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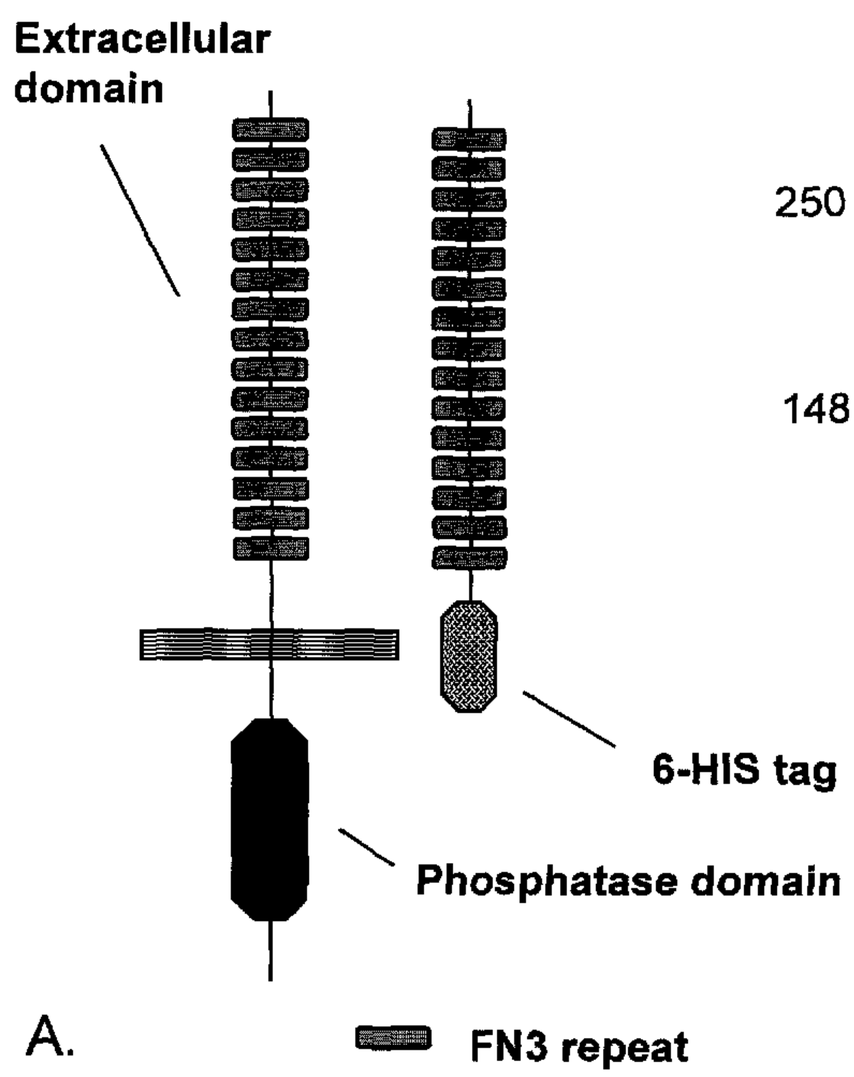
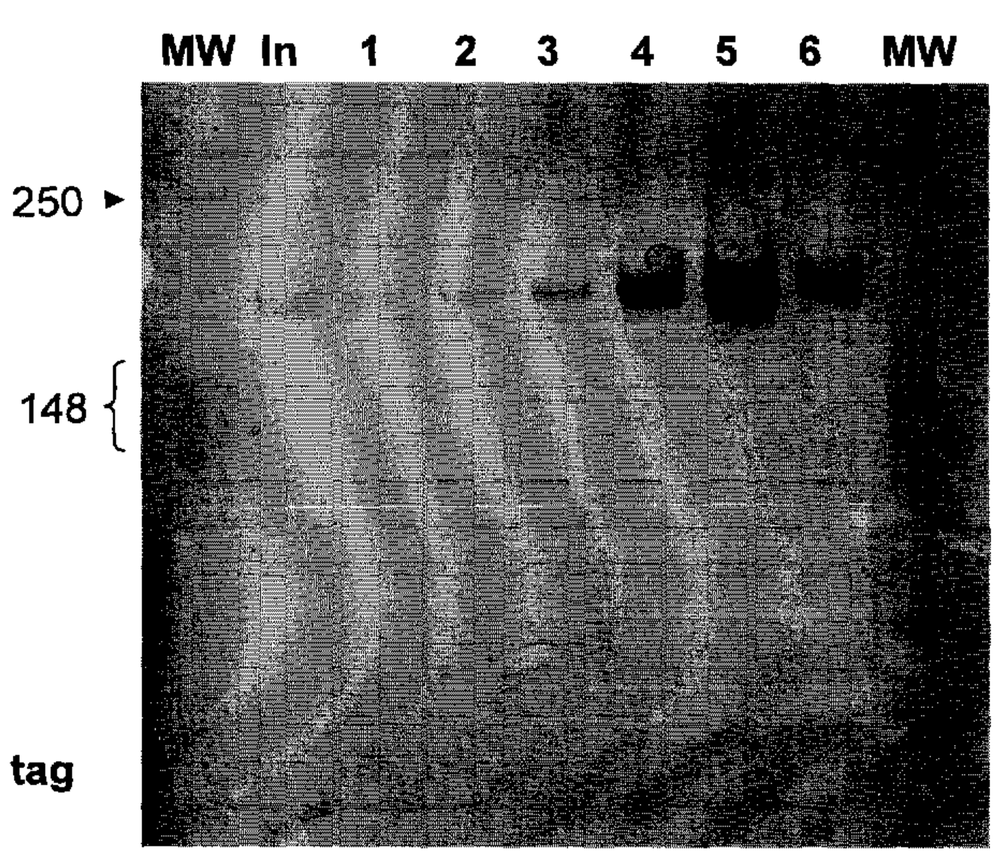
(54) **Titre : ANTICORPS SE LIANT A LA PROTEINE TYROSINE PHOSPHATASE BETA HUMAINE (HPTPBETA) ET UTILISATIONS CORRESPONDANTES**

(54) **Title: ANTIBODIES THAT BIND HUMAN PROTEIN TYROSINE PHOSPHATASE BETA (HPTPBETA) AND USES THEREOF**





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Antibodies and antigen binding fragments thereof that bind to human protein tyrosine phosphatase beta (HPTPβ), and uses thereof.

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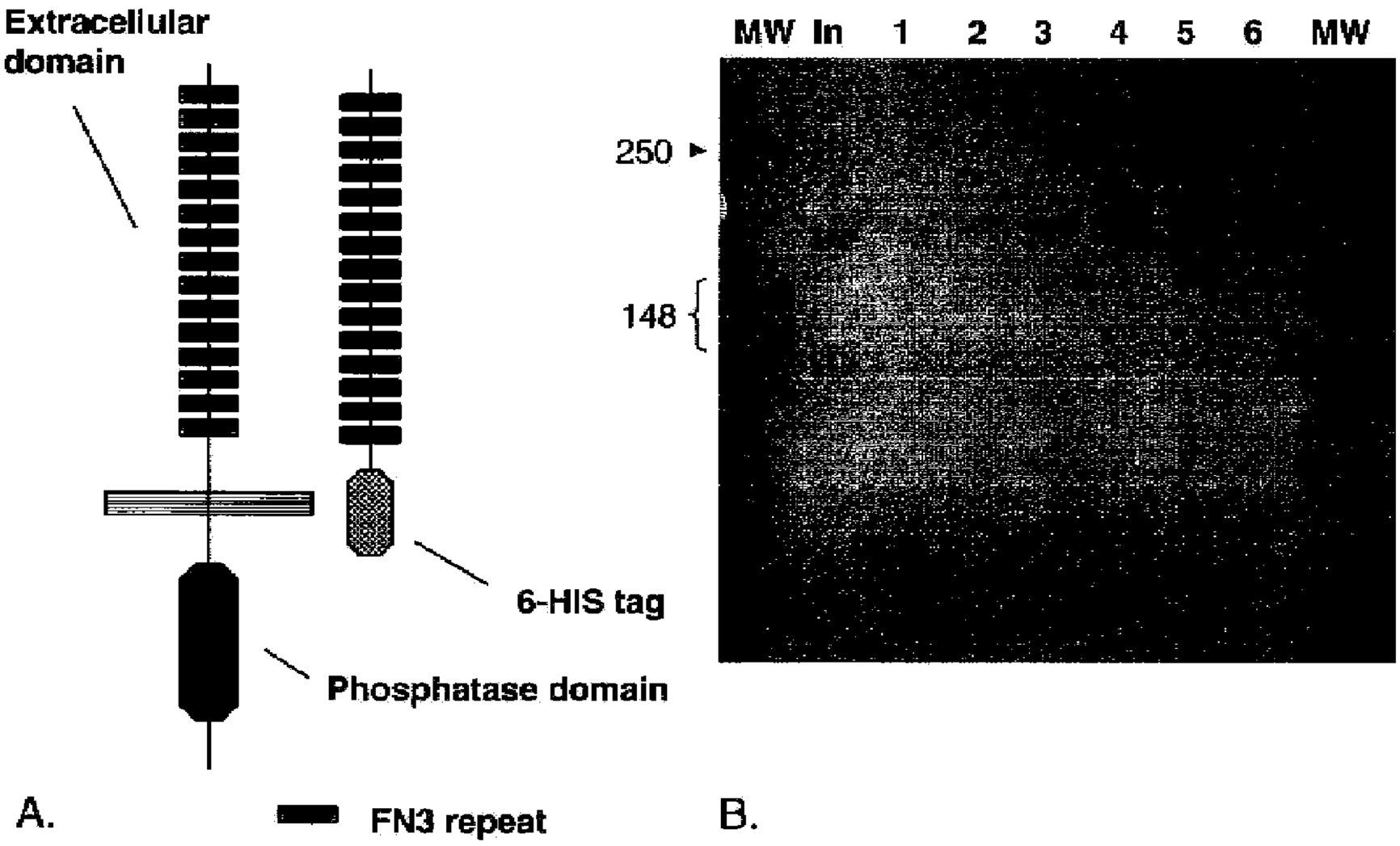
(54) Title: ANTIBODIES THAT BIND HUMAN PROTEIN TYROSINE PHOSPHATASE BETA (HPTPBETA) AND USES THEREOF



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ANTIBODIES THAT BIND HUMAN PROTEIN TYROSINE PHOSPHATASE BETA (HPTPBETA) AND USES THEREOF

FIELD OF INVENTION

This invention relates to antibodies and antigen binding fragments thereof that bind to human protein tyrosine phosphatase beta (HPTPbeta) and uses thereof.

BACKGROUND OF THE INVENTION

Angiogenesis, the sprouting of new blood vessels from the pre-existing vasculature, plays an important role in a wide range of physiological and pathological processes (Nguyen, L.L. et al, Int. Rev. Cytol., 204, 1-48, (2001)). Angiogenesis is a complex process, mediated by communication between the endothelial cells that line blood vessels and their surrounding environment. In the early stages of angiogenesis, tissue or tumor cells produce and secrete pro-angiogenic growth factors in response to environmental stimuli such as hypoxia. These factors diffuse to nearby endothelial cells and stimulate receptors that lead to the production and secretion of proteases that degrade the surrounding extracellular matrix. The activated endothelial cells begin to migrate and proliferate into the surrounding tissue toward the source of these growth factors (Bussolino, F., Trends Biochem. Sci., 22, 251-256, (1997)). Endothelial cells then stop proliferating and differentiate into tubular structures, which is the first step in the formation of stable, mature blood vessels. Subsequently, periendothelial cells, such as pericytes and smooth muscle cells, are recruited to the newly formed vessel in a further step toward vessel maturation.

Angiogenesis is regulated by a balance of naturally occurring pro- and anti-angiogenic factors. Vascular endothelial growth factor, fibroblast growth factor, and angiopoeitin represent a few of the many potential pro-angiogenic growth factors. These ligands bind to their respective receptor tyrosine kinases on the endothelial cell surface and transduce signals that promote cell migration and proliferation. Whereas many regulatory factors have been identified, the molecular mechanisms that drive this process are still not fully understood.

There are many disease states driven by persistent unregulated or improperly regulated angiogenesis. In such disease states, unregulated or improperly regulated angiogenesis may either cause a particular disease or exacerbate an existing pathological condition. For example, ocular

neovascularization has been implicated as the most common cause of blindness and underlies the pathology of approximately 20 eye diseases. In certain previously existing conditions, such as arthritis, newly formed capillary blood vessels invade the joints and destroy cartilage. In diabetes, new capillaries formed in the retina invade the vitreous humor, causing bleeding and blindness.

Both the growth and metastasis of solid tumors may also be angiogenesis-dependent, Folkman et al., "Tumor Angiogenesis," Chapter 10, 206-32, in The Molecular Basis of Cancer, Mendelsohn et al., eds., W. B. Saunders, (1995). It has been shown that tumors which enlarge to greater than 2 mm in diameter must obtain their own blood supply and do so by inducing the growth of new capillary blood vessels. After these new blood vessels become embedded in the tumor, they provide nutrients and growth factors essential for tumor growth as well as a means for tumor cells to enter the circulation and metastasize to distant sites, such as liver, lung or bone (Weidner, New Eng. J. Med., 324, 1, 1-8 (1991)). When used as drugs in tumor-bearing animals, natural inhibitors of angiogenesis may prevent the growth of small tumors (O'Reilly et al., Cell, 79, 315-28 (1994)). In some protocols, the application of such inhibitors leads to tumor regression and dormancy even after cessation of treatment (O'Reilly et al., Cell, 88, 277-85 (1997)). Moreover, supplying inhibitors of angiogenesis to certain tumors may potentiate their response to other therapeutic regimens (see, e.g., Teischer et al., Int. J. Cancer, 57, 920-25 (1994)).

Although many disease states are driven by persistent unregulated or improperly regulated angiogenesis, some disease states may be treated by enhancing angiogenesis. Tissue growth and repair are biologic events wherein cellular proliferation and angiogenesis occur. Thus an important aspect of wound repair is the revascularization of damaged tissue by angiogenesis.

Chronic, non-healing wounds are a major cause of prolonged morbidity in the aged human population. This is especially the case in bedridden or diabetic patients who develop severe, non-healing skin ulcers. In many of these cases, the delay in healing is a result of inadequate blood supply either as a result of continuous pressure or of vascular blockage. Poor capillary circulation due to small artery atherosclerosis or venous stasis contributes to the failure to repair damaged tissue. Such tissues are often infected with microorganisms that proliferate unchallenged by the innate defense systems of the body which require well vascularized tissue to effectively eliminate pathogenic organisms. As a result, most therapeutic intervention centers on

restoring blood flow to ischemic tissues thereby allowing nutrients and immunological factors access to the site of the wound.

Atherosclerotic lesions in large vessels may cause tissue ischemia that could be ameliorated by modulating blood vessel growth to the affected tissue. For example, atherosclerotic lesions in the coronary arteries may cause angina and myocardial infarction that could be prevented if one could restore blood flow by stimulating the growth of collateral arteries. Similarly, atherosclerotic lesions in the large arteries that supply the legs may cause ischemia in the skeletal muscle that limits mobility and in some cases necessitates amputation, which may also be prevented by improving blood flow with angiogenic therapy.

Other diseases such as diabetes and hypertension are characterized by a decrease in the number and density of small blood vessels such as arterioles and capillaries. These small blood vessels are important for the delivery of oxygen and nutrients. A decrease in the number and density of these vessels contributes to the adverse consequences of hypertension and diabetes including claudication, ischemic ulcers, accelerated hypertension, and renal failure. These common disorders and many other less common ailments, such as Burgers disease, could be ameliorated by increasing the number and density of small blood vessels using angiogenic therapy.

Thus, there is a continuing need to identify regulators of angiogenesis.

In view of the foregoing, there is a need to identify biochemical targets in the treatment of angiogenesis mediated disorders. However, angiogenesis involves the action of multiple growth factors and their cognate receptor tyrosine kinases (RTKs), Yancopoulos et al., Nature, 407,242-248, 2000). Vascular endothelial growth factor (VEGF), for example, is important for the differentiation of endothelial cells into nascent blood vessels in the embryonic vasculature. Further, VEGF enhances blood vessel development in the adult vasculature. Administration of exogenous VEGF enhances the development of the collateral vasculature and improves blood flow to ischemic tissues.

To date, three VEGF RTKs have been identified, VEGFR1 (FLT-1), VEGFR2 (KDR), and VEGFR3 (FLT-4). Although these receptors are highly conserved, based on biochemical characterization and biological activity, each has specific and non-overlapping functions. Of the three receptors, VEGFR2 is believed to play the predominant role in mediating VEGF actions in the developing vasculature and during angiogenesis in adults. However, both VEGFR1 and VEGFR3 are required for normal development of the embryonic vasculature and may also be

important for angiogenesis in adult tissues. Upon VEGF binding and dimerization, a conformational change in the VEGFR2 kinase domain enhances its kinase activity resulting in "autophosphorylation" of the other member of the pair on specific tyrosine residues. These autophosphorylation events serve to further enhance the kinase activity and provide anchor points for the association of intracellular signaling molecules.

However, activation of a single angiogenic pathway may not be sufficient to produce persistent and functional vessels that provide adequate perfusion to ischemic tissue. These findings, together with fact that multiple RTKs are involved in the assembly of embryonic vasculature, indicate that biochemical targets that modulate multiple angiogenic pathways will have advantages over administration of a single growth factor.

Protein tyrosine phosphatases (PTPs) comprise a large family of closely related enzymes that dephosphorylate proteins that contain phosphotyrosine residues. Recent evidence suggests that one function of PTPs is to limit the phosphorylation and activation of RTKs. For example, HCPTPA, a low molecular weight protein tyrosine phosphatase, was shown to associate with VEGFR2 and negatively regulate its activation in cultured endothelial cells and its biological activity in angiogenesis assays, (Huang et al., Journal of Biological Chemistry, 274, 38183-38185, 1999).

In addition to VEGFR2, signaling input from another RTK, Tie-2, the receptor for the angiopoietins (Ang1 and Ang2), is also important. Deletion of either the Ang1 or Tie-2 gene in mice may result in embryonic lethality secondary to abnormalities in the developing vasculature (Yancopoulos et al., Nature, 407, 242-248, 2000). In addition, overexpression of Ang1 in the skin increases skin vascularity and administration of exogenous Ang1 increases blood flow to ischemic skeletal muscle (Suri et al., Science, 282, 468-471, 1998). Moreover, inhibiting the activation of Tie-2 inhibits angiogenesis and limits tumor progression in animal models of cancer, (Lin et al., J Clin. Invest., 100, 2072-2078, 1997). In addition to its angiogenic activities, activation of Tie-2 by exogenous administration of Ang1 blocks VEGF mediated vascular leak and pro-inflammatory effects, but enhances its angiogenic effects (Thurston et al., Nature Medicine, 6, 460-463, 2000). Therefore, biological targets that modulate both VEGFR2 and Tie-2 signaling may yield superior proangiogenic or antiangiogenic therapies.

HPTPbeta (first described in Kruegar et al., EMBO J., 9, (1990)) has been suggested for modulating the activity of angiopoietin receptor-type tyrosine kinase Tie-2, e.g., WO 00/65088).

HPTPbeta is also suggested for regulating activities of VEGFR2, e.g., US Pat. Pub. No. 2004/0077065.

It would be desirable to develop antibodies, e.g., a humanized monoclonal antibody, which selectively regulate the activity of HPTPbeta and thereby enhance angiogenic signaling, stimulate blood vessel growth (angiogenesis), and/or increase blood flow in ischemic tissue, or reduce angiogenic signaling, reduce blood vessel growth, and/or decrease blood flow to the effected tissue. Herein are described antibodies and fragments thereof that bind HPTPbeta and regulate angiogenic cell signaling, which in turn, regulates angiogenesis.

SUMMARY OF THE INVENTION

The present invention relates to antibodies that bind human protein tyrosine phosphatase beta HPTPbeta and thereby regulate angiogenic cell signaling, which in turn, regulates angiogenesis.

In one embodiment, the invention relates to an isolated antibody or antigen-binding fragment thereof which binds to human protein tyrosine phosphatase beta, wherein said antibody or antigen-binding fragment thereof regulates angiogenic cell signaling, which in turn, regulates angiogenesis.

In another embodiment, the invention relates to an antibody that binds the N-terminal portion of human protein tyrosine phosphatase beta.

In another embodiment, the invention relates to an antibody that binds the first FN3 repeat of human protein tyrosine phosphatase beta.

In another embodiment, the invention relates to an antibody that binds the first FN3 repeat of human protein tyrosine phosphatase beta, wherein the first FN3 repeat of human protein tyrosine phosphatase beta has the sequence as shown in SEQ ID NO: 11, or a portion thereof.

In another embodiment, the invention relates to an antibody wherein the antibody is a monoclonal antibody.

In another embodiment, the invention relates to an antibody wherein the antibody is the monoclonal antibody R15E6 (Mouse hybridoma, Balbc spleen cells (B cells) deposited with American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108 USA on 04 May 2006, assigned ATCC No. PTA-7580).

In another embodiment, the invention relates to an antibody having the same, or substantially the same, biological characteristics of R15E6.

In another embodiment, the invention relates to an antibody, wherein the antibody or the antigen binding fragment is humanized.

In another embodiment, the invention relates to an antibody, wherein the antibody comprises antigen binding region residues from the monoclonal antibody R15E6 and is humanized.

In another embodiment, the invention relates to an antigen binding fragment of an antibody, wherein the fragment comprises heavy and light chain variable regions.

In another embodiment, the invention relates to an antigen binding fragment of an antibody, wherein the antigen-binding fragment is selected from the group consisting of an Fv fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

In another embodiment, the invention relates to an a method of treating an angiogenesis regulated disorder in a subject, comprising: identifying a subject in need of regulation of angiogenesis; and administering to the subject an effective amount of an antibody or antigen-binding fragment thereof which binds HPTPbeta and regulates angiogenesis.

In another embodiment, the invention relates to a method of treating an angiogenesis regulated disorder in a subject, wherein the angiogenesis regulated disorder is an angiogenesis elevated disorder, and is selected from the group consisting of diabetic retinopathy, macular degeneration, cancer, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndrome, toxoplasmosis, trauma and post-laser complications, diseases associated with rubeosis, and proliferative vitreoretinopathy.

In another embodiment, the invention relates to a method of treating an angiogenesis regulated disorder in a subject, wherein the angiogenesis regulated disorder is an angiogenesis elevated disorder, and is selected from the group including but not limited to diabetic retinopathy, macular degeneration, cancer, rheumatoid arthritis, hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, and solid or blood borne tumors

In another embodiment, the invention relates to a method of treating an angiogenesis regulated disorder in a subject, wherein the angiogenesis regulated disorder is an angiogenesis elevated disorder, and is selected from the group consisting of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis, rheumatoid arthritis, hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

In another embodiment, the invention relates to a method of treating an angiogenesis regulated disorder in a subject, wherein the angiogenesis regulated disorder is an angiogenesis reduced disorder and is selected from the group including but not limited to skeletal muscle or myocardial ischemia, stroke, coronary artery disease, peripheral vascular disease, coronary artery disease, cerebrovascular disease, diabetic neuropathy and wound healing.

In another embodiment, the invention relates to a method of treating an angiogenesis regulated disorder in a subject, wherein the angiogenesis regulated disorder is an angiogenesis reduced disorder and is selected from the group consisting of skeletal muscle and myocardial ischemia, stroke, coronary artery disease, peripheral vascular disease, coronary artery disease.

In another embodiment, the invention relates to a method of treating an angiogenesis reduced disorder in a subject, wherein the angiogenesis reduced disorder is peripheral vascular disease.

In another embodiment, the invention relates to a method of treating an angiogenesis reduced disorder in a subject, wherein the angiogenesis reduced disorder is coronary artery disease.

In another embodiment, the invention relates to a pharmaceutical composition, comprising: an antibody or a fragment thereof which binds to human protein tyrosine phosphatase beta; and a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to a pharmaceutical composition, comprising: an antibody or a fragment thereof which binds to human protein tyrosine phosphatase beta, wherein the antibody is the monoclonal antibody R15E6; and a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to a pharmaceutical composition, comprising: an antibody or a fragment thereof which binds to human protein tyrosine phosphatase beta, wherein the antibody is a monoclonal antibody having the same, or substantially the same, biological characteristics of R15E6; and a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to a pharmaceutical composition, comprising: an antibody or a fragment thereof which binds to human protein tyrosine phosphatase beta, wherein the antibody or the antigen binding fragment is humanized; and a pharmaceutically acceptable carrier.

In another embodiment, the invention relates to a pharmaceutical composition, comprising: an antibody or a fragment thereof which binds to human protein tyrosine phosphatase beta, wherein the antibody comprises antigen binding region residues from the monoclonal antibody R15E6 and is humanized; and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Design and production of the HPTPβ ECD protein. (Panel A) Schematic representation of the full-length HPTPβ and the HPTPβ extracelllular domain-6His fusion protein. (Panel B) Silver stain of Imidazole eluates from a Ni-NTA column loaded with supernatant from HEK293 cells transfected with a vector directing the expression of βECD-6His. A single high molecular weight band consistent with the HPTPβ extracelllular domain-6His protein is detected.

Figure 2. R15E6 Recognizes Endogenous HPTPβ on Endothelial Cells. (Panel A) Endothelial cell lysates are immunoprecipitated with a control antibody (Lane 1), with R15E6 (Lane 2) or with a mixture of anti-Tie2 and anti-VEGFR2 antibodies (Lane 3). Immunoprecipitates are resolved by SDS-PAGE, transferred to a PVD membrane and probed by western blot with a mixture of R15E6, anti-Tie2 and anti-VEGFR2 antibodies. A single major high molecular weight band consistent with HPTPβ is seen with R15E6 (Lane 2) and not with the control antibody (Lane 1) or the mixture of anti-Tie2 and anti-VEGFR2 (Lane 3). (Panel B) Endothelial cells are subjected to FACS analysis with R15E6 (white peak) or a no primary antibody control (black peak). The robust shift in fluorescence indicates that R15E6 binds to HPTPβ on the surface of intact endothelial cells.

Figure 3. R15E6 Enhances Tie2 Receptor Activation in HUVEC's. Tie2 activation is measured in human endothelial cells as described in Example 4. R15E6 dose dependently enhances both basal and Ang1-induced Tie2 activation.

Figure 4. R15E6 Enhances HUVEC Survival. Survival of serum starved human endothelial cells is measured as described in Example 4. Consistent with its effects on Tie2 activation, R15E6 dose dependently enhances both basal and Ang1-induced endothelial cell survival (Panel A). In addition, R15E6 also dose dependently enhances VEGF and FGF-mediated endothelial cell survival (Panels B and C). A control antibody fails to enhance endothelial cell survival (Panel D).

Figure 5. R15E6 Enhances HUVEC Migration. Migration of human endothelial cells is measured as described in Example 4. R15E6 dose dependently enhances both basal and VEGF-induced endothelial cell migration.

Figure 6. R15E6 Enhances Capillary Morphogenesis in the HUVEC/Bead Sprouting Assay. Capillary morphogenesis of human endothelial cells is measured in the bead sprouting assay as described in Example 4. R15E6 enhances both basal and VEGF-induced endothelial cell capillary morphogenesis.

Figure 7. Western blot analysis localizes the R15E6 binding epitope to the N-terminal FN3 repeat of the HPTPβ extracellular domain. (Panel A) By western analysis, R15E6 binds to all of the C-terminal truncation mutants demonstrating that the binding epitope is located in the N-terminal 2 FN3 repeats. (Panel B) Analysis of mouse/human chimeric proteins further localizes the R15E6 binding epitope to the HPTPβ N-terminal FN3 repeat.

Figure 8. MSD analysis confirms localization of the R15E6 binding epitope to the N-terminal FN3 repeat of the HPTPβ extracellular domain. (Panel A) By MSD analysis, R15E6 binds to all of the C-terminal truncation mutants confirming that the binding epitope is located in the N-terminal 2 FN3 repeats. (Panel B) Analysis of mouse/human chimeric proteins further confirms the localization of the R15E6 binding epitope to the HPTPβ N-terminal FN3 repeat.

Figure 9. MSD analysis demonstrates that the monovalent R15E6 Fab fragment also binds the N-terminal FN3 repeat of HPTPβ. (Panel A) Similar to the intact R15 E6 antibody, the R15E6 Fab fragment binds to all of the C-terminal truncation mutants confirming that the binding epitope is

located in the N-terminal 2 FN3 repeats. (Panel B) Analysis of mouse/human chimeric proteins further localizes the binding epitope of the R15E6 Fab fragment to the HPTPβ N-terminal FN3 repeat.

Figure 10. The monovalent R15E6 Fab fragment fails to enhance Tie2 activation and blocks Tie2 activation by intact R15E6.

Figure 11. The R15E6 Fab fragment potently inhibits endothelial cell survival. (Panel A) Compared to a control Fab fragment, the R15E6 Fab fragment potently inhibits endothelial cell survival. (Panel B) The inhibitory effect of the R15E6 Fab fragment is rescued by competition with intact R15E6.

Figure 12. The R15E6 Fab fragment inhibits VEGF mediated endothelial cell migration.

SEQUENCE LISTING DESCRIPTION

Each of the nucleotide and protein sequences in the sequence listing, along with the corresponding Genbank or Derwent accession number(s), where applicable, and species from which it is derived, is shown in Table I.

Table I

Sequence Description	SEQ ID NOs: Nucleotide, Protein	Species	Equivalent Genbank Acc. No.
Extracellular domain of HPTPbeta with His and Gly tag	1, 2	*Homo Sapiens*	
Extracellular domain of full-length HPTPbeta	3	*Homo Sapiens*	X54131 NM_002837
1/2 (AA1-730, 8 FN3's)775 aa	4	*Homo Sapiens*	
1/4 (AA1-376, 4 FN3's)421 aa	5	*Homo Sapiens*	
1/8 (AA1-202, 2 FN3's)247 aa	6	*Homo Sapiens*	
Mouse full length ECD1632 aa	7	*Mus musculus*	NM_029928

Sequence Description	SEQ ID NOs: Nucleotide, Protein	Species	Equivalent Genbank Acc. No.
First human FN3-Mouse ½	8	Human-mouse chimera	
Second human FN3-Mouse ½	9	Human-mouse chimera	
First two human FN3, - Mouse ½	10	Human-mouse chimera	
Human FN3, first repeat	11	*Homo sapines*	

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to antibodies that bind HPTPbeta and uses thereof.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The techniques and procedures are generally performed according to conventional methods known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Protein," is used herein interchangeably with peptide and polypeptide. HPTPbeta is human protein tyrosine phosphatase as defined in the sequence listing. In some of the embodiments, various fragments of HPTPbeta are used. Homologs, orthologs, fragments, variants, and mutants of HPTPbeta protein and gene, as described below, are considered as within the scope of the term "HPTPbeta".

By "fragment" is intended a portion of the nucleotide or protein sequence. Fragments may retain the biological activity of the native protein. Fragments of a nucleotide sequence are also useful as hybridization probes and primers or to regulate expression of a gene, e.g.,

antisense, siRNA, or micro RNA. A biologically active portion may be prepared by isolating a portion of one of the nucleotide sequences of the invention, expressing the encoded portion (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded protein.

One of skill in the art would also recognize that genes and proteins from species other than those listed in the sequence listing, particularly vertebrate species, may be useful. Such species include, but are not limited to, mice, rats, guinea pigs, rabbits, dogs, pigs, goats, cows, monkeys, chimpanzees, sheep, hamsters, and zebrafish. One of skill in the art would further recognize that by using probes from the known species' sequences, cDNA or genomic sequences homologous to the known sequence could be obtained from the same or alternate species by known cloning methods. Such homologs and orthologs are contemplated to be useful in practicing the invention.

By "variants" are intended similar sequences. For example, conservative variants may include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the polypeptides of the invention. Naturally occurring allelic variants, and splice variants may be identified with the use of known techniques, e.g., with polymerase chain reaction (PCR), single nucleotide polymorphism (SNP) analysis, and hybridization techniques. To isolate orthologs and homologs, generally stringent hybridization conditions are utilized, dictated by specific sequences, sequence length, guanine + cytosine (GC) content, and other parameters. Variant nucleotide sequences also include synthetically derived nucleotide sequences, e.g., derived by using site-directed mutagenesis. Variants may contain additional sequences from the genomic locus alone or in combination with other sequences.

The molecules of the invention also include truncated and/or mutated proteins wherein regions of the protein not required for ligand binding or signaling have been deleted or modified. Similarly, they may be mutated to modify their ligand binding or signaling activities. Such mutations may involve non-conservative mutations, deletions, or additions of amino acids or protein domains. Variant proteins may or may not retain biological activity. Such variants may result from, e.g., genetic polymorphism or from human manipulation.

Fusions proteins are also contemplated herein. Using known methods, one of skill in the art would be able to make fusion proteins of the proteins of the invention; that, while different from native form, may be useful. For example, the fusion partner may be a signal (or leader) polypeptide sequence that co-translationally or post-translationally directs transfer of the protein from its site of synthesis to another site (e.g., the yeast α-factor leader). Alternatively, it may be

added to facilitate purification or identification of the protein of the invention (e.g., poly-His, Flag peptide, or fluorescent proteins).

The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally is capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "epitope" includes any antigenic determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or a T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings such as amino acids, sugars, lipids, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. An antibody is also said to specifically bind an antigen when it exhibits higher affinity to the antigen than other related and/or unrelated molecules.

The term “antibody” (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (e.g. bispecific antibodies), single chain antibodies, e.g., antibodies from llama and camel, antibody fragments, e.g., variable regions and/or constant region fragments, so long as they exhibit a desired biological activity, e.g., antigen-binding activity. The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein.

An “isolated antibody” is one which has been identified, and/or separated, and/or recovered from its natural environment.

The basic four-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer unit along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies may polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain). In the case of IgGs, the four-chain unit is generally about 150 kilo Daltons (kDa). Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H)

followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species may be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins may be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

Members of the Camelidae family, e.g., llama, camel, and dromedaries, contain a unique type of antibody, that are devoid of light chains, and further lack the C_{H1} domain (Muyldermans, S., Rev. Mol. Biotechnol., 74, 277-302 (2001)). The variable region of these heavy chain antibodies are termed V_{HH} or VHH, and constitute the smallest available intact antigen binding fragment (15 kDa) derived from a functional immunoglobulin.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FR) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the

hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 1-35 (H1), 50-65 (H2) and 95-102 (H3) in the V_H; Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop".

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. In contrast to polyclonal antibody preparations which include different antibodies directed against different epitopes, each monoclonal antibody is directed against a single epitope, i.e., a single antigenic determinant. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries, using the available techniques, e.g., Clackson et al., *Nature,* 352:624-628 (1991).

The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA,* 81, 6851-6855 (1984)).

An "antibody fragment" comprises a portion of a multimeric antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, dimmers and trimers of Fab conjugates, Fv, scFv, minibodies,; dia-, tria-, and tetrabodies; linear antibodies (See Hudson et al, Nature Med. 9, 129-134 (2003)).

"Fv" is the minimum antibody fragment which contains a complete antigen binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, and are therefore included in the definition of Fv.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding.

The terms "dia-, tria-, and tetrabodies" refer to small antibody fragments prepared by constructing sFv fragments with short linkers (about 5-10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a multivalent fragment.

The term "humanized antibody" or "human antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Means for making chimeric, CDR-grafted and humanized antibodies are known to those of ordinary skill in the art (see, e.g., U.S. Pat. Nos. 4,816,567 and 5,225,539). One method for making human antibodies employs the use of transgenic animals, such as a transgenic mouse. These transgenic animals contain a substantial portion of the human antibody producing genome inserted into their own genome and the animal's own endogenous antibody production is rendered deficient in the production of antibodies. Methods for making such transgenic animals are known in the art. Such transgenic animals may be made using XenoMouse^{RTM} technology or by using a

"minilocus" approach. Methods for making XenoMice^{RTM} are described in U.S. Patent Nos. 6,162,963, 6,150,584, 6,114,598 and 6,075,181. Methods for making transgenic animals using the "minilocus" approach are described in U.S. Pat. Nos. 5,545,807, 5,545,806 and 5,625,825, and WO 93/12227.

Humanization of a non-human antibody has become routine in recent years, and is now within the knowledge of one skilled in the art. Several companies provide services to make a humanized antibody, e.g., Xoma, Aries, Medarex, PDL, and Cambridge Antibody Technologies. Humanization protocols are extensively described in technical literature, e.g., Kipriyanov and Le Gall, Molecular Biotechnol, Vol. 26, pp 39-60 (2004), Humana Press, Totowa, NJ; Lo, Methods Mol. Biol., Vol. 248, pp 135-159 (2004), Humana Press, Totowa, NJ; Wu et al, J. Mol. Biol. 294, 151-162 (1999).

In certain embodiments, antibodies of the present invention may be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies may be used for transformation of a suitable mammalian host cell by known methods for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector), or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399, 216, 4,912,040, 4,740,461, and 4,959,455. The transformation procedure used may depend upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include; but are not limited to, dextran-mediated trasfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, mixing nucleic acid with positively-charged lipids, and direct microinjection of the DNA into nuclei.

A nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of an antibody, or a fragment thereof in a suitable combination if desired, is/are inserted into an appropriate expression vector using standard ligation techniques. The antibody heavy chain or light chain constant region may be appended to the C terminus of the appropriate variable region and is ligated into an expression vector. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene may occur). For a

review of expression vectors, see Methods Enzymol. vol. 185 (Goeddel, ed.), 1990, Academic Press.

Antibodies and fragments thereof of the present invention bind HPTPbeta and regulate angiogenesis. As defined above, the term antibody is used as to denote an antigen binding fragment. The uses of such antibodies and antigen binding fragments are further described below.

Screening Assays using in vitro and in vivo models of angiogenesis

Antibodies of the invention may be screened in angiogenesis assays that are known in the art. Such assays include *in vitro* assays that measure surrogates of blood vessel growth in cultured cells or formation of vascular structures from tissue explants and *in vivo* assays that measure blood vessel growth directly or indirectly (Auerbach,R., et al. (2003). Clin Chem 49, 32-40, Vailhe,B., et al. (2001). Lab Invest 81, 439-452).

In vitro models of angiogenesis

Most of these assays employ cultured endothelial cells or tissue explants and measure the effect of agents on "angiogenic" cell responses or on the formation of blood capillary-like structures. Examples of *in vitro* angiogenesis assays include but are not limited to endothelial cell migration and proliferation, capillary tube formation, endothelial sprouting, the aortic ring explant assay and the chick aortic arch assay.

In vivo models of angiogenesis

In these assays agents or antibodies are administered locally or systemically in the presence or absence of growth factors (i.e. VEGF or angiopoietin 1) and new blood vessel growth is measured by direct observation or by measuring a surrogate marker such as hemoglobin content or a fluorescent indicator. Examples of angiogenesis include but are not limited to chick chorioallantoic membrane assay, the corneal angiogenesis assay, and the MATRIGEL^{TM} plug assay.

Treatment of angiogenesis regulated disorders

The term "regulate" is defined as in its accepted dictionary meanings. Thus, the meaning of the term "regulate" includes, but is not limited to, up-regulate or down-regulate, to fix, to bring order or uniformity, to govern, or to direct by various means. In one aspect, an antibody may be used in a method for the treatment of an "angiogenesis elevated disorder" or "angiogenesis reduced disorder". As used herein, an "angiogenesis elevated disorder" is one that involves unwanted or elevated angiogenesis in the biological manifestation of the disease, disorder, and/or condition; in the biological cascade leading to the disorder; or as a symptom of the disorder. Similarly, the "angiogenesis reduced disorder" is one that involves wanted or reduced angiogenesis in the biological manifestations. This "involvement" of angiogenesis in an angiogenesis elevated/reduced disorder includes, but is not limited to, the following:

(1) The angiogenesis as a "cause" of the disorder or biological manifestation, whether the level of angiogenesis is elevated or reduced genetically, by infection, by autoimmunity, trauma, biomechanical causes, lifestyle, or by some other causes.

(2) The angiogenesis as part of the observable manifestation of the disease or disorder. That is, the disease or disorder is measurable in terms of the increased or reduced angiogenesis. From a clinical standpoint, angiogenesis indicates the disease; however, angiogenesis need not be the "hallmark" of the disease or disorder.

(3) The angiogenesis is part of the biochemical or cellular cascade that results in the disease or disorder. In this respect, regulation of angiogenesis may interrupt the cascade, and may control the disease. Non-limiting examples of angiogenesis regulated disorders that may be treated by the present invention are herein described below.

Antibodies of the present invention may be used to treat diseases associated with retinal/choroidal neovascularization that include, but are not limited to, diabetic retinopathy, macular degeneration, cancer, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularlation of the iris) and diseases caused by the abnormal

proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Antibodies of the present invention may be used to treat diseases associated with chronic inflammation. Diseases with symptoms of chronic inflammation include inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis and rheumatoid arthritis. Angiogenesis is a key element that these chronic inflammatory diseases have in common. The chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells produce granulomas and thus, maintain the chronic inflammatory state. Inhibition of angiogenesis by the compositions and methods of the present invention would prevent the formation of the granulomas and alleviate the disease.

Crohn's disease and ulcerative colitis are characterized by chronic inflammation and angiogenesis at various sites in the gastrointestinal tract. Crohn's disease is characterized by chronic granulomatous inflammation throughout the gastrointestinal tract consisting of new capillary sprouts surrounded by a cylinder of inflammatory cells. Prevention of angiogenesis inhibits the formation of the sprouts and prevents the formation of granulomas. Crohn's disease occurs as a chronic transmural inflammatory disease that most commonly affects the distal ileum and colon but may also occur in any part of the gastrointestinal tract from the mouth to the anus and perianal area. Patients with Crohn's disease generally have chronic diarrhea associated with abdominal pain, fever, anorexia, weight loss and abdominal swelling. Ulcerative colitis is also a chronic, nonspecific, inflammatory and ulcerative disease arising in the colonic mucosa and is characterized by the presence of bloody diarrhea.

The inflammatory bowel diseases also show extraintestinal manifestations such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and may occur at many sites other than the gastrointestinal tract. Antibodies of the present invention may be capable of treating these lesions by preventing the angiogenesis, thus reducing the influx of inflammatory cells and the lesion formation.

Sarcoidosis is another chronic inflammatory disease that is characterized as a multisystem granulomatous disorder. The granulomas of this disease may form anywhere in the body and thus the symptoms depend on the site of the granulomas and whether the disease active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells.

Antibodies of the present invention may also treat the chronic inflammatory conditions associated with psoriasis. Psoriasis, a skin disease, is another chronic and recurrent disease that is characterized by papules and plaques of various sizes. Prevention of the formation of the new blood vessels necessary to maintain the characteristic lesions leads to relief from the symptoms.

Rheumatoid arthritis is a chronic inflammatory disease characterized by nonspecific inflammation of the peripheral joints. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis. Other diseases that may be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

Antibodies of the present invention may also be used to treat an "angiogenesis reduced disorder". As used herein, an "angiogenesis reduced disorder" is one that angiogenesis would be considered beneficial to treat a disease, disorder, and/or condition. The disorder is one characterized by tissue that is suffering from or at risk of suffering from ischemic damage, infection, and/or poor healing, which results when the tissue is deprived of an adequate supply of oxygenated blood due to inadequate circulation. As used herein, "tissue" is used in the broadest sense, to include, but not limited to, the following: cardiac tissue, such as myocardium and cardiac ventricles; erectile tissue; skeletal muscle; neurological tissue, such as from the cerebellum; internal organs, such as the brain, heart, pancreas, liver, spleen, and lung; or generalized area of the body such as entire limbs, a foot, or distal appendages such as fingers or toes.

Methods of vascularizing ischemic tissue

In one aspect, antibodies may be used in a method of vascularizing ischemic tissue. As used herein, "ischemic tissue," means tissue that is deprived of adequate blood flow. Examples of ischemic tissue include, but are not limited to, tissue that lack adequate blood supply resulting from myocardial and cerebral infarctions, mesenteric or limb ischemia, or the result of a vascular occlusion or stenosis. In one example, the interruption of the supply of oxygenated blood may be caused by a vascular occlusion. Such vascular occlusion may be caused by arteriosclerosis,

trauma, surgical procedures, disease, and/or other etiologies. Standard routine techniques are available to determine if a tissue is at risk of suffering ischemic damage from undesirable vascular occlusion. For example, in myocardial disease these methods include a variety of imaging techniques (e.g., radiotracer methodologies, x-ray, and MRI) and physiological tests. Therefore, induction of angiogenesis is an effective means of preventing or attenuating ischemia in tissues affected by or at risk of being affected by a vascular occlusion. Further, the treatment of skeletal muscle and myocardial ischemia, stroke, coronary artery disease, peripheral vascular disease, coronary artery disease is fully contemplated.

A person skilled in the art of using standard techniques may measure the vascularization of tissue. Non-limiting examples of measuring vascularization in a subject include SPECT (single photon emission computed tomography); PET (positron emission tomography); MRI (magnetic resonance imaging); and combination thereof, by measuring blood flow to tissue before and after treatment. Angiography may be used as an assessment of macroscopic vascularity. Histologic evaluation may be used to quantify vascularity at the small vessel level. These and other techniques are discussed in Simons, et al., "Clinical trials in coronary angiogenesis," Circulation, 102, 73-86 (2000).

Methods of repairing tissue

In one aspect, antibodies may be used in a method of repairing tissue. As used herein, "repairing tissue" means promoting tissue repair, regeneration, growth, and/or maintenance including, but not limited to, wound repair or tissue engineering. One skilled in the art appreciates that new blood vessel formation is required for tissue repair. In turn, tissue may be damaged by, including, but not limited to, traumatic injuries or conditions including arthritis, osteoporosis and other skeletal disorders, and burns. Tissue may also be damaged by injuries due to surgical procedures, irradiation, laceration, toxic chemicals, viral infection or bacterial infections, or burns. Tissue in need of repair also includes non-healing wounds. Examples of non-healing wounds include non-healing skin ulcers resulting from diabetic pathology; or fractures that do not heal readily.

Antibodies may also be used in tissue repair in the context of guided tissue regeneration (GTR) procedures. Such procedures are currently used by those skilled in the arts to accelerate wound healing following invasive surgical procedures.

Antibodies may be used in a method of promoting tissue repair characterized by enhanced tissue growth during the process of tissue engineering. As used herein, "tissue engineering" is defined as the creation, design, and fabrication of biological prosthetic devices, in combination with synthetic or natural materials, for the augmentation or replacement of body tissues and organs. Thus, the present methods may be used to augment the design and growth of human tissues outside the body for later implantation in the repair or replacement of diseased tissues. For example, antibodies may be useful in promoting the growth of skin graft replacements that are used as a therapy in the treatment of burns.

In another aspect of tissue engineering, antibodies of the present invention may be included in cell-containing or cell-free devices that induce the regeneration of functional human tissues when implanted at a site that requires regeneration. As previously discussed, biomaterial-guided tissue regeneration may be used to promote bone regrowth in, for example, periodontal disease. Thus, antibodies may be used to promote the growth of reconstituted tissues assembled into three-dimensional configurations at the site of a wound or other tissue in need of such repair.

In another aspect of tissue engineering, antibodies may be included in external or internal devices containing human tissues designed to replace the function of diseased internal tissues. This approach involves isolating cells from the body, placing them with structural matrices, and implanting the new system inside the body or using the system outside the body. For example, antibodies may be included in a cell-lined vascular graft to promote the growth of the cells contained in the graft. It is envisioned that the methods of the invention may be used to augment tissue repair, regeneration and engineering in products such as cartilage and bone, central nervous system tissues, muscle, liver, and pancreatic islet (insulin-producing) cells.

Pharmaceutical Formulations and Methods for Use

The antibodies of the invention may be administered to individuals to treat or to prevent diseases or disorders that are regulated by genes and proteins of the invention. The term “treatment” is used herein to mean that administration of a compound of the present invention mitigates a disease or a disorder in a host. Thus, the term “treatment” includes, preventing a disorder from occurring in a host, particularly when the host is predisposed to acquiring the disease, but has not yet been diagnosed with the disease; inhibiting the disorder; and/or alleviating or reversing the disorder. Insofar as the methods of the present invention are directed to preventing disorders, it is understood that the term “prevent” does not require that the disease

state be completely thwarted. (See Webster's Ninth Collegiate Dictionary.) Rather, as used herein, the term preventing refers to the ability of the skilled artisan to identify a population that is susceptible to disorders, such that administration of the compounds of the present invention may occur prior to onset of a disease. The term does not imply that the disease state be completely avoided. The compounds identified by the screening methods of the present invention may be administered in conjunction with other compounds.

Safety and therapeutic efficacy of compounds identified may be determined by standard procedures using *in vitro* or *in vivo* technologies. Compounds that exhibit large therapeutic indices may be preferred, although compounds with lower therapeutic indices may be useful if the level of side effects is acceptable. The data obtained from the *in vitro* and *in vivo* toxicological and pharmacological techniques may be used to formulate the range of doses.

Effectiveness of a compound may further be assessed either in animal models or in clinical trials of patients with unregulated or improperly regulated angiogenesis.

As used herein, "pharmaceutically acceptable carrier" is intended to include all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, such media may be used in the compositions of the invention. Supplementary active compounds may also be incorporated into the compositions. A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application may include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH may be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water-soluble), or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, CREMOPHOR EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Systemic administration may also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration may be accomplished using nasal sprays or suppositories. For

transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds may also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials may also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) may also be used as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and are directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

EXAMPLES

Example 1. Production of the HPTPβ extracellular domain protein.

Methods: Full length HPTPβ is cloned from a human placental library according to the manufacturer's (Origene) instructions. The clone is identical to a previously reported cDNA clone (Genbank accession # X54131) except it is missing FNIII repeat # 5. A cDNA encoding the entire soluble extracellular domain (ECD) of HPTPβ is cloned by PCR from the full length cDNA (see sequence below) coding for AA 1-1534 with an added c-terminal His-His-His-His-

His-His-Gly (6His-Gly) (SEQ ID NO: 1). The resulting cDNA is cloned into mammalian expression vectors for transient (pShuttle-CMV) or stable (pcDNA3.1(-))expression in HEK293 cells. To obtain purified HPTPβ ECD (βECD), HEK293 cells transfected with a βECD expression vector are incubated in OptiMEM-serum free (Gibco) for 24 hours under normal growth conditions. The conditioned media is then recovered, centrifuged to remove debris (1000 rpm x 5 minutes), and 1 mL of washed Ni-NTA agarose (Qiagen) (500 µL packed material) is added to each 10 mL of cleared media and allowed to rock overnight at 4°C. On the following day, the mixture is loaded into a column and washed with 20 bed volumes of 50 mM NaH2PO4, 300 mM NaCl, 20 mM Imidazole, pH 8. The purified HPTPβ extracellular domain protein (SEQ ID NO: 2) is then eluted in six fractions with 200 µL/elution in 50 mM NaH2PO4, 300 mM NaCl, 250 mM Imidazole, pH 8. Fractions are analyzed for protein content using reducing-denaturing SDS-polyacrylimide gel electrophoresis and detected by silver stain (Invitrogen) and confirmed by mass spectrometry.

Results: To develop an antibody to the extracellular domain of HPTPβ , expression vectors directing the expression of a 6-His tagged HPTPβ extracellular domain protein (Figure 1, Panel A) are developed. Subsequently, the 6-His tagged HPTPβ extracellular domain protein is purified to near homogeneity (Figure 1, Panel B) from the conditioned media of HEK293 cells transfected with the expression vector.

Example 2. Generation of monoclonal antibodies to HPTPβ extracellular domain.

Methods: For production of the HPTPβ extracellular domain immunogen, the purified HPTPβ extracellular domain-6His protein is conjugated to porcine thyroglobulin (Sigma) using EDC coupling chemistry (Hockfield, S. et al, (1993) Cold Spring Habor Laboratory Press. Volume 1 pp. 111-201, Immunocytochemistry). The resulting HPTPβ extracellular domain-thyroglobulin conjugate is dialyzed against PBS, pH 7.4. Adult Balb/c mice are then immunized subcutaneously with the conjugate (100-200 µg) and complete Freund's adjuvant in a 1:1 mixture. After 2-3 weeks, the mice are injected intraperitoneally or subcutaneously with incomplete Freund's adjuvant and the conjugate in a 1:1 mixture. The injection is repeated at 4-6 weeks. Sera are collected from mice 7 days post-third-injection and assayed for immunoreactivity to HPTPβ extracellular domain antigen by ELISA and western blotting. Mice that display a good response to the antigen are boosted by a single intra-spleen injection with 50 µl of purified

HPTPβ extracellular domain protein mixed 1:1 with Alum hydroxide using a 31 gauge extra long needle (Goding, J. W., (1996) Monoclonal Antibodies: Principles and Practices. Third Edition, Academic Press Limited. p.145). Briefly, mice are anesthetized with 2.5% avertin, and a 1 centimeter incision is created on the skin and left oblique body wall. The antigen mixture is administered by inserting the needle from the posterior portion to the anterior portion of the spleen in a longitudinal injection. The body wall is sutured and the skin is sealed with two small metal clips. Mice are monitored for safe recovery. Four days after surgery the mouse spleen is removed and single cell suspensions are made for fusion with mouse myeloma cells for the creation of hybridoma cell lines (Spitz, M., (1986) Methods In Enzymology, Volume 121. Eds. John J, Lagone and Helen Van Vunakis. PP.33-41 (Academic Press, New York, NY)). Resulting hybridomas are cultured in Dulbeccos modified media (Gibco) supplemented with 15 % fetal calf serum (Hyclone) and hypoxathine, aminopterin and thymidine.

Screening for positive hybridomas begins 8 days after the fusion and continues for 15 days. Hybridomas producing anti-HPTPβ extracellular domain antibodies are identified by ELISA on two sets of 96-well plates: one coated with the histidine tagged-HPTPβ extracellular domain and another one coated with a histidine-tagged bacterial MurA protein as a negative control. The secondary antibody is a donkey anti-mouse IgG labeled with horseradish peroxidase (HRP) (Jackson Immunoresearch). Immunoreactivity is monitored in wells using color development initiated by ABTS tablets dissolved in TBS buffer, pH 7.5. The individual HRP reaction mixtures are terminated by adding 100 microliters of 1% SDS and reading absorbance at 405 nm with a spectrophotometer. Hybridomas producing antibodies that interact with HPTPβ extracellular domain-6His, and not with the murA-6His protein are used for further analysis. Limiting dilutions (0.8 cells per well) are performed twice on positive clones in 96 well plates, with clonality defined as having greater than 99% of the wells with positive reactivity. Isotypes of antibodies are determined using the iso-strip technology (Roche). To obtain purified antibody for further evaluation, tissue culture supernatants are affinity purified using a protein A or protein G columns.

Results: Five monoclonal antibodies immunoreactive to HPTPβ extracellular domain protein are isolated and given the following nomenclature, R15E6, R12A7, R3A2, R11C3, R15G2 and R5A8.

The monoclonal antibody R15E6 is deposited with American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108 USA on 04 May 2006.

Example 3. R15E6 binds to endogenous HPTPβ on human endothelial cells.

A. R15E6 binds endogenous HPTPβ as demonstrated by immunoprecipitation and western blot.

Materials: Human umbilical vein endothelial cells (HUVECs), EGM media, and trypsin neutralizing solution from Cambrex; OPTIMEM I (Gibco), bovine serum albumin (BSA; Santa Cruz), phosphate buffered saline (PBS; Gibco), Growth Factors including Angiopoietin 1 (Ang1), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (R&D Systems), Tie2 monoclonal antibody (Duke University/P&GP), VEGF receptor 2 (VEGFR2) polyclonal antibody (Whitaker et. al), protein A/G agarose (Santa Cruz), Tris-Glycine pre-cast gel electrophoresis/transfer system (6-8%) (Invitrogen), PVDF membranes (Invitrogen), lysis buffer (20 mm Tris-HCl, 137 mm NaCl, 10% glycerol, 1% triton-X-100, 2 mM EDTA, 1 mM NaOV, 1 mM NaF, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin).

Method: HUVECs are pre-treated for 30 min with antibody (in OPTIMEM) or OPTIMEM I alone. After removal of pre-treatment, cells are treated with Ang1 (100 ng/ml) for 6 minutes in PBS + 0.2% BSA and lysed in lysis buffer. Lysates are run directly on a Tris-Glycine gel or immunoprecipitated with 2-5 μg/ml Tie-2 antibody or 10 μg/ml R15E6 antibody and protein A/G agarose. Immunoprecipitated samples are rinsed 1x with lysis buffer and boiled for 5 min in 1 x sample buffer. Samples are resolved on a Tris-Glycine gel, transferred to a PVDF membrane, and detected by western blot using the indicated antibodies (pTYR Ab (PY99, Santa Cruz), Tie-2, VEGFR2 and/or R15E6).

Results: By IP/western blotting, R15E6 recognizes a major, high molecular weight band consistent with the size of HPTPβ (Figure 2, Panel A, Lane 2). The less intense, lower molecular weight bands likely represent less glycosylated precursor forms of HPTPβ. An immunoprecipitation (IP) with control, non-immune IgG shows no bands in the molecular weight range of HPTPβ (Figure 2, Panel A, Lane 1), and a combined Tie2/VEGFR2 IP shows bands of the expected molecular weight (Figure 2, Panel A, Lane 3). This result demonstrates that R15E6 recognizes and is specific for HPTPβ.

B. R15E6 binds endogenous HPTPβ as demonstrated by FACS analysis

Materials: HUVECs, EGM media, and trypsin neutralizing solution from Cambrex; Secondary Alexfluor 488-tagged antibody from Molecular Probes; Hanks balanced salt solution (Gibco); FACSCAN flow cytometer and CellQuest software from Becton Dickenson.

Method: HUVECs are trypsinized, treated with trypsin neutralizing solution and rinsed with HBSS. R15E6 antibody (0.6 µg) is added to 250,000 cells in 50 µl of HBSS and incubated on ice for 20 minutes. Cells are rinsed with 1 ml HBSS followed by adding 2 µg of fluorescent-conjugated secondary antibody for 20 minutes on ice. Cells are rinsed and resuspended in 1 ml HBSS then analyzed on the FACSCAN flow cytometer with CellQuest software. Control cells are treated with fluorescent-conjugated secondary antibody only.

Results: By FACS analysis, intact HUVECs, R15E6 causes a robust shift (>90% of cells) in the fluorescence signal compared to the secondary antibody alone (Figure 2, Panel B). This result indicates that R15E6 binds to endogenous HPTPβ presented on the surface of intact endothelial cells.

Example 4. R15E6 enhances Tie2 activation, and promotes multiple angiogenic responses (endothelial cell survival, migration and capillary morphogenesis).

A. R15E6 enhances Tie2 phosphorylation in the absence and presence of the angiopoietin 1 (Ang1), the Tie2 ligand.

Methods: HUVECs are cultured in serum free media as described above in the presence or absence of various concentrations of R15E6 and with or without added Ang1. Lysates are prepared, immunoprecipitated with a Tie2 antibody, resolved by polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Membrane-bound immunoprecipitated proteins are then serially western blotted with an antiphosphotyrosine antibody to quantify Tie2 phosphorylation followed by a Tie2 antibody to quantify total Tie2. Tie2 phosphorylation is expressed as the ratio of the antiphosphotyrosine signal over the total Tie2 signal.

Results: R15E6 enhances Tie2 phosphorylation both in the absence and presence of Ang1 (Figure 3). This result indicates that binding of R15E6 to HPTPβ on the surface of endothelial cells modulates its biological function resulting in enhanced activation of Tie2 in the absence or presence of ligand.

B. R15E6 enhances endothelial cell survival in the absence and in the presence of endothelial growth factors.

Materials: HUVECs, EGM media, and trypsin neutralizing solution from Cambrex; DMEM (Cell Gro), Delipidized BSA (BD Falcon), Cell Titer Glo ATP Assay (Promega), Growth Factors (Ang1 , VEGF 165, and FGF) (R&D Systems), Victor V Multilabel plate reader (Perkin Elmer Wallac).

Method: HUVECs are plated at 10,000 cells/well, serum starved in DMEM/0.2% BSA and treated for 72h in the presence or absence of growth factor (Ang1, VEGF, or FGF), with or without various concentrations of R15E6 antibody. After 72 hours, the cells are rinsed with DMEM and surviving cells are quantified by measuring ATP levels using the Cell Titer Glo Luminescence Assay according to manufacturer's instructions (Promega).

Results: Consistent with the results of the Tie2 activation assay, R15E6 enhances endothelial cell survival in the absence of added growth factor at concentrations between 0.5 and 5 nM (Figure 4, Panel A). Similarly, R15E6 enhances Ang1 mediated endothelial cell survival (Figure 4, Panel A) as well as cell survival mediated by VEGF and FGF (Figure 4, Panels B and C). No enhanced survival is seen with a control monoclonal antibody (Figure 4, Panel D). These results demonstrate that R15E6 binding to HPTPβ on the endothelial cell surface enhances baseline endothelial cell survival as well as cell survival mediated by multiple angiogenic pathways (Ang1, VEGF, and FGF).

C. R15E6 enhances endothelial cell migration in the absence and in the presence of VEGF.

Materials: HUVECs, EGM media, and trypsin neutralizing solution from Cambrex; EBM-phenol red free (PRF-EBM, Cambrex), Delipidized BSA (BD Falcon), BD Falcon Biocoat Endothelial Cell Migration system (BD Falcon), Calcein AM (Molecular Probes); Growth Factors (VEGF 165) (R&D Systems), Victor V Multilabel plate reader (Perkin Elmer Wallac).

Method: HUVECs are resuspended in PRF-EBM+0.1% BSA and plated at 50,000 cells/transwell (BD Bioscience, 3 μm pore size). Growth Factor/ R15E6 is placed in the bottom well of the transwell chamber and incubated 4-22h. Cells migrating through the membrane are detected by labeling with 4μg/ml Calcein AM for 90'. Fluorescence is measured using a Victor V instrument (485/535).

Results: Consistent with the results in the survival study, R15E6 enhances both baseline and VEGF-mediated endothelial cell migration (Figure 5).

D. R15E6 enhances endothelial cell sprouting and capillary morphogenesis in the absence and in the presence of endothelial growth factors.

Materials: HUVECs and EGM media from Cambrex; Cytodex™ beads and type I collagen from Sigma; Dulbecco's PBS and M199 media from Gibco; VEGF from R&D.

Method: HUVECs passage 4 ($2x10^6$ cells) are cultured with 5 mg of Cytodex™ beads in 10 ml of EGM in 100 mm non-tissue culture treated bacteriological dishes for 48 hours with occasional swirling. Cell coated beads are transferred to a 50 ml conical tube and resuspended in 380 µl D-PBS. Collagen gels are prepared by adding 71.4 µl of cell coated beads to 2.8 ml of a matrix solution consisting of 3 mg/ml collagen in M199 media supplemented with 0.005 NNaOH, 20 mM HEPES, and 26 mM $NaHCO_3$. Three hundred and fifty microliters of the beads are dispensed into a well on a 24 well tissue culture plate and the matrix is allowed to solidify for 1 hour at 37°C/5% CO_2. One ml of EGM medium with or without VEGF (10 ng/ml) or R15E6 (7.5 µg/ml) is added per well and returned to the incubator. After 48 hours, a blinded observer visualizes the sprouts with a phase contrast inverted microscope and observes 50 beads per well, in triplicate wells, for the presence of endothelial cell sprouts. Results are expressed as the number of sprouts per bead.

Results: Consistent with the results in the other assays, R15E6 also enhances baseline and VEGF mediated capillary morphogenesis in the endothelial bead sprouting assay (Figure 6).

Example 5. The binding epitope for R15E6 is in the N-terminal FN3 repeat of the human HPTPβ extracellular domain.

A. Western blot analysis of recombinant c-terminal truncation mutants and mouse/human chimeric proteins show that the R15E6 binding epitope is in the N-terminal FN3 repeat of the HPTPβ extracellular domain.

Methods: HEK293 cells are transfected with expression vectors encoding the indicated HPTPβ truncation mutant or mouse/human chimera. Transfected cells are then incubated in OptiMEM for an additional 24 hours after which conditioned media containing the indicated HPTPβ extracellular domain is harvested and either stored for future use or used immediately for

western blot or ECL (see below) studies. For western blot analysis, 20 µl of conditioned media containing the indicated HPTPβ protein or no recombinant protein (Mock, empty vector transfected) is resolved by PAGE, transferred to a PVDF membrane and probed with R15E6.

Results: By western blot analysis, R15E6 binds all of the HPTPβ C-terminal deletion mutants (Figure 7A) indicating that the binding epitope is within the first two N-terminal FN3 repeats. R15E6 fails to bind murine HPTPβ (SEQ ID NO: 7) extracellular domain demonstrating specificity for the human protein (Figure 7B lane 6 vs. lane 2). Replacing the murine 1st or 1st and 2nd N-terminal FN3 repeats with the human sequences restored R15E6 binding (Figure 7B lanes 3 and 5). Conversely, replacing only the murine 2nd FN3 repeat with the human sequence fails to restore binding (Figure 7B lane 4). Taken together, these findings localize the binding epitope of R15E6 to the N-terminal FN3 repeat (~100 amino acids) of human HPTPβ.

B. ECL (electrochemiluminescent) analysis of -terminal truncation mutants and mouse/human chimeric proteins confirms that the R15E6 binding epitope is in the N-terminal FN3 repeat of the HPTPβ extracellular domain.

Methods: Supernatants containing the indicated HPTPβ protein are coated on a 96 well High bind MSD (Meso Scale Discovery) plate, allowed to dry, and blocked with 3% BSA for 1 h. The protein is then incubated with the R15E6 monoclonal antibody or the R15E6 Fab fragment (10 nM or 1.5 µg/ml) for 1 h, rinsed, and incubated with a goat anti-mouse antibody with an MSD-Tag label (10 nM) for 1 h. The excess antibody is rinsed off and MSD read buffer is added. Light emission is measured using the Sector 2400 reader (MSD). MSD utilizes electrochemiluminescent detection to detect binding events on patterned arrays. Meso Scale Discovery's technology uses proprietary MULTI-ARRAY™ and MULTI-SPOT™ microplates with electrodes integrated into the bottom of the plate. MSD's electrodes are made from carbon and biological reagents may be attached to the carbon simply by passive adsorption and retain a high level of biological activity. MSD assays use electrochemiluminescent labels for ultra-sensitive detection. These electrochemiluminescent labels emit light when electrochemically stimulated. The detection process is initiated at the built in electrodes located in the bottom of MSD's microplates and only labels near the electrode are excited and light detected at 620 nm.

Results: Consistent with the western blot studies, R15E6 binds to all of the HPTPβ C-terminal truncation proteins by MSD analysis (Figure 8A). Also consistent with the western blot

analysis, R15E6 fails to bind the murine HPTPβ extracellular domain but binding is restored by replacing the murine N-terminal FN3 repeat with the human N-terminal FN3 domain (Figure 8B). These data confirm that the binding epitope of R15E6 is in the N-terminal FN3 repeat of human HPTPβ. As expected, the binding epitope of the monovalent R15E6 Fab fragment could also be mapped to the N-terminal most FN3 repeat of human HPTPβ (Figure 9).

Example 6. A monovalent R15E6 Fab fragment blocks R15E6 mediated Tie2 activation and inhibits endothelial cell survival and migration.

Methods: Tie2 activation and endothelial cell survival and migration assays are performed as described above in example 4. Monovalent R15E6 Fab fragments are prepared as previously described. Purified R15E6 is dialyzed in 0.1M Tris-HCL, pH 8.0, containing 2 mM EDTA and 1 mM dithiothreitol. Papain (Pierce) at 1-2 mg/ml is activated in the aforementioned buffer for 15 minutes at 37˚C. R15E6 at 10 mg/ml is incubated with papain in the same buffer using an enzyme:substrate ratio of 1:100, for 1h at 37˚C. The digestion is terminated by the addition of iodoacetamide (final concentration 20 mM, and held on ice for 1 h, protected from light. The papain digested material is dialyzed overnight against phosphate-buffered saline, to remove iodoacetamide. The extent of digestion is monitored by SDS-PAGE with the disappearance of the gamma heavy chain (MW 55,000 kDa) and the appearance of the Fc fragment of gamma (MW 27,000 kDa) and light chains (MW 22,000-25,000 kDa).

Results: Unlike the intact R15E6 antibody, the Fab fragments fails to enhance Tie2 activation (Figure 10). Moreover, in the presence of excess Fab fragment, R15E6-mediated Tie2 activation is blocked (Figure 10). Surprisingly, the R15E6 Fab fragment markedly inhibits endothelial cell survival compared to a control Fab (Figure 11A) and this effect could be rescued by the addition of intact R15E6 (Figure 11B). Consistent with the negative effect on endothelial survival, the R15E6 Fab also blocks VEGF mediated endothelial cell migration (Figure 12). These findings demonstrate that the intact, dimeric R15E6 is required for the enhancement of angiogenic signaling and that monomeric R15E6 blocks these actions and actually has a negative effect on angiogenic endothelial cell responses.

Except as otherwise noted, all amounts including quantities, percentages, portions, and proportions, are understood to be modified by the word "about", and amounts are not intended to indicate significant digits.

Except as otherwise noted, the articles "a", "an", and "the" mean "one or more".

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Thr Val Ser Ala Phe Arg His Ser Gln Lys Val Asp Ser Gln Thr Ile
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ccc aag cac gtc ttt gag cac acg ttc cac aga ctg gag gcc ggg gag      2640
Pro Lys His Val Phe Glu His Thr Phe His Arg Leu Glu Ala Gly Glu
865                 870                 875                 880

cag tac cag atc atg att gcc tca gtc agc ggg tcc ctg aag aat cag      2688
Gln Tyr Gln Ile Met Ile Ala Ser Val Ser Gly Ser Leu Lys Asn Gln
                885                 890                 895
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ata aat gtg gtt ggg cgg aca gtt cca gca tct gtc caa gga gta att      2736
Ile Asn Val Val Gly Arg Thr Val Pro Ala Ser Val Gln Gly Val Ile
            900                 905                 910

gca gac aat gca tac agc agt tat tcc tta ata gta agt tgg caa aaa      2784
Ala Asp Asn Ala Tyr Ser Ser Tyr Ser Leu Ile Val Ser Trp Gln Lys
        915                 920                 925

gct gct ggt gtg gca gaa aga tat gat atc ctg ctt cta act gaa aat      2832
Ala Ala Gly Val Ala Glu Arg Tyr Asp Ile Leu Leu Leu Thr Glu Asn
    930                 935                 940

gga atc ctt ctg cgc aac aca tca gag cca gcc acc act aag caa cac      2880
Gly Ile Leu Leu Arg Asn Thr Ser Glu Pro Ala Thr Thr Lys Gln His
945                 950                 955                 960

aaa ttt gaa gat cta aca cca ggc aag aaa tac aag ata cag atc cta      2928
Lys Phe Glu Asp Leu Thr Pro Gly Lys Lys Tyr Lys Ile Gln Ile Leu
                965                 970                 975

act gtc agt gga ggc ctc ttt agc aag gaa gcc cag act gaa ggc cga      2976
Thr Val Ser Gly Gly Leu Phe Ser Lys Glu Ala Gln Thr Glu Gly Arg
            980                 985                 990

aca gtc cca gca gct gtc acc gac  ctg agg atc aca gag  aac tcc acc    3024
Thr Val Pro Ala Ala Val Thr Asp  Leu Arg Ile Thr Glu  Asn Ser Thr
        995                 1000                1005

agg cac  ctg tcc ttc cgc tgg  acc gcc tca gag ggg  gag ctc agc       3069
Arg His  Leu Ser Phe Arg Trp  Thr Ala Ser Glu Gly  Glu Leu Ser
    1010                1015                1020

tgg tac  aac atc ttt ttg tac  aac cca gat ggg aat  ctc cag gag       3114
Trp Tyr  Asn Ile Phe Leu Tyr  Asn Pro Asp Gly Asn  Leu Gln Glu
    1025                1030                1035

aga gct  caa gtt gac cca cta  gtc cag agc ttc tct  ttc cag aac       3159
Arg Ala  Gln Val Asp Pro Leu  Val Gln Ser Phe Ser  Phe Gln Asn
    1040                1045                1050

ttg cta  caa ggc aga atg tac  aag atg gtg att gta  act cac agt       3204
Leu Leu  Gln Gly Arg Met Tyr  Lys Met Val Ile Val  Thr His Ser
    1055                1060                1065

ggg gag  ctg tct aat gag tct  ttc ata ttt ggt aga  aca gtc cca       3249
Gly Glu  Leu Ser Asn Glu Ser  Phe Ile Phe Gly Arg  Thr Val Pro
    1070                1075                1080

gcc tct  gtg agt cat ctc agg  ggg tcc aat cgg aac  acg aca gac       3294
Ala Ser  Val Ser His Leu Arg  Gly Ser Asn Arg Asn  Thr Thr Asp
    1085                1090                1095

agc ctt  tgg ttc aac tgg agt  cca gcc tct ggg gac  ttt gac ttt       3339
Ser Leu  Trp Phe Asn Trp Ser  Pro Ala Ser Gly Asp  Phe Asp Phe
    1100                1105                1110
```

```
tat gag  ctg att ctc tat aat  ccc aat ggc aca aag  aag gaa aac      3384
Tyr Glu  Leu Ile Leu Tyr Asn  Pro Asn Gly Thr Lys  Lys Glu Asn
    1115                 1120                 1125

tgg aaa  gac aag gac ctg acg  gag tgg cgg ttt caa  ggc ctt gtt      3429
Trp Lys  Asp Lys Asp Leu Thr  Glu Trp Arg Phe Gln  Gly Leu Val
    1130                 1135                 1140

cct gga  agg aag tac gtg ctg  tgg gtg gta act cac  agt gga gat      3474
Pro Gly  Arg Lys Tyr Val Leu  Trp Val Val Thr His  Ser Gly Asp
    1145                 1150                 1155

ctc agc  aat aaa gtc aca gcg  gag agc aga aca gct  cca agt cct      3519
Leu Ser  Asn Lys Val Thr Ala  Glu Ser Arg Thr Ala  Pro Ser Pro
    1160                 1165                 1170

ccc agt  ctt atg tca ttt gct  gac att gca aac aca  tcc ttg gcc      3564
Pro Ser  Leu Met Ser Phe Ala  Asp Ile Ala Asn Thr  Ser Leu Ala
    1175                 1180                 1185

atc acg  tgg aaa ggg ccc cca  gac tgg aca gac tac  aac gac ttt      3609
Ile Thr  Trp Lys Gly Pro Pro  Asp Trp Thr Asp Tyr  Asn Asp Phe
    1190                 1195                 1200

gag ctg  cag tgg ttg ccc aga  gat gca ctt act gtc  ttc aac ccc      3654
Glu Leu  Gln Trp Leu Pro Arg  Asp Ala Leu Thr Val  Phe Asn Pro
    1205                 1210                 1215

tac aac  aac aga aaa tca gaa  gga cgc att gtg tat  ggt ctt cgt      3699
Tyr Asn  Asn Arg Lys Ser Glu  Gly Arg Ile Val Tyr  Gly Leu Arg
    1220                 1225                 1230

cca ggg  aga tcc tat caa ttc  aac gtc aag act gtc  agt ggt gat      3744
Pro Gly  Arg Ser Tyr Gln Phe  Asn Val Lys Thr Val  Ser Gly Asp
    1235                 1240                 1245

tcc tgg  aaa act tac agc aaa  cca att ttt gga tct  gtg agg aca      3789
Ser Trp  Lys Thr Tyr Ser Lys  Pro Ile Phe Gly Ser  Val Arg Thr
    1250                 1255                 1260

aag cct  gac aag ata caa aac  ctg cat tgc cgg cct  cag aac tcc      3834
Lys Pro  Asp Lys Ile Gln Asn  Leu His Cys Arg Pro  Gln Asn Ser
    1265                 1270                 1275

acg gcc  att gcc tgt tct tgg  atc cct cct gat tct  gac ttt gat      3879
Thr Ala  Ile Ala Cys Ser Trp  Ile Pro Pro Asp Ser  Asp Phe Asp
    1280                 1285                 1290

ggt tat  agt att gaa tgc cgg  aaa atg gac acc caa  gaa gtt gag      3924
Gly Tyr  Ser Ile Glu Cys Arg  Lys Met Asp Thr Gln  Glu Val Glu
    1295                 1300                 1305

ttt tcc  aga aag ctg gag aaa  gaa aaa tct ctg ctc  aac atc atg      3969
Phe Ser  Arg Lys Leu Glu Lys  Glu Lys Ser Leu Leu  Asn Ile Met
    1310                 1315                 1320
```

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atg cta  gtg ccc cat aag agg  tac ctg gtg tcc atc  aaa gtg cag      4014
Met Leu  Val Pro His Lys Arg  Tyr Leu Val Ser Ile  Lys Val Gln
    1325                  1330                  1335

tcg gcc  ggc atg acc agc gag  gtg gtt gaa gac agc  act atc aca      4059
Ser Ala  Gly Met Thr Ser Glu  Val Val Glu Asp Ser  Thr Ile Thr
    1340                  1345                  1350

atg ata  gac cgc ccc cct cct  cca ccc cca cac att  cgt gtg aat      4104
Met Ile  Asp Arg Pro Pro Pro  Pro Pro Pro His Ile  Arg Val Asn
    1355                  1360                  1365

gaa aag  gat gtg cta att agc  aag tct tcc atc aac  ttt act gtc      4149
Glu Lys  Asp Val Leu Ile Ser  Lys Ser Ser Ile Asn  Phe Thr Val
    1370                  1375                  1380

aac tgc  agc tgg ttc agc gac  acc aat gga gct gtg  aaa tac ttc      4194
Asn Cys  Ser Trp Phe Ser Asp  Thr Asn Gly Ala Val  Lys Tyr Phe
    1385                  1390                  1395

aca gtg  gtg gtg aga gag gct  gat ggc agt gat gag  ctg aag cca      4239
Thr Val  Val Val Arg Glu Ala  Asp Gly Ser Asp Glu  Leu Lys Pro
    1400                  1405                  1410

gaa cag  cag cac cct ctc cct  tcc tac ctg gag tac  agg cac aat      4284
Glu Gln  Gln His Pro Leu Pro  Ser Tyr Leu Glu Tyr  Arg His Asn
    1415                  1420                  1425

gcc tcc  att cgg gtg tat cag  act aat tat ttt gcc  agc aaa tgt      4329
Ala Ser  Ile Arg Val Tyr Gln  Thr Asn Tyr Phe Ala  Ser Lys Cys
    1430                  1435                  1440

gcc gaa  aat cct aac agc aac  tcc aag agt ttt aac  att aag ctt      4374
Ala Glu  Asn Pro Asn Ser Asn  Ser Lys Ser Phe Asn  Ile Lys Leu
    1445                  1450                  1455

gga gca  gag atg gag agc cta  ggt gga aaa tgc gat  ccc act cag      4419
Gly Ala  Glu Met Glu Ser Leu  Gly Gly Lys Cys Asp  Pro Thr Gln
    1460                  1465                  1470

caa aaa  ttc tgt gat gga cca  ctg aag cca cac act  gcc tac aga      4464
Gln Lys  Phe Cys Asp Gly Pro  Leu Lys Pro His Thr  Ala Tyr Arg
    1475                  1480                  1485

atc agc  att cga gct ttt aca  cag ctc ttt gat gag  gac ctg aag      4509
Ile Ser  Ile Arg Ala Phe Thr  Gln Leu Phe Asp Glu  Asp Leu Lys
    1490                  1495                  1500

gaa ttc  aca aag cca ctc tat  tca gac aca ttt ttt  tct tta ccc      4554
Glu Phe  Thr Lys Pro Leu Tyr  Ser Asp Thr Phe Phe  Ser Leu Pro
    1505                  1510                  1515

atc act  act gaa tca gag ccc  ttg ttt gga gct att  gaa cgc ggc      4599
Ile Thr  Thr Glu Ser Glu Pro  Leu Phe Gly Ala Ile  Glu Arg Gly
    1520                  1525                  1530
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cgc cat  cat cat cat cat cac  gga                                    4623
Arg His  His His His His His  Gly
    1535                 1540
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<210> 2
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<212> PRT
<213> Homo sapiens

<400> 2

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Met Leu Ser His Gly Ala Gly Leu Ala Leu Trp Ile Thr Leu Ser Leu
1               5                  10                  15

Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
            20                  25                  30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
        35                  40                  45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
    50                  55                  60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65                  70                  75                  80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
                85                  90                  95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
            100                 105                 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
        115                 120                 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
    130                 135                 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145                 150                 155                 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
                165                 170                 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
            180                 185                 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
        195                 200                 205
```

Ile Gly Ile Ser Thr Lys Ala Asn Ser Leu Leu Ile Ser Trp Ser His
210 215 220

Gly Ser Gly Asn Val Glu Arg Tyr Arg Leu Met Leu Met Asp Lys Gly
225 230 235 240

Ile Leu Val His Gly Gly Val Val Asp Lys His Ala Thr Ser Tyr Ala
245 250 255

Phe His Gly Leu Thr Pro Gly Tyr Leu Tyr Asn Leu Thr Val Met Thr
260 265 270

Glu Ala Ala Gly Leu Gln Asn Tyr Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Ser Leu Thr
290 295 300

Ser Leu Lys Val Lys Trp Gln Arg Pro Pro Gly Asn Val Asp Ser Tyr
305 310 315 320

Asn Ile Thr Leu Ser His Lys Gly Thr Ile Lys Glu Ser Arg Val Leu
325 330 335

Ala Pro Trp Ile Thr Glu Thr His Phe Lys Glu Leu Val Pro Gly Arg
340 345 350

Leu Tyr Gln Val Thr Val Ser Cys Val Ser Gly Glu Leu Ser Ala Gln
355 360 365

Lys Met Ala Val Gly Arg Thr Phe Pro Leu Ala Val Leu Gln Leu Arg
370 375 380

Val Lys His Ala Asn Glu Thr Ser Leu Ser Ile Met Trp Gln Thr Pro
385 390 395 400

Val Ala Glu Trp Glu Lys Tyr Ile Ile Ser Leu Ala Asp Arg Asp Leu
405 410 415

Leu Leu Ile His Lys Ser Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe
420 425 430

Thr Asp Leu Val Pro Gly Arg Lys Tyr Met Ala Thr Val Thr Ser Ile
435 440 445

Ser Gly Asp Leu Lys Asn Ser Ser Ser Val Lys Gly Arg Thr Val Pro
450 455 460

Ala Gln Val Thr Asp Leu His Val Ala Asn Gln Gly Met Thr Ser Ser
465 470 475 480

Leu Phe Thr Asn Trp Thr Gln Ala Gln Gly Asp Val Glu Phe Tyr Gln
485 490 495

Val Leu Leu Ile His Glu Asn Val Val Ile Lys Asn Glu Ser Ile Ser

```
                500                 505                 510

Ser Glu Thr Ser Arg Tyr Ser Phe His Ser Leu Lys Ser Gly Ser Leu
        515                 520                 525

Tyr Ser Val Val Val Thr Thr Val Ser Gly Gly Ile Ser Ser Arg Gln
    530                 535                 540

Val Val Val Glu Gly Arg Thr Val Pro Ser Ser Val Ser Gly Val Thr
545                 550                 555                 560

Val Asn Asn Ser Gly Arg Asn Asp Tyr Leu Ser Val Ser Trp Leu Leu
                565                 570                 575

Ala Pro Gly Asp Val Asp Asn Tyr Glu Val Thr Leu Ser His Asp Gly
            580                 585                 590

Lys Val Val Gln Ser Leu Val Ile Ala Lys Ser Val Arg Glu Cys Ser
        595                 600                 605

Phe Ser Ser Leu Thr Pro Gly Arg Leu Tyr Thr Val Thr Ile Thr Thr
    610                 615                 620

Arg Ser Gly Lys Tyr Glu Asn His Ser Phe Ser Gln Glu Arg Thr Val
625                 630                 635                 640

Pro Asp Lys Val Gln Gly Val Ser Val Ser Asn Ser Ala Arg Ser Asp
                645                 650                 655

Tyr Leu Arg Val Ser Trp Val His Ala Thr Gly Asp Phe Asp His Tyr
            660                 665                 670

Glu Val Thr Ile Lys Asn Lys Asn Asn Phe Ile Gln Thr Lys Ser Ile
        675                 680                 685

Pro Lys Ser Glu Asn Glu Cys Val Phe Val Gln Leu Val Pro Gly Arg
    690                 695                 700

Leu Tyr Ser Val Thr Val Thr Thr Lys Ser Gly Gln Tyr Glu Ala Asn
705                 710                 715                 720

Glu Gln Gly Asn Gly Arg Thr Ile Pro Glu Pro Val Lys Asp Leu Thr
                725                 730                 735

Leu Arg Asn Arg Ser Thr Glu Asp Leu His Val Thr Trp Ser Gly Ala
            740                 745                 750

Asn Gly Asp Val Asp Gln Tyr Glu Ile Gln Leu Leu Phe Asn Asp Met
        755                 760                 765

Lys Val Phe Pro Pro Phe His Leu Val Asn Thr Ala Thr Glu Tyr Arg
    770                 775                 780

Phe Thr Ser Leu Thr Pro Gly Arg Gln Tyr Lys Ile Leu Val Leu Thr
785                 790                 795                 800
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Ile Ser Gly Asp Val Gln Gln Ser Ala Phe Ile Glu Gly Phe Thr Val
805 810 815

Pro Ser Ala Val Lys Asn Ile His Ile Ser Pro Asn Gly Ala Thr Asp
820 825 830

Ser Leu Thr Val Asn Trp Thr Pro Gly Gly Gly Asp Val Asp Ser Tyr
835 840 845

Thr Val Ser Ala Phe Arg His Ser Gln Lys Val Asp Ser Gln Thr Ile
850 855 860

Pro Lys His Val Phe Glu His Thr Phe His Arg Leu Glu Ala Gly Glu
865 870 875 880

Gln Tyr Gln Ile Met Ile Ala Ser Val Ser Gly Ser Leu Lys Asn Gln
885 890 895

Ile Asn Val Val Gly Arg Thr Val Pro Ala Ser Val Gln Gly Val Ile
900 905 910

Ala Asp Asn Ala Tyr Ser Ser Tyr Ser Leu Ile Val Ser Trp Gln Lys
915 920 925

Ala Ala Gly Val Ala Glu Arg Tyr Asp Ile Leu Leu Leu Thr Glu Asn
930 935 940

Gly Ile Leu Leu Arg Asn Thr Ser Glu Pro Ala Thr Thr Lys Gln His
945 950 955 960

Lys Phe Glu Asp Leu Thr Pro Gly Lys Lys Tyr Lys Ile Gln Ile Leu
965 970 975

Thr Val Ser Gly Gly Leu Phe Ser Lys Glu Ala Gln Thr Glu Gly Arg
980 985 990

Thr Val Pro Ala Ala Val Thr Asp Leu Arg Ile Thr Glu Asn Ser Thr
995 1000 1005

Arg His Leu Ser Phe Arg Trp Thr Ala Ser Glu Gly Glu Leu Ser
1010 1015 1020

Trp Tyr Asn Ile Phe Leu Tyr Asn Pro Asp Gly Asn Leu Gln Glu
1025 1030 1035

Arg Ala Gln Val Asp Pro Leu Val Gln Ser Phe Ser Phe Gln Asn
1040 1045 1050

Leu Leu Gln Gly Arg Met Tyr Lys Met Val Ile Val Thr His Ser
1055 1060 1065

Gly Glu Leu Ser Asn Glu Ser Phe Ile Phe Gly Arg Thr Val Pro
1070 1075 1080

Ala Ser Val Ser His Leu Arg Gly Ser Asn Arg Asn Thr Thr Asp

1085 1090 1095

Ser Leu Trp Phe Asn Trp Ser Pro Ala Ser Gly Asp Phe Asp Phe
1100 1105 1110

Tyr Glu Leu Ile Leu Tyr Asn Pro Asn Gly Thr Lys Lys Glu Asn
1115 1120 1125

Trp Lys Asp Lys Asp Leu Thr Glu Trp Arg Phe Gln Gly Leu Val
1130 1135 1140

Pro Gly Arg Lys Tyr Val Leu Trp Val Val Thr His Ser Gly Asp
1145 1150 1155

Leu Ser Asn Lys Val Thr Ala Glu Ser Arg Thr Ala Pro Ser Pro
1160 1165 1170

Pro Ser Leu Met Ser Phe Ala Asp Ile Ala Asn Thr Ser Leu Ala
1175 1180 1185

Ile Thr Trp Lys Gly Pro Pro Asp Trp Thr Asp Tyr Asn Asp Phe
1190 1195 1200

Glu Leu Gln Trp Leu Pro Arg Asp Ala Leu Thr Val Phe Asn Pro
1205 1210 1215

Tyr Asn Asn Arg Lys Ser Glu Gly Arg Ile Val Tyr Gly Leu Arg
1220 1225 1230

Pro Gly Arg Ser Tyr Gln Phe Asn Val Lys Thr Val Ser Gly Asp
1235 1240 1245

Ser Trp Lys Thr Tyr Ser Lys Pro Ile Phe Gly Ser Val Arg Thr
1250 1255 1260

Lys Pro Asp Lys Ile Gln Asn Leu His Cys Arg Pro Gln Asn Ser
1265 1270 1275

Thr Ala Ile Ala Cys Ser Trp Ile Pro Pro Asp Ser Asp Phe Asp
1280 1285 1290

Gly Tyr Ser Ile Glu Cys Arg Lys Met Asp Thr Gln Glu Val Glu
1295 1300 1305

Phe Ser Arg Lys Leu Glu Lys Glu Lys Ser Leu Leu Asn Ile Met
1310 1315 1320

Met Leu Val Pro His Lys Arg Tyr Leu Val Ser Ile Lys Val Gln
1325 1330 1335

Ser Ala Gly Met Thr Ser Glu Val Val Glu Asp Ser Thr Ile Thr
1340 1345 1350

Met Ile Asp Arg Pro Pro Pro Pro Pro Pro His Ile Arg Val Asn
1355 1360 1365

Glu Lys Asp Val Leu Ile Ser Lys Ser Ser Ile Asn Phe Thr Val
1370 1375 1380

Asn Cys Ser Trp Phe Ser Asp Thr Asn Gly Ala Val Lys Tyr Phe
1385 1390 1395

Thr Val Val Val Arg Glu Ala Asp Gly Ser Asp Glu Leu Lys Pro
1400 1405 1410

Glu Gln Gln His Pro Leu Pro Ser Tyr Leu Glu Tyr Arg His Asn
1415 1420 1425

Ala Ser Ile Arg Val Tyr Gln Thr Asn Tyr Phe Ala Ser Lys Cys
1430 1435 1440

Ala Glu Asn Pro Asn Ser Asn Ser Lys Ser Phe Asn Ile Lys Leu
1445 1450 1455

Gly Ala Glu Met Glu Ser Leu Gly Gly Lys Cys Asp Pro Thr Gln
1460 1465 1470

Gln Lys Phe Cys Asp Gly Pro Leu Lys Pro His Thr Ala Tyr Arg
1475 1480 1485

Ile Ser Ile Arg Ala Phe Thr Gln Leu Phe Asp Glu Asp Leu Lys
1490 1495 1500

Glu Phe Thr Lys Pro Leu Tyr Ser Asp Thr Phe Phe Ser Leu Pro
1505 1510 1515

Ile Thr Thr Glu Ser Glu Pro Leu Phe Gly Ala Ile Glu Arg Gly
1520 1525 1530

Arg His His His His His His Gly
1535 1540

<210> 3
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<212> PRT
<213> Homo sapiens

<400> 3

Met Leu Ser His Gly Ala Gly Leu Ala Leu Trp Ile Thr Leu Ser Leu
1 5 10 15

Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

```
Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
    50                  55                  60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65                  70                  75                  80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
                85                  90                  95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
            100                 105                 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
        115                 120                 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
    130                 135                 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145                 150                 155                 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
                165                 170                 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
            180                 185                 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
        195                 200                 205

Ile Gly Ile Ser Thr Lys Ala Asn Ser Leu Leu Ile Ser Trp Ser His
    210                 215                 220

Gly Ser Gly Asn Val Glu Arg Tyr Arg Leu Met Leu Met Asp Lys Gly
225                 230                 235                 240

Ile Leu Val His Gly Gly Val Val Asp Lys His Ala Thr Ser Tyr Ala
                245                 250                 255

Phe His Gly Leu Ser Pro Gly Tyr Leu Tyr Asn Leu Thr Val Met Thr
            260                 265                 270

Glu Ala Ala Gly Leu Gln Asn Tyr Arg Trp Lys Leu Val Arg Thr Ala
        275                 280                 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Ser Leu Thr
    290                 295                 300

Ser Leu Lys Val Lys Trp Gln Arg Pro Pro Gly Asn Val Asp Ser Tyr
305                 310                 315                 320

Asn Ile Thr Leu Ser His Lys Gly Thr Ile Lys Glu Ser Arg Val Leu
                325                 330                 335

Ala Pro Trp Ile Thr Glu Thr His Phe Lys Glu Leu Val Pro Gly Arg
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340 345 350

Leu Tyr Gln Val Thr Val Ser Cys Val Ser Gly Glu Leu Ser Ala Gln
355 360 365

Lys Met Ala Val Gly Arg Thr Phe Pro Asp Lys Val Ala Asn Leu Glu
370 375 380

Ala Asn Asn Asn Gly Arg Met Arg Ser Leu Val Val Ser Trp Ser Pro
385 390 395 400

Pro Ala Gly Asp Trp Glu Gln Tyr Arg Ile Leu Leu Phe Asn Asp Ser
405 410 415

Val Val Leu Leu Asn Ile Thr Val Gly Lys Glu Glu Thr Gln Tyr Val
420 425 430

Met Asp Asp Thr Gly Leu Val Pro Gly Arg Gln Tyr Glu Val Glu Val
435 440 445

Ile Val Glu Ser Gly Asn Leu Lys Asn Ser Glu Arg Cys Gln Gly Arg
450 455 460

Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn Glu
465 470 475 480

Thr Ser Leu Ser Ile Met Trp Gln Thr Pro Val Ala Glu Trp Glu Lys
485 490 495

Tyr Ile Ile Ser Leu Ala Asp Arg Asp Leu Leu Leu Ile His Lys Ser
500 505 510

Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Val Pro Gly
515 520 525

Arg Lys Tyr Met Ala Thr Val Thr Ser Ile Ser Gly Asp Leu Lys Asn
530 535 540

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

His Val Ala Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp Thr
565 570 575

Gln Ala Gln Gly Asp Val Glu Phe Tyr Gln Val Leu Leu Ile His Glu
580 585 590

Asn Val Val Ile Lys Asn Glu Ser Ile Ser Ser Glu Thr Ser Arg Tyr
595 600 605

Ser Phe His Ser Leu Lys Ser Gly Ser Leu Tyr Ser Val Val Val Thr
610 615 620

Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Val Glu Gly Arg
625 630 635 640

Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly Arg
645 650 655

Asn Asp Tyr Leu Ser Val Ser Trp Leu Leu Ala Pro Gly Asp Val Asp
660 665 670

Asn Tyr Glu Val Thr Leu Ser His Asp Gly Lys Val Val Gln Ser Leu
675 680 685

Val Ile Ala Lys Ser Val Arg Glu Cys Ser Phe Ser Ser Leu Thr Pro
690 695 700

Gly Arg Leu Tyr Thr Val Thr Ile Thr Thr Arg Ser Gly Lys Tyr Glu
705 710 715 720

Asn His Ser Phe Ser Gln Glu Arg Thr Val Pro Asp Lys Val Gln Gly
725 730 735

Val Ser Val Ser Asn Ser Ala Arg Ser Asp Tyr Leu Arg Val Ser Trp
740 745 750

Val His Ala Thr Gly Asp Phe Asp His Tyr Glu Val Thr Ile Lys Asn
755 760 765

Lys Asn Asn Phe Ile Gln Thr Lys Ser Ile Pro Lys Ser Glu Asn Glu
770 775 780

Cys Val Phe Val Gln Leu Val Pro Gly Arg Leu Tyr Ser Val Thr Val
785 790 795 800

Thr Thr Lys Ser Gly Gln Tyr Glu Ala Asn Glu Gln Gly Asn Gly Arg
805 810 815

Thr Ile Pro Glu Pro Val Lys Asp Leu Thr Leu Arg Asn Arg Ser Thr
820 825 830

Glu Asp Leu His Val Thr Trp Ser Gly Ala Asn Gly Asp Val Asp Gln
835 840 845

Tyr Glu Ile Gln Leu Leu Phe Asn Asp Met Lys Val Phe Pro Pro Phe
850 855 860

His Leu Val Asn Thr Ala Thr Glu Tyr Arg Phe Thr Ser Leu Thr Pro
865 870 875 880

Gly Arg Gln Tyr Lys Ile Leu Val Leu Thr Ile Ser Gly Asp Val Gln
885 890 895

Gln Ser Ala Phe Ile Glu Gly Phe Thr Val Pro Ser Ala Val Lys Asn
900 905 910

Ile His Ile Ser Pro Asn Gly Ala Thr Asp Ser Leu Thr Val Asn Trp
915 920 925

Thr Pro Gly Gly Gly Asp Val Asp Ser Tyr Thr Val Ser Ala Phe Arg
930 935 940

His Ser Gln Lys Val Asp Ser Gln Thr Ile Pro Lys His Val Phe Glu
945 950 955 960

His Thr Phe His Arg Leu Glu Ala Gly Glu Gln Tyr Gln Ile Met Ile
965 970 975

Ala Ser Val Ser Gly Ser Leu Lys Asn Gln Ile Asn Val Val Gly Arg
980 985 990

Thr Val Pro Ala Ser Val Gln Gly Val Ile Ala Asp Asn Ala Tyr Ser
995 1000 1005

Ser Tyr Ser Leu Ile Val Ser Trp Gln Lys Ala Ala Gly Val Ala
1010 1015 1020

Glu Arg Tyr Asp Ile Leu Leu Leu Thr Glu Asn Gly Ile Leu Leu
1025 1030 1035

Arg Asn Thr Ser Glu Pro Ala Thr Thr Lys Gln His Lys Phe Glu
1040 1045 1050

Asp Leu Thr Pro Gly Lys Lys Tyr Lys Ile Gln Ile Leu Thr Val
1055 1060 1065

Ser Gly Gly Leu Phe Ser Lys Glu Ala Gln Thr Glu Gly Arg Thr
1070 1075 1080

Val Pro Ala Ala Val Thr Asp Leu Arg Ile Thr Glu Asn Ser Thr
1085 1090 1095

Arg His Leu Ser Phe Arg Trp Thr Ala Ser Glu Gly Glu Leu Ser
1100 1105 1110

Trp Tyr Asn Ile Phe Leu Tyr Asn Pro Asp Gly Asn Leu Gln Glu
1115 1120 1125

Arg Ala Gln Val Asp Pro Leu Val Gln Ser Phe Ser Phe Gln Asn
1130 1135 1140

Leu Leu Gln Gly Arg Met Tyr Lys Met Val Ile Val Thr His Ser
1145 1150 1155

Gly Glu Leu Ser Asn Glu Ser Phe Ile Phe Gly Arg Thr Val Pro
1160 1165 1170

Ala Ser Val Ser His Leu Arg Gly Ser Asn Arg Asn Thr Thr Asp
1175 1180 1185

Ser Leu Trp Phe Asn Trp Ser Pro Ala Ser Gly Asp Phe Asp Phe
1190 1195 1200

Tyr Glu Leu Ile Leu Tyr Asn Pro Asn Gly Thr Lys Lys Glu Asn

1205 1210 1215

Trp Lys Asp Lys Asp Leu Thr Glu Trp Arg Phe Gln Gly Leu Val
1220 1225 1230

Pro Gly Arg Lys Tyr Val Leu Trp Val Val Thr His Ser Gly Asp
1235 1240 1245

Leu Ser Asn Lys Val Thr Ala Glu Ser Arg Thr Ala Pro Ser Pro
1250 1255 1260

Pro Ser Leu Met Ser Phe Ala Asp Ile Ala Asn Thr Ser Leu Ala
1265 1270 1275

Ile Thr Trp Lys Gly Pro Pro Asp Trp Thr Asp Tyr Asn Asp Phe
1280 1285 1290

Glu Leu Gln Trp Leu Pro Arg Asp Ala Leu Thr Val Phe Asn Pro
1295 1300 1305

Tyr Asn Asn Arg Lys Ser Glu Gly Arg Ile Val Tyr Gly Leu Arg
1310 1315 1320

Pro Gly Arg Ser Tyr Gln Phe Asn Val Lys Thr Val Ser Gly Asp
1325 1330 1335

Ser Trp Lys Thr Tyr Ser Lys Pro Ile Phe Gly Ser Val Arg Thr
1340 1345 1350

Lys Pro Asp Lys Ile Gln Asn Leu His Cys Arg Pro Gln Asn Ser
1355 1360 1365

Thr Ala Ile Ala Cys Ser Trp Ile Pro Pro Asp Ser Asp Phe Asp
1370 1375 1380

Gly Tyr Ser Ile Glu Cys Arg Lys Met Asp Thr Gln Glu Val Glu
1385 1390 1395

Phe Ser Arg Lys Leu Glu Lys Glu Lys Ser Leu Leu Asn Ile Met
1400 1405 1410

Met Leu Val Pro His Lys Arg Tyr Leu Val Ser Ile Lys Val Gln
1415 1420 1425

Ser Ala Gly Met Thr Ser Glu Val Val Glu Asp Ser Thr Ile Thr
1430 1435 1440

Met Ile Asp Arg Pro Pro Pro Pro Pro Pro His Ile Arg Val Asn
1445 1450 1455

Glu Lys Asp Val Leu Ile Ser Lys Ser Ser Ile Asn Phe Thr Val
1460 1465 1470

Asn Cys Ser Trp Phe Ser Asp Thr Asn Gly Ala Val Lys Tyr Phe

1475 1480 1485

Thr Val Val Val Arg Glu Ala Asp Gly Ser Asp Glu Leu Lys Pro
1490 1495 1500

Glu Gln Gln His Pro Leu Pro Ser Tyr Leu Glu Tyr Arg His Asn
1505 1510 1515

Ala Ser Ile Arg Val Tyr Gln Thr Asn Tyr Phe Ala Ser Lys Cys
1520 1525 1530

Ala Glu Asn Pro Asn Ser Asn Ser Lys Ser Phe Asn Ile Lys Leu
1535 1540 1545

Gly Ala Glu Met Glu Ser Leu Gly Gly Lys Arg Asp Pro Thr Gln
1550 1555 1560

Gln Lys Phe Cys Asp Gly Pro Leu Lys Pro His Thr Ala Tyr Arg
1565 1570 1575

Ile Ser Ile Arg Ala Phe Thr Gln Leu Phe Asp Glu Asp Leu Lys
1580 1585 1590

Glu Phe Thr Lys Pro Leu Tyr Ser Asp Thr Phe Phe Ser Leu Pro
1595 1600 1605

Ile Thr Thr Glu Ser Glu Pro Leu Phe Gly Ala Ile Glu
1610 1615 1620

<210> 4
<211> 775
<212> PRT
<213> Homo sapiens

<400> 4

Met Leu Ser His Gly Ala Gly Leu Ala Leu Trp Ile Thr Leu Ser Leu
1 5 10 15

Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
50 55 60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65 70 75 80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile

85 90 95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
100 105 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
115 120 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
130 135 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145 150 155 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
165 170 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
180 185 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
195 200 205

Ile Gly Ile Ser Thr Lys Ala Asn Ser Leu Leu Ile Ser Trp Ser His
210 215 220

Gly Ser Gly Asn Val Glu Arg Tyr Arg Leu Met Leu Met Asp Lys Gly
225 230 235 240

Ile Leu Val His Gly Gly Val Val Asp Lys His Ala Thr Ser Tyr Ala
245 250 255

Phe His Gly Leu Thr Pro Gly Tyr Leu Tyr Asn Leu Thr Val Met Thr
260 265 270

Glu Ala Ala Gly Leu Gln Asn Tyr Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Ser Leu Thr
290 295 300

Ser Leu Lys Val Lys Trp Gln Arg Pro Pro Gly Asn Val Asp Ser Tyr
305 310 315 320

Asn Ile Thr Leu Ser His Lys Gly Thr Ile Lys Glu Ser Arg Val Leu
325 330 335

Ala Pro Trp Ile Thr Glu Thr His Phe Lys Glu Leu Val Pro Gly Arg
340 345 350

Leu Tyr Gln Val Thr Val Ser Cys Val Ser Gly Glu Leu Ser Ala Gln
355 360 365

Lys Met Ala Val Gly Arg Thr Phe Pro Leu Ala Val Leu Gln Leu Arg
370 375 380

Val Lys His Ala Asn Glu Thr Ser Leu Ser Ile Met Trp Gln Thr Pro
385 390 395 400

Val Ala Glu Trp Glu Lys Tyr Ile Ile Ser Leu Ala Asp Arg Asp Leu
405 410 415

Leu Leu Ile His Lys Ser Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe
420 425 430

Thr Asp Leu Val Pro Gly Arg Lys Tyr Met Ala Thr Val Thr Ser Ile
435 440 445

Ser Gly Asp Leu Lys Asn Ser Ser Ser Val Lys Gly Arg Thr Val Pro
450 455 460

Ala Gln Val Thr Asp Leu His Val Ala Asn Gln Gly Met Thr Ser Ser
465 470 475 480

Leu Phe Thr Asn Trp Thr Gln Ala Gln Gly Asp Val Glu Phe Tyr Gln
485 490 495

Val Leu Leu Ile His Glu Asn Val Val Ile Lys Asn Glu Ser Ile Ser
500 505 510

Ser Glu Thr Ser Arg Tyr Ser Phe His Ser Leu Lys Ser Gly Ser Leu
515 520 525

Tyr Ser Val Val Val Thr Thr Val Ser Gly Gly Ile Ser Ser Arg Gln
530 535 540

Val Val Val Glu Gly Arg Thr Val Pro Ser Ser Val Ser Gly Val Thr
545 550 555 560

Val Asn Asn Ser Gly Arg Asn Asp Tyr Leu Ser Val Ser Trp Leu Leu
565 570 575

Ala Pro Gly Asp Val Asp Asn Tyr Glu Val Thr Leu Ser His Asp Gly
580 585 590

Lys Val Val Gln Ser Leu Val Ile Ala Lys Ser Val Arg Glu Cys Ser
595 600 605

Phe Ser Ser Leu Thr Pro Gly Arg Leu Tyr Thr Val Thr Ile Thr Thr
610 615 620

Arg Ser Gly Lys Tyr Glu Asn His Ser Phe Ser Gln Glu Arg Thr Val
625 630 635 640

Pro Asp Lys Val Gln Gly Val Ser Val Ser Asn Ser Ala Arg Ser Asp
645 650 655

Tyr Leu Arg Val Ser Trp Val His Ala Thr Gly Asp Phe Asp His Tyr
660 665 670

Glu Val Thr Ile Lys Asn Lys Asn Asn Phe Ile Gln Thr Lys Ser Ile
675 680 685

Pro Lys Ser Glu Asn Glu Cys Val Phe Val Gln Leu Val Pro Gly Arg
690 695 700

Leu Tyr Ser Val Thr Val Thr Thr Lys Ser Gly Gln Tyr Glu Ala Asn
705 710 715 720

Glu Gln Gly Asn Gly Arg Thr Ile Pro Glu Lys Gly Asn Ser Ala Asp
725 730 735

Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu
740 745 750

Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr
755 760 765

Gly His His His His His His
770 775

<210> 5
<211> 421
<212> PRT
<213> Homo sapiens

<400> 5

Met Leu Ser His Gly Ala Gly Leu Ala Leu Trp Ile Thr Leu Ser Leu
1 5 10 15

Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
50 55 60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65 70 75 80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
85 90 95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
100 105 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
115 120 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
130 135 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145 150 155 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
165 170 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
180 185 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
195 200 205

Ile Gly Ile Ser Thr Lys Ala Asn Ser Leu Leu Ile Ser Trp Ser His
210 215 220

Gly Ser Gly Asn Val Glu Arg Tyr Arg Leu Met Leu Met Asp Lys Gly
225 230 235 240

Ile Leu Val His Gly Gly Val Val Asp Lys His Ala Thr Ser Tyr Ala
245 250 255

Phe His Gly Leu Thr Pro Gly Tyr Leu Tyr Asn Leu Thr Val Met Thr
260 265 270

Glu Ala Ala Gly Leu Gln Asn Tyr Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Ser Leu Thr
290 295 300

Ser Leu Lys Val Lys Trp Gln Arg Pro Pro Gly Asn Val Asp Ser Tyr
305 310 315 320

Asn Ile Thr Leu Ser His Lys Gly Thr Ile Lys Glu Ser Arg Val Leu
325 330 335

Ala Pro Trp Ile Thr Glu Thr His Phe Lys Glu Leu Val Pro Gly Arg
340 345 350

Leu Tyr Gln Val Thr Val Ser Cys Val Ser Gly Glu Leu Ser Ala Gln
355 360 365

Lys Met Ala Val Gly Arg Thr Phe Lys Gly Asn Ser Ala Asp Ile Gln
370 375 380

His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly Lys
385 390 395 400

Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His
405 410 415

His His His His His
420

<210> 6
<211> 247
<212> PRT
<213> Homo sapiens

<400> 6

Met Leu Ser His Gly Ala Gly Leu Ala Leu Trp Ile Thr Leu Ser Leu
1 5 10 15

Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
50 55 60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65 70 75 80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
85 90 95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
100 105 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
115 120 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
130 135 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145 150 155 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
165 170 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
180 185 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Lys Gly Asn Ser Ala Asp
195 200 205

Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu
210 215 220

Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr
225 230 235 240

Gly His His His His His His
245

<210> 7

<211> 1632

<212> PRT

<213> Mus musculus

<400> 7

Met Leu Arg His Gly Ala Leu Thr Ala Leu Trp Ile Thr Leu Ser Val
1 5 10 15

Val Gln Thr Gly Val Ala Glu Gln Val Lys Cys Asn Phe Thr Leu Leu
20 25 30

Glu Ser Arg Val Ser Ser Leu Ser Ala Ser Ile Gln Trp Arg Thr Phe
35 40 45

Ala Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Ser Gly
50 55 60

Pro Met Trp Cys His Pro Ile Arg Ile Asp Asn Phe Thr Tyr Gly Cys
65 70 75 80

Asn Pro Lys Asp Leu Gln Ala Gly Thr Val Tyr Asn Phe Arg Ile Val
85 90 95

Ser Leu Asp Gly Glu Glu Ser Thr Leu Val Leu Gln Thr Asp Pro Leu
100 105 110

Pro Pro Ala Arg Phe Glu Val Asn Arg Glu Lys Thr Ala Ser Thr Thr
115 120 125

Leu Gln Val Arg Trp Thr Pro Ser Ser Gly Lys Val Ser Trp Tyr Glu
130 135 140

Val Gln Leu Phe Asp His Asn Asn Gln Lys Ile Gln Glu Val Gln Val

145 150 155 160

Gln Glu Ser Thr Thr Trp Ser Gln Tyr Thr Phe Leu Asn Leu Thr Glu
165 170 175

Gly Asn Ser Tyr Lys Val Ala Ile Thr Ala Val Ser Gly Glu Lys Arg
180 185 190

Ser Phe Pro Val Tyr Ile Asn Gly Ser Thr Val Pro Ser Pro Val Lys
195 200 205

Asp Leu Gly Ile Ser Pro Asn Pro Asn Ser Leu Leu Ile Ser Trp Ser
210 215 220

Arg Gly Ser Gly Asn Val Glu Gln Tyr Arg Leu Val Leu Met Asp Lys
225 230 235 240

Gly Ala Ile Val Gln Asp Thr Asn Val Asp Arg Arg Asp Thr Ser Tyr
245 250 255

Ala Phe His Glu Leu Thr Pro Gly His Leu Tyr Asn Leu Thr Ile Val
260 265 270

Thr Met Ala Ser Gly Leu Gln Asn Ser Arg Trp Lys Leu Val Arg Thr
275 280 285

Ala Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Arg Leu
290 295 300

Thr Ser Leu Asn Val Lys Trp Gln Lys Pro Pro Gly Asp Val Asp Ser
305 310 315 320

Tyr Ser Ile Thr Leu Ser His Gln Gly Thr Ile Lys Glu Ser Lys Thr
325 330 335

Leu Ala Pro Pro Val Thr Glu Thr Gln Phe Lys Asp Leu Val Pro Gly
340 345 350

Arg Leu Tyr Gln Val Thr Ile Ser Cys Ile Ser Gly Glu Leu Ser Ala
355 360 365

Glu Lys Ser Ala Ala Gly Arg Thr Val Pro Glu Lys Val Arg Asn Leu
370 375 380

Val Ser Tyr Asn Glu Ile Trp Met Lys Ser Phe Thr Val Asn Trp Thr
385 390 395 400

Pro Pro Ala Gly Asp Trp Glu His Tyr Arg Ile Val Leu Phe Asn Glu
405 410 415

Ser Leu Val Leu Leu Asn Thr Thr Val Gly Lys Glu Glu Thr His Tyr
420 425 430

Ala Leu Asp Gly Leu Glu Leu Ile Pro Gly Arg Gln Tyr Glu Ile Glu
435 440 445

Val Ile Val Glu Ser Gly Asn Leu Arg Asn Ser Glu Arg Cys Gln Gly
450 455 460

Arg Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn
465 470 475 480

Glu Thr Ser Leu Gly Ile Thr Trp Arg Ala Pro Leu Gly Glu Trp Glu
485 490 495

Lys Tyr Ile Ile Ser Leu Met Asp Arg Glu Leu Leu Val Ile His Lys
500 505 510

Ser Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Met Pro
515 520 525

Gly Arg Asn Tyr Lys Ala Thr Val Thr Ser Met Ser Gly Asp Leu Lys
530 535 540

Gln Ser Ser Ser Ile Lys Gly Arg Thr Val Pro Ala Gln Val Thr Asp
545 550 555 560

Leu His Val Asn Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp
565 570 575

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

Glu Asn Val Val Val Lys Asn Glu Ser Val Ser Ser Asp Thr Ser Arg
595 600 605

Tyr Ser Phe Arg Ala Leu Lys Pro Gly Ser Leu Tyr Ser Val Val Val
610 615 620

Thr Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Ala Glu Gly
625 630 635 640

Arg Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly
645 650 655

Arg Asn Asp Tyr Leu Ser Val Ser Trp Leu Pro Ala Pro Gly Glu Val
660 665 670

Asp His Tyr Val Val Ser Leu Ser His Glu Gly Lys Val Asp Gln Phe
675 680 685

Leu Ile Ile Ala Lys Ser Val Ser Glu Cys Ser Phe Ser Ser Leu Thr
690 695 700

Pro Gly Arg Leu Tyr Asn Val Thr Val Thr Thr Lys Ser Gly Asn Tyr
705 710 715 720

Ala Ser His Ser Phe Thr Glu Glu Arg Thr Val Pro Asp Lys Val Gln
725 730 735

Gly Ile Ser Val Ser Asn Ser Ala Arg Ser Asp Tyr Leu Lys Val Ser
740 745 750

Trp Val His Ala Thr Gly Asp Phe Asp His Tyr Glu Val Thr Ile Lys
755 760 765

Asn Arg Glu Ser Phe Ile Gln Thr Lys Thr Ile Pro Lys Ser Glu Asn
770 775 780

Glu Cys Glu Phe Ile Glu Leu Val Pro Gly Arg Leu Tyr Ser Val Thr
785 790 795 800

Val Ser Thr Lys Ser Gly Gln Tyr Glu Ala Ser Glu Gln Gly Thr Gly
805 810 815

Arg Thr Ile Pro Glu Pro Val Lys Asp Leu Thr Leu Leu Asn Arg Ser
820 825 830

Thr Glu Asp Leu His Val Thr Trp Ser Arg Ala Asn Gly Asp Val Asp
835 840 845

Gln Tyr Glu Val Gln Leu Leu Phe Asn Asp Met Lys Val Phe Pro His
850 855 860

Ile His Leu Val Asn Thr Ala Thr Glu Tyr Lys Phe Thr Ala Leu Thr
865 870 875 880

Pro Gly Arg His Tyr Lys Ile Leu Val Leu Thr Ile Ser Gly Asp Val
885 890 895

Gln Gln Ser Ala Phe Ile Glu Gly Leu Thr Val Pro Ser Thr Val Lys
900 905 910

Asn Ile His Ile Ser Ala Asn Gly Ala Thr Asp Arg Leu Met Val Thr
915 920 925

Trp Ser Pro Gly Gly Gly Asp Val Asp Ser Tyr Val Val Ser Ala Phe
930 935 940

Arg Gln Asp Glu Lys Val Asp Ser Gln Thr Ile Pro Lys His Ala Ser
945 950 955 960

Glu His Thr Phe His Arg Leu Glu Ala Gly Ala Lys Tyr Arg Ile Ala
965 970 975

Ile Val Ser Val Ser Gly Ser Leu Arg Asn Gln Ile Asp Ala Leu Gly
980 985 990

Gln Thr Val Pro Ala Ser Val Gln Glu Val Val Ala Ala Asn Ala Tyr
995 1000 1005

Ser Ser Asn Ser Leu Thr Val Ser Trp Gln Lys Ala Leu Gly Val
1010 1015 1020

Ala Glu Arg Tyr Asp Ile Leu Leu Leu Asn Glu Asn Gly Leu Leu
1025 1030 1035

Leu Ser Asn Val Ser Glu Pro Ala Thr Ala Arg Gln His Lys Phe
1040 1045 1050

Glu Asp Leu Thr Pro Gly Lys Lys Tyr Lys Met Gln Ile Leu Thr
1055 1060 1065

Val Ser Gly Gly Leu Phe Ser Lys Glu Ser Gln Ala Glu Gly Arg
1070 1075 1080

Thr Val Pro Ala Ala Val Thr Asn Leu Arg Ile Thr Glu Asn Ser
1085 1090 1095

Ser Arg Tyr Leu Ser Phe Gly Trp Thr Ala Ser Glu Gly Glu Leu
1100 1105 1110

Ser Trp Tyr Asn Ile Phe Leu Tyr Asn Pro Asp Arg Thr Leu Gln
1115 1120 1125

Glu Arg Ala Gln Val Asp Pro Leu Val Gln Ser Phe Ser Phe Gln
1130 1135 1140

Asn Leu Leu Gln Gly Arg Met Tyr Lys Met Val Ile Val Thr His
1145 1150 1155

Ser Gly Glu Leu Ser Asn Glu Ser Phe Ile Phe Gly Arg Thr Val
1160 1165 1170

Pro Ala Ala Val Asn His Leu Lys Gly Ser His Arg Asn Thr Thr
1175 1180 1185

Asp Ser Leu Trp Phe Ser Trp Ser Pro Ala Ser Gly Asp Phe Asp
1190 1195 1200

Phe Tyr Glu Leu Ile Leu Tyr Asn Pro Asn Gly Thr Lys Lys Glu
1205 1210 1215

Asn Trp Lys Glu Lys Asp Val Thr Glu Trp Arg Phe Gln Gly Leu
1220 1225 1230

Val Pro Gly Arg Lys Tyr Thr Leu Tyr Val Val Thr His Ser Gly
1235 1240 1245

Asp Leu Ser Asn Lys Val Thr Gly Glu Gly Arg Thr Ala Pro Ser
1250 1255 1260

Pro Pro Ser Leu Leu Ser Phe Ala Asp Val Ala Asn Thr Ser Leu
1265 1270 1275

Ala Ile Thr Trp Lys Gly Pro Pro Asp Trp Thr Asp Tyr Asn Asp
1280 1285 1290

Phe Glu Leu Gln Trp Phe Pro Gly Asp Ala Leu Thr Ile Phe Asn

1295 1300 1305

Pro Tyr Ser Ser Arg Lys Ser Glu Gly Arg Ile Val Tyr Gly Leu
1310 1315 1320

His Pro Gly Arg Ser Tyr Gln Phe Ser Val Lys Thr Val Ser Gly
1325 1330 1335

Asp Ser Trp Lys Thr Tyr Ser Lys Pro Ile Ser Gly Ser Val Arg
1340 1345 1350

Thr Lys Pro Asp Lys Ile Gln Asn Leu His Cys Arg Pro Gln Asn
1355 1360 1365

Ser Thr Ala Ile Ala Cys Ser Trp Ile Pro Pro Asp Ser Asp Phe
1370 1375 1380

Asp Gly Tyr Ser Ile Glu Cys Arg Lys Met Asp Thr Gln Glu Ile
1385 1390 1395

Glu Phe Ser Arg Lys Leu Glu Lys Glu Lys Ser Leu Leu Asn Ile
1400 1405 1410

Met Met Leu Val Pro His Lys Arg Tyr Leu Val Ser Ile Lys Val
1415 1420 1425

Gln Ser Ala Gly Met Thr Ser Glu Val Val Glu Asp Ser Thr Ile
1430 1435 1440

Thr Met Ile Asp Arg Pro Pro Gln Pro Pro Pro His Ile Arg Val
1445 1450 1455

Asn Glu Lys Asp Val Leu Ile Ser Lys Ser Ser Ile Asn Phe Thr
1460 1465 1470

Val Asn Cys Ser Trp Phe Ser Asp Thr Asn Gly Ala Val Lys Tyr
1475 1480 1485

Phe Ala Val Val Val Arg Glu Ala Asp Ser Met Asp Glu Leu Lys
1490 1495 1500

Pro Glu Gln Gln His Pro Leu Pro Ser Tyr Leu Glu Tyr Arg His
1505 1510 1515

Asn Ala Ser Ile Arg Val Tyr Gln Thr Asn Tyr Phe Ala Ser Lys
1520 1525 1530

Cys Ala Glu Ser Pro Asp Ser Ser Ser Lys Ser Phe Asn Ile Lys
1535 1540 1545

Leu Gly Ala Glu Met Asp Ser Leu Gly Gly Lys Cys Asp Pro Ser
1550 1555 1560

Gln Gln Lys Phe Cys Asp Gly Pro Leu Lys Pro His Thr Ala Tyr
1565 1570 1575

Arg Ile Ser Ile Arg Ala Phe Thr Gln Leu Phe Asp Glu Asp Leu
1580 1585 1590

Lys Glu Phe Thr Lys Pro Leu Tyr Ser Asp Thr Phe Phe Ser Met
1595 1600 1605

Pro Ile Thr Thr Glu Ser Glu Pro Leu Phe Gly Val Ile Glu Arg
1610 1615 1620

Gly Arg His His His His His His Gly
1625 1630

<210> 8
<211> 774
<212> PRT
<213> Artificial

<220>
<223> human-mouse chimeric molecule

<400> 8

Met Leu Arg His Gly Ala Leu Thr Ala Leu Trp Ile Thr Leu Ser Val
1 5 10 15

Val Gln Thr Gly Val Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
50 55 60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65 70 75 80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
85 90 95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
100 105 110

Pro Ala Arg Phe Glu Val Asn Arg Glu Lys Thr Ala Ser Thr Thr Leu
115 120 125

Gln Val Arg Trp Thr Pro Ser Ser Gly Lys Val Ser Trp Tyr Glu Val
130 135 140

Gln Leu Phe Asp His Asn Asn Gln Lys Ile Gln Glu Val Gln Val Gln
145 150 155 160

Glu Ser Thr Thr Trp Ser Gln Tyr Thr Phe Leu Asn Leu Thr Glu Gly
165 170 175

Asn Ser Tyr Lys Val Ala Ile Thr Ala Val Ser Gly Glu Lys Arg Ser
180 185 190

Phe Pro Val Tyr Ile Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
195 200 205

Leu Gly Ile Ser Pro Asn Pro Asn Ser Leu Leu Ile Ser Trp Ser Arg
210 215 220

Gly Ser Gly Asn Val Glu Gln Tyr Arg Leu Val Leu Met Asp Lys Gly
225 230 235 240

Ala Ile Val Gln Asp Thr Asn Val Asp Arg Arg Asp Thr Ser Tyr Ala
245 250 255

Phe His Glu Leu Thr Pro Gly His Leu Tyr Asn Leu Thr Ile Val Thr
260 265 270

Met Ala Ser Gly Leu Gln Asn Ser Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Arg Leu Thr
290 295 300

Ser Leu Asn Val Lys Trp Gln Lys Pro Pro Gly Asp Val Asp Ser Tyr
305 310 315 320

Ser Ile Thr Leu Ser His Gln Gly Thr Ile Lys Glu Ser Lys Thr Leu
325 330 335

Ala Pro Pro Val Thr Glu Thr Gln Phe Lys Asp Leu Val Pro Gly Arg

340 345 350

Leu Tyr Gln Val Thr Ile Ser Cys Ile Ser Gly Glu Leu Ser Ala Glu
355 360 365

Lys Ser Ala Ala Gly Arg Thr Val Pro Glu Lys Val Arg Asn Leu Val
370 375 380

Ser Tyr Asn Glu Ile Trp Met Lys Ser Phe Thr Val Asn Trp Thr Pro
385 390 395 400

Pro Ala Gly Asp Trp Glu His Tyr Arg Ile Val Leu Phe Asn Glu Ser
405 410 415

Leu Val Leu Leu Asn Thr Thr Val Gly Lys Glu Glu Thr His Tyr Ala
420 425 430

Leu Asp Gly Leu Glu Leu Ile Pro Gly Arg Gln Tyr Glu Ile Glu Val
435 440 445

Ile Val Glu Ser Gly Asn Leu Arg Asn Ser Glu Arg Cys Gln Gly Arg
450 455 460

Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn Glu
465 470 475 480

Thr Ser Leu Gly Ile Thr Trp Arg Ala Pro Leu Gly Glu Trp Glu Lys
485 490 495

Tyr Ile Ile Ser Leu Met Asp Arg Glu Leu Leu Val Ile His Lys Ser
500 505 510

Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Met Pro Gly
515 520 525

Arg Asn Tyr Lys Ala Thr Val Thr Ser Met Ser Gly Asp Leu Lys Gln
530 535 540

Ser Ser Ser Ile Lys Gly Arg Thr Val Pro Ala Gln Val Thr Asp Leu
545 550 555 560

His Val Asn Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp Thr
565 570 575

Lys Ala Leu Gly Asp Val Glu Phe Tyr Gln Val Leu Leu Ile His Glu
580 585 590

Asn Val Val Val Lys Asn Glu Ser Val Ser Ser Asp Thr Ser Arg Tyr
595 600 605

Ser Phe Arg Ala Leu Lys Pro Gly Ser Leu Tyr Ser Val Val Val Thr
610 615 620

Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Ala Glu Gly Arg
625 630 635 640

Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly Arg
645 650 655

Asn Asp Tyr Leu Ser Val Ser Trp Leu Pro Ala Pro Gly Glu Val Asp
660 665 670

His Tyr Val Val Ser Leu Ser His Glu Gly Lys Val Asp Gln Phe Leu
675 680 685

Ile Ile Ala Lys Ser Val Ser Glu Cys Ser Phe Ser Ser Leu Thr Pro
690 695 700

Gly Arg Leu Tyr Asn Val Thr Val Thr Thr Lys Ser Gly Asn Tyr Ala
705 710 715 720

Ser His Ser Phe Thr Glu Glu Arg Thr Lys Gly Asn Ser Ala Asp Ile
725 730 735

Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly
740 745 750

Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly
755 760 765

His His His His His His
770

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<211> 775
<212> PRT
<213> Artificial

<220>
<223> human-mouse chimeric molecule

<400> 9

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1 5 10 15

Val Gln Thr Gly Val Ala Glu Gln Val Lys Cys Asn Phe Thr Leu Leu
20 25 30

Glu Ser Arg Val Ser Ser Leu Ser Ala Ser Ile Gln Trp Arg Thr Phe
35 40 45

Ala Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Ser Gly
50 55 60

Pro Met Trp Cys His Pro Ile Arg Ile Asp Asn Phe Thr Tyr Gly Cys
65 70 75 80

Asn Pro Lys Asp Leu Gln Ala Gly Thr Val Tyr Asn Phe Arg Ile Val
85 90 95

Ser Leu Asp Gly Glu Glu Ser Thr Leu Val Leu Gln Thr Asp Pro Leu
100 105 110

Pro Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly
115 120 125

Leu His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu
130 135 140

Val Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile
145 150 155 160

Gln Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala
165 170 175

Gly Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg
180 185 190

Ser Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys
195 200 205

Asp Leu Gly Ile Ser Pro Asn Pro Asn Ser Leu Leu Ile Ser Trp Ser
210 215 220

Arg Gly Ser Gly Asn Val Glu Gln Tyr Arg Leu Val Leu Met Asp Lys
225 230 235 240

Gly Ala Ile Val Gln Asp Thr Asn Val Asp Arg Arg Asp Thr Ser Tyr
245 250 255

Ala Phe His Glu Leu Thr Pro Gly His Leu Tyr Asn Leu Thr Ile Val
260 265 270

Thr Met Ala Ser Gly Leu Gln Asn Ser Arg Trp Lys Leu Val Arg Thr
275 280 285

Ala Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Arg Leu
290 295 300

Thr Ser Leu Asn Val Lys Trp Gln Lys Pro Pro Gly Asp Val Asp Ser
305 310 315 320

Tyr Ser Ile Thr Leu Ser His Gln Gly Thr Ile Lys Glu Ser Lys Thr
325 330 335

Leu Ala Pro Pro Val Thr Glu Thr Gln Phe Lys Asp Leu Val Pro Gly
340 345 350

Arg Leu Tyr Gln Val Thr Ile Ser Cys Ile Ser Gly Glu Leu Ser Ala
355 360 365

Glu Lys Ser Ala Ala Gly Arg Thr Val Pro Glu Lys Val Arg Asn Leu
370 375 380

Val Ser Tyr Asn Glu Ile Trp Met Lys Ser Phe Thr Val Asn Trp Thr
385 390 395 400

Pro Pro Ala Gly Asp Trp Glu His Tyr Arg Ile Val Leu Phe Asn Glu
405 410 415

Ser Leu Val Leu Leu Asn Thr Thr Val Gly Lys Glu Glu Thr His Tyr
420 425 430

Ala Leu Asp Gly Leu Glu Leu Ile Pro Gly Arg Gln Tyr Glu Ile Glu
435 440 445

Val Ile Val Glu Ser Gly Asn Leu Arg Asn Ser Glu Arg Cys Gln Gly
450 455 460

Arg Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn
465 470 475 480

Glu Thr Ser Leu Gly Ile Thr Trp Arg Ala Pro Leu Gly Glu Trp Glu
485 490 495

Lys Tyr Ile Ile Ser Leu Met Asp Arg Glu Leu Leu Val Ile His Lys
500 505 510

Ser Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Met Pro
515 520 525

Gly Arg Asn Tyr Lys Ala Thr Val Thr Ser Met Ser Gly Asp Leu Lys
530 535 540

Gln Ser Ser Ser Ile Lys Gly Arg Thr Val Pro Ala Gln Val Thr Asp
545 550 555 560

Leu His Val Asn Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp
565 570 575

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

Glu Asn Val Val Val Lys Asn Glu Ser Val Ser Ser Asp Thr Ser Arg
595 600 605

Tyr Ser Phe Arg Ala Leu Lys Pro Gly Ser Leu Tyr Ser Val Val Val
610 615 620

Thr Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Ala Glu Gly
625 630 635 640

Arg Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly
645 650 655

Arg Asn Asp Tyr Leu Ser Val Ser Trp Leu Pro Ala Pro Gly Glu Val
660 665 670

Asp His Tyr Val Val Ser Leu Ser His Glu Gly Lys Val Asp Gln Phe
675 680 685

Leu Ile Ile Ala Lys Ser Val Ser Glu Cys Ser Phe Ser Ser Leu Thr
690 695 700

Pro Gly Arg Leu Tyr Asn Val Thr Val Thr Thr Lys Ser Gly Asn Tyr
705 710 715 720

Ala Ser His Ser Phe Thr Glu Glu Arg Thr Lys Gly Asn Ser Ala Asp
725 730 735

Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu
740 745 750

Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr
755 760 765

Gly His His His His His His
770 775

<210> 10
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<220>
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<400> 10

Met Leu Arg His Gly Ala Leu Thr Ala Leu Trp Ile Thr Leu Ser Val
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Val Gln Thr Gly Val Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
20 25 30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
35 40 45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
50 55 60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65 70 75 80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
85 90 95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
100 105 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
115 120 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
130 135 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145 150 155 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
165 170 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
180 185 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
195 200 205

Leu Gly Ile Ser Pro Asn Pro Asn Ser Leu Leu Ile Ser Trp Ser Arg
210 215 220

Gly Ser Gly Asn Val Glu Gln Tyr Arg Leu Val Leu Met Asp Lys Gly
225 230 235 240

Ala Ile Val Gln Asp Thr Asn Val Asp Arg Arg Asp Thr Ser Tyr Ala
245 250 255

Phe His Glu Leu Thr Pro Gly His Leu Tyr Asn Leu Thr Ile Val Thr

260 265 270

Met Ala Ser Gly Leu Gln Asn Ser Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Arg Leu Thr
290 295 300

Ser Leu Asn Val Lys Trp Gln Lys Pro Pro Gly Asp Val Asp Ser Tyr
305 310 315 320

Ser Ile Thr Leu Ser His Gln Gly Thr Ile Lys Glu Ser Lys Thr Leu
325 330 335

Ala Pro Pro Val Thr Glu Thr Gln Phe Lys Asp Leu Val Pro Gly Arg
340 345 350

Leu Tyr Gln Val Thr Ile Ser Cys Ile Ser Gly Glu Leu Ser Ala Glu
355 360 365

Lys Ser Ala Ala Gly Arg Thr Val Pro Glu Lys Val Arg Asn Leu Val
370 375 380

Ser Tyr Asn Glu Ile Trp Met Lys Ser Phe Thr Val Asn Trp Thr Pro
385 390 395 400

Pro Ala Gly Asp Trp Glu His Tyr Arg Ile Val Leu Phe Asn Glu Ser
405 410 415

Leu Val Leu Leu Asn Thr Thr Val Gly Lys Glu Glu Thr His Tyr Ala
420 425 430

Leu Asp Gly Leu Glu Leu Ile Pro Gly Arg Gln Tyr Glu Ile Glu Val
435 440 445

Ile Val Glu Ser Gly Asn Leu Arg Asn Ser Glu Arg Cys Gln Gly Arg
450 455 460

Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn Glu
465 470 475 480

Thr Ser Leu Gly Ile Thr Trp Arg Ala Pro Leu Gly Glu Trp Glu Lys
485 490 495

Tyr Ile Ile Ser Leu Met Asp Arg Glu Leu Leu Val Ile His Lys Ser
500 505 510

Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Met Pro Gly
515 520 525

Arg Asn Tyr Lys Ala Thr Val Thr Ser Met Ser Gly Asp Leu Lys Gln
530 535 540

Ser Ser Ser Ile Lys Gly Arg Thr Val Pro Ala Gln Val Thr Asp Leu
545 550 555 560

His Val Asn Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp Thr
565 570 575

Lys Ala Leu Gly Asp Val Glu Phe Tyr Gln Val Leu Leu Ile His Glu
580 585 590

Asn Val Val Val Lys Asn Glu Ser Val Ser Ser Asp Thr Ser Arg Tyr
595 600 605

Ser Phe Arg Ala Leu Lys Pro Gly Ser Leu Tyr Ser Val Val Val Thr
610 615 620

Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Ala Glu Gly Arg
625 630 635 640

Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly Arg
645 650 655

Asn Asp Tyr Leu Ser Val Ser Trp Leu Pro Ala Pro Gly Glu Val Asp
660 665 670

His Tyr Val Val Ser Leu Ser His Glu Gly Lys Val Asp Gln Phe Leu
675 680 685

Ile Ile Ala Lys Ser Val Ser Glu Cys Ser Phe Ser Ser Leu Thr Pro
690 695 700

Gly Arg Leu Tyr Asn Val Thr Val Thr Thr Lys Ser Gly Asn Tyr Ala
705 710 715 720

Ser His Ser Phe Thr Glu Glu Arg Thr Lys Gly Asn Ser Ala Asp Ile
725 730 735

Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly
740 745 750

Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly
755 760 765

His His His His His His
770

<210> 11
<211> 89
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Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala Glu Ser Lys Ala
1 5 10 15

Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu Gly Ser Pro Cys
20 25 30

Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly Ala Ala Leu Cys
35 40 45

Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys Asn Leu Gln Asp
50 55 60

Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile Ser Leu Asp Glu
65 70 75 80

Glu Arg Thr Val Val Leu Gln Thr Asp
85

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An isolated antibody or antigen-binding fragment thereof which binds specifically to human protein tyrosine phosphatase beta (HPTPβ), wherein said antibody or antigen-binding fragment thereof regulates angiogenesis, and wherein the antibody or antigen-binding fragment thereof binds the N-terminal portion of HPTPβ.

2. The antibody or antigen-binding fragment thereof according to Claim 1, wherein the antibody or antigen-binding fragment thereof binds the first FN3 repeat of HPTPβ.

3. The antibody or antigen-binding fragment thereof according to Claim 2, wherein the first FN3 repeat of HPTPβ has the sequence as shown in SEQ ID NO:11, or a fragment thereof.

4. The antibody or antigen-binding fragment thereof according to any one of Claims 1 to 3, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof.

5. The monoclonal antibody according to Claim 4, produced by hybridoma cell line ATCC No. PTA-7580, or the antigen-binding fragment of the monoclonal antibody according to Claim 4 produced by hybridoma cell line ATCC No. PTA-7580.

6. The antigen-binding fragment according to Claim 5, wherein the fragment comprises heavy and light chain variable regions.

7. The antigen-binding fragment according to Claim 5, wherein the antigen-binding fragment is selected from the group consisting of an Fv fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

8. A use of an antibody or antigen-binding fragment thereof which binds specifically to HPTPβ and regulates angiogenesis, and wherein the antibody or antigen-binding

fragment thereof binds the N-terminal portion of HPTPβ in the manufacture of a medicament for use in treating an angiogenesis regulated disorder.

9. The use according to Claim 8, wherein the angiogenesis regulated disorder is an angiogenesis reduced disorder selected from skeletal muscle and myocardial ischemia, stroke, coronary artery disease, and peripheral vascular disease.

10. The use according to claim 9, wherein the angiogenesis reduced disorder is peripheral vascular disease.

11. The use according to claim 9, wherein the angiogenesis reduced disorder is coronary artery disease.

12. A use of an antibody or antigen-binding fragment thereof which binds specifically to HPTPβ and regulates angiogenesis, and wherein the antibody or antigen-binding fragment thereof binds the N-terminal portion of HPTPβ for treating an angiogenesis regulated disorder.

13. The use according to Claim 12, wherein the angiogenesis regulated disorder is an angiogenesis reduced disorder selected from skeletal muscle and myocardial ischemia, stroke, coronary artery disease, and peripheral vascular disease.

14. The use according to Claim 13, wherein the angiogenesis reduced disorder is peripheral vascular disease.

15. The use according to Claim 13, wherein the angiogenesis reduced disorder is coronary artery disease.

16. The monoclonal antibody R15E6 produced by hybridoma cell line ATCC No. PTA-7580 or an antigen-binding fragment thereof which binds specifically to human protein tyrosine phosphatise beta (HPTPβ).

17. A pharmaceutical composition, comprising:

a) the antibody or a fragment thereof according to any one of Claims 1-7 and 16; and

b) a pharmaceutically acceptable carrier.

18. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for enhancing Tie-2 signaling.

19. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for promoting endothelial cell migration.

20. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for promoting endothelial cell survival.

21. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for enhancing Tie-2 signaling.

22. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for promoting endothelial cell migration.

23. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for promoting endothelial cell survival.

24. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for decreasing Tie-2 signaling.

25. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for decreasing endothelial cell migration.

26. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for decreasing endothelial cell survival.

27. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for decreasing Tie-2 signaling.

28. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for decreasing endothelial cell migration.

29. A use of an isolated antibody or antigen-binding fragment thereof according to any one of Claims 1-7 and 16, for making a medicament for decreasing endothelial cell survival.

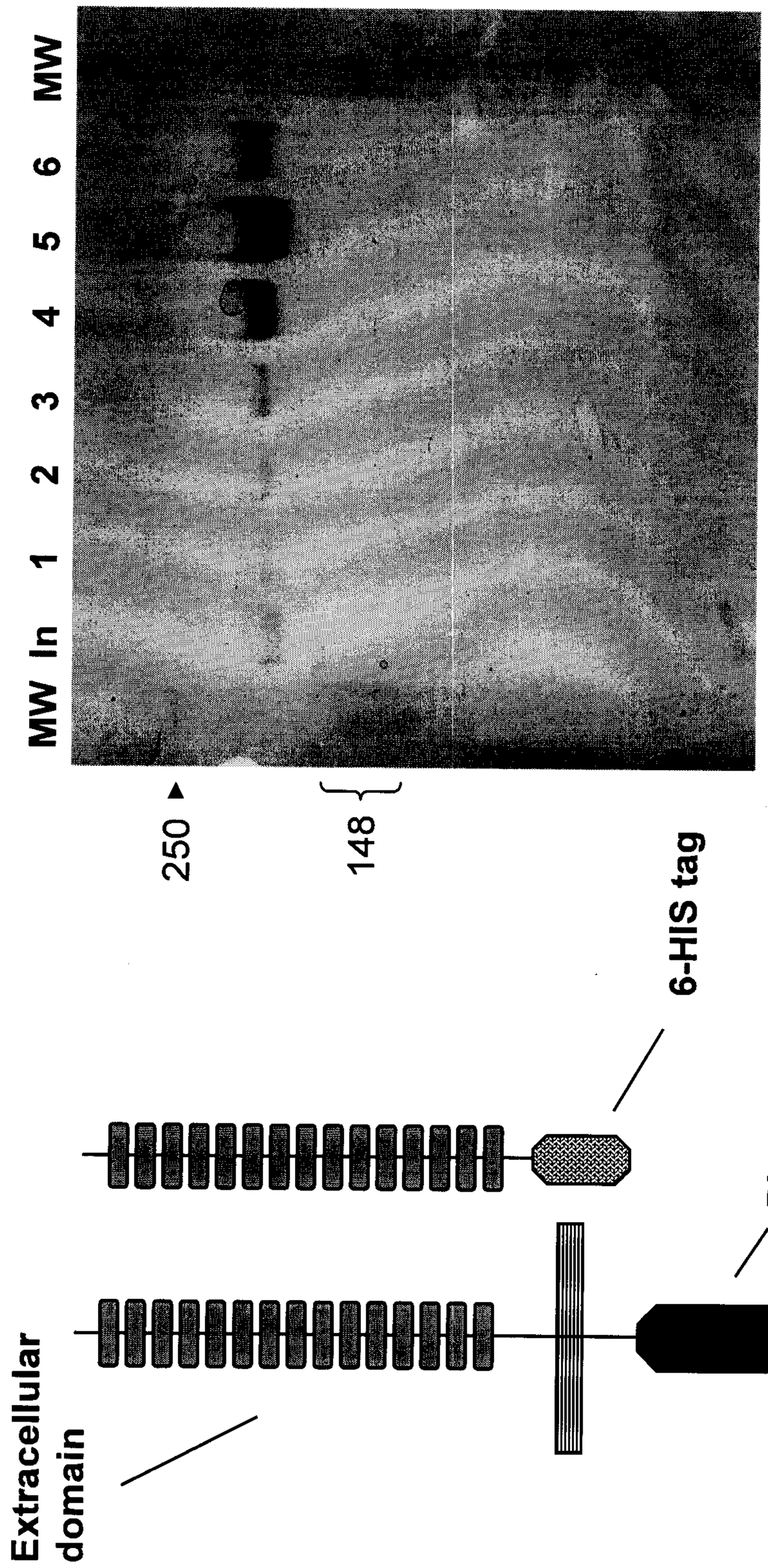

MW In 1 2 3 4 5 6 MW
250
148
6-HIS tag
Phosphatase domain
FN3 repeat
Extracellular domain
A.
B.

FIGURE 1.

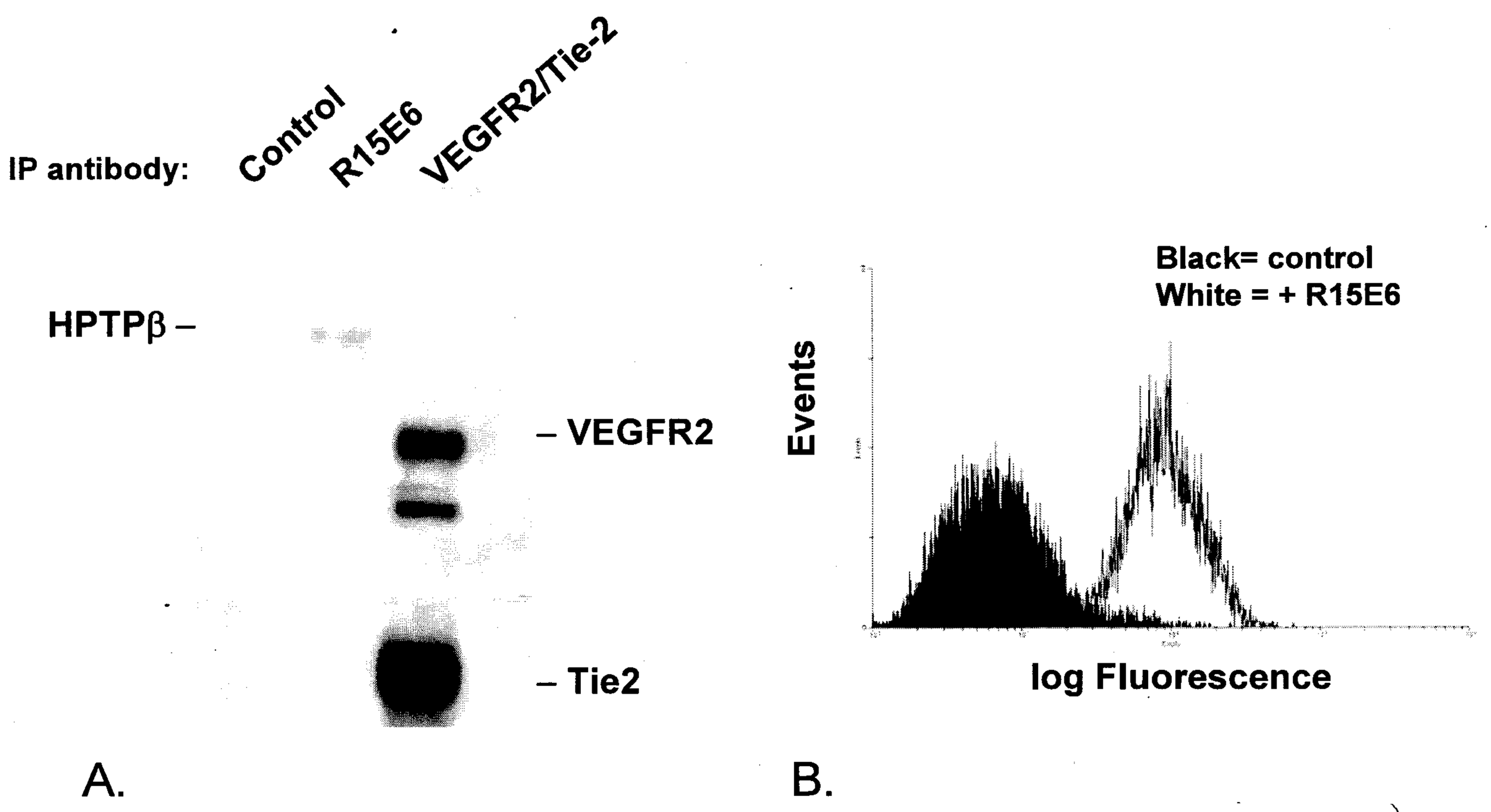


FIGURE 2.

CA 02648284 2009-04-03

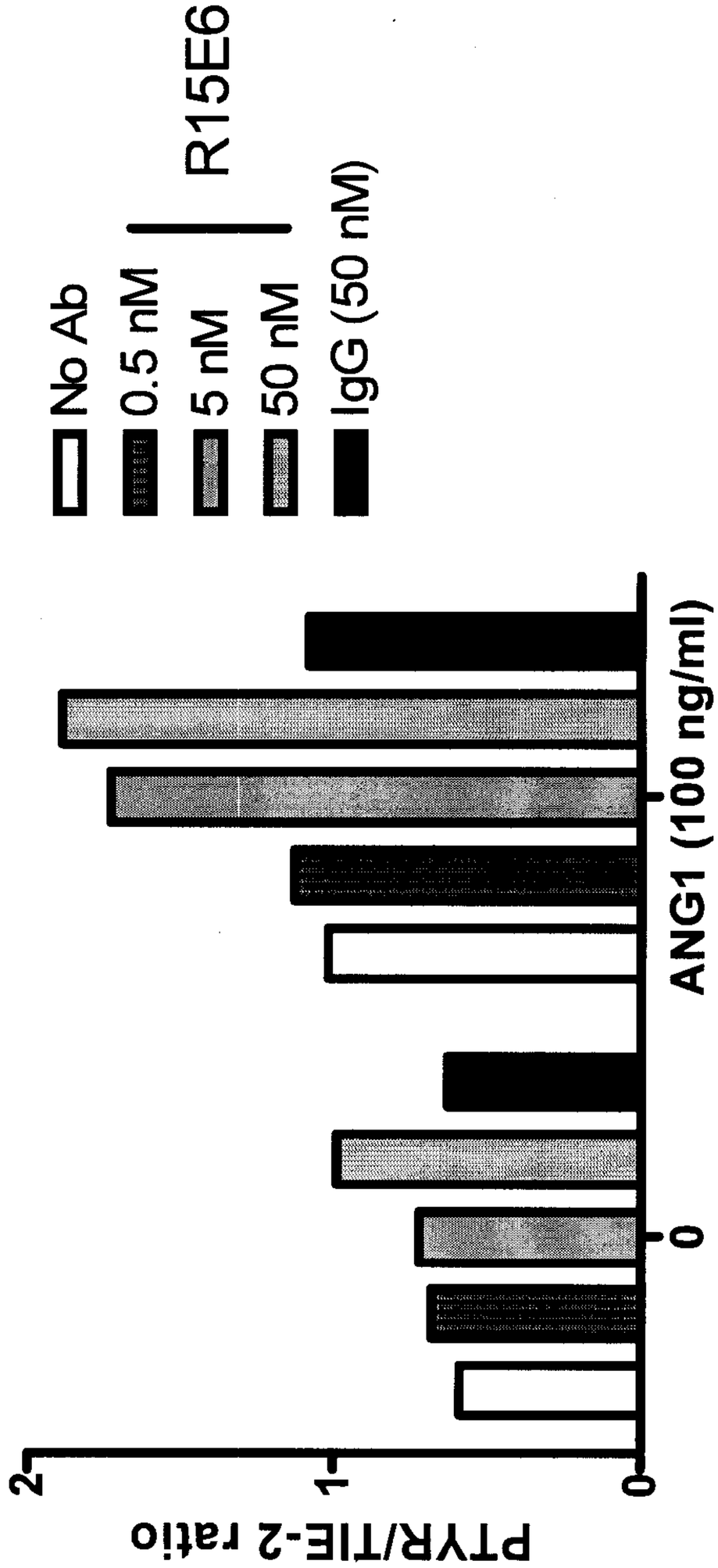

15E6
No Ab
0.5 nM
5 nM
50 nM
IgG (50 nM)
R
ANG1 (100 ng/ml)
0
PTYR/TIE-2 ratio
2
1
0

FIGURE 3.

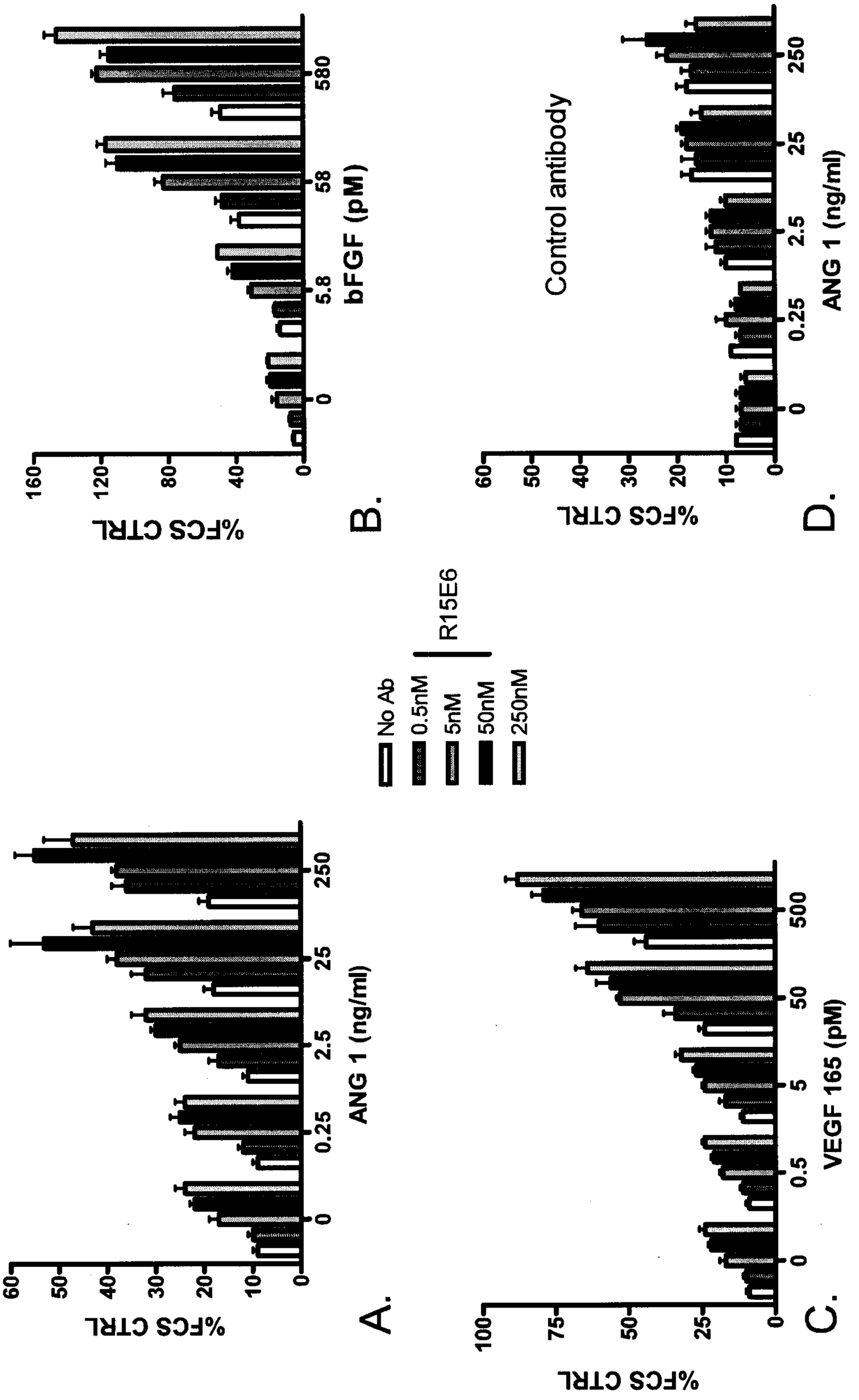

%FCS CTRL
0
40
80
120
160
bFGF (pM)
0
5.8
58
580
B.
%FCS CTRL
0
10
20
30
40
50
60
ANG 1 (ng/ml)
0
0.25
2.5
25
250
D.
R15E6
No Ab
0.5nM
5nM
50nM
250nM
%FCS CTRL
0
25
50
75
100
VEGF 165 (pM)
0
0.5
5
50
500
C.
%FCS CTRL
0
10
20
30
40
50
60
ANG 1 (ng/ml)
0
0.25
2.5
25
250
A.

FIGURE 4.

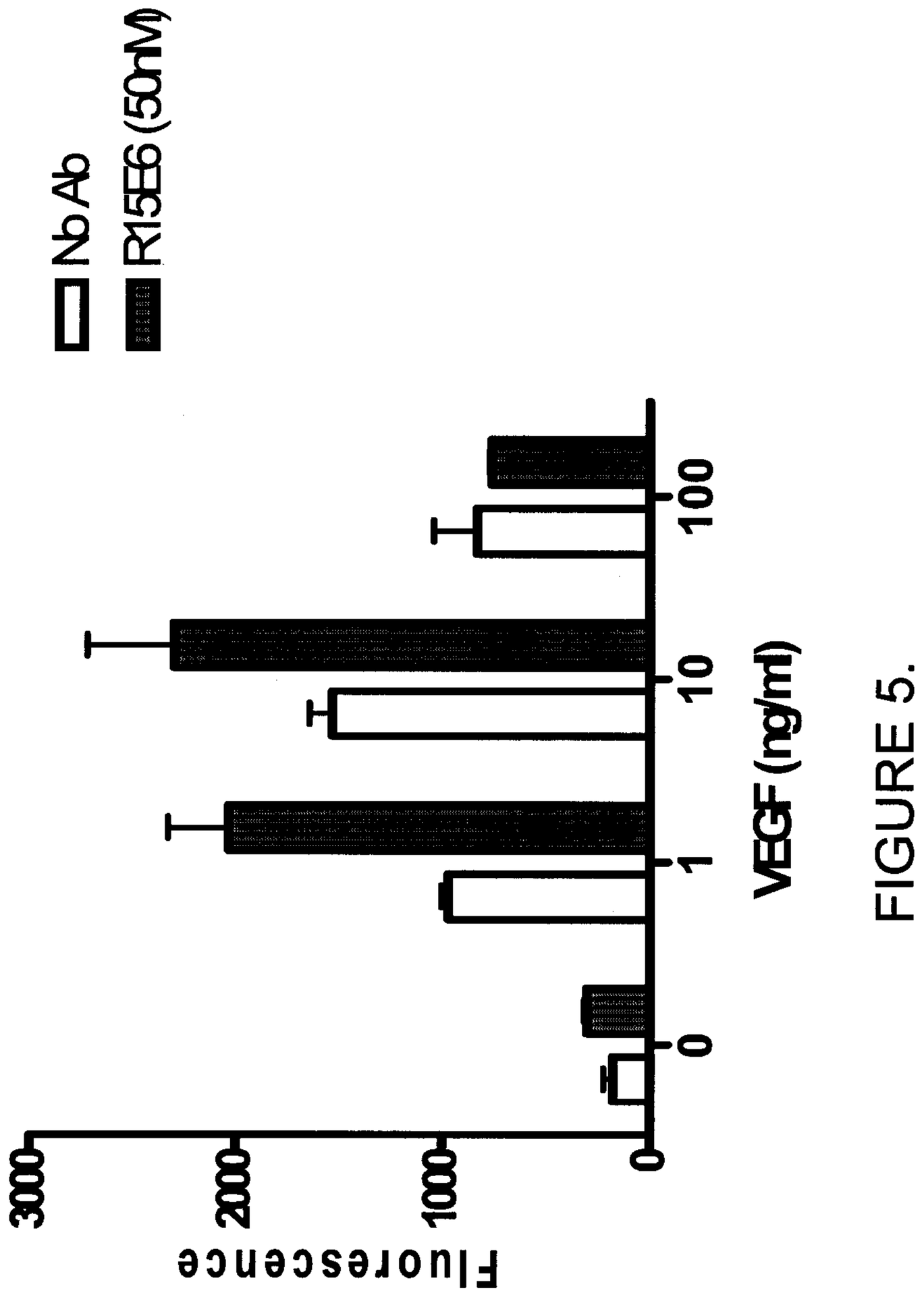

NbAb (50nM)
R15E6
Fluorescence
3000
2000
1000
0
-1
0
1
10
100
VEGF (ng/ml)

FIGURE 5.

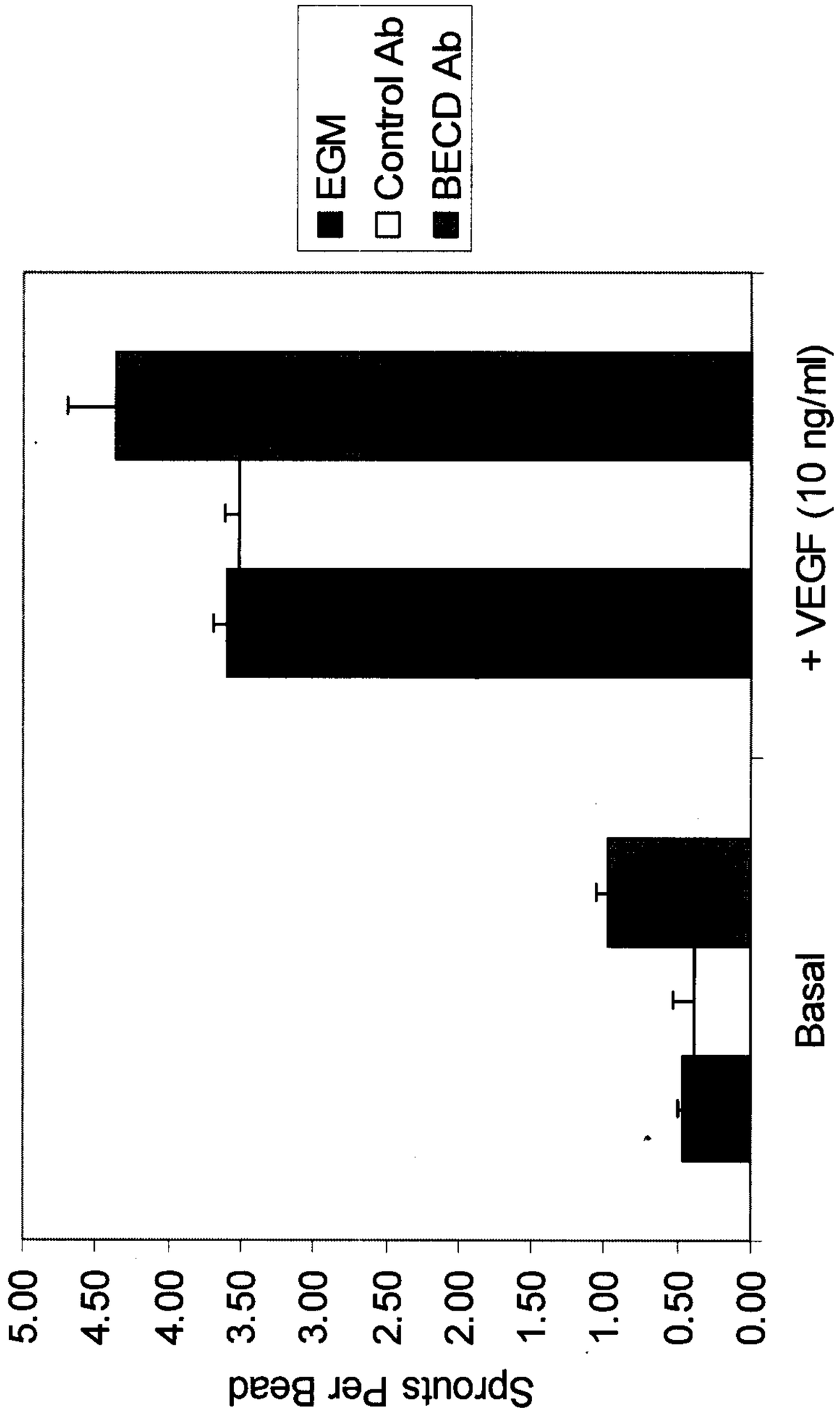
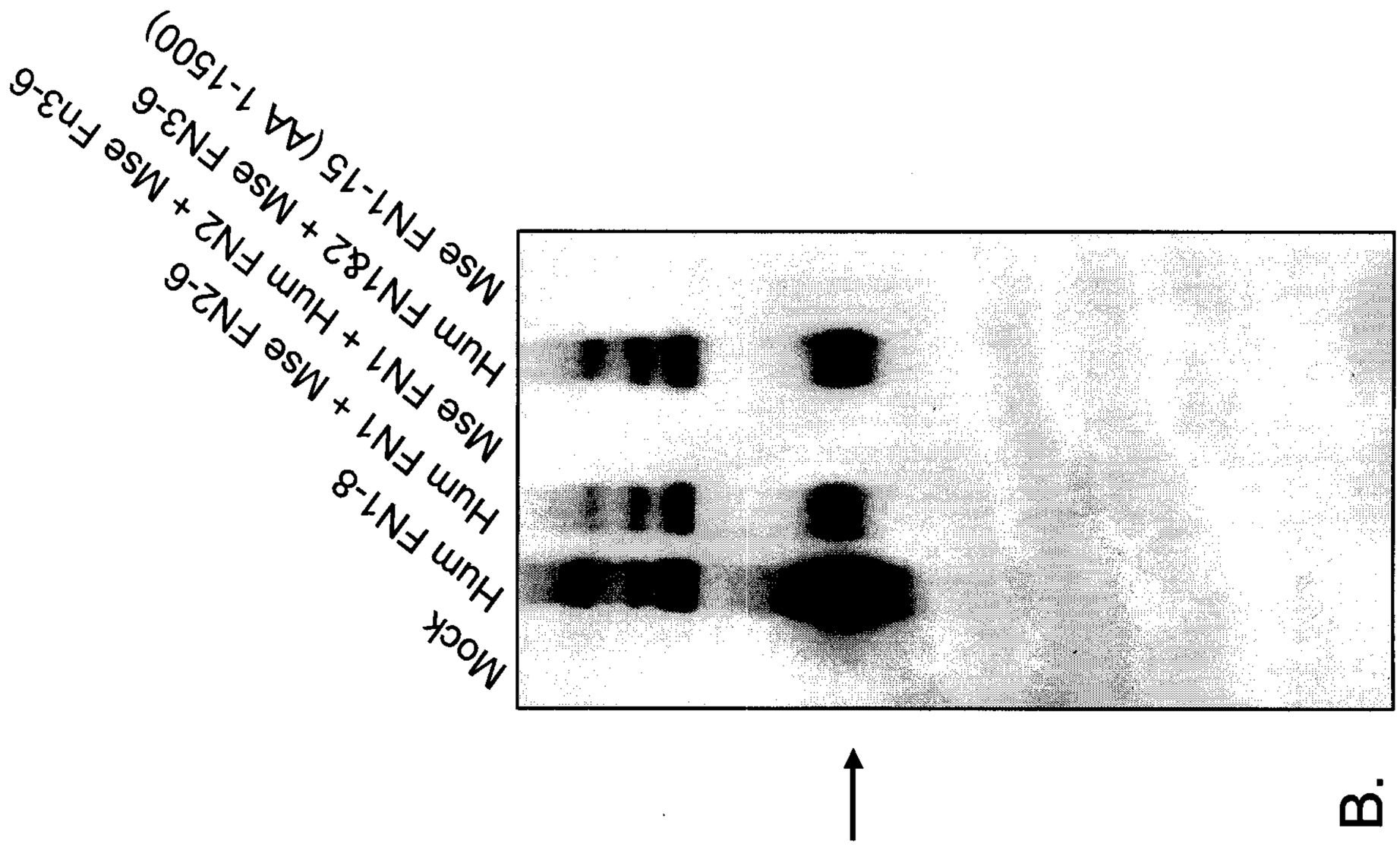

EGM
Control Ab
BECD Ab
Basal
+ VEGF (10 ng/ml)
Sprouts Per Bead
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00

FIGURE 6.


Mock
Hum FN1-8
Hum FN1 + Mse FN2-6
Mse FN1 + Hum FN2 + Mse Fn3-6
Hum FN1&2 + Mse FN3-6
Mse FN1-15 (AA 1-150
B.

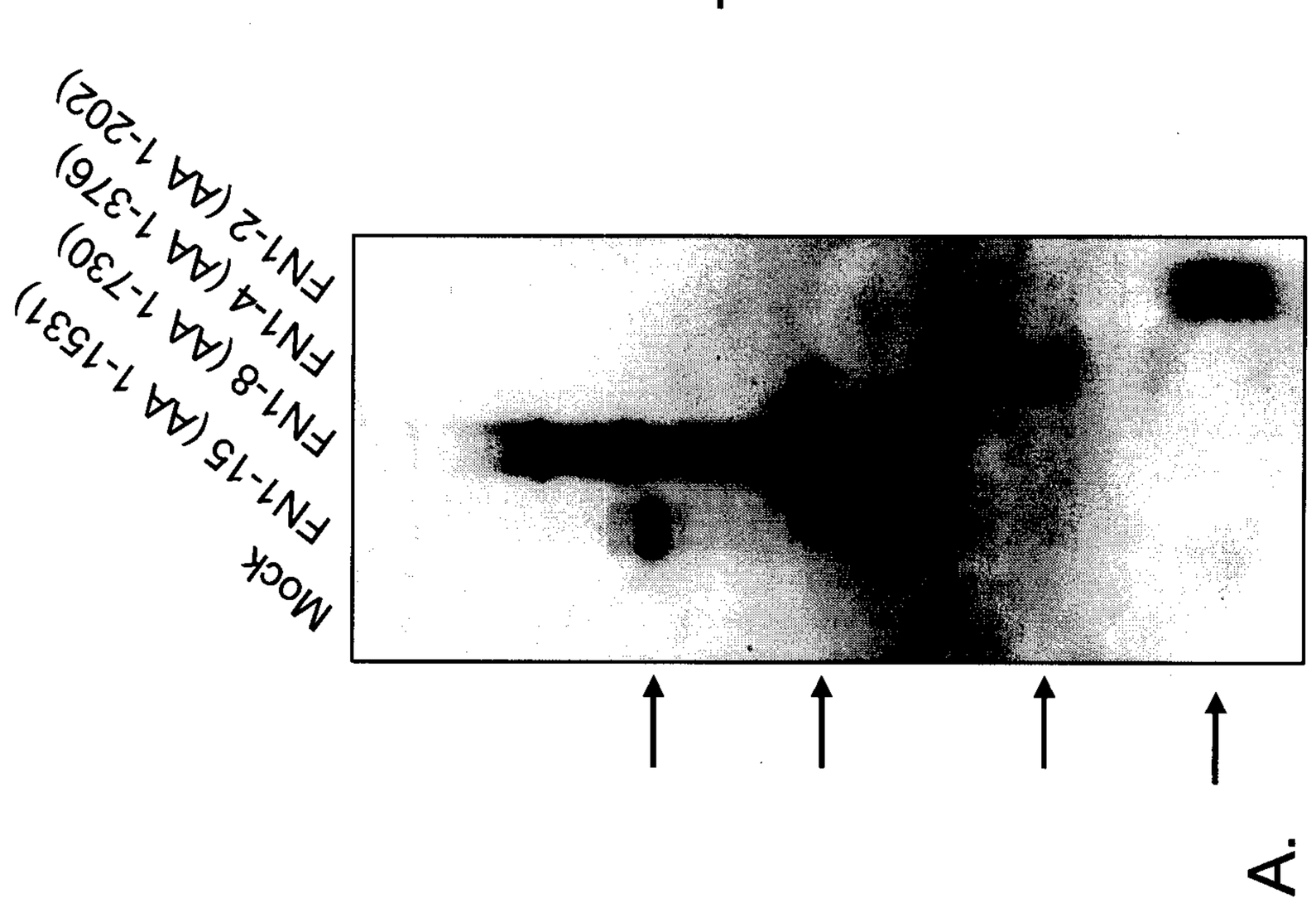
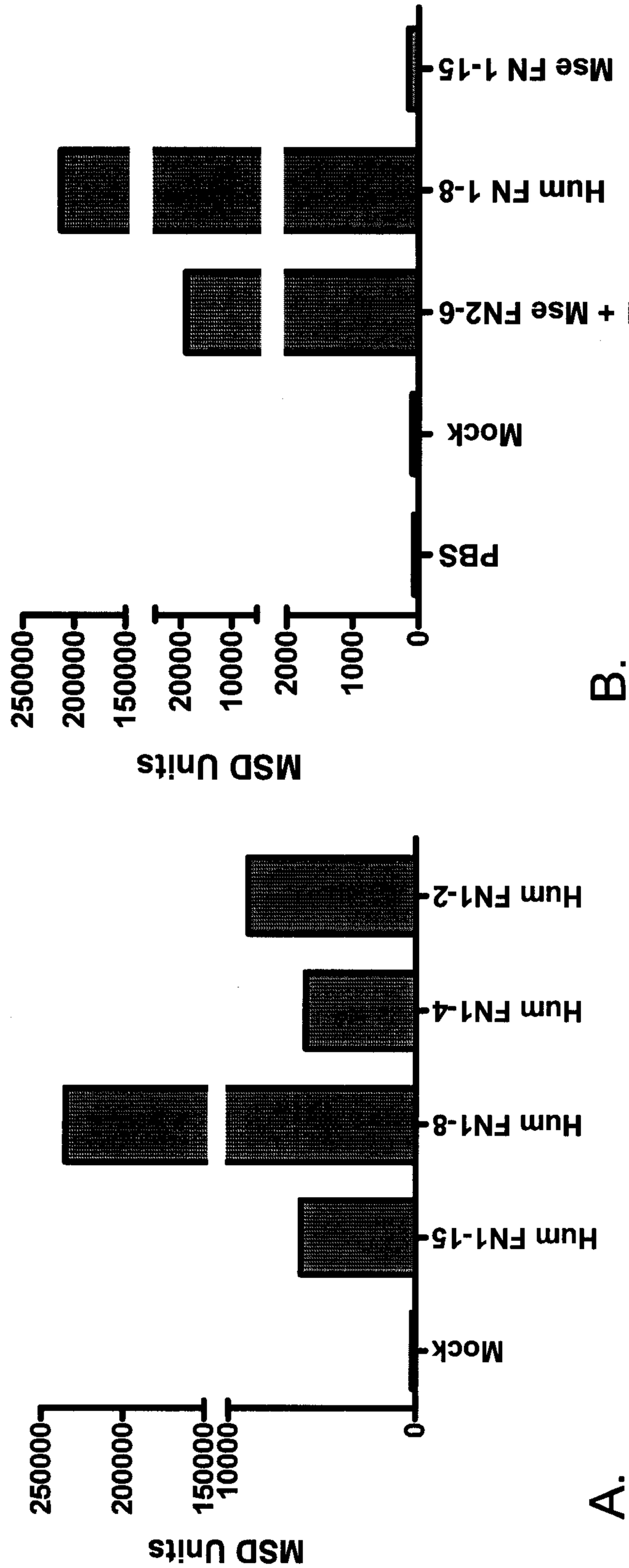

Mock
FN1-15 (AA 1-1531)
FN1-8 (AA 1-730)
FN1-4 (AA 1-376)
FN1-2 (AA 1-202)
A.

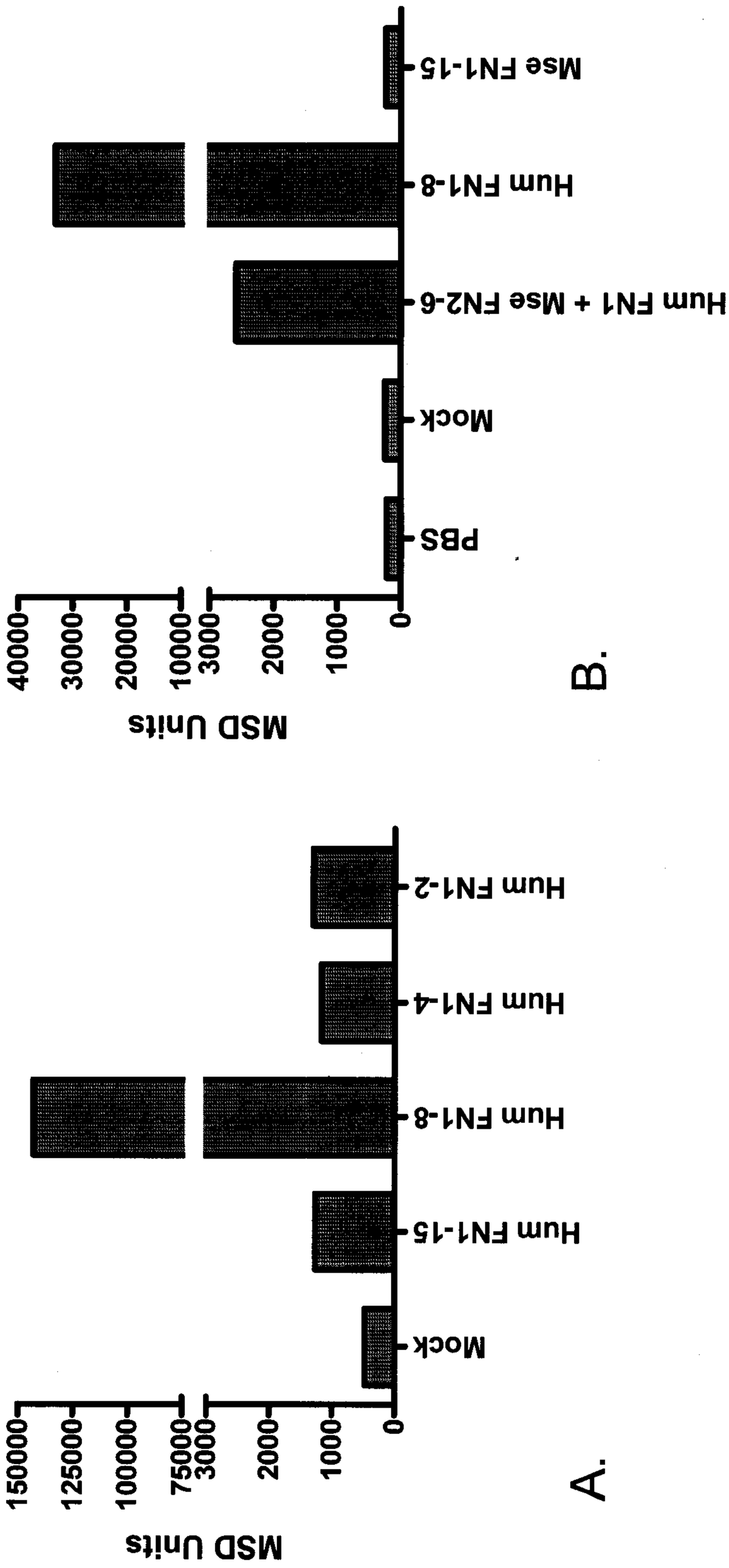
FIGURE 7.


MSD Units
250000
200000
150000
20000
10000
2000
1000
0
PBS
Mock
Hum FN1 + Mse FN2-6
Hum FN1-8
Mse FN 1-15
Hum FN1-2
Hum FN1-4
Hum FN1-8
Hum FN1-15
Mock
MSD Units
250000
200000
150000
10000
0

B.

A.

FIGURE 8.


MSD Units
40000
30000
20000
10000
3000
2000
1000
0
PBS
Mock
Hum FN1 + Mse FN2-6
Hum FN1-8
Mse FN1-15
Mock
Hum FN1-15
Hum FN1-8
Hum FN1-4
Hum FN1-2
MSD Units
150000
125000
100000
75000
3000
2000
1000
0

B.

A.

FIG 9.

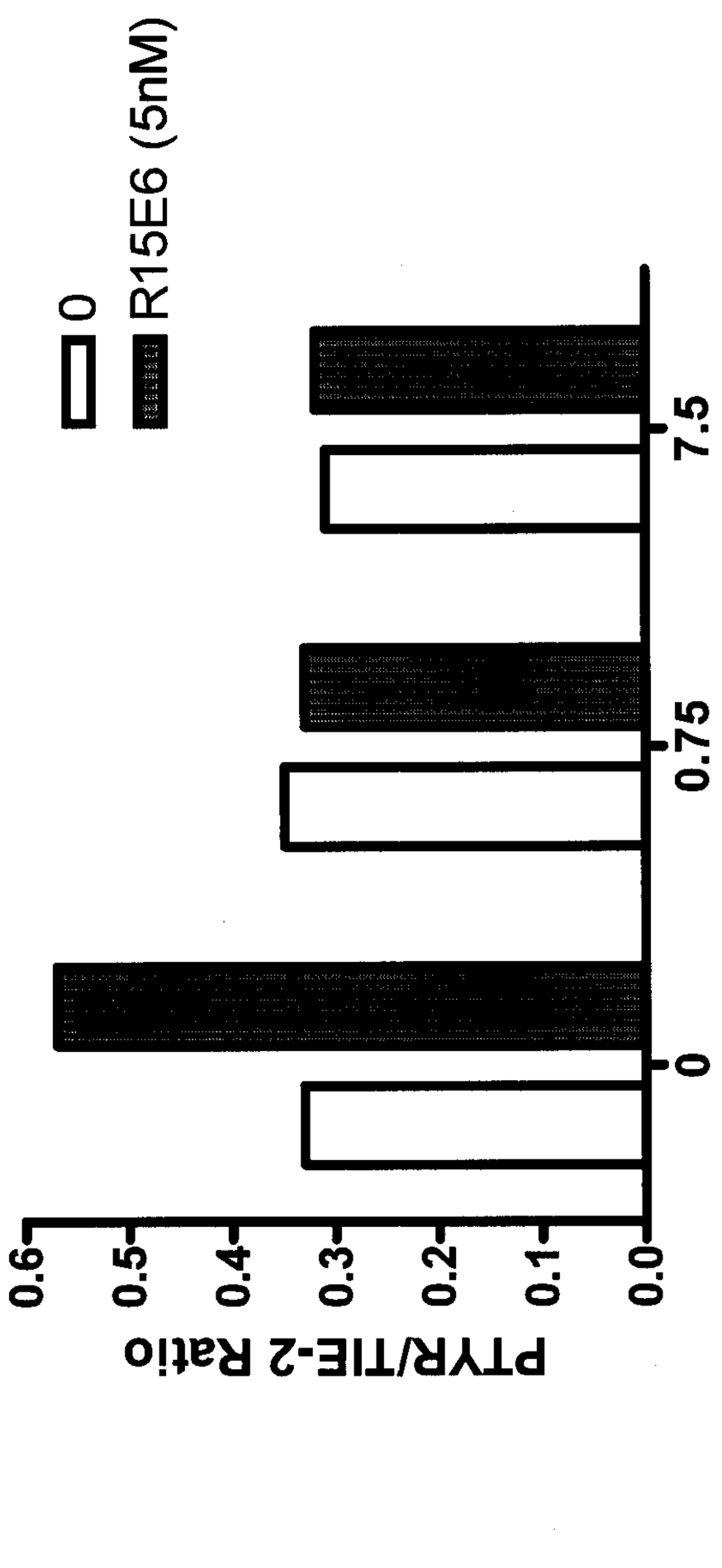
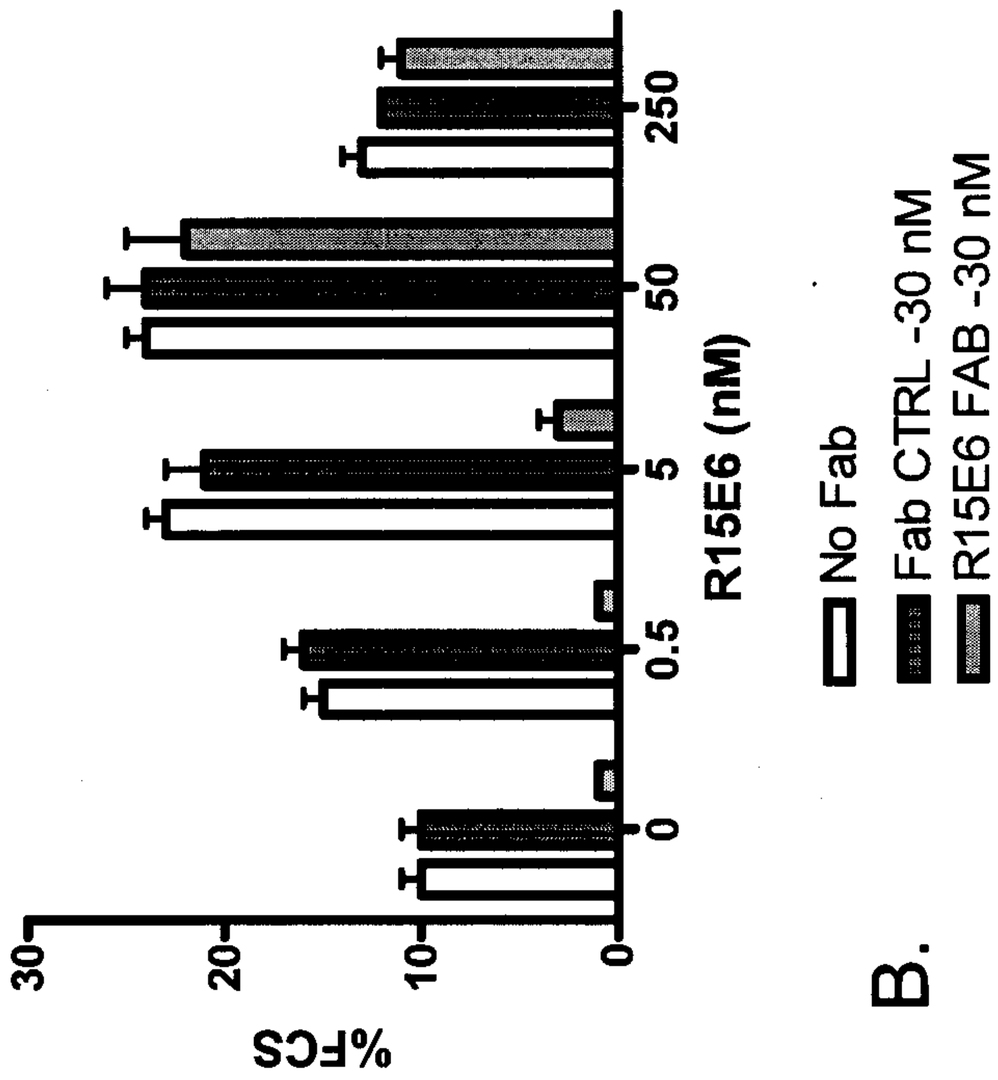
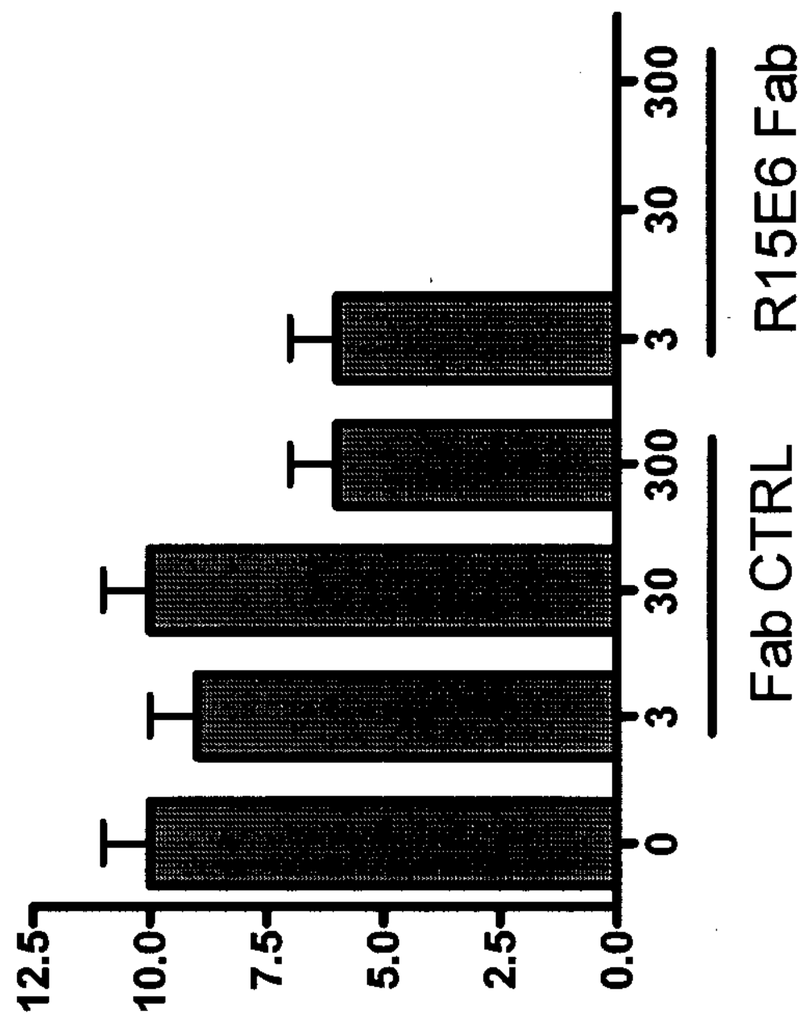

0
R15E6 (5nM)
PTYR/TIE-2 Ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
0.75
7.5
R15E6 Fab (µg/ml)

FIGURE 10.


%FCS
30
20
10
0
0
0.5
5
50
250
R15E6 (nM)
No Fab
Fab CTRL -30 nM
R15E6 FAB -30 nM

B.


%FCS
12.5
10.0
7.5
5.0
2.5
0.0
0
3
30
300
0
3
30
300
Fab CTRL
R15E6 Fab
Fab (nM)

A.

FIGURE 11.

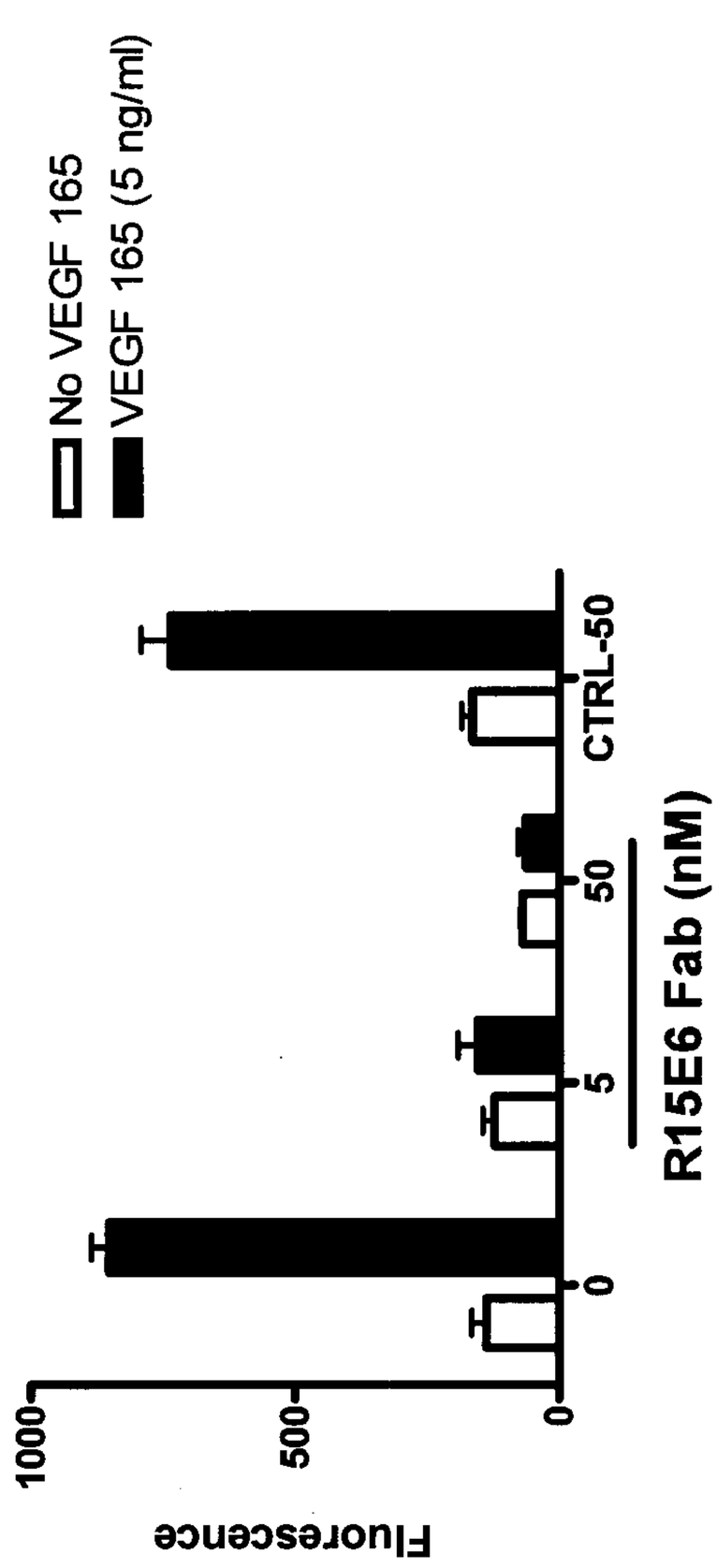
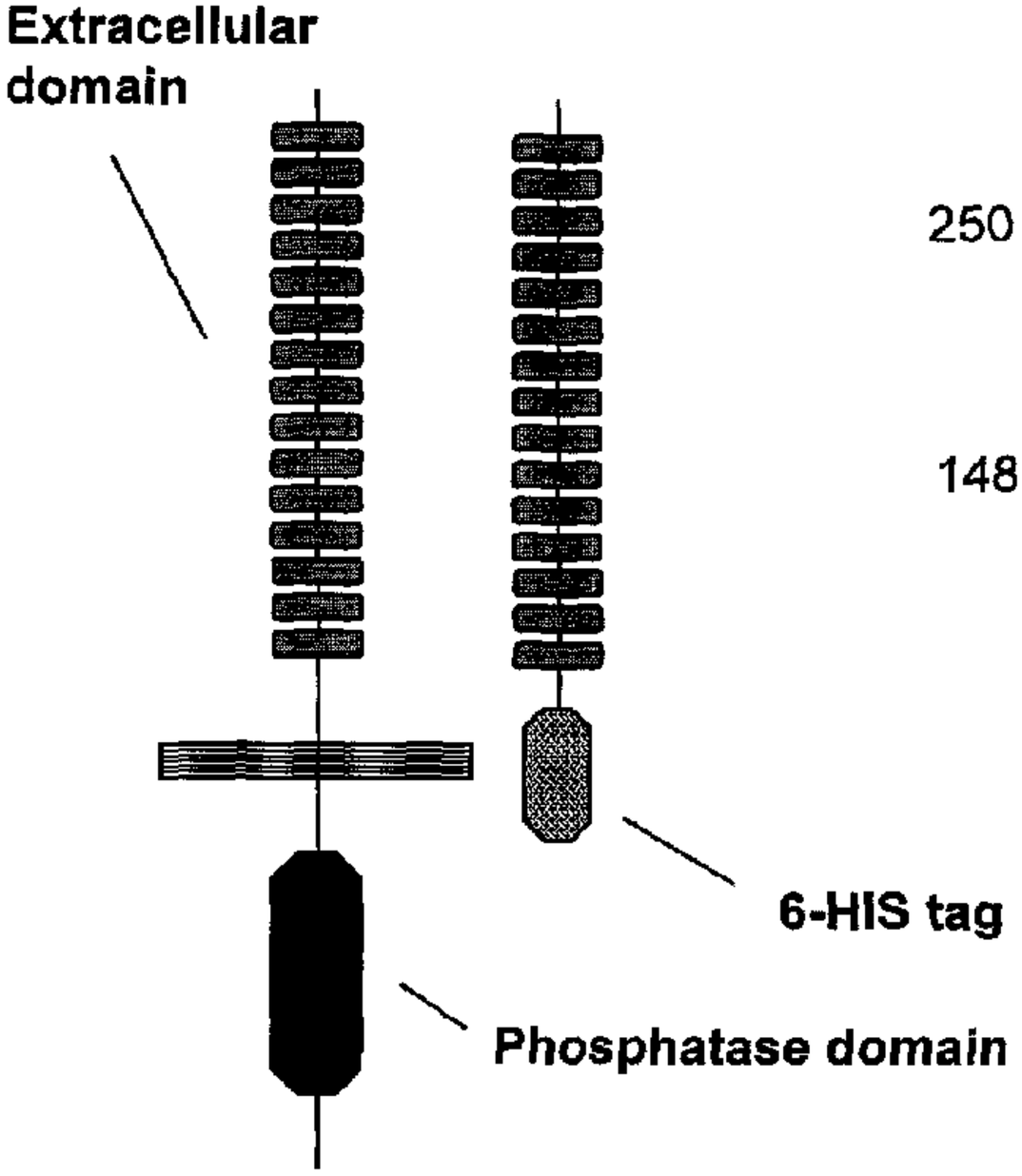
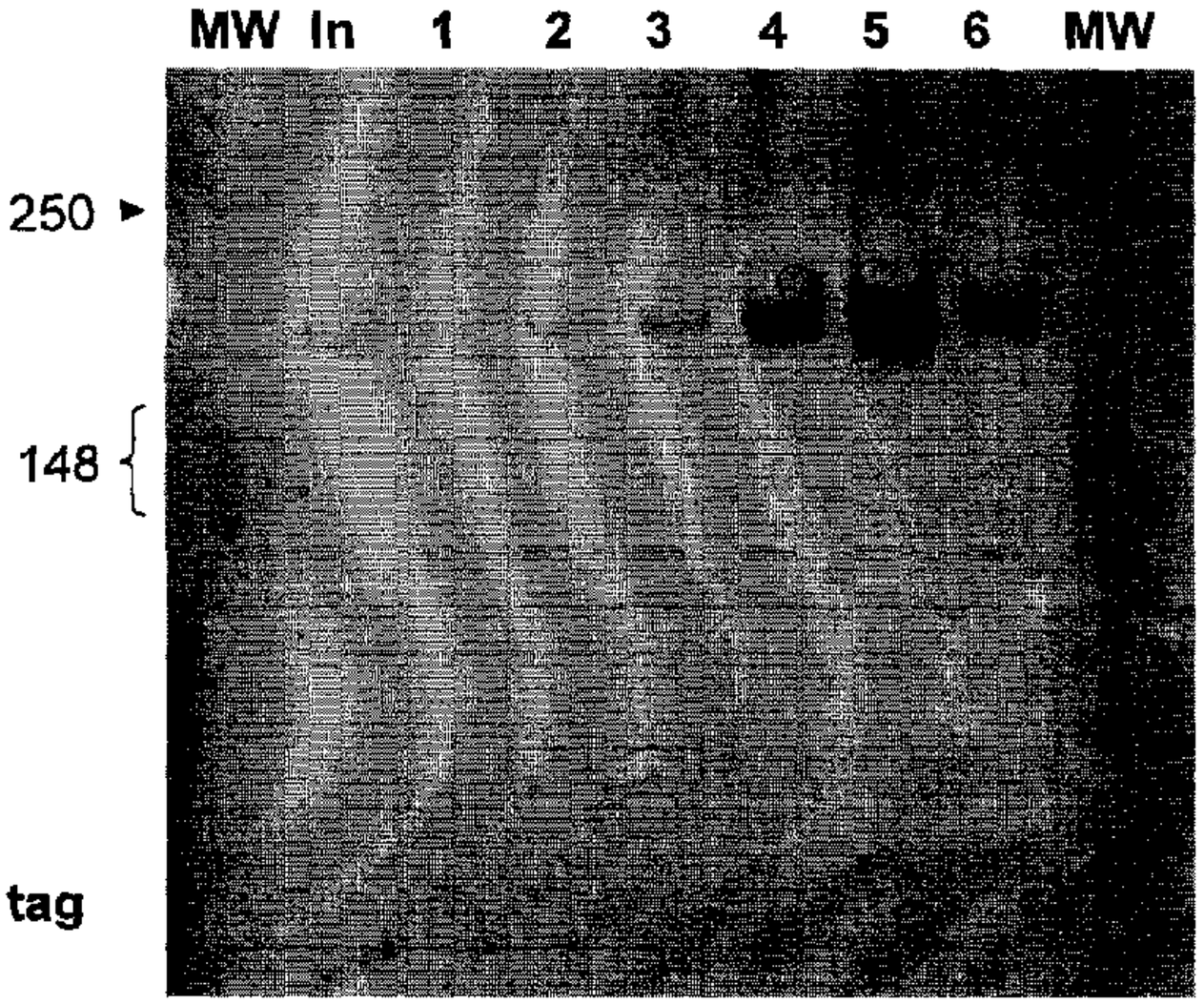

No VEGF 165
VEGF 165 (5 ng/ml)
Fluorescence
1000
500
0
0
5
50
R15E6 Fab (nM)
CTRL-50

FIGURE 12.


Extracellular domain
6-HIS tag
Phosphatase domain
FN3 repeat

A.


MW
In
1
2
3
4
5
6
MW
250
148

B.