant E3 recognition motif. The specificity in IkB recognition is supported by the context of the phosphorylated substrate: an associated cellular complex masks the substrate from non-specific E3s. This feature restricts the NF- κ B inhibitor degradation to the post-stimulation phase, at which it is exposed through site-specific phosphorylation event(s) to the specific ligase. NF- κ B activation and its resultant
 15 function can be specifically abolished by *in vivo* inhibition of the IkB ligase, using a modulating agent as provided herein.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration,
 20 various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SUMMARY OF SEQUENCE LISTING

SEQ ID NO:1 is amino acid sequence of IkB α
 SEQ ID NO:2 is DNA sequence of IkB α
 25 SEQ ID NO:3 is amino acid sequence of IkB β
 SEQ ID NO:4 is DNA sequence of IkB β
 SEQ ID NO:5 is amino acid sequence of pp7
 SEQ ID NO:6 is amino acid sequence of pp11
 SEQ ID NO:7 is amino acid sequence of pp15

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SEQ ID NO:8 is amino acid sequence of pp19

SEQ ID NO:9 is amino acid sequence of pp21

SEQ ID NO:10 is amino acid sequence of phospho-Fos peptide

SEQ ID NO:11 is amino acid sequence of pp21 S/A

SEQ ID NO:12 is amino acid sequence of HA-tagged I κ B α

SEQ ID NO:13 is amino acid sequence of HA-tagged S32, 36 I κ B α

SEQ ID NO:14 is amino acid sequence of HA-tagged I κ B β

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Alkalay, Irit
Ben-Neriah, Yinon
Ciechanover, Aaron
Manning, Anthony
Mercurio, Frank
Yaron, Avraham
- (ii) TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR MODULATING
CELLULAR NF- κ B ACTIVATION
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: SEED and BERRY LLP
 - (B) STREET: 6300 Columbia Center, 701 Fifth Avenue
 - (C) CITY: Seattle
 - (D) STATE: Washington
 - (E) COUNTRY: USA
 - (F) ZIP: 98104
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 14-FEB-1997
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Maki, David J.
 - (B) REGISTRATION NUMBER: 31,392
 - (C) REFERENCE/DOCKET NUMBER: 860098.418
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (206) 622-4900
 - (B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 317 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Phe Gln Ala Ala Glu Arg Pro Gln Glu Trp Ala Met Glu Gly Pro

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1	5	10	15
Arg Asp Gly Leu Lys Lys Glu Arg Leu Leu Asp Asp Arg His Asp Ser	20	25	30
Gly Leu Asp Ser Met Lys Asp Glu Glu Tyr Glu Gln Met Val Lys Glu	35	40	45
Leu Gln Glu Ile Arg Leu Glu Pro Gln Glu Val Pro Arg Gly Ser Glu	50	55	60
Pro Trp Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu	65	70	75
Ala Ile Ile His Glu Glu Lys Ala Leu Thr Met Glu Val Ile Arg Gln	85	90	95
Val Lys Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln	100	105	110
Thr Pro Leu His Leu Ala Val Ile Thr Asn Gln Pro Glu Ile Ala Glu	115	120	125
Ala Leu Leu Gly Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly	130	135	140
Asn Thr Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val	145	150	155
Gly Val Leu Thr Gln Ser Cys Thr Thr Pro His Leu His Ser Ile Leu	165	170	175
Lys Ala Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Ile	180	185	190
His Gly Tyr Leu Gly Ile Val Glu Leu Leu Val Ser Leu Gly Ala Asp	195	200	205
Val Asn Ala Gln Glu Pro Cys Asn Gly Arg Thr Ala Leu His Leu Ala	210	215	220
Val Asp Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly	225	230	235
Ala Asp Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu	245	250	255
Thr Trp Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu	260	265	270
Thr Leu Glu Asn Leu Gln Met Leu Pro Glu Ser Glu Asp Glu Glu Ser	275	280	285
Tyr Asp Thr Glu Ser Glu Phe Thr Glu Phe Thr Glu Asp Glu Leu Pro	290	295	300
Tyr Asp Asp Cys Val Phe Gly Gly Gln Arg Leu Thr Leu	305	310	315

(2) INFORMATION FOR SEQ ID NO:2:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1550 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCCGCCGTC CCGCCCGCCA GCGCCCCAGC GAGGAAGCAG CGCGCAGCCC GCGGCCCCAGC	60
GCACCCGCAG CAGCGCCCGC AGCTCGTCCG CGCCATGTTC CAGGCGGCCG AGCGCCCCCA	120
GGAGTGGGCC ATGGAGGGCC CCCGCGACGG GCTGAAGAAG GAGCGGCTAC TGGACGACCG	180
CCACGACAGC GGCCTGGACT CCATGAAAGA CGAGGAGTAC GAGCAGATGG TCAAGGAGCT	240
GCAGGAGATC CGCCTCGAGC CGCAGGAGGT GCCGCGCGGC TCGGAGCCCT GGAAGCAGCA	300
GCTCACCGAG GACGGGGACT CGTTCCTGCA CTTGGCCATC ATCCATGAAG AAAAGGCACT	360
GACCATGGAA GTGATCCGCC AGGTGAAGGG AGACCTGGCT TTCCTCAACT TCCAGAACAA	420
CCTGCAGCAG ACTCCACTCC ACTTGGCTGT GATCACCAAC CAGCCAGAAA TTGCTGAGGC	480
ACTTCTGGGA GCTGGCTGTG ATCCTGAGCT CCGAGACTTT CGAGGAAATA CCCCCCTACA	540
CCTTGCCTGT GAGCAGGGCT GCCTGGCCAG CGTGGGAGTC CTGACTCAGT CCTGCACCAC	600
CCCGCACCTC CACTCCATCC TGAAGGCTAC CAACTACAAT GGCCACACGT GTCTACACTT	660
AGCCTCTATC CATGGCTACC TGGGCATCGT GGAGCTTTTG GTGTCCTTGG GTGCTGATGT	720
CAATGCTCAG GAGCCCTGTA ATGGCCGGAC TGCCCTTCAC CTCGCAGTGG ACCTGCAAAA	780
TCCTGACCTG GTGTCACTCC TGTTGAAGTG TGGGGCTGAT GTCAACAGAG TTACCTACCA	840
GGGCTATTCT CCCTACCAGC TCACCTGGGG CCGCCCAAGC ACCCGGATAC AGCAGCAGCT	900
GGGCCAGCTG AACTAGAAA ACCTTCAGAT GCTGCCAGAG AGTGAGGATG AGGAGAGCTA	960
TGACACAGAG TCAGAGTTCA CGGAGTTCAC AGAGGACGAG CTGCCCTATG ATGACTGTGT	1020
GTTTGGAGGC CAGCGTCTGA CGTTATGAGT GCAAAGGGGC TGAAAGAACA TGGACTTGTA	1080
TATTTGTACA AAAAAAAGT TTTATTTTTC TAAAAAAGA AAAAAGAAGA AAAAATTAA	1140
AGGGTGTACT TATATCCACA CTGCACACTG CCTAGCCCAA AACGTCTTAT TGTGGTAGGA	1200
TCAGCCCTCA TTTTGTGCT TTTGTGAACT TTTGTAGGG GACGAGAAAG ATCATTGAAA	1260
TTCTGAGAAA ACTTCTTTTA AACCTCACCT TTGTGGGGTT TTTGGAGAAG GTTATCAAAA	1320
ATTCATGGA AGGACCACAT TTTATATTTA TTGTGCTTCG AGTGACTGAC CCCAGTGGTA	1380
TCCTGTGACA TGTAACAGCC AGGAGTGTTA AGCGTTCAGT GATGTGGGGT GAAAAGTTAC	1440
TACCTGTCAA GGTTTGTGTT ACCCTCCTGT AAATGGTGTA CATAATGTAT TGTTGGTAAT	1500

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TATTTTGGTA CTTTATGAT GTATATTTAT TAAAGAGATT TTTACAAATG

1550

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Ala	Gly	Val	Ala	Cys	Leu	Gly	Lys	Thr	Ala	Asp	Ala	Asp	Glu	Trp	1	5	10	15
Cys	Asp	Ser	Gly	Leu	Gly	Ser	Leu	Gly	Pro	Asp	Ala	Ala	Ala	Pro	Gly	20	25	30	
Gly	Pro	Gly	Leu	Gly	Ala	Glu	Leu	Gly	Pro	Glu	Leu	Ser	Trp	Ala	Pro	35	40	45	
Leu	Val	Phe	Gly	Tyr	Val	Thr	Glu	Asp	Gly	Asp	Thr	Ala	Leu	His	Leu	50	55	60	
Ala	Val	Ile	His	Gln	His	Glu	Pro	Phe	Leu	Asp	Phe	Leu	Leu	Gly	Phe	65	70	75	80
Ser	Ala	Gly	His	Glu	Tyr	Leu	Asp	Leu	Gln	Asn	Asp	Leu	Gly	Gln	Thr	85	90	95	
Ala	Leu	His	Leu	Ala	Ala	Ile	Leu	Gly	Glu	Ala	Ser	Thr	Val	Glu	Lys	100	105	110	
Leu	Tyr	Ala	Ala	Gly	Ala	Gly	Val	Leu	Val	Ala	Glu	Arg	Gly	Gly	His	115	120	125	
Thr	Ala	Leu	His	Leu	Ala	Cys	Arg	Val	Arg	Ala	His	Thr	Cys	Ala	Cys	130	135	140	
Val	Leu	Leu	Gln	Pro	Arg	Pro	Ser	His	Pro	Arg	Asp	Ala	Ser	Asp	Thr	145	150	155	160
Tyr	Leu	Thr	Gln	Ser	Gln	Asp	Cys	Thr	Pro	Asp	Thr	Ser	His	Ala	Pro	165	170	175	
Ala	Ala	Val	Asp	Ser	Gln	Pro	Asn	Pro	Glu	Asn	Glu	Glu	Glu	Pro	Arg	180	185	190	
Asp	Glu	Asp	Trp	Arg	Leu	Gln	Leu	Glu	Ala	Glu	Asn	Tyr	Asp	Gly	His	195	200	205	
Thr	Pro	Leu	His	Val	Ala	Val	Ile	His	Lys	Asp	Ala	Glu	Met	Val	Arg	210	215	220	
Leu	Leu	Arg	Asp	Ala	Gly	Ala	Asp	Leu	Asn	Lys	Pro	Glu	Pro	Thr	Cys	225	230	235	240

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Gly Arg Thr Pro Leu His Leu Ala Val Glu Ala Gln Ala Ala Ser Val
 245 250 255
 Leu Glu Leu Leu Leu Lys Ala Gly Ala Asp Pro Thr Ala Arg Met Tyr
 260 265 270
 Gly Gly Arg Thr Pro Leu Gly Ser Ala Leu Leu Arg Pro Asn Pro Ile
 275 280 285
 Leu Ala Arg Leu Leu Arg Ala His Gly Ala Pro Glu Pro Glu Asp Glu
 290 295 300
 Asp Asp Lys Leu Ser Pro Cys Ser Ser Ser Gly Ser Asp Ser Asp Ser
 305 310 315 320
 Asp Asn Arg Asp Glu Gly Asp Glu Tyr Asp Asp Ile Val Val His Ser
 325 330 335
 Gly Arg Ser Gln Asn Arg Gln Pro Pro Ser Pro Ala Ser Lys Pro Leu
 340 345 350
 Pro Asp Asp Pro Asn Pro Ala
 355

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1212 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGCACTGGA GCTCATCGCA GAGCCCAGCG ACAGGCAGGC GACCACAGGG GGCCACCCGA	60
GGTGGCTGGG GCCATGGCCG GGGTCGCGTG CTTGGGGAAA ACTGCGGATG CCGATGAATG	120
GTGCGACAGC GGCCTGGGCT CTCTAGGTCC CGACGCAGCG GCTCCCGGAG GACCAGGTCT	180
GGGCGCAGAG CTTGGCCCAG AGCTGTCGTG GCGGCCCTTA GTCTTTGGCT ACGTCACTGA	240
GGATGGGGAC ACAGCCCTGC ACTTGGCTGT GATTCATCAG CATGAGCCCT TCCTGGATTT	300
CCTCCTGGGC TTTTCCGCCG GCCACGAGTA CCTTGACCTG CAGAATGACC TAGGCCAAAC	360
AGCCCTGCAT CTAGCAGCCA TCCTTGGGGA GGCATCTACA GTAGAGAAGT TGTATGCAGC	420
CGGTGCAGGA GTGTTGGTGG CTGAGAGAGG GGGCCACACG GCATTGCACT TGGCCTGCCG	480
GGTCAGGGCA CACACGTGCG CGTGCGTACT GCTCCAGCCC CGTCCCAGCC ACCCAAGAGA	540
TGCCTCAGAT ACCTACCTCA CTCAGAGCCA GGACTGTACC CCAGACACCA GCCATGCCCC	600
TGCTGCCGTG GATTCCCAAC CCAACCCAGA GAACGAAGAG GAGCCGCGTG ATGAAGACTG	660

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GAGGCTACAA CTAGAAGCTG AAAACTATGA TGGCCATACC CCACTCCATG TAGCTGTCAT	720
CCACAAAGAT GCAGAGATGG TCCGGCTGCT CAGGGATGCC GGAGCCGACC TCAATAAACC	780
GGAGCCTACG TGTGGCCGGA CCCCTCTGCA CCTGGCAGTA GAAGCCCAGG CAGCCAGCGT	840
GCTGGAACTT CTCCTGAAAG CCGGTGCTGA CCCACCGCC CGCATGTATG GGGGCCGCAC	900
CCCGCTTGGC AGTGCCCTGC TCCGGCCCAA CCCCATCCTT GCCCGCCTCC TCCGTGCACA	960
TGGGGCCCCT GAACCTGAGG ACGAGGACGA TAAGCTTAGC CCTTGCAGCA GCAGCGGCAG	1020
CGACAGTGAC AGTGACAACA GAGATGAGGG CGATGAATAT GATGACATCG TGGTTCACAG	1080
TGGCAGGAGC CAAAACCGAC AACCGCCTTC CCCGGCATCC AAACCTCTTC CTGATGACCC	1140
CAACCCTGCC TGACTIONAAGT GCTAATATTA ATATAATTTC CAACTTAATA AAATTGCAGA	1200
CCTGACAACC AG	1212

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys	Asp	Ser	Gly	Leu	Asp	Ser	Met
1				5			

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys	Asp	Asp	Arg	His	Asp	Ser	Gly	Leu	Asp	Ser	Met
1				5					10		

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys	Asp	Asp	Arg	His	Asp	Ser	Gly	Leu	Asp	Ser	Met	Lys	Asp	Glu	Glu
1				5				10						15	

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys	Glu	Arg	Leu	Leu	Asp	Asp	Arg	His	Asp	Ser	Gly	Leu	Asp	Ser	Met
1				5				10						15	
Lys Asp Glu Glu															
20															

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys	Lys	Lys	Glu	Arg	Leu	Leu	Asp	Asp	Arg	His	Asp	Ser	Gly	Leu	Asp
1				5				10						15	
Ser Met Lys Asp Glu Glu															
20															

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile	Gly	Arg	Arg	Gly	Lys	Val	Glu	Gln	Leu	Ser	Pro	Glu	Glu	Glu	Glu
1				5					10					15	
Lys Arg Arg															

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys	Lys	Lys	Glu	Arg	Leu	Leu	Asp	Asp	Arg	His	Asp	Ala	Gly	Leu	Asp
1				5					10					15	
Ala Met Lys Asp Glu Glu															
20															

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 347 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Met	Tyr	Pro	Tyr	Asp	Val
1				5					10					15	
Pro Asp Tyr Ala Met Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Met Phe															
20 25 30															
Gln Ala Ala Glu Arg Pro Gln Glu Trp Ala Met Glu Gly Pro Arg Asp															
35 40 45															
Gly Leu Lys Lys Glu Arg Leu Leu Asp Asp Arg His Asp Ser Gly Leu															
50 55 60															
Asp Ser Met Lys Asp Glu Glu Tyr Glu Gln Met Val Lys Glu Leu Gln															
65 70 75 80															
Glu Ile Arg Leu Glu Pro Gln Glu Val Pro Arg Gly Ser Glu Pro Trp															
85 90 95															

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Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu Ala Ile
 100 105 110
 Ile His Glu Glu Lys Ala Leu Thr Met Glu Val Ile Arg Gln Val Lys
 115 120 125
 Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln Thr Pro
 130 135 140
 Leu His Leu Ala Val Ile Thr Asn Gln Pro Glu Ile Ala Glu Ala Leu
 145 150 155 160
 Leu Gly Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly Asn Thr
 165 170 175
 Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val Gly Val
 180 185 190
 Leu Thr Gln Ser Cys Thr Thr Pro His Leu His Ser Ile Leu Lys Ala
 195 200 205
 Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Ile His Gly
 210 215 220
 Tyr Leu Gly Ile Val Glu Leu Leu Val Ser Leu Gly Ala Asp Val Asn
 225 230 235 240
 Ala Gln Glu Pro Cys Asn Gly Arg Thr Ala Leu His Leu Ala Val Asp
 245 250 255
 Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly Ala Asp
 260 265 270
 Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu Thr Trp
 275 280 285
 Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu Thr Leu
 290 295 300
 Glu Asn Leu Gln Met Leu Pro Glu Ser Glu Asp Glu Glu Ser Tyr Asp
 305 310 315 320
 Thr Glu Ser Glu Phe Thr Glu Phe Thr Glu Asp Glu Leu Pro Tyr Asp
 325 330 335
 Asp Cys Val Phe Gly Gly Gln Arg Leu Thr Leu
 340 345

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 347 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Met	Tyr	Pro	Tyr	Asp	Val	1	5	10	15
Pro	Asp	Tyr	Ala	Met	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Met	Phe	20	25	30	
Gln	Ala	Ala	Glu	Arg	Pro	Gln	Glu	Trp	Ala	Met	Glu	Gly	Pro	Arg	Asp	35	40	45	
Gly	Leu	Lys	Lys	Glu	Arg	Leu	Leu	Asp	Asp	Arg	His	Asp	Ala	Gly	Leu	50	55	60	
Asp	Ala	Met	Lys	Asp	Glu	Glu	Tyr	Glu	Gln	Met	Val	Lys	Glu	Leu	Gln	65	70	75	80
Glu	Ile	Arg	Leu	Glu	Pro	Gln	Glu	Val	Pro	Arg	Gly	Ser	Glu	Pro	Trp	85	90	95	
Lys	Gln	Gln	Leu	Thr	Glu	Asp	Gly	Asp	Ser	Phe	Leu	His	Leu	Ala	Ile	100	105	110	
Ile	His	Glu	Glu	Lys	Ala	Leu	Thr	Met	Glu	Val	Ile	Arg	Gln	Val	Lys	115	120	125	
Gly	Asp	Leu	Ala	Phe	Leu	Asn	Phe	Gln	Asn	Asn	Leu	Gln	Gln	Thr	Pro	130	135	140	
Leu	His	Leu	Ala	Val	Ile	Thr	Asn	Gln	Pro	Glu	Ile	Ala	Glu	Ala	Leu	145	150	155	160
Leu	Gly	Ala	Gly	Cys	Asp	Pro	Glu	Leu	Arg	Asp	Phe	Arg	Gly	Asn	Thr	165	170	175	
Pro	Leu	His	Leu	Ala	Cys	Glu	Gln	Gly	Cys	Leu	Ala	Ser	Val	Gly	Val	180	185	190	
Leu	Thr	Gln	Ser	Cys	Thr	Thr	Pro	His	Leu	His	Ser	Ile	Leu	Lys	Ala	195	200	205	
Thr	Asn	Tyr	Asn	Gly	His	Thr	Cys	Leu	His	Leu	Ala	Ser	Ile	His	Gly	210	215	220	
Tyr	Leu	Gly	Ile	Val	Glu	Leu	Leu	Val	Ser	Leu	Gly	Ala	Asp	Val	Asn	225	230	235	240
Ala	Gln	Glu	Pro	Cys	Asn	Gly	Arg	Thr	Ala	Leu	His	Leu	Ala	Val	Asp	245	250	255	
Leu	Gln	Asn	Pro	Asp	Leu	Val	Ser	Leu	Leu	Leu	Lys	Cys	Gly	Ala	Asp	260	265	270	
Val	Asn	Arg	Val	Thr	Tyr	Gln	Gly	Tyr	Ser	Pro	Tyr	Gln	Leu	Thr	Trp	275	280	285	
Gly	Arg	Pro	Ser	Thr	Arg	Ile	Gln	Gln	Gln	Leu	Gly	Gln	Leu	Thr	Leu	290	295	300	
Glu	Asn	Leu	Gln	Met	Leu	Pro	Glu	Ser	Glu	Asp	Glu	Glu	Ser	Tyr	Asp	305	310	315	320

Val Asp Ser Gln Pro Asn Pro Glu Asn Glu Glu Glu Pro Arg Asp Glu

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210	215	220
Asp Trp Arg Leu Gln Leu Glu Ala Glu Asn Tyr Asp Gly His Thr Pro 225 230 235 240		
Leu His Val Ala Val Ile His Lys Asp Ala Glu Met Val Arg Leu Leu 245 250 255		
Arg Asp Ala Gly Ala Asp Leu Asn Lys Pro Glu Pro Thr Cys Gly Arg 260 265 270		
Thr Pro Leu His Leu Ala Val Glu Ala Gln Ala Ala Ser Val Leu Glu 275 280 285		
Leu Leu Leu Lys Ala Gly Ala Asp Pro Thr Ala Arg Met Tyr Gly Gly 290 295 300		
Arg Thr Pro Leu Gly Ser Ala Leu Leu Arg Pro Asn Pro Ile Leu Ala 305 310 315 320		
Arg Leu Leu Arg Ala His Gly Ala Pro Glu Pro Glu Asp Glu Asp Asp 325 330 335		
Lys Leu Ser Pro Cys Ser Ser Ser Gly Ser Asp Ser Asp Ser Asp Asn 340 345 350		
Arg Asp Glu Gly Asp Glu Tyr Asp Asp Ile Val Val His Ser Gly Arg 355 360 365		
Ser Gln Asn Arg Gln Pro Pro Ser Pro Ala Ser Lys Pro Leu Pro Asp 370 375 380		
Asp Pro Asn Pro Ala 385		

CLAIMS

1. A method for assaying the ability of an I κ B polypeptide to undergo ubiquitination *in vivo*, comprising:
 - (a) incubating an I κ B polypeptide with a cellular extract, wherein the step of incubating is carried out under conditions and for a time sufficient to allow phosphorylation of the I κ B polypeptide and formation of a complex comprising phosphorylated I κ B polypeptide and NF- κ B;
 - (b) subsequently subjecting the complex to *in vitro* ubiquitination; and
 - (c) subsequently evaluating the extent of ubiquitination of the complex, and thereby assaying the ability of the I κ B polypeptide to undergo ubiquitination *in vivo*.
2. A method for identifying an agent that modulates ubiquitination of I κ B α and/or I κ B β , comprising:
 - (a) incubating a candidate agent with an I κ B polypeptide and a cellular extract, wherein the step of incubating is carried out under conditions and for a time sufficient to allow phosphorylation of the I κ B polypeptide and formation of a complex comprising phosphorylated I κ B polypeptide and NF- κ B; and
 - (b) subsequently measuring the ability of the candidate agent to modulate ubiquitination of the complex, and therefrom identifying an agent that modulates ubiquitination of I κ B α and/or I κ B β .
3. A method according to claim 2, wherein the agent is a peptide.
4. A method according to claim 3, wherein the peptide comprises a recognition domain for E3 ubiquitin ligase.
5. An agent that modulates ubiquitination of I κ B α and/or I κ B β .

6. An agent according to claim 5, wherein the agent is a peptide.
7. An agent according to claim 6, wherein the agent comprises a recognition domain for E3 ubiquitin ligase.
8. An agent according to claim 7, wherein the agent comprises an amino acid sequence recited in at least one of SEQ ID NO:5 - SEQ ID NO:9.
9. An isolated DNA molecule encoding an agent according to at least one of claims 6-8.
10. A recombinant expression vector comprising a DNA molecule according to claim 9.
11. A host cell transformed or transfected with an expression vector according to claim 10.
12. A host cell according to claim 11, wherein the host cell is selected from the group consisting of bacteria, yeast, baculovirus infected insect cells and mammalian cells.
13. A pharmaceutical composition comprising an agent that modulates ubiquitination of I κ B α and/or I κ B β in combination with a pharmaceutically acceptable carrier.
14. A method for modulating NF- κ B activity in a patient, comprising administering to a patient a pharmaceutical composition according to claim 13.
15. An agent, according to any one of claims 5-8, for use in the manufacture of a medicament for treating a patient afflicted with a disorder associated with NF- κ B activation.

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16. An agent according to claim 15, wherein the disorder is selected from the group consisting of inflammatory diseases, autoimmune diseases, cancer and viral infection.

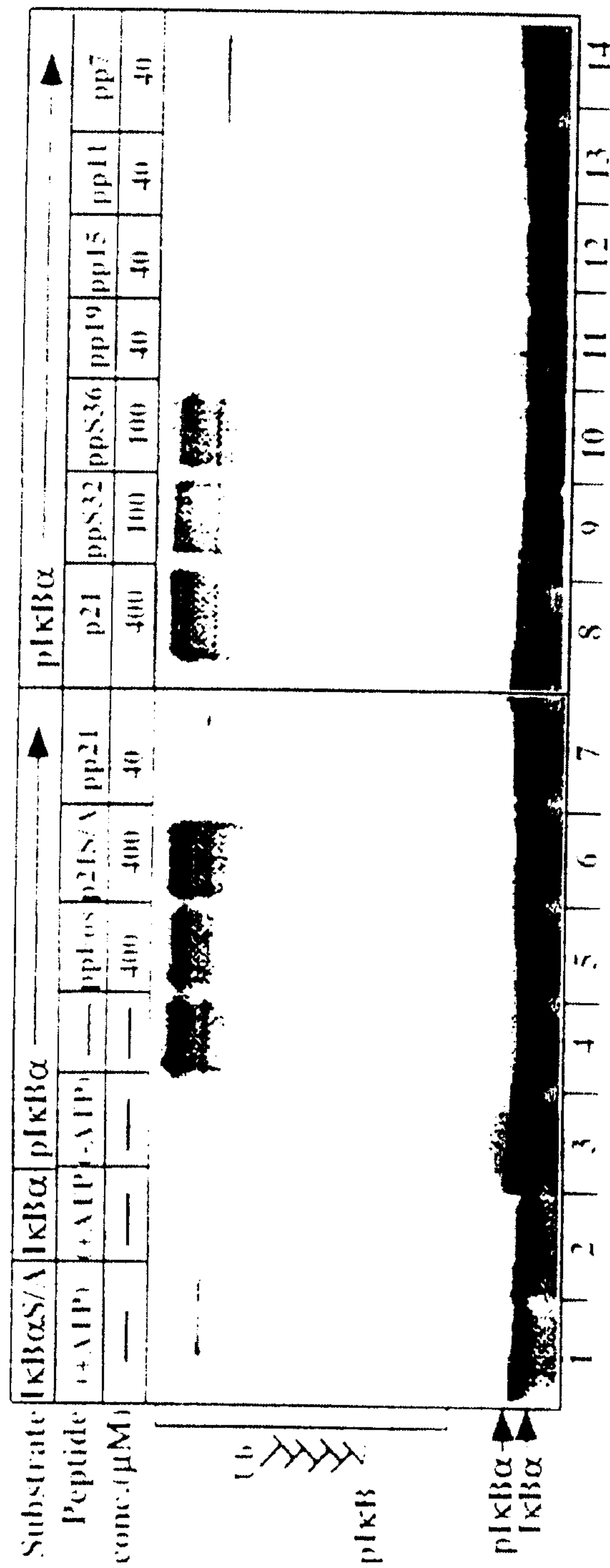


Fig. 1A

Fig. 1B

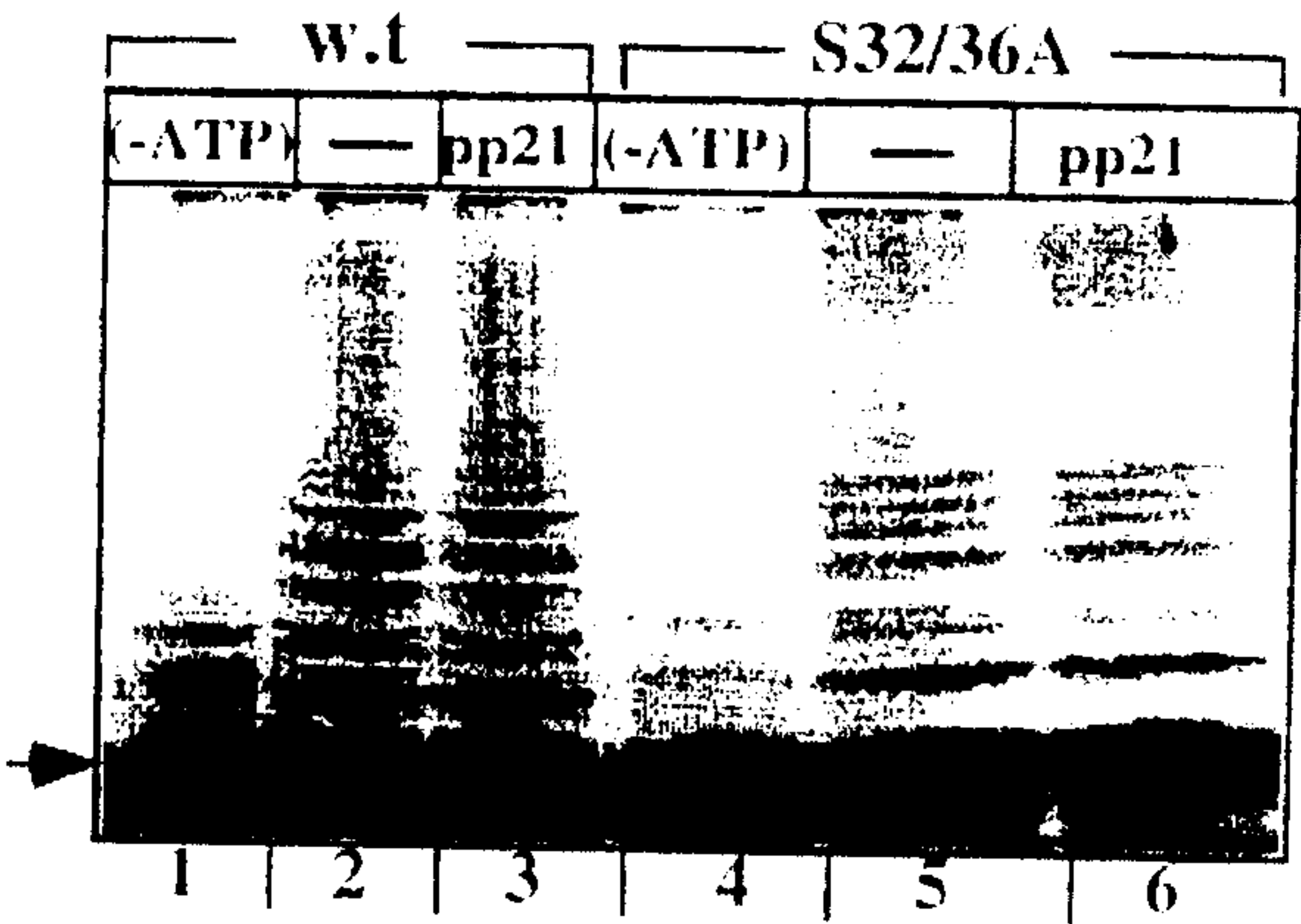


Fig. 1C

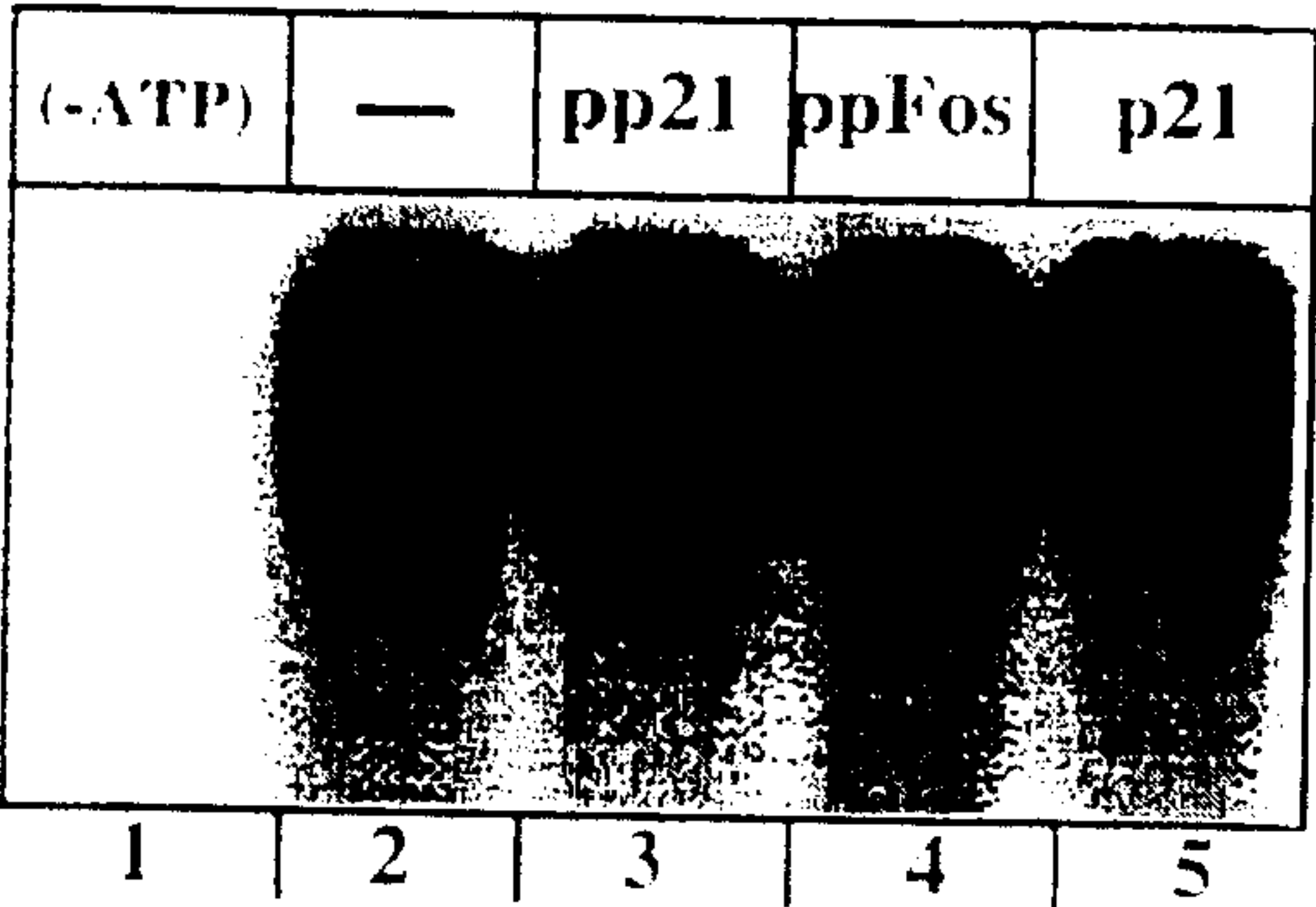
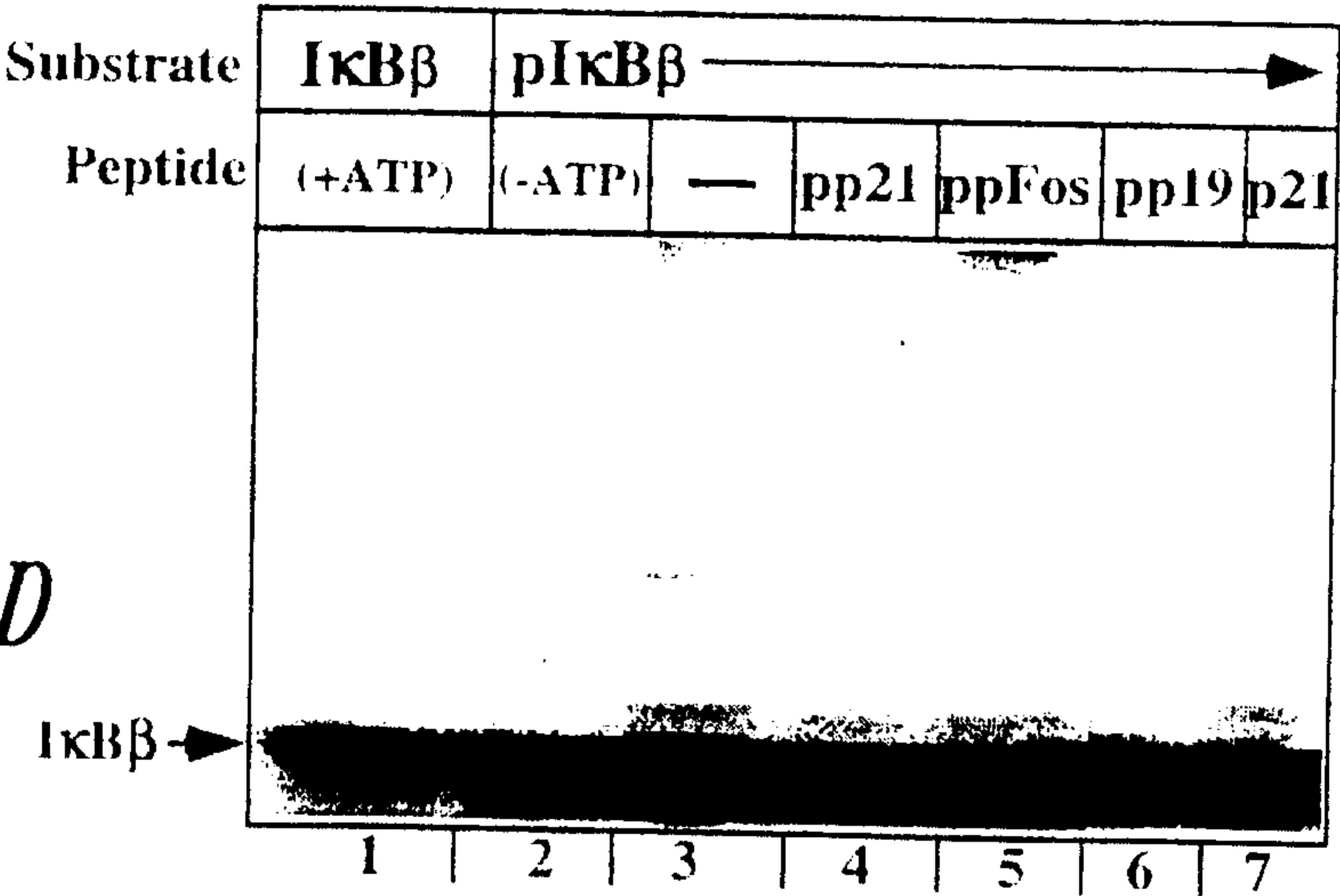


Fig. 1D



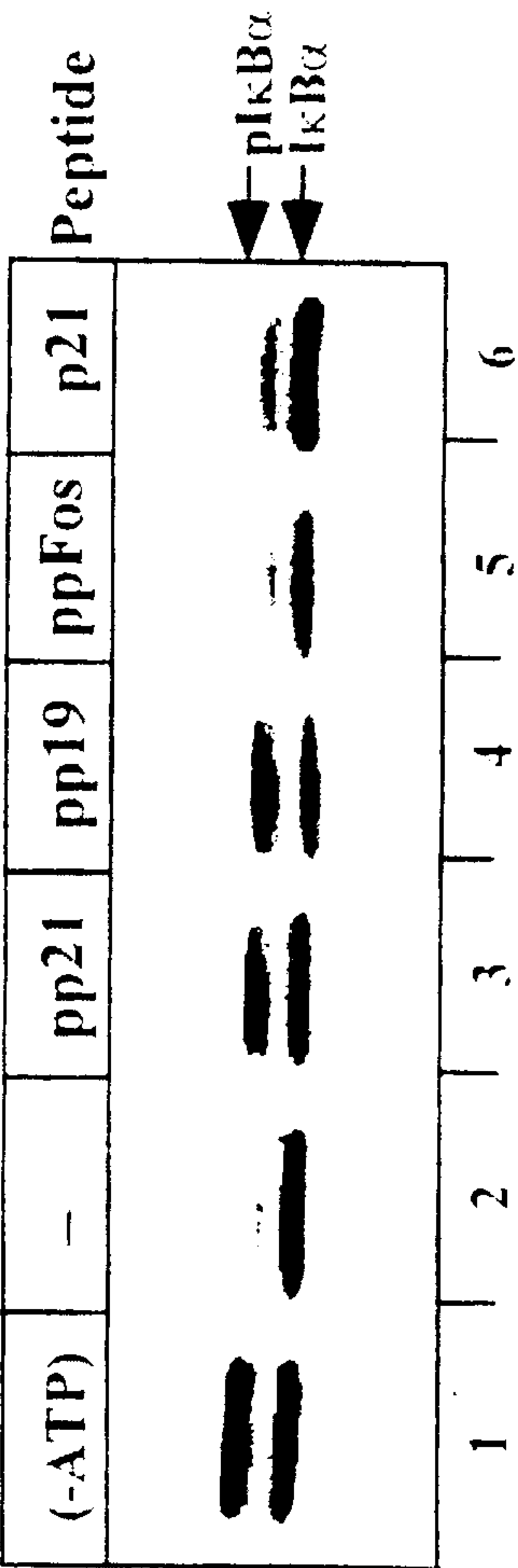


Fig. 2

Fig. 3A

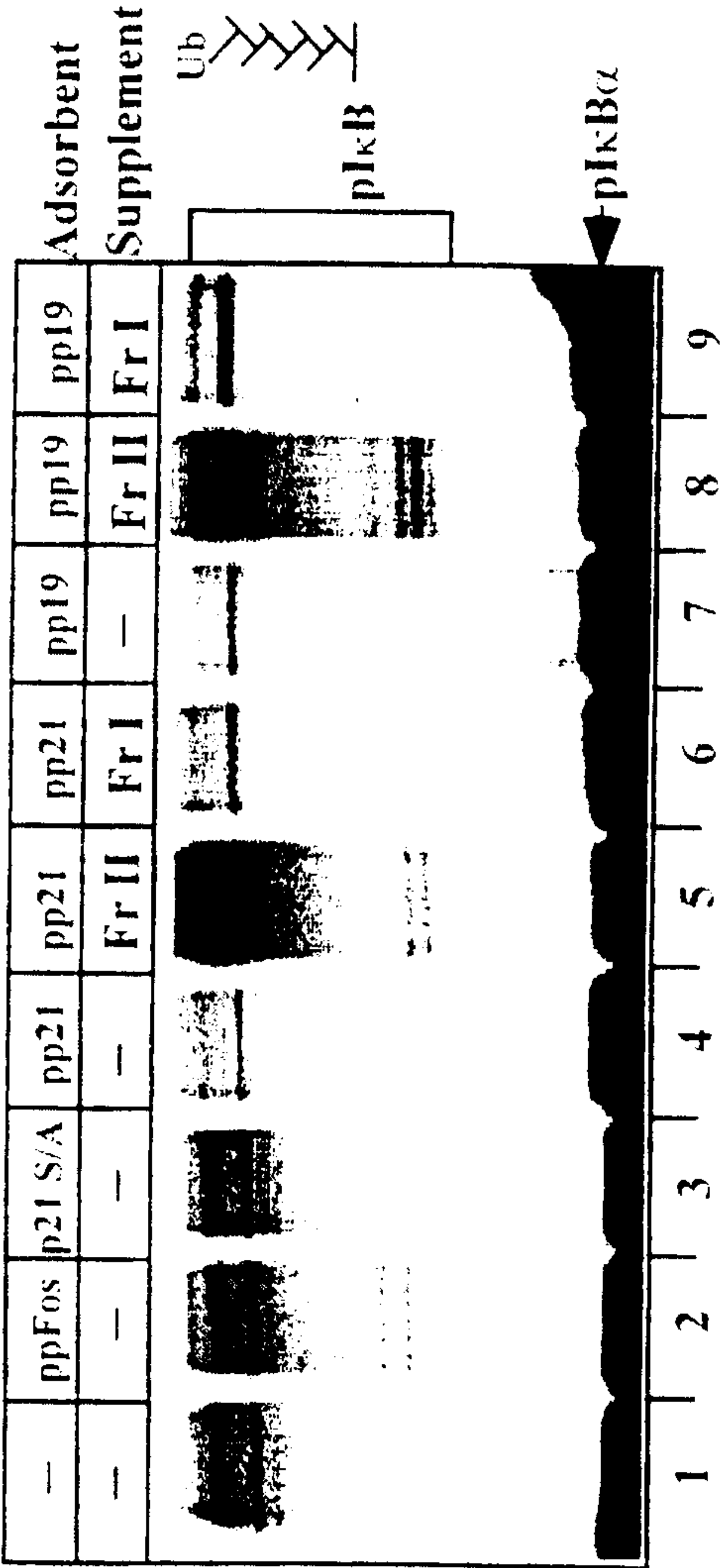
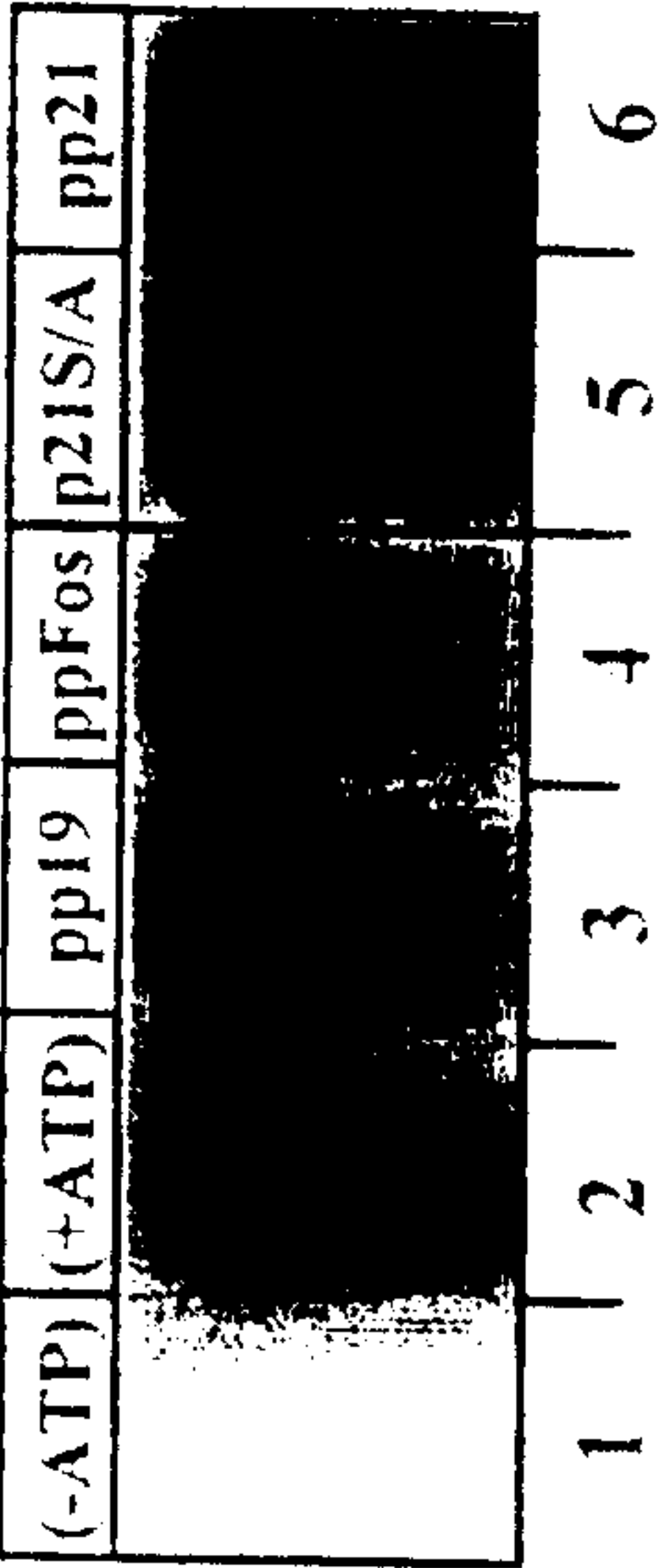


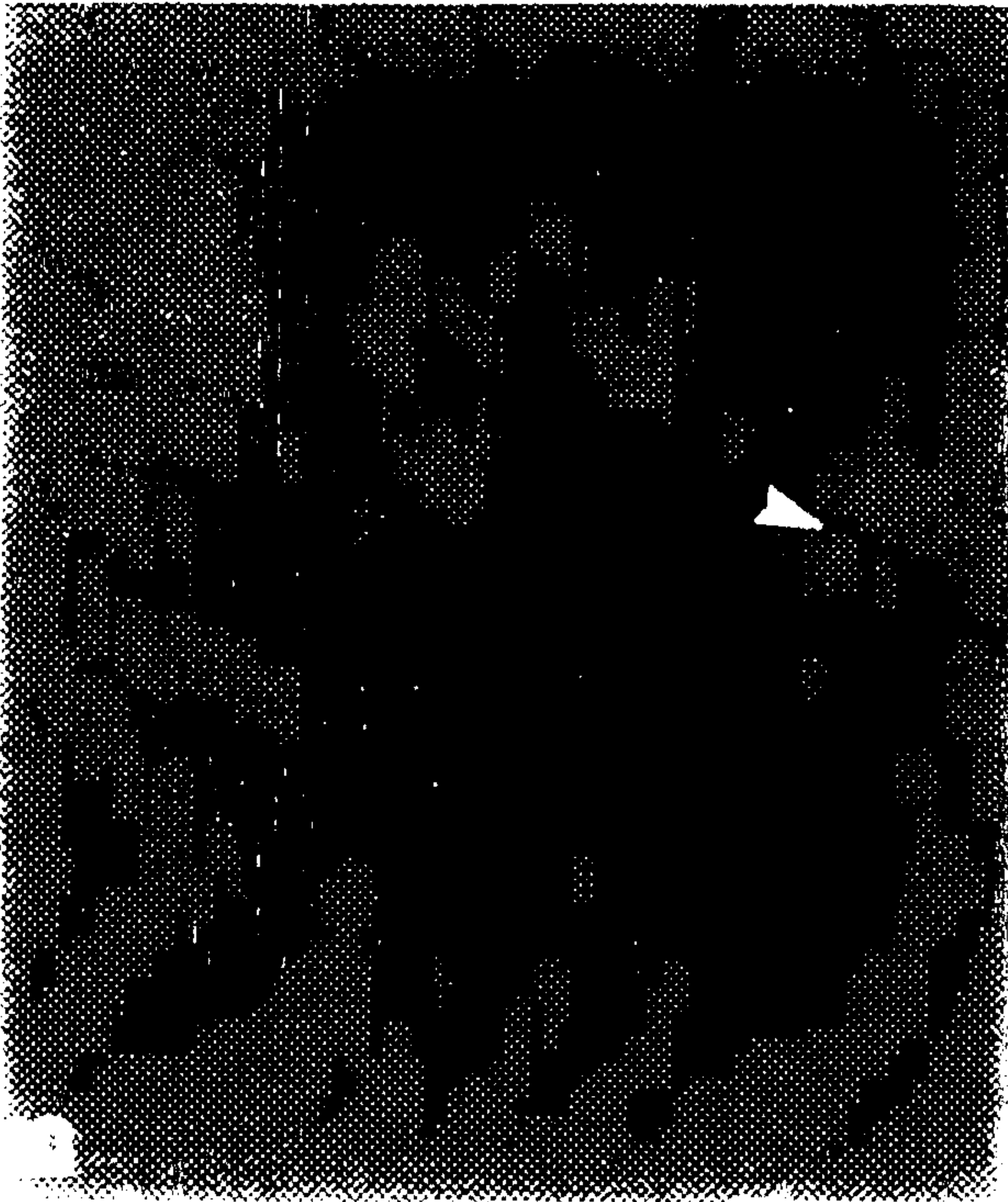
Fig. 3B



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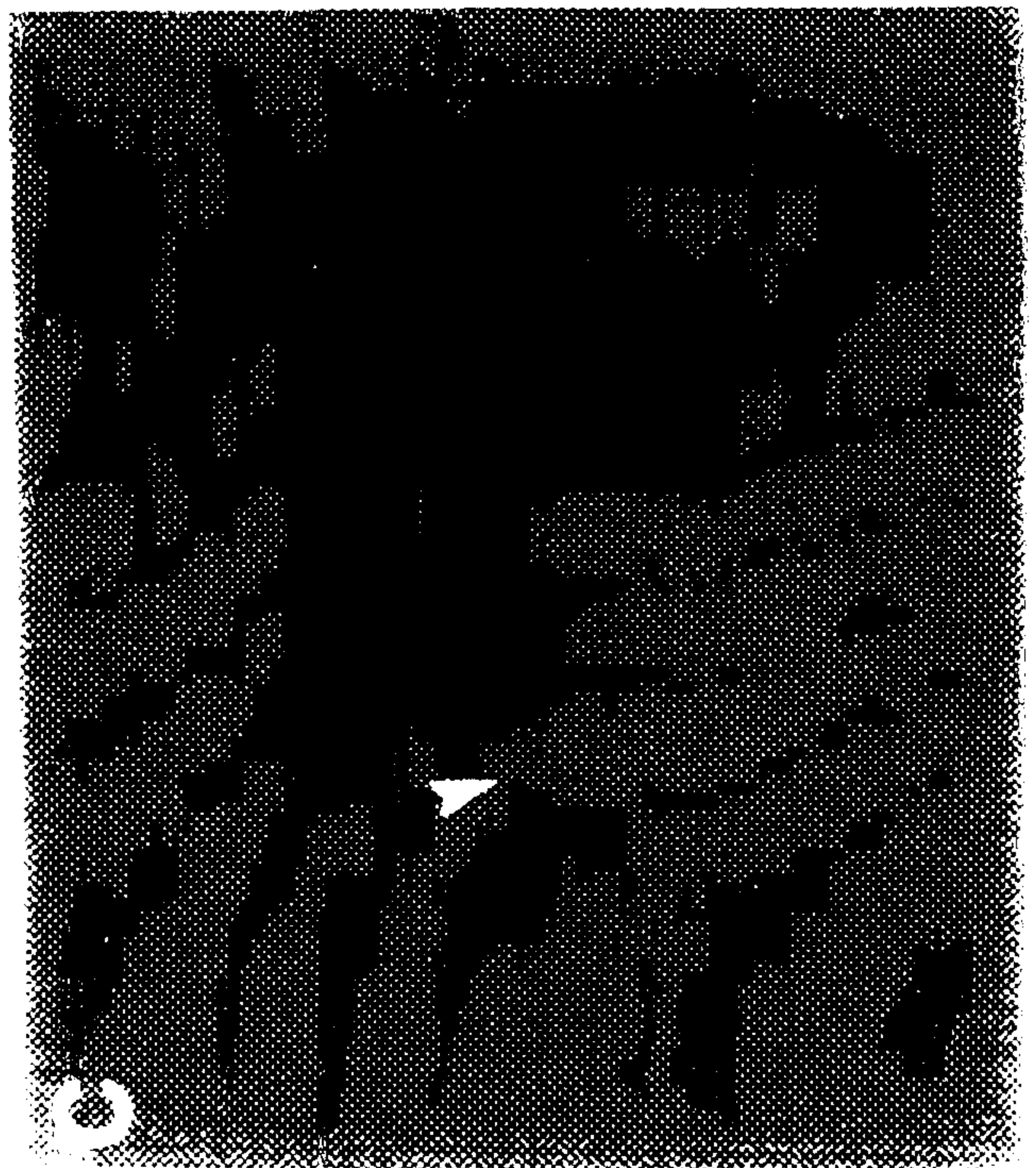
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4B

Fig. 1



4B

Fig. 1

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Fig. 4B

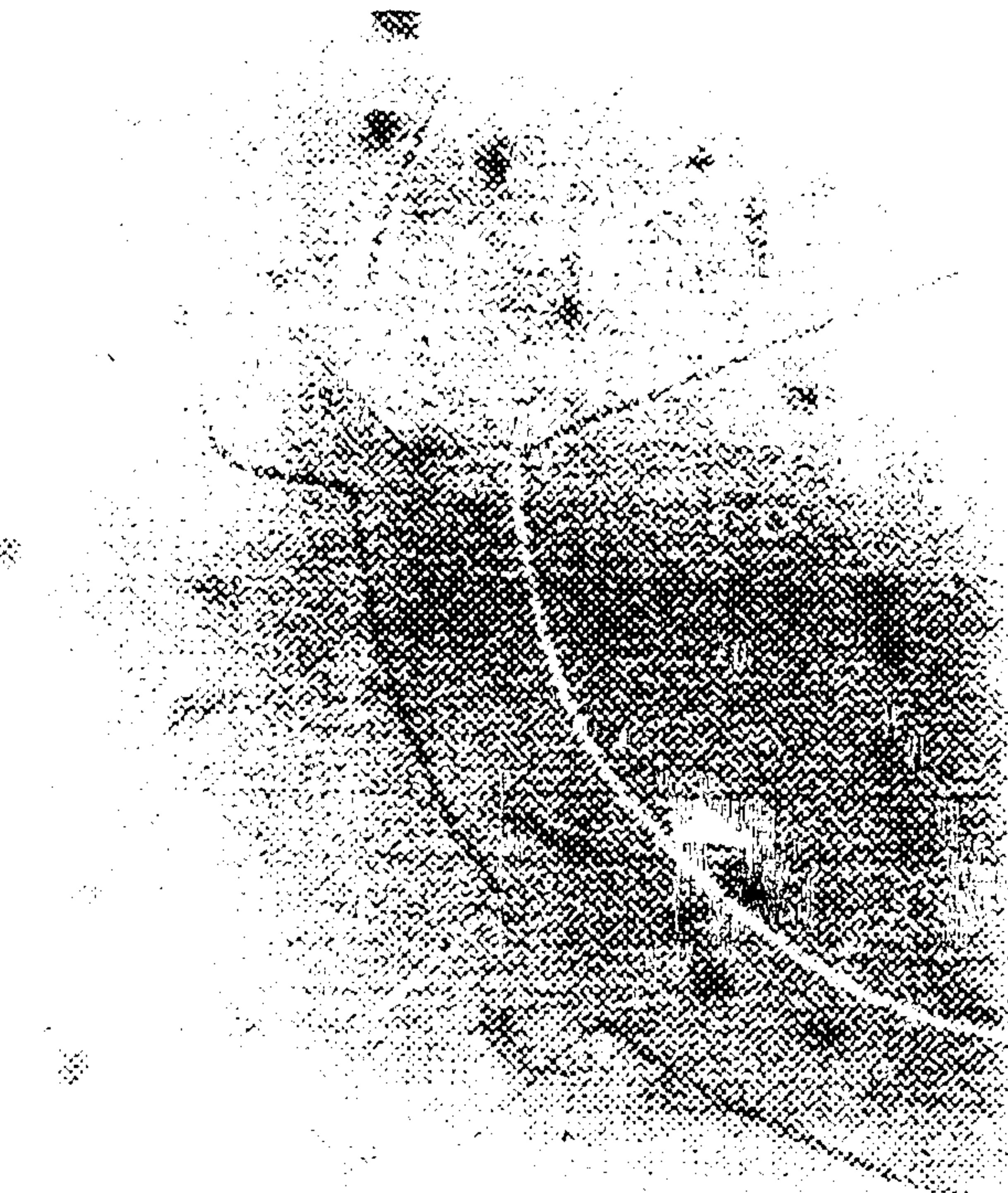


Fig. 4C

Fig. 4D

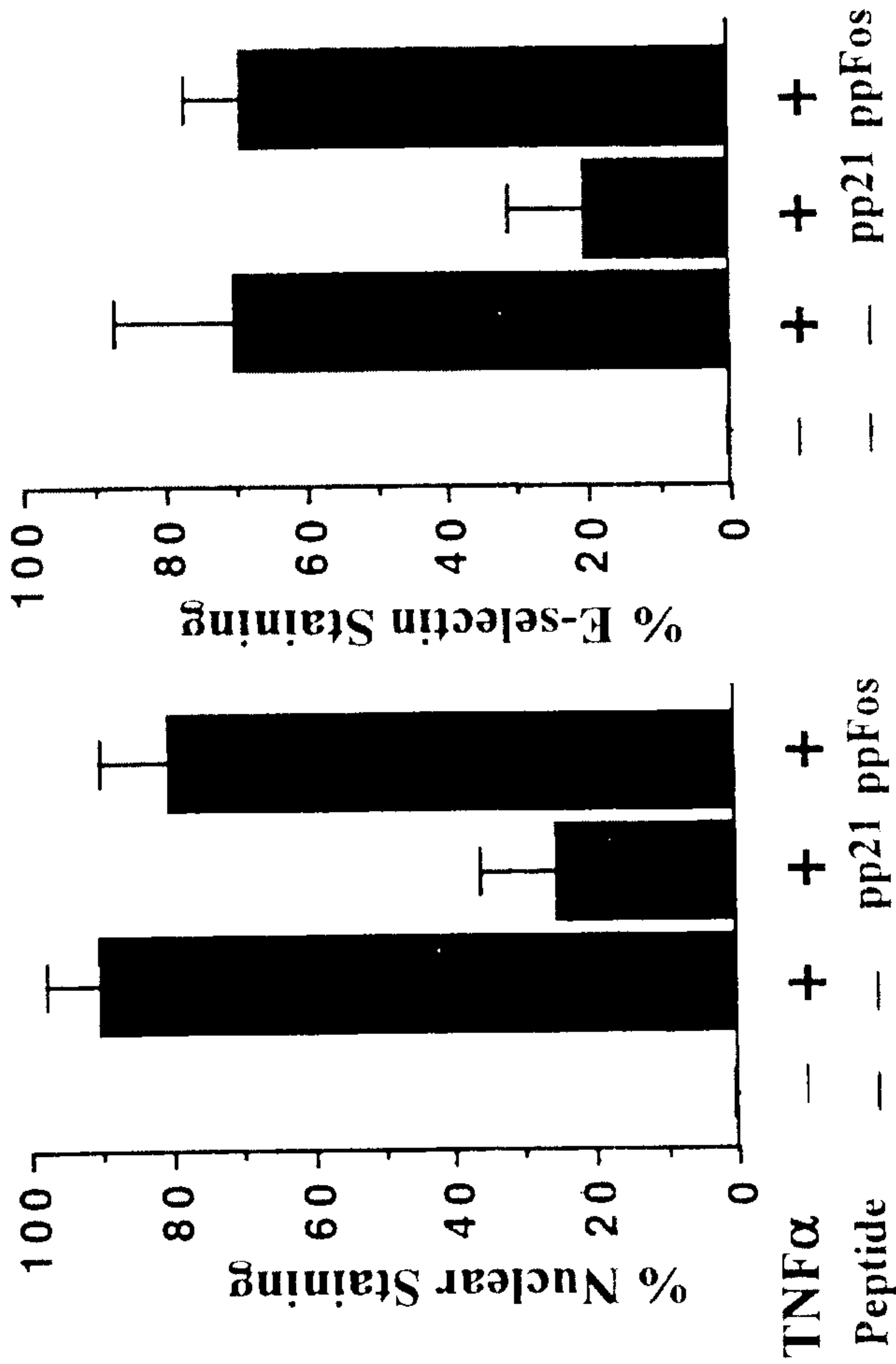


Fig. 4G

Fig. 4H