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(54) Titre : POLYPEPTIDE A ACTIVITE ENTREtenant LA PROLIFERATION OU LA SURVIE DES CELLULES
SOUCHES HEMATOPOIETIQUES ET DES PROGENITEURS HEMATOPOIETIQUES, ET ADN CODANT CE
POLYPEPTIDE

(54) Title: POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF
HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

(57) **Abrégé/Abstract:**

A gene encoding a polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is isolated by comparing expressed genes between cells which support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells and cells which do not support the proliferation or survival. Proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is supported by using stromal cells in which the isolated gene is expressed or a gene product of the isolated gene.



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(54) Title: POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF HEMATOPOIETIC
STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

(57) Abstract: A gene encoding a polypeptide having an activity to support proliferation or survival of hematopoietic stem cells
or hematopoietic progenitor cells is isolated by comparing expressed genes between cells which support proliferation or survival of
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or survival of hematopoietic stem cells or hematopoietic progenitor cells is supported by using stromal cells in which the isolated
gene is expressed or a gene product of the isolated gene.



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DESCRIPTION

POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION
OR SURVIVAL OF HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC
PROGENITOR CELL, AND DNA CODING FOR THE SAME

5 Background of the InventionField of the Invention

The present invention relates to a polypeptide
having an activity to support proliferation or survival
of hematopoietic stem cells or hematopoietic progenitor
10 cells, a DNA coding the polypeptide, and a
pharmaceutical composition comprising the polypeptide as
active ingredient.

Description of the Related Art

15 Fully differentiated mature hematopoietic cells
have limited short lives. Homeostasis of the blood is
maintained due to supply of the mature blood cells
caused by continuous differentiation of hematopoietic
progenitor cells. The hematopoietic progenitor cells
20 are giving rise from more undifferentiated
hematopoietic stem cells. The hematopoietic stem cells
have potential of differentiating into all of the
differentiation lineages (totipotency) and have
potential of self-renew with retaining the totipotency
25 so as to supply the hematopoietic cells through life.
That is, the hematopoietic stem cells are known to
generate totipotent stem cells by the self-renew and to

differentiate in parts to a variety of the mature blood cells through the hematopoietic progenitor cells.

This differentiation of the blood cells is regulated by a variety of cytokines. Erythropoietin is known to
5 promote the differentiation of the erythrocytic lineages. G-CSF and thrombopoietin are also known to promote the differentiation of the neutrophils, and the megakaryocytes and the platelet productive cells, respectively. However, a factor required for the self-
10 renew of the hematopoietic stem cell with retaining the totipotency has not been clear. Although SCF/MGF (Williams, D.E., *Cell*, 63: 167-174, 1990; Zsebo, K.M., *Cell*, 63: 213-224, 1990), SCGF (WO98/08869), and the like are reported as growth factors for the
15 hematopoietic stem cells, none of them have potency to sufficiently retain the totipotency of the hematopoietic stem cells. Although attempts to culture the hematopoietic stem cells in the presence of combinations of known cytokines, a system for efficient amplification
20 of the hematopoietic stem cells was not realized (Miller, C. L., *Proc. Natl. Acad. Sci. USA*, 94: 13648-13653, 1997; Yagi, M., *Proc. Natl. Acad. Sci. USA*, 96: 8126-8131, 1999; Shih, C.C., *Blood*, 94: 5 1623-1636, 1999).

On the other hand, attempts to allow the
25 hematopoietic stem cells to survive or proliferate without differentiation by using stromal cells which supply an environment suitable for survival or

proliferation of the hematopoietic stem cells were reported (Moore K.A., *Blood*, 89: 12, 4337-4347, 1997). In addition, WO99/03980 discloses a stromal cell line capable of supporting proliferation or survival of
5 hematopoietic stem cells and hematopoietic progenitor cells, which are established from an AGM (Aorta-Gonad-Mesonephros) region of a fetal mouse.

It is postulated that there should be more peptides that efficiently facilitate hematopoietic stem cell and
10 progenitor cell amplification by themselves or in combination with stromal cells or stimulating factors such as cytokines, in addition to known factors affecting hematopoietic cells.

15 Summary of the Invention

Since the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells *in vitro* can be supported by co-culture of stromal cells and hematopoietic stem cells and hematopoietic progenitor
20 cells, the stromal cells are expected to produce factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells. An object of the present invention is to provide a factor supporting the proliferation or survival of
25 hematopoietic stem cells or hematopoietic progenitor cells, which is derived from the stromal cells.

The inventor of the present invention has assumed

that the mouse stromal cells produce factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, as mentioned above. Attention is given that there are two kinds of stromal cells. One has a ability to support the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells (hereafter sometimes referred to as "activity to support hematopoietic stem cells"). The other does not have the activity to support hematopoietic stem cells. The inventor of the present invention has assumed that this difference in the ability is due to the fact that expression of genes encoding the factors is increased in the supporting stromal cells and that the expression is low in non-supporting stromal cells. Thus the inventor think it can be found the factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells among the genes expressed higher in the supporting cells compared to in the non-supporting cells. In this context, the inventor has identified genes of which expressions are high in AGM-s3-A9 cell line which has the activity to support hematopoietic stem cells, and low or undetected in AGM-s3-A7 cell line which does not have the activity to support hematopoietic stem cells, and has determined the activities to support hematopoietic stem cells, of cells in which these gene groups are highly expressed. As a result, the present

invention has been completed.

That is, the present invention provides the followings.

(1) A DNA coding for a polypeptide of the following (A) or (B):

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(2) The DNA according to (1), which is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, and the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a probe prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic

progenitor cells.

(3) The DNA according to (2), the stringent condition is 6 x SSC, 5 x Denhardt, 0.5% SDS and 68°C (SSC: 3 M NaCl, 0.3 M sodium citrate; 50 x Denhardt: 1% BSA, 1% polyvinyl pyrrolidone, 1% Ficoll 400), or 6 x SSC, 5 x Denhardt, 0.5% SDS, 50% formamide and 42°C.

(4) A expression vector which comprises the DNA of any one of (1) to (3) in such a manner that the DNA can be expressed.

10 (5) A cell into which the DNA of any one of (1) to (3) is introduced in such a manner that the DNA can be expressed.

(6) A polypeptide which is an expression product of the DNA of any one of (1) to (3), the polypeptide 15 having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(7) The polypeptide according to (6), which comprises an amino acid sequence selected from the group 20 consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25, or an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence.

(8) The polypeptide according to (6) or (7), 25 which is modified with one or more modifying agents selected from the group consisting of polyethylene glycol (PEG), dextran, poly(N-vinyl-pyrrolidone),

polypropylene glycol homopolymer, copolymer of polypropylene oxide/ethylene oxide, polyoxyethylated polyol and polyvinyl alcohol.

(9) An monoclonal antibody which binds to the polypeptide of any one of (6) to (8).

(10) A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of co-culturing stromal cells in which a DNA coding for a polypeptide of the following (A) or (B) is expressed, with hematopoietic stem cells or progenitor cells,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(11) The method according to (10), wherein the DNA is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 1671 of SEQ ID NO: 8, the

nucleotide sequence of nucleotides 1 to 1674 of SEQ ID NO: 10, the nucleotide sequence of nucleotides 1 to 366 of SEQ ID NO: 12, the nucleotide sequence of nucleotides 84 to 1121 of SEQ ID NO: 14, the nucleotide sequence of nucleotides 1 to 1035 of SEQ ID NO: 16, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 20, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24, the nucleotide sequence of nucleotides 1 to 2487 of SEQ ID NO: 26, and the nucleotide sequence of nucleotides 1 to 2496 of SEQ ID NO: 28; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a probe prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(12) A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of culturing hematopoietic stem cells or progenitor cells in the presence of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when the hematopoietic

stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(13) A pharmaceutical composition having an effect to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, which comprises an effective amount of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when hematopoietic stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23,

SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

Terms used in this specification are defined as follows.

10 A hematopoietic stem cell is defined as a cell having totipotency, that is, ability to differentiate into all the cell lineages of the blood cells, and having a potency of self-renew with retaining the totipotency. A hematopoietic progenitor cell is defined
15 as a cell which can differentiate a single cell lineage of the blood cell or plural cell lineages but cannot differentiate into all of the cell lineages. A stromal cell is defined as a cell which can be co-cultured together with the hematopoietic stem cells to construct
20 a hematopoietic environment simulating *in vivo* hematopoietic environment *in vitro*. Cells derived from any origin can be used as long as the cells can be co-cultured with the hematopoietic cells *in vitro*.

Erythrocyte progenitor cells hardly survive and
25 proliferate in *in vitro* culture environments and rapidly disappear. If the survival and proliferation of the erythrocyte progenitor cells are observed, continuous

production of the erythrocyte progenitor cells is predicted to occur due to the survival and proliferation of the more immature hematopoietic stem cells or the hematopoietic progenitor cells. Therefore, in an
5 assessment system of human hematopoietic stem cells, proliferation of hematopoietic stem cells or immature hematopoietic progenitor cells can be determined by using the survival and proliferation of the erythrocyte progenitor cells (BFU-E, CFU-E, and CFU-E mix) as an
10 index.

Brief Explanation of the Drawings

Fig. 1 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined
15 by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-s3 subclone A9, A7, or D11 cells for two weeks.

Fig. 2 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined
20 by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-s3 subclone A9, A7, or OP9 cells for two weeks.

Fig. 3 shows time course of donor derived lymphoid lineage cells or myeloid lineage cells reconstitution in
25 irradiated recipient mice that received the hematopoietic stem cells co-cultured with stromal cells.

Fig. 4 shows proliferation statuses of hematopoietic

stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-2 is highly expressed (A9/SCR-2), AGM-S3-A9
5 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 5 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive
10 hematopoietic stem cells with AGM-S3-A7 cells in which a gene SCR-2 is highly expressed (A7/SCR-2), AGM-S3-A7 cells into which a control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells (A7) for two weeks.

Fig. 6 shows time course of donor derived lymphoid
15 lineage cells or myeloid lineage cells reconstitution in peripheral blood of irradiated recipient mice that received the hematopoietic stem cells co-cultured with AGM-S3-A7 cells in which a gene SCR-3 is highly expressed (A7/SCR-3), AGM-S3-A7 cells into which a
20 control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells.

Fig. 7 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive
25 hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-4 is highly expressed (A9/SCR-4), AGM-S3-A9 cells into which a control vector is introduced

(A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 8 shows time course of donor derived lymphoid lineage cells or myeloid lineage cells reconstitution in peripheral blood of irradiated recipient mice that
5 received the hematopoietic stem cells co-cultured with AGM-S3-A7 cells in which a gene SCR-5 is highly expressed (A7/SCR-5), AGM-S3-A7 cells into which a control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells.

10 Fig. 9 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-6 is highly expressed (A9/SCR-6), AGM-S3-A9
15 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 10 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture
20 of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-7 is highly expressed (A9/SCR-7), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

25 Fig. 11 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture

of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-8 is highly expressed (A9/SCR-8), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two 5 weeks.

Detailed Description of the Invention

Hereafter, the present invention will be described in detail below.

10 The following genes are those identified as genes of which expressions are high in AGM-s3-A9 cell line which has the activity to support hematopoietic stem cells, and low or undetected in AGM-s3-A7 cell line which does not have the activity to support hematopoietic stem 15 cells, and determined to have the activities to support hematopoietic stem cells, of cells in which these gene groups are highly expressed.

Gene SCR-2

20 The gene is the same gene as a mouse gene, *Mus musculus* glypican-1 (GPC-1) of a GenBank accession number AF185613.

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide 25 sequence are shown in SEQ ID NO: 8. Only the amino acid sequence is shown in SEQ ID NO: 9.

The human amino acid sequence of GPC-1 is recorded

in GenBank under an accession number P35052, and the human nucleotide sequence of GPC-1 is recorded in GenBank database under an accession number AX020122. It is predicted that the similar activity is detected in the human gene.

The nucleotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11.

Glypican is a major heparan sulfate proteoglycan existing on a cell surface, and have a characteristic structure such as cysteine rich globular domain, short glycosaminoglycan binding domain, glycosylphosphatidyl-inositol membrane binding domain. Six family genes from glypican-1 to glypican-6 have been found (J Biol Chem 1999 Sep 17;274(38):26968-77, Glypican-6, a new member of the glypican family of cell surface heparan sulfate proteoglycans. Veugelers M, De Cat B, Ceulemans H, Bruystens AM, Coomans C, Durr J, Vermeesch J, Marynen P, David G).

With respect to biological activities of GPC-1, there are a number of reports: To regulate growth stimulating activity of heparin binding growth factors (fibroblast growth factor 2 (FGF2), heparin-binding EGF-like growth factor (HB-EGF)) to promote proliferation of cancer cells showing autocrine proliferation by stimulation by the growth factors (J Clin Invest 1998

Nov 1; 102(9):1662-73, The cell-surface heparan sulfate
proteoglycan glypican-1 regulates growth factor action
in pancreatic carcinoma cells and is overexpressed in
human pancreatic cancer., Kleeff J, Ishiwata T, Kumbasar
5 A, Friess H, Buchler MW, Lander AD, Korc M).

To bind HGF (hepatocyte growth factor) to promote
reactivity with cytokines, of antigen-specific B cells.
To participate in association of a cell with an adhesive
molecule to involve in invasion of the cell (J Biol Chem
10 1998 Aug 28;273(35):22825-32, Heparan sulfate
proteoglycans as adhesive and anti-invasive molecules.
Syndecans and glypican have distinct functions., Liu W,
Litwack ED, Stanley MJ, Langford JK, Lander AD,
Sanderson RD). These findings show that GPC-1 involves
15 in activity expression of various cell-stimulating
factors. Also, there is a report that expression of the
glypican family gene in bone marrow is confirmed
(Biochem J 1999 Nov 1;343 Pt 3:663-8, Expression of
proteoglycan core proteins in human bone marrow stroma.,
20 Schofield KP, Gallagher JT, David G
reports, it is not described about effects of GPC-1 on
hematopoietic stem cells or hematopoietic progenitor
cells.

25 Gene SCR-3

The gene is the same gene as mouse genes, *Mus*
musculus chemokine MMRP2 mRNA of a GenBank accession

number U15209, *Mus musculus* C10-like chemokine mRNA of U19482 and mouse macrophage inflammatory protein-1gamma mRNA of U49513.

The nucleotide sequence of the gene from mouse and
5 the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 12. Only the amino acid sequence is shown in SEQ ID NO: 13.

Gene SCR-4

10 The nucleotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

It has been found that the sequence has a high
15 homology to *Homo sapiens* clone 25077 mRNA of a GenBank accession number AF131820, and that it is considered to be a mouse ortholog. This sequence is described in WO 00/66784.

The nucleotide sequence of the gene from human and
20 the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 16. Only the amino acid sequence is shown in SEQ ID NO: 17.

Gene SCR-5

25 The nucleotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 18. Only the amino

acid sequence is shown in SEQ ID NO: 19.

It has been found that the sequence has a high
homology with *Homo sapiens* esophageal cancer related
gene 4 protein (ECRG4) mRNA of a GenBank accession
5 number AF325503, and that it is considered to be a mouse
ortholog of AF325503.

The nucleotide sequence of the gene from human and
the amino acid sequence deduced from the nucleotide
sequence are shown in SEQ ID NO: 20. Only the amino
10 acid sequence is shown in SEQ ID NO: 21.

Gene SCR-6

The nucleotide sequence of the gene from mouse and
the amino acid sequence deduced from the nucleotide
15 sequence are shown in SEQ ID NO: 22. Only the amino
acid sequence is shown in SEQ ID NO: 23.

Gene SCR-7

The nucleotide sequence of the gene from mouse and
20 the amino acid sequence deduced from the nucleotide
sequence are shown in SEQ ID NO: 24. Only the amino
acid sequence is shown in SEQ ID NO: 25.

Gene SCR-8

25 The gene is the same gene as *Mus musculus* mRNA for
ADAM23 of a GenBank accession number AB009673.

The nucleotide sequence of the gene from mouse and

the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 26. Only the amino acid sequence is shown in SEQ ID NO: 27.

The sequence has a high homology with a sequence
5 described by JP 11155574-A and the sequence described by JP 11155574-A is considered to be a human ortholog.

The nucleotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 28. Only the amino
10 acid sequence is shown in SEQ ID NO: 29.

Polypeptides which are products of these genes have an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor
15 cells. The expression that a polypeptide has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells means that proliferation or survival of hematopoietic stem cells or hematopoietic progenitor
20 cells is supported in the presence of the polypeptide or in the presence of stroma cells expressing the polypeptide.

Therefore, the present invention provides use of the polypeptides and DNAs encoding the polypeptides and
25 novel polypeptides among the polypeptides and DNAs encoding the novel polypeptides.

A stem cell proliferation-supporting factor which is

a polypeptide encoded by the DNA can be produced by introducing the DNA into a suitable host to prepare a transformant cell, and allowing the DNA to be expressed in the transformant cell.

5 The DNA may encode the above described factors which have amino acid sequences including substitution, deletion or insertion of one or several amino acids, as long as the activity of the stem cell proliferation-supporting factor to be encoded is not lost. DNAs
10 encoding substantially equivalent polypeptides to this stem cell proliferation-supporting factor can be obtained by modifying the nucleotide sequences so as to include substitution, deletion, insertion, addition, or inversion of amino acid residues in a specific region
15 using site-directed mutagenesis.

 The DNAs including the above described mutation can be expressed in appropriate cells and the activity to support hematopoietic stem cells, of the expressed products can be examined, so that the DNAs encoding the
20 polypeptide having functions which are substantially equivalent to this stem cell proliferation-supporting factor are obtained. In addition, the DNAs encoding substantially equivalently active protein as this stem cell proliferation-supporting factor can be obtained by
25 isolating DNAs which hybridize with DNAs including, for example, the nucleotide sequence as described in SEQ ID NO: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 28 from the

cells having the DNA, or probes prepared from these DNAs under the stringent condition; and which encode proteins possessing the activity to support hematopoietic stem cells. The length of the probe is usually 30 to 1000
5 nucleotides. The stringent condition is, for example, one in which DNAs having homology (determinable with homology search in the compare function of DNASIS version 3.7 (Hitachi Software Engineering)) at not less than 70%, preferably at not less than 80%, are
10 hybridized each other and DNAs having less homology than those are not hybridized each other. The above described stringent condition may be 6 × SSC, 5 × Denhardt, 0.5% SDS, 68°C (SSC; 3 M NaCl, 0.3 M sodium citrate) (50 × Denhardt; 1% BSA, 1% polyvinyl
15 pyrrolidone, 1% Ficoll 400) or 6 × SSC, 5 × Denhardt, 0.5% SDS, 50% Formamide, 42°C, or the like.

Microorganisms such as *Escherichia coli* and yeast, culture cells derived from animals or plants, and the like are used for host cells for expressing the DNA.
20 Preferably, culture cells derived from mammals are used as the host cells. In the case that prokaryotic cells are used as the host cells, the expression is preferably performed in a condition in which a signal peptide region is replaced with a leader sequence suitable for
25 the prokaryotic cells such as β-lactamase (*bla*), alkaline phosphatase (*phoA*), and outer membrane protein A (*ompA*) and the like, or in a form in which a

methionine residue is added to the N-terminal site of the mature protein.

The introduction of the DNA to the host cell can be carried out by, for example, incorporating the DNA into
5 a vector suitable for the host in an expressible form, and introducing the resultant recombinant vector to the host cell.

Examples of the culture cells derived from mammals include CHO cell, 293 cell, COS7 cell, and the like.
10 Gene expression regulatory sequence such as a promoter to express the DNA may be originated from the gene itself, or may be derived from other genes such as cytomegalovirus promoter and elongation factor 1 promoter and the like.

15 Examples of a vector for animal culture cells include plasmid vectors, retrovirus vectors, adenovirus vectors (Neering, S.J., *Blood*, 88: 1147, 1996), herpes virus vectors (Dilloo, D., *Blood*, 89: 119, 1997), HIV vectors, and the like.

20 In order to introduce the recombinant vector into culture cells, the conventional methods which are usually employed for transformation of culture cells such as calcium phosphate transfection, the liposome method, the DEAE dextran method, the electroporation
25 method and the microinjection method are employed.

The polypeptides of the present invention also comprise polypeptides having amino acid sequences in

which one or several amino acids are substituted,
deleted or inserted in the amino acid sequence
represented in SEQ ID NO: 9, 11, 13, 15, 17, 19, 21, 23,
25, 27 or 29, and having activity to support
5 hematopoietic stem cells in addition to the polypeptides
having the amino acid sequence represented in SEQ ID NO:
9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29. That is,
even if mouse and human stem cell proliferation-
supporting factors are modified by substitution,
10 deletion, insertion or the like, polypeptides holding
essential functions as a stem cell proliferation-
supporting factor can be considered to be substantially
equivalent to the stem cell proliferation-supporting
factor.

15 These modified stem cell proliferation-supporting
factors can be obtained by treating DNA encoding the
stem cell proliferation-supporting factor or host cells
including the above mentioned DNA with a mutagen, or by
mutating the above mentioned DNA so as to substitute,
20 delete, or insert an amino acid at a specific site using
site-directed mutagenesis. The residual of the activity
to support the hematopoietic stem cells in the obtained
mutant polypeptide is confirmed by transferring
hematopoietic stem cells cultured in the presence of the
25 mutant polypeptides into irradiated mice, and monitoring
peripheral hematological cellularity over time, as in
the examples described below.

As for the amino acid deletion, the polypeptide may be a fragment which lacks an amino acid sequence at the N-terminal end and/or the C-terminal end. The fragment lacking the amino acid sequence at the N-terminal end and/or the C-terminal end can be obtained by a usual method, and the hematopoietic stem cell-supporting activity of the fragment can be determined by a similar way to that described with respect to the mutated polypeptide. In particular, if there is a portion predicted as a signal sequence or a transmembrane region in the amino acid sequence, a fragment having the hematopoietic stem cell-supporting activity is predicted by using it as an index. For example, a protein encoded by human SCR-8 is a transmembrane protein of type I passing through the membrane once, and it is therefore predicted that even if it made to be a soluble protein lacking the transmembrane region, it has the activity to support to proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells. The transmembrane region can be predicted with a known program based on the amino acid sequence. For example, if it is predicted with a program called PSORT II (available through the Internet, URL: <http://psort.nibb.ac.jp/index.html>), the transmembrane region is amino acids at positions 790 to 806 in SEQ ID NO: 29, and it is predicted that even if a fragment up to position 789, the fragment has activity to support

proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

Since the nucleotide sequences of the above described DNAs have been clarified by the present invention, the DNAs can be also obtained by isolating the corresponding DNAs from mouse or human cDNA or chromosome DNA libraries using PCR in which the oligonucleotides prepared based on these nucleotide sequences are used as primers or using hybridization in which the oligonucleotides prepared based on these nucleotide sequences are used as probes.

In order to complete the present invention, the DNAs of the present invention have been isolated from cDNA library of AGM-s3-A9 cells which are a mouse stromal cell line having the activity to support the hematopoietic stem cells, using SBH (Sequencing By Hybridization) method (Drmanac, S., *Nat. Biotechnol.*, 16, 54, 1998; Drmanac, R., *Methods. Enzymol.*, 303, 165, 1999) as described below. The mouse stromal cell lines having the activity to support the hematopoietic stem cells can be obtained using the method disclosed in WO99/03980 or from Cell Bank of Institute of Physical and Chemical Research (RIKEN) or ATCC.

An outline of SBH method will be described below. Probes having eight or nine nucleotides whose sequences are different from each other are prepared. When the nucleotide sequences corresponding to those of the probe

exist in a targeted gene, the probes can hybridize with the gene. The hybridization can be easily detected with utilization of radio isotope- or fluorescence-labelled probes. Each clone in the library is picked up, and
5 blotted on a membrane. Then, the repeated hybridizations are performed with the each of above described probes, so that one can identify the combination of probes that hybridize to each clone. Since the combination of hybridized probes depends on
10 genes, the combination of probes which hybridize to an identical gene is the same. That is, the same gene can be identified as one group (cluster) according to the the combination of the hybridized probes. The number of clones of each gene in the cDNA library can be
15 determined by classifying each clone in the library based on patterns of the hybridized probes and counting the classified clones. Thus, frequency of expression of each gene in the library can be determined.

cDNA libraries are prepared from cells having an
20 activity to support the hematopoietic stem cells and from cells not having the activity. Clustering is performed for the cDNA libraries. Statuses of expressed genes among cells are compared, so that the genes highly expressed with specificity to the supporting cells are
25 selected. The expression statuses of these genes in each of above described cells are further examined by Northern blot analysis, so that genes which are highly

expressed in the cells having the activity to support the hematopoietic stem cells are obtained.

The above mentioned genes are the genes which are highly expressed with specificity to the supporting
5 cells and which are obtained through the above described process. Full-length genes have been cloned from the cDNA library derived from AGM-s3-A9 cell.

Further, in order to determine an ability of gene products to support hematopoiesis, a gene fragment
10 including gene ORF was transferred into stromal cells using a retrovirus vector, and the change in the activity to support the hematopoietic stem cells of the stromal cells was determined. Specifically, after the stromal cells into which the gene was not introduced or
15 into which a control vector was introduced and those into which the gene was introduced were each co-cultured with the mouse hematopoietic stem cells, the hematopoietic cells were transplanted into irradiated mice. The engraftment of the co-cultured hematopoietic
20 cells in recipient mice were examined. As a result, the mice into which the hematopoietic stem cells co-cultured with the gene-introduced cells were transplanted, showed increased chimerism after the transplantation compared with co-culture with the cells into which the gene was
25 not introduced. This result shows that in the gene-expressed stromal cells, an activity to support the proliferation or survival of the hematopoietic stem

cells or the hematopoietic progenitor cells is increased or imparted. As a result, it has become evident that expression of the above described genes has a function to increase the above described activity in the stromal
5 cells or impart the activity to the stromal cells.
Therefore, it is revealed that products of the genes affect hematopoietic stem cells or hematopoietic progenitor cells to show an activity to support the survival or the proliferation thereof, or affect stromal
10 cells to show an activity to increase an activity to support the hematopoietic stem cells therein or impart the activity thereto.

The polypeptides of the present invention can be used as a medicine to proliferate or support human
15 hematopoietic stem cells or human hematopoietic progenitor cells when they affect hematopoietic stem cells or hematopoietic progenitor cells to show an activity to support survival or proliferation thereof, in other words, the polypeptides have an activity to
20 support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells if the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the presence of the polypeptides.
The pharmaceutical composition can be used for
25 supporting proliferation or survival of human hematopoietic stem cells or human hematopoietic progenitor cells *in vitro*. For hematopoietic stem cell

transplantation therapies such as peripheral blood stem cell transplantation and cord blood stem cell transplantation, a sufficient amount of the hematopoietic stem cells sometimes cannot be collected and the transplantation may not be performed. Even if the enough amount of the stem cells can not be collected, the enough amount of the hematopoietic stem cells can be obtained and transplanted by amplification of the hematopoietic stem cells *in vitro* using this polypeptides. That is, the hematopoietic stem cells can be amplified without differentiation by culturing the hematopoietic stem cells in culture medium including these polypeptides. It may be considered the hematopoietic stem cells are able to be amplified more efficiently with addition of a variety of cytokines to the medium.

When the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the medium including the polypeptides of the present invention, the hematopoietic stem cells or the hematopoietic progenitor cells used may be isolated one of these cell types alone or may be both of the cell types. In addition, the cells may include at least the hematopoietic stem cells or the hematopoietic progenitor cells, and include other hematopoietic cells. Further, it can be used a fraction containing hematopoietic stem cells or progenitor cells fractionated from the cell

population that contain the hematopoietic stem cells or progenitor cells.

Examples of sources of the hematopoietic stem cells and the hematopoietic progenitor cells in the method of the present invention include a fetal liver, bone marrow, fetal bone marrow, peripheral blood, the peripheral blood from persons whose stem cells are mobilized by administration of cytokines and/or antitumor drugs, cord blood, and the like of mammals such as human and mouse and the like. Any sources may be used as long as the tissue includes the hematopoietic stem cells.

A culture method using petri dishes and flasks for culture may be employed to culture the hematopoietic stem cells or the hematopoietic progenitor cells. The cultivation of the hematopoietic stem cells and/or progenitor cells may be improved by mechanically controlling medium composition, pH, and the like, and using a bioreactor capable of high density cultivation (Schwartz, *Proc. Natl. Acad. Sci. U.S.A.*, 88: 6760, 1991; Koller, M.R., *Bio/Technology*, 11: 358, 1993; Koller, M.R., *Blood*, 82: 378, 1993; Palsson, B.O., *Bio/Technology*, 11: 368, 1993).

The stromal cells in which DNAs encoding the polypeptide of the present invention can be obtained as described with respect to the expression of the DNAs.

The co-culture of the stromal cells and the hematopoietic cells can be performed simply after the

collection of the bone marrow cells without further separation. Furthermore, co-culture can be performed by separating components such as stromal cells, hematopoietic cells and other cell populations from collected bone marrow and combining them with the hematopoietic cells and stromal cells which are not from the individual from which the bone marrow is collected. Furthermore, after stromal cells are cultured to grow to the stromal cells, hematopoietic cells can be added to perform co-culture with the hematopoietic stem cells. At this time, cell stimulating factors can be added to the culture system of stromal cells to more effectively support proliferation and survival. Concrete examples of the cell stimulating factor include a growth factor which is typically a cytokine such as SCF (stem cell factor), IL-3 (interleukin 3), GM-CSF (granulocyte/macrophage colony-stimulating factor), IL-6 (interleukin 6), TPO (thrombopoietin), G-CSF (granulocyte colony-stimulating factor), TGF- β (transforming growth factor- β), MIP-1 α (Davatelis, G., J. Exp. Med. 167: 1939, 1988); a differentiation and proliferation control factor such as hematopoietic hormones such as EPO (erythropoietin), chemokine, Wnt gene product, and notch ligand; and a development control factor.

In addition, when the polypeptide of the present invention affects hematopoietic stem cells or

hematopoietic progenitor cells to show an activity to support survival or proliferation thereof, in other words, the polypeptide has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells if the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the presence of the polypeptide, the proliferation and the survival of the hematopoietic stem cells or the hematopoietic progenitor cells can be retained by allowing the recombinant polypeptide of the present invention alone or in combination with the cell stimulating factors to affect hematopoietic stem cells or hematopoietic progenitor cells, without stromal cells. Examples of the cell stimulating factors used in this case are above described cell stimulating factors and the like.

Medium used for the culture is not specially restricted as long as the proliferation or the survival of the hematopoietic stem cells or the hematopoietic progenitor cells is not harmed. Preferable media are, for example, MEM- α medium (GIBCO BRL), SF-02 medium (Sanko Junyaku), Opti-MEM medium (GIBCO BRL), IMDM medium (GIBCO BRL), and PRMI1640 medium (GIBCO BRL). A culture temperature is usually ranging from 25 to 39°C, and preferably ranging from 33 to 39°C. Examples of additives to the medium are fetal bovine serum, human serum, horse serum, insulin, transferrin, lactoferrin,

ethanolamine, sodium selenite, monothiolglycerol, 2-mercaptoethanol, bovine serum albumin, sodium pyruvate, polyethylene glycol, a variety of vitamins, and a variety of amino acids. A concentration of CO₂ is usually ranging from four to six percent, and preferably five percent.

Since the hematopoietic stem cells can differentiate into all the hematopoietic cell lineages, the hematopoietic stem cells can be amplified and differentiated into a specific cell type *in vitro*, and then the specific cells can be transplanted. For example, when erythrocytes are necessary, after the cultivation of the patient's stem cells to amplify them, the hematopoietic cells whose main component is the erythrocyte can be artificially produced using an erythrocyte differentiation induction-promoting factor such as EPO.

The hematopoietic stem cells or the hematopoietic progenitor cells cultured using the polypeptides of the present invention can be used as a graft for blood cell transplantation replacing the conventional bone marrow transplantation or cord blood transplantation. Transplantation of the hematopoietic stem cells is superior to the conventional blood cell transplantation therapy, since the engraftment can last semipermanently.

The transplantation of the hematopoietic stem cells can be employed as therapy for a variety of diseases in

addition to combination therapy with total body X-ray irradiation therapy or advanced chemotherapy for leukemia. For example, when therapy accompanied with myelosuppression as an adverse reaction, such as

5 chemotherapy, radiation therapy, and the like is performed for the patient with solid cancer, the patient can get benefit of early recovery and stronger chemotherapy than the conventional one can be performed to improve the therapeutic effect of the chemotherapy by

10 collecting the bone marrow before the therapy, allowing the hematopoietic stem cells or the hematopoietic progenitor cells to be amplified *in vitro* and returning the amplified cells to the patient after the therapy.

In addition, by allowing the hematopoietic stem cells or

15 the hematopoietic progenitor cells obtained according to the present invention to be differentiated into a variety of hematopoietic cells and transplanting these cells into a patient with hypoplasia of a given hematopoietic cells, the patient's deficient status can

20 be improved. In addition, this therapy can improve dyshemopoietic anemia to develop anemia such as aplastic anemia caused by bone marrow hypoplasia. Furthermore, examples of diseases in which the transplantation of the hematopoietic stem cells according to the present

25 invention is effective include immunodeficiency syndrome such as chronic granulomatous disease, duplicated immunodeficiency syndrome, agammaglobulinemia, Wiskott-

Aldrich syndrome, acquired immunodeficiency syndrome (AIDS), and the like, thalassemia, hemolytic anemia due to an enzyme defect, congenital anemia such as sickle cell anemia, Gaucher's disease, lysosomal storage disease such as mucopolysaccharidosis, adrenoleukodystrophy, a variety of cancers and tumors, and the like.

Transplantation of the hematopoietic stem cells may be performed in the same manner as the conventional bone marrow transplantation or cord blood transplantation other than the differences of the cells used.

The source of the hematopoietic stem cells which may be used for the above described hematopoietic stem cell transplantation is not restricted to the bone marrow, and the above described fetal liver, the fetal bone marrow, the peripheral blood, the peripheral blood with stem cells mobilized by administration of cytokines and/or antitumor drugs, the cord blood, and the like may be used.

The graft may be a composition including buffer solution and the like in addition to the hematopoietic stem cells and the hematopoietic progenitor cells produced by the method according to the present invention.

The hematopoietic stem cells or the hematopoietic progenitor cells produced according to the present invention may be used for *ex vivo* gene therapy. Because of the low frequency of recombination of target genes to

the chromosome because the stem cells are in the resting state, differentiation of the stem cells during the culture period, and the like, the gene therapy to the hematopoietic stem cells has been hard to be established.

5 However, the present invention can amplify the stem cells without differentiation, so that efficacy of gene transfer is expected to be remarkably improved. In this gene therapy, a foreign gene (a gene for therapy) is transferred into the hematopoietic stem cells or the

10 hematopoietic progenitor cells, and then the obtained gene-transferred cells are used. The foreign gene to be transferred is appropriately selected according to disease. Examples of diseases in which the target cells of the gene therapy are the hematopoietic cells include

15 immunodeficiency syndrome such as chronic granulomatous disease, duplicated immunodeficiency syndrome, agammaglobulinemia, Wiskott-Aldrich syndrome, acquired immunodeficiency syndrome (AIDS), and the like, thalassemia, hemolytic anemia due to an enzyme defect,

20 congenital anemia such as sickle cell anemia, Gaucher's disease, lysosomal storage disease such as mucopolysaccharidosis, adrenoleukodystrophy, a variety of cancers and tumors, and the like.

A usual method used for transfer of a gene into

25 animal cells is employed for the transfer of the gene for the therapy into the hematopoietic stem cells or the hematopoietic progenitor cells. Examples include a

method using a vector for animal cells derived from virus utilized for a gene therapy such as retrovirus vectors such as Moloney mouse leukemia virus, adenovirus vectors, adeno-associated virus (AAV) vectors, herpes simplex virus vectors, and HIV vectors (with respect to a vector for gene therapy, see Verma, I.M., Nature, 389: 239, 1997); calcium phosphate transfection, DEAE-dextran transfection, electroporation, the liposome method, the lipofection method, the microinjection method, and the like. Among them, the method using the retrovirus vector, the adeno-associated virus vector, or the HIV vector is preferable, since permanent expression of a gene is expected due to insertion into the chromosome DNA of a target cell.

For example, the adeno-associated virus (AAV) vector can be prepared as follows. First, a vector plasmid in which a gene for therapy is inserted into ITR (inverted terminal repeat) at both ends of wild-type adeno-associated virus DNA and a helper plasmid for supplementing virus proteins are transfected into 293 cell line. Next, adenovirus as helper virus is infected, so that virus particles including the AAV vector are produced. Alternatively, instead of adenovirus, a plasmid which expresses adenovirus gene having helper function may be transfected. The hematopoietic stem cells or the hematopoietic progenitor cells are infected with the obtained virus particles. Preferably,

appropriate promoter, enhancer, insulator and the like are inserted into the upstream region of the target gene in the vector DNA, so that the expression of the gene is regulated. When a marker gene such as a drug resistant gene is used in addition to the gene for therapy, cells into which the gene for therapy are transferred are easily selected. The gene for therapy may be a sense gene or an antisense gene.

A composition for gene therapy may include a buffer solution and a novel active ingredient and the like in addition to the hematopoietic stem cells or the hematopoietic progenitor cells by the method according to the present invention.

A vector for gene therapy can be produced by incorporating the DNA of the present invention in an expression vector using a usual method. This vector for gene therapy is useful to treat diseases which need survival and proliferation of the human hematopoietic stem cells. That is, the vector for gene therapy is transferred into the hematopoietic stem cells and the cells are cultured *in vitro*, so that the hematopoietic stem cells or the hematopoietic progenitor cells can proliferate dominantly. The proliferation of hematopoietic stem cells *in vivo* can be caused by returning these hematopoietic stem cells thus treated. The proliferation of hematopoietic stem cells *in vivo* can significantly promoted by introducing this vector

for gene therapy into the body. Alternatively, the bone marrow cells derived from a patient are cultured as it is and this vector for gene therapy is transferred thereto, so that the hematopoietic stem cells or the
5 hematopoietic progenitor cells can be proliferated in a culture system. Alternatively, this vector for gene therapy is transferred into the stromal cells and mesenchymal stem cells obtained by isolating and culturing stromal cells from the bone marrow, so that
10 the activity to support the hematopoietic stem cells can be added or increased.

As shown in Examples, since it is possible that by introducing the DNA of the present invention into the stromal cells without the activity to support the
15 hematopoietic stem cells, this activity can be imparted, stromal cells having the activity to support the hematopoietic stem cells can be prepared by gene transfer to stromal cells derived from human or mouse. The stromal cells expressing the DNA of the present
20 invention and the hematopoietic stem cells or the hematopoietic progenitor cells are co-cultured, so that the hematopoietic stem cells or the hematopoietic progenitor cells can survive and proliferate so as to be useful for a variety treatment.

25 Since the hematopoietic stem cells or the hematopoietic progenitor cells can survive and proliferate by expression of the DNA of the present

invention in the stromal cell, an activity to support the hematopoietic stem cells of the stromal cells can be determined using the expression of the DNA of the present invention as an index. The expression of the DNA of the present invention in the stromal cells can be confirmed using an antibody against a polypeptide encoded by the DNA of the present invention. Also, PCR primers can be prepared based on nucleotide sequences, and RNA is prepared from the stromal cells of interest, and RT-PCR is performed, so that the expression of the DNA of the present invention can be confirmed. The antibody will be described below.

The pharmaceutical composition of the present invention can be administered to human. The pharmaceutical composition having an activity to proliferate or to support the human hematopoietic stem cells or the hematopoietic progenitor cells can be produced by mixing medically acceptable diluent, stabilizer, carrier, and/or other additives with the polypeptides of the present invention. At this time, in order to increase the stability of the protein *in vivo*, the polypeptides of the present invention may be modified by a modifying agent. Examples of the modifying agent include polyethylene glycol (PEG), dextran, poly(N-vinyl-pyrrolidone), polypropylene glycol homopolymer, polypropylene oxide/ethylene oxide copolymer, polyoxyethylated polyol, polyvinyl alcohol,

and the like. The modification of the protein with PEG can be performed by, for example, a method in which activated ester derivatives of PEG is reacted with the protein, a method in which aldehyde derivatives at the terminal portion of PEG is reacted with the protein in the presence of a reducing agent, and the like.

Japanese Patent Application Laid-Open No. 10-510980 discloses such protein modification in detail.

When the pharmaceutical composition of the present invention is administered to human, recovery from hematological suppression due to an adverse drug reaction of carcinostatics; early recovery of hematopoietic cells at bone marrow transplantation, peripheral blood stem cell transplantation, or cord blood transplantation; and recovery of hematopoietic function at pancytopenia such as aplastic anemia (AA) and myelodysplastic syndrome (MDS) are expected.

The antibodies of the present invention react specifically to the above described polypeptides of the present invention. The antibodies of the present invention may be monoclonal antibodies or polyclonal antibodies as long as they react specifically to the above described polypeptides.

The antibodies of the present invention can be prepared according to usual methods. For example, the antibodies can be prepared either *in vivo* method in which animals are additionally immunized by an antigen

together with adjuvant once or several times at an interval of several weeks or *in vitro* method in which immune cells are isolated and sensitized in an appropriate culture system. Examples of immune cells
5 which can produce the antibodies of the present invention include splenic cells, tonsillar cells, lymph gland cells, and the like.

The whole polypeptide according to the present invention is not necessarily used as an antigen. A part
10 of this polypeptide may be used as an antigen. When the antigen is a short peptide, particularly, a peptide made of about 20 amino acid residues, it may be used by binding it to a carrier protein having high antigenicity such as keyhole limpet hemocyanin or bovine serum
15 albumin using chemical modification and the like. Alternatively, the antigen may be used by covalently binding it to peptide having branching skeleton such as lysine core MAP peptide instead of the carrier protein (Posnett *et al.*, *J. Biol. Chem.*, 263, 1719-1725, 1988;
20 Lu *et al.*, *Mol. Immunol.*, 28, 623-630, 1991; Briand *et al.*, *J. Immunol. Methods*, 156, 255-265, 1992).

Examples of adjuvant include Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide gel, and the like. Antigen-given animals are,
25 for example, mouse, rat, rabbit, sheep, goat, chicken, bovine, horse, guinea pig, hamster, and the like. The blood is collected from these animals and the serum is

separated. Then, immunoglobulin is purified from the serum using an ammonium sulfate precipitation method, anion exchange chromatography, protein A chromatography, or protein G chromatography to obtain polyclonal
5 antibodies.

With respect to chicken, antibodies can be purified from an egg. Monoclonal antibodies can be prepared by purification from supernatant of culture of hybridoma cells which are made by fusion of the immune cells
10 sensitized *in vitro*, or immune cells from the above described animals with parent cells capable of cultivation, or ascites from animals which received intraperitoneal administration of hybridoma cells. Examples of parent cells include X63, NS-1, P3U1,
15 X63.653, SP2/O, Y3, SK0-007, GM1500, UC729-6, HM2.0, NP4-1 cell lines, and the like. Preparation may be performed by cultivating the immortalized antibody-forming cells obtained by sensitization *in vitro*, or infection of a proper virus such as EB virus to the
20 immune cells of the above described animals.

In addition to these cell engineering methods, the antibodies can be obtained using gene engineering methods. For example, the antibody gene obtained from the *in vitro* sensitized cells or immune cells derived
25 from the above described animals is amplified by PCR (polymerase chain reaction) and isolated, and the amplified genes are transferred into microorganisms such

as *E. coli* to produce the antibodies. Alternatively, the antibodies may be expressed on surfaces of phages as fused proteins.

By measuring polypeptides *in vivo* using the antibodies of the present invention, the relationship between the polypeptides and pathological status of a variety of diseases can be clarified. Moreover, the antibodies can be used for diagnosis and treatment of diseases, and efficient affinity purification of the polypeptides.

The present invention provides polypeptides having an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells by effecting thereon, or an activity to impart an activity to support the hematopoietic stem cells to stromal cells by effecting thereon, and also provides DNAs encoding thereof. The polypeptides of the present invention can efficiently maintain the proliferation or the survival of the hematopoietic stem cells or the hematopoietic progenitor cells.

Best Mode for Carrying out the Invention

Hereafter, the present invention will be described in detail by reference to examples.

25

Example 1 Preparation of fragment of gene which is specifically expressed in hematopoietic stem cell-

supporting cells

(I) Preparation of stromal cell line derived from mouse AGM

(1) Isolation of AGM region from fetal mouse

5 C3H/HeNSLc mice of both genders (purchased from Japan SLC INC.) were kept under a SPF (specific pathogen-free) environment. One or two female mice and one male mouse were placed in the same cage over a night. In the next morning, the female mice in which the
10 existence of a vaginal plug was observed were transferred to other cages and kept. The day when the existence of the vaginal plug was observed was defined to be the 0.5th day of pregnancy. On the 10.5th day of the pregnancy, after mouse was sacrificed by cervical
15 dislocation, fetuses were extirpated. Isolation of AGM regions was performed according to the method by Godin et al. (Godin, I., *Proc. Natl. Acad. Sci. U.S.A.*, 92: 773-777, 1995) and the method by Medvinsky et al. (Medvinsky, A.L., *Blood*, 87: 557-565, 1996). The
20 fetuses were placed in a culture dishes to which PBS(-) (phosphate buffered saline) (produced by Nissui Seiyaku) was added in a volume just sufficient to cover the fetuses. After the AGM regions were carefully excised so as not to include other regions under a stereoscopic
25 microscope, they were put in another 24-well culture dish (Nunc).

(2) Establishment of cell lines derived from AGM

One drop of MEM medium (Sigma) containing 10% FCS (Hyclone) was added to the AGM regions in the 24-well culture dish (Nunc), and AGM regions were cultured in an incubator overnight. The culture was performed in the MEM medium (Sigma) containing 10% FCS (Hyclone) at 37°C, in an atmosphere of 5% CO₂, and at a humidity of 100%. When the cells of the AGM regions adhered to the culture dish due to overnight cultivation, two milliliters of MEM medium containing 10% FCS was further added.

Stromal cells began to appear around the AGM region tissue fragment after the continuous cultivation. After one-week cultivation, adhesive cells were separated by trypsin treatment (0.05% trypsin in PBS containing 0.53 mM EDTA (Gibco BRL) at 37°C for three to five minutes).

The stromal cells were then washed twice with the medium, and seeded on 6-well culture dish (Nunc). On the next day, the cells which did not adhere to the culture dish and the medium were removed, and then, fresh medium was added. Two weeks after transfer to the 6-well culture dish, cells were γ -ray-irradiated at 900 Rad to eliminate endogenous hematopoietic cells. An attempt of the direct cell cloning by limiting dilution from this culture system was made, but no cell proliferation was observed and the cloning ended in failure. Then, after the number of seeded cells in one well was increased and cells were adapted so as to be able to proliferate from a small number of cells, the cells were cloned by

limiting dilution.

Specifically, the AGM was extirpated and cultured in the same manner as described above. The culture system two weeks after the γ -ray radiation was trypsinized
5 (0.05% trypsin in PBS containing 0.53 mM EDTA at 37°C for three to five minutes) to suspend the cells, and the cells were seeded in a 24-well culture dish at 50 to 100 cells/well. After the culture was continued for three weeks, the cells were seeded in a 96-well culture dish
10 (Nunc) by means of limiting dilution, at 0.3 cells/well. The cells which were grown from the well in which only one cell was seeded were allowed to enlarge culture. As a result, the cells were successfully cloned to obtain fibroblast-like cells and cobble stone-like cells.

15 A CD34-positive cell fraction derived from the human cord blood was co-cultured with the fibroblast-like cells for two weeks to examine the presence of colony-forming cells during the culture. Colony-forming cells could not be found in the co-culture system with the
20 fibroblast-like cells. Then, the similar examination was performed for seven cell clones showing the cobble-stone-like form. Three clones having an activity to support proliferation of human hematopoietic stem cells were obtained and were named AGM-s1, AGM-s2, and AGM-s3.
25 (II) Preparation of hematopoietic stem cells from mouse bone marrow

Bone marrow was collected from a femur of C57BL/6-

Ly5.1 pep (eight- to ten-week age, and male) (the gift from Professor K. Nakauchi, University of Tsukuba), and suspended in PBS. After the mouse bone marrow mononuclear cells were concentrated by specific gravity centrifugation according to the usual method (S. Kouzu, Fundamental techniques for immunology, YODOSHA, 1995), the cells were suspended in staining buffer (PBS containing 5% FCS and 0.05% NaN₃), and a hematopoietic stem cell fraction was obtained as follows (Osawa, M. et al., Science 273: 242-245, 1996).

An FITC-conjugated anti-CD34 antibody, a phycoerythrin-conjugated anti-Sca-1 antibody, an allophycocyanin anti-c-Kit antibody (all purchased from Pharmingen) and six biotylated anti-differentiation antigen antibodies (CD45R, CD4, CD8, Gr-1, Ter119, and CD11c, all purchased from Pharmingen) as molecular markers (Lin), were added to a suspension of the bone marrow mononuclear cells and incubated for 20 min on ice to cause reaction. After the cells were washed twice with staining buffer, CD34-negative, Sca-1-positive, c-Kit-positive, and Lin-negative cells were isolated on a cell sorter (FACS Vantage, Becton Dickinson).

(III) Subcloning of mouse stromal cell line and determination of activity to support hematopoietic stem cells of a variety of cell lines

(1) Subcloning of mouse stromal cell line

1) Isolation of AGM-s3 subclone

Stromal cell line AGM-s3 derived from AGM, which was subcultured in MEM α medium (GIBCO BRL) including inactivated 10% FCS (bovine fetal serum, Hyclone), was suspended in PBS containing 5% FCS (PBS-FCS). Clone
5 sorting was performed in a 96-well culture dish (Falcon) at one cell/well using a cell sorter (FACS Vantage; Becton Dickinson). Among cells in the 96 wells, cultures of the cells which grew were expanded, so that thirteen kinds of AGM-s3 subclones were obtained. The
10 activity to support the hematopoietic cells of these AGM-s3 subclones were examined.

2) Isolation of human cord blood CD34-positive stem cell

The human cord blood was collected at normal delivery according to the criteria approved by Ethics
15 committee of Kirin Beer Iyaku Tansaku Kenkyusho. The cord blood was collected using a heparin-added syringe so as not to coagulate. The heparin treated cord blood was overlaid on Lymphoprep (NYCOMED PHARMA), and mononuclear cells were separated by specific gravity
20 centrifugation (at 400G, at room temperature, and for 30 minutes). Erythrocytes contaminated in the mononuclear cell fraction were lysed by treatment with an ammonium chloride buffer solution (0.83% NH₄Cl-Tris HCl, 20 mM, pH 6.8) at room temperature for two minutes. After the
25 mononuclear cells were washed with PBS-FCS, ten milligrams of human IgG was added thereto and the mixture was allowed to stand on ice for ten minutes.

Then, the cells were further washed with PBS-FCS. Biotinylated antibodies against the antigens specific to the human differentiated blood cells, that is, the antibodies against CD2, CD11c (purified from ATCC hybridoma), CD19 (PharMingen), CD15, and CD41 (Leinco Technologies Inc.), and Glycophorin A (Cosmo Bio) were added thereto, and the mixture was allowed to stand on ice for 20 min. After washing with PBS-FCS, the cells were suspended in one milliliter of PBS containing 5% FCS, 10 mM EDTA, and 0.05% NaN_3 (PBS-FCS-EDTA- NaN_3). Streptavidin-bound magnetic beads (BioMag. Per Septive Diagnostics) were added thereto, and the mixture was allowed to stand on ice for 40 min. The differentiated blood cells which expressed differentiation antigens were removed using a magnetic separator (DynaL MPC-1 Dynal). An FITC-labeled anti-CD34 antibody (Immunotech S.A., Marseilles, France) was added to the remaining differentiated blood cell antigen-negative cell fraction. After incubation on ice for 20 min., a CD34-positive fraction was recovered using a cell sorter. This cell population was defined as a hematopoietic stem cell population derived from the human cord blood.

3) Co-culture of the human hematopoietic stem cells and AGM-s3 subclone

After 13 kinds of AGM-s3 subclones and stromal cell line MS-5 derived from the mouse bone marrow were each seeded in a 24-well culture dish (Falcon) at 1×10^4

cells/well, and cells were cultured in one milliliter of MEM α medium containing 10% FCS and allowed to grow until the cells covered all over the bottom surfaces of the wells. CD34-positive hematopoietic stem cells derived from the human cord blood were placed on the above described stromal cells at 500 cells/well, and co-cultured in one milliliter of MEM α medium containing 10% FCS. One week after the start of the co-culture, one milliliter of the same medium was further added. Two weeks after the start of the co-culture, the stromal cells and the human blood cells were trypsinized (0.05% trypsin in PBS containing 0.5 mM EDTA (GIBCO BRL) at 37°C; standing for two to five min.) to simultaneously separate them from the culture dish. An activity to support the hematopoietic stem cells was determined with a clonogenic assay.

4) Assessment of proliferation statuses of the hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

The cells which proliferated in the above described co-culture system were appropriately diluted, and subjected to one milliliter of methylcellulose culture system to be analyzed. The analysis using the methylcellulose culture system was performed using a 6-well culture dish (Falcon) in MethoCult H4230 (Stem Cell Technologies Inc.) to which 10 ng/ml of human SCF, human IL-3, human IL-6, human G-CSF, and human TPO, and 2

IU/ml of EPO were added. All of a variety of the above described hematopoietic factors were recombinants and pure. After two-week culture, developed colonies were observed under a microscope to count numbers of CFU-GM (granulocyte-macrophage colony-forming unit), BFU-E (erythroid burst forming unit), and CFU-E mix (erythrocyte mixed colony-forming unit).

Fig. 1 shows the result of two-week co-culture of the CD34-positive hematopoietic stem cells and the AGM-s3 subclone A9, A7, or D11. As a result of the co-culture, A9 and D11 subclones among 13 kinds of AGM-s3 subclones supported proliferation of all three series of CFU-GM, BFU-E, and CFU-E mix. Especially, although BFU-E and CFU-E mix, that is, the progenitor cells of erythrocytes were hardly to be supported in usual, their proliferations were observed in the co-culture system with A9 or D11 cells. The results showed that proliferation or maintenance of the hematopoietic stem cells or the hematopoietic progenitor cells occurred in the co-culture with A9 or D11 cells and the progenitor cells of the erythrocyte were continuously supplied. In contrast, although cellular morphology of A7 was similar to that of A9, A7 did not support CFU-GM, BFU-E, and CFU-E mix.

5) Comparison of an activity to support the human hematopoietic stem cells between A9 and a stromal cell line OP9 derived from mouse fetus

Comparison of an activity to support the CD34-positive hematopoietic stem cells derived from the human cord blood between AGM-s3 subclones A9 and A7, and a stromal cell line OP9 derived from mouse fetus (RCB1124, the Cell Development Bank of RIKEN) were performed with CFU-GM, BFU-E, CFU-E and CFU-E mix as indexes, using the above described determination system. Fig. 2 shows the result of the two-week co-culture. In the A7 cell culture system, CFU-GM, BFU-E, and CFU-E were significantly decreased and CFU-E mix was completely disappeared. In contrast, with OP9 cells, a variety of blood cell progenitor cells including CFU-E mix were supported, although the supporting ability was less than that of A9 cells. Therefore, it has been found that OP9 cells possess the activity to support the hematopoietic stem cells.

(2) Assessment of activity to support the hematopoietic stem cells in a variety of cell lines

The above described stromal cell lines (AGM-s3-A9, AGM-s3-A7, and AGM-s3-G1), 3T3Swiss (ATCC), OP9, and NIH3T3 (ATCC) were seeded in a 24-well culture dish (Falcon) at 5×10^4 cells/well. The cell lines were cultured in MEM α medium (GIBCO BRL) containing inactivated 10% FCS (bovine fetal serum, Hyclone) for one day and allowed to proliferate until the cells covered all over the bottom surfaces of the wells. Then, the medium was replaced to one milliliter of fresh

medium, thirty cells of the mouse hematopoietic stem cells (derived from C57BL/6-Ly5.1) obtained in the above (II) were placed on this cell layer, and co-culture was started.

5 On seventh day of the cultivation, the cells were trypsinized (0.05% trypsin in PBS containing 0.5 mM EDTA (GIBCO BRL) at 37°C for two to five minutes) to separate and recover all the cells on the culture dish. The recovered whole cells of each cell line and 200,000
10 cells of whole bone marrow cells (derived from C57BL/6-Ly5.2 mouse, Charles River) were transplanted into C57BL/6-Ly5.2 mice (eight weeks age and male, Charles River) irradiated with X-ray at 8.5 Gy through the tail vein. After the transplantation, peripheral blood was
15 collected from orbit at intervals, and the ratio in number of cells derived from the C57BL/6-Ly5.1 prep mouse was determined with FACS. The peripheral blood was analyzed according to the usual method (S. Kouzu, Fundamental techniques for immunology, YODOSHA, 1995).
20 Three hundreds and fifty μ L of distilled water was added to 50 μ L of the peripheral blood, and the mixture was allowed to stand for 30 seconds so as to lyse the erythrocytes. Then, PBS at twice concentrations was added and the mixture was centrifuged to recover white
25 blood cells. After the cells were washed once using the staining buffer (PBS containing 5% FCS and 0.05% NaN_3), anti-CD16 antibody, anti-Ly5.1 (CD45.1) antibody labeled

with FITC, anti-Gr-1 and anti-CD11c antibodies labeled with phycoerythrin, and anti-CD45R (B220) and anti-CD90 (Thy1) antibodies labeled with allophycocyanin (all of these were purchased from Pharmingen) were added. After 5 these cells were allowed to stand for reaction in the ice bath for 30 minutes, they were washed with the staining buffer and FACS analysis was performed.

Change in the number of cells capable of reconstitution during the hematopoietic stem cell 10 culture was determined by calculating the proportions of Ly5.1-positive cells in the Gr-1- or CD11c-positive cells (myeloid cells) and Ly5.1-positive cells in the CD90- or CD45R-positive cells (lymphoid cells) in the peripheral blood at intervals after transplantation.

15 Fig. 3 shows the results. When the cells were co-cultured with AGM-s3-A9 cells, OP9 cells, or 3T3Swiss cells, high chimerism of donor cells were maintained after the transplantation. Therefore, these stromal cells were considered to have a high activity to support 20 the hematopoietic stem cells. In contrast, when the cells were co-cultured with AGM-s3-A7 cells, AGM-s3-G1 cells, or NIH3T3 cells, high chimerism derived from the transplanted cells was not observed. Therefore, these stromal cells were low in an activity to support the 25 hematopoietic stem cells or the hematopoietic progenitor cells.

(IV) Identification of sequences of genes which

specifically express in hematopoietic stem cell-supporting cells

AGM-s3-A9 cells, AGM-s3-A7 cells and OP9 cells were each dissolved in 20 mL of ISOGEN (Nippon gene, Japan) and total RNAs were prepared according to the attachment. Messenger RNAs were prepared from one milligram of the total RNAs according to the protocol of the mRNA purification kit (Amersham Pharmacia, U.S.A.). cDNAs were synthesized from the mRNAs and cDNA libraries (hereinafter, also called as AGM-s3-A9 cDNA, AGM-s3-A7 cDNA and OP9 cDNA, respectively) were constructed using pSPORT1 (GIBCO Lifetech, U.S.A.). A clone harboring a cDNA fragment which highly expresses specifically to AGM-s3-A9 cells or OP9 cells compared with AGM-s3-A7 cells was obtained from the libraries with SBH method (Hyseq, U.S.A.). A nucleotide sequence of the obtained clone was determined using ABI377 DNA sequencer (Perkin Elmer, U.S.A.).

As a result, it has been found that expression of genes comprising nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or parts thereof in AGM-s3-A9 or OP9 cells is higher than that in AGM-s3-A7 cells. These genes were named as SCR-2, SCR-3, SCR-4, SCR-5, SCR-6, SCR-7 and SCR-8, respectively.

Example 2 Cloning of SCR-2 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 1 with BLAST, it has been found that SCR-2 is the same gene as a mouse gene, *Mus musculus* glypican-1 (Gpc-1) of an accession number AF185613. The nucleotide sequence of ORF (Open Reading Frame) of SCR-2 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 8. Only the amino acid sequence is shown in SEQ ID NO: 9.

10 The human nucleotide sequence of Gpc-1 is recorded in GenBank database under an accession number AX020122. The nucleotide sequence of ORF of AX020122 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is
15 shown in SEQ ID NO: 11.

Determination of the activity to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of
20 mouse SCR-2

Based on the nucleotide sequence of SCR-2 ORF, SCR-2Fsal1 and SCR-2Reco primers having the following nucleotide sequences were prepared, and PCR was performed using OP9 cDNA as a template.

25 SCR-2Fsal

CCGGTCGACCACCatggaactccggacccgaggctgg (SEQ ID NO: 30)

SCR-2Reco

CCGAATTCTtaccgccacctgggcctggctgc (SEQ ID NO: 31)

An amplified fragment was digested with restriction enzymes *EcoRI* and *Sall*. After electrophoresis, a DNA
5 fragment was purified using JETSORB (Genomed, Germany). The purified DNA fragment was ligated with pMX-IRES-GFP vector digested with *EcoRI* and *XhoI* (gift from Professor T. Kitamura, TOKYO UNIV. INST. OF MEDICAL SCIENCE, Japan). The pMX-IRES-GFP vector is a plasmid obtained
10 by inserting sequences encoding IRES (Internal Ribosome Entry Site) and GFP (Green Fluorescence Protein) into the retrovirus vector pMX. IRES (Internal Ribosome Entry Site) enables ribosome to access to the middle of the mRNA. Therefore, two genes can be expressed from
15 one mRNA by ligation of upward and downward genes separated by IRES in one transcription unit during the construction of an expression vector. With respect to the above-described plasmid, SCR-2 cDNA was inserted in the upward site and GFP (Green Fluorescence Protein) was
20 inserted in the downward site. Thus, the expression of SCR-2 could be monitored by detecting the expression status of GFP using FACS.

The obtained recombinant vector was introduced into *E. coli* DH5 α , and was seeded on LB agar medium
25 containing 100 μ g/ml of ampicillin, so that independent colonies were formed. After the isolated colony was cultured in 100 mL of LB medium containing 100 μ g/ml of

ampicillin, plasmid was purified using QIAGENtip100 (QIAGEN, U.S.A.). The sequence of the inserted gene was determined using a conventional method, so that the sequence was confirmed to be identical to the nucleotide
5 sequence of SCR-2 ORF.

(2) Preparation of stromal cells highly expressing SCR-2
BOSC23 cells were seeded on a collagen type I-coated 60-mm dish (Asahi technoglass) at 2×10^6 cells/dish, and cultured in DMEM medium containing 10% FCS at 37°C,
10 under an atmosphere of 5% CO₂, and at a humidity of 100%. Twelve to 18 hours after the start of the culture, the medium was replaced by two milliliters of OPTI MEM medium (GIBCO BRL).

About 3 µg of plasmid obtained by inserting SCR-2
15 into the above described pMX-IRES-GFP was added to 18 µl of LIPOFECTAMINE Reagent (GIBCO BRL) diluted with 100 µl of OPTI MEM medium, and the mixture was allowed to stand at room temperature for 30 min. The prepared DNA solution was added to the prepared BOSC23 cell culture
20 solution. After about five hours, two milliliters of DMEM medium containing 20% FCS (GIBCO BRL) was added.

After about 24 hours, the medium was replaced by 4 ml of DMEM containing 10% FCS. Further, after about 48 hours, the culture medium was harvested. After the
25 culture medium was filtrated through 0.45-µm filter, the filtrate was centrifuged at 1,200g for 16 hours and the supernatant was removed to obtain the virus

precipitation.

AGM-s3-A7 or AGM-s3-A9 cells were cultured in one milliliter of MEM α medium containing 10% FCS (GIBCO BRL) on a 24-well culture dish (FALCON) at 1×10^4 cells/well. 5 After 12 to 18 hours, the virus precipitation was suspended in one milliliter of MEM α medium containing 10% FCS, and the stromal cell culture medium was replaced by the virus suspension. Next, POLYBRENE (Sigma, SEQUA-BRENE) was added to be 10 μ g/ml. After 10 the culture dish was centrifuged at 700g for 45 minutes, the cells were cultured at 37°C, under an atmosphere of 5% CO₂, and at a humidity of 100%. After 48 hours, the medium was replaced by one milliliter of MEM α medium containing 10% FCS. After 24 hours, the cells were 15 subcultured on a 6-well culture dish (FALCON) and cultured in three milliliters of MEM α medium containing 10% FCS. Forty-eight hours after the subculturing, GFP expression in the stromal cells was detected using a cell sorter (FACSVantage, Becton Dickinson) to 20 indirectly confirm that not less than 80% of cells expressed SCR-2.

Also, the same procedures were repeated by using pMX-IRES-GFP vector instead of the plasmid obtained by inserting SCR-2 into pMX-IRES-GFP to prepare stromal 25 cells into which a control vector was introduced.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-2, and determination

of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 or AGM-s3-A7 cells in which SCR-2 was highly expressed through retrovirus, AGM-s3-A9 or AGM-s3-A7 cells into which a control vector was introduced, or AGM-s3-A9 or AGM-s3-A7 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 4 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-2 was highly expressed (A9/SCR-2), AGM-S3-A9 cells into which a control vector was introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks. Also, Fig. 5 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A7 cells in which SCR-2 was highly expressed, AGM-S3-A7 cells into which a control vector was introduced or AGM-S3-A7 cells for two weeks. As a result, by the co-culture with AGM-S3-A9 cells in which SCR-2 was highly expressed or AGM-S3-A7 cells in which SCR-2 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 or AGM-S3-

A7 increases by allowing SCR-2 to be highly expressed.

From the results, it has been revealed that a gene

product of SCR-2 has an activity to support survival or proliferation of hematopoietic stem cells or

5 hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

10 Example 3 Cloning of SCR-3 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 2 with BLAST, it has been found that SCR-3 is the same gene as mouse genes, *Mus*

15 *musculus* chemokine MMRP2 mRNA of an accession number U15209, *Mus musculus* C10-like chemokine mRNA of U19482 and mouse macrophage inflammatory protein-1gamma mRNA of U49513. The nucleotide sequence of SCR-3 ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 12. Only the amino acid
20 sequence is shown in SEQ ID NO: 13.

Determination of the activity of SCR-3 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of
25 mouse SCR-3

Based on the nucleotide sequence of SCR-3 ORF, SCR-3F_xhoI and SCR-3Reco primers having the following

nucleotide sequences were prepared, and PCR was performed using AGM-s3-A9 cDNA as a template. An amplified fragment was inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-3F_{xho}I

ccgCTCGAGccaccATGAAGCCTTTTCATACTGCC (SEQ ID NO: 32)

SCR-3Reco

tccGAATTCttattgtttgtaggtccgtgg (SEQ ID NO: 33)

10

(2) Preparation of stromal cells highly expressing SCR-3
AGM-s3-A7 cells in which SCR-3 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

15 (3) Determination of activity to support hematopoietic stem cells of stromal cells in which SCR-3 is highly expressed

In the same manner as described in (III) (2) of Example 1, determination of the activity to support hematopoietic stem cells was performed except that AGM-S3-A7 cells, AGM-S3-A7 cells in which SCR-3 was highly expressed through retrovirus, and AGM-S3-A7 cells into which a control vector was introduced were seeded in a 24-well culture dish (Falcon) at 1×10^5 cells/well.

25 The results are shown in Fig. 6. Hematopoietic cells co-cultured with AGM-s3-A7 cells in which SCR-3 was highly expressed (A7/SCR-3) showed high chimerism in

recipient individuals after the transplantation compared with the parent cell lines or hematopoietic cells co-cultured with the cells into which a control vector was introduced. The high chimerism was observed in myeloid and lymphoid cells two months after the transplantation. Therefore, it is revealed that hematopoietic stem cells and hematopoietic progenitor cells which can reconstitute the hematopoietic system in bodies of irradiated mice have maintained and amplified superiorly to the co-culture with cells into which SCR-3 is not introduced, during the co-culture period. From the results, it is revealed that an activity of stromal cells to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells is increased by high expression of SCR-3. Therefore, it is revealed that a gene product of SCR-3 has an activity to affect hematopoietic stem cells or hematopoietic progenitor cells to support survival or proliferation thereof or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

Example 4 Cloning of SCR-4 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 3 with BLAST, it has been found that SCR-4 has a high homology to *Homo sapiens*

clone 25077 mRNA of an accession number AF131820, and that SCR-4 is a mouse ortholog. This sequence is described in WO 00/66784.

The nucleotide sequence of ORF of AF131820 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 16. Only the amino acid sequence is shown in SEQ ID NO: 17.

The nucleotide sequence of ORF of SCR-4 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

Determination of the activity of SCR-4 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of human SCR-4

From 3 µg of mRNA derived from fetal liver (CLONETEC, U.S.A.), cDNA was synthesized by using oligo-dT primer and reverse transcriptase (SuperscriptII, GIBCO-BRL). Using the cDNA as a template, the ORF region of human SCR-4 was amplified by PCR with HSCR-4F_{XhoI} and HSCR-4RecoRV primers having the following nucleotide sequences. An amplified fragment was digested with *XhoI* and inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2. For the insertion, the pMX-IRES-GFP was digested with a restriction enzyme *EcoRI*, blunt-ended with KOD DNA

synthase (TOYOBO, Japan) and digested with a restriction enzyme *XhoI*.

HSCR-4F*xhoI*

CCGCTCGAGCCACCCatggttggtgcaaggctggtgt (SEQ ID NO: 34)

5 HSCR-4RecoRV

CCGGATATCtcatttcttttctggtgcctcca (SEQ ID NO: 35)

(2) Preparation of stromal cells highly expressing human SCR-4

10 AGM-s3-A9 cells in which human SCR-4 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing human SCR-4, and
15 determination of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-4 was
20 highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and
25 hematopoietic progenitor cells are determined.

Fig. 6 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9

cells in which human SCR-4 was highly expressed, AGM-S3-A9 cells into which a control vector was introduced or AGM-S3-A9 cells for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which human SCR-4 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 increases by allowing human SCR-4 to be highly expressed. From the results, it has been revealed that human SCR-4 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to impart a hematopoietic cell-supporting activity to the stromal cells.

Example 5 Cloning of SCR-5 and activity determination

In the nucleotide sequence of SEQ ID NO: 4 obtained by the SBH analysis, the presence of ORF was predicted. The nucleotide sequence of ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 18. Only the amino acid sequence is shown in SEQ ID NO: 19.

By searching GenBank database for the nucleotide sequence of SEQ ID NO: 18 with BLAST, it has been found that SCR-5 has a high homology with *Homo sapiens* esophageal cancer related gene 4 protein (ECRG4) mRNA of

an accession number AF325503, and that SCR-5 is a mouse ortholog of AF325503. The nucleotide sequence of ORF of AF325503 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 20. Only
5 the amino acid sequence is shown in SEQ ID NO: 21.

Determination of the activity of SCR-5 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of
10 mouse SCR-5

Based on the nucleotide sequence of SCR-5 ORF, SCR-5F_{XhoI} and SCR-5R_{blunt} primers having the following nucleotide sequences were prepared for retrovirus cloning, and PCR was performed using DNA having the
15 nucleotide sequence shown in SEQ ID NO: 23 as a template. An amplified fragment was digested with a restriction enzyme *XhoI* and inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2. For the insertion, the pMX-IRES-GFP was
20 digested with a restriction enzyme *EcoRI*, blunt-ended with KOD DNA synthase (TOYOBO, Japan) and digested with a restriction enzyme *XhoI*.

SCR-5F_{XhoI}

ccgCTCGAGccaccatgagcacctcgtctgcgcg (SEQ ID NO: 36)

25 SCR-5R_{blunt}

tccGTTAACTtaatagtcatcatagttca (SEQ ID NO: 37)

(2) Preparation of stromal cells highly expressing SCR-5
AGM-s3-A7 cells in which SCR-5 was highly expressed
were prepared by using the above retrovirus vector in
the same manner as (2) of Example 2.

5 (3) Determination of activity to support hematopoietic
stem cells of stromal cells in which SCR-5 is highly
expressed

In the same manner as described in (3) of Example
3, determination of the activity to support
10 hematopoietic stem cells was performed.

The results are shown in Fig. 8. Hematopoietic
cells co-cultured with AGM-s3-A7 cells in which SCR-5
was highly expressed (A7/SCR-5) showed high chimerism in
recipient individuals after the transplantation compared
15 with the parent cell lines or hematopoietic cells co-
cultured with the cells into which a control vector was
introduced. The high chimerism was observed in myeloid
and lymphoid cells two months after the transplantation.
Therefore, it is revealed that hematopoietic stem cells
20 and hematopoietic progenitor cells which can
reconstitute the hematopoietic system in bodies of
irradiated mice have maintained and amplified superiorly
to the co-culture with cells into which SCR-5 is not
introduced, during the co-culture period. From the
25 results, it is revealed that an activity of stromal
cells to support survival or proliferation of
hematopoietic stem cells or hematopoietic progenitor

cells is increased by high expression of SCR-5.

Therefore, it is revealed that a gene product of SCR-5 has an activity to affect hematopoietic stem cells or hematopoietic progenitor cells to support survival or proliferation thereof or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

10 Example 6 Cloning of SCR-6 and activity determination

Based on the nucleotide sequence of SEQ ID NO: 5, a probe was prepared and AGM-s3-A9 cDNA was screened by hybridization to obtain a gene containing ORF of mouse SCR-6.

15 AGM-s3-A9 cells (1.4×10^8 cells) were dissolved in 20 mL of ISOGEN (Nippon gene, Japan) and total RNAs were prepared according to the attachment. Messenger RNAs were prepared from one milligram of the total RNAs according to the protocol of the mRNA purification kit (Amersham Pharmacia, U.S.A.). By using SMART cDNA library construction kit (CLONTECH, U.S.A.), cDNA libraries divided to 15 fractions were prepared from the 20 2 μ g of the prepared mRNAs according to the attachment. The libraries contained about 400,000 of independent clones in total. For each fraction, PCR was performed under the following conditions to identify a fraction containing SCR-6 cDNA.

Based on the sequence of a partial fragment of the mouse SCR-6 gene, the following primers were prepared, and PCR was performed with 35 cycles of 94°C, 30 seconds, 55°C, 30 seconds and 72°C, 1 minute, by using each
5 fraction of AGM-s3-A9 cDNA libraries as a template.

SCR-6F

AGCTCATTACTGTATATTTA (SEQ ID NO: 22; 1971-1990)

(SEQ ID NO: 38)

SCR-6R

10 GCTATATTTTCATAAGTCATC (SEQ ID NO: 22; 2330-2349)

(SEQ ID NO: 39)

The PCR product was subjected to 2% agarose gel electrophoresis and a fraction from which the PCR
15 product having the expected size was obtained was identified. For each of two fractions among the positive fractions, 50,000 plaques were seeded on two 15-cm petri dishes and incubated 37°C for 10 hours. Then, plaques of each petri dish were replicated to a
20 sheet of Biodyne nylon filter (Pall, U.S.A.). The replicated nylon filter was subjected to DNA fixation treatment according to the attachment, and screening with ³²P-labeled DNA probe was performed.

The probe was prepared as follows. PCR was
25 performed with 35 cycles of 94°C, 30 seconds, 55°C, 30 seconds and 72°C, 1 minute, by using SCR-6F and SCR-6R and the plasmid containing a partial fragment of the

mouse SCR-6 gene as a template. The PCR product was subjected to 2% agarose gel electrophoresis and the amplified fragment was purified by JETSORB. By using 25 ng of the obtained PCR fragment, ³²P-labeled DNA probe was prepared with Megaprime labeling kit (Amersham Pharmacia, U.S.A.).

Hybridization and washing were performed with ExpressHybSolution (CLONETECH, U.S.A.) according to the attachment. An X-ray film was exposed to the filter and developed with a Fuji film auto developer to analyze the result. A plaque at a position corresponding to the resultant strongly exposed portion was scraped from the petri dish, and seeded again so that about 200 of plaques should appear on 10-cm petri dish. Screening was again performed according to the above-mentioned method to isolate a single plaque. The obtained clone was transfected to *E. coli* strain BM25.8 according to the attachment of SMART cDNA library construction kit, and the transfected cells were cultured on LB agar medium containing 50 µg/ml ampicilin to form colonies. A single colony of the transfected *E. coli* was inoculated to 3 ml of LB medium containing 50 µg/ml ampicilin and cultured at 30°C overnight. Plasmid was extracted with RPM kit (BIO101, U.S.A.) to obtain about 10 mg of plasmid.

Sequencing the both ends of the inserted fragment with an ABI377 DNA sequencer by using λTriplEx5'LD-

Insert Screening Amplimer (CTCGGGAAGCGCGCCATTGTGTTGGT
(SEQ ID NO: 40); CLONTECH, U.S.A.) revealed that it
included cDNA containing the nucleotide sequence from
nucleotide 1 of SEQ ID NO: 5. The full-length
5 nucleotide sequence was also determined with the ABI377
DNA sequencer. The nucleotide sequence and the amino
acid sequence deduced from a nucleotide sequence
predicted as ORF in the nucleotide sequence are shown in
SEQ ID NO: 22. Only the amino acid sequence is shown in
10 SEQ ID NO: 23.

Determination of the activity of SCR-6 to support
the hematopoietic stem cells or hematopoietic progenitor
cells was performed as follows.

(1) Construction of retrovirus vector for expression of
15 mouse SCR-6

Based on the nucleotide sequence of SCR-6 ORF, SCR-
6F_XhoI and SCR-6Reco primers having the following
sequences were prepared for retrovirus cloning, and PCR
was performed by using DNA having the nucleotide
20 sequence shown in SEQ ID NO: 22 as a template. An
amplified fragment was inserted to the retrovirus vector
pMX-IRES-GFP in the same manner as described in (1) of
Example 2.

SCR-6F_XhoI

25 ccgctcgagccaccATGCGTTTTTGCCTCTTCTC (SEQ ID NO: 41)

SCR-6Reco

cggaattcTTATTGGTTCACTCTGTCTG (SEQ ID NO: 42)

(2) Preparation of stromal cells highly expressing SCR-6
AGM-s3-A9 cells in which SCR-6 was highly expressed
were prepared by using the above retrovirus vector in
5 the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and
stromal cells highly expressing SCR-6, and determination
of proliferation statuses of hematopoietic stem cells
and hematopoietic progenitor cells by clonogenic assay
10 In the same manner as described in (III) (1) 3) to
4) of Example 1, AGM-s3-A9 cells in which SCR-6 was
highly expressed through retrovirus, AGM-s3-A9 cells
into which a control vector was introduced, or AGM-s3-A9
cells were co-cultured with CD34-positive hematopoietic
15 stem cells derived from human cord blood, and
proliferation statuses of hematopoietic stem cells and
hematopoietic progenitor cells are determined.

Fig. 9 shows results when the CD34-positive
hematopoietic stem cells were co-cultured with AGM-S3-A9
20 cells in which SCR-6 was highly expressed (A9/SCR-9),
AGM-S3-A9 cells into which a control vector was
introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two
weeks. As a result, the co-culture with AGM-S3-A9 cells
in which SCR-6 was highly expressed, increases of BFU-E
25 and CFU-C were observed. Therefore, it has been
revealed that the activity to support hematopoietic stem
cells or hematopoietic progenitor cells, of AGM-S3-A9

increases by allowing SCR-6 to be highly expressed.

From the results, it has been revealed that the gene product of SCR-6 has an activity to support survival or proliferation of hematopoietic stem cells or

5 hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

10 Example 7 Cloning of SCR-7 and activity determination

In the nucleotide sequence of SEQ ID NO: 6 obtained by the SBH analysis, the presence of ORF was predicted. The nucleotide sequence of ORF and the amino acid sequence deduced from the nucleotide sequence are shown
15 in SEQ ID NO: 24. Only the amino acid sequence is shown in SEQ ID NO: 25.

Determination of the activity of SCR-7 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

20 (1) Construction of retrovirus vector for expression of mouse SCR-7

Based on the nucleotide sequence of SCR-7 ORF, SCR-7FsaI and SCR-7Reco primers having the following nucleotide sequences were prepared for retrovirus
25 cloning, and PCR was performed using DNA having the nucleotide sequence shown in SEQ ID NO: 24 as a template. An amplified fragment was inserted to the retrovirus

vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-7FSalI

acgcgtcgacccaccATGCCCCGCTACGAGTTG (SEQ ID NO: 43)

5 SCR-7Reco

attGAATTCTCACTTCTTCCTCCTCTTTG (SEQ ID NO: 44)

(2) Preparation of stromal cells highly expressing SCR-7
AGM-s3-A9 cells in which SCR-7 was highly expressed
10 were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-7, and determination of proliferation statuses of hematopoietic stem cells
15 and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-7 was highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9
20 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 10 shows results when the CD34-positive
25 hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-7 was highly expressed (A9/SCR-7), AGM-S3-A9 cells into which a control vector was

introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which SCR-7 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been
5 revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 increases by allowing SCR-7 to be highly expressed. From the results, it has been revealed that the gene product of SCR-7 has an activity to support survival or
10 proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

15

Example 8 Cloning of SCR-8 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 7 with BLAST, it has been found that SCR-8 is the same gene as *Mus musculus* mRNA
20 for ADAM23 of an accession number AB009673. The nucleotide sequence of SCR-8 ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 26. Only the amino acid sequence is shown in SEQ ID NO: 27.

25 Also, the sequence encoding Human MDC3 protein [*Homo sapiens*] described by JP 11155574-A has a homology of not less than 90% with SCR-8 and, therefore, is a human

ortholog of SCR-8. The nucleotide sequence of this ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 28. Only the amino acid sequence is shown in SEQ ID NO: 29.

5 Determination of the activity of SCR-8 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-8

10 Based on the nucleotide sequence of SCR-8 ORF, SCR-8FxhoI and SCR-8Reco primers having the following nucleotide sequences were prepared, and PCR was performed using AGM-s3-A9 cDNA as a template. An amplified fragment was inserted to the retrovirus vector
15 pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-8FxhoI

ccgctcgagccaccATGAAGCCGCCCGGCAGCATC (SEQ ID NO: 45)

SCR-8Reco

20 cggaattcTCAGATGGGGCCTTGCTGAGT (SEQ ID NO: 46)

(2) Preparation of stromal cells highly expressing SCR-8
AGM-s3-A9 cells in which SCR-8 was highly expressed were prepared by using the above retrovirus vector in
25 the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-8, and determination

of proliferation statuses of hematopoietic stem cells
and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to
4) of Example 1, AGM-s3-A9 cells in which SCR-8 was
5 highly expressed through retrovirus, AGM-s3-A9 cells
into which a control vector was introduced, or AGM-s3-A9
cells were co-cultured with CD34-positive hematopoietic
stem cells derived from human cord blood, and
proliferation statuses of hematopoietic stem cells and
10 hematopoietic progenitor cells are determined.

Fig. 11 shows results when the CD34-positive
hematopoietic stem cells were co-cultured with AGM-S3-A9
cells in which SCR-8 was highly expressed, AGM-S3-A9
cells into which a control vector was introduced or
15 AGM-S3-A9 cells for two weeks. As a result, the co-
culture with AGM-S3-A9 cells in which SCR-8 was highly
expressed, increases of BFU-E and CFU-C were observed.
Therefore, it has been revealed that the activity to
support hematopoietic stem cells or hematopoietic
20 progenitor cells, of AGM-S3-A9 increases by allowing
SCR-8 to be highly expressed. From the results, it has
been revealed that the gene product of SCR-8 has an
activity to support survival or proliferation of
hematopoietic stem cells or hematopoietic progenitor
25 cells or an activity to affect stromal cells to enhance
a hematopoietic cell-supporting activity of the stromal
cells or impart the activity to the stromal cells.

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SEQUENCE LISTING

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Leu Ala Pro Glu Cys Ser Arg Ala Ile Met Lys Leu Val Tyr Cys Ala			
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cat tgc cgg gga gtc ccg ggc gcc cgg ccc tgc ccc gac tat tgc cga			816
His Cys Arg Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys Arg			
260	265	270	
aat gtg ctc aaa ggc tgc ctt gcc aac cag gcc gac ctg gat gcc gag			864
Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala Glu			
275	280	285	

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tgg agg aac ctc ctg gac tcc atg gtg ctc atc act gac aag ttc tgg	912
Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe Trp	
290 295 300	
ggc ccg tcg ggt gcg gag agt gtc att ggc ggt gtg cac gtg tgg ctg	960
Gly Pro Ser Gly Ala Glu Ser Val Ile Gly Gly Val His Val Trp Leu	
305 310 315 320	
gcg gag gcc atc aac gcc ctc cag gac aac aag gac aca ctc aca gct	1008
Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Lys Asp Thr Leu Thr Ala	
325 330 335	
aag gtc atc cag gcc tgt gga aac ccc aag gtc aat ccc cac ggc tct	1056
Lys Val Ile Gln Ala Cys Gly Asn Pro Lys Val Asn Pro His Gly Ser	
340 345 350	
ggg ccc gag gag aag cgt cgc cgt ggc aaa ttg gca ctg cag gag aag	1104
Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Leu Gln Glu Lys	
355 360 365	
ccc tcc aca ggt act ctg gaa aaa ctg gtc tct gag gcc aag gcc cag	1152
Pro Ser Thr Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala Gln	
370 375 380	
ctc cga gac att cag gac ttc tgg atc agc ctc cca ggg aca ctg tgc	1200
Leu Arg Asp Ile Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu Cys	
385 390 395 400	
agt gag aag atg gcc atg agt cct gcc agt gat gac cgc tgc tgg aat	1248
Ser Glu Lys Met Ala Met Ser Pro Ala Ser Asp Asp Arg Cys Trp Asn	
405 410 415	
gga att tcc aag ggc cgg tac cta cca gag gtg atg ggt gac ggg ctg	1296
Gly Ile Ser Lys Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly Leu	
420 425 430	
gcc aac cag atc aac aac cct gag gtg gaa gtg gac atc acc aag cca	1344
Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys Pro	
435 440 445	
gac atg acc atc cgc cag cag att atg cag ctc aag atc atg acc aac	1392
Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr Asn	
450 455 460	
cgt tta cgt ggc gcc tat ggc ggc aac gac gtg gac ttc cag gat gct	1440
Arg Leu Arg Gly Ala Tyr Gly Gly Asn Asp Val Asp Phe Gln Asp Ala	

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465		470		475		480	
agt gat gac ggc	agt ggc tcc ggc	agc ggt ggc gga	tgc cca gat gac				1488
Ser Asp Asp Gly	Ser Gly Ser Gly	Ser Gly Gly Gly	Cys Pro Asp Asp				
	485	490	495				
acc tgt ggc cgg	agg gtc agc aag	aag agt tcc agc	tcc cgg acc ccc				1536
Thr Cys Gly Arg	Arg Val Ser Lys	Lys Ser Ser Ser	Arg Thr Pro				
	500	505	510				
ttg acc cat gcc	ctc ccc ggc ctg	tca gaa cag gag	gga cag aag acc				1584
Leu Thr His Ala	Leu Pro Gly Leu	Ser Glu Gln Glu	Gly Gln Lys Thr				
	515	520	525				
tca gct gcc acc	tgc cca gag ccc	cac agc ttc ttc	ctg ctc ttc ctc				1632
Ser Ala Ala Thr	Cys Pro Glu Pro	His Ser Phe Phe	Leu Leu Phe Leu				
	530	535	540				
gtc acc ttg gtc	ctt gcg gca gcc	agg ccc agg tgg	cgg taa				1674
Val Thr Leu Val	Leu Ala Ala Ala	Arg Pro Arg Trp	Arg				
545	550	555					

<210> 9
 <211> 557
 <212> PRT
 <213> Mus musculus

<400> 9
 Met Glu Leu Arg Thr Arg Gly Trp Trp Leu Leu Cys Ala Ala Ala Ala
 1 5 10 15

Leu Val Val Cys Ala Arg Gly Asp Pro Ala Ser Lys Ser Arg Ser Cys
 20 25 30

Ser Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
 35 40 45

Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
 50 55 60

Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn His

11/64

65

70

75

80

Ser Arg Met Glu Leu Glu Ser Ala Leu His Asp Ser Ser Arg Ala Leu
 85 90 95

Gln Ala Thr Leu Ala Thr Gln Leu His Gly Ile Asp Asp His Phe Gln
 100 105 110

Arg Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Glu Ala Phe Pro Gly
 115 120 125

Ala Phe Gly Asp Leu Tyr Thr Gln Asn Thr Arg Ala Phe Arg Asp Leu
 130 135 140

Tyr Val Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
 145 150 155 160

Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
 165 170 175

Gln Leu His Pro Gln Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu Gly
 180 185 190

Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Asp Ala Pro Arg Glu Leu
 195 200 205

Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val Gln
 210 215 220

Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val Pro
 225 230 235 240

Leu Ala Pro Glu Cys Ser Arg Ala Ile Met Lys Leu Val Tyr Cys Ala
 245 250 255

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His Cys Arg Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys Arg
260 265 270

Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala Glu
275 280 285

Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe Trp
290 295 300

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

Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Lys Asp Thr Leu Thr Ala
325 330 335

Lys Val Ile Gln Ala Cys Gly Asn Pro Lys Val Asn Pro His Gly Ser
340 345 350

Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Leu Gln Glu Lys
355 360 365

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

Leu Arg Asp Ile Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu Cys
385 390 395 400

Ser Glu Lys Met Ala Met Ser Pro Ala Ser Asp Asp Arg Cys Trp Asn
405 410 415

Gly Ile Ser Lys Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly Leu
420 425 430

Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys Pro

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435

440

445

Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr Asn
450 455 460

Arg Leu Arg Gly Ala Tyr Gly Gly Asn Asp Val Asp Phe Gln Asp Ala
465 470 475 480

Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Gly Gly Cys Pro Asp Asp
485 490 495

Thr Cys Gly Arg Arg Val Ser Lys Lys Ser Ser Ser Ser Arg Thr Pro
500 505 510

Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys Thr
515 520 525

Ser Ala Ala Thr Cys Pro Glu Pro His Ser Phe Phe Leu Leu Phe Leu
530 535 540

Val Thr Leu Val Leu Ala Ala Ala Arg Pro Arg Trp Arg
545 550 555

<210> 10
<211> 1677
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1674)

<400> 10
atg gag ctc cgg gcc cga ggc tgg tgg ctg cta tgt gcg gcc gca gcg 48
Met Glu Leu Arg Ala Arg Gly Trp Trp Leu Leu Cys Ala Ala Ala Ala
1 5 10 15

ctg gtc gcc tgc gcc cgc ggg gac ccg gcc agc aag agc cgg agc tgc 96

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Leu	Val	Ala	Cys	Ala	Arg	Gly	Asp	Pro	Ala	Ser	Lys	Ser	Arg	Ser	Cys	
			20					25					30			
ggc	gag	gtc	cgc	cag	atc	tac	gga	gcc	aag	ggc	ttc	agc	ctg	agc	gac	144
Gly	Glu	Val	Arg	Gln	Ile	Tyr	Gly	Ala	Lys	Gly	Phe	Ser	Leu	Ser	Asp	
		35					40					45				
gtg	ccc	cag	gcg	gag	atc	tcg	ggt	gag	cac	ctg	cgg	atc	tgt	ccc	cag	192
Val	Pro	Gln	Ala	Glu	Ile	Ser	Gly	Glu	His	Leu	Arg	Ile	Cys	Pro	Gln	
	50					55					60					
ggc	tac	acc	tgc	tgc	acc	agc	gag	atg	gag	gag	aac	ctg	gcc	aac	cgc	240
Gly	Tyr	Thr	Cys	Cys	Thr	Ser	Glu	Met	Glu	Glu	Asn	Leu	Ala	Asn	Arg	
65					70				75						80	
agc	cat	gcc	gag	ctg	gag	acc	gcg	ctc	cgg	gac	agc	agc	cgc	gtc	ctg	288
Ser	His	Ala	Glu	Leu	Glu	Thr	Ala	Leu	Arg	Asp	Ser	Ser	Arg	Val	Leu	
			85					90						95		
cag	gcc	atg	ctt	gcc	acc	cag	ctg	cgc	agc	ttc	gat	gac	cac	ttc	cag	336
Gln	Ala	Met	Leu	Ala	Thr	Gln	Leu	Arg	Ser	Phe	Asp	Asp	His	Phe	Gln	
		100					105						110			
cac	ctg	ctg	aac	gac	tcg	gag	cgg	acg	ctg	cag	gcc	acc	ttc	ccc	ggc	384
His	Leu	Leu	Asn	Asp	Ser	Glu	Arg	Thr	Leu	Gln	Ala	Thr	Phe	Pro	Gly	
		115				120						125				
gcc	ttc	gga	gag	ctg	tac	acg	cag	aac	gcg	agg	gcc	ttc	cgg	gac	ctg	432
Ala	Phe	Gly	Glu	Leu	Tyr	Thr	Gln	Asn	Ala	Arg	Ala	Phe	Arg	Asp	Leu	
	130					135					140					
tac	tca	gag	ctg	cgc	ctg	tac	tac	cgc	ggt	gcc	aac	ctg	cac	ctg	gag	480
Tyr	Ser	Glu	Leu	Arg	Leu	Tyr	Tyr	Arg	Gly	Ala	Asn	Leu	His	Leu	Glu	
145					150					155					160	
gag	acg	ctg	gcc	gag	ttc	tgg	gcc	cgc	ctg	ctc	gag	cgc	ctc	ttc	aag	528
Glu	Thr	Leu	Ala	Glu	Phe	Trp	Ala	Arg	Leu	Leu	Glu	Arg	Leu	Phe	Lys	
			165				170						175			
cag	ctg	cac	ccc	cag	ctg	ctg	ctg	cct	gat	gac	tac	ctg	gac	tgc	ctg	576
Gln	Leu	His	Pro	Gln	Leu	Leu	Leu	Pro	Asp	Asp	Tyr	Leu	Asp	Cys	Leu	
			180				185						190			
ggc	aag	cag	gcc	gag	gcg	ctg	cgg	ccc	ttc	ggg	gag	gcc	ccg	aga	gag	624
Gly	Lys	Gln	Ala	Glu	Ala	Leu	Arg	Pro	Phe	Gly	Glu	Ala	Pro	Arg	Glu	
		195				200						205				

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ctg cgc ctg cgg gcc acc cgt gcc ttc gtg gct gct cgc tcc ttt gtg	672
Leu Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val	
210 215 220	
cag ggc ctg ggc gtg gcc agc gac gtg gtc cgg aaa gtg gct cag gtc	720
Gln Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val	
225 230 235 240	
ccc ctg ggc ccg gag tgc tgc aga gct gtc atg aag ctg gtc tac tgt	768
Pro Leu Gly Pro Glu Cys Ser Arg Ala Val Met Lys Leu Val Tyr Cys	
245 250 255	
gct cac tgc ctg gga gtc ccc ggc gcc agg ccc tgc cct gac tat tgc	816
Ala His Cys Leu Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys	
260 265 270	
cga aat gtg ctc aag ggc tgc ctt gcc aac cag gcc gac ctg gac gcc	864
Arg Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala	
275 280 285	
gag tgg agg aac ctc ctg gac tcc atg gtg ctc atc acc gac aag ttc	912
Glu Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe	
290 295 300	
tgg ggt aca tgc ggt gtg gag agt gtc atc ggc agc gtg cac acg tgg	960
Trp Gly Thr Ser Gly Val Glu Ser Val Ile Gly Ser Val His Thr Trp	
305 310 315 320	
ctg gcg gag gcc atc aac gcc ctc cag gac aac agg gac acg ctc acg	1008
Leu Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Arg Asp Thr Leu Thr	
325 330 335	
gcc aag gtc atc cag ggc tgc ggg aac ccc aag gtc aac ccc cag gcc	1056
Ala Lys Val Ile Gln Gly Cys Gly Asn Pro Lys Val Asn Pro Gln Gly	
340 345 350	
cct ggg cct gag gag aag cgg cgc cgg ggc aag ctg gcc ccg cgg gag	1104
Pro Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Pro Arg Glu	
355 360 365	
agg cca cct tca ggc acg ctg gag aag ctg gtc tct gaa gcc aag gcc	1152
Arg Pro Pro Ser Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala	
370 375 380	
cag ctc cgc gac gtc cag gac ttc tgg atc agc ctc cca ggg aca ctg	1200

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Gln	Leu	Arg	Asp	Val	Gln	Asp	Phe	Trp	Ile	Ser	Leu	Pro	Gly	Thr	Leu		
385					390					395					400		
tgc	agt	gag	aag	atg	gcc	ctg	agc	act	gcc	agt	gat	gac	cgc	tgc	tgg		1248
Cys	Ser	Glu	Lys	Met	Ala	Leu	Ser	Thr	Ala	Ser	Asp	Asp	Arg	Cys	Trp		
				405					410					415			
aac	ggg	atg	gcc	aga	ggc	cgg	tac	ctc	ccc	gag	gtc	atg	ggg	gac	ggc		1296
Asn	Gly	Met	Ala	Arg	Gly	Arg	Tyr	Leu	Pro	Glu	Val	Met	Gly	Asp	Gly		
			420					425					430				
ctg	gcc	aac	cag	atc	aac	aac	ccc	gag	gtg	gag	gtg	gac	atc	acc	aag		1344
Leu	Ala	Asn	Gln	Ile	Asn	Asn	Pro	Glu	Val	Glu	Val	Asp	Ile	Thr	Lys		
		435					440					445					
ccg	gac	atg	acc	atc	cgg	cag	cag	atc	atg	cag	ctg	aag	atc	atg	acc		1392
Pro	Asp	Met	Thr	Ile	Arg	Gln	Gln	Ile	Met	Gln	Leu	Lys	Ile	Met	Thr		
	450					455					460						
aac	cgg	ctg	cgc	agc	gcc	tac	aac	ggc	aac	gac	gtg	gac	ttc	cag	gac		1440
Asn	Arg	Leu	Arg	Ser	Ala	Tyr	Asn	Gly	Asn	Asp	Val	Asp	Phe	Gln	Asp		
465					470					475					480		
gcc	agt	gac	gac	ggc	agc	ggc	tcg	ggc	agc	ggg	gat	ggc	tgt	ctg	gat		1488
Ala	Ser	Asp	Asp	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Asp	Gly	Cys	Leu	Asp		
				485				490						495			
gac	ctc	tgc	ggc	cgg	aag	gtc	agc	agg	aag	agc	tcc	agc	tcc	cgg	acg		1536
Asp	Leu	Cys	Gly	Arg	Lys	Val	Ser	Arg	Lys	Ser	Ser	Ser	Ser	Arg	Thr		
			500					505					510				
ccc	ttg	acc	cat	gcc	ctc	cca	ggc	ctg	tca	gag	cag	gaa	gga	cag	aag		1584
Pro	Leu	Thr	His	Ala	Leu	Pro	Gly	Leu	Ser	Glu	Gln	Glu	Gly	Gln	Lys		
		515					520					525					
acc	tcg	gct	gcc	agc	tgc	ccc	cag	ccc	ccg	acc	ttc	ctc	ctg	ccc	ctc		1632
Thr	Ser	Ala	Ala	Ser	Cys	Pro	Gln	Pro	Pro	Thr	Phe	Leu	Leu	Pro	Leu		
	530					535				540							
ctc	ctc	ttc	ctg	gcc	ctt	aca	gta	gcc	agg	ccc	cgg	tgg	cgg	taa			1677
Leu	Leu	Phe	Leu	Ala	Leu	Thr	Val	Ala	Arg	Pro	Arg	Trp	Arg				
545					550				555								

<210> 11

<211> 558

17/64

<212> PRT

<213> Homo sapiens

<400> 11

Met Glu Leu Arg Ala Arg Gly Trp Trp Leu Leu Cys Ala Ala Ala Ala
1 5 10 15

Leu Val Ala Cys Ala Arg Gly Asp Pro Ala Ser Lys Ser Arg Ser Cys
20 25 30

Gly Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
35 40 45

Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
50 55 60

Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn Arg
65 70 75 80

Ser His Ala Glu Leu Glu Thr Ala Leu Arg Asp Ser Ser Arg Val Leu
85 90 95

Gln Ala Met Leu Ala Thr Gln Leu Arg Ser Phe Asp Asp His Phe Gln
100 105 110

His Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Ala Thr Phe Pro Gly
115 120 125

Ala Phe Gly Glu Leu Tyr Thr Gln Asn Ala Arg Ala Phe Arg Asp Leu
130 135 140

Tyr Ser Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
145 150 155 160

Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
165 170 175

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Gln Leu His Pro Gln Leu Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu
180 185 190

Gly Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Glu Ala Pro Arg Glu
195 200 205

Leu Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val
210 215 220

Gln Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val
225 230 235 240

Pro Leu Gly Pro Glu Cys Ser Arg Ala Val Met Lys Leu Val Tyr Cys
245 250 255

Ala His Cys Leu Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys
260 265 270

Arg Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala
275 280 285

Glu Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe
290 295 300

Trp Gly Thr Ser Gly Val Glu Ser Val Ile Gly Ser Val His Thr Trp
305 310 315 320

Leu Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Arg Asp Thr Leu Thr
325 330 335

Ala Lys Val Ile Gln Gly Cys Gly Asn Pro Lys Val Asn Pro Gln Gly
340 345 350

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Pro Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Pro Arg Glu
355 360 365

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

Gln Leu Arg Asp Val Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu
385 390 395 400

Cys Ser Glu Lys Met Ala Leu Ser Thr Ala Ser Asp Asp Arg Cys Trp
405 410 415

Asn Gly Met Ala Arg Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly
420 425 430

Leu Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys
435 440 445

Pro Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr
450 455 460

Asn Arg Leu Arg Ser Ala Tyr Asn Gly Asn Asp Val Asp Phe Gln Asp
465 470 475 480

Ala Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Asp Gly Cys Leu Asp
485 490 495

Asp Leu Cys Gly Arg Lys Val Ser Arg Lys Ser Ser Ser Ser Arg Thr
500 505 510

Pro Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys
515 520 525

Thr Ser Ala Ala Ser Cys Pro Gln Pro Pro Thr Phe Leu Leu Pro Leu
530 535 540

20/64

Leu Leu Phe Leu Ala Leu Thr Val Ala Arg Pro Arg Trp Arg
 545 550 555

<210> 12
 <211> 369
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(366)

<400> 12
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 Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala
 1 5 10 15
 ctt gga atc tgg gcc cag atc aca cat gca aca gag aca aaa gaa gtc 96
 Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val
 20 25 30
 cag agc agt ctg aag gca cag caa ggg ctt gaa att gaa atg ttt cac 144
 Gln Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His
 35 40 45
 atg ggc ttt caa gac tct tca gat tgc tgc ctg tcc tat aac tca cgg 192
 Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg
 50 55 60
 att cag tgt tca aga ttt ata ggt tat ttt ccc acc agt ggt ggg tgt 240
 Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys
 65 70 75 80
 acc agg ccg ggc atc atc ttt atc agc aag agg ggg ttc cag gtc tgt 288
 Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys
 85 90 95
 gcc aac ccc agt gat cgg aga gtt cag aga tgc att gaa aga ttg gag 336
 Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu
 100 105 110
 caa aac tca caa cca cgg acc tac aaa caa taa 369
 Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln

21/64

115

120

<210> 13
<211> 122
<212> PRT
<213> Mus musculus

<400> 13
Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala
1 5 10 15

Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val
20 25 30

Gln Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His
35 40 45

Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg
50 55 60

Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys
65 70 75 80

Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys
85 90 95

Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu
100 105 110

Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln
115 120

<210> 14
<211> 1223
<212> DNA
<213> Mus musculus

22/64

<220>

<221> CDS

<222> (84)..(1121)

<400> 14

gtgacccgga agggagcccc gtggttagagg tgaccggagc tgagcatttc agatctgctt 60

agtaaaccgg tgtatcgccc acc atg ttg gct gca agg ctt gtg tgt ctc cgg 113

Met Leu Ala Ala Arg Leu Val Cys Leu Arg

1

5

10

aca cta cct tcc agg gtt ttc cag ccc act ttc atc acc aag gcc tct 161

Thr Leu Pro Ser Arg Val Phe Gln Pro Thr Phe Ile Thr Lys Ala Ser

15

20

25

cca ctt gtg aag aat tcc atc aca aag aac caa tgg ctc gta aca ccc 209

Pro Leu Val Lys Asn Ser Ile Thr Lys Asn Gln Trp Leu Val Thr Pro

30

35

40

agc agg gaa tat gct acc aag aca aga att agg act cac cgt ggg aaa 257

Ser Arg Glu Tyr Ala Thr Lys Thr Arg Ile Arg Thr His Arg Gly Lys

45

50

55

act gga caa gaa ctg aaa gag gca gcc ttg gaa cca tca atg gaa aaa 305

Thr Gly Gln Glu Leu Lys Glu Ala Ala Leu Glu Pro Ser Met Glu Lys

60

65

70

atc ttt aaa atc gat caa atg gga agg tgg ttt gtt gct gga gga gca 353

Ile Phe Lys Ile Asp Gln Met Gly Arg Trp Phe Val Ala Gly Gly Ala

75

80

85

90

gct gtt ggt ctt gga gcg ctc tgc tac tat ggc ttg gga atg tct aat 401

Ala Val Gly Leu Gly Ala Leu Cys Tyr Tyr Gly Leu Gly Met Ser Asn

95

100

105

gag att gga gct atc gaa aag gct gta att tgg cct cag tat gta aag 449

Glu Ile Gly Ala Ile Glu Lys Ala Val Ile Trp Pro Gln Tyr Val Lys

110

115

120

gat aga att cat tct act tac atg tac tta gca gga agg tat tgt tta 497

Asp Arg Ile His Ser Thr Tyr Met Tyr Leu Ala Gly Arg Tyr Cys Leu

125

130

135

aca gct ttg tct gcc ttg gca gta gcc aga aca cct gct ctc atg aac 545

Thr Ala Leu Ser Ala Leu Ala Val Ala Arg Thr Pro Ala Leu Met Asn

140

145

150

23/64

ttc atg atg aca ggc tct tgg gtg aca att ggt gcg acc ttt gca gcc	593
Phe Met Met Thr Gly Ser Trp Val Thr Ile Gly Ala Thr Phe Ala Ala	
155 160 165 170	
atg att gga gct gga atg ctt gta cac tca ata tca tat gag cag agc	641
Met Ile Gly Ala Gly Met Leu Val His Ser Ile Ser Tyr Glu Gln Ser	
175 180 185	
cca ggc cca aag cat ctg gct tgg atg ctg cat tct ggt gtg atg ggt	689
Pro Gly Pro Lys His Leu Ala Trp Met Leu His Ser Gly Val Met Gly	
190 195 200	
gca gtt gtg gct cct ctg acg atc tta ggg ggg cct ctt ctc ctg aga	737
Ala Val Val Ala Pro Leu Thr Ile Leu Gly Gly Pro Leu Leu Leu Arg	
205 210 215	
gcc gca tgg tac acc gct ggt att gtg gga ggc ctc tct act gtg gcc	785
Ala Ala Trp Tyr Thr Ala Gly Ile Val Gly Gly Leu Ser Thr Val Ala	
220 225 230	
atg tgt gcg cct agt gag aag ttt ctg aac atg gga gca ccc ctg gga	833
Met Cys Ala Pro Ser Glu Lys Phe Leu Asn Met Gly Ala Pro Leu Gly	
235 240 245 250	
gtg ggc ctg ggt ctt gtc ttt gcg tct tct ctg ggg tct atg ttt ctt	881
Val Gly Leu Gly Leu Val Phe Ala Ser Ser Leu Gly Ser Met Phe Leu	
255 260 265	
ccc cct acc tct gtg gct ggt gcc act ctg tac tca gtg gca atg tat	929
Pro Pro Thr Ser Val Ala Gly Ala Thr Leu Tyr Ser Val Ala Met Tyr	
270 275 280	
ggt gga tta gtt ctt ttc agc atg ttc ctt ctg tat gat act cag aaa	977
Gly Gly Leu Val Leu Phe Ser Met Phe Leu Leu Tyr Asp Thr Gln Lys	
285 290 295	
gta atc aaa cgt gca gaa ata aca ccc atg tat gga gct caa aag tat	1025
Val Ile Lys Arg Ala Glu Ile Thr Pro Met Tyr Gly Ala Gln Lys Tyr	
300 305 310	
gat ccc atc aat tcg atg ttg aca atc tac atg gat aca tta aat ata	1073
Asp Pro Ile Asn Ser Met Leu Thr Ile Tyr Met Asp Thr Leu Asn Ile	
315 320 325 330	
ttt atg cga gtt gca act atg cta gca act gga agc aac aga aag aaa	1121

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Phe Met Arg Val Ala Thr Met Leu Ala Thr Gly Ser Asn Arg Lys Lys
335 340 345

tgaagtaacc gcttgatg tctccgctca ctgatgtctt gcttgtttaa taggagcaga 1181

tagtcattac agtttgcac agcagaattc ccgcgcggcc gc 1223

<210> 15

<211> 346

<212> PRT

<213> Mus musculus

<400> 15

Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
1 5 10 15

Phe Gln Pro Thr Phe Ile Thr Lys Ala Ser Pro Leu Val Lys Asn Ser
20 25 30

Ile Thr Lys Asn Gln Trp Leu Val Thr Pro Ser Arg Glu Tyr Ala Thr
35 40 45

Lys Thr Arg Ile Arg Thr His Arg Gly Lys Thr Gly Gln Glu Leu Lys
50 55 60

Glu Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln
65 70 75 80

Met Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala
85 90 95

Leu Cys Tyr Tyr Gly Leu Gly Met Ser Asn Glu Ile Gly Ala Ile Glu
100 105 110

Lys Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr
115 120 125

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Tyr Met Tyr Leu Ala Gly Arg Tyr Cys Leu Thr Ala Leu Ser Ala Leu
130 135 140

Ala Val Ala Arg Thr Pro Ala Leu Met Asn Phe Met Met Thr Gly Ser
145 150 155 160

Trp Val Thr Ile Gly Ala Thr Phe Ala Ala Met Ile Gly Ala Gly Met
165 170 175

Leu Val His Ser Ile Ser Tyr Glu Gln Ser Pro Gly Pro Lys His Leu
180 185 190

Ala Trp Met Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu
195 200 205

Thr Ile Leu Gly Gly Pro Leu Leu Leu Arg Ala Ala Trp Tyr Thr Ala
210 215 220

Gly Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu
225 230 235 240

Lys Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val
245 250 255

Phe Ala Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Ser Val Ala
260 265 270

Gly Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe
275 280 285

Ser Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu
290 295 300

Ile Thr Pro Met Tyr Gly Ala Gln Lys Tyr Asp Pro Ile Asn Ser Met
305 310 315 320

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Leu Thr Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr
 325 330 335

Met Leu Ala Thr Gly Ser Asn Arg Lys Lys
 340 345

<210> 16
 <211> 1038
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(1035)

<400> 16
 atg ttg gct gca agg ctg gtg tgt ctc cgg aca cta cct tct agg gtt 48
 Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
 1 5 10 15

ttc cac cca gct ttc acc aag gcc tcc cct gtt gtg aag aat tcc atc 96
 Phe His Pro Ala Phe Thr Lys Ala Ser Pro Val Val Lys Asn Ser Ile
 20 25 30

acg aag aat caa tgg ctg tta aca cct agc agg gaa tat gcc acc aaa 144
 Thr Lys Asn Gln Trp Leu Leu Thr Pro Ser Arg Glu Tyr Ala Thr Lys
 35 40 45

aca aga att ggg atc cgg cgt ggg aga act ggc caa gaa ctc aaa gag 192
 Thr Arg Ile Gly Ile Arg Arg Gly Arg Thr Gly Gln Glu Leu Lys Glu
 50 55 60

gca gca ttg gaa cca tcg atg gaa aaa ata ttt aaa att gat cag atg 240
 Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln Met
 65 70 75 80

gga aga tgg ttt gtt gct gga ggg gct gct gtt ggt ctt gga gca ttg 288
 Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala Leu
 85 90 95

tgc tac tat ggc ttg gga ctg tct aat gag att gga gct att gaa aag 336
 Cys Tyr Tyr Gly Leu Gly Leu Ser Asn Glu Ile Gly Ala Ile Glu Lys

27/64

100	105	110	
gct gta att tgg cct cag tat gtc aag gat aga att cat tcc acc tat			384
Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr Tyr			
115	120	125	
atg tac tta gca ggg agt att ggt tta aca gct ttg tct gcc ata gca			432
Met Tyr Leu Ala Gly Ser Ile Gly Leu Thr Ala Leu Ser Ala Ile Ala			
130	135	140	
atc agc aga acg cct gtt ctc atg aac ttc atg atg aga ggc tct tgg			480
Ile Ser Arg Thr Pro Val Leu Met Asn Phe Met Met Arg Gly Ser Trp			
145	150	155	160
gtg aca att ggt gtg acc ttt gca gcc atg gtt gga gct gga atg ctg			528
Val Thr Ile Gly Val Thr Phe Ala Ala Met Val Gly Ala Gly Met Leu			
165	170	175	
gta cga tca ata cca tat gac cag agc cca ggc cca aag cat ctt gct			576
Val Arg Ser Ile Pro Tyr Asp Gln Ser Pro Gly Pro Lys His Leu Ala			
180	185	190	
tgg ttg cta cat tct ggt gtg atg ggt gca gtg gtg gct cct ctg aca			624
Trp Leu Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu Thr			
195	200	205	
ata tta ggg ggt cct ctt ctc atc aga gct gca tgg tac aca gct ggc			672
Ile Leu Gly Gly Pro Leu Leu Ile Arg Ala Ala Trp Tyr Thr Ala Gly			
210	215	220	
att gtg gga ggc ctc tcc act gtg gcc atg tgt gcg ccc agt gaa aag			720
Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu Lys			
225	230	235	240
ttt ctg aac atg ggt gca ccc ctg gga gtg ggc ctg ggt ctc gtc ttt			768
Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val Phe			
245	250	255	
gtg tcc tca ttg gga tct atg ttt ctt cca cct acc acc gtg gct ggt			816
Val Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Thr Val Ala Gly			
260	265	270	
gcc act ctt tac tca gtg gca atg tac ggt gga tta gtt ctt ttc agc			864
Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe Ser			
275	280	285	

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atg ttc ctt ctg tat gat acc cag aaa gta atc aag cgt gca gaa gta 912
 Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu Val
 290 295 300

tca cca atg tat gga gtt caa aaa tat gat ccc att aac tcg atg ctg 960
 Ser Pro Met Tyr Gly Val Gln Lys Tyr Asp Pro Ile Asn Ser Met Leu
 305 310 315 320

agt atc tac atg gat aca tta aat ata ttt atg cga gtt gca act atg 1008
 Ser Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr Met
 325 330 335

ctg gca act gga ggc aac aga aag aaa tga 1038
 Leu Ala Thr Gly Gly Asn Arg Lys Lys
 340 345

<210> 17
 <211> 345
 <212> PRT
 <213> Homo sapiens

<400> 17
 Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
 1 5 10 15

Phe His Pro Ala Phe Thr Lys Ala Ser Pro Val Val Lys Asn Ser Ile
 20 25 30

Thr Lys Asn Gln Trp Leu Leu Thr Pro Ser Arg Glu Tyr Ala Thr Lys
 35 40 45

Thr Arg Ile Gly Ile Arg Arg Gly Arg Thr Gly Gln Glu Leu Lys Glu
 50 55 60

Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln Met
 65 70 75 80

Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala Leu
 85 90 95

29/64

Cys Tyr Tyr Gly Leu Gly Leu Ser Asn Glu Ile Gly Ala Ile Glu Lys
100 105 110

Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr Tyr
115 120 125

Met Tyr Leu Ala Gly Ser Ile Gly Leu Thr Ala Leu Ser Ala Ile Ala
130 135 140

Ile Ser Arg Thr Pro Val Leu Met Asn Phe Met Met Arg Gly Ser Trp
145 150 155 160

Val Thr Ile Gly Val Thr Phe Ala Ala Met Val Gly Ala Gly Met Leu
165 170 175

Val Arg Ser Ile Pro Tyr Asp Gln Ser Pro Gly Pro Lys His Leu Ala
180 185 190

Trp Leu Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu Thr
195 200 205

Ile Leu Gly Gly Pro Leu Leu Ile Arg Ala Ala Trp Tyr Thr Ala Gly
210 215 220

Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu Lys
225 230 235 240

Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val Phe
245 250 255

Val Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Thr Val Ala Gly
260 265 270

Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe Ser

30/64

275	280	285	
Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu Val			
290	295	300	
Ser Pro Met Tyr Gly Val Gln Lys Tyr Asp Pro Ile Asn Ser Met Leu			
305	310	315	320
Ser Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr Met			
	325	330	335
Leu Ala Thr Gly Gly Asn Arg Lys Lys			
	340	345	
<210> 18			
<211> 447			
<212> DNA			
<213> Mus musculus			
<220>			
<221> CDS			
<222> (1)..(444)			
<400> 18			
atg agc acc tcg tct gcg cgg cct gca gtc ctg gcc ctt gcc ggg ctg			48
Met Ser Thr Ser Ser Ala Arg Pro Ala Val Leu Ala Leu Ala Gly Leu			
1	5	10	15
gct ctg ctc ctt ctg ctg tgc ctg ggt cca gat ggc ata agt gga aac			96
Ala Leu Leu Leu Leu Leu Cys Leu Gly Pro Asp Gly Ile Ser Gly Asn			
	20	25	30
aaa ctc aag aag atg ctc cag aaa cga gaa gga cct gtc ccg tca aag			144
Lys Leu Lys Lys Met Leu Gln Lys Arg Glu Gly Pro Val Pro Ser Lys			
	35	40	45
act aat gta gct gta gcc gag aac aca gca aag gaa ttc cta ggt ggc			192
Thr Asn Val Ala Val Ala Glu Asn Thr Ala Lys Glu Phe Leu Gly Gly			
	50	55	60
ctg aag cgt gcc aaa cga cag ctg tgg gac cgt acg cgg cct gag gta			240

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Leu	Lys	Arg	Ala	Lys	Arg	Gln	Leu	Trp	Asp	Arg	Thr	Arg	Pro	Glu	Val		
65					70				75						80		
cag	cag	tgg	tac	cag	cag	ttc	ctc	tac	atg	ggc	ttt	gat	gag	gct	aaa		288
Gln	Gln	Trp	Tyr	Gln	Gln	Phe	Leu	Tyr	Met	Gly	Phe	Asp	Glu	Ala	Lys		
				85					90					95			
ttt	gaa	gat	gat	gtc	aac	tat	tgg	cta	aac	aga	aat	cga	aac	ggc	cat		336
Phe	Glu	Asp	Asp	Val	Asn	Tyr	Trp	Leu	Asn	Arg	Asn	Arg	Asn	Gly	His		
				100				105						110			
gac	tac	tat	ggt	gac	tac	tac	cag	cgt	cat	tat	gat	gaa	gat	gcg	gcc		384
Asp	Tyr	Tyr	Gly	Asp	Tyr	Tyr	Gln	Arg	His	Tyr	Asp	Glu	Asp	Ala	Ala		
			115				120					125					
att	ggt	ccc	cac	agc	cgg	gaa	agc	ttc	agg	cat	gga	gcc	agt	gtg	aac		432
Ile	Gly	Pro	His	Ser	Arg	Glu	Ser	Phe	Arg	His	Gly	Ala	Ser	Val	Asn		
	130					135					140						
tat	gat	gac	tat	taa													447
Tyr	Asp	Asp	Tyr														
145																	

<210> 19
 <211> 148
 <212> PRT
 <213> Mus musculus

<400> 19
 Met Ser Thr Ser Ser Ala Arg Pro Ala Val Leu Ala Leu Ala Gly Leu
 1 5 10 15

Ala Leu Leu Leu Leu Leu Cys Leu Gly Pro Asp Gly Ile Ser Gly Asn
 20 25 30

Lys Leu Lys Lys Met Leu Gln Lys Arg Glu Gly Pro Val Pro Ser Lys
 35 40 45

Thr Asn Val Ala Val Ala Glu Asn Thr Ala Lys Glu Phe Leu Gly Gly
 50 55 60

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Leu Lys Arg Ala Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

Phe Glu Asp Asp Val Asn Tyr Trp Leu Asn Arg Asn Arg Asn Gly His
100 105 110

Asp Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ala Ala
115 120 125

Ile Gly Pro His Ser Arg Glu Ser Phe Arg His Gly Ala Ser Val Asn
130 135 140

Tyr Asp Asp Tyr
145

<210> 20
<211> 447
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(444)

<400> 20
atg gct gcc tcc ccc gcg cgg cct gct gtc ctg gcc ctg acc ggg ctg 48
Met Ala Ala Ser Pro Ala Arg Pro Ala Val Leu Ala Leu Thr Gly Leu
1 5 10 15

gcg ctg ctc ctg ctc ctg tgc tgg ggc cca ggt ggc ata agt gga aat 96
Ala Leu Leu Leu Leu Leu Cys Trp Gly Pro Gly Gly Ile Ser Gly Asn
20 25 30

aaa ctc aag ctg atg ctt caa aaa cga gaa gca cct gtt cca act aag 144
Lys Leu Lys Leu Met Leu Gln Lys Arg Glu Ala Pro Val Pro Thr Lys
35 40 45

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act aaa gtg gcc gtt gat gag aat aaa gcc aaa gaa ttc ctt ggc agc 192
 Thr Lys Val Ala Val Asp Glu Asn Lys Ala Lys Glu Phe Leu Gly Ser
 50 55 60

ctg aag cgc cag aag cgg cag ctg tgg gac cgg act cgg ccc gag gtg 240
 Leu Lys Arg Gln Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
 65 70 75 80

cag cag tgg tac cag cag ttt ctc tac atg ggc ttt gac gaa gcg aaa 288
 Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
 85 90 95

ttt gaa gat gac atc acc tat tgg ctt aac aga gat cga aat gga cat 336
 Phe Glu Asp Asp Ile Thr Tyr Trp Leu Asn Arg Asp Arg Asn Gly His
 100 105 110

gaa tac tat ggc gat tac tac caa cgt cac tat gat gaa gac tct gca 384
 Glu Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ser Ala
 115 120 125

att ggt ccc cgg agc ccc tac ggc ttt agg cat gga gcc agc gtc aac 432
 Ile Gly Pro Arg Ser Pro Tyr Gly Phe Arg His Gly Ala Ser Val Asn
 130 135 140

tac gat gac tac taa 447
 Tyr Asp Asp Tyr
 145

<210> 21

<211> 148

<212> PRT

<213> Homo sapiens

<400> 21

Met Ala Ala Ser Pro Ala Arg Pro Ala Val Leu Ala Leu Thr Gly Leu
 1 5 10 15

Ala Leu Leu Leu Leu Leu Cys Trp Gly Pro Gly Gly Ile Ser Gly Asn
 20 25 30

Lys Leu Lys Leu Met Leu Gln Lys Arg Glu Ala Pro Val Pro Thr Lys
 35 40 45

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Thr Lys Val Ala Val Asp Glu Asn Lys Ala Lys Glu Phe Leu Gly Ser
50 55 60

Leu Lys Arg Gln Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

Phe Glu Asp Asp Ile Thr Tyr Trp Leu Asn Arg Asp Arg Asn Gly His
100 105 110

Glu Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ser Ala
115 120 125

Ile Gly Pro Arg Ser Pro Tyr Gly Phe Arg His Gly Ala Ser Val Asn
130 135 140

Tyr Asp Asp Tyr
145

<210> 22
<211> 3132
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (630)..(1358)

<400> 22
gggggtctgc atctccatcg gaaagtgcgc tggccacatc ccttcggcct ccgggcagtg 60
ttctgtctcc cttagctcag gcagcgagaa acttcagctg tgaagtgggtg gtggagagag 120
ccctgggagc agcgactgga cccggacacc aagaagagag tggacgcgcc cctcgactag 180
gaatcgctct cgcaggcgga gaccagcat ctcagcgcct gcggtcgcgc ttgcccggcc 240

35/64

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gcgcgcctttt gctagggcgcc gccagcccccg aaggaccctc ggggtccgcg gacccttctg      300
cagccggcgcg aatcctaaag ctgccaagag ctcccggcgg gtgtcggcaa actttttccg      360
agcccacgtg ctgaccaaac agcccggctc gcttccagag cctggcatgg agcgccgcgc      420
ctaggcacgc cgtgcagccc gagagacgcg agcgcacggt tcaccgtgga gggagagatg      480
ctcatcgagc caaattgata attgcagccc cagggcagtg acatctgtct ctgagtcctc      540
cctaggagcg cgacccgcac tgtctccttc caggagcccc tcatttcctc gacttttgag      600
aggtgtctct cccagccccg accgtccag atg cgt ttt tgc ctc ttc tca ttt      653
                               Met Arg Phe Cys Leu Phe Ser Phe
                               1                               5

gcc ctc atc att ctg aac tgt atg gat tac agc cag tgc caa ggc aac      701
Ala Leu Ile Ile Leu Asn Cys Met Asp Tyr Ser Gln Cys Gln Gly Asn
    10                               15                               20

cga tgg aga cgc aat aag cga gct agt tat gta tca aat ccc att tgc      749
Arg Trp Arg Arg Asn Lys Arg Ala Ser Tyr Val Ser Asn Pro Ile Cys
    25                               30                               35                               40

aag ggt tgt ttg tct tgt tcg aag gac aat ggt tgc agc cga tgt caa      797
Lys Gly Cys Leu Ser Cys Ser Lys Asp Asn Gly Cys Ser Arg Cys Gln
                               45                               50                               55

cag aag ttg ttc ttt ttc ctt cga aga gaa gga atg cgt cag tat gga      845
Gln Lys Leu Phe Phe Phe Leu Arg Arg Glu Gly Met Arg Gln Tyr Gly
                               60                               65                               70

gag tgc ctg cat tcc tgc cca tca ggg tat tat gga cac cga gcc cca      893
Glu Cys Leu His Ser Cys Pro Ser Gly Tyr Tyr Gly His Arg Ala Pro
                               75                               80                               85

gat atg aac aga tgt gca cga tgc aga ata gaa aac tgt gat tct tgc      941
Asp Met Asn Arg Cys Ala Arg Cys Arg Ile Glu Asn Cys Asp Ser Cys
                               90                               95                               100

ttt agc aaa gac ttt tgt acg aag tgc aaa gta ggc ttt tat ttg cat      989
Phe Ser Lys Asp Phe Cys Thr Lys Cys Lys Val Gly Phe Tyr Leu His
    105                               110                               115                               120

aga ggc cgc tgc ttt gat gaa tgt cca gat ggt ttt gca ccg tta gat      1037

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36/64

Arg Gly Arg Cys Phe Asp Glu Cys Pro Asp Gly Phe Ala Pro Leu Asp	
125 130 135	
gag act atg gaa tgt gta gaa ggt tgt gaa gtt ggt cat tgg agc gaa	1085
Glu Thr Met Glu Cys Val Glu Gly Cys Glu Val Gly His Trp Ser Glu	
140 145 150	
tgg gga acg tgt agc aga aac aac cgc acg tgt gga ttt aaa tgg ggt	1133
Trp Gly Thr Cys Ser Arg Asn Asn Arg Thr Cys Gly Phe Lys Trp Gly	
155 160 165	
ctg gaa acc aga aca cgg cag att gtt aaa aag cca gca aaa gac aca	1181
Leu Glu Thr Arg Thr Arg Gln Ile Val Lys Lys Pro Ala Lys Asp Thr	
170 175 180	
ata cca tgt ccg acc att gcg gag tcc agg aga tgc aag atg gcc atg	1229
Ile Pro Cys Pro Thr Ile Ala Glu Ser Arg Arg Cys Lys Met Ala Met	
185 190 195 200	
agg cac tgt cca gga gga aag aga aca cca aag gca aaa gag aag aga	1277
Arg His Cys Pro Gly Gly Lys Arg Thr Pro Lys Ala Lys Glu Lys Arg	
205 210 215	
aac aag aag aag agg cgg aag ctg att gag aga gcc caa gag cag cac	1325
Asn Lys Lys Lys Arg Arg Lys Leu Ile Glu Arg Ala Gln Glu Gln His	
220 225 230	
agc gtc ttc ctc gct aca gac aga gtg aac caa taaaatacaa gaaatagctg	1378
Ser Val Phe Leu Ala Thr Asp Arg Val Asn Gln	
235 240	
gggcattttg aggttttctg ttttgtttat gttgttgttt tgcaaaagtg cacaagcta	1438
ctctccagtc cacactggtg gacagcattc ctgatcctct gaccagtatc cattttcagt	1498
aatgctgcag agggaggtgc ccaagcatgg actcagcgtt atttatgctt tgattggaat	1558
ctggggcctg tgatggcagg agcttgttga gctgagtcag cgggagctga tgcattctgta	1618
ctcttgat gatcacagtg tgtcataaga acctgtccct ggcacggtgg acccacagga	1678
ggcacaaggc tgtagatcac caccagagaa tgcacctgtg cctattttga tggatggcaa	1738
tgctaagcaa gcaagcactg ttacttggtg actttcattt ctcacactgt gcaactgtcaa	1798
agacaaatgt gcatggaaaa atgttttagtg tcacctcatg gcgttctcag catcagtgc	1858

37/64

cttcaaacgg tcctacaatg agactgtggt ctagctaggg gtagtctgtg gaaattcctg 1918
ctacatttca tcttagtgct aacatgtaca gattctgctg cgctacattc aaagctcatt 1978
actgtatatt tatgctttct ctgtgtaaca agttatacct gataagatgt cactttgttt 2038
ctagtgattc ttaaccatgg tctggtacat ggctattcta gttttggaaa ttaacaagtg 2098
ttttgttgcc tcttgttttc tttgttcct atcatttttg gcggggggtg ggtgggcttg 2158
attctaaccg taagtatagg ataagctagt tttgtatata gagtcaaagc actgatgtca 2218
gaggatcagt gctgatagaa cttccccagt tcatgtcacg atacacacag agagaaagca 2278
gcatgaggca tcttgccatc agaagccaaa tttcttttga gtcccaaat tgatgactta 2338
tgaaatatag ctgaaaacaa gatttggtg tagttacttg tatttattat acaatttcca 2398
attacatttt ttttcaaact caaaataacc catgactttg agtgataggt cacttggtgca 2458
tgttcttgaa ttactgggga agctgtgtgc actaagataa tgagagagaa aatagaatgg 2518
cttcgcccaa gtgagagcca catcttacct ttctctgttg aatcggaatc aactatatta 2578
gaacagaagc ctgatagaag ctttctagtt aacacacaca aggccatggg ttcaaaaaca 2638
tctttgtccc cttaggtcag tttgtcctta gattatgaat tggcaggttc taattgcatt 2698
atttccttgg ctgatccagg aaaaagttag aacaaaataa gttgcatagt tttgaggaaa 2758
catccaaagc aaggcgaagc ctttccttgc cttgcattgg caaaactacc tcttttagcat 2818
ttatgttgat tcagaaacat cttgctgata tgtgtagatg ttttaagctt cattgtgaaa 2878
atattgatgc aagataagcc atatatgaat gttgtattca actttagggc ttgaaattaa 2938
tcctaaagtg ttcacctctc tccatgtcta tttacactct gttcctatth actaagaggg 2998
taggggtctc cttaatatca tacttcattg ttaataagtc aatgcttggt atgtttcttg 3058
gctgttggtt ttgtgcatta aaaactcaaa attggaaaaa aaaaaaaaaa aaaaaaaaaa 3118
aaaaaaaaaa aaaa 3132

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<210> 23

<211> 243

<212> PRT

<213> Mus musculus

<400> 23

Met Arg Phe Cys Leu Phe Ser Phe Ala Leu Ile Ile Leu Asn Cys Met
1 5 10 15

Asp Tyr Ser Gln Cys Gln Gly Asn Arg Trp Arg Arg Asn Lys Arg Ala
20 25 30

Ser Tyr Val Ser Asn Pro Ile Cys Lys Gly Cys Leu Ser Cys Ser Lys
35 40 45

Asp Asn Gly Cys Ser Arg Cys Gln Gln Lys Leu Phe Phe Phe Leu Arg
50 55 60

Arg Glu Gly Met Arg Gln Tyr Gly Glu Cys Leu His Ser Cys Pro Ser
65 70 75 80

Gly Tyr Tyr Gly His Arg Ala Pro Asp Met Asn Arg Cys Ala Arg Cys
85 90 95

Arg Ile Glu Asn Cys Asp Ser Cys Phe Ser Lys Asp Phe Cys Thr Lys
100 105 110

Cys Lys Val Gly Phe Tyr Leu His Arg Gly Arg Cys Phe Asp Glu Cys
115 120 125

Pro Asp Gly Phe Ala Pro Leu Asp Glu Thr Met Glu Cys Val Glu Gly
130 135 140

Cys Glu Val Gly His Trp Ser Glu Trp Gly Thr Cys Ser Arg Asn Asn
145 150 155 160

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Arg Thr Cys Gly Phe Lys Trp Gly Leu Glu Thr Arg Thr Arg Gln Ile
 165 170 175

Val Lys Lys Pro Ala Lys Asp Thr Ile Pro Cys Pro Thr Ile Ala Glu
 180 185 190

Ser Arg Arg Cys Lys Met Ala Met Arg His Cys Pro Gly Gly Lys Arg
 195 200 205

Thr Pro Lys Ala Lys Glu Lys Arg Asn Lys Lys Lys Arg Arg Lys Leu
 210 215 220

Ile Glu Arg Ala Gln Glu Gln His Ser Val Phe Leu Ala Thr Asp Arg
 225 230 235 240

Val Asn Gln

<210> 24
 <211> 843
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (132)..(506)

<400> 24
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 cttcctctgc gcccgcgctc cccgccctcg cgaccccggc tctcctggac tcggcgccgc 120
 caacctgggc g atg ccc cgc tac gag ttg gct ttg att ctg aaa gcc atg 170
 Met Pro Arg Tyr Glu Leu Ala Leu Ile Leu Lys Ala Met
 1 5 10
 cgg cgg cca gag acc gct gct gct ttg aaa cgt aca ata gaa tcc ctg 218
 Arg Arg Pro Glu Thr Ala Ala Ala Leu Lys Arg Thr Ile Glu Ser Leu
 15 20 25

```
<400> 25
Met Pro Arg Tyr Glu Leu Ala Leu Ile Leu Lys Ala Met Arg Arg Pro
1          5          10         15
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Glu Thr Ala Ala Ala Leu Lys Arg Thr Ile Glu Ser Leu Met Asp Arg
 20 25 30

Gly Ala Ile Val Arg Asn Leu Glu Ser Leu Gly Glu Arg Ala Leu Pro
 35 40 45

Tyr Arg Ile Ser Ser His Ser Gln Gln His Ser Arg Gly Gly Tyr Phe
 50 55 60

Leu Val Asp Phe Tyr Ala Pro Thr Ser Ala Val Glu Asn Ile Leu Glu
 65 70 75 80

His Leu Ala Arg Asp Ile Asp Val Val Arg Pro Asn Ile Val Lys His
 85 90 95

Pro Leu Thr Gln Glu Val Lys Glu Cys Asp Gly Ile Val Pro Val Pro
 100 105 110

Leu Glu Glu Lys Leu Tyr Ser Thr Lys Arg Arg Lys Lys
 115 120 125

<210> 26
 <211> 2490
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(2487)

<400> 26
 atg aag ccg ccc ggc agc atc tcc cgg cgg ccg acc ctg acg ggt tgc 48
 Met Lys Pro Pro Gly Ser Ile Ser Arg Arg Pro Thr Leu Thr Gly Cys
 1 5 10 15

agc ctt ccc ggc gcc tcc tgc ggc ccc ggc cgc tgc ccc gcc ggc ccg 96
 Ser Leu Pro Gly Ala Ser Cys Gly Pro Gly Arg Cys Pro Ala Gly Pro
 20 25 30

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gtg ccg gcc cgc gcg ccg ccc tgc cgc ctg ctc ctc gtc ctt ctc ctg	144
Val Pro Ala Arg Ala Pro Pro Cys Arg Leu Leu Leu Val Leu Leu Leu	
35 40 45	
cta cct gcg ctc gcc acc tca tcc cgg ccc cgt gcc cgg ggg gcc gct	192
Leu Pro Ala Leu Ala Thr Ser Ser Arg Pro Arg Ala Arg Gly Ala Ala	
50 55 60	
gcg ccc agc gct ccg cac tgg aat gaa act gca gaa aaa acc ctg gga	240
Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu Lys Thr Leu Gly	
65 70 75 80	
gtc ctg gca gat gaa gac aac aca ttg caa caa aat agc agc agc aga	288
Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn Ser Ser Ser Arg	
85 90 95	
aat acc agc tac agc agt gca gtg caa aaa gaa atc aca ctg cct tca	336
Asn Thr Ser Tyr Ser Ser Ala Val Gln Lys Glu Ile Thr Leu Pro Ser	
100 105 110	
aga ctg gtg tat tac atc aac cag gac tca gaa agc ccc tat cat gtt	384
Arg Leu Val Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro Tyr His Val	
115 120 125	
ctt gac aca aag gcc aga cac caa cag aaa cac aat aag gct gtg cat	432
Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys Ala Val His	
130 135 140	
ctg gcc cag gca agc ttc cag atc gaa gct ttc ggc tcc aag ttc att	480
Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser Lys Phe Ile	
145 150 155 160	
ctt gac ctc aca ctg aac aat ggt ttg cta tct tct gac tac gtg gag	528
Leu Asp Leu Thr Leu Asn Asn Gly Leu Leu Ser Ser Asp Tyr Val Glu	
165 170 175	
atc cac tat gaa gac ggg aag cag atg tac tct aag ggt gga gag cac	576
Ile His Tyr Glu Asp Gly Lys Gln Met Tyr Ser Lys Gly Gly Glu His	
180 185 190	
tgt tac tac cac gga agc atc aga ggc gtc aag gat tcc agg gtg gct	624
Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser Arg Val Ala	
195 200 205	
cta tcg acc tgc aat gga ctc cat ggc atg ttt gag gat gac acc ttt	672

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Leu	Ser	Thr	Cys	Asn	Gly	Leu	His	Gly	Met	Phe	Glu	Asp	Asp	Thr	Phe		
210						215					220						
gtg	tat	atg	ata	gag	cct	ctg	gaa	ctg	act	gat	gat	gag	aaa	agc	aca		720
Val	Tyr	Met	Ile	Glu	Pro	Leu	Glu	Leu	Thr	Asp	Asp	Glu	Lys	Ser	Thr		
225					230					235					240		
ggc	cga	ccg	cac	ata	atc	cag	aaa	acc	ttg	gca	gga	cag	tat	tct	aag		768
Gly	Arg	Pro	His	Ile	Ile	Gln	Lys	Thr	Leu	Ala	Gly	Gln	Tyr	Ser	Lys		
			245						250					255			
cag	atg	aag	aat	ctc	agc	aca	gat	ggc	agt	gac	cag	tgg	cct	ttg	cta		816
Gln	Met	Lys	Asn	Leu	Ser	Thr	Asp	Gly	Ser	Asp	Gln	Trp	Pro	Leu	Leu		
			260					265					270				
cct	gaa	tta	caa	tgg	ctg	aga	aga	agg	aaa	aga	gcg	gtc	aat	cca	tct		864
Pro	Glu	Leu	Gln	Trp	Leu	Arg	Arg	Arg	Lys	Arg	Ala	Val	Asn	Pro	Ser		
	275					280						285					
cgt	ggt	gtg	ttt	gaa	gaa	atg	aag	tat	ttg	gag	ctt	atg	att	gtt	aat		912
Arg	Gly	Val	Phe	Glu	Glu	Met	Lys	Tyr	Leu	Glu	Leu	Met	Ile	Val	Asn		
	290					295				300							
gat	cac	aag	acg	tat	aag	aag	cac	cgc	tct	tct	cac	gcg	cat	acc	aac		960
Asp	His	Lys	Thr	Tyr	Lys	Lys	His	Arg	Ser	Ser	His	Ala	His	Thr	Asn		
305					310				315					320			
aac	ttc	gca	aag	tct	gtg	gtc	aac	ctt	gta	gat	tct	att	tac	aag	gaa		1008
Asn	Phe	Ala	Lys	Ser	Val	Val	Asn	Leu	Val	Asp	Ser	Ile	Tyr	Lys	Glu		
			325					330						335			
cag	ctc	aac	acc	agg	gtt	gtc	ctg	gtg	gct	gtc	gag	acc	tgg	acc	gag		1056
Gln	Leu	Asn	Thr	Arg	Val	Val	Leu	Val	Ala	Val	Glu	Thr	Trp	Thr	Glu		
			340				345						350				
aag	gat	cac	att	gac	atc	acc	atc	aac	ccc	gtg	cag	atg	cta	cat	gac		1104
Lys	Asp	His	Ile	Asp	Ile	Thr	Ile	Asn	Pro	Val	Gln	Met	Leu	His	Asp		
		355				360					365						
ttc	tcc	aag	tac	cgg	cag	cga	atc	aaa	cag	cac	gct	gac	gcg	gtc	cac		1152
Phe	Ser	Lys	Tyr	Arg	Gln	Arg	Ile	Lys	Gln	His	Ala	Asp	Ala	Val	His		
	370				375				380								
ctc	atc	tcg	cgc	gtg	aca	ttc	cat	tat	aag	aga	agc	agt	ctg	agt	tac		1200
Leu	Ile	Ser	Arg	Val	Thr	Phe	His	Tyr	Lys	Arg	Ser	Ser	Leu	Ser	Tyr		
385					390				395						400		

44/64

ttt gga ggc gtg tgt tct cga ata aga ggg gtt ggt gtg aat gag tat	1248
Phe Gly Gly Val Cys Ser Arg Ile Arg Gly Val Gly Val Asn Glu Tyr	
405 410 415	
ggg ctt cca atg gcg gtg gca caa gta tta tca cag agc ctg gct caa	1296
Gly Leu Pro Met Ala Val Ala Gln Val Leu Ser Gln Ser Leu Ala Gln	
420 425 430	
aac ctt gga atc cag tgg gaa cct tcg agc agg aag cca aaa tgt gaa	1344
Asn Leu Gly Ile Gln Trp Glu Pro Ser Ser Arg Lys Pro Lys Cys Glu	
435 440 445	
tgc ata gag tcc tgg ggc ggc tgc atc atg gaa gaa aca ggg gtg tcc	1392
Cys Ile Glu Ser Trp Gly Gly Cys Ile Met Glu Glu Thr Gly Val Ser	
450 455 460	
cac tct cga aag ttc tca aag tgc agc att ttg gag tac aga gac ttt	1440
His Ser Arg Lys Phe Ser Lys Cys Ser Ile Leu Glu Tyr Arg Asp Phe	
465 470 475 480	
tta cag aga ggt ggc gga gca tgt ctt ttc aat agg cca act aag ctg	1488
Leu Gln Arg Gly Gly Gly Ala Cys Leu Phe Asn Arg Pro Thr Lys Leu	
485 490 495	
ttt gag ccc acg gaa tgt gga aat gga tat gtg gag gcc ggg gag gaa	1536
Phe Glu Pro Thr Glu Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu Glu	
500 505 510	
tgc gac tgt ggt ttc cat gtg gaa tgc tat gga gtt tgc tgt aag aag	1584
Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Val Cys Cys Lys Lys	
515 520 525	
tgt tcg ctc tcc aat ggg gcc cac tgc agt gac ggc ccc tgc tgt aac	1632
Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys Asn	
530 535 540	
aac acc tca tgt ctt ttt cag tca cga ggg tat gaa tgt cgg gat gcc	1680
Asn Thr Ser Cys Leu Phe Gln Ser Arg Gly Tyr Glu Cys Arg Asp Ala	
545 550 555 560	
gta aac agc tgt gat atc acc gag tac tgc act gga gac tct ggc cag	1728
Val Asn Ser Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly Gln	
565 570 575	
tgc cca ccg aac ctc cat aaa caa gat ggc tat agc tgc aat caa aat	1776

45/64

Cys	Pro	Pro	Asn	Leu	His	Lys	Gln	Asp	Gly	Tyr	Ser	Cys	Asn	Gln	Asn		
			580					585					590				
cag	ggt	cgc	tgc	tac	aat	ggc	gag	tgc	aag	aca	agg	gac	aat	caa	tgc		1824
Gln	Gly	Arg	Cys	Tyr	Asn	Gly	Glu	Cys	Lys	Thr	Arg	Asp	Asn	Gln	Cys		
		595					600					605					
cag	tac	atc	tgg	ggg	aca	aag	gct	gcg	ggg	tca	gac	aag	ttc	tgc	tat		1872
Gln	Tyr	Ile	Trp	Gly	Thr	Lys	Ala	Ala	Gly	Ser	Asp	Lys	Phe	Cys	Tyr		
	610					615					620						
gaa	aag	ctg	aac	acg	gaa	ggc	acc	gag	aag	ggc	aat	tgt	gga	aag	gat		1920
Glu	Lys	Leu	Asn	Thr	Glu	Gly	Thr	Glu	Lys	Gly	Asn	Cys	Gly	Lys	Asp		
625					630					635					640		
gga	gac	cgg	tgg	atc	ccg	tgc	agc	aag	cat	gat	gtg	ttc	tgt	gga	ttt		1968
Gly	Asp	Arg	Trp	Ile	Pro	Cys	Ser	Lys	His	Asp	Val	Phe	Cys	Gly	Phe		
			645						650					655			
ctg	ctt	tgc	acc	aat	ctt	acc	cga	gct	cca	cgt	atc	ggt	caa	ctt	caa		2016
Leu	Leu	Cys	Thr	Asn	Leu	Thr	Arg	Ala	Pro	Arg	Ile	Gly	Gln	Leu	Gln		
			660					665					670				
gga	gag	atc	atc	ccg	act	tcc	ttc	tat	cat	caa	ggc	cga	gtg	att	gac		2064
Gly	Glu	Ile	Ile	Pro	Thr	Ser	Phe	Tyr	His	Gln	Gly	Arg	Val	Ile	Asp		
		675					680					685					
tgc	agt	ggt	gct	cat	gta	gtt	tta	gac	gat	gat	aca	gac	gtg	ggt	tac		2112
Cys	Ser	Gly	Ala	His	Val	Val	Leu	Asp	Asp	Asp	Thr	Asp	Val	Gly	Tyr		
	690					695					700						
gtt	gaa	gat	ggg	act	ccg	tgt	ggc	ccc	tcc	atg	atg	tgc	tta	gat	cgg		2160
Val	Glu	Asp	Gly	Thr	Pro	Cys	Gly	Pro	Ser	Met	Met	Cys	Leu	Asp	Arg		
705					710					715					720		
aag	tgc	cta	cag	att	caa	gcc	ctg	aat	atg	agc	agc	tgc	cca	ctt	gac		2208
Lys	Cys	Leu	Gln	Ile	Gln	Ala	Leu	Asn	Met	Ser	Ser	Cys	Pro	Leu	Asp		
			725					730					735				
tca	agg	ggt	aaa	gtc	tgc	tcc	ggc	cac	ggg	gtg	tgt	agc	aac	gaa	gcc		2256
Ser	Arg	Gly	Lys	Val	Cys	Ser	Gly	His	Gly	Val	Cys	Ser	Asn	Glu	Ala		
			740				745						750				
acc	tgc	atc	tgt	gat	ttc	act	tgg	gca	ggc	aca	gac	tgc	agc	atc	cgg		2304
Thr	Cys	Ile	Cys	Asp	Phe	Thr	Trp	Ala	Gly	Thr	Asp	Cys	Ser	Ile	Arg		
		755					760					765					

46/64

gat cca gtt cgg aac ccc aac ccc cct aag gat gaa ggc cct aag ggt 2352
 Asp Pro Val Arg Asn Pro Asn Pro Pro Lys Asp Glu Gly Pro Lys Gly
 770 775 780

cct agc gcc acc aat ctc ata ata ggc tcc atc gct ggt gcc atc ctg 2400
 Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly Ala Ile Leu
 785 790 795 800

gta gca gct att gtc ctt ggg ggc aca ggc tgg gga ttt aaa aac gtc 2448
 Val Ala Ala Ile Val Leu Gly Gly Thr Gly Trp Gly Phe Lys Asn Val
 805 810 815

aag aag agg aga ttc gat ccc act cag caa ggc ccc atc tga 2490
 Lys Lys Arg Arg Phe Asp Pro Thr Gln Gln Gly Pro Ile
 820 825

<210> 27

<211> 829

<212> PRT

<213> Mus musculus

<400> 27

Met Lys Pro Pro Gly Ser Ile Ser Arg Arg Pro Thr Leu Thr Gly Cys
 1 5 10 15

Ser Leu Pro Gly Ala Ser Cys Gly Pro Gly Arg Cys Pro Ala Gly Pro
 20 25 30

Val Pro Ala Arg Ala Pro Pro Cys Arg Leu Leu Leu Val Leu Leu Leu
 35 40 45

Leu Pro Ala Leu Ala Thr Ser Ser Arg Pro Arg Ala Arg Gly Ala Ala
 50 55 60

Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu Lys Thr Leu Gly
 65 70 75 80

Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn Ser Ser Ser Arg
 85 90 95

47/64

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

Arg Leu Val Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro Tyr His Val
115 120 125

Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys Ala Val His
130 135 140

Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser Lys Phe Ile
145 150 155 160

Leu Asp Leu Thr Leu Asn Asn Gly Leu Leu Ser Ser Asp Tyr Val Glu
165 170 175

Ile His Tyr Glu Asp Gly Lys Gln Met Tyr Ser Lys Gly Gly Glu His
180 185 190

Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser Arg Val Ala
195 200 205

Leu Ser Thr Cys Asn Gly Leu His Gly Met Phe Glu Asp Asp Thr Phe
210 215 220

Val Tyr Met Ile Glu Pro Leu Glu Leu Thr Asp Asp Glu Lys Ser Thr
225 230 235 240

Gly Arg Pro His Ile Ile Gln Lys Thr Leu Ala Gly Gln Tyr Ser Lys
245 250 255

Gln Met Lys Asn Leu Ser Thr Asp Gly Ser Asp Gln Trp Pro Leu Leu
260 265 270

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Pro Glu Leu Gln Trp Leu Arg Arg Arg Lys Arg Ala Val Asn Pro Ser
275 280 285

Arg Gly Val Phe Glu Glu Met Lys Tyr Leu Glu Leu Met Ile Val Asn
290 295 300

Asp His Lys Thr Tyr Lys Lys His Arg Ser Ser His Ala His Thr Asn
305 310 315 320

Asn Phe Ala Lys Ser Val Val Asn Leu Val Asp Ser Ile Tyr Lys Glu
325 330 335

Gln Leu Asn Thr Arg Val Val Leu Val Ala Val Glu Thr Trp Thr Glu
340 345 350

Lys Asp His Ile Asp Ile Thr Ile Asn Pro Val Gln Met Leu His Asp
355 360 365

Phe Ser Lys Tyr Arg Gln Arg Ile Lys Gln His Ala Asp Ala Val His
370 375 380

Leu Ile Ser Arg Val Thr Phe His Tyr Lys Arg Ser Ser Leu Ser Tyr
385 390 395 400

Phe Gly Gly Val Cys Ser Arg Ile Arg Gly Val Gly Val Asn Glu Tyr
405 410 415

Gly Leu Pro Met Ala Val Ala Gln Val Leu Ser Gln Ser Leu Ala Gln
420 425 430

Asn Leu Gly Ile Gln Trp Glu Pro Ser Ser Arg Lys Pro Lys Cys Glu
435 440 445

Cys Ile Glu Ser Trp Gly Gly Cys Ile Met Glu Glu Thr Gly Val Ser
450 455 460

49/64

His Ser Arg Lys Phe Ser Lys Cys Ser Ile Leu Glu Tyr Arg Asp Phe
465 470 475 480

Leu Gln Arg Gly Gly Gly Ala Cys Leu Phe Asn Arg Pro Thr Lys Leu
485 490 495

Phe Glu Pro Thr Glu Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu Glu
500 505 510

Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Val Cys Cys Lys Lys
515 520 525

Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys Asn
530 535 540

Asn Thr Ser Cys Leu Phe Gln Ser Arg Gly Tyr Glu Cys Arg Asp Ala
545 550 555 560

Val Asn Ser Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly Gln
565 570 575

Cys Pro Pro Asn Leu His Lys Gln Asp Gly Tyr Ser Cys Asn Gln Asn
580 585 590

Gln Gly Arg Cys Tyr Asn Gly Glu Cys Lys Thr Arg Asp Asn Gln Cys
595 600 605

Gln Tyr Ile Trp Gly Thr Lys Ala Ala Gly Ser Asp Lys Phe Cys Tyr
610 615 620

Glu Lys Leu Asn Thr Glu Gly Thr Glu Lys Gly Asn Cys Gly Lys Asp
625 630 635 640

50/64

Gly Asp Arg Trp Ile Pro Cys Ser Lys His Asp Val Phe Cys Gly Phe
645 650 655

Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly Gln Leu Gln
660 665 670

Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg Val Ile Asp
675 680 685

Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp Val Gly Tyr
690 695 700

Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys Leu Asp Arg
705 710 715 720

Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys Pro Leu Asp
725 730 735

Ser Arg Gly Lys Val Cys Ser Gly His Gly Val Cys Ser Asn Glu Ala
740 745 750

Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys Ser Ile Arg
755 760 765

Asp Pro Val Arg Asn Pro Asn Pro Pro Lys Asp Glu Gly Pro Lys Gly
770 775 780

Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly Ala Ile Leu
785 790 795 800

Val Ala Ala Ile Val Leu Gly Gly Thr Gly Trp Gly Phe Lys Asn Val
805 810 815

Lys Lys Arg Arg Phe Asp Pro Thr Gln Gln Gly Pro Ile
820 825

51/64

<210> 28
 <211> 2499
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(2496)

<400> 28
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 Met Lys Pro Pro Gly Ser Ser Ser Arg Gln Pro Pro Leu Ala Gly Cys
 1 5 10 15

agc ctt gcc ggc gct tcc tgc ggc ccc caa cgc ggc ccc gcc ggc tcg 96
 Ser Leu Ala Gly Ala Ser Cys Gly Pro Gln Arg Gly Pro Ala Gly Ser
 20 25 30

gtg cct gcc agc gcc ccg gcc cgc acg ccg ccc tgc cgc ctg ctt ctc 144
 Val Pro Ala Ser Ala Pro Ala Arg Thr Pro Pro Cys Arg Leu Leu Leu
 35 40 45

gtc ctt ctc ctg ctg cct ccg ctc gcc gcc tcg tcc cgg ccc cgc gcc 192
 Val Leu Leu Leu Leu Pro Pro Leu Ala Ala Ser Ser Arg Pro Arg Ala
 50 55 60

tgg ggg gct gct gcg ccc agc gct ccg cat tgg aat gaa act gca gaa 240
 Trp Gly Ala Ala Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu
 65 70 75 80

aaa aat ttg gga gtc ctg gca gat gaa gac aat aca ttg caa cag aat 288
 Lys Asn Leu Gly Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn
 85 90 95

agc agc agt aat atc agt tac agc aat gca atg cag aaa gaa atc aca 336
 Ser Ser Ser Asn Ile Ser Tyr Ser Asn Ala Met Gln Lys Glu Ile Thr
 100 105 110

ctg cct tca aga ctc ata tat tac atc aac caa gac tcg gaa agc cct 384
 Leu Pro Ser Arg Leu Ile Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro
 115 120 125

tat cac gtt ctt gac aca aag gca aga cac cag caa aaa cat aat aag 432
 Tyr His Val Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys

52/64

130	135	140	
gct gtc cat ctg gcc cag gca agc ttc cag att gaa gcc ttc ggc tcc			480
Ala Val His Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser			
145	150	155	160
aaa ttc att ctt gac ctc ata ctg aac aat ggt ttg ttg tct tct gat			528
Lys Phe Ile Leu Asp Leu Ile Leu Asn Asn Gly Leu Leu Ser Ser Asp			
	165	170	175
tat gtg gag att cac tac gaa aat ggg aaa cca cag tac tct aag ggt			576
Tyr Val Glu Ile His Tyr Glu Asn Gly Lys Pro Gln Tyr Ser Lys Gly			
	180	185	190
gga gag cac tgt tac tac cat gga agc atc aga ggc gtc aaa gac tcc			624
Gly Glu His Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser			
	195	200	205
aag gtg gct ctg tca acc tgc aat gga ctt cat ggc atg ttt gaa gat			672
Lys Val Ala Leu Ser Thr Cys Asn Gly Leu His Gly Met Phe Glu Asp			
	210	215	220
gat acc ttc gtg tat atg ata gag cca cta gag ctg gtt cat gat gag			720
Asp Thr Phe Val Tyr Met Ile Glu Pro Leu Glu Leu Val His Asp Glu			
225	230	235	240
aaa agc aca ggt cga cca cat ata atc cag aaa acc ttg gca gga cag			768
Lys Ser Thr Gly Arg Pro His Ile Ile Gln Lys Thr Leu Ala Gly Gln			
	245	250	255
tat tct aag caa atg aag aat ctc act atg gaa aga ggt gac cag tgg			816
Tyr Ser Lys Gln Met Lys Asn Leu Thr Met Glu Arg Gly Asp Gln Trp			
	260	265	270
ccc ttt ctc tct gaa tta cag tgg ttg aaa aga agg aag aga gca gtg			864
Pro Phe Leu Ser Glu Leu Gln Trp Leu Lys Arg Arg Lys Arg Ala Val			
	275	280	285
aat cca tca cgt ggt ata ttt gaa gaa atg aaa tat ttg gaa ctt atg			912
Asn Pro Ser Arg Gly Ile Phe Glu Glu Met Lys Tyr Leu Glu Leu Met			
	290	295	300
att gtt aat gat cac aaa acg tat aag aag cat cgc tct tct cat gca			960
Ile Val Asn Asp His Lys Thr Tyr Lys Lys His Arg Ser Ser His Ala			
305	310	315	320

53/64

cat acc aac aac ttt gca aag tcc gtg gtc aac ctt gtg gat tct att	1008
His Thr Asn Asn Phe Ala Lys Ser Val Val Asn Leu Val Asp Ser Ile	
325 330 335	
tac aag gag cag ctc aac acc agg gtt gtc ctg gtg gct gta gag acc	1056
Tyr Lys Glu Gln Leu Asn Thr Arg Val Val Leu Val Ala Val Glu Thr	
340 345 350	
tgg act gag aag gat cag att gac atc acc acc aac cct gtg cag atg	1104
Trp Thr Glu Lys Asp Gln Ile Asp Ile Thr Thr Asn Pro Val Gln Met	
355 360 365	
ctc cat gag ttc tca aaa tac cgg cag cgc att aag cag cat gct gat	1152
Leu His Glu Phe Ser Lys Tyr Arg Gln Arg Ile Lys Gln His Ala Asp	
370 375 380	
gct gtg cac ctc atc tcg cgg gtg aca ttt cac tat aag aga agc agt	1200
Ala Val His Leu Ile Ser Arg Val Thr Phe His Tyr Lys Arg Ser Ser	
385 390 395 400	
ctg agt tac ttt gga ggt gtc tgt tct cgc aca aga gga gtt ggt gtg	1248
Leu Ser Tyr Phe Gly Gly Val Cys Ser Arg Thr Arg Gly Val Gly Val	
405 410 415	
aat gag tat ggt ctt cca atg gca gtg gca caa gta tta tcg cag agc	1296
Asn Glu Tyr Gly Leu Pro Met Ala Val Ala Gln Val Leu Ser Gln Ser	
420 425 430	
ctg gct caa aac ctt gga atc caa tgg gaa cct tct agc aga aag cca	1344
Leu Ala Gln Asn Leu Gly Ile Gln Trp Glu Pro Ser Ser Arg Lys Pro	
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aaa tgt gac tgc aca gaa tcc tgg ggt ggc tgc atc atg gag gaa aca	1392
Lys Cys Asp Cys Thr Glu Ser Trp Gly Gly Cys Ile Met Glu Glu Thr	
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ggg gtg tcc cat tct cga aaa ttt tca aag tgc agc att ttg gag tat	1440
Gly Val Ser His Ser Arg Lys Phe Ser Lys Cys Ser Ile Leu Glu Tyr	
465 470 475 480	
aga gac ttt tta cag aga gga ggt gga gcc tgc ctt ttc aac agg cca	1488
Arg Asp Phe Leu Gln Arg Gly Gly Gly Ala Cys Leu Phe Asn Arg Pro	
485 490 495	
aca aag cta ttt gag ccc acg gaa tgt gga aat gga tac gtg gaa gct	1536
Thr Lys Leu Phe Glu Pro Thr Glu Cys Gly Asn Gly Tyr Val Glu Ala	

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ggg	gag	gag	tgt	gat	tgt	ggt	ttt	cat	gtg	gaa	tgc	tat	gga	tta	tgc	1584
Gly	Glu	Glu	Cys	Asp	Cys	Gly	Phe	His	Val	Glu	Cys	Tyr	Gly	Leu	Cys	
		515					520					525				
tgt	aag	aaa	tgt	tcc	ctc	tcc	aac	ggg	gct	cac	tgc	agc	gac	ggg	ccc	1632
Cys	Lys	Lys	Cys	Ser	Leu	Ser	Asn	Gly	Ala	His	Cys	Ser	Asp	Gly	Pro	
		530					535				540					
tgc	tgt	aac	aat	acc	tca	tgt	ctt	ttt	cag	cca	cga	ggg	tat	gaa	tgc	1680
Cys	Cys	Asn	Asn	Thr	Ser	Cys	Leu	Phe	Gln	Pro	Arg	Gly	Tyr	Glu	Cys	
545						550				555					560	
cgg	gat	gct	gtg	aac	gag	tgt	gat	att	act	gaa	tat	tgt	act	gga	gac	1728
Arg	Asp	Ala	Val	Asn	Glu	Cys	Asp	Ile	Thr	Glu	Tyr	Cys	Thr	Gly	Asp	
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tct	ggt	cag	tgc	cca	cca	aat	ctt	cat	aag	caa	gac	gga	tat	gca	tgc	1776
Ser	Gly	Gln	Cys	Pro	Pro	Asn	Leu	His	Lys	Gln	Asp	Gly	Tyr	Ala	Cys	
			580					585					590			
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Asn	Gln	Asn	Gln	Gly	Arg	Cys	Tyr	Asn	Gly	Glu	Cys	Lys	Thr	Arg	Asp	
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Asn	Gln	Cys	Gln	Tyr	Ile	Trp	Gly	Thr	Lys	Ala	Ala	Gly	Ser	Asp	Lys	
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ttc	tgc	tat	gaa	aag	ctg	aat	aca	gaa	ggc	act	gag	aag	gga	aac	tgc	1920
Phe	Cys	Tyr	Glu	Lys	Leu	Asn	Thr	Glu	Gly	Thr	Glu	Lys	Gly	Asn	Cys	
625						630				635					640	
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Cys	Gly	Phe	Leu	Leu	Cys	Thr	Asn	Leu	Thr	Arg	Ala	Pro	Arg	Ile	Gly	
			660					665					670			
caa	ctt	cag	ggt	gag	atc	att	cca	act	tcc	ttc	tac	cat	caa	ggc	cgg	2064
Gln	Leu	Gln	Gly	Glu	Ile	Ile	Pro	Thr	Ser	Phe	Tyr	His	Gln	Gly	Arg	
		675					680					685				

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gtg att gac tgc agt ggt gcc cat gta gtt tta gat gat gat acg gat	2112
Val Ile Asp Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp	
690 695 700	
gtg ggc tat gta gaa gat gga acg cca tgt ggc ccg tct atg atg tgt	2160
Val Gly Tyr Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys	
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tta gat cgg aag tgc cta caa att caa gcc cta aat atg agc agc tgt	2208
Leu Asp Arg Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys	
725 730 735	
cca ctc gat tcc aag ggt aaa gtc tgt tgc ggc cat ggg gtg tgt agt	2256
Pro Leu Asp Ser Lys Gly Lys Val Cys Ser Gly His Gly Val Cys Ser	
740 745 750	
aat gaa gcc acc tgc att tgt gat ttc acc tgg gca ggg aca gat tgc	2304
Asn Glu Ala Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys	
755 760 765	
agt atc cgg gat cca gtt agg aac ctt cac ccc ccc aag gat gaa gga	2352
Ser Ile Arg Asp Pro Val Arg Asn Leu His Pro Pro Lys Asp Glu Gly	
770 775 780	
ccc aag ggt cct agt gcc acc aat ctc ata ata ggc tcc atc gct ggt	2400
Pro Lys Gly Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly	
785 790 795 800	
gcc atc ctg gta gca gct att gtc ctt ggg ggc aca ggc tgg gga ttt	2448
Ala Ile Leu Val Ala Ala Ile Val Leu Gly Gly Thr Gly Trp Gly Phe	
805 810 815	
aaa aat gtc aag aag aga agg ttc gat cct act cag caa ggc ccc atc	2496
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Val	Pro	Ala	Ser	Ala	Pro	Ala	Arg	Thr	Pro	Pro	Cys	Arg	Leu	Leu	Leu		
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Val	Leu	Leu	Leu	Leu	Pro	Pro	Leu	Ala	Ala	Ser	Ser	Arg	Pro	Arg	Ala		
	50					55						60					
Trp	Gly	Ala	Ala	Ala	Pro	Ser	Ala	Pro	His	Trp	Asn	Glu	Thr	Ala	Glu		
65						70				75					80		
Lys	Asn	Leu	Gly	Val	Leu	Ala	Asp	Glu	Asp	Asn	Thr	Leu	Gln	Gln	Asn		
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			100					105					110				
Leu	Pro	Ser	Arg	Leu	Ile	Tyr	Tyr	Ile	Asn	Gln	Asp	Ser	Glu	Ser	Pro		
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Tyr	His	Val	Leu	Asp	Thr	Lys	Ala	Arg	His	Gln	Gln	Lys	His	Asn	Lys		
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Lys	Phe	Ile	Leu	Asp	Leu	Ile	Leu	Asn	Asn	Gly	Leu	Leu	Ser	Ser	Asp		
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Tyr	Val	Glu	Ile	His	Tyr	Glu	Asn	Gly	Lys	Pro	Gln	Tyr	Ser	Lys	Gly		
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Gly Glu His Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser
195 200 205

Lys Val Ala Leu Ser Thr Cys Asn Gly Leu His Gly Met Phe Glu Asp
210 215 220

Asp Thr Phe Val Tyr Met Ile Glu Pro Leu Glu Leu Val His Asp Glu
225 230 235 240

Lys Ser Thr Gly Arg Pro His Ile Ile Gln Lys Thr Leu Ala Gly Gln
245 250 255

Tyr Ser Lys Gln Met Lys Asn Leu Thr Met Glu Arg Gly Asp Gln Trp
260 265 270

Pro Phe Leu Ser Glu Leu Gln Trp Leu Lys Arg Arg Lys Arg Ala Val
275 280 285

Asn Pro Ser Arg Gly Ile Phe Glu Glu Met Lys Tyr Leu Glu Leu Met
290 295 300

Ile Val Asn Asp His Lys Thr Tyr Lys Lys His Arg Ser Ser His Ala
305 310 315 320

His Thr Asn Asn Phe Ala Lys Ser Val Val Asn Leu Val Asp Ser Ile
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Tyr Lys Glu Gln Leu Asn Thr Arg Val Val Leu Val Ala Val Glu Thr
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Trp Thr Glu Lys Asp Gln Ile Asp Ile Thr Thr Asn Pro Val Gln Met
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Leu His Glu Phe Ser Lys Tyr Arg Gln Arg Ile Lys Gln His Ala Asp

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370

375

380

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Cys Cys Asn Asn Thr Ser Cys Leu Phe Gln Pro Arg Gly Tyr Glu Cys
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Cys Gly Phe Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly
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Gln Leu Gln Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg
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690 695 700

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740

745

750

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Pro Lys Gly Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly
 785 790 795 800

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64/64

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cggaattctc agatggggcc ttgctgagt

29

CLAIMS

1. A DNA coding for a polypeptide of the following (A) or (B):

(A) a polypeptide which comprises an amino acid
5 sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence
10 as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

2. The DNA according to claim 1, which is a DNA of the following (a) or (b):

15 (a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, and the nucleotide sequence of nucleotides 132
20 to 506 of SEQ ID NO: 24; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a probe prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or
25 survival of hematopoietic stem cells or hematopoietic progenitor cells.

3. The DNA according to claim 2, the stringent

condition is 6 x SSC, 5 x Denhardt, 0.5% SDS and 68°C
(SSC: 3 M NaCl, 0.3 M sodium citrate; 50 x Denhardt: 1%
BSA, 1% polyvinyl pyrrolidone, 1% Ficoll 400), or 6 x
SSC, 5 x Denhardt, 0.5% SDS, 50% formamide and 42°C.

5 4. A expression vector which comprises the DNA
of any one of claims 1 to 3 in such a manner that the
DNA can be expressed.

 5. A cell into which the DNA of any one of
claims 1 to 3 is introduced in such a manner that the
10 DNA can be expressed.

 6. A polypeptide which is an expression product
of the DNA of any one of claims 1 to 3, the polypeptide
having an activity to support proliferation or survival
of hematopoietic stem cells or hematopoietic progenitor
15 cells.

 7. The polypeptide according to claim 6, which
comprises an amino acid sequence selected from the group
consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID
NO: 25, or an amino acid sequence including deletion,
20 substitution or insertion of one or several amino acids
in the amino acid sequence.

 8. The polypeptide according to claim 6 or 7,
which is modified with one or more modifying agents
selected from the group consisting of polyethylene
25 glycol (PEG), dextran, poly(N-vinyl-pyrrolidone),
polypropylene glycol homopolymer, copolymer of
polypropylene oxide/ethylene oxide, polyoxyethylated

polyol and polyvinyl alcohol.

9. An monoclonal antibody which binds to the polypeptide of any one of claims 6 to 8.

10. A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of co-culturing stromal cells in which a DNA coding for a polypeptide of the following (A) or (B) is expressed, with hematopoietic stem cells or progenitor cells,

10 (A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

15 (B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or
20 hematopoietic progenitor cells.

11. The method according to claim 10, wherein the DNA is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide
25 sequence of nucleotides 1 to 1671 of SEQ ID NO: 8, the nucleotide sequence of nucleotides 1 to 1674 of SEQ ID NO: 10, the nucleotide sequence of nucleotides 1 to 366

of SEQ ID NO: 12, the nucleotide sequence of nucleotides
84 to 1121 of SEQ ID NO: 14, the nucleotide sequence of
nucleotides 1 to 1035 of SEQ ID NO: 16, the nucleotide
sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the
5 nucleotide sequence of nucleotides 1 to 444 of SEQ ID
NO: 20, the nucleotide sequence of nucleotides 630 to
1358 of SEQ ID NO: 22, the nucleotide sequence of
nucleotides 132 to 506 of SEQ ID NO: 24, the nucleotide
sequence of nucleotides 1 to 2487 of SEQ ID NO: 26, and
10 the nucleotide sequence of nucleotides 1 to 2496 of SEQ
ID NO: 28; or

(b) a DNA which is hybridizable with a DNA comprising
the nucleotide sequence as defined in (a) or a probe
prepared from said DNA, under the stringent condition,
15 and which has an activity to support proliferation or
survival of hematopoietic stem cells or hematopoietic
progenitor cells.

12. A method for supporting proliferation or
survival of hematopoietic stem cells or hematopoietic
20 progenitor cells, comprising the step of culturing
hematopoietic stem cells or progenitor cells in the
presence of a polypeptide of the following (A) or (B),
said polypeptide having an activity to support
proliferation or survival of hematopoietic stem cells or
25 hematopoietic progenitor cells when the hematopoietic
stem cells or hematopoietic progenitor cells are
cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, 5 SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support 10 proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

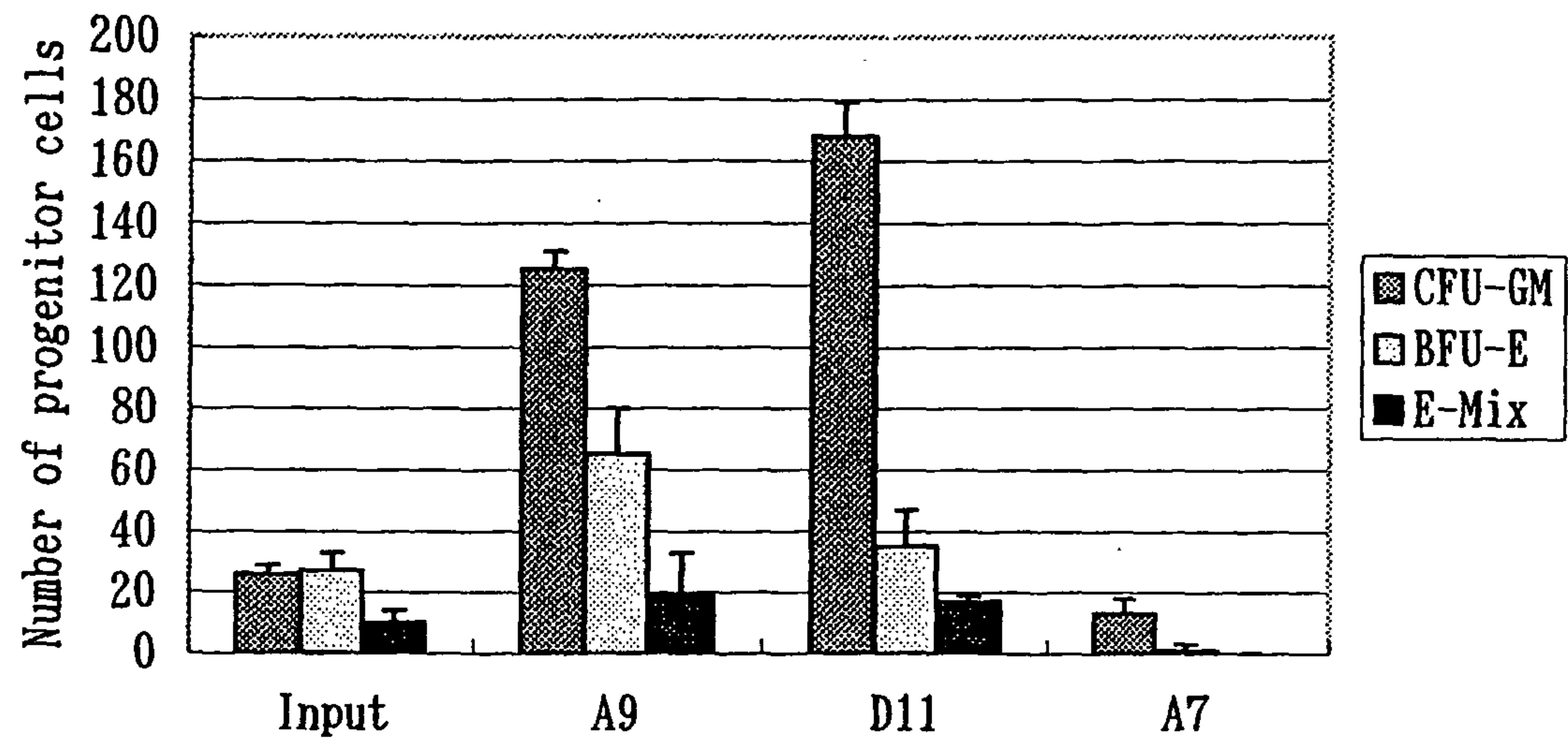
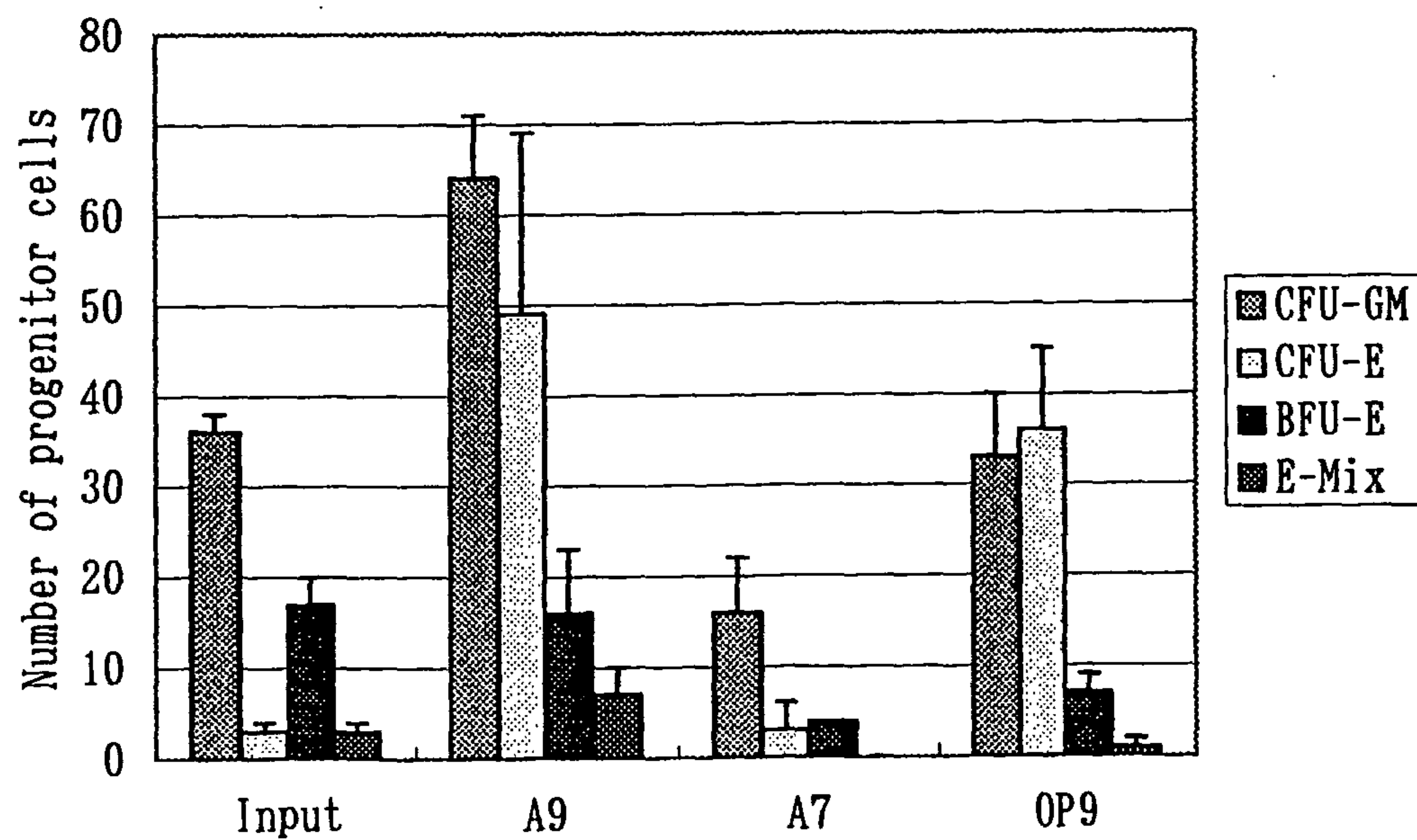
13. A pharmaceutical composition having an effect to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor 15 cells, which comprises an effective amount of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when hematopoietic stem cells or 20 hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ 25 ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

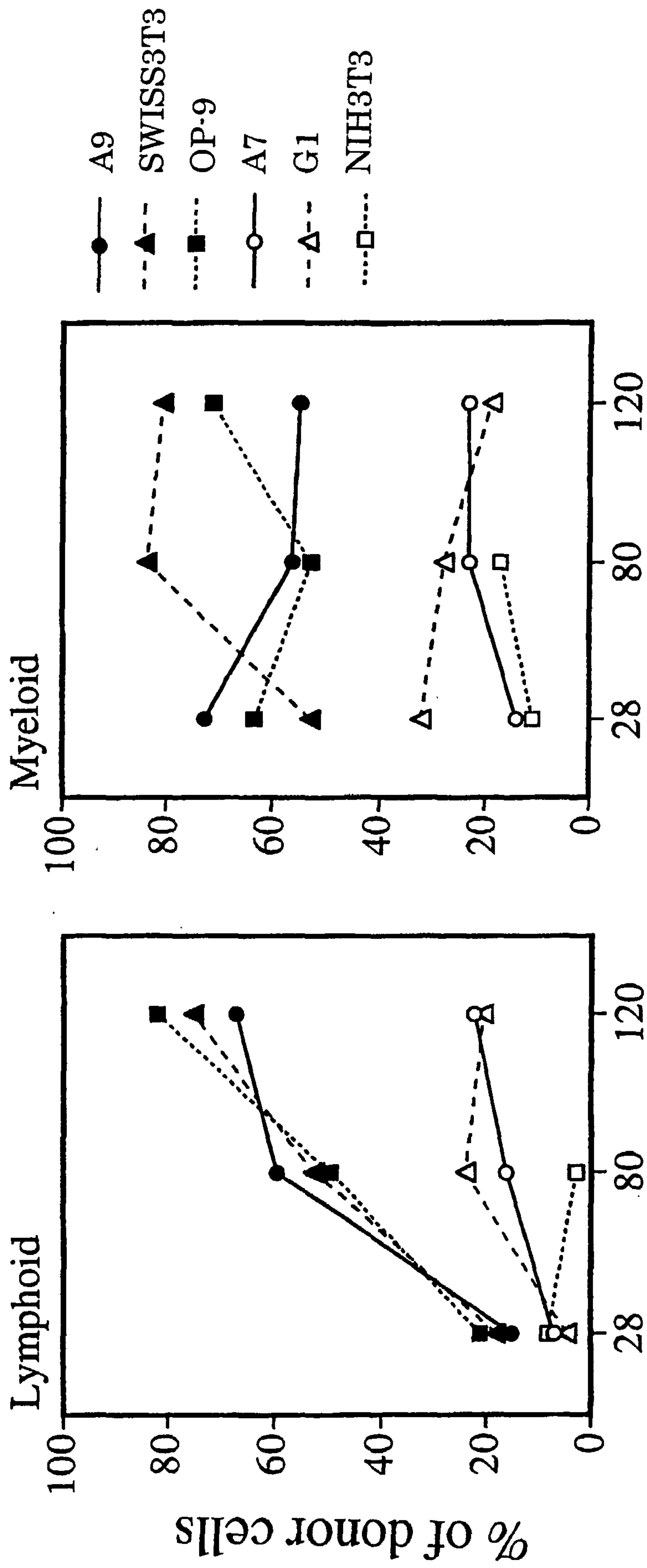
(B) a polypeptide which comprises an amino acid

sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or
5 hematopoietic progenitor cells.

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*Fig.1**Fig.2*

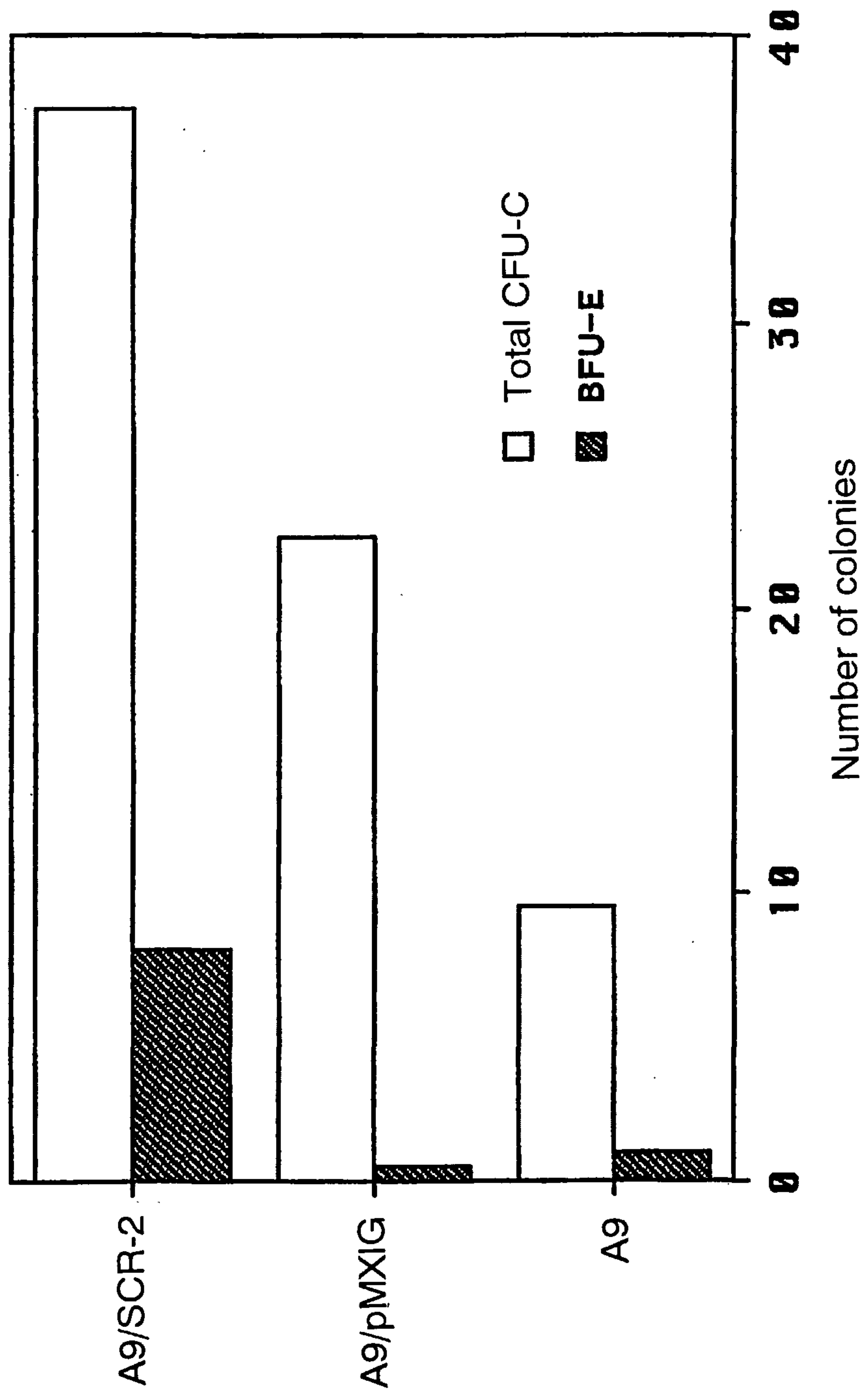
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Days after transplantation

Fig.3

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*Fig. 4*

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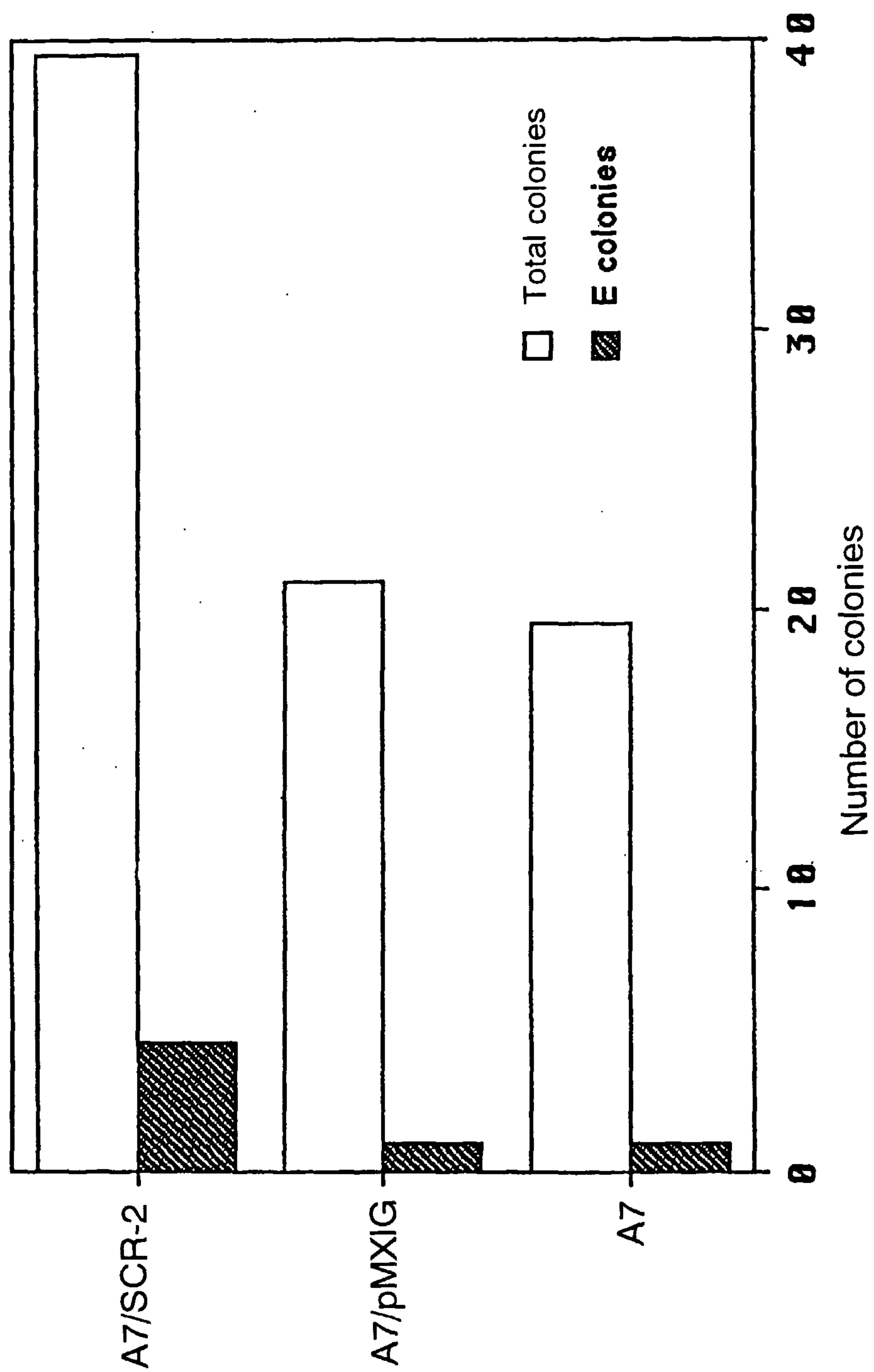


Fig. 5

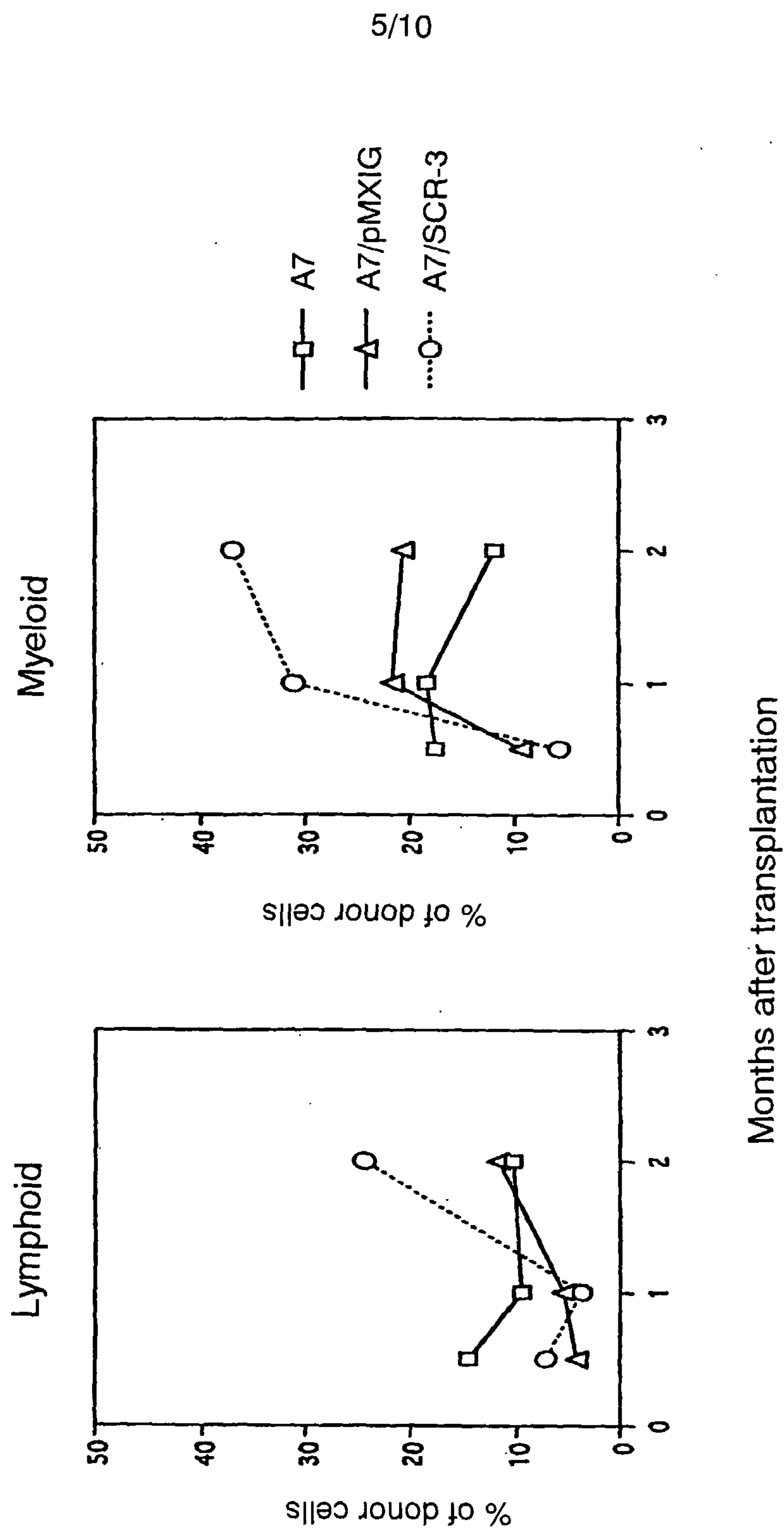
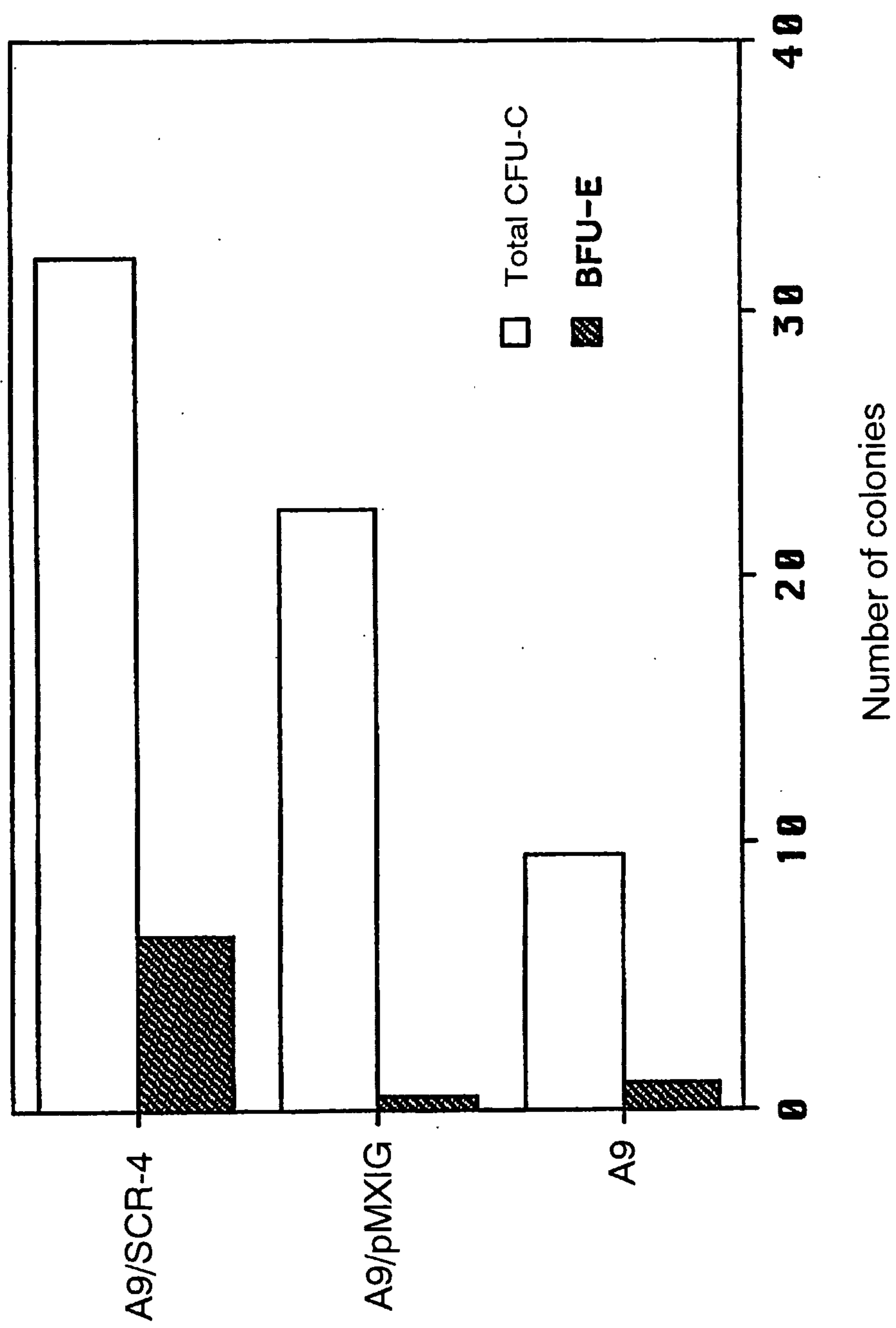


Fig. 6

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*Fig. 7*

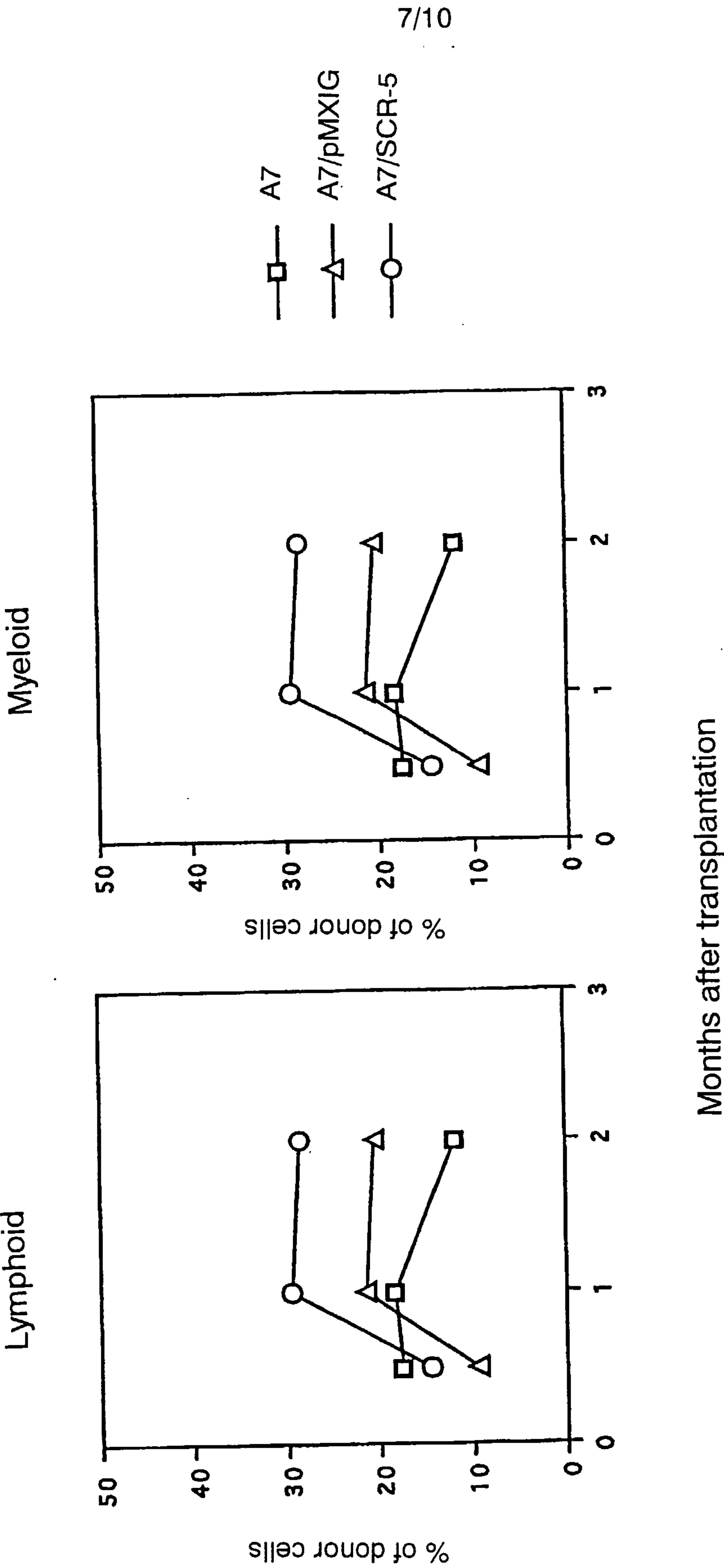
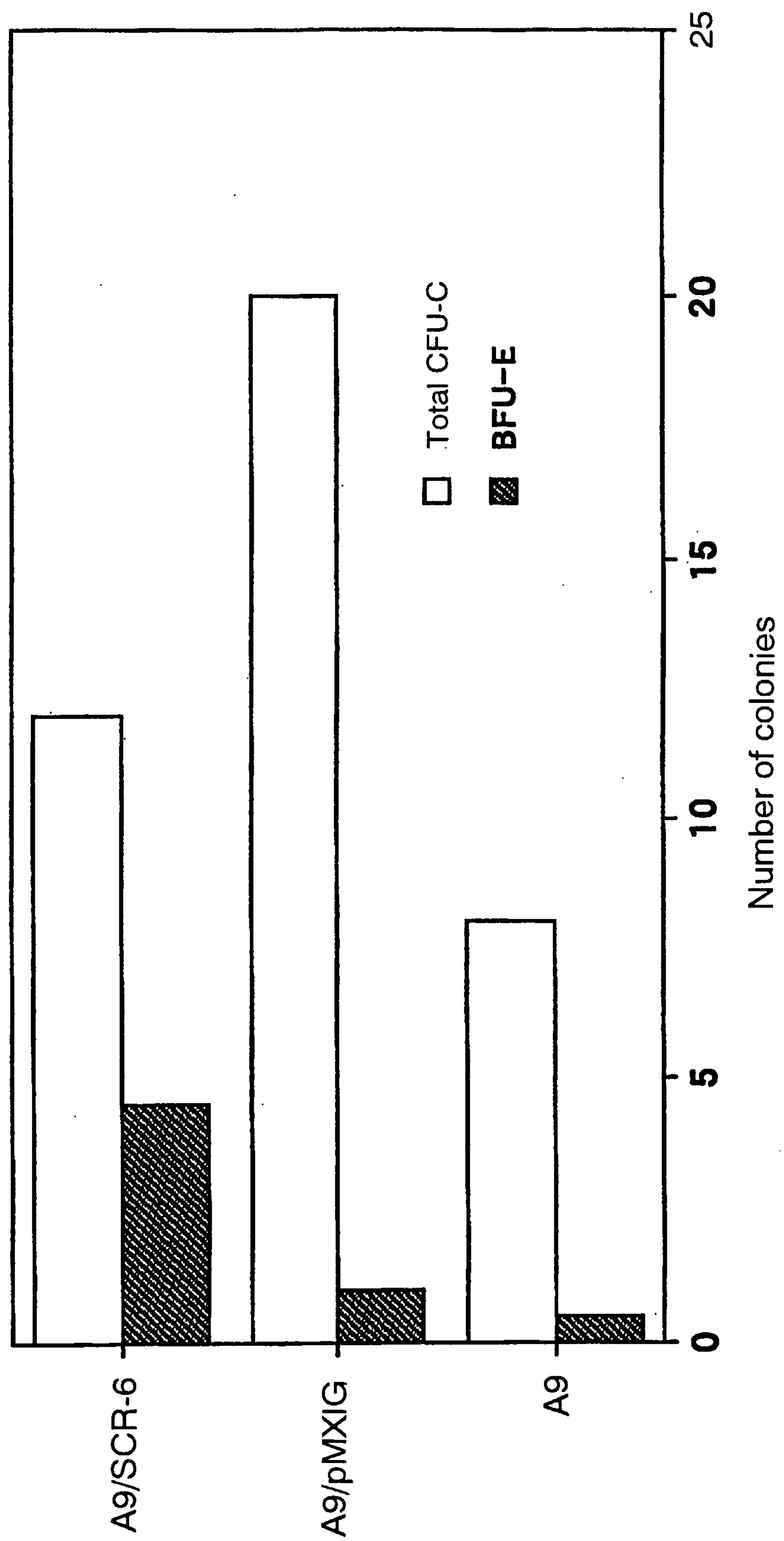


Fig. 8

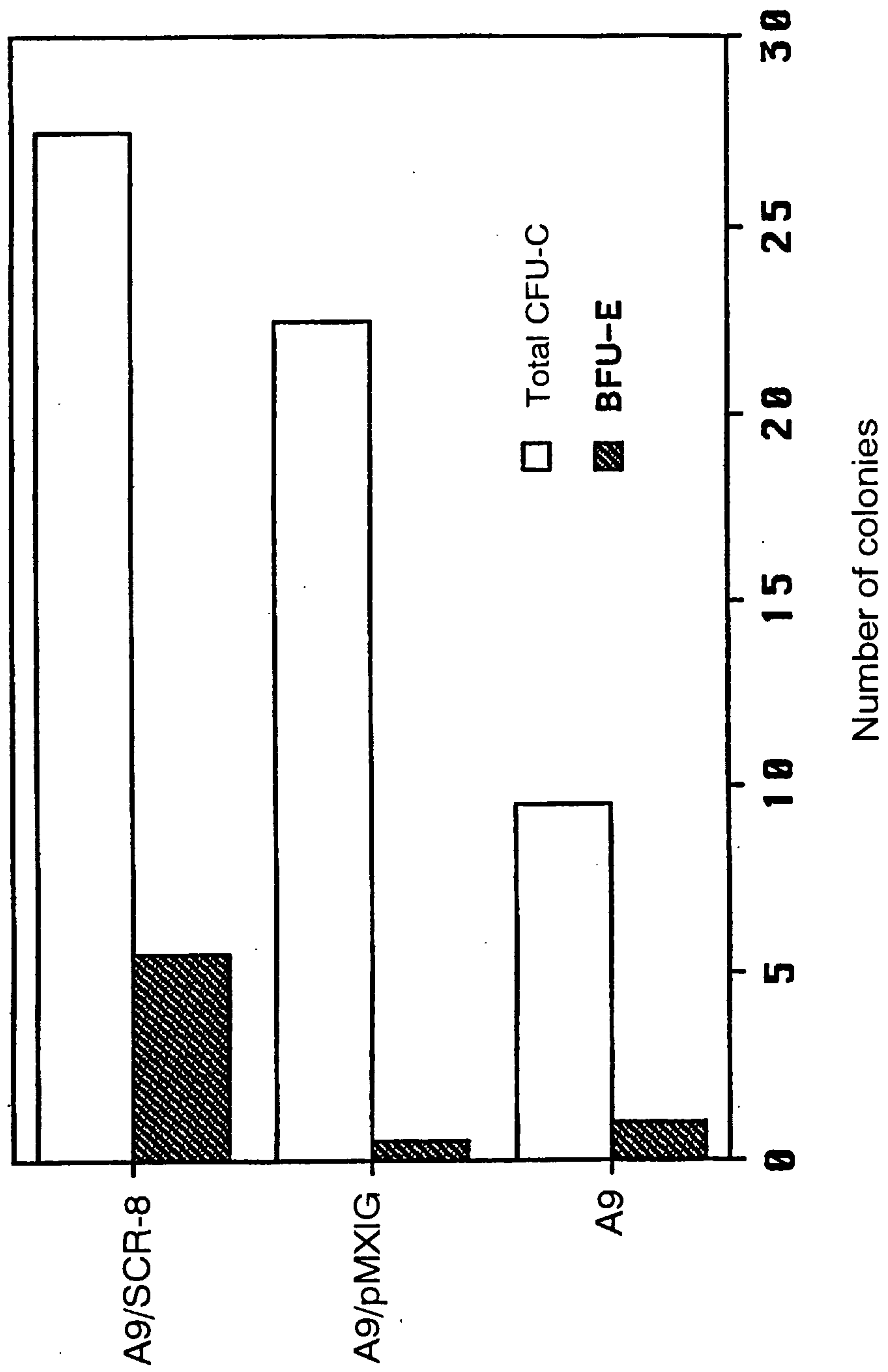
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*Fig. 9*

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*Fig. 10*

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*Fig. 11*