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CA 2465731 A1 2003/05/15

(21) **2 465 731**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2002/11/01
(87) Date publication PCT/PCT Publication Date: 2003/05/15
(85) Entrée phase nationale/National Entry: 2004/04/30
(86) N° demande PCT/PCT Application No.: EP 2002/012272
(87) N° publication PCT/PCT Publication No.: 2003/040726
(30) Priorité/Priority: 2001/11/09 (60/344,504) US

(51) Cl.Int.⁷/Int.Cl.⁷ G01N 33/576
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(54) Titre : CRIBLAGE DESTINE A IDENTIFIER DES INHIBITEURS DE PENETRATION DU VIRUS DE L'HEPATITE C
(54) Title: SCREENING FOR HEPATITIS C VIRUS ENTRY INHIBITORS

(57) **Abrégé/Abstract:**

The present invention features methods of screening for compounds that inhibit HCV binding to a cell, methods of inhibiting HCV entry into a cell, and methods of actively or prophylactically treating against an HCV infection. The different methods are based on the identification of the scavenger receptor class B type I as a target site for HCV E2 binding to a cell.



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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 May 2003 (15.05.2003)

PCT

(10) International Publication Number
WO 03/040726 A3

(51) International Patent Classification⁷: **G01N 33/576**

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(21) International Application Number: PCT/EP02/12272

(22) International Filing Date:
1 November 2002 (01.11.2002)

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(25) Filing Language: English

(81) Designated States (*national*): CA, JP, US.

(26) Publication Language: English

(84) Designated States (*regional*): European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR).

(30) Priority Data:
60/344,504 9 November 2001 (09.11.2001) US

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Published:
— with international search report

(88) Date of publication of the international search report:
18 December 2003

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SCREENING FOR HEPATITIS C VIRUS ENTRY INHIBITORS

(57) Abstract: The present invention features methods of screening for compounds that inhibit HCV binding to a cell, methods of inhibiting HCV entry into a cell, and methods of actively or prophylactically treating against an HCV infection. The different methods are based on the identification of the scavenger receptor class B type I as a target site for HCV E2 binding to a cell.



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TITLE OF THE INVENTION

SCREENING FOR HEPATITIS C VIRUS ENTRY INHIBITORS

RELATED APPLICATIONS

5 The present application claims priority to provisional application U.S. Serial No. 60/344,504, filed November 9, 2001, which is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited in the present application are not admitted to be prior art to the claimed invention.

 It is estimated that about 3% of the world's population is infected with the hepatitis C virus (HCV). (Wasley *et al.*, *Semin. Liver Dis.* 20:1-16, 2000.) HCV exposure results in an overt acute disease in a small percentage of cases, while in most
15 instances the virus establishes a chronic infection causing liver inflammation and slowly progresses into liver failure and cirrhosis. (Strader *et al.*, *ILAR J.* 42:107-116, 2001.) Epidemiological surveys indicate an important role for HCV in the onset of hepatocellular carcinoma. (Strader *et al.*, *ILAR J.* 42:107-116, 2001.)

 HCV can be classified into a number of distinct genotypes (1 to 6), and
20 subtypes (a to c). The distribution of the genotypes and subtypes varies both geographically and between risk groups. (Robertson *et al.*, *Arch Virol.* 143:2493-2503, 1998.)

 The HCV genome consists of a single strand RNA about 9.5 kb encoding a precursor polyprotein of about 3000 amino acids. (Choo *et al.*, *Science*
25 244:362-364, 1989, Choo *et al.*, *Science* 244:359-362, 1989.) The HCV polyprotein contains the viral proteins in the order: C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B. Cleavage of the precursor polyprotein results in mature structural and non-structural viral proteins. (Neddermann *et al.*, *Biol. Chem.* 378:469-476, 1997.)

 As part of its infection cycle, HCV enters into a cell. The LDL
30 receptor and CD81 molecule have been identified as putative HCV receptors. The LDL receptor has been suggested to mediate virus internalization via binding to LDL particles that are virus-associated. (Agnello *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 96:12766-12771, 1999.) The CD81 molecule has been suggested to bind HCV E2 based on recombinant envelope protein E2 from HCV genotype 1a. (Pileri *et al.*,
35 *Science* 282:938-941, 1998.)

SUMMARY OF THE INVENTION

The present invention features methods of screening for compounds that inhibit HCV binding to a cell, methods of inhibiting HCV entry into a cell, and
5 methods of actively or prophylactically treating against an HCV infection. The different methods are based on the identification of the scavenger receptor class B type I (SR-BI) as a target site for HCV E2 binding to a cell.

Targeting the SR-BI to inhibit HCV entry into a cell can be achieved by inhibiting one or more of the following: (a) activities relating to HCV binding to
10 SR-BI, (b) activities related to HCV internalization mediated by SR-BI, including activities downstream from SR-BI binding to HCV, and (c) activities related to functional surface expression of SR-BI.

Thus, a first aspect of the present invention features a method of screening for a compound that inhibits the ability of a HCV E2 polypeptide to bind to
15 a cell. The method involves contacting SR-BI or a functional derivative thereof with a polypeptide that binds to the SR-BI HCV E2 binding site and with a test compound, and measuring the ability of the test compound to inhibit binding of the polypeptide to SR-BI or the functional derivative thereof.

A “compound” or “test compound” refers to a discrete chemical entity.
20 The term compounds includes molecules of different sizes and compositions. Examples of compounds include small molecules, peptides, polypeptides, antibodies, and nucleic acid.

The “SR-BI HCV E2 binding site” is the site to which at least the HCV E2 polypeptide from HCV 1a binds SR-BI. An example of such an HCV E2
25 polypeptide from HCV 1a is provided in the Examples *infra*. Reference to the ability of HCV 1a to bind SR-BI does not exclude binding of HCV E2 from other HCV strains to SR-BI. For example, HCV E2 from other HCV strains such as HCV 1b can bind to the naturally occurring human SR-BI HCV E2 binding site.

SR-BI and functional derivatives of SR-BI contain a SR-BI amino acid
30 sequence region of at least 20 contiguous amino acids as that present in SEQ. ID. NO. 1 and can bind at least HCV E2 from HCV 1a.

SEQ. ID. NO. 1 provides a human SR-BI sequence. The presence of at least 20 contiguous amino acids as provided in SEQ. ID. NO. 1 provides a structural tag distinguishing SR-BI or a functional derivative thereof from other proteins.

Reference to “inhibit” or “inhibiting” indicates a detectable reduction in activity. Preferably, there is at least about a 50%, at least about 75%, or at least about 95% percent reduction in activity.

Another aspect of the present invention describes a method of
5 screening for a compound that inhibits SR-BI activity. The method involves the steps of (a) contacting a cell capable of expressing a SR-BI or a functional derivative thereof with a polypeptide that binds to the SR-BI HCV E2 binding site and with a test compound, and (b) measuring the ability of the test compound to inhibit one or more of the following: (i) activities related to HCV binding to SR-BI or a functional
10 derivative thereof, (ii) activities related to HCV internalization, and (iii) functional surface expression of SR-BI or a functional derivative thereof.

In an embodiment of the present invention, the test compound is pre-incubated with SR-BI prior to adding the polypeptide that binds to the SR-BI HCV E2 binding site. Pre-incubation with a test compound is a preferred method for assaying
15 SR-BI functional surface expression inhibitors.

Reference to “capable of expressing” a polypeptide indicates that in the absence of an expression inhibitor, the polypeptide will be expressed in detectable amounts and has detectable activity related to HCV binding or HCV internalization. Expression inhibitors include compounds such as antisense nucleic acid and
20 ribozymes able to decrease activity of nucleic acid encoding for SR-BI and compounds that can modulate functional surface expression of SR-BI at the transcriptional or post-transcriptional levels.

Compounds modulating functional surface expression at the post-transcriptional level include compounds acting on lipid rafts membrane compartments
25 (referred to as “raft domains”) to alter SR-BI activity. SR-BI activity that can be altered by such compounds include HCV binding and internalization.

Another aspect of the present invention features a method of inhibiting entry of a HCV into a cell. The method involves the step of contacting the cell with a SR-BI E2 binding antagonist.

30 A “SR-BI E2 binding antagonist” can at least inhibit binding of a naturally occurring HCV E2 to the SR-BI of SEQ. ID. NO. 1. Preferably, the SR-BI E2 binding antagonist inhibits at least binding of HCV E2 from HCV 1a.

Another aspect of the present invention features a method of treating an HCV infected patient. The method involves the step of decreasing SR-BI activity
35 or functional surface expression.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention.

5 Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate immunoblot detection of HCV E2

10 obtained from genotype 1a (Figure 1A) and biotinylated cell surface proteins interacting with HCV E2 (Figure 1B). Biotinylated HepG2 cells were incubated in presence (lanes 1 and 3) or absence of HCV E2 recombinant protein (lanes 2 and 4). The bound species were cross-linked with DTSSP and the complexes were immunoprecipitated with an antibody against the His tag of the HCV E2 recombinant

15 protein. Samples were eluted both under non-reducing condition, (lanes 1 and 2) and reducing conditions (lanes 3 and 4), that allowed the cleavage between the cross-linked molecular species, and loaded on 10% SDS-PAGE. In Figure 1A, HCV E2 protein is detected with anti-E2 rat mAb followed by anti-rat HRP conjugated as a monomer under reducing conditions (lane 3) and at higher molecular weight under

20 non-reducing conditions (lane 1). In Figure 1B, the reactivity with streptavidin HRP conjugated reveals under reducing conditions (lane 3) a biotinylated protein band at 82 kDa.

Figure 2 illustrates silver staining of a 7.5% SDS-PAGE loaded with samples obtained after a purification step with Con-A sepharose and the

25 deglycosylation step with the PNG-ase F enzyme. The arrows show the purified receptor migrating at 82 kDa (lane 1) before PNG-ase treatment (-) and migrating at 54 kDa (lane 2) after the deglycosylation (+). In the control samples cross-linking was performed in absence of HCV E2 (lanes 3 and 4). Fetuin was loaded on the SDS-PAGE before deglycosylation and after (lane 5 and lane 6) as a control for the PNG-

30 ase F enzymatic activity.

Figure 3 illustrates a Western blot analysis of the HCV E2 receptor purified on Con-A sepharose and deglycosylated with PNG-ase F (+). Rabbit anti-SR-BI polyclonal antibodies incubation was followed by anti-rabbit HRP conjugated for detection of both the glycosylated (lane 1) and deglycosylated (lane 2) receptor

protein. Lanes 3 and 4 represent the control experiment where cross-linking was performed in absence of HCV E2.

Figures 4A and 4B illustrates a FACS analysis of the binding of HCV E2 recombinant protein from strain H to mock transfected (Figure 4A) or SR-BI
5 transfected BHK-21 cells (Figure 4B). Dot plot analysis showed that 10% of the cells transfected with pcDNA3-SR-BI show binding for HCV E2.

Figures 5A-F illustrate CHO cells transfected with the plasmid pcDNA3 and pcDNA3 encoding the human SR-BI or the mouse SR-BI. Figures 5A-C illustrate FACS analysis of the anti-SR-BI (Novus Biologicals NB 400-104)
10 binding to CHO transfected cells. Figures 5D-F illustrate the analysis of the E2 protein binding to transfected cells.

Figure 6 illustrates competition of E2 binding to HepG2 cells and CHO cells transfected with human SR-BI (CHO-SR-BI) by the anti-HVR1 mAb 9/27. Binding was detected by FACS analysis and expressed as percentage of the median
15 fluorescence intensity values obtained in absence of competitor. Binding to HepG2 (open triangle) and CHO-SR-BI (open square) of E2 from H isolate. Binding of E2 from BK isolate to HepG2 (filled triangle) and to CHO-SR-BI (filled square). E2 recombinant proteins were used at half saturating concentration.

20 DETAILED DESCRIPTION OF THE INVENTION

SR-BI is identified herein as a HCV receptor. SR-BI binding to HCV is independent from CD81. Without being limited to any particular theory, SR-BI may provide a privileged HCV entry site by mediating E2 viral glycoprotein interaction with raft domains.

25 Naturally occurring SR-BI is highly expressed in the liver hepatocytes and steroidogenic tissues, and mediates the selective cellular uptake of cholesterol and phospholipids. (Acton *et al.*, *Science* 271:518-520, 1996, Urban *et al.*, *J. Biol. Chem.* 275:33409-33415, 2000.) SR-BI and other scavenger receptors recognize modified lipid particles both acetylated LDL and oxidized LDL. In contrast to other scavenger
30 receptors, SR-BI also binds with high affinity to native HDL and LDL. (Acton *et al.*, *Science* 271:518-520, 1996.)

SR-BI has been located into raft domains. Rafts domains are thought to represent a specific physical state of lipid bilayer, the liquid order phase. (Brown *et al.*, *Annu. Rev. Cell. Dev. Biol.* 14:11-136, 1998, van der Goot *et al.*, *Semin. Immunol.*
35 13:89-97, 2001.) Proteins localized into raft domains are resistant to cold non-ionic

detergent extraction (detergent resistant membranes). (London *et al.*, *Biochim. Biophys Acta.* 1508:182-195, 2000.)

Proteins localized in raft domains are either GPI membrane anchored or fatty acylated. (Brown *et al.*, *Annu. Rev. Cell. Dev Biol.* 14:11-136, 1998.) SR-BI
5 is a fatty acylated protein. (Babitt *et al.*, *J. Biol. Chem.* 272:13242-13249, 1997.)

Rafts domains may represent a preferential entry site for pathogens providing them a way to escape from the classical degradative pathway. (van der Goot *et al.*, *Semin. Immunol.* 13:89-97, 2001). Examples of pathogens that may enter a cell by targeting raft domain components include SV40, echoviruses, HIV, and
10 HTLV-1. (Bergelson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 91:6245-6249, 1994, Manes *et al.*, *EMBO Rep.* 1:190-196, 2000, Niyogi *et al.*, *J. Virol.* 75:7351-7361, 2001, Parton *et al.*, *Immunol. Rev.* 168:23-31, 1999, van der Goot *et al.*, *Semin. Immunol.* 13:89-97, 2001.)

The identification of SR-BI as a site for HCV E2 binding provides a
15 target that can be modulated to study the HCV infection cycle and to inhibit HCV replication or infection. The ability of a test compound to modulate the interaction between SR-BI and HCV E2 can be performed for example, using assays employing a naturally occurring SR-BI or derivative thereof that binds HCV E2, a compound that binds to the SR-BI HCV E2 binding site, and the test compound.

20 Test compounds found to inhibit HCV E2 interaction with SR-BI can be used, for example, to study the effect of modulating SR-BI HCV E2 interaction on HCV replication or infection. Those test compounds having appropriate pharmacological properties such as efficacy and lack of unacceptable toxicity may be used to treat or inhibit HCV infection in a patient.

25

Scavenger Receptor Class B Type I (SR-BI)

SR-BI and functional derivatives thereof used to screen for modulators of SR-BI interaction with HCV E2 can bind at least HCV E2 from HCV 1a. SR-BI and functional derivative of SR-BI contain a SR-BI amino acid sequence region of at
30 least 20 contiguous amino acids as that present in SEQ. ID. NO. 1. The presence of at least 20 contiguous amino acids as provided in SEQ. ID. NO. 1 provides a tag distinguishing SR-BI from other proteins.

SR-BI can be obtained from mammalian sources such as human, hamster, mouse and rat. The ability of SR-BI obtained from a particular source to
35 bind HCV E2 can be confirmed using techniques such as those described in the

Examples *infra*. Examples of naturally occurring SR-BI amino acid and nucleic acid sequences are provided for by SEQ. ID. NO. 1, SEQ. ID. NO. 2, and in references such as Acton U.S. Patent No. 5,998,141, Calvo *et al.*, *J. Biol. Chem.* 268:18929-18935, 1993, Acton *et al.*, *J. Biol. Chem.* 269:21003-21009, 1994, Cao *et al.*, *J. Biol.*
5 *Chem.* 272:33068-33076, 1997, and Webb *et al.*, *J. Biol. Chem.* 24:15241-15248, 1998.

Methods screening for compounds inhibiting the ability of a HCV E2 polypeptide to bind to a cell, or inhibiting SR-B1 activity, preferably employ human SR-B1 or a functional derivative thereof. Human SR-BI is also referred to in the
10 literature as CLA-1. Splice variants or isoforms, and different polymorphic forms of SR-BI that bind to HCV E2 are included within the definition of SR-BI by reference to the presence of an at least 20 amino acid tag.

Based on SR-BI sequences known in the art, additional naturally occurring SR-BI encoding nucleic acid, preferably of human origin, can be obtained.
15 Cloning techniques well known in the art, such as those employing probes, primers, and degenerative probes and primers, can be used to clone SR-BI.

Sets of degenerative probes and primers can be produced taking into account the degeneracy of the genetic code. Hybridization conditions can be selected to control probe or primer specificity to allow for hybridization to nucleic acids
20 having similar sequences.

Techniques employed for hybridization detection and PCR cloning are well known in the art. Nucleic acid detection techniques are described, for example, in Sambrook, *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989. PCR cloning techniques are described, for
25 example, in White, *Methods in Molecular Cloning*, volume 67, Humana Press, 1997.

A naturally occurring SR-BI that binds HCV E2 can be used to produce functional variants. Variants include naturally occurring SR-BI with one or more amino acid alterations. Amino acid alterations are substitutions, additions and deletions. SR-BI activity, such as binding to HCV, SR-BI functional expression, and
30 HCV internalization can be measured based on the guidance described herein.

Differences in naturally occurring amino acids R groups may be taken into account in designing variants. An R group affects different properties of an amino acid such as physical size, charge, and hydrophobicity. Amino acids can be divided into different groups as follows: neutral and hydrophobic (alanine, valine,
35 leucine, isoleucine, proline, tyrtophan, phenylalanine, and methionine); neutral and

polar (glycine, serine, threonine, tryosine, cysteine, asparagine, and glutamine); basic (lysine, arginine, and histidine); and acidic (aspartic acid and glutamic acid).

Generally, in substituting different amino acids it is preferable to exchange amino acids having similar properties. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine,
5 and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Changes outside of different amino acid groups can also be made. Preferably, such changes are made taking into account the position of the amino acid to be substituted in the polypeptide. For example, arginine can substitute more freely
10 for nonpolar amino acids in the interior of a polypeptide than glutamate because of its long aliphatic side chain. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

SEQ. ID. NO. 1 provides a reference sequence for SR-BI including
15 SR-BI functional variants. In different embodiments SR-BI or a functional variant thereof contains at least contiguous 50 amino acids as that present in SEQ. ID. NO. 1, contains at least contiguous 75 amino acids as that present in SEQ. ID. NO. 1; contains SEQ. ID. NO. 1 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid alterations; or contains a substantially similar sequence to SEQ. ID. NO. 1.

20 A “substantially similar sequence” indicates an identity of at least about 65% to a reference sequence. Thus, for example, polypeptides having an amino acid sequence substantially similar to SEQ. ID. NO. 1 have an overall amino acid identity of at least about 65% to SEQ. ID. NO. 1. In different embodiments, substantially similar sequence refers to a sequence identity of at least about 75%, at least about 85%, at least
25 about 95%, or 100%.

Amino acid sequence identity can be determined by methods well known in the art that compare the amino acid sequence of one polypeptide to the amino acid sequence of a second polypeptide and generate a sequence alignment. Amino acid identity can be calculated from the alignment by counting the number of
30 aligned residue pairs that have identical amino acids.

Methods for determining sequence identity include those described by Schuler, G.D. in *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*, Baxevanis, A.D. and Ouelette, B.F.F., eds., John Wiley & Sons, Inc, 2001; Yona *et al.*, in *Bioinformatics: Sequence, Structure and Databanks*, Higgins, D. and
35 Taylor, W. eds., Oxford University Press, 2000; and *Bioinformatics: Sequence and*

Genome Analysis, Mount, D.W., ed., Cold Spring Harbor Laboratory Press, 2001). Methods to determine amino acid sequence identity are codified in publicly available computer programs such as GAP (Wisconsin Package Version 10.2, Genetics Computer Group (GCG), Madison, Wisc.), BLAST (Altschul *et al.*, *J. Mol. Biol.* 215(3):403-10, 1990), and FASTA (Pearson, *Methods in Enzymology* 183:63-98, 1990, R.F. Doolittle, ed.).

In an embodiment of the present invention sequence identity between two polypeptides is determined using the GAP program (Wisconsin Package Version 10.2, Genetics Computer Group (GCG), Madison, Wisc.). GAP uses the alignment method of Needleman and Wunsch. (Needleman *et al.*, *J. Mol. Biol.* 48:443-453, 1970.) GAP considers all possible alignments and gap positions between two sequences and creates a global alignment that maximizes the number of matched residues and minimizes the number and size of gaps. A scoring matrix is used to assign values for symbol matches. In addition, a gap creation penalty and a gap extension penalty are required to limit the insertion of gaps into the alignment. Default program parameters for polypeptide comparisons using GAP are the BLOSUM62 (Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10915-10919, 1992) amino acid scoring matrix (MATrix=blosum62.cmp), a gap creation parameter (GAPweight=8), and a gap extension parameter (LENgthweight=2).

Polypeptide Bindings to the SR-BI HCV E2 Binding Site

Polypeptides capable of binding to the SR-BI HCV E2 binding site contain a region able bind to the same site as HCV E2. The polypeptide region that binds to SR-BI includes naturally occurring E2 regions or binding fragments thereof, derivatives of such E2 regions or binding fragments thereof, and E2 mimotopes.

Polypeptides capable of binding to the SR-BI HCV E2 binding site can also contain regions not involved in SR-BI binding. Non-binding regions, if present, do not prevent the polypeptide from binding to at least the human SR-BI of SEQ. ID. NO. 1. Preferred non-binding regions are additional HCV regions and regions that facilitate detection of binding.

Regions facilitating detection of binding include detectable labels and regions that can be detected. Examples of detectable labels include moieties such as radiolabels, luminescent molecules, haptens and enzyme substrates. Examples of regions that can be detected include regions that provide an epitope for antibody binding or that provide a specific binding region for other types of compounds.

HCV E2 binding mimotopes have a primary structure unrelated to HCV E2, but share binding characteristics with HCV E2. References describing techniques for producing mimotopes in general and describing different HCV E2 mimotopes include Felici *et al.*, U.S. Patent No. 5,994,083 and Nicosia *et al.*,
5 International Application Number WO 99/60132.

The ability of a polypeptide to bind to the SR-BI E2 binding site can be determined, for example, by competition experiments with a HCV E2 polypeptide already shown to bind to SR-BI. Such experiments can be performed employing a polypeptide that may bind to the SR-BI HCV E2 binding site as a test compound.

10

Recombinant SR-BI Expression

Screening for compounds inhibiting HCV binding to SR-BI is facilitated using recombinant nucleic acid expressing SR-BI or a functional derivative thereof. Recombinantly expressed receptors offers several advantages in screening for
15 compounds active at a polypeptide, such as the ability to express the polypeptide in a cell having little or no endogenous expression of the polypeptide and using the same cell without recombinantly expressed polypeptide as a control. For example, SR-BI can be expressed in BHK-21 or CHO cells using an expression vector, wherein the same cell line without the expression vector can act as a control. Additional cell lines
20 lacking SR-BI expression can be identified using techniques such as those employing SR-BI antibodies or those measuring SR-BI RNA.

A recombinant "nucleic acid" refers to an artificial combination of two or more nucleotide sequence regions. The artificial combination is not found in nature. Recombinant nucleic acid includes nucleic acid having a first coding region
25 and a regulatory element or a second coding region not naturally associated with the first coding region. The recombinant nucleotide sequence can be present in a cellular genome or can be part of an expression vector.

Preferably, expression is achieved in a host cell using an expression vector. An expression vector contains recombinant nucleic acid encoding a
30 polypeptide along with regulatory elements for proper transcription and processing. The regulatory elements that may be present include those naturally associated with the nucleotide sequence encoding the polypeptide and exogenous regulatory elements not naturally associated with the nucleotide sequence.

Generally, the regulatory elements that are present in an expression
35 vector include a transcriptional promoter, a ribosome binding site, a terminator, and

an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high
 5 copy number. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

An alternative means to produce recombinant nucleic acid is by altering the cellular genome. One type of alteration that can increase cellular expression is the use of a strong promoter such as the immediate early human
 10 cytomegalovirus promoter. Alterations to the cellular genome can be performed, for example, using techniques described by Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998.

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid
 15 sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
 C=Cys=Cysteine: codons UGC, UGU
 20 D=Asp=Aspartic acid: codons GAC, GAU
 E=Glu=Glutamic acid: codons GAA, GAG
 F=Phe=Phenylalanine: codons UUC, UUU
 G=Gly=Glycine: codons GGA, GGC, GGG, GGU
 H=His=Histidine: codons CAC, CAU
 25 I=Ile=Isoleucine: codons AUA, AUC, AUU
 K=Lys=Lysine: codons AAA, AAG
 L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
 M=Met=Methionine: codon AUG
 N=Asn=Asparagine: codons AAC, AAU
 30 P=Pro=Proline: codons CCA, CCC, CCG, CCU
 Q=Gln=Glutamine: codons CAA, CAG
 R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
 S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
 T=Thr=Threonine: codons ACA, ACC, ACG, ACU
 35 V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Nucleic acid having a desired sequence can be synthesized using chemical and biochemical techniques. Examples of chemical techniques are
5 described in Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Biochemical synthesis techniques involve the use of a nucleic acid template and appropriate enzymes such as DNA and/or RNA polymerases. Examples
10 of such techniques include *in vitro* amplification techniques such as PCR and transcription based amplification, and *in vivo* nucleic acid replication. Examples of suitable techniques are provided by Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, and Kacian *et al.*, U.S.
15 Patent No. 5,480,784.

Assay Formats

A variety of different assay formats may be employed to measure activities related to HCV binding to SR-BI or a functional derivative thereof, activities
20 related to HCV internalization, and activities related to functional surface expression of SR-BI or a functional derivative thereof. Depending on the activity being assayed, assays can be performed using whole cells, membrane preparations, and purified SR-BI.

Techniques for detecting binding to SR-BI include those employing a
25 HCV E2 polypeptide containing a detectable label or region, and those involving the use of secondary antibodies to a region distinct from the SR-BI HCV E2 binding region. Assay formats that may be employed include FACS analysis, a scintillation proximity assay ("SPA"), and sandwich type assays where a detectable antibody is targeted to a region distinct from the SR-BI HCV E2 binding region. An example of
30 techniques that can be employed for a FACS analysis are provided in Example 1 *infra*.

Techniques for performing a SPA are well known in the art. SPA can be performed using a bead or a plate coated with a scintillant fluid and a radiolabeled molecule. Proximity of the radiolabeled molecule to the scintillant fluid stimulates
35 light emission. SPA can be used to measure binding to SR-BI by, for example,

joining SR-BI to a SPA bead or growing cells expressing SR-BI on a SPA plate (cytostar), radiolabeling a HCV E2 polypeptide, and measuring the ability of a test compound to inhibit light production from the radiolabeled polypeptide.

Antibodies binding to a region distinct from the SR-BI HCV E2 binding site can be employed to detect binding using capture assays formats. For example, cell membranes expressing SR-BI or purified SR-BI protein are attached to a solid support and a HCV E2 polypeptide is added to the solid support in presence of the test compound, the solid support is washed, and the presence of E2 on the support is determined using an antibody with a detectable label that binds to E2.

Activities related to HCV internalization can be assayed by, for example, incubating cells with the HCV virus at 37°C and measuring the intracytoplasmic HCV RNA by *in situ* hybridization (ISH). Examples of ISH techniques are provided by Agnello *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 96:12766-12771, 1999.

Activities related to functional surface expression of SR-BI or a functional derivative thereof can be assayed by, for example, measuring surface expression of SR-BI. Surface expression of SR-BI can be measured using compounds binding to SR-BI such as SR-BI antibodies or a labeled polypeptide that binds to SR-BI.

Inhibition of SR-BI Expression

SR-BI nucleic acid activity can be inhibited using compounds affecting the ability of such nucleic acid to be transcribed or translated. Inhibition of SR-BI nucleic acid activity can be used, for example, in target validation studies, as a tool to study HCV infection, and to inhibit HCV infection or replication.

A preferred target for inhibiting SR-BI translation is mRNA. The ability of mRNA encoding SR-BI to be translated into a protein can be affected by compounds such as anti-sense nucleic acid and enzymatic nucleic acid.

Anti-sense nucleic acid can hybridize to a complementary region of a target mRNA. Depending on the structure of the anti-sense nucleic acid, anti-sense activity can be brought about by different mechanisms such as blocking the initiation of translation, preventing processing of mRNA, hybrid arrest, and degradation of mRNA by RNase H activity.

Enzymatic nucleic acid can recognize and cleave another nucleic acid molecule. Preferred enzymatic nucleic acids are ribozymes. Ribozymes targeting particular nucleic acid motifs are well known in the art.

Modified and unmodified nucleic acids can be used as anti-sense molecules and ribozymes. Different types of modifications can affect certain anti-sense activities such as the ability to be cleaved by RNase H, and can affect nucleic acid stability. Examples of references describing different anti-sense molecules and ribozymes, and the use of such molecules, are provided in U.S. Patent Nos. 5,849,902, 5,859,221, and 5,852,188.

10

Administration

Compounds for treating HCV can be formulated and administered to a patient using the guidance provided herein along with techniques well known in the art. The preferred route of administration ensures that an effective amount of compound reaches the target. Guidelines for pharmaceutical administration in general are provided in, for example, *Remington's Pharmaceutical Sciences 18th Edition*, Ed. Gennaro, Mack Publishing, 1990, and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990, both of which are hereby incorporated by reference herein.

15

A "patient" refers to a mammal capable of being infected with HCV. A patient may or may not be infected with HCV. Examples of patients are humans and chimpanzees.

Depending upon the structure of a particular compound, the compound may be prepared as an acidic or basic salt. Pharmaceutically acceptable salts (in the form of water- or oil-soluble or dispersible products) include conventional non-toxic salts or the quaternary ammonium salts that are formed, *e.g.*, from inorganic or organic acids or bases. Examples of such salts include acid addition salts such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate; and base salts such as ammonium salts, alkali metal salts such as sodium and potassium

30

salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine.

Compounds can be administered using different routes such as by injection. When administered by injection, the injectable solution or suspension may be formulated using suitable non-toxic, parenterally-acceptable diluents or solvents, such as Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the desired effect; and the particular compound employed.

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug. The daily dose for a patient is expected to be between 0.01 and 1,000 mg per day.

EXAMPLES

Examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: Materials and Methods

This example describes different materials and methods that were employed to study HCV E2 binding to SR-BI.

Cells

Molt-4 (human T-cell leukaemia) cells were obtained from the MRC ADP Repository. HepG2 (human hepatoma), HEK-293 (human embryonic kidney) BHK-21 (baby hamster kidney) and CHO (chinese hamster ovary) cell lines were obtained from the ATCC Repository. Cells were grown under standard conditions in presence of 10% fetal calf serum.

Cloning and Expression of E2 Glycoproteins

HCV E2 protein representative of genotype 1a (strain H) and genotype 1b (strain BK) were cloned into VIJnsTPA as described by Meola *et al.*, *J. Virol.* 74:5933-5938, 2000. The cloned E2 fragment contains the coding sequence for E2
5 from amino acid 384 to amino acid 661 of the HCV polyprotein and a tag of 6 His at the C-terminal. 293 cells were transfected by calcium phosphate method. Recombinant proteins produced by transfected HEK 293 cells were collected from culture supernatant harvested 48 hours after transfection, concentrated 40 times using filter devices (Millipore Centricon Plus-80) and supplemented with protease inhibitor
10 cocktail tablets (Boehringer Mannheim) and 10% glycerol. The amount of E2 in the extracts was quantified by using a GNA (lectin from *Galanthus nivalis*) capture assay as described by Flint *et al.*, *J. Virol.* 73:6782-6790, 1999.

FACS Analysis of Anti-CD81 and E2 Glycoproteins Binding to Cell Lines

15 Cells were washed twice in phosphate-buffered saline 1% FCS, 0.05% sodium azide (washing buffer). Then, 2×10^5 cells were allowed to bind at room temperature for 1 hour with E2 concentrated supernatants or, as control, with the concentrated supernatant of HEK 293 cells transfected with VIJnsTPA plasmid (mock). After one wash in washing buffer the anti-His mouse monoclonal antibody
20 (mAb, Quiagen) was added at the concentration of 2 $\mu\text{g/ml}$ for 1 hour at room temperature. Binding to anti-CD81 was also performed at room temperature for 1 hour by using the mouse monoclonal antibody (1.3.3.22 Santa Cruz Biotechnology) diluted 1:200.

Cell bound mAbs were visualized with anti-mouse IgG1-phycoerythrin
25 (PE) conjugate (Serotec). Flow cytometry data acquisition was performed using a Becton-Dickinson FACS Vantage flow cytometer. Dead cells were detected as Sytox Green dye (Molecular Probes) stained positive and were excluded from analyses.

Selection of a HepG2 Sub-Population with Increased E2-Binding Capacity

30 HepG2 cells were incubated with a saturating concentration of 1a-derived E2 recombinant protein for 2 hours at room temperature. The E2 bound protein was revealed as described above and analyzed in a FACS Vantage (Becton Dickinson) flow cytometer. The cells that showed the highest fluorescence intensity were sorted and expanded. The procedure was repeated four times.
35

Coating of Anti-His antibody onto Dynabeads

Dynabeads M-450 rat anti-mouse IgG1, (Dyna, Oxoid) were washed once with PBS 0.1% BSA before the addition of 15 µg of the anti-His Mab in 4 ml volume of PBS 0.1% BSA. After 1 hour of coating, the beads were washed twice
5 with PBS 0.1% BSA and once with 0.2 M triethanolamine pH 9. To cross-link the bound antibody dimethylpimlidate dihydrochlorite (DMP) was added at a 20 mM concentration to the Dynabeads in 10 ml volume of 0.2 M triethanolamine pH 9. The reaction was stopped by adding 0.2 M Tris HCl at pH 8. Beads were washed first with PBS 0.5% Triton and then with PBS 0.1% BSA. Once prepared the Dynabeads
10 were stored at 4°C in presence of 0.1% sodium azide.

Cell Surface Biotinylation

HepG2s4 cells (1×10^8) were harvested by trypsinization and washed once with cold PBS in 250 ml volume. Cells were incubated in the dark for 15
15 minutes with a freshly prepared solution of 2 mM sodium periodate at the concentration of 1×10^7 cell/ml. After this mild oxidation, cells were washed twice and incubated with 5 mM biotin-LC hydrazide for 10 minutes at room temperature at the concentration of 5×10^7 cell/ml. Biotin-LC hydrazide was freshly prepared by solubilization in DMSO at a 50 mM concentration.

20 After biotinylation two washes in 250 ml volume of cold PBS were performed, and cells were incubated with the concentrated supernatant containing E2 recombinant protein or as a control with the supernatant obtained from the mock transfection. Incubation with E2 lasted 1 hour at room temperature. Staining of bound E2 was performed as described above and analyzed by FACS.

25

E2-Receptor Cross-Linking and Immunoprecipitation of Complexes

DTSSP (dithiobis-sulfosuccinimidylpropionate, Pierce,) was used as a cross linker. DTSSP is a water soluble cross-linker homobifunctional N-hydroxysuccinimide ester and is thiol cleavable.

30 HepG2s4 cells (5×10^7 cell/ml) were washed once after binding to E2 and incubated with the DTSSP at 2 mM concentration in PBS for 20 minutes at room temperature. The reaction was stopped by incubation with Tris HCl 50 mM at pH 7.5. Cells were lysed in PBS 1% Triton in the presence of protease inhibitor cocktail (Boehringer Mannheim) for 20 minutes at 37°C. E2-receptor complexes were
35 immunoprecipitated with the anti-His Dynabeads by incubation overnight at 4°C.

After 5 washes in PBS 1% Triton elution was performed either boiling directly the beads in sodium dodecyl sulfate (SDS) sample buffer or incubating beads 30 minutes at 37°C in 50 mM dithiothreitol (DTT), 50 mM NaCl, 50 mM Tris HCl pH 8.

Samples obtained from boiling the beads were loaded on SDS-Page
 5 and analyzed by Western blot for detection of biotinylated proteins using streptavidin horseradish peroxidase conjugated (HRP; Pierce) diluted 1:25.000 in Tris Buffer Saline 0.05% Tween 20 (TBST), 2% bovine serum albumin (BSA). For detecting recombinant E2 protein of genotype 1a the rat monoclonal antibody 6-1/a (Flint *et al.*, *J. Virol.* 73:6782-6790, 1990) was used diluted 1:50 in TBST 5% low fat milk,
 10 followed by incubation with anti-rat HRP (Dako) conjugated 1:2000. The chemiluminescent Super Signal West Pico (Pierce) substrate was used and immunoreactive proteins were detected by exposure on X ray film (Kodak Biomax ML).

15 *Con-A Sepharose Purification and Enzymatic Deglycosylation of the E2 Receptor*

Eluates obtained from the immunoprecipitation with anti-His Dynabeads were diluted 1:2 with 1 M NaCl, 0.2% Triton and incubated with Con-A beads for two hours at room temperature. After three washes in incubation buffer, elution was performed under denaturing conditions in 1% SDS, 1% β -
 20 mercaptoethanol and 100 mM phosphate pH 7.5 at 95°C for 10 minutes. The eluates were diluted 1:10 in the PNGase F incubation buffer containing 0.1% SDS, 0.5% NP40, 10 mM EDTA, and 100 mM Na phosphate pH 7.5. Eluates were divided into aliquots one of which was treated with the enzyme PNGase F (Bio-rad) for 3 hours at 37°C.

25 Samples were loaded on 7.5% SDS pre-cast gel (Bio-rad) and silver stained. Immunoreactivity of the purified samples was probed in Western blot by using rabbit anti-SR-BI purified polyclonal antibodies (Novus Biologicals NB 400-104) diluted 1:1500 in TBST 2% BSA followed by anti-rabbit HRP (Pierce) diluted 1:20.000. The chemiluminescent substrate Super Signal West Pico (Pierce) was used
 30 for detection.

Cloning of SR-BI Coding Sequence and Transfection in BHK Cell Line

RNA was extracted from HepG2s4 cells with TRIzol reagent (Gibco BRL) following manufacturer's instructions. First-strand cDNA was produced
 35 mixing total RNA (2 μ g) with 10 pmol of the antisense primer SR-BI 5'-

CCAGTCTAGACAGTTTTGCTTCCTGCAGCACAGAGCCC-3' (SEQ. ID. NO. 3).

The restriction site for *Xba I* is in italics. The reaction was performed by using Superscript II reverse transcriptase (Gibco BRL) as described by Meola *et al.*, *J. Virol.* 74:5933-5938, 2000.

5 An aliquot of the cDNA was amplified by PCR using Platinum *Pfx* DNA polymerase (Gibco BRL) following manufacturer's instructions. The primers used were the antisense SR-BI primer and the sense SR-BI 5'-
AGGCAAGCTTGCCGCCATGGGCTGCTCCGCCAAAGCGCGCTGGG-3', (SEQ.
ID. NO. 4) where the restriction site for *Hind III* is in italics. PCR was performed in a
10 Perkin-Elmer 2400 thermocycler, denaturing samples for 4 minutes at 94°C and then running 35 cycles of incubation at 94°C for 30 seconds, at 50°C for 30 seconds, and 68° for 2 minutes.

The PCR product was digested with the restriction enzymes *Hind III* and *Xba I* for directional cloning in the vector pcDNA3. Clones were sequenced with
15 the Big Dye Terminator Cycle Sequencing Kit using AmpliTaq (Applied Biosystems) and an Applied Biosystem Model 373A Sequencer. Sequences were analyzed by the Vector NTI program.

The BHK-21 cell line was used as recipient for transient transfection. Transfection was performed by mixing plasmid DNA with the FuGENE 6 transfection
20 reagent (Roche). Cells were harvested 24 hours after transfection and analyzed by FACS for E2 binding.

Example 2: Identification of SR-BI as an E2 Receptor

SR-BI was identified as an E2 receptor by examining the ability of E2
25 to bind to HepG2 cells, enriching for HepG2 cells having increased ability to bind E2, and determining that SR-BI binds E2. HepG2 cells were used to search for the E2 receptor because they were found to lack CD81 and retain the ability to bind HCV E2.

Characterization of CD81-Independent Binding to E2 Glycoproteins

30 The ability of HepG2 cells to bind E2 independent of CD81 was determined using a FACS analysis. The absence of the CD81 molecule from HepG2 cells was determined using an anti-CD81 mouse monoclonal antibody, and a secondary antibody anti-mouse IgG1-phycoerythrin conjugate. Binding of recombinant E2 proteins derived from genotype 1a and 1b to cells was detected by

antibodies reactive against a 6-His tag present in the recombinant proteins followed by incubation with an anti-mouse IgG1-phycoerythrin conjugate.

FACS analysis for CD81 expression was performed using HepG2 cells and Molt-4 cells as described in Example 1. HepG2 cells were negative for CD81
5 expression. In contrast, Molt-4 cells showed high levels of CD81 on the cell surface.

FACS analysis for E2 binding was performed using HepG2 cells and Molt-4 cells as described in Example 1. Both cell lines tested showed binding to recombinant E2 derived from genotype 1a. However, the E2 recombinant glycoprotein derived from 1b genotype showed a much reduced binding to Molt-4
10 cells, while the binding to HepG2 cells was comparable to that obtained with 1a derived E2 recombinant protein.

Cross-Linking of Recombinant E2 Protein to the Cellular Receptor

Enrichment of HepG2 cells expressing high levels of the E2 receptor
15 was achieved with four subsequent rounds of sorting. A subpopulation of HepG2 cells "HepG2s4" showed upon binding to E2, a mean fluorescence intensity value 3.5 times higher than the original cell population. The observed phenotype was stable after several weeks of cell culture.

Surface labeling of HepG2s4 was performed using a biotin-LC-
20 hydrazide reagent. (Kahne *et al.*, *J. Immunol. Methods*. 168:209-218, 1994.) The hydrazides are reactive with the aldehyde groups obtained by mild oxidation with sodium periodate of hydroxyl groups of the glycoprotein carbohydrate moieties. This biotinylation method is compatible with the subsequent step of cross-linking involving a primary amine as a target for a NHS (N-hydroxysuccinimide) ester cross-
25 linker.

Biotinylated HepG2s4 cells were incubated with the E2 glycoprotein 1a. The reactivity of E2 to the putative receptor was unaffected by the biotinylation procedure as detected by flow cytometric analysis after staining of bound E2.

Cross-linking was performed after the E2 binding by addition of the
30 DTSSP cross-linker, that is cleavable by thiol. Cells were finally lysed in PBS 1% Triton and the E2-receptor complexes were immunoprecipitated under non-reducing conditions by Dynabeads conjugated with an antibody reactive against the His tag of recombinant E2. The experiment was run in parallel with a control sample where the biotinylated HepG2s4 cells were incubated with the concentrated supernatant from the
35 mock transfected 293 cells before the cross-linking.

Immunoprecipitated samples were eluted directly in sample buffer both under reducing and non-reducing conditions and loaded on SDS-page. Immunoblot was performed by using an anti-E2 rat monoclonal antibody, 6/1a, specific for genotype 1a protein (Flint *et al.*, *J. Virol.* 73:6782-6790, 1990), followed by an anti-
5 rat secondary antibody conjugated with peroxidase for enhanced chemiluminescence detection.

E2 protein was detected as a diffuse band of the expected molecular weight for the monomer species under reducing condition. Under non-reducing conditions most of E2 protein was detected at higher molecular weight probably
10 representing the E2 receptor-complexes and additional aggregated forms of E2 (Fig. 1A).

For detection of biotinylated species Western blot were developed with streptavidin peroxidase conjugate. By thiol cleavage of the E2/receptor complexes, it was possible to detect only under reducing condition a predominant biotinylated band
15 with an apparent molecular weight of 82 kDa, probably corresponding to the biotinylated receptor (Fig. 1B).

Purification and Enzymatic Deglycosylation of the Putative Receptor

The cross-linking experiment indicated that the receptor involved in E2
20 binding was an 82 kd glycoprotein, since only glycoproteins could have been biotinylated by the biotin hydrazide reagent. The protein involved in E2 binding was identified by performing cross-linking in the absence of the surface labeling step with biotin due to cellular toxicity associated with sodium periodate. Treatment with sodium periodate was quite toxic for the cells increasing significantly the number of
25 cells lost before binding.

Cells were harvested (6×10^8 cells) to perform binding to E2 followed by cross-linking. The E2/receptor complexes were purified from the lysate with anti-His Dynabeads as described in Example 1, and eluting the receptor molecule from the complex by incubation in 50 mM DTT at 37°C for 30 minutes. The analysis of the
30 eluate on SDS page by silver staining indicated that several molecular species were eluted together with the putative receptor.

A second purification step was performed using Concanavalin A lectin (Con-A). Con-A is a commonly used lectin for purification of glycoproteins that binds Asn-linked glycans. Samples eluted from the anti-His Dynabeads were
35 incubated with Con-A sepharose for a second immunoprecipitation.

After several washes in the incubation buffer, elution from the Con-A beads was performed under denaturing condition compatible with the enzymatic activity of PNGase F. PNGase F is an enzyme that releases all Asn-linked oligosaccharides from the glycoproteins.

5 The eluted sample was enzymatically deglycosylated by using the PNGase F enzyme. Analysis of samples on silver stained gel visualized the glycosylated receptor band with an apparent molecular weight of 82 kDa and the deglycosylated form migrating at 54 kDa compatible with the presence of 10 potential Asn glycosylation sites (Figure 2).

10

Identification of the Putative Receptor

Based on preliminary data, SR-BI was suspected of being an HCV receptor. Preliminary data on inhibition of the E2 binding to HepG2 cells with β -cyclodextrins suggested that cholesterol may play a role in the observed binding. β -cyclodextrins can selectively remove cholesterol from cell membranes. (Yancey *et al.*, *J. Biol. Chem.* 271:16026-16034, 1996.) Additionally, the migration pattern on SDS page of the glycosylated and deglycosylated receptor were very similar to that for SR-BI.

20 SR-BI was confirmed to be the receptor binding HCV E2 using anti-SR-BI antibodies. The reactivity of the purified proteins in Western blot with antibodies against SR-BI is shown in Figure 3.

Example 3: Transfection of the SR-BI Coding Sequence in BHK-21 Cell Line

25 The coding sequence for the human SR-BI was amplified from RNA of HepG2s4 cells and cloned in a vector suitable for transfection. Transfection was performed in BHK-21 recipient cells since they were negative for E2 binding. FACS analysis of cells 24 hours after transfection indicated that SR-BI transfected cells acquired binding for E2 (Figure 4).

30 Example 4. Transfection of Human and Mouse SR-BI Coding Sequence in CHO Cell Line

The human SR-BI coding sequence in Example 3 was amplified with the sense primer (SEQ. ID. NO. 4) and the antisense primer (SEQ. ID. NO. 3). The stop codon in this construct was provided by the cloning vector 36 nucleotides after the SR-BI coding sequence, and therefore 12 amino acids were added to the carboxyl

35

terminal of the SR-BI natural sequence. To obtain the human SR-BI protein of its natural size ending with the leu 509 (SEQ. ID. NO. 1), the coding sequence for human SR-BI was PCR amplified by using the sense primer (SEQ. ID. NO. 4) and a novel antisense primer (SEQ. ID. NO. 5). The new primer contains a stop codon in the primer sequence.

The mouse SR-BI sequence was amplified from IMAGE clone BC004656 by using the sense primer (SEQ. ID. NO. 6), and the antisense primer (SEQ. ID. NO. 7). The human and the mouse sequences were cloned in pcDNA3 vector and clones obtained were sequenced.

The hamster CHO cell line negative for binding to HCV E2 protein was transfected with the plasmids using lipofectamine 2000 reagent (Invitrogen). The combination of CHO cells and lipofectamine reagent gave improved transfection efficiency. Transfected cells were harvested 24 hours after transfection and analyzed by FACS, for receptor expression and E2 binding capability.

Results demonstrate that the human and the mouse receptors, were expressed at comparable levels (Figures 5A-C), but only cells transfected with the human SR-BI acquired the ability to bind HCV E2 (Figures 5D-F). Moreover, the mouse SR-BI, showing 80% of homology at amino acid level to the human receptor, doesn't bind to HCV E2, mirroring the species specificity of HCV infection (Figures 5D-F).

Example 5. A monoclonal antibody against the hypervariable region 1 (HVR1) of HCV E2 glycoprotein inhibits the E2 binding to SR-BI.

A biological relevant question concerns the ability of antibodies against the hypervariable region 1 to neutralize HCV virus. A monoclonal antibody (9/27; Flint, *et al.*, 2000, *J. Virol.*, 74, 702-709), obtained upon immunization with E2 and reactive against the HVR1 of E2 derived from H isolate was used to inhibit E2 binding to SR-BI. The antibody showed a dose dependent inhibitory activity for the binding of the E2 protein from genotype 1a to HepG2 cells and to CHO cells stably transfected with SR-BI with an apparent IC₅₀ of about 500nM (Figure 6). The antibody was not effective on the binding of the E2 protein derived from genotype 1b, BK strain, consistent with its lack of reactivity with this variant (Figure 6).

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

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Val	Gln	Asn	Ile	Ser	Arg	Ile	His	Leu	Val	Asp	Lys	Trp	Asn	Gly	Leu	225	230	235	240
Ser	Lys	Val	Asp	Phe	Trp	His	Ser	Asp	Gln	Cys	Asn	Met	Ile	Asn	Gly	245	250	255	

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Cys	Pro	Cys	Leu	Glu	Ser	Gly	Ile	Gln	Asn	Val	Ser	Thr	Cys	Arg	Phe
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Ser	Ala	Pro	Leu	Phe	Leu	Ser	His	Pro	His	Phe	Leu	Asn	Ala	Asp	Pro
			340					345					350		
Val	Leu	Ala	Glu	Ala	Val	Thr	Gly	Leu	His	Pro	Asn	Gln	Glu	Ala	His
		355					360					365			
Ser	Leu	Phe	Leu	Asp	Ile	His	Pro	Val	Thr	Gly	Ile	Pro	Met	Asn	Cys
	370					375					380				
Ser	Val	Lys	Leu	Gln	Leu	Ser	Leu	Tyr	Met	Lys	Ser	Val	Ala	Gly	Ile
385					390					395					400
Gly	Gln	Thr	Gly	Lys	Ile	Glu	Pro	Val	Val	Leu	Pro	Leu	Leu	Trp	Phe
				405					410					415	
Ala	Glu	Ser	Gly	Ala	Met	Glu	Gly	Glu	Thr	Leu	His	Thr	Phe	Tyr	Thr
			420					425					430		
Gln	Leu	Val	Leu	Met	Pro	Lys	Val	Met	His	Tyr	Ala	Gln	Tyr	Val	Leu
		435					440					445			
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WHAT IS CLAIMED IS:

1. A method of screening for a compound that inhibits the ability of a hepatitis C virus E2 polypeptide to bind to a cell comprising the steps of:
 - 5 a) contacting a human scavenger receptor class B type I (SR-BI) or functional derivative thereof with a polypeptide that binds to the SR-BI HCV E2 binding site and with a test compound, and
 - b) measuring the ability of said test compound to inhibit binding of said polypeptide to said SR-BI.
- 10 2. The method of claim 1, wherein said SR-BI is present as a soluble protein.
3. The method of claim 1, wherein said SR-BI is present in a
15 membrane preparation.
4. A method of screening for a compound that inhibits SR-BI activity comprising the steps of:
 - 20 a) contacting a cell capable of expressing a human SR-BI or functional derivative thereof with a polypeptide that binds to the HCV SR-BI E2 binding site and with a test compound, and
 - b) measuring the ability of said test compound to inhibit one or more of the following: (i) binding of said polypeptide to said SR-BI or functional derivative thereof, (ii) HCV internalization, and (iii) functional surface expression of said SR-BI
25 or functional derivative thereof.
5. The method of claim 4, wherein said cell is pre-incubated with said test compound prior to adding said polypeptide.
- 30 6. The method of claim 4, wherein said SR-BI or functional derivative thereof has an amino acid sequence substantially similar to SEQ ID NO: 1.
7. The method of claim 6, wherein said step (b) measures the ability of said test compound to inhibit binding of said polypeptide to said cell.

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8. The method of claim 7, wherein said cell comprises recombinant nucleic acid capable of expressing said SR-BI or functional derivative thereof.

5 9. The method of claim 8, wherein said cell is a mammalian cell.

10. The method of claim 9, wherein said mammalian cell does not endogenously express said SR-BI or functional derivative thereof.

10 11. The method of claim 10, wherein said SR-BI is the human SR-BI of SEQ ID NO: 1.

12. The method of claim 11, wherein said polypeptide comprises a naturally occurring E2 region.

15 13. The method of claim 12, wherein said polypeptide comprises a E2 region from either HCV 1a or HCV 1b.

14. A method of inhibiting entry of a hepatitis C virus into a cell comprising the step of contacting said cell with an SR-BI E2 binding antagonist.

15 15. The method of claim 14, further comprising the step of using said antagonist as the test compound in the method of any one of claims 1-13 prior to inhibiting entry of said hepatitis C virus.

16. A method of treating an HCV infected patient comprising the step of decreasing SR-BI activity or functional surface expression.

17. The method of claim 16, wherein method inhibits the ability of SR-BI to bind HCV.

18. The method of claim 16, wherein said step of decreasing SR-BI activity or functional surface expression is performed using a compound identified as inhibiting binding to SR-BI or a functional derivative thereof or inhibiting SR-BI activity using the method of any one of claims 1-13.

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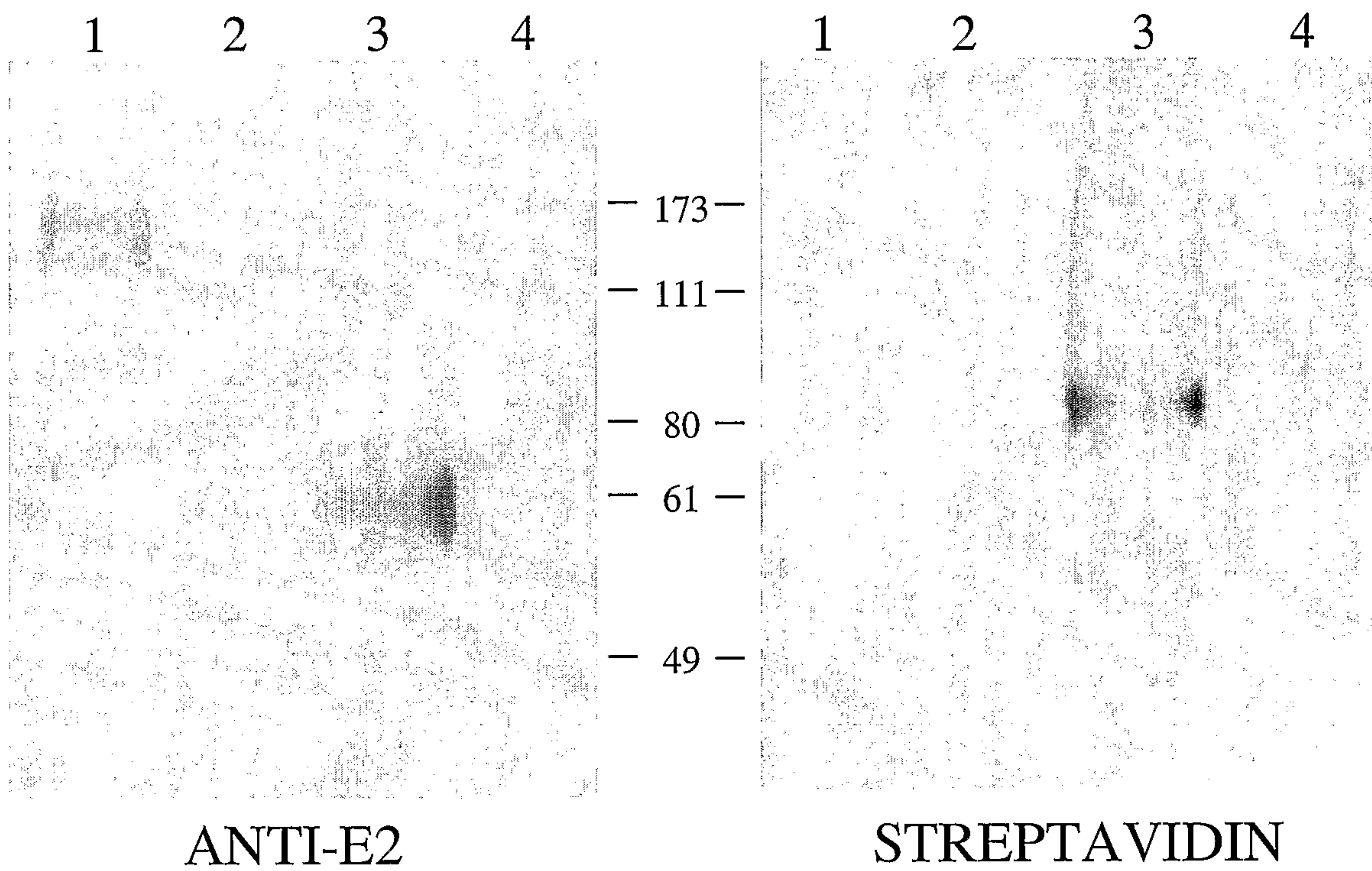


FIG. 1A

FIG. 1B

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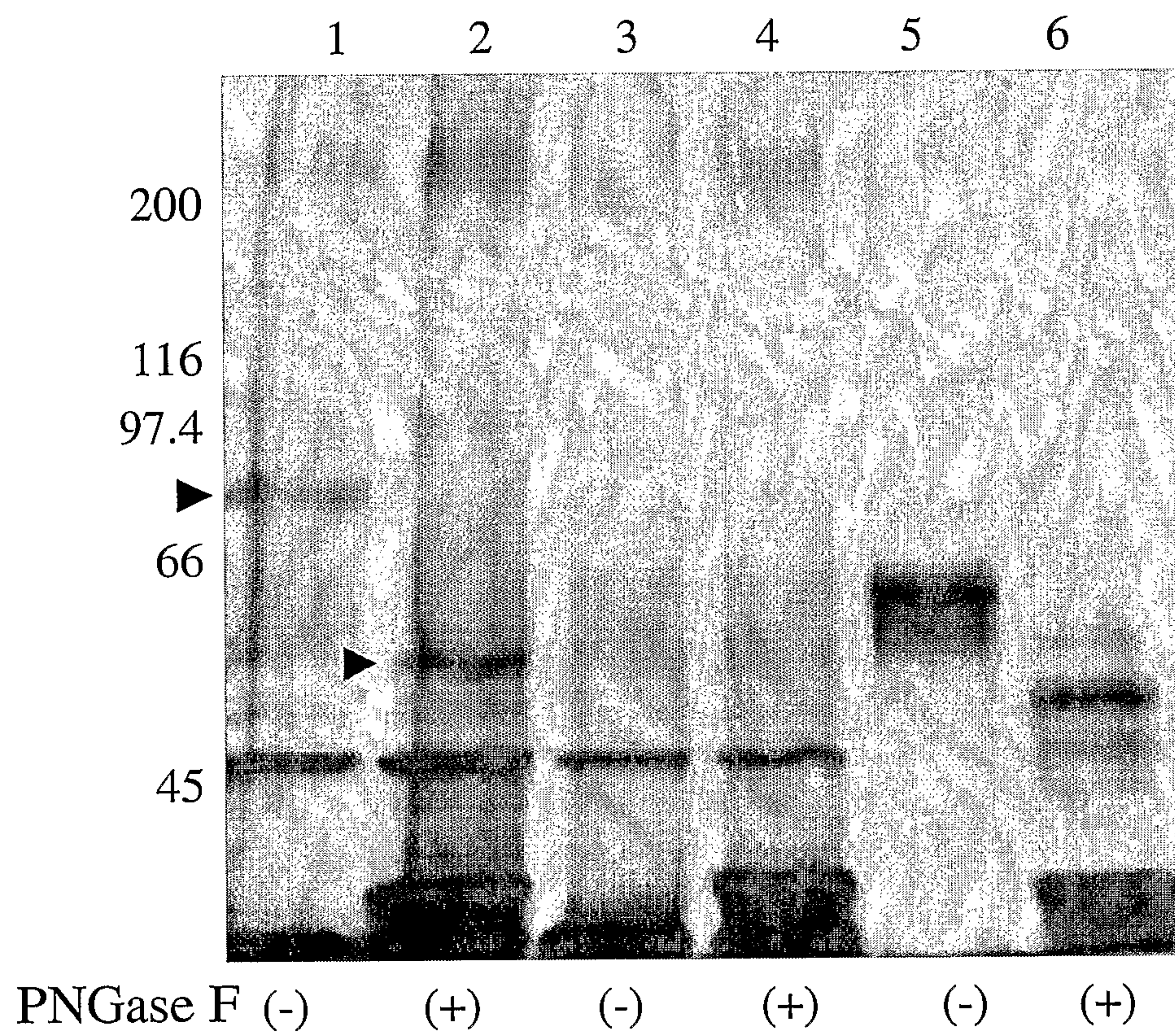


FIG. 2

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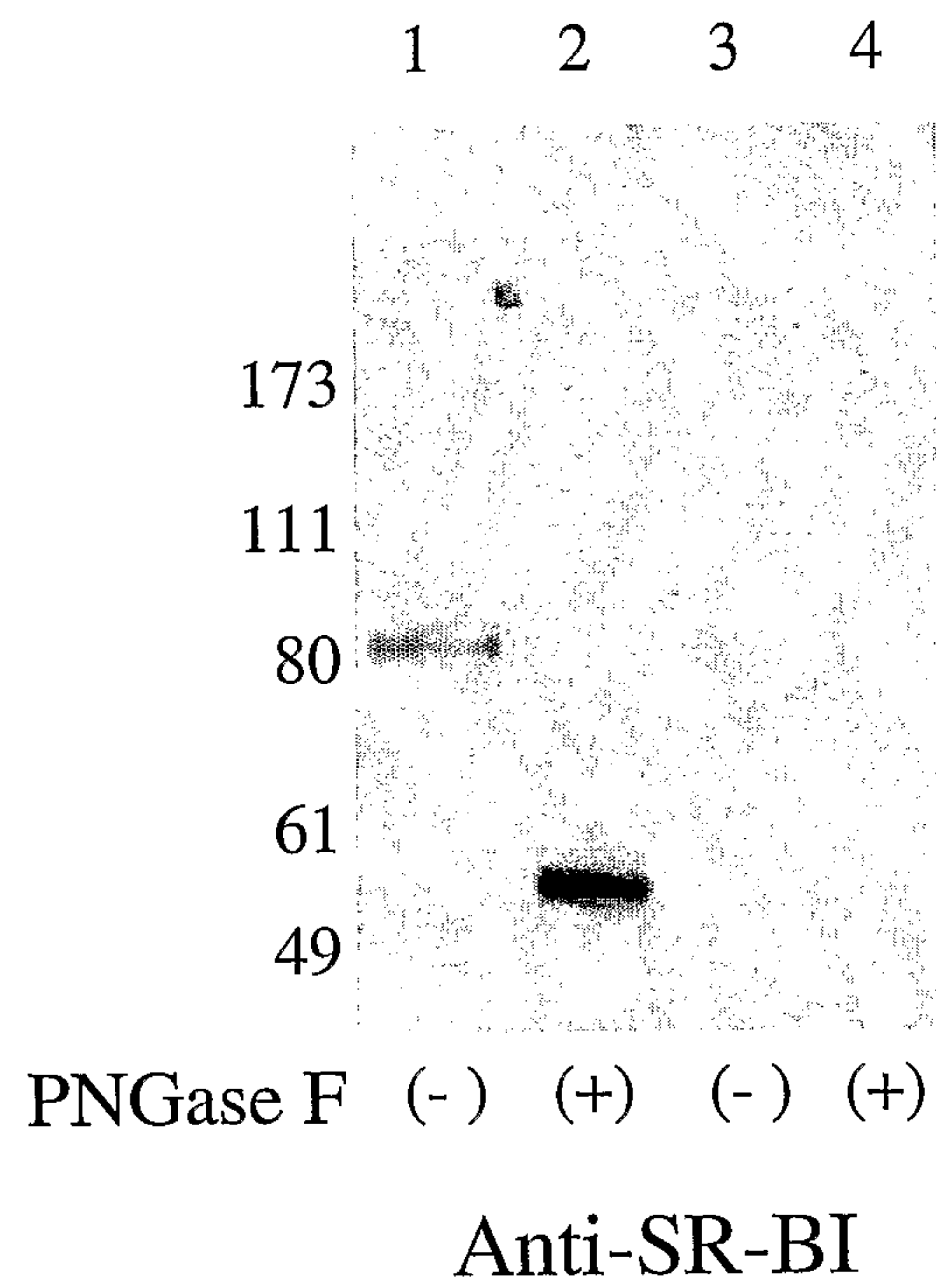


FIG. 3

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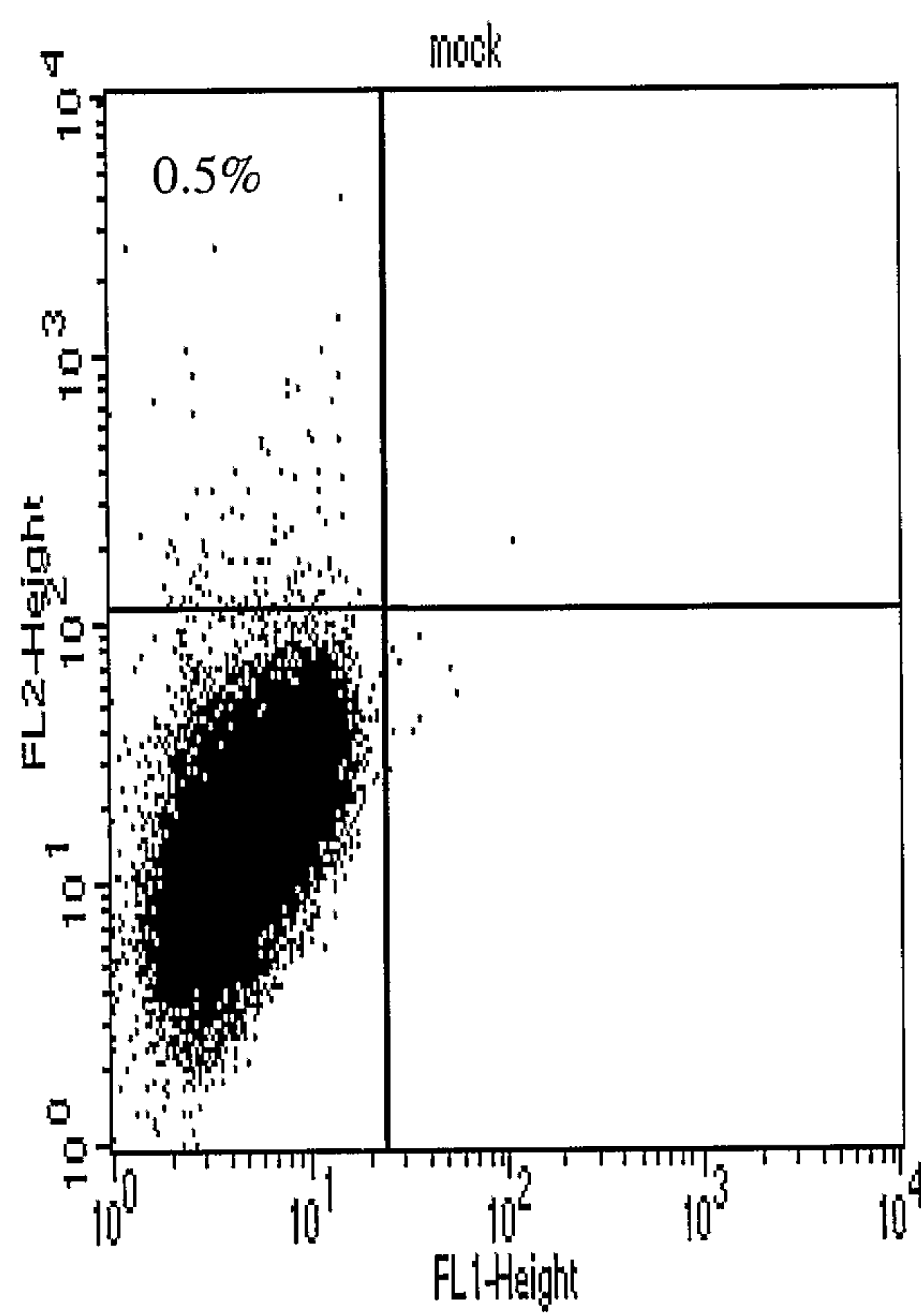


FIG. 4A

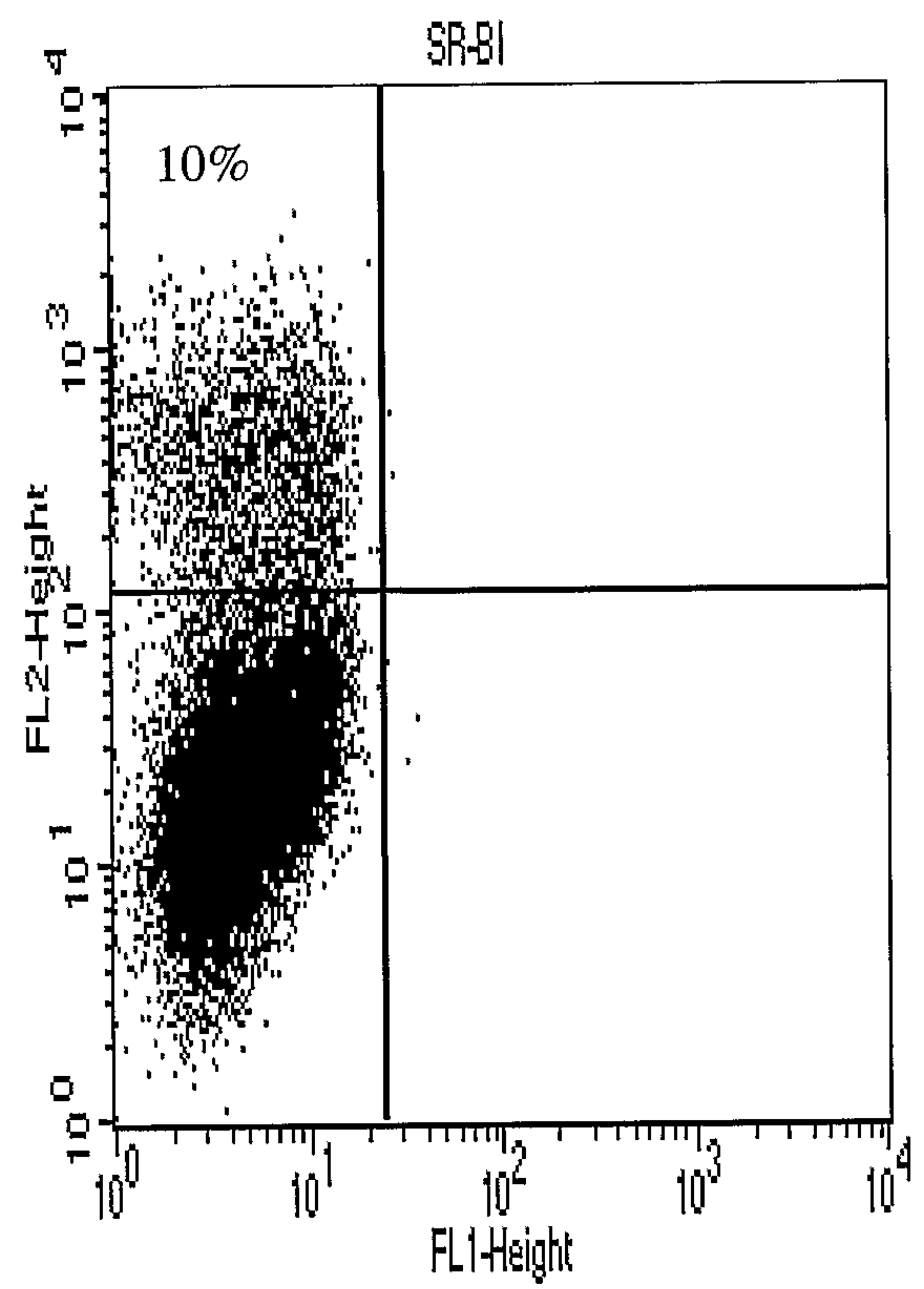


FIG. 4B

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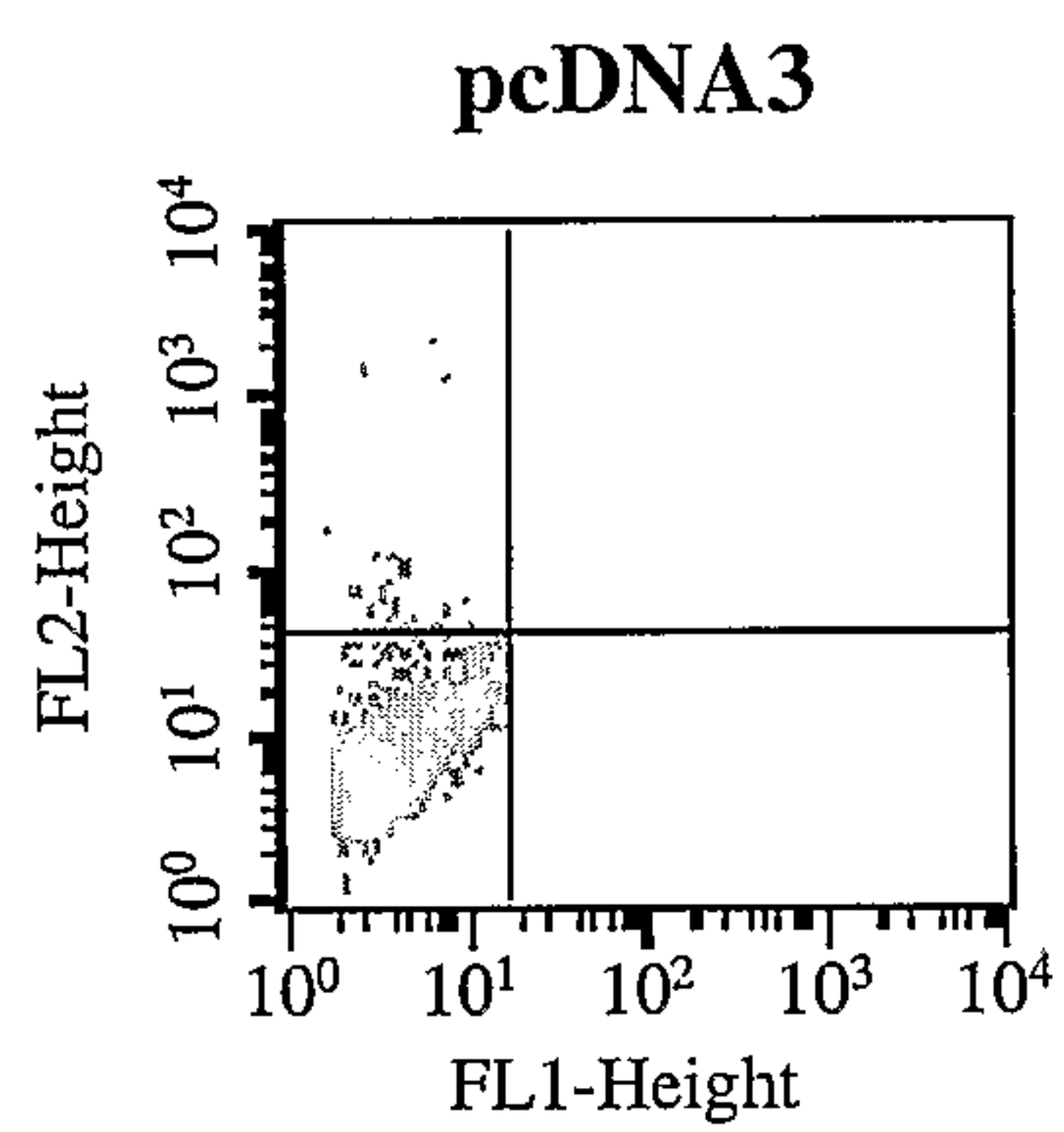


Fig. A

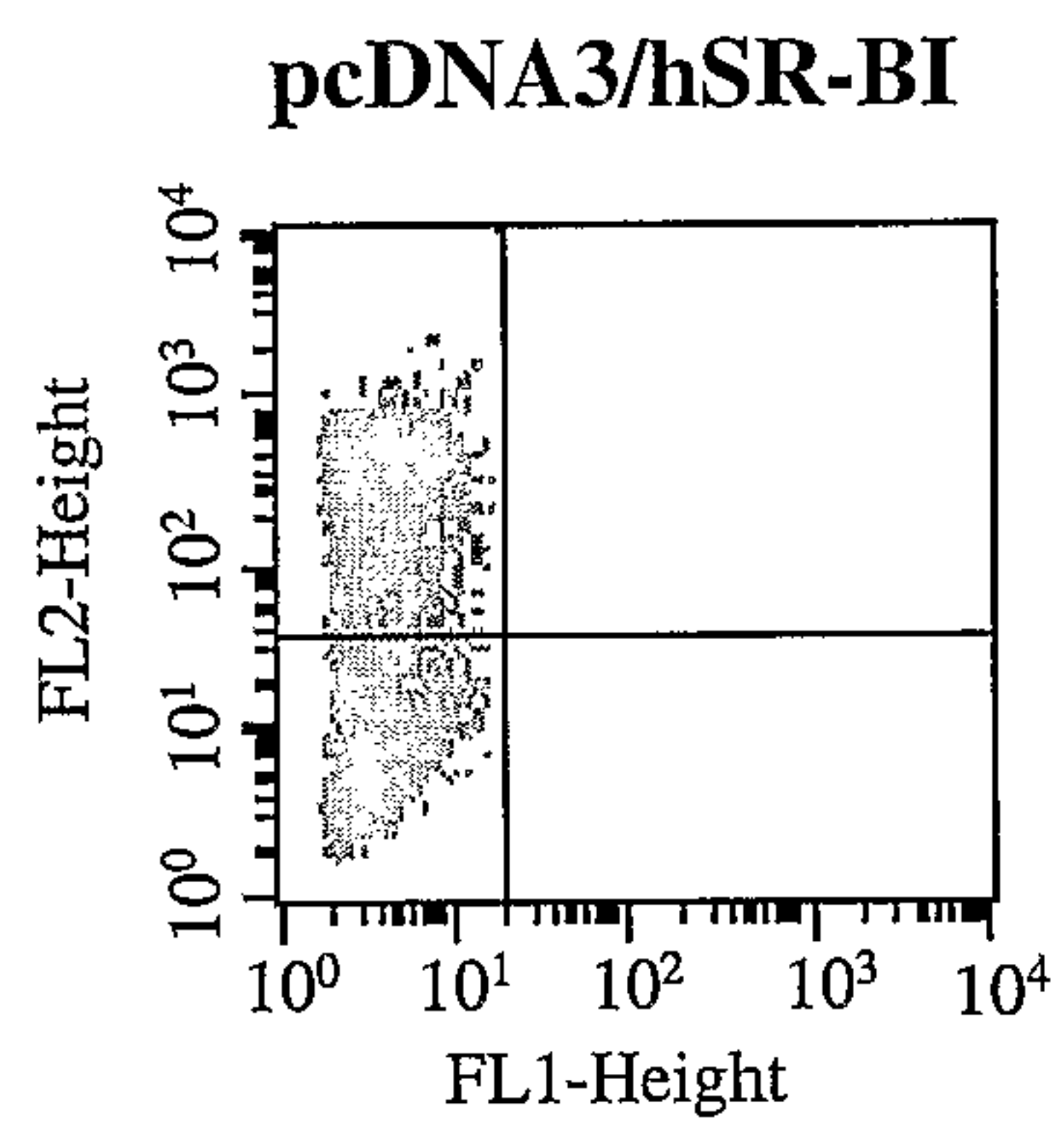


Fig. B

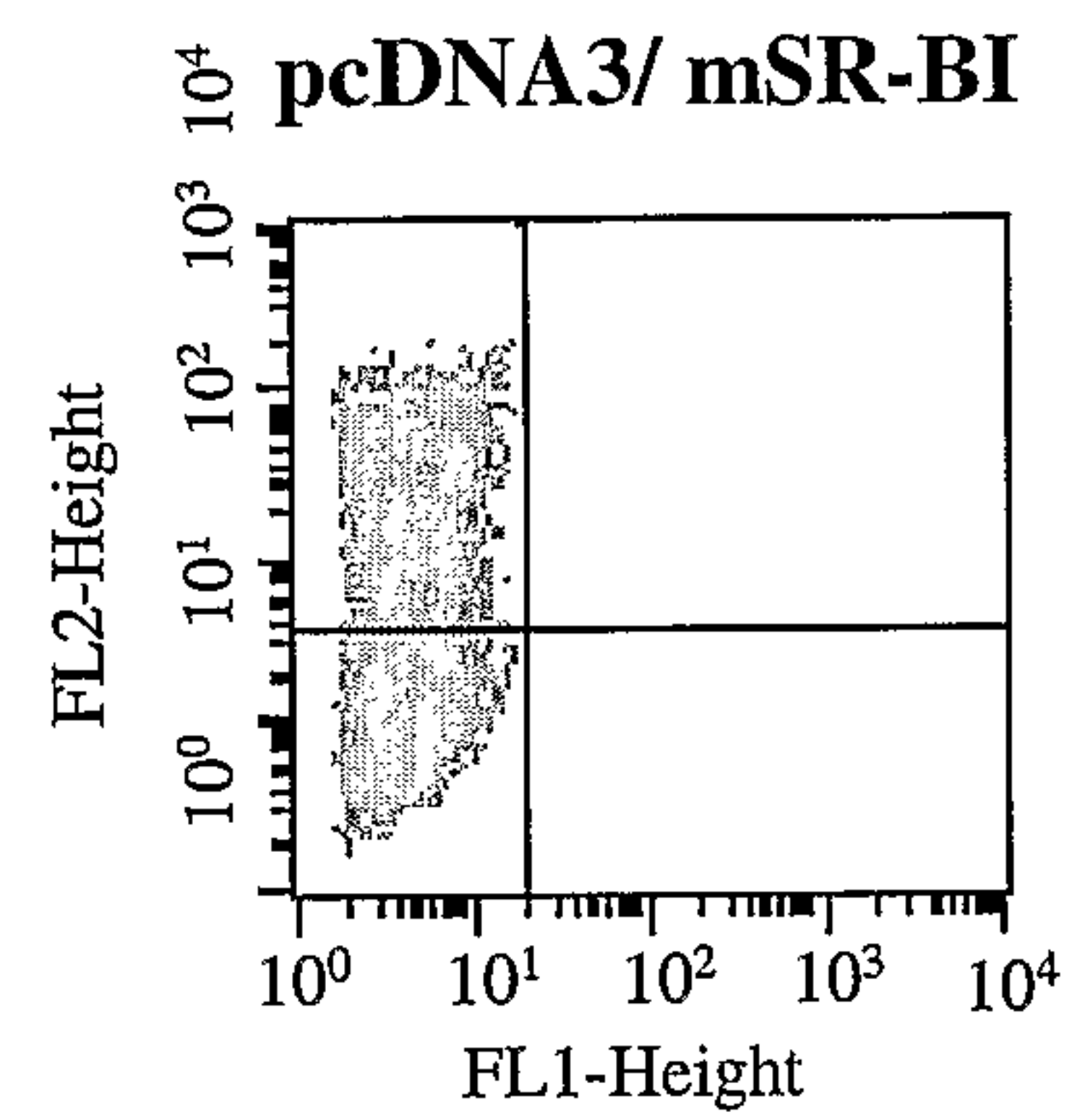


Fig. C

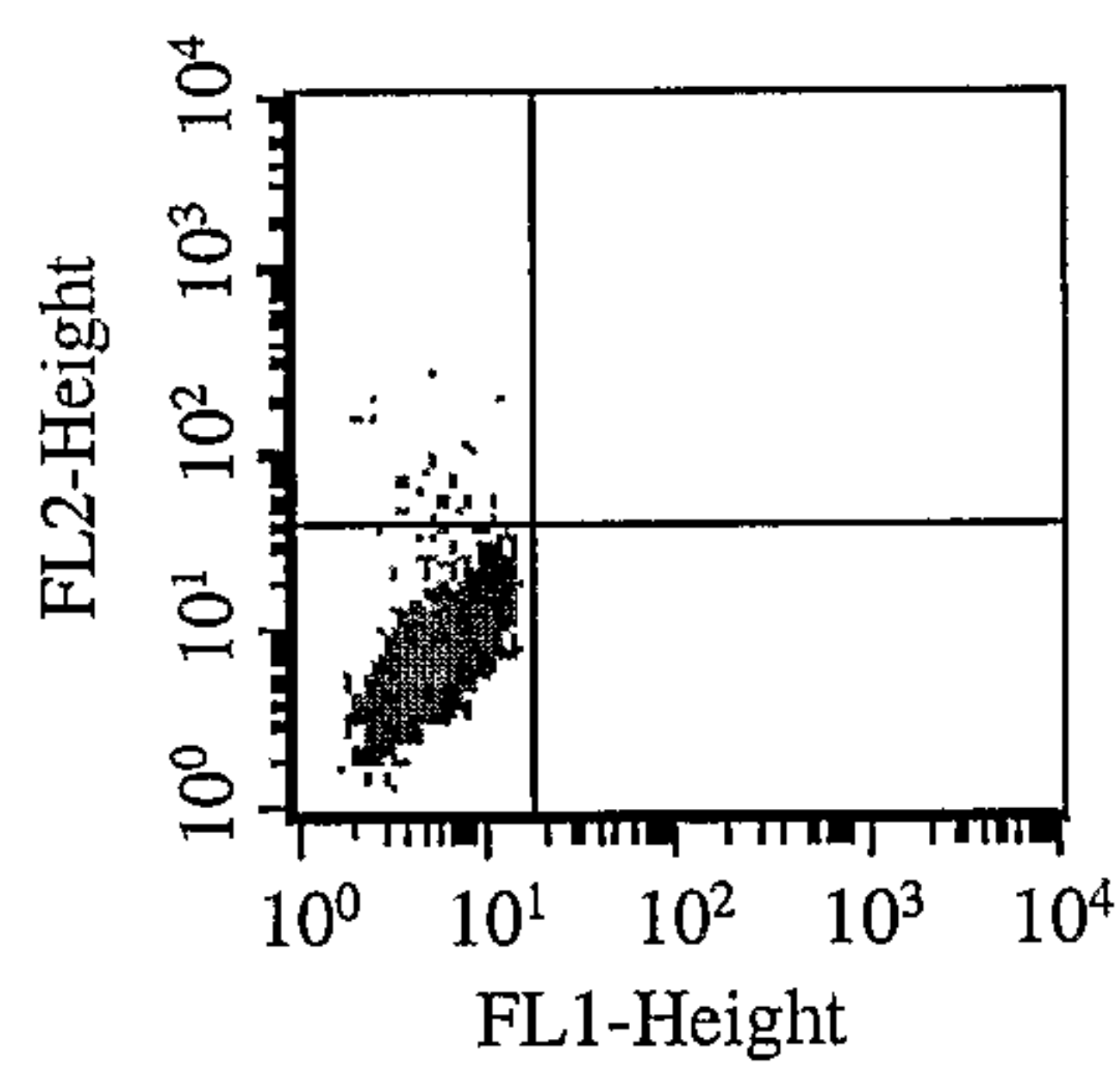


Fig. D

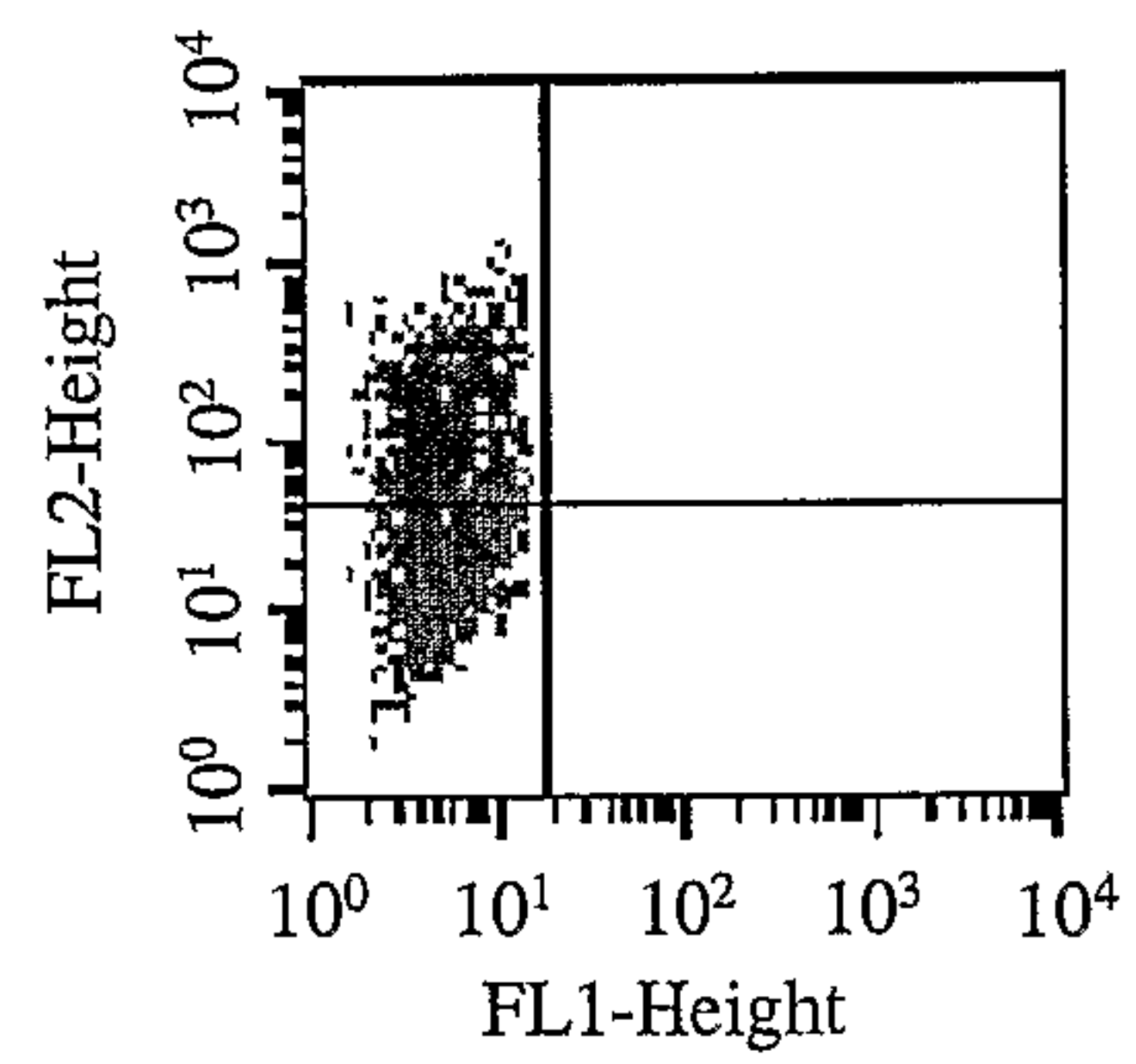


Fig. E

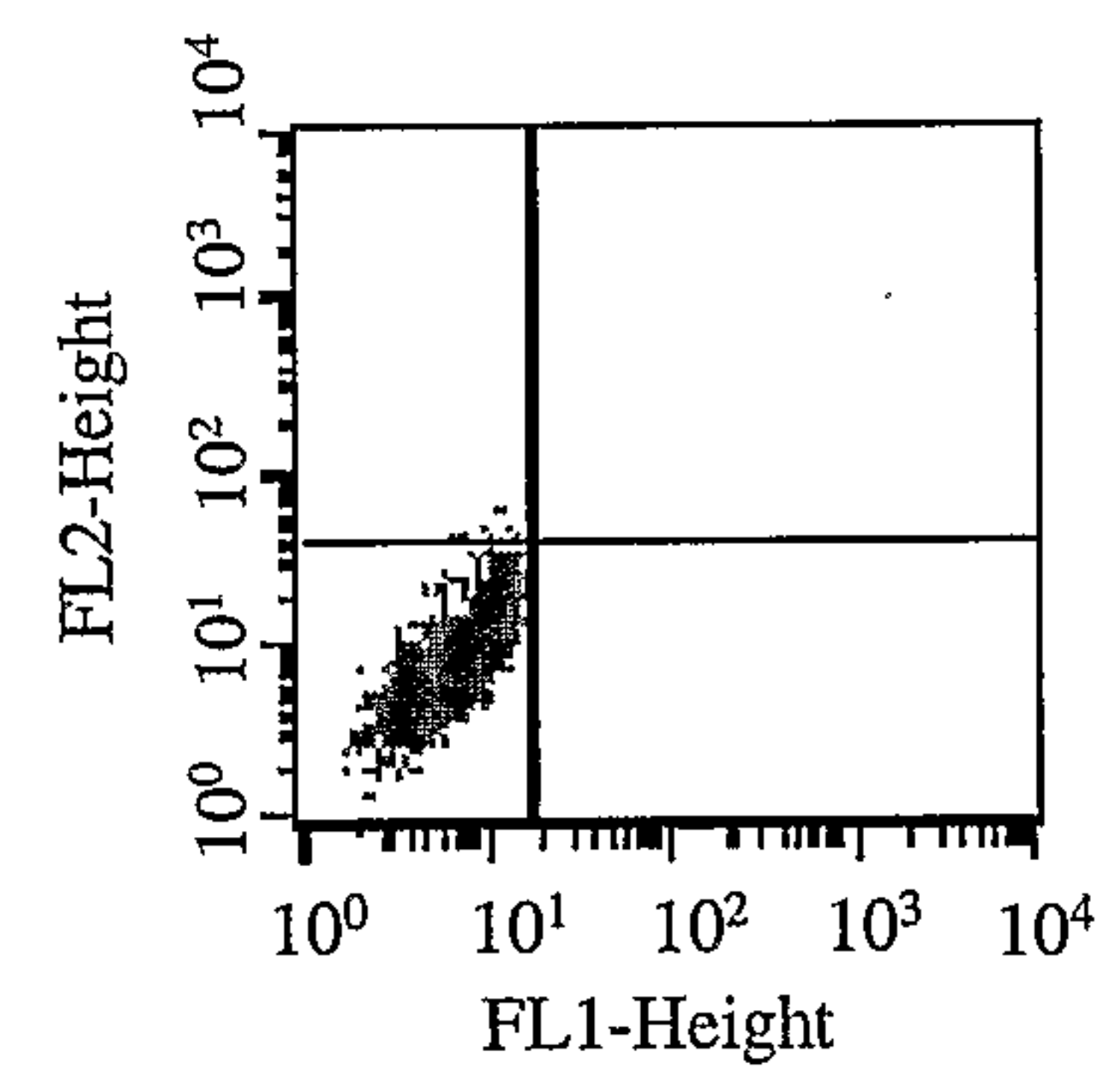


Fig. F

FIG. 5

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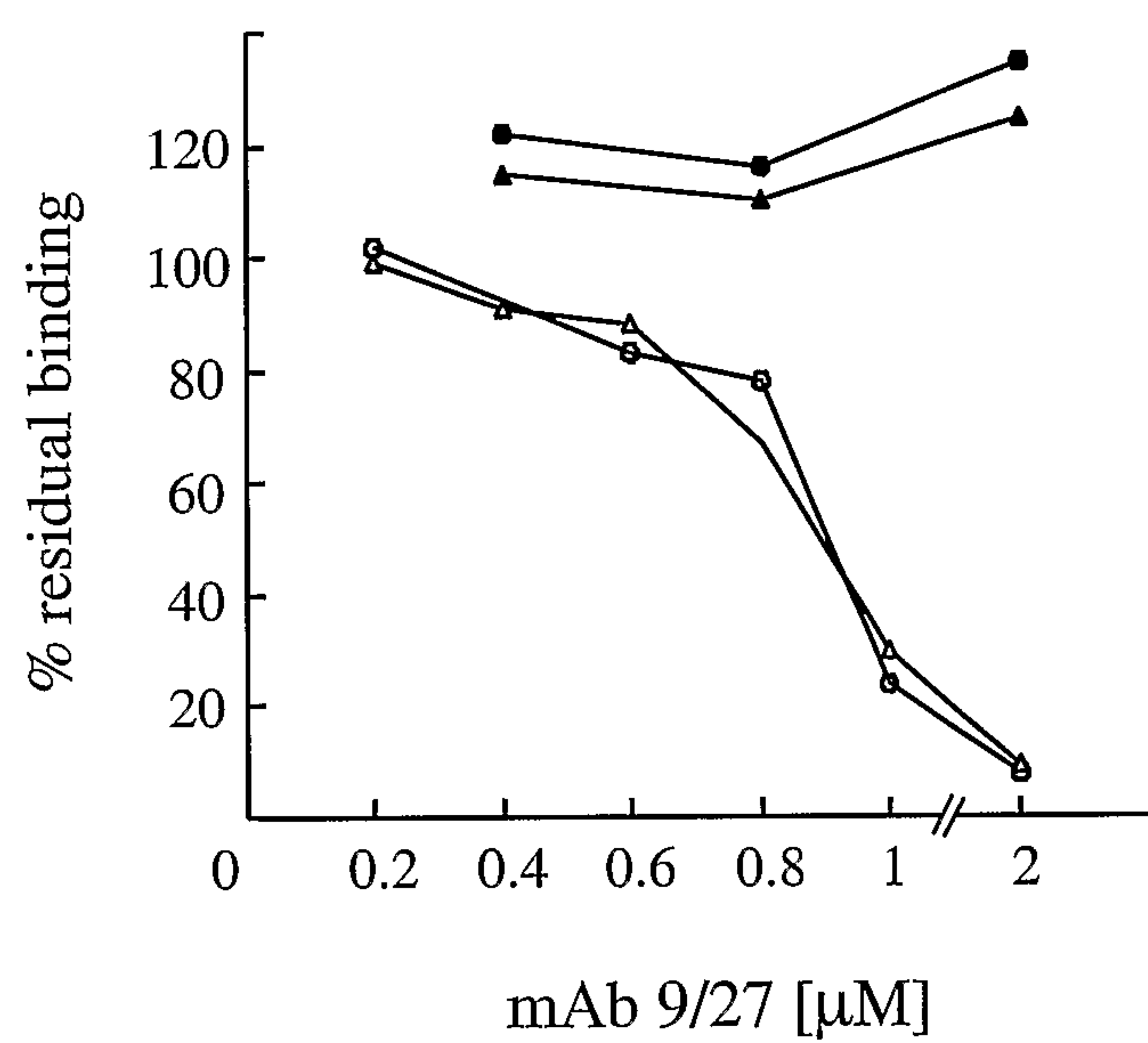


FIG. 6