

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 February 2009 (12.02.2009)

PCT

(10) International Publication Number
WO 2009/019117 A1

(51) International Patent Classification:

C07K 14/31 (2006.01) **G01N 33/68** (2006.01)
C07K 14/65 (2006.01) **A61K 51/08** (2006.01)
A61K 38/30 (2006.01)

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(21) International Application Number:

PCT/EP2008/059391

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(22) International Filing Date: 17 July 2008 (17.07.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/963,223 3 August 2007 (03.08.2007) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(54) Title: IGF-1R BINDING POLYPEPTIDES AND THEIR USE

IGF1R binding Z variants on MCF-7 cells

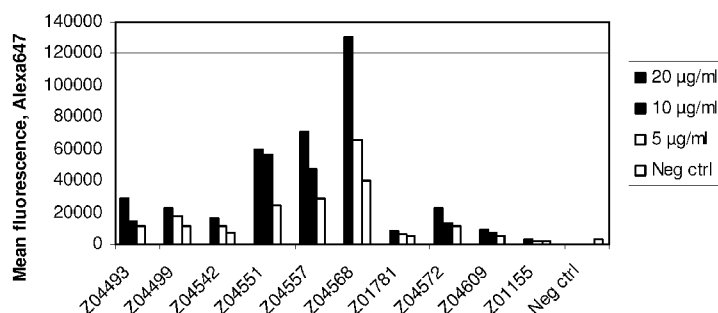


FIGURE 9

(57) Abstract: This invention relates to polypeptides which bind to IGF-1R and to applications of those polypeptides in medicine, veterinary medicine, diagnostics and imaging.

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IGF-1R BINDING POLYPEPTIDES AND THEIR USE

Field of the Invention

This invention relates to polypeptides which bind to insulin-like growth factor 1 receptor (IGF-1R). The polypeptides have industrial applications for example in medicine, veterinary medicine, imaging, separation techniques and diagnostics.

5

Background

The insulin-like growth factor 1 receptor (IGF-1R) is a membrane-spanning tyrosine kinase. It is a hetero-tetrameric complex consisting of two extracellular α chains and two membrane-spanning β chains, which are interconnected by several disulphide bridges. Binding of insulin-like growth factor 1 (IGF-1), and to a lesser extent insulin-like growth factor 2 and insulin, activates the receptor and leads to an autophosphorylation of tyrosines in the intracellular kinase domain in the β -chains. The activated receptor subsequently submits a mitogenic signal through, among others, the ras signaling pathway (McCormick, Nature 363:15-16 (1993)).

IGF-1R is expressed in most cell types and involved in cell growth, but it is not an absolute requirement for growth (Baker *et al*, Cell 75:73-82 (1993); Sell *et al*, Mol Cell Biol 14:3604-3612 (1994)). The over-expression and hyperactivation of IGF-1R, however, is implicated in transformation and tumorigenesis. Increased expression of IGF-1R has been observed in many human malignancies, including carcinomas of the lung, breast, thyroid, gastrointestinal tract and prostate, glioblastomas, neuroblastomas, rhabdomyosarcomas and leukemias (Macaulay, Br J Cancer 65:311-320 (1992)). IGF-1R also functions as a positive regulator of the invasive/metastatic phenotype (Long *et al*, Exp Cell Res 238:116-121 (1998)).

Based on those features, IGF-1R is an attractive target for cancer therapy. A number of antibodies to IGF-1R have been developed, and some of them have been found to block IGF-1 binding and inhibit growth of several cancer cells (Rohlik *et al*, Biochem Biophys Res Commun 149:276-281 (1987); Scotlandi *et al*, Cancer Res 58:4127-4131 (1998)). It was also able to enhance the antitumor activity of conventional chemotherapy (Benini *et al*, Clin Cancer Res 7:1790-1797 (2001)). The mouse monoclonal antibodies MAB 391 and mAb 4G11 were able to down-regulate IGF-1R expression (Hailey *et al*, Mol Cancer Ther 1:1349-1353 (2002); Jackson-Booth *et al*, Horm Metab Res 35:850-856 (2003)) and cause reversal of tumor phenotype (Burtrum *et al*, Cancer Res 63:8912-8921 (2003)). An

engineered humanized antibody, mAb1H7, was shown to suppress tumor growth of MCF7 xenografts (Sachdev *et al*, Cancer Res 63:627-635 (2003)). The fully human antibody A12 could block IGF-1 binding, deactivating IGF-1R and inducing receptor degradation (Burtrum *et al*, supra). One antibody (CP-751,871) is
 5 currently subject to a phase I clinical trial for the treatment of multiple myeloma (Miller *et al*, Cancer Res 65:10123-10127 (2005)).

Despite the comparable success of currently used IGF-1R antibodies, a substantial number of important questions remain concerning the future of this
 10 strategy. As a consequence, the continued provision of agents with a comparable affinity for IGF-1R remains a matter of substantial interest within the field, as well as the provision of uses of such molecules in the treatment and diagnosis of disease. It is therefore an object of the invention to provide new IGF-1R-binding agents, that could for example be used for diagnostic, *in vitro* or *in vivo* imaging,
 15 and therapeutic applications.

Summary of the Invention

According to one aspect thereof, the invention provides an insulin-like growth factor 1 receptor (IGF-1R) binding polypeptide, comprising an insulin-like
 20 growth factor 1 receptor binding motif, *IBM*, which motif consists of an amino acid sequence selected from:

i) $EX_2X_3X_4AX_6X_7EIX_{10} X_{11}LPNLX_{16}X_{17}X_{18}QX_{20} X_{21}AFIX_{25}SLX_{28}D$,

25 wherein, independently of each other,
 X_2 is selected from G, P and K;
 X_3 is selected from F and Y;
 X_4 is selected from Y and F;
 X_6 is selected from A, L, S and G;
 30 X_7 is selected from I, L, M and V;
 X_{10} is selected from I, L, and V;
 X_{11} is selected from T, A, S, G, L, Q, I and V;
 X_{16} is selected from N and T;
 X_{17} is selected from S, Q, H, R, N, K, P, A, D, E, G and T;
 35 X_{18} is selected from S, Q, H, R, N, K, D, P, T and G;
 X_{20} is selected from S, Q, H, R, and W;
 X_{21} is selected from T, G, R, A, N, D, Q, E, K and S;

X₂₅ is selected from T, G, R, A, N, D, Q, E, K, H and S; and

X₂₈ is selected from E, N, G, S, A, T, K, P, Q and D;

and

5

ii) an amino acid sequence which has at least 85 % identity to the sequence defined in i).

The above definition of a class of sequence related, IGF-1R-binding polypeptides according to the invention is based on an analysis of a number of random polypeptide variants of a parent scaffold, that were selected for their interaction with IGF-1R in selection experiments. The identified IGF-1R-binding motif, or "*IBM*", corresponds to the target binding region of the parent scaffold, which region constitutes two alpha helices within a three-helical bundle protein domain. In the parent scaffold, the varied amino acid residues of the two *IBM* helices constitute a binding surface for interaction with the constant Fc part of antibodies. In the present invention, the random variation of binding surface residues and the subsequent selection of variants have replaced the Fc interaction capacity with a capacity for interaction with IGF-1R.

20 As the skilled person will realize, the function of any polypeptide, such as the IGF-1R-binding capacity of the polypeptides according to the invention, is dependent on the tertiary structure of the polypeptide. It is therefore possible to make minor changes to the sequence of amino acids in a polypeptide without affecting the function thereof. Thus, the invention encompasses modified variants of the *IBM* of i), which are such that the resulting sequence is at least 85 % identical to a sequence belonging to the class defined by i). For example, it is possible that an amino acid residue belonging to a certain functional grouping of amino acid residues (e.g. hydrophobic, hydrophilic, polar etc) could be exchanged for another amino acid residue from the same functional group.

30 In one embodiment of the polypeptide according to the invention, X₂ is G.
In one embodiment of the polypeptide according to the invention, X₃ is F.
In one embodiment of the polypeptide according to the invention, X₄ is Y.
In one embodiment of the polypeptide according to the invention, X₆ is A.
In a more specific embodiment of the polypeptide according to the invention, X₃X₄ is FY and X₆ is A.

35 In one embodiment of the polypeptide according to the invention, X₁₀ is L.

In a more specific embodiment of the polypeptide according to the invention, $X_2X_3X_4$ is GFY, X_6 is A and X_{10} is L.

In one embodiment of the polypeptide according to the invention, X_7 is I.

5 In a more specific embodiment of the polypeptide according to the invention, $X_2X_3X_4$ is GFY, X_6X_7 is AI and X_{10} is L.

In one embodiment of the polypeptide according to the invention, X_{16} is N.

In one embodiment of the polypeptide according to the invention, X_{17} is H.

In one embodiment of the polypeptide according to the invention, X_{18} is selected from K and S, and may in particular be S.

10 In one embodiment of the polypeptide according to the invention, X_{20} is R.

In one embodiment of the polypeptide according to the invention, X_{21} is selected from T and G, and may in particular be T.

In one embodiment of the polypeptide according to the invention, X_{25} is T.

15 In a more specific embodiment of the polypeptide according to the invention, $X_{20}X_{21}$ is RG and X_{25} is T.

In a more specific embodiment of the polypeptide according to the invention, X_{11} is S, $X_{17}X_{18}$ is HS, $X_{20}X_{21}$ is RG and X_{25} is T.

In one embodiment of the polypeptide according to the invention, X_{28} is E.

20 In a more specific embodiment of the polypeptide according to the invention, X_{11} is A, $X_{17}X_{18}$ is RK, $X_{20}X_{21}$ is ST and X_{28} is E.

As described in detail in the experimental section to follow, the selection of IGF-1R-binding variants has led to the identification of individual IGF-1R-binding motif (*IBM*) sequences. These sequences constitute individual embodiments of the *IBM* sequence i) in the definition of IGF-1R-binding polypeptides according to this aspect of the present invention. The sequences of individual IGF-1R-binding motifs are presented in Figure 1 and as SEQ ID NO:1-197. In embodiments of this aspect of the invention, the *IBM* sequence i) may in particular be selected from SEQ ID NO:1-2, 52, 58, 101, 110, 115, 126, 130 and 166. In a more specific embodiment, the *IBM* sequence i) is selected from SEQ ID NO:110, 115 and 126.

30 In embodiments of the present invention, the *IBM* may form part of a three-helix bundle protein domain. For example, the *IBM* may essentially constitute or form part of two alpha helices with an interconnecting loop, within said three-helix bundle protein domain.

35 In particular embodiments of the invention, such a three-helix bundle protein domain is selected from domains of bacterial receptor proteins. Non-limiting examples of such domains are the five different three-helical domains of protein A from *Staphylococcus aureus*, and derivatives thereof. Thus, an IGF-1R-

binding polypeptide according to the invention may comprise an amino acid sequence selected from:

ADNNFNK-[IBM]-DPSQSANLLSEAKKLNESQAPK (*IBM* within domain A of staphylococcal protein A);

5 ADNKFNK-[IBM]-DPSQSANLLAEAKKLNDAQAPK (*IBM* within domain B of staphylococcal protein A);

ADNKFNK-[IBM]-DPSVSKEILAEAKKLNDAQAPK (*IBM* within domain C of staphylococcal protein A);

10 ADAQQNNFNK-[IBM]-DPSQSTNVLGEAKKLNESQAPK (*IBM* within domain D of staphylococcal protein A);

AQHDE-[IBM]-DPSQSANVLGEAQKLNDSQAPK (*IBM* within domain E of staphylococcal protein A); and

VDNKFNK-[IBM]-DPSQSANLLAEAKKLNDAQAPK (*IBM* within the protein Z derivative of domain B of staphylococcal protein A);

15 wherein [IBM] is an IGF-1R-binding motif as defined above.

According to another alternative aspect thereof, the invention provides an IGF-1R-binding polypeptide, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following: iii) it is selected from SEQ ID NO:198-394, and iv) it is an amino acid sequence having 85 % or greater
20 identity to a sequence selected from SEQ ID NO: 198-394. In embodiments of this aspect of the invention, the IGF-1R-binding polypeptide may in particular comprise a sequence selected from SEQ ID NO:198-199, 249, 255, 298, 307, 312, 323, 327 and 363, and sequences having 85 % or greater identity thereto. In one specific embodiment, the IGF-1R-binding polypeptide may in particular comprise a
25 sequence selected from SEQ ID NO: 307, 312 and 323, and sequences having 85 % or greater identity thereto.

An IGF-1R-binding polypeptide according to any aspect of the invention may bind to IGF-1R such that the K_D value of the interaction is at most 1×10^{-6} M, for example at most 1×10^{-7} M.

30 When reference is made herein to the degree of identity between the amino acid sequences of different polypeptides, the lower limit of 85 % identity to a sequence disclosed herein is given. In some embodiments, the inventive polypeptide may have a sequence which is at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least
35 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 % or at least 99 % identical to the sequence described herein. The comparison may be performed over a window corresponding to the shortest of the sequences being compared, or

over a window corresponding to an IGF-1R-binding motif in at least one of the sequences being compared.

The polypeptides are advantageous in that they bind well to an IGF-1R. The polypeptides may for example bind to the ectodomain of IGF-1R, such as the
5 ectodomain corresponding to SEQ ID NO:397. Typically, the polypeptides can be relatively short and by virtue of their small size they should have better penetration in tumor tissue than antibodies while at the same time have better systemic circulation properties than conventional low molecular weight IGF-1R-binding substances (often too short half-lives) and monoclonal antibodies (often too long
10 circulation times). In addition to the development of marketed molecular imaging agents, applications include use in the drug development and screening procedure where specific imaging agents are desired to measure outcome of treatment in *in vivo* models and subsequently during clinical development. Molecular imaging provides a direct read-out of efficacy of a pharmaceutical aimed to down-regulate
15 a growth factor receptor, as well as for assessing the anti-tumor effect.

A polypeptide in accordance with the invention may be about 53-58 amino acids in length. However, the length can be greater or smaller. The length of the polypeptide can for example be reduced at the N terminus by up to four amino acids.

20 The use of the term "position" is relative. In a polypeptide in accordance with the invention which has as many amino acid residues as a specifically disclosed polypeptide, i.e. those described above, the positions of amino acids in the polypeptide correspond exactly to those in the disclosed polypeptides. In a situation where there is, for example, an N terminal extension compared to the
25 disclosed polypeptides, those amino acid residues in the extended peptide that correspond to those of the non-extended peptide have the same position numbers. For example, if there is a six amino acid residue extension on the extended polypeptide, then amino acid number seven of that modified polypeptide, counting from the N terminus, corresponds to the amino acid in position number
30 one of the disclosed polypeptide.

Accordingly, a polypeptide according to the invention may be used as an alternative to conventional antibodies or low molecular weight substances in various medical, veterinary, diagnostic and imaging applications. For example, the IGF-1R-binding polypeptides of the invention may be used in the treatment of IGF-
35 1R-related cancers such as those caused by over-expression of IGF-1R described above, e.g. carcinomas of the lung, breast, thyroid, gastrointestinal tract and prostate, glioblastomas, neuroblastomas, rhabdomyosarcomas and leukemias.

The IGF-1R-binding polypeptides of the invention may be used to inhibit cell signaling by binding to an IGF-1R on a cell surface. Such blocking of receptor function may be utilized to obtain a therapeutic effect, in analogy with antibodies that compete with IGF-1 for binding to the receptor. The IGF-1R-binding polypeptides of the invention may also be used in the diagnosis of cancer, both *in vivo* and *in vitro*, in targeting agents to cells which express IGF-1R, particularly cells which over-express IGF-1R, in histochemical methods for the detection of IGF-1R, in methods of separation and other applications. In addition to the development of molecular imaging agents for the clinic, an application exists for specific preclinical imaging agents to measure outcome of treatment in *in vivo* models and subsequently during clinical development. Molecular imaging should provide a direct read-out of the efficacy of a pharmaceutical aimed to down-regulate a growth factor receptor e.g. IGF-1R, as well as for assessing the anti-tumor effect. The polypeptides of the invention may be useful in any method which relies on affinity for IGF-1R of a reagent. Thus, the polypeptides may be used as a detection reagent, a capture reagent or a separation reagent in such methods, but also as a therapeutic or diagnostic agent in their own right or as a means for targeting other therapeutic or diagnostic agents, with direct (e.g. toxic molecules, toxins) or indirect effects (e.g. cancer vaccines, immunostimulatory molecules) to the IGF-1R protein.

Methods that employ the polypeptides in accordance with the invention *in vitro* may be performed in different formats, such as microtitre plates, in protein arrays, on biosensor surfaces, on beads, in flow cytometry, on tissue sections, and so on.

The skilled addressee will appreciate that various modifications and/or additions can be made to a polypeptide according to the invention in order to tailor the polypeptide to a specific application without departing from the scope of the present invention. These modifications and additions are described in more detail below and may include additional amino acids in the same polypeptide chain, or labels and/or therapeutic agents that are chemically conjugated or otherwise bound to the polypeptide of the invention.

Furthermore, the invention also encompasses fragments of IGF-1R-binding polypeptides according to the invention that retain IGF-1R-binding. The possibility of creating fragments of a wild-type *Staphylococcus aureus* protein A domain with retained binding specificity was shown by Braisted AC *et al* in Proc Natl Acad Sci USA 93:5688-5692 (1996). In the experiments described in that paper, using a structure-based design and phage display methods, the binding domain of a three-

helix bundle of 59 residues was reduced to a resulting two-helix derivative of 33 residues. This was achieved by stepwise selection of random mutations from different regions, which caused the stability and binding affinity to be iteratively improved. Following the same reasoning, with the polypeptides of the present invention, the skilled addressee will be able to obtain a "minimized" IGF-1R-binding polypeptide with the same binding properties as that of the "parent" IGF-1R-binding polypeptide. Thus, a polypeptide constituting a fragment of a polypeptide according to the invention, which fragment retains target binding, is within the scope of the invention. Such a fragment may for example comprise an N-terminal reduction, such as by up to four amino acid residues, of a polypeptide as described above.

The terms "IGF-1R-binding" and "binding affinity for IGF-1R" as used in this specification refers to a property of a polypeptide which may be tested for example by the use of surface plasmon resonance technology, such as in a Biacore instrument (Biacore AB). For example as described in the examples below, IGF-1R-binding affinity may be tested in an experiment in which IGF-1R, or a fragment of IGF-1R such as the ectodomain comprising amino acid residues 1-901 (Jansson *et al*, J Biol Chem 272:8189-8197 (1997)), is immobilized on a sensor chip of the instrument, and the sample containing the polypeptide to be tested is passed over the chip. Alternatively, the polypeptide to be tested is immobilized on a sensor chip of the instrument, and a sample containing IGF-1R, or fragment thereof, is passed over the chip. Thus, IGF-1R may, in this regard, be a polypeptide comprising the amino acid sequence SEQ ID NO:396, and its ectodomain may be a polypeptide comprising the amino acid sequence SEQ ID NO:397. The skilled person may then interpret the results obtained by such experiments to establish at least a qualitative measure of the binding affinity of the polypeptide for IGF-1R. If a qualitative measure is desired, for example to determine a K_D value for the interaction, surface plasmon resonance methods may also be used. Binding values may for example be defined in a Biacore 2000 instrument (Biacore AB). IGF-1R is immobilized on a sensor chip of the measurement, and samples of the polypeptide whose affinity is to be determined are prepared by serial dilution and injected in random order. K_D values may then be calculated from the results using for example the 1:1 Langmuir binding model of the BIAevaluation 4.1 software provided by the instrument manufacturer.

Where amino acid substitutions are introduced, these should not affect the basic structure of the polypeptide. For example, the overall folding of the $C\alpha$ backbone of the central three-helix bundle of the polypeptide can be essentially

the same as that of a Z "wild-type" domain to which it may be related, i.e. having the same elements of secondary structure in the same order. Thus polypeptides having this basic structure will have similar CD spectra to the Z "wild-type" domain. The skilled addressee is aware of other parameters that may be relevant. The
5 requirement of conserving the basic structure, places restrictions on which positions of the amino acid sequence may be subject to substitution. For example, it is preferred that amino acid residues located on the surface of the polypeptide are substituted, whereas amino acid residues buried within the core of the polypeptide "three-helix bundle" should be kept constant in order to preserve the
10 structural properties of the molecule. The same reasoning applies to fragments of polypeptides of the invention.

The invention also covers polypeptides in which the IGF-1R-binding polypeptide described above is present as an IGF-1R-binding domain to which additional amino acid residues have been added at either terminal. These
15 additional amino acid residues may play a role in enhancing the binding of IGF-1R by the polypeptide, but may equally well serve other purposes, related for example to improving one or more of the production, purification, stabilization *in vivo* and/or *in vitro*, coupling or detection of the polypeptide. Such additional amino acid residues may comprise one or more amino acid residues added for the purpose of
20 chemical coupling. One example of this, is the addition of a cysteine residue at the very first or very last position in the polypeptide chain, i.e. at the N or C terminus. Such additional amino acid residues may also provide a "tag" for purification or detection of the polypeptide such as a His₆ tag or a "myc" (c-myc) tag or a "FLAG" tag for interaction with antibodies specific to the tag.

25 The present invention also covers IGF-1R-binding polypeptides in which a IGF-1R-binding polypeptide as described above is present as an IGF-1R-binding domain to which additional peptides or proteins or other functional groups are coupled N- or C-terminally or to any other residues (specifically or non-specifically) by means of chemical conjugation (using known organic chemistry methods).

30 The "additional amino acid residues" discussed above may also provide one or more polypeptide domains with any desired function, such as the same binding function as the first, IGF-1R-binding domain, or another binding function, or an enzymatic function, toxic function (e.g. an immunotoxin), or a fluorescent signaling function, or combinations thereof.

35 The polypeptide of the invention may be in monomeric or multimeric forms. Multimeric forms of the polypeptide may be advantageous in that they may have enhanced binding properties. Preferred multimeric forms include dimeric and

trimeric forms. Multimeric forms of the polypeptides may comprise a suitable number of polypeptides of the invention. These polypeptides essentially form domains within the multimer. These domains may all have the same amino acid sequence, but alternatively, they may have different amino acid sequences. The polypeptides may be joined by covalent coupling using known organic chemistry methods, or expressed as one or more fusion polypeptides in a system for recombinant expression of polypeptides, or joined in any other fashion, either directly or via a linker, for example an amino acid linker.

Additionally, fusion polypeptides, in which the IGF-1R-binding polypeptide of the invention provides a first domain or moiety, and second or further moieties have other functions than binding IGF-1R are also contemplated and within the scope of the present invention. The second or further moieties of such a fusion polypeptide may comprise a binding domain with an affinity for another target molecule than IGF-1R. Such a binding domain may be another, similar polypeptide binder. For example, the polypeptide binder may be a Z variant. This makes it possible to create multi-specific reagents that may be used in several types of applications such as medicine, veterinary medicine, diagnosis, separation, and imaging. The preparation of such multi-specific fusion polypeptides may be performed as generally described above.

In other embodiments of the invention, the second or further moieties may comprise an unrelated, naturally occurring or recombinant protein (or a fragment thereof which retains the binding or other ability of the naturally-occurring or recombinant protein) having a binding affinity for a target. For example, an IGF-1R-binding polypeptide in accordance with the invention may be joined to an albumin-binding domain, such as the albumin binding domain GA3 of protein G from *Streptococcus* strain G148 or a derivative thereof, or any other polypeptide with affinity for a serum protein to prolong the half-life of the IGF-1R-binding polypeptide for use in applications *in vivo*, such as diagnostic or therapeutic applications.

The IGF-1R-binding polypeptides of the present invention may be provided in the form of other fusion polypeptides. For example the IGF-1R-binding polypeptide, or fragment thereof, may be covalently coupled to a second or further moiety or moieties, which in addition to, or instead of target binding, exhibit other functions. One example would be a fusion between one or more IGF-1R-binding polypeptides and an enzymatically active polypeptide serving as a reporter or effector moiety. Examples of reporter enzymes, which may be coupled to the IGF-1R-binding polypeptide to form a fusion protein, are well-known to the skilled

person and include enzymes such as β -galactosidase, alkaline phosphatase, horseradish peroxidase, carboxypeptidase. Other options for the second and further moiety or moieties of a fusion polypeptide according to the invention include fluorescent polypeptides, such as green fluorescent protein, red fluorescent protein, luciferase and variants thereof.

Other options for the second and further moiety or moieties of a fusion polypeptide according to the invention include a moiety or moieties for therapeutic applications. In therapeutic applications, other molecules can also be coupled, covalently or non-covalently, to the IGF-1R-binding polypeptide of the invention by other means. For example, other molecules such as enzymes for "ADEPT" (Antibody-Directed Enzyme Prodrug Therapy) applications using the polypeptide of the invention to direct the effector enzyme (e.g. carboxypeptidase) or RNase or DNase fusions; proteins for recruitment of effector cells and other components of the immune system; cytokines, such as IL-2, IL-12, TNF α , IP-10; pro coagulant factors, such as tissue factor, von Willebrand factor; toxins, such as ricin A, Pseudomonas exotoxins, calicheamicin, maytansinoid, toxic small molecules, such as auristatin analogues, doxorubicin.

The above-described additional amino acids (particularly hexahistidine, cysteine) can be used to comprise a chelator to facilitate incorporation of a radionuclide for the purposes of using an inventive IGF-1R-binding polypeptide in imaging *in vivo* or *in vitro*. Those, or other amino acid residues on the surface of the polypeptide itself may also be used as an attachment site for indirect labeling with a molecule containing a radionuclide (often used with radio-halogens) or as an attachment site for a chelating moiety which is subsequently used to capture a radio-metal. Non-limiting examples of radionuclides for diagnosis are ^{68}Ga , ^{76}Br , ^{111}In , $^{99\text{m}}\text{Tc}$ and ^{125}I , and non-limiting examples of radionuclides for therapy are ^{90}Y , ^{131}I , ^{211}At and ^{177}Lu .

The invention also embraces polypeptides in which the IGF-1R-binding polypeptide described above has been provided with a label group, such as at least one fluorophore, biotin or radioactive isotope, for example for the purposes of detection of the polypeptide *in vitro* and/or *in vivo*.

With regard to the description above of fusion polypeptides and proteins incorporating an IGF-1R-binding polypeptide of the invention, it should be noted that the designation of first, second and further moieties is made for the purposes of clarity to distinguish between the IGF-1R-binding moiety or moieties on the one hand, and moieties exhibiting other functions on the other hand. These designations are not intended to refer to the actual order of the different domains

in the polypeptide chain of the fusion protein or polypeptide. Thus, for example, a first moiety may be appear at the N-terminal end, in the middle, or at the C-terminal end of the fusion protein or polypeptide.

Further preferred aspects and embodiments of the invention are apparent
5 from the appended claims.

Brief description of the figures

Figure 1 is a listing of the amino acid sequences of examples of IGF-1R binding motifs comprised in IGF-1R binding polypeptides of the invention (SEQ ID
10 NO:1-197), examples of IGF-1R binding polypeptides according to the invention (SEQ ID NO:198-394), the protein Z derivative of domain B of *Staphylococcus aureus* protein A (SEQ ID NO:395), entire human IGF-1R (SEQ ID NO:396) and the ectodomain of human IGF-1R (SEQ ID NO:397).

Figure 2 shows the result of an SDS-PAGE gel electrophoresis of purified Z
15 variants in the following order from left to right: Z01778, Z01779, Z01780, Z01781, Z01997, Z01998, Z01999, Z02001, Z02002, Z02003 and Z02008.

Figure 3: MCF-7 cells were stained with biotinylated Z variants at a concentration of 1 µg/ml. This was followed by incubation with neutravidin labeled with Alexa Fluor 488 and subsequently with Amplification kit A and B from
20 Molecular Probes. The cells were analyzed in a flow cytometer. Thin lines correspond to non-stained cells. Thick lines correspond to stained cells.

Figure 4. Non-stained MCF-7 cells (dashed line), and MCF-7 cells stained with Z variants (thin line). Biotinylated and non-biotinylated Z variants at a ratio of 1:10 were mixed and used to stain MCF-7 cells (thick line). Staining was otherwise
25 as in Figure 3. Variants, for which a 10-fold excess (thick line) is able reduce the fluorescent shift significantly, are characterized as having specific binding, whereas variants where the shift is not reduced stick non-specifically to cells.

Figure 5. MCF-7 cells were stained (as in Figure 3) with different concentrations of selected Z variants as well as with IGF-1. Fluorescent shift was
30 plotted against the logarithmic concentration of the variants. The experiment was repeated three times; the figure shows the result of a representative experiment. The inflection points of the curves correspond to where half the receptors have bound Z variants, i.e. the K_D concentration.

Figure 6 shows the result of fluorescent microscopic analysis of MCF-7 cells
35 stained with the IGF-1R binding Z variant Z01781.

Figure 7 is an overview of the IGF-1R selection described in Example 2, showing target concentrations and number of washes. Track 1 (divided into tracks

1a and b in round 4): High target protein concentration. Track 2 (divided into tracks 2a and b in round 4): Low target protein concentration. Track 3 (divided into tracks 3a and b in round 4): High target protein concentration, and pre-selection against IR preceding each round.

5 Figures 8A-8E are dose response curves from the ELISA experiment described in Example 4 for the nine indicated IGF-1R specific Z variants and for Z00810 (negative control). Concentration of the indicated Z variants ranges from 400 ng/ml to 0.2 ng/ml (X-axis). The Z variant molecule concentration is plotted against the absorbance at 450 nm.

10 Figure 9 shows the result of the experiment described in Example 5, i.e. flow cytometry analysis of MCF-7 cells stained with the ten indicated Z variants in 3 different concentrations. Mean fluorescence of 20000 cells is shown. "Neg ctrl" (negative control) corresponds to goat IgG and Alexa647 anti-goat only.

15

Example 1

Selection and characterization of IGF-1R binding polypeptides

Materials and methods

20 General: Escherichia coli strain RR1ΔM15 (Rüther, Nucleic Acids Res 10:5765–5772, 1982) was used throughout this work except where otherwise noted. The nucleotide sequence of all DNA constructs was verified by cycle sequencing using an ABI9600 sequencing machine (Applied Biosystems).

Production and purification of IGF-1R: HEK-293 cells expressing the
25 ectodomain of the IGF-1R with a C-terminal His₆-tag (IGF-1R) (Jansson *et al*, J Biol Chem 272:8189-8197 (1997)) were cultivated continuously and the culture medium was collected and stored at –80 °C. Upon purification, 675 ml medium was thawed and concentrated by ultracentrifugation to a total volume of 55 ml. The medium was desalted on a 245 ml Sephadex G25 column (GE Healthcare) and
30 then purified on a 1 ml Histrap FF column (GE Healthcare) using the His₆ purification tag. Elution from the column was performed using 300 mM imidazole, and eluted material was desalted to 10 mM HEPES, pH 7.4, and stored until use.

Selection of IGF-1R binding polypeptides: A library of random variants of protein Z (Nilsson *et al*, Prot Eng 1:107-113, 1987) displayed on bacteriophage
35 was used to select IGF-1R binding polypeptides. The library was constructed essentially as described in Nord *et al*, Prot Eng 8:601-608 (1995). Selection was performed as follows: Four approaches with four cycles each were performed for

selection. To avoid non-specific binding, all tubes used for selection were blocked with 0.1 % gelatin in PBST (PBS: 10 mM phosphate, 137 mM NaCl, pH 7.2, supplemented with 0.1 % Tween 20). For the first two cycles of selection, the phage stock, prepared according to previously described procedures (Nord *et al*,
5 Nat Biotech 15:772-777 (1997); Hansson *et al*, Immunotechnology 4:237-252 (1999)) was pre-incubated with Talon™ beads (Clontech). The resulting supernatant was mixed with non-biotinylated IGF-1R at 10 nM (for selection 1) or 100 nM (for selections 2, 3 and 4) and 20 ml (for selection 1) or 200 ml (for selections 2, 3 and 4) of fresh Talon™ beads, followed by incubation under
10 continuous rotation (end over end) at room temperature for 2 h. After wash for increasing times with PBST (1 % rather than 0.1 % of Tween 20 was supplemented for selection 3), the bound phage were eluted with 500 ml of 200 mM imidazole in PBS, pH 7.2 (imidazole) for selections 1, 2 and 3 and with 76 ml of IGF-1 at 1 mg/ml (10 mM) for selection 4. For cycles 3 and 4 of selection, the
15 phage stocks were pre-incubated with M-280 paramagnetic beads with immobilized streptavidin (Dynabeads®; Invitrogen). The supernatant was mixed with 10 nM (for selection 1) or 20 nM biotinylated IGF-1R supplemented with 75 ml (for selection 1) or 150 ml (for selections 2, 3 and 4) of fresh M-280 beads. Biotinylation of IGF-1R was performed using EZ-Link™ Sulfo-NHS-LC-Biotin
20 (Pierce). A 15-fold molar excess of biotin was added to IGF-1R in PBS and the mixture was incubated on ice for 2 h followed by extensive dialysis against PBS at 4 °C. 500 ml of 50 mM glycine hydrochloride, pH 2.2, was used for elution of bound phage for selections 1, 2 and 3, while 15 ml of the above IGF-1 (2 mM) was used for elution in selection 4. The eluted phages were amplified in *E. coli* strain
25 RR1ΔM15 between the cycles. After the fourth cycle of selection, a number of colonies were isolated and grown in 1 ml cultures. Their Z variant-encoding inserts were expressed by addition of IPTG. Expressed Z variants fused to an albumin binding domain (ABD; the albumin binding domain GA3 of protein G from *Streptococcus* strain G148) were released from cells by the freeze/thaw method.
30 To test if the variants could indeed interact with the IGF-1R, an ELISA was performed. 100 µl of a solution containing 6 µg/ml human serum albumin was added to each well of an 96-well ELISA plate and incubated over night. The HSA solution was poured off and the wells were blocked with 200 µl of 2 % non-fat dry milk solution for 1 h. 100 µl of released Z variant solution from each of the
35 cultivations were added to each well and incubated 1.5 h at room temperature with slow shaking. The supernatants were poured off and the wells were washed 5 times with PBST. 100 µl biotinylated IGF-1R (1 µg/ml) were added to each well

and incubation was performed for 90 min with slow shaking. The wells were washed with PBST 5 times and a conjugate of streptavidin and horseradish peroxidase was added to the wells followed by development with a TMB substrate (Pierce) according to the manufacturer's recommendations.

- 5 Sub-cloning and expression of Z variants: Selected Z variants were sub-cloned in the pAY442 vector (Grönwall *et al*, J Biotechnol 128:162-183 (2007)) using sticky-end cloning essentially as described previously. The variants were expressed by addition of 1 mM IPTG and purified by immobilized metal affinity chromatography on a Talon™ resin (Clontech). The purified Z variants were
- 10 subsequently biotinylated using EZ-Link™ Sulfo-NHS-LC-Biotin (Pierce) according to the manufacturer's recommendations.

- Flow cytometry: Sub-confluent MCF-7 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen; no ACC115) were detached with a solution of 0.05 % trypsin and 0.53 mM EDTA (Invitrogen). For every sample, 150 000 cells
- 15 were collected, washed once with PBSB (PBS: 100 mM Na phosphate, pH 7.5, 100 mM NaCl, supplemented with 1 % bovine serum albumin). Staining of cells with biotinylated Z variants was performed by incubation with 70 ml of Z variant solution at 1 µg/ml unless otherwise noted for 1 h at room temperature. The cells were washed once with PBSB and incubated with 70 µl of FITC-labeled
- 20 neutravidin (1 µg/ml) for 30 min on ice. The cells were washed again and treated with the Alexa Fluor® 488 Signal-amplification kit (Molecular Probes) essentially as recommended by the manufacturer. After staining with Z variants, the cells were washed once with PBSB and re-suspended in PBSB and analyzed in a FacsVantage SE flow cytometer (BD Biosciences) fitted with a 100 mm nozzle.
- 25 10 000 events were recorded for each sample, and the population corresponding to single cells was gated and analyzed as histogram plots.

- Immunofluorescence: MCF-7 cells (*supra*) were grown over night on multi-well slides (Histolab, Sweden) and subjected to staining the following day after a brief wash in PBS. The cells were stained with the Z variant Z01781 at a
- 30 concentration of 10 µg/ml. Z01781 was detected with a purified goat IgG against an epitope common for all Z variants (Anti-ZBirk005), followed by Alexa Fluor® 488 labeled anti-goat IgG (Molecular Probes, Invitrogen).

Results

- 35 Phage selection from Z variant library: To generate IGF-1R binding Z variant polypeptides, a library displayed on bacteriophage was subjected to selection using the ectodomain of the receptor as target or "bait". Three parallel

selections were carried out to evaluate how receptor concentration and the amount of Tween 20 during selection affected the outcome. Two cycles of selection were performed where the receptor was incubated with the phagemid library followed by isolation of receptor/phagemid complexes by a solid support containing Co^{2+} ions that could specifically interact with the hexahistidine tag present in the receptor. The receptor/phagemid complexes were subsequently eluted by imidazole, followed by amplification in *E. coli*. In an attempt to minimize the ability of phagemid particles that bound unspecifically to the Co^{2+} containing matrix to co-purify with the receptor/phagemid complexes, the Co^{2+} containing solid support was replaced with a solid support containing streptavidin in cycles 3 and 4. The target receptor used during these cycles was also biotinylated *in vitro* to allow capture by streptavidin. During these cycles, the phagemids were eluted by lowering the pH to break the bond between the phagemid and the receptor.

To enrich for Z variants that interact with an epitope over-lapping the IGF-1 site of interaction, a fourth selection was performed where elution was carried out using an excess of IGF-1. Capturing using a Co^{2+} containing solid support during cycles 1 and 2 and a streptavidin containing solid support during cycles 3 and 4 was performed in an analogous manner as described above.

In all four selections, an increase in the number of eluted phagemids was observed as the rounds progressed, indicating an enrichment for phagemids interacting specifically with the receptor and/or unspecifically with other components in the system. After the fourth round of selection, randomly picked clones were cultivated and their gene fusion of Z variant and ABD was expressed. Cell lysates from these cultures were prepared and analyzed for binding to the receptor in an ELISA experiment. A number of clones showed interaction with the receptor and the DNA sequence of the Z variant-encoding nucleic acid in these clones was determined. Z variants interacting with the ectodomain of IGF-1R were successfully isolated in all four selection experiments. Each individual Z variant was given a unique identification number #####, and individual variants are referred to as Z#####.

Of the sequences determined, a selected number of sequences were grouped together in a cluster of similar sequences. Interestingly, the selected cluster of similar sequences were identified in the experiment using competitive elution with IGF-1, indicating that these IGF-1R binding polypeptides interact with the same or overlapping epitopes on IGF-1R as does IGF-1. The predicted amino acid sequences of the corresponding polypeptides and their IGF-1R binding motifs were deduced, which yielded a number of sequences of IGF-1R binding

polypeptides according to the invention. The amino acid sequences of deduced IGF-1R binding motifs are listed in Figure 1 and in the sequence listing as SEQ ID NO:1-7, whereas the amino acid sequences of the corresponding full-length Z variants are listed in Figure 1 and in the sequence listing as SEQ ID NO:198-204.

5 Analysis of interaction with MCF-7 cells: After the selection and initial screening of Z variants with an affinity for the recombinantly produced ectodomain of IGF-1R, it was of importance to determine if they could also bind to the full receptor in its native state, as part of a cellular membrane. Eleven Z variants from the selection were each sub-cloned in an *E. coli* expression vector, where they
10 were also fitted with a hexahistidine purification tag. They were expressed and subsequently purified by immobilized metal affinity chromatography. Samples of the purified Z variant molecules were separated by SDS-PAGE. A photograph of the gel is displayed in Figure 2. All variants could be produced and purified to a high level of purity.

15 To evaluate the ability of the Z variants to bind to endogenous IGF-1R, the breast cancer cell line MCF-7 was used, which has previously been shown to contain a high number of IGF-1R (Yee, Breast Cancer Res Treat. 32:85-95 (1994)). Biotin was covalently attached to the purified Z variants, and they were subsequently used as a primary reagent to stain MCF-7 cells. The bound Z
20 variants were detected by a neutravidin-FITC/anti-FITC antibody sandwich followed by determination of cellular fluorescence by flow cytometry (Figure 3). The experiment shows that Z variants Z01778, Z01781 (SEQ ID NO:198), Z01997, Z01998, Z01999, Z02002, Z02003 (SEQ ID NO:199) and Z02008 are able to interact with the cells, whereas Z01779, Z01780 and Z02001 are not. Biotinylated
25 IGF-1 was used as a positive control and could interact with the cells. Two negative controls showed no interaction with the cells: Zwt (also designated Z00000 (SEQ ID NO:395)), which specifically interacts with certain classes of IgG:s (Nilsson *et al, supra*), and Z_{control}, which was isolated in a previous selection effort against an unrelated target protein that was not expected to be present on
30 the surface of MCF-7 cells.

Evaluation of the specificity of interaction of Z variants with IGF-1R: To investigate whether the selected Z variants with the ability to bind MCF-7 cells as determined above were specific in their interaction, a competition experiment was performed. Each variant was used as a primary reagent to stain MCF-7 cells in the
35 presence or absence of a 100-fold excess of a non-biotinylated version of the Z variant. If the Z variant was able to bind specifically to the cells, an excess of non-biotinylated Z variant was expected to out-compete the biotinylated Z variant,

resulting in a lower amount of bound molecule. Signals from biotinylated Z variants were again detected by a neutravidin-FITC/anti-FITC antibody sandwich followed by determination of cellular fluorescence by flow cytometry (Figure 4). For Z01781 (SEQ ID NO:198), Z02002, Z02003 (SEQ ID NO:199) and Z02008, a clear
5 quenching of the signal is observed, indicating specific cell binding. For Z01778, Z01997, Z01998 and Z01999 the quenching of the signal is not complete, suggesting that these variants to some extent can interact with cells unspecifically.

Determination of the affinity constant for selected Z variants to IGF1R over-expressing cells: To quantify the affinity constant of the Z variants for the IGF-1R over-expressing cell line MCF-7, serial dilutions of biotinylated Z variants as well
10 as IGF-1 were used to stain cells, followed by detection with a neutravidin-FITC/anti-FITC antibody sandwich, which in turned was followed by determination of cellular fluorescence by flow cytometry. The measured fluorescence was plotted against the logarithmic concentration of the Z variant or IGF-1 used during the
15 staining (Figure 5). The measured points were fitted to a sigmoidal curve where the inflection point corresponds to a state where half the number of available receptors in the system are occupied. The concentration of Z variant or IGF-1 at this point corresponds to the dissociation concentration. The values obtained were 1.2 nM for Z01781, 805 nM for Z01999, 1934 nM for Z02008 and 3.6 nM for IGF-1.

Immunofluorescence: IGF-1R, expressed on the membrane of the human
20 breast carcinoma cells MCF-7, was detected by immunofluorescence staining using the Z variant Z01781. The results are shown in Figure 6. The picture shows strong immunofluorescence staining of membrane localized IGF-1R on MCF-7 cells.

25

Example 2

Phage display selection of additional IGF-1R binding polypeptides

30 *Materials and methods*

Library design: Based on the results of the selection experiments described in Example 1, a new phage display library of Z variants was constructed. In the new library, 14 variable positions in the Z molecule scaffold were biased towards certain amino acid residues, according to a strategy based on the Z variant
35 sequences defined by SEQ ID NO:198-204. A degenerated oligonucleotide encoding the possible variants was obtained from Scandinavian Gene Synthesis

AB and denoted AFFI-1300. The theoretical frequencies and distributions of amino acid residues in the new library for the 14 variable Z positions are given in Table 1.

Table 1: Frequencies and distribution of amino acids in varied positions

Amino acid	X2	X3	X4	X6	X7	X10	X11	X16	X17	X18	X20	X21	X25	X28	Amino acid
Ala (A)	16	0,0	0,0	49	0	0	20	0	1	1	1	12	12	10	Ala (A)
Arg (R)	6	0,0	0,0	1,25	0	0	6	0	26	26	26	16	16	6	Arg (R)
Asn (N)	2	2,4	2,4	1,75	0	0	4	50	12,5	12,5	12,5	6	6	12	Asn (N)
Asp (D)	8	2,4	2,4	4,9	0	0	3,2	0	2,5	2,5	2,5	3	3	15	Asp (D)
Cys (C)	0	0,0	0,0	0	0	0	0	0	0	0	0	0	0	0	Cys (C)
Gln (Q)	0	0,1	0,1	0,15	0	0	0,2	0	10	10	10	1	1	3	Gln (Q)
Glu (E)	8	0,1	0,1	2,1	0	0	0,8	0	2,5	2,5	2,5	3	3	15	Glu (E)
Gly (G)	48	0,0	0,0	7	0	0	12	0	4	4	4	12	12	10	Gly (G)
His (H)	0	2,4	2,4	0,35	0	0	0,8	0	10	10	10	1	1	3	His (H)
Ile (I)	0	2,4	2,4	1,75	20	20	4	0	0	0	0	0	0	0	Ile (I)
Leu (L)	0	4,6	4,6	0,5	20	20	1	0	0	0	0	0	0	0	Leu (L)
Lys (K)	2	0,1	0,1	0,75	0	0	1	0	12,5	12,5	12,5	6	6	12	Lys (K)
Met (M)	0	0,1	0,1	0,75	20	20	1	0	0	0	0	0	0	0	Met (M)
Phe (F)	0	40,4	40,4	0	20	20	0	0	0	0	0	0	0	0	Phe (F)
Pro (P)	0	0,0	0,0	3,5	0	0	5	0	4	4	4	4	4	2	Pro (P)
Ser (S)	6	0,0	0,0	1,75	0	0	12	0	10	10	10	12	12	4	Ser (S)
Thr (T)	4	0,0	0,0	17,5	0	0	25	50	5	5	5	24	24	8	Thr (T)
Trp (w)	0	0,0	0,0	0	0	0	0	0	0	0	0	0	0	0	Trp (w)
Try (Y)	0	40,4	40,4	0	0	0	0	0	0	0	0	0	0	0	Try (Y)
Val (V)	0	2,5	2,5	7	20	20	4	0	0	0	0	0	0	0	Val (V)
Stop (.)	0	2,1	2,1	0	0	0	0	0	0	0	0	0	0	0	Stop (.)

5

Library construction: The degenerated oligonucleotide AFFI-1300 (5'-ctcgaggtagacaacaaattcaacaaagaannkrrstgggcgctgrbygagatckkggnkttacctaacttaaa csagvrscatgggnkgccttcacnnkagtttamrwatgacccaagccaaagc) was amplified by PCR using 0.1 pmol template, 50 pmol AFFI-50 (5'-ccccctgctagcaagtttagcgcttgg-cttgggtcatc) and AFFI-976 (5'-ccccccccctcgaggtagacaacaaattcaa) primers, 2 mM dNTP mix, 2.5 U AmpliTaq Gold polymerase (Applied Biosystems #4311816), 1x PCR buffer (Applied Biosystems #N8080006). Primers AFFI-50 and AFFI-976 included restriction sites for *NheI* and *XhoI*, respectively. Ninety-six PCR reactions à 50 µl were performed on a Mastercycler epgradient S (Eppendorf), using the program: 10 min @ 95 °C, 20 cycles of (20 s @ 95 °C, 30 s @ 50 °C, 60 s @ 72 °C] and 10 min @ 72 °C. The amplified fragments were cleaved by 2 h incubation at 37 °C with 650 U *NheI* (New England Biolabs #R0131M) and 60 U *XhoI* (New England Biolabs #R0146), BSA (New England Biolabs #B9001S) and NEB2 buffer (New England Biolabs #B7002S). The cleaved fragments were concentrated and purified using QIAquick PCR Purification Kit (Qiagen) and size exclusion separation on 1 % agarose gel followed by purification with QIAquick Gel Extraction Kit (Qiagen).

When ligated to a Z variant fragment, phagemid vector pAY00065 encodes the construct Z-ABD-pIII, comprising the Z variant fused to the albumin binding domain GA3 of protein G from *Streptococcus* strain G148 (ABD), in turn fused to the phage coat protein pIII; with the proviso that the pIII fusion domain is not present unless an *amber* suppressor host is used for expression, due to the presence of an *amber* stop codon between the ABD and pIII encoding portions. The vector is also denoted pAffi1 and described in Grönwall et al, J Biotechnol

128:162-183, 2007. Ligation of 20 µg phagemid vector pAY00065 and 3.2 µg fragment was performed in 2 h incubation with T4 DNA ligase (New England Biolabs). The ligation was subjected to a standard DNA extraction using phenol:chloroform:isoamylalcohol (25:24:1, Invitrogen #15593-031) and
5 chloroform:isoamylalcohol (24:1, Sigma C0549-1PT) followed by ethanol precipitation. The ligation reactions were transformed into electrocompetent *Escherichia coli* RR1ΔM15 cells (Rüther, Nucleic Acids Res 10:5765–5772, 1982) followed by immediate incubation in SOC medium (tryptic soy broth 30 g/l, yeast extract 5 g/l, 1 mM MgCl₂, 1 mM MgSO₄, 1 mM NaCl, 0.25 mM KCl and 1 %
10 glucose) at 37 °C for 50 min. The transformed cells were amplified over night in three 5 l cultivation flasks containing 1000 ml tryptic soy broth and yeast extract (TSB-YE), 2 % glucose and 100 mg/ml ampicillin, by incubation at 37 °C and 80 rpm. The cells were stored at –80 °C in 40 % glycerol as a glycerol stock. The size of the library was determined by titration.

15 Preparation of phage stock: Cells from the glycerol stock containing the phagemid vector were grown in TSB-YE supplemented with 2 % glucose and 100 mg/ml ampicillin at 37°C and 80 rpm, starting at an optical density (OD) of 0.1. When the cells reached log phase (at OD 0.5-0.8), the same amount of cells as initially inoculated was infected using a 10x excess of M13K07 helper phage (New
20 England Biolabs) and incubated for 20 min at 37 °C. The infected cells were harvested at 2500 g for 10 min at room temperature. The pellet was re-suspended and incubated over night at 30 °C and 90 rpm in TSB-YE containing 100 µg/ml ampicillin, 25 µg/ml kanamycin and 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG).

25 Precipitation of phage particles: In order to separate phage particles from *E. coli* cells, the phage particle cultivation was spun in a centrifuge for 10 min at 3300 g. Phage particles were precipitated by adding 1/5 volume 20 % PEG in 2.5 M NaCl and incubating for 1 h on ice. The precipitation was pelleted 30 min at 10700 g at 4 °C. The pellet was re-suspended in sterile H₂O and precipitated once more
30 by adding 1/5 volume 20 % PEG in 2.5 M NaCl and incubating for 45 min on ice. The precipitation was spun for 30 min at 17500 g at 4 °C and the pellet containing phages was re-suspended in phosphate buffered saline (PBS: 10 mM phosphate, 2.68 mM KCl, 137 mM NaCl, pH 7.4) with 0.1 % Tween (PBST 0.1) and 0.1 % gelatine. Remaining cells and cell debris were spun down and the supernatant
35 was filtered using a 0.45 µm filter. Phage particles were stored for short term at 4 °C and for long term at –80 °C in 50 % glycerol.

Biotinylation of target protein: Recombinant human IGF-1R (R&D Systems #391-GR) in PBS was biotinylated with a 10x molar excess of Sulfo-NHS-LC-Biotin (Pierce #21327) under 30 min incubation at room temperature. In order to remove any excess biotin, a buffer exchange was made on spin columns (Pierce #89849) pre-equilibrated with PBS according to the manufacturer's instructions.

5 The biotinylated IGF-1R was stored at 4 °C.

Phage display – General strategy: The selection was performed in 5 rounds initially divided into 3 tracks. In round 4, these were further divided into 6 tracks (see Figure 7 for an overview). In each round, the target protein concentration was

10 decreased and the number of washes was increased, making the selection more stringent. Preceding the first round, a pre-selection against streptavidin was made on all phages entering the selection. In track 3, which was subdivided into tracks 3a and 3b in round 4, pre-selection was made against the insulin receptor (IR) before each selection round. The selection was performed in liquid phase, and

15 complexes of target bound to phage were captured on streptavidin coated magnetic beads (Dynabeads® M-280, Invitrogen).

Phage display – Selection: All tubes used in the selection were pre-incubated in selection buffer (PBST 0.1 with 0.1 % gelatine) to avoid nonspecific binding to plastic. The streptavidin coated beads that were used to capture the

20 target protein-phage complex after selection were also pre-incubated in selection buffer in order to avoid nonspecific binding to the beads.

A volume of phage stock corresponding to 100 copies per Z variant was used in the selection. The phage stock was precipitated with PEG/NaCl as described above to remove glycerol. The pre-selection against streptavidin coated

25 beads was performed by incubating the phages rotating at room temperature for 1 h with 1 mg washed (2x in PBST 0.1) beads in PBST 0.1 in a total volume of 1 ml. The pre-selection against streptavidin was only performed before the first selection round.

Pre-selection against IR done in selection track 3 (divided into tracks 3a and 3b in round 4) was done by incubating a high binding tube (Nunc ImmunoTube) with a coating solution containing 60 pmol IR (R&D Systems #1544-IR) in 50 mM carbonate buffer pH 9.6 at 37 °C for 1 h or at 4 °C over night. The coating solution was discarded and the phages incubated rotating in the IR coated tube for 1 h at room temperature. In round 5, track 3a, 6.25 µg IR was also present

30 during the selection.

Pre-selected phages and biotinylated target protein were diluted in selection buffer to a total volume of 1 ml. Selection took place in a blocked tube incubated in

an end-over-end rotator for 1 h 45 min at room temperature. To capture the phage-protein complexes, the selection solution was incubated for an additional 15 min with dry streptavidin beads. Unbound phages were removed by washing with 1 ml PBST 0.1 according to the selection scheme in Figure 7. Bound phages were
5 eluted using a low pH strategy where 500 µl of 50 mM glycine-HCl pH 2.2 was added to the streptavidin beads. After 10 min incubation at room temperature, the solution was neutralized by addition of 450 µl PBS and 50 µl 1 M Tris-HCl, pH 8.

Phage display – Phage particle titration: The concentrations of phages entering selections as well as phages eluted after selections were determined by
10 titration in each round. *E. coli* RR1ΔM15 cells in log phase were infected by phage particles in serial dilutions. After 5 min incubation, 5 µl cultivation from each dilution step was transferred to Tryptone yeast extract plates (TYE plates: 10 g/l Tryptone (Merck), 5 g/l yeast extract (Merck), 100 µg/ml ampicillin, 3 mg/ml NaCl and 2 % glucose) and incubated at 37 °C over night. The titer was calculated from
15 the number of colonies formed. If a subsequent round was started before the results of the titer from previous round was available, the amount of phages was calculated by an estimation that 1 ml over night culture gives rise to 1×10^{11} cfu.

Phage display – Amplification of phage particles: Log phase *E. coli* RR1ΔM15 cells were infected with 950 µl eluted phage particles for 20 min at
20 37 °C. The cells were pelleted for 10 min at 3300 g and the pellet was re-suspended in TSB, distributed on TYE plates and incubated over night at 37 °C.

The cells from the TYE plates were re-suspended in TSB-YE medium and the concentration was determined by optical density measurement. In an attempt to make sure that all phage variants were represented, a 1000x excess with
25 respect to phage output titration was inoculated to TSB-YE containing 2 % glucose and 100 µg/ml ampicillin. Culture volume was adjusted to a starting OD₆₀₀ of approximately 0.1 and incubated at 37 °C and 90 rpm until log phase was reached. The same amount of cells as inoculated before, using an assumption that OD=1 corresponds to 5×10^8 cells, was infected with a 10x excess of M13K07
30 helper phages for 30 min at 37 °C. The cells were pelleted in a centrifuge for 10 min at 3300 g and the pellet was re-suspended in TSB-YE medium containing 100 µg/ml ampicillin and 50 µg/ml kanamycin, and 0.1 mM IPTG to induce the production of Z variant molecules. The culture was incubated over night at 37 °C and 90 rpm. The phage particles were harvested by precipitation as described
35 earlier.

Screening of selected Z variants: From rounds 4 and 5, 744 randomly picked colonies were inoculated and grown over night at 37 °C and 200 rpm in

deep well plates containing 1 ml TSB-YE supplemented with 100 µg/ml ampicillin and 1 mM IPTG. After the over night incubation, replica plates were made by transferring a fraction of the cells to square well storage plates with 15 % glycerol for storage at -20 °C. The deep well plates were spun for 10 min at 3000 g, the
5 supernatant was discarded and the pellets were re-suspended in 400 µl PBST 0.1. The plates were frozen over night at -80 °C and thawed to release the Z variant molecules in the periplasmic fraction of the cells. The plates were spun for 15 min at 3700 g, after which the supernatants could be utilized for ELISA screening.

ELISA characterization of selected Z variants: Half area 96 well ELISA
10 plates (Costar #3690) were coated over night at 4 °C with 50 µl, 1 µg/ml IGF-1R in 50 mM carbonate buffer, pH 9.6. The plates were rinsed in tap water and blocked with 0.5 % casein in PBST 0.1 for 1 h at room temperature. The blocking solution was discarded and 50 µl of Z variant containing periplasmic solution was added. After incubation with supernatants, the plates were washed in 4x175 µl PBS with
15 0.05 % Tween (PBST 0.05) using a Skan Washer 300 (Skatron) washing system. 50 µl IgG rabbit antibody directed against all Z variants (0.7 mg/ml, produced in-house by Affibody AB) and diluted 1:5000 was added and incubated for 2 h. The plates were washed as described above and 50 µl anti-IgG-rabbit-HRP (Dako #P0448) diluted 1:5 000 was added and incubated for 1 h. A commercial IGF-1R
20 antibody (Abcam ab32823-100) detected with a commercial secondary antibody conjugated with HRP (Jackson #703-036-155) was utilized as a positive control. After washing, 50 µl ImmunoPure TMB substrate (Pierce #34021) was added and incubated for 10 min in darkness before the reaction was stopped by addition of 50 ml 2 M sulphuric acid and absorbance measured at 450 nm in an ELISA reader
25 Tecan Ultra 384 (Tecan Group LTD) and evaluated with Magellan v. 5.0 software (Tecan).

Sequencing: From the ELISA screening, individual clones were picked for sequencing based on absorbance values. The Z variant inserts from the pAY00065 vector were amplified by PCR by adding cells from individual clones to
30 1x PCR buffer with MgCl₂ (Applied Biosystems #8080006), 0.4 µM dNTP mix (Applied Biosystems #N8080260) and 0.1 µM AmpliTaq Gold DNA polymerase (Applied Biosystems #4311816) and 5 pmol Affi21 (5'-tgctccggctcgtatgtgtgtg) and Affi22 (5'-cggaaccagagccaccaccgg) primers. The PCR program, performed on a Mastercycler epgradient S (Eppendorf), was 10 min @ 96 °C, 30 cycles of [15 s @
35 96 °C, 30 s @ 55 °C, 90 s @ 72 °C] and 7 min @ 72 °C.

A new PCR plate was prepared with 5 pmol Affi72 biotinylated primer (biotin-5'-cggaaccagagccaccaccgg) and Big Dye Terminator v.3.1 Cycle

sequencing Kit (Applied Biosystems) to which 1 µl of the amplified inserts was added and 30 cycles of [30 s @ 96 °C, 15 s @ 55 °C, 60 s @ 60 °C] were run. The biotinylated PCR sequence products were purified on a Magnatrix 8000 workstation using streptavidin Dynabeads® REGEN (Magnetic Biosolution
5 #11103). Sequencing of the purified fragments was performed on an ABI Prism® 3130xl Genetic Analyzer (Applied Biosystems).

Results

Construction of a new phagemid library: The new library was designed
10 based on a primary set of IGF-1R-binding Z variants with verified cell binding properties. The design of the new library resulted in a theoretical size of 1×10^8 different Z variant molecules. The actual size of the library, determined by titration after transformation to *E. coli* RR1ΔM15 cells, was 2.6×10^8 transformants, thus covering the estimated number of unique variants in the new library. From
15 sequencing of 96 of the clones in the obtained matured library, correspondence with the library design was confirmed.

Phage display selection: Phage display selections were performed against IGF-1R from the new library of Z variant molecules. The selection was performed in 5 rounds where the tracks differed in target concentration and pre-selection as
20 follows; one with high target concentration, one with low target concentration and one with high target concentration and pre-selection against IR. In round 4, the 3 tracks were divided in two tracks respectively, resulting in 6 tracks in total where tracks 1b, 2b and 3b involved rather harsh selection conditions with low target concentrations and several washes, including one over night wash. Phages eluted
25 from these tracks were too few to continue on a fifth selection round. On the other hand, the amounts of phages eluted from tracks 1a, 2a and 3a in round 4 were sufficient to proceed to a fifth selection round.

ELISA: A direct ELISA was utilized to screen for IGF-1R binders among 352 of the clones derived from the selections. Four negative controls for the Z variant
30 ELISA were applied, two where the Z variant was excluded, one where the primary antibody was excluded and one where the secondary antibody was excluded. A commercial IGF-1R antibody detected with a commercial secondary antibody conjugated with HRP was utilized as a positive control for the IGF-1R coating in two wells. Negative controls were also included, one omitting the primary antibody
35 and one omitting the secondary antibody. Nearly all the screened clones demonstrated affinity for IGF-1R with low background signals at this concentration.

Sequencing: Sequencing of clones analyzed by ELISA resulted in 195 unique sequences, one clone of which appeared twice. 190 unique clones were considered positive in the ELISA experiment, defined as for example an absorbance value of above 0.4. The predicted amino acid sequences of the corresponding polypeptides and their IGF-1R binding motifs were deduced, which yielded a number of sequences of IGF-1R binding polypeptides according to the invention. The obtained sequences all corresponded to the design of the library with regard to the amino acid residues allowed in each variable position. As in Example 1, each individual Z variant was given a unique identification number #####, and individual variants are referred to as Z#####. The amino acid sequences of deduced IGF-1R binding motifs are listed in Figure 1 and in the sequence listing as SEQ ID NO:8-197, whereas the amino acid sequences of the corresponding full-length Z variants are listed in Figure 1 and in the sequence listing as SEQ ID NO:205-394.

Example 3

Sub-cloning and expression of a subset of IGF-1R binding polypeptides

Sub-cloning

Gene fragments corresponding to eight of the Z variants selected in Example 2 were sub-cloned in a modified pET-T7 expression vector that included sequence encoding an N-terminal His₆-tag and a C-terminal Cys-residue. The IGF-1R binding Z variants were sub-cloned as monomers, and the constructs encoded by the expression vectors were

MGSSHHHHHLQ-[Z#####]-VDC,

wherein Z##### was selected from Z04493 (SEQ ID NO:249), Z04499 (SEQ ID NO:255), Z04542 (SEQ ID NO:298), Z04551 (SEQ ID NO:307), Z04557 (SEQ ID NO:312), Z04568 (SEQ ID NO:323), Z04572 (SEQ ID NO:327) and Z04609 (SEQ ID NO:363).

Expression and purification

The eight IGF-1R-binding Z variants listed in the previous section, as well as the Z variant Z01781 from Example 1 (SEQ ID NO:197), were expressed and purified to homogeneity by IMAC using the His₆ tag provided in the construct. In addition, in order to block the C-terminal cysteine, an aliquot of NEM conjugated Z variant molecules were produced. The identity of the Z variant molecules were

confirmed by LC/MS analyses and the purity was assessed by SDS-PAGE analysis. The melting temperatures (T_m) and reversibility of folding were determined by CD spectropolarity measurements. The melting temperatures ranged from 48 to 62 °C and the molecules in general folded back to the three
5 helical bundle structure when the temperature was lowered from 90°C to 20 °C.

Example 4

ELISA assessment of expressed IGF-1R binding polypeptides

10

Materials and methods

Microtiter plate (Costar #3690) wells were coated with 3 µg/ml IGF-1R, 50 µl/well, in 50 mM carbonate buffer, pH 9.6, and incubated at 4 °C over night. The wells were blocked with phosphate buffered saline (PBS: 10 mM phosphate, 2.68
15 mM KCl, 137 mM NaCl, pH 7.4) supplemented with 0.5 % casein (PBSC) during 1 h at room temperature. After removal of blocking, 100 µl of 400 ng/ml of the Z variant molecules Z01781, Z04499, Z04542, Z04551, Z04493, Z04557, Z04568, Z04572, Z04609 (all produced according to Example 3) and Z00810 (a Z variant molecule with affinity for an unrelated target; used as negative control), bearing an
20 N-terminal hexa-histidine tag and a C-terminal cysteine (NEM), were diluted in PBSC and added in triplicates to column 1 of the microplate. Columns 2-12 were filled with 50 µl PBSC. The Z variants were diluted stepwise 1:2 from column 1 to 11, leaving 50 µl of diluted Z variant molecule in each well. The plates were then incubated for 1.5 h at room temperature.

25 A goat IgG antibody against an epitope common to all Z variants (developed in house by Affibody AB) was diluted to 1.5 µg/ml in PBSC, 50 µl were added to each well, and incubated for 1.5 h. Complexes of Z variants and goat IgG were detected with 50 µl rabbit anti-goat IgG conjugated with HRP (SIGMA P0449) diluted 1:5000 in PBSC and incubated for 1 h at room temperature. The
30 plates were washed four times with PBS with 0.05 % Tween (PBST0.05) before incubation with primary and secondary antibody. Developing solution was prepared by mixing an equal volume of ImmunoPure TMB substrate solution (Thermo Scientific #34021) A and B and 50 µl were added to each well. After 20 min incubation, 50 µl 2 M H₂SO₄ were added and the plates were read at 450 nm
35 in an ELISA reader (Tecan Ultra 384, Tecan Group Ltd) and evaluated with Magellan v. 5.0 software (Tecan).

As described above, a negative control, the Z variant Z00810, was used in the same set up as the IGF-1R specific Z variants.

Curves were plotted in Microsoft Excel, and EC50 values were calculated using the log(agonist) vs. response (Variable slope) model, setting the bottom value to 0, using GraphPad software (GraphPad Software Inc.).

Results

Dose response curves were created for 9 Z variant molecules using an ELISA assay. IGF-1R was coated in microplate wells and Z variants were allowed to bind to the target. Bound Z variants were detected with specific antibodies. The dose response curves are presented as Figures 8A-8E, and clearly show specific binding of all tested IGF-1R binding polypeptides to IGF-1R. As expected, there is no binding by the negative control Z00810.

EC50 values, i.e the concentration of Z variant that provokes a response halfway between the baseline (bottom) and maximum response (top), were calculated for the IGF-1R binding Z variants using the log(agonist) vs. response (variable slope) model with the GraphPad software. The results are shown in Table 2.

Table 2: EC50 values for IGF-1R binding Z variants based on dose response curves with the bottom line set to 0

Molecule	EC50 (ng/ml)
Z01781	15.0
Z04493	16.2
Z04499	13.4
Z04542	13.1
Z04551	23.8
Z04557	20.5
Z04568	33.6
Z04572	21.3
Z04609	16.7

Example 5

Flow cytometry analysis of expressed IGF-1R binding polypeptides

Materials and methods

The Z variant molecules Z01781, Z04499, Z04542, Z04551, Z04493, Z04557, Z04568, Z04572, Z04609 (all produced according to Example 3) and Z01155 (a Z variant molecule with affinity for an unrelated target; used as negative

control), bearing an N-terminal hexa-histidine tag and a C-terminal cysteine, were tested.

300000 MCF-7 cells (ATCC HTB-22) per tube were incubated with 100 µl of Z variant diluted to 20, 10 and 5 µg/ml in ice cold PBS + 0.1 % BSA. After one
5 hour on ice, the cells were washed twice in ice cold PBS and incubated with a goat IgG antibody directed towards an epitope common for all Z variants, 5 µg/ml diluted in ice cold PBS + 0.1 % BSA, for one hour on ice. The cells were washed again and Alexa647 chicken anti-goat antibody (Molecular Probes A21469) was added, diluted in ice cold PBS + 0.1 % BSA to 15 µg/ml. After one hour on ice in
10 the dark, the cells were washed twice and analyzed in a FACS apparatus. 20000 cells per tube were analyzed.

An IGF-1R binding antibody was used in another experiment to confirm that the cells indeed do express the IGF-1 receptor. This antibody (chicken anti-human IGF-1R, α subunit; Abcam ab32823) was detected with Alexa488 anti-chicken
15 antibody (Molecular Probes A41658). Incubations and washes were done as described above. Since this detection system differs from that of the IGF-1R binding Z variant molecules, the antibody control was excluded in further experiments.

20 *Results*

The results are shown in Figure 9. All IGF-1R binding Z variants bound to the MCF-7 cells, and the signals from Z variants selected according to Example 2 were stronger than those from Z01781 described in Example 1. Among the Z variants from Example 2, the strongest signals were seen with Z04568, Z04557
25 and Z04551. Intermediate signals were seen with Z04493, Z04499 and Z04572, and the weakest signals were seen with Z04542 and Z04609. Z01781 rendered the weakest signals of all IGF-1R binding Z variants, and the signals from the unrelated variant Z01155 were similar to that of the negative control (goat anti-Z antibody and Alexa647 anti-goat antibody only).

CLAIMS

1. Insulin-like growth factor 1 receptor (IGF-1R) binding polypeptide,
comprising an insulin-like growth factor 1 receptor binding motif, which motif
5 consists of an amino acid sequence selected from:

i) $EX_2X_3X_4AX_6X_7EIX_{10} X_{11}LPNLX_{16}X_{17}X_{18}QX_{20} X_{21}AFIX_{25}SLX_{28}D$,

wherein, independently of each other,

- 10 X_2 is selected from G, P and K;
 X_3 is selected from F and Y;
 X_4 is selected from Y and F;
 X_6 is selected from A, L, S and G;
 X_7 is selected from I, L, M and V;
15 X_{10} is selected from I, L and V;
 X_{11} is selected from T, A, S, G, L, Q, I and V;
 X_{16} is selected from N and T;
 X_{17} is selected from S, Q, H, R, N, K, P, A, D, E, G and T;
 X_{18} is selected from S, Q, H, R, N, K, D, P, T and G;
20 X_{20} is selected from S, Q, H, R and W;
 X_{21} is selected from T, G, R, A, N, D, Q, E, K and S;
 X_{25} is selected from T, G, R, A, N, D, Q, E, K, H and S; and
 X_{28} is selected from E, N, G, S, A, T, K, P, Q and D;
25 and

ii) an amino acid sequence which has at least 85 % identity to the sequence defined in i).

- 30 2. IGF-1R-binding polypeptide according to claim 1, wherein X_2 is G.

3. IGF-1R-binding polypeptide according to any preceding claim, wherein X_3 is F.

4. IGF-1R-binding polypeptide according to any preceding claim, wherein X_4 is Y.
5. IGF-1R-binding polypeptide according to any preceding claim, wherein X_6 is A.
6. IGF-1R-binding polypeptide according to any one of claims 3-5, wherein X_3X_4 is FY and X_6 is A.
7. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{10} is L.
8. IGF-1R-binding polypeptide according to claim 7, wherein $X_2X_3X_4$ is GFY, X_6 is A and X_{10} is L.
9. IGF-1R-binding polypeptide according to any preceding claim, wherein X_7 is I.
10. IGF-1R-binding polypeptide according to claim 9, wherein $X_2X_3X_4$ is GFY, X_6X_7 is AI and X_{10} is L.
11. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{16} is N.
12. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{17} is H.
13. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{18} is selected from K and S.
14. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{20} is R.

15. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{21} is selected from T and G.

5 16. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{25} is T.

17. IGF-1R-binding polypeptide according to claim 16, wherein $X_{20}X_{21}$ is RG and X_{25} is T.

10 18. IGF-1R-binding polypeptide according to claim 17, wherein X_{11} is S, $X_{17}X_{18}$ is HS, $X_{20}X_{21}$ is RG and X_{25} is T.

15 19. IGF-1R-binding polypeptide according to any preceding claim, wherein X_{28} is E.

20. IGF-1R-binding polypeptide according any one of claims 1-11, wherein X_{11} is A, $X_{17}X_{18}$ is RK, $X_{20}X_{21}$ is ST and X_{28} is E.

20 21. IGF-1R-binding polypeptide according to any preceding claim, wherein the amino acid sequence i) is selected from SEQ ID NO:1-197.

25 22. IGF-1R-binding polypeptide according to claim 21, wherein the amino acid sequence i) is selected from SEQ ID NO:1-2, 52, 58, 101, 110, 115, 126, 130 and 166.

23. IGF-1R-binding polypeptide according to any preceding claim, in which said IGF-1R-binding motif forms part of a three-helix bundle protein domain.

30 24. IGF-1R-binding polypeptide according to claim 23, in which said IGF-1R-binding motif essentially forms part of two alpha helices and a loop connecting them, within said three-helix bundle protein domain.

25. IGF-1R-binding polypeptide according to claim 24, in which said three-helix bundle protein domain is selected from domains of bacterial receptor proteins.

5 26. IGF-1R-binding polypeptide according to claim 25, in which said three-helix bundle protein domain is selected from domains of protein A from *Staphylococcus aureus* or derivatives thereof.

10 27. IGF-1R-binding polypeptide according to claim 26, which comprises an amino acid sequence selected from:

ADNNFNK-[*IBM*]-DPSQSANLLSEAKKLNESQAPK;
ADNKFNK-[*IBM*]-DPSQSANLLAEAKKLNDQAQAPK;
ADNKFNK-[*IBM*]-DPSVSKEILAEAKKLNDQAQAPK;
ADAQQNNFNK-[*IBM*]-DPSQSTNVLGEAKKLNESQAPK;
15 AQHDE-[*IBM*]-DPSQSANVLGEAQKLNDSDQAQAPK; and
VDNKFNK-[*IBM*]-DPSQSANLLAEAKKLNDQAQAPK;

wherein [*IBM*] is an IGF-1R-binding motif as defined in any one of claims 1-22.

20 28. IGF-1R-binding polypeptide, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following:

- iii) it is selected from SEQ ID NO:198-394;
- iv) it is an amino acid sequence having 85 % or greater identity to a sequence selected from SEQ ID NO: 198-394.

25 29. IGF-1R-binding polypeptide according to claim 28, whose amino acid sequence comprises a sequence which fulfils one definition selected from the following:

- v) it is selected from SEQ ID NO:198-199, 249, 255, 298, 307, 312, 323, 327 and 363;
- 30 vi) it is an amino acid sequence having 85 % or greater identity to a sequence selected from SEQ ID NO:198-199, 249, 255, 298, 307, 312, 323, 327 and 363.

30. IGF-1R-binding polypeptide according to any preceding claim comprising additional amino acid residues C terminally and/or N terminally with respect to said IGF-1R-binding polypeptide.

5 31. IGF-1R-binding polypeptide according to claim 30 in which the or each amino acid extension enhances binding of IGF-1R by the polypeptide.

32. IGF-1R-binding polypeptide according to claim 30 or 31 in which the or each amino acid extension improves production, purification, stabilization *in vivo* or
10 *in vitro*, coupling, or detection of the polypeptide.

33. IGF-1R-binding polypeptide according to claim 32 in which the extension comprises an albumin-binding domain, or a derivative thereof, which prolongs the half life of the IGF-1R-binding polypeptide in applications *in vivo*.

15

34. IGF-1R-binding polypeptide according to claim 33, in which the albumin-binding domain is domain GA3 of protein G from *Streptococcus* strain G148, or a derivative thereof, which prolongs the half life of the IGF-1R-binding polypeptide in applications *in vivo*.

20

35. IGF-1R-binding polypeptide according to any preceding claim, which binds to IGF-1R such that the K_D value of the interaction is at most 1×10^{-6} M.

36. IGF-1R-binding polypeptide according to claim 35, which binds to IGF-
25 1R such that the K_D value of the interaction is at most 1×10^{-7} M.

37. IGF-1R-binding polypeptide according to any preceding claim which binds to the ectodomain of IGF-1R.

30 38. IGF-1R-binding polypeptide according to claim 37 which binds to a portion of the ectodomain of IGF-1R corresponding to SEQ ID NO:397.

39. IGF-1R-binding polypeptide comprising a fragment of an IGF-1R-binding polypeptide according to any preceding claim, which fragment retains IGF-1R binding.

5 40. IGF-1R-binding polypeptide according to claim 39 in which the fragment comprises an N terminal reduction of a polypeptide according to any one of claims 1-38.

10 41. IGF-1R-binding polypeptide according to claim 40 in which the N terminal reduction is by up to four amino acid residues.

15 42. IGF-1R-binding polypeptide according to any preceding claim in multimeric form, comprising at least two IGF-1R-binding polypeptide monomer units, whose amino acid sequences may be the same or different.

 43. IGF-1R-binding polypeptide according to claim 42, in which the IGF-1R-binding polypeptide monomer units are covalently coupled together.

20 44. IGF-1R-binding polypeptide according to claim 42, in which the IGF-1R-binding polypeptide monomer units are expressed as a fusion protein.

 45. A polynucleotide encoding a polypeptide according to any preceding claim.

25 46. Method of producing a polypeptide according to any one of claims 1-44, the method comprising expressing a polynucleotide according to claim 45.

 47. Combination of an IGF-1R-binding polypeptide according to any one of claims 1-44 and a detectable agent.

30 48. Combination according to claim 47, in which the detectable agent is a molecule comprising a radionuclide for use in molecular imaging.

49. Combination according to claim 48, in which the detectable agent is a chelating entity in which a radionuclide is chelated.

50. Combination according to claim 48, in which the detectable agent is a
5 single radionuclide.

51. Combination according to claim 47, in which the detectable agent is an enzyme.

10 52. Combination of an IGF-1R-binding polypeptide according to any one of claims 1-44 and a therapeutic agent.

53. Combination according to any one of claims 47-52, in which the IGF-1R-binding polypeptide and detectable agent or therapeutic agent are covalently
15 coupled together.

54. Combination according to any one of claims 47-52, in which the IGF-1R-binding polypeptide and detectable agent or therapeutic agent are expressed as a fusion protein.

20

55. Method of molecular imaging, in which a combination according to any one of claims 47-54 is used as an imaging agent.

56. Method of detection of IGF-1R, comprising providing a sample
25 suspected to contain an IGF-1R, contacting the sample with an IGF-1R-binding polypeptide according to any one of claims 1-44, or a combination according to any one of claims 47-54 and detecting binding of the polypeptide or combination to indicate the presence of an IGF-1R in the sample.

30 57. Method of separating or capturing IGF-1R from a sample, the method comprising contacting the sample with an IGF-1R-binding polypeptide according to any one of claims 1-44 or a combination according to any one of claims 47-54, whereby IGF-1R binds to the polypeptide and can be removed from the sample.

58. Method for determining the presence of an IGF-1R in a mammalian subject, the method including contacting the subject, or a sample derived from the subject, with an IGF-1R-binding polypeptide according to any one of claims 1-44
5 or a combination according to any one of 47-54 and detecting binding of the polypeptide or combination.

59. Method according to claim 58, in which the subject is human.

10 60. Method according to claim 58 or 59, in which the method is performed *in vivo*.

61. Method according to claim 58 or 59, in which the method is performed on a sample *in vitro*.

15

62. Method of treatment of an IGF-1R-related condition in a mammalian subject or in material derived from a mammalian subject, in which the subject or material is treated with an IGF-1R-binding polypeptide according to any one of claims 1-44 or a combination according to any one of claims 47-54.

20

63. Method of treatment according to claim 62, in which binding of said IGF-1R-binding polypeptide or said combination to an IGF-1R of the subject, or in the material, inhibits or stimulates activation of the receptor.

25 64. Method of treatment according to claim 62 or 63, in which binding of the IGF-1R-binding polypeptide to an IGF-1R of the subject, or in the material, inhibits receptor signaling.

65. Method of treatment according to any one of claims 62-64, in which the
30 IGF-1R-related condition is a cancer.

66. Method of treatment according to claim 65, in which the cancer is selected from carcinomas of the lung, breast, thyroid, gastrointestinal tract and prostate, glioblastomas, neuroblastomas, rhabdomyosarcomas and leukemias.

5 67. Method according to any one of claims 62-66, in which said subject is human.

68. An IGF-1R-binding polypeptide according to any one of claims 1-44 for use as a medicament.

10

69. An IGF-1R-binding polypeptide according to any one of claims 1-44 for use as a medicament for the treatment of cancer.

70. A combination according to any one of claims 47-54 for use as a
15 medicament.

71. A combination according to any one of claims 47-54 for use as a medicament for the treatment of cancer.

20 72. Use of an IGF-1R-binding polypeptide according to any one of claims 1-44 or a combination according to any one of claims 47-54 for the manufacture of a medicament for the treatment of cancer.

73. An IGF-1R-binding polypeptide according to any one of claims 1-44 for
25 use as a diagnostic agent.

74. An IGF-1R-binding polypeptide according to any one of claims 1-44 for use as a diagnostic agent for the diagnosis of cancer *in vivo*.

30 75. A combination according to any one of claims 47-54 for use as a diagnostic agent.

76. A combination according to any one of claims 47-54 for use as a diagnostic agent for the diagnosis of cancer *in vivo*.

5 77. Use of an IGF-1R-binding polypeptide according to any one of claims 1-44 or a combination according to any one of claims 47-54 for the manufacture of a diagnostic agent for the diagnosis of cancer *in vivo*.

Polypeptide	Amino acid sequence	SEQ ID NO:
IBM01781	EGFYAAIEIL ALPNLNKQKQ TAFISLED	1
IBM02003	EGFYAAIEIL SLPNLNHSQR GAFITSLTD	2
IBM02998	EKFYAAIEIL ALPNLNKQKQ TAFIRSLED	3
IBM02215	EPFYAAAMEIL TLPNLNNGQR RAFITSLND	4
IBM _{4:2:64}	EGFYAALEII LLPNLNPSQW TAFITSLGD	5
IBM _{4:2:35}	EGFYAALEIL VLPNLNTQQR GAFITSLSD	6
IBM02987	EGFYAAIEIL SLPNLNHSQR GAFITSLTD	7
IBM04279	EGFYAAAMEIL ALPNLNSRQS KAFINSLTD	8
IBM04280	EGFYAAVEIL GLPNLNARQR TAFINSLED	9
IBM04281	EGFYAAAMEIV ILPNLTHRQR AAFIGSLTD	10
IBM04282	EGFYAAIEIL SLPNLTKQKH TAFIGSLTD	11
IBM04283	EGFYAAAMEIL ALPNLNGRQR SAFITSLND	12
IBM04284	EGFYAAIEIL TLPNLNRRQR SAFITSLSD	13
IBM04285	EGFYAALEIL ALPNLNKQKQ TAFISSLDD	14
IBM04286	EGFYAALEIV SLPNLTERQR AAFIGSLDD	15
IBM04287	EGFYAAVEIV TLPNLTKGQR EAFIGSLGD	16
IBM04288	EGFYAALEIL TLPNLTKQH RAFITSLGD	17
IBM04289	EGFYAAIEIV ALPNLNRRQR SAFIRSLED	18
IBM04290	EGFYAALEIV ALPNLTPRQR TAFIGSLED	19
IBM04291	EGFYAGIEIL ILPNLNERQR AAFIRSLED	20
IBM04292	EGFYAALEIV ALPNLTSKQR EAFIGSLGD	21
IBM04293	EGFYAAIEIL ILPNLNGSQR RAFISSIAD	22
IBM04294	EGFYAALEIL ALPNLNTQR SAFISSLPD	23
IBM04295	EGFYAALEIL VLPNLTORQR TAFITSLSD	24
IBM04296	EGFYAAIEIL TLPNLNRRQR TAFITSLPD	25
IBM04297	EGFYAAVEIL VLPNLTSRQR TAFIGSLTD	26
IBM04298	EGFYAAIEIL ALPNLNHRQR GAFISSIGD	27
IBM04299	EGFYAALEIV SLPNLNQRQR TAFITSLSD	28
IBM04300	EGFYAAIEIL TLPNLNERQR GAFIGSLSD	29
IBM04301	EGFYAALEIL ILPNLNRRQR TAFISSLGD	30
IBM04302	EGFYAAIEIL TLPNLNRRQR DAFITSLND	31

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
IBM04303	EGFYAAIEIM SLPNLTRRQR SAFIGSLD	32
IBM04304	EGFYAALEIL VLPNLNSHQ R AFIASLED	33
IBM04305	EGFYAGIEIL TLPNLNRRQR EAFIGSLND	34
IBM04306	EGFYAAVEIL TLPNLTRRQR SAFIGSLGD	35
IBM04307	EGFYAAIEIV ALPNLTGKQR SAFIKSLGD	36
IBM04308	EGFYAALEIV TLPNLNERQR RAFIASLTD	37
IBM04309	EGFYAAIEIV ALPNLNARQR TAFIGSLTD	38
IBM04310	EGFYAALEII TLPNLTHRQQ RAFITSLED	39
IBM04311	EGFYAAIEIV ALPNLTRHQQ RAFINSLGD	40
IBM04312	EGFYAAVEII ILPNLTEKQR SAFIGSLGD	41
IBM04313	EGFYAALEIL TLPNLNQRQH RAFISSLGD	42
IBM04314	EGFYAALEIV TLPNLNRQQ R DAFISSLND	43
IBM04315	EGFYAALEII TLPNLNAQQR RAFIGSLHD	44
IBM04316	EGFYAAVEIL VLPNLNKSQR DAFIGSLND	45
IBM04317	EGFYAAIEIV ALPNLNNQR TAFIRSLGD	46
IBM04319	EGFYAAIEIL VLPNLNRRQR TAFIGSLQD	47
IBM04320	EGFYAALEIL GLPNLNRQR SAFISSLED	48
IBM04490	EGFYAAIEIL SLPNLNPGQR TAFINSLED	49
IBM04491	EGFYAAIEIL TLPNLNSGQR SAFIRSLTD	50
IBM04492	EGFYAAIEIL TLPNLTRNQR SAFIGSLGD	51
IBM04493	EGFYAALEIV ALPNLNRQR TAFITSLTD	52
IBM04494	EGFYAAIEIV ALPNLNRKQR SAFIRSLGD	53
IBM04495	EGFYAAIEIV SLPNLNGRQR RAFISSLGD	54
IBM04496	EGFYAAIEIL TLPNLNNTQR KAFITSLED	55
IBM04497	EGFYAALEIV ALPNLNRKQR GAFIDSND	56
IBM04498	EGFYAAIEIL SLPNLNSKQH RAFINSLTD	57
IBM04499	EGFYAAIEIL TLPNLNANQR TAFIGSLGD	58
IBM04500	EGFYAALEIV ALPNLTNRQR SAFITSLED	59
IBM04501	EGFYAAIEIL TLPNLNRRQH EAFIASLGD	60
IBM04502	EGFYAAIEIL VLPNLTORQR EAFITSND	61
IBM04503	EGFYAAIEIV ALPNLNSNQR TAFIRSLTD	62

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
IBM04504	EGFYAAIEIV ALPNLTQRQ AFIRSLAD	63
IBM04505	EGFYAAIEIL SLPNLNKHQR KAFISLED	64
IBM04506	EGFYAAIEIV ALPNLNQQQR RAFIQSLGD	65
IBM04507	EGFYAAVEIL ALPNLNQRQR TAFITSLSD	66
IBM04508	EGFYAAIEII SLPNLNRRQR GAFIASLTD	67
IBM04509	EGFYAAIEIV ALPNLNSKQR RAFITSLSD	68
IBM04510	EGFYAAIEII ALPNLTQRQR TAFIRSLSD	69
IBM04511	EGFYAAIEIL VLPNLNESQR SAFIKSLGD	70
IBM04512	EGFYAAIEIL VLPNLTSQR RAFITSLSD	71
IBM04513	EGFYAAIEIL TLPNLNKKQH RAFINSLED	72
IBM04514	EGFYAAIEIL TLPNLNQRQR TAFITSLGD	73
IBM04515	EGFYAAIEIL VLPNLNSRQR NAFIHSLED	74
IBM04516	EGFYAAIEIL ALPNLNTKQR TAFINSLED	75
IBM04517	EGFYAAIEIL TLPNLNPRQR TAFIESLTD	76
IBM04518	EGFYAAIEIL TLPNLNPRQR RAFITSLDD	77
IBM04519	EGFYAAIEII TLPNLNTRQR TAFIRSLGD	78
IBM04520	EGFYAAIEIL SLPNLNQRQR GAFIASLGD	79
IBM04521	EGFYAAIEIV TLPNLNRRQR DAFIASLGD	80
IBM04522	EGFYAAIEIL ILPNLNHGQR SAFIGSLTD	81
IBM04523	EGFYAAIEII TLPNLTHRQH AAFINSLED	82
IBM04524	EGFYAAIEIL ILPNLNRGQH AAFIRSLTD	83
IBM04525	EGFYAAIEII ALPNLTKRQR TAFISSLAD	84
IBM04526	EGFYAAIEIL ALPNLTSKQR EAFIGSLGD	85
IBM04527	EGFYASIEIL VLPNLNSRQR SAFISSLGD	86
IBM04528	EGFYAAIEIV ALPNLTRRQR AAFIASLDD	87
IBM04529	EGFYAAIEIL TLPNLNQRQR GAFINSLED	88
IBM04530	EGFYAAIEII ALPNLTQRQH RAFITSLND	89
IBM04531	EGFYAAIEII TLPNLTKRQR AAFIGSLSD	90
IBM04532	EGFYAAIEIL VLPNLTTQRH KAFINSLED	91
IBM04533	EGFYAAIEIV SLPNLTSRQR TAFIHSLED	92
IBM04534	EGFYAAIEIV ALPNLNRNQR TAFIRSLTD	93

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
IBM04535	EGFYAAIEII SLPNLRKQR GAFIASLTD	94
IBM04536	EGFYAAIEIL ILPNLRKQS RAFIASLED	95
IBM04537	EGFYAAIEIL SLPNLRKQR TAFITSLED	96
IBM04538	EGFYAAIEIL VLPNLNQHQ R GAFISSLSD	97
IBM04539	EGFYAALEIV SLPNLRNQR GAFIQSLTD	98
IBM04540	EGFYAAIEII SLPNLTKQR RAFITS LGD	99
IBM04541	EGFYAAIEIV ALPNLTHKQR RAFIES LGD	100
IBM04542	EGFYAALEIL ALPNLNHRQR TAFIGSLED	101
IBM04543	EGFYAAIEIL SLPNLNRRQR TAFIGS LGD	102
IBM04544	EGFYAAIEIL TLPNLNSRQR DAFIGS LGD	103
IBM04545	EGFYAALEIV SLPNLTPRQR GAFISSLGD	104
IBM04546	EGFYAAIEIV SLPNLNRRQR EAFITSLED	105
IBM04547	EGFYAALEIL TLPNLTGRQR TAFIRS LGD	106
IBM04548	EGFYAAIEIL VLPNLNKKQR SAFIKSLDD	107
IBM04549	EGFYAAIEIL TLPNLTSRQH RAFIASLED	108
IBM04550	EGFYAALEII TLPNLNQQR QAFIAS LGD	109
IBM04551	EGFYAALEIL ILPNLTQQR GAFISSLSD	110
IBM04552	EGFYAAIEII ILPNLTPRQR AAFITSLED	111
IBM04553	EGFYAAIEIL VLPNLNSKQR TAFIASLSD	112
IBM04554	EGFYAAVEIL GLPNLRKQR SAFIASLDD	113
IBM04555	EGFYAAIEIL ALPNLNGGQR SAFIRSLED	114
IBM04557	EGFYAAIEIL TLPNLTNKQR RAFIGSLED	115
IBM04558	EGFYAALEIL ILPNLNDQR RAFIGSLND	116
IBM04559	EGFYAALEIV TLPNLNPKQR SAFISSLSD	117
IBM04560	EGFYAAIEIL SLPNLNSRQR QAFIGSLED	118
IBM04561	EGFYAALEIV ILPNLNTRQR DAFIRS LAD	119
IBM04562	EGFYAAIEIV SLPNLNQQR TAFISSIAD	120
IBM04563	EGFYAGIEII ALPNLNQHQ R EAFIKSLND	121
IBM04564	EGFYAAIEIL SLPNLNSRQH RAFITS LAD	122
IBM04565	EGFYAALEIV SLPNLNSRQR TAFITS LSD	123
IBM04566	EGFYALIEIV SLPNLNKKQR NAFIRS LND	124

FIGURE 1

Polypeptide	Amino acid sequence		SEQ ID NO:
IBM04567	EGFYAALEIV	ALPNLNPRQR TAFIRSLED	125
IBM04568	EGFYAAIEIL	TLPNLNRRQR SAFIASLGD	126
IBM04569	EGFYAALEIV	GLPNLTGRQR TAFIQSLGD	127
IBM04570	EGFYAAEMEIV	ALPNLNQRQR RAFIRSLED	128
IBM04571	EGFYAAIEIV	TLPNLTGNQR TAFIRSLED	129
IBM04572	EGFYAALEIL	TLPNLTRHQR TAFIDSLGD	130
IBM04573	EGFYASMEIL	SLPNLNGKQR TAFITSLED	131
IBM04574	EGFYAALEIL	TLPNLNTKQR TAFIGSLGD	132
IBM04575	EGFYAAEMEIV	ALPNLNRRQR TAFINSLTD	133
IBM04576	EGFYAAIEII	ILPNLTPRQR SAFIDSLED	134
IBM04577	EGFYAAEMEIL	QLPNLTNRQR AAFISSLPD	135
IBM04578	EGFYAAIEIL	VLPNLNQDQR TAFITSLTD	136
IBM04579	EGFYAALEIL	ILPNLNSSQR TAFIGSLED	137
IBM04580	EGFYAAIEIL	ALPNLNTRQH RAFIRSLED	138
IBM04581	EGFYAALEIL	VLPNLNKKQR TAFIGSLED	139
IBM04582	EGFYAAIEIL	SLPNLTRRQR GAFITSLED	140
IBM04583	EGFYAAIEII	SLPNLNARQR TAFIASLGD	141
IBM04584	EGFYAAIEIL	ALPNLNRTQR SAFIGSLAD	142
IBM04585	EGFYAAIEII	SLPNLNPRQR TAFITSLED	143
IBM04586	EGFYAAIEIL	VLPNLNKRQR AAFIGSLED	144
IBM04587	EGFYAAIEIL	ALPNLNKKQR TAFIQSLDD	145
IBM04588	EGFYAALEII	SLPNLTQRQH EAFIRSLED	146
IBM04589	EGFYAAIEIL	TLPNLTQNQH TAFIRSLED	147
IBM04590	EGFYAAIEIL	TLPNLNQKQH RAFIASLGD	148
IBM04591	EGFYAAEMEIV	ALPNLTSKQR TAFIRSLED	149
IBM04592	EGFYAAIEIL	ILPNLNTRQR GAFIGSLND	150
IBM04593	EGFYAAIEIL	SLPNLNRRQR SAFISSLED	151
IBM04594	EGFYAALEIL	SLPNLTKKQR TAFIRSLED	152
IBM04595	EGFYAALEIL	TLPNLNQNQR SAFIRSLED	153
IBM04596	EGFYAAIEIL	TLPNLNRPQR DAFISSLED	154
IBM04597	EGFYAAIEII	TLPNLTQGQR TAFIRSLED	155

FIGURE 1

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Polypeptide	Amino acid sequence	SEQ ID NO:
IBM04598	EGFYAAAEIL ILPNLTRGQR RAFIASLGD	156
IBM04599	EGFYAAAEIL TLPNLNQKQR DAFITSLGD	157
IBM04600	EGFYAAAEIL TLPNLNKRQR TAFIESLTD	158
IBM04601	EGFYAAAEII ALPNLTRRQR TAFITSLED	159
IBM04602	EGFYAAAEIV TLPNLTHRQR SAFIGSLGD	160
IBM04604	EGFYAAAEIL TLPNLNQQR TAFITSLGD	161
IBM04605	EGFYAAAEII TLPNLTORQR SAFINSLPD	162
IBM04606	EGFYAAAEIL TLPNLTGRQR TAFIRSLED	163
IBM04607	EGFYAAAEII SLPNLTRRQR AAFIRSLED	164
IBM04608	EGFYAAAEIV SLPNLTSKQR GAFITS LTD	165
IBM04609	EGFYAAAEIV SLPNLTORQR TAFITSLED	166
IBM04610	EGFYAAAEIL ILPNLTQHQR TAFITSLGD	167
IBM04611	EGFYAAAEIL TLPNLTSQQR RAFITSLGD	168
IBM04612	EGFYAAAEII TLPNLNPRQR TAFINSLTD	169
IBM04614	EGFYAAAEIL TLPNLNKRQR SAFIRSLED	170
IBM04615	EGFYAAAEIL VLPNLNTQQR RAFIKSLED	171
IBM04616	EGFYAAAEIV SLPNLNTRQR SAFITSLGD	172
IBM04617	EGFYAAAEIL VLPNLNKRQR EAFIGSLTD	173
IBM04618	EGFYAAAEIL VLPNLNQKQR RAFINSLGD	174
IBM04619	EGFYAAAEIL TLPNLTRRQR SAFITSLQD	175
IBM04620	EGFYAAAEIL SLPNLTGRQH RAFITSLED	176
IBM04621	EGFYAAAEII ALPNLRRRQR SAFIRSLED	177
IBM04623	EGFYAAAEIL VLPNLTRQR KAFIESLPD	178
IBM04624	EGFYAAAEIL TLPNLNSRQR TAFIGSLGD	179
IBM04625	EGFYAAAEIL VLPNLNPKQR TAFIDSLSD	180
IBM04626	EGFYASAEIL VLPNLNGRQR TAFITSLAD	181
IBM04627	EGFYAAAEIL TLPNLNSRQR RAFIGSLQD	182
IBM04628	EGFYAAAEIL TLPNLNRKQR SAFIGSLTD	183
IBM04629	EGFYAAAEIL ILPNLNQRQR SAFIGSLED	184
IBM04630	EGFYAAAEIL ALPNLNRQR SAFINSLGD	185
IBM04631	EGFYAAAEIV ALPNLNRKQR TAFISSLTD	186

FIGURE 1

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Polypeptide	Amino acid sequence	SEQ ID NO:
IBM04632	EGFYAAAEIL GLPNLNNRQR RAFITSLTD	187
IBM04633	EGFYAAVEIL TLPNLTRRQH RAFITSLGD	188
IBM04634	EGFYAAIEIV ALPNLTTKQR SAFIRSLGD	189
IBM04635	EGFYAALEIL VLPNLITGNQR TAFITSLTD	190
IBM04636	EGFYAALEII ALPNLNQKQR SAFIQSLGD	191
IBM04637	EGFYAAIEIL VLPNLNSRQR DAFIRSLSD	192
IBM04638	EGFYAALEIL ILPNLNQQR DAFITSLGD	193
IBM04639	EGFYAAEIL ILPNLTRRQH RAFITSLD	194
IBM04640	EGFYAALEIL SLPNLTPKQR SAFITSLTD	195
IBM04641	EGFYAAIEIV VLPNLTPRQR SAFIKSLDD	196
IBM04642	EGFYAAIEIL TLPNLNPQR TAFIKSLED	197
Z01781	VDNKFNKEGF YAAIEILALP NLNRKQSTAF ISSLEDDPSQ SANLLAEAKK LNDQAQAPK	198
Z02003	VDNKFNKEGF YAAIEILSLP NLNHSQRGAF ITSLTDDPSQ SANLLAEAKK LNDQAQAPK	199
Z02998	VDNKFNKEKF YAAIEILALP NLNRKQSTAF IRSLEDDPSQ SANLLAEAKK LNDQAQAPK	200
Z02215	VDNKFNKEPF YAAIEILTL P NLNNGQRRAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	201
Z _{4:2:64}	VDNKFNKEGF YAAIEIILLP NLNPSQWTAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	202
Z _{4:2:35}	VDNKFNKEGF YAAIEILVLP NLNTQQRGAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	203
Z02987	VDNKFNKEGF YAAIEILSLP NLNHSQRGAF ITSLTDDPSQ SANLLAEAKK LNDQAQAPK	204
Z04279	VDNKFNKEGF YAAIEILALP NLNSRQSKAF INSLEDDPSQ SANLLAEAKK LNDQAQAPK	205
Z04280	VDNKFNKEGF YAAVEILGLP NLNARQRTAF INSLEDDPSQ SANLLAEAKK LNDQAQAPK	206
Z04281	VDNKFNKEGF YAAIEIVILP NLTHRQRAAF IGSLTDDPSQ SANLLAEAKK LNDQAQAPK	207
Z04282	VDNKFNKEGF YAAIEILSLP NLTKQHTAF IGSLTDDPSQ SANLLAEAKK LNDQAQAPK	208
Z04283	VDNKFNKEGF YAAIEILALP NLNGRQSAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	209
Z04284	VDNKFNKEGF YAAIEILTL P NLNRQRSAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	210
Z04285	VDNKFNKEGF YAAIEILALP NLNRKQRTAF ISSLEDDPSQ SANLLAEAKK LNDQAQAPK	211
Z04286	VDNKFNKEGF YAAIEIVSLP NLTERQRAAF IGSLDDDDPSQ SANLLAEAKK LNDQAQAPK	212
Z04287	VDNKFNKEGF YAAVEIVTL P NLTKGQREAF IGSLDDDDPSQ SANLLAEAKK LNDQAQAPK	213
Z04288	VDNKFNKEGF YAAIEILTL P NLNTKQHRAF ITSLEDDPSQ SANLLAEAKK LNDQAQAPK	214
Z04289	VDNKFNKEGF YAAIEIVALP NLNRQRSAF IRSLEDDPSQ SANLLAEAKK LNDQAQAPK	215
Z04290	VDNKFNKEGF YAAIEIVALP NLTPRQRTAF IGSLDDDDPSQ SANLLAEAKK LNDQAQAPK	216
Z04291	VDNKFNKEGF YAGIEILILP NLNERQRAAF IRSLEDDPSQ SANLLAEAKK LNDQAQAPK	217

FIGURE 1

Polypeptide	Amino acid sequence		SEQ ID NO:
Z04292	VDNKFNKEGF YAALEIVSLP NLTSKQREAF IGSIGDDPSQ	SANLLAEAKK LNDQAQPK	218
Z04293	VDNKFNKEGF YAAIEILILP NLNGSQRRAF ISSLADDPSP	SANLLAEAKK LNDQAQPK	219
Z04294	VDNKFNKEGF YAALEILALP NLNTRQRSF ISSLPDDPSQ	SANLLAEAKK LNDQAQPK	220
Z04295	VDNKFNKEGF YAALEILVLP NLTQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	221
Z04296	VDNKFNKEGF YAAIEILTLN LNRRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	222
Z04297	VDNKFNKEGF YAAVEILVLP NLTSRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	223
Z04298	VDNKFNKEGF YAAIEILALP NLNHRQGF ISSLGGDDPSQ	SANLLAEAKK LNDQAQPK	224
Z04299	VDNKFNKEGF YAALEIVSLP NLNQRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	225
Z04300	VDNKFNKEGF YAAIEILTLN NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	226
Z04301	VDNKFNKEGF YAALEILILP NLNRQRTAF ISSLGGDDPSQ	SANLLAEAKK LNDQAQPK	227
Z04302	VDNKFNKEGF YAAIEILTLN NLNRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	228
Z04303	VDNKFNKEGF YAAIEIMSLP NLRRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	229
Z04304	VDNKFNKEGF YAALEILVLP NLNHRQAF IASLEDDPSQ	SANLLAEAKK LNDQAQPK	230
Z04305	VDNKFNKEGF YAGIEILTLN NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	231
Z04306	VDNKFNKEGF YAAVEILTLN NLRRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	232
Z04307	VDNKFNKEGF YAAIEIVLP NLTKQRTAF IKSLEDDPSQ	SANLLAEAKK LNDQAQPK	233
Z04308	VDNKFNKEGF YAAIEIVTLN NLNRQRTAF IASLEDDPSQ	SANLLAEAKK LNDQAQPK	234
Z04309	VDNKFNKEGF YAAIEIVLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	235
Z04310	VDNKFNKEGF YAAIEITLP NLTHRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	236
Z04311	VDNKFNKEGF YAAIEIVLP NLTHRQRTAF INSLEDDPSQ	SANLLAEAKK LNDQAQPK	237
Z04312	VDNKFNKEGF YAAVEIILP NLTKQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	238
Z04313	VDNKFNKEGF YAAIEITLP NLNRQRTAF ISSLEDDPSQ	SANLLAEAKK LNDQAQPK	239
Z04314	VDNKFNKEGF YAAIEIVLP NLNRQRTAF ISSLEDDPSQ	SANLLAEAKK LNDQAQPK	240
Z04315	VDNKFNKEGF YAAIEITLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	241
Z04316	VDNKFNKEGF YAAVEILVLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	242
Z04317	VDNKFNKEGF YAAIEIVLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	243
Z04319	VDNKFNKEGF YAAIEILVLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	244
Z04320	VDNKFNKEGF YAAIEILGLP NLNRQRTAF ISSLEDDPSQ	SANLLAEAKK LNDQAQPK	245
Z04490	VDNKFNKEGF YAAIEILSLP NLNRQRTAF INSLEDDPSQ	SANLLAEAKK LNDQAQPK	246
Z04491	VDNKFNKEGF YAAIEITLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	247
Z04492	VDNKFNKEGF YAAIEITLP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	248

FIGURE 1

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Polypeptide	Amino acid sequence		SEQ ID NO:
Z04493	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLTDDPSQ SANLLAEAKK LNDQAQPK	249
Z04494	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IRSLGDDPSQ SANLLAEAKK LNDQAQPK	250
Z04495	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ISSLGDDPSQ SANLLAEAKK LNDQAQPK	251
Z04496	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLGDDPSQ SANLLAEAKK LNDQAQPK	252
Z04497	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IDSLNDDPSQ SANLLAEAKK LNDQAQPK	253
Z04498	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	INSLTDDPSQ SANLLAEAKK LNDQAQPK	254
Z04499	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IGSLGDDPSQ SANLLAEAKK LNDQAQPK	255
Z04500	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	256
Z04501	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IASLGDDPSQ SANLLAEAKK LNDQAQPK	257
Z04502	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	258
Z04503	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IRSLTDDPSQ SANLLAEAKK LNDQAQPK	259
Z04504	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IRSLADDPSQ SANLLAEAKK LNDQAQPK	260
Z04505	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ISSLEDDPSQ SANLLAEAKK LNDQAQPK	261
Z04506	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IQSLGDDPSQ SANLLAEAKK LNDQAQPK	262
Z04507	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLSDDPSQ SANLLAEAKK LNDQAQPK	263
Z04508	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IASLTDDPSQ SANLLAEAKK LNDQAQPK	264
Z04509	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	265
Z04510	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IRSLADDPSQ SANLLAEAKK LNDQAQPK	266
Z04511	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IKSLGDDPSQ SANLLAEAKK LNDQAQPK	267
Z04512	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	268
Z04513	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	INSLEDDPSQ SANLLAEAKK LNDQAQPK	269
Z04514	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	270
Z04515	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IHSLGDDPSQ SANLLAEAKK LNDQAQPK	271
Z04516	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	INSLEDDPSQ SANLLAEAKK LNDQAQPK	272
Z04517	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IESLTDDPSQ SANLLAEAKK LNDQAQPK	273
Z04518	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	ITSLEDDPSQ SANLLAEAKK LNDQAQPK	274
Z04519	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IRSLGDDPSQ SANLLAEAKK LNDQAQPK	275
Z04520	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IASLGDDPSQ SANLLAEAKK LNDQAQPK	276
Z04521	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IASLGDDPSQ SANLLAEAKK LNDQAQPK	277
Z04522	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	IGSLTDDPSQ SANLLAEAKK LNDQAQPK	278
Z04523	VDNKFNKEGF YAAIEIVSLP NLNRQRRTAF	INSLEDDPSQ SANLLAEAKK LNDQAQPK	279

FIGURE 1

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Polypeptide	Amino acid sequence		SEQ ID NO:
Z04524	VDNKFNKEGF YAALEILILP NLNRGQHAFF IRSLTDDPSQ	SANLLAEAKK LNDQAQPK	280
Z04525	VDNKFNKEGF YAAMEIIALP NLTKRQRTAF ISSLADDPSP	SANLLAEAKK LNDQAQPK	281
Z04526	VDNKFNKEGF YAALEILALP NLTSKQREAF IGS LGDDPSQ	SANLLAEAKK LNDQAQPK	282
Z04527	VDNKFNKEGF YASIEILVLP NLNSRQSAF ISS LGDDPSQ	SANLLAEAKK LNDQAQPK	283
Z04528	VDNKFNKEGF YAAIEIVLP NLTRRQRAAF IASLDDDPSP	SANLLAEAKK LNDQAQPK	284
Z04529	VDNKFNKEGF YAAIEILTP NLNQQRGAF INSLEDDPSQ	SANLLAEAKK LNDQAQPK	285
Z04530	VDNKFNKEGF YAAIEIIALP NLTRQHRAF ITS LNDDPSQ	SANLLAEAKK LNDQAQPK	286
Z04531	VDNKFNKEGF YAAIEIITLP NLTKRQRAAF IGS LSDDPSP	SANLLAEAKK LNDQAQPK	287
Z04532	VDNKFNKEGF YAAIEILVLP NLTRQHKAF INS LGDDPSQ	SANLLAEAKK LNDQAQPK	288
Z04533	VDNKFNKEGF YAAIEIVSLP NLTSRQRTAF IHS LQDDPSQ	SANLLAEAKK LNDQAQPK	289
Z04534	VDNKFNKEGF YAAIEIVLP NLNRNQRTAF IRS LTDDPSQ	SANLLAEAKK LNDQAQPK	290
Z04535	VDNKFNKEGF YAAIEIISLP NLNRKQRAAF IAS LTDDPSQ	SANLLAEAKK LNDQAQPK	291
Z04536	VDNKFNKEGF YAAIEILIP NLNRKQSAF IAS LEDDPSP	SANLLAEAKK LNDQAQPK	292
Z04537	VDNKFNKEGF YAAMEIISLP NLNKQRRTAF ITS LEDDPSP	SANLLAEAKK LNDQAQPK	293
Z04538	VDNKFNKEGF YAAIEILVLP NLNQHQGAF ISS LSDDPSP	SANLLAEAKK LNDQAQPK	294
Z04539	VDNKFNKEGF YAAIEIVSLP NLNRNQGAF IQS LTDDPSQ	SANLLAEAKK LNDQAQPK	295
Z04540	VDNKFNKEGF YAAMEIISLP NLTKQRRAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	296
Z04541	VDNKFNKEGF YAAIEIVLP NLTHKQRRF IES LGDDPSQ	SANLLAEAKK LNDQAQPK	297
Z04542	VDNKFNKEGF YAALEILALP NLNHRQRTAF IGS LEDDPSP	SANLLAEAKK LNDQAQPK	298
Z04543	VDNKFNKEGF YAAIEILSLP NLNRQRRTAF IGS LGDDPSQ	SANLLAEAKK LNDQAQPK	299
Z04544	VDNKFNKEGF YAAIEILTP NLNSRQRDAF IGS LGDDPSQ	SANLLAEAKK LNDQAQPK	300
Z04545	VDNKFNKEGF YAAIEIVSLP NLTPRQRGAF ISS LGDDPSQ	SANLLAEAKK LNDQAQPK	301
Z04546	VDNKFNKEGF YAAIEIVSLP NLNRQRREAF ITS LEDDPSP	SANLLAEAKK LNDQAQPK	302
Z04547	VDNKFNKEGF YAALEILTP NLTRQRTAF IRS LGDDPSQ	SANLLAEAKK LNDQAQPK	303
Z04548	VDNKFNKEGF YAAIEILVLP NLNKKQSAF IKS LDDDPSP	SANLLAEAKK LNDQAQPK	304
Z04549	VDNKFNKEGF YAAIEILTP NLTSRQHRAF IAS LEDDPSP	SANLLAEAKK LNDQAQPK	305
Z04550	VDNKFNKEGF YAALEIITLP NLNQQRQAF IAS LGDDPSQ	SANLLAEAKK LNDQAQPK	306
Z04551	VDNKFNKEGF YAALEILIP NLTKQQRGAF ISS LSDDPSP	SANLLAEAKK LNDQAQPK	307
Z04552	VDNKFNKEGF YAAMEIITLP NLTPRQRAAF ITS LEDDPSP	SANLLAEAKK LNDQAQPK	308
Z04553	VDNKFNKEGF YAAIEILVLP NLNSKQRTAF IAS LSDDPSP	SANLLAEAKK LNDQAQPK	309
Z04554	VDNKFNKEGF YAAVEILGLP NLNRKQSAF IAS LDDDPSP	SANLLAEAKK LNDQAQPK	310

FIGURE 1

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Polypeptide	Amino acid sequence		SEQ ID NO:
Z04555	VDNKFNKEGF YAAIEILALP NLNGGQSAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	311
Z04557	VDNKFNKEGF YAAIEILTLNLTNKQRRAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	312
Z04558	VDNKFNKEGF YAAIEILILP NLNDRQRRAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	313
Z04559	VDNKFNKEGF YAAIEIVTLNLPKQSAF ISSLSDDPSQ	SANLLAEAKK LNDQAQPK	314
Z04560	VDNKFNKEGF YAAIEILSLP NLNSRQSAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	315
Z04561	VDNKFNKEGF YAAIEIVLP NLNTRQDAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	316
Z04562	VDNKFNKEGF YAAIEIVSLP NLNQRTAF ISSLEDDPSQ	SANLLAEAKK LNDQAQPK	317
Z04563	VDNKFNKEGF YAGIEIILP NLNQHREAF IKSLEDDPSQ	SANLLAEAKK LNDQAQPK	318
Z04564	VDNKFNKEGF YAAIEILSLP NLNSRQHRAE ITSLADDPQ	SANLLAEAKK LNDQAQPK	319
Z04565	VDNKFNKEGF YAAIEIVSLP NLNSRQRTAF ITSLSDDPQ	SANLLAEAKK LNDQAQPK	320
Z04566	VDNKFNKEGF YALIEIVSLP NLNHKQRTAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	321
Z04567	VDNKFNKEGF YAAIEIVLP NLNPRQRTAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	322
Z04568	VDNKFNKEGF YAAIEILTLNLTNRQSAF IASLGDDPSQ	SANLLAEAKK LNDQAQPK	323
Z04569	VDNKFNKEGF YAAIEIVGLP NLTGRTAF IQSLGDDPSQ	SANLLAEAKK LNDQAQPK	324
Z04570	VDNKFNKEGF YAAIEIVLP NLNGRQRRAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	325
Z04571	VDNKFNKEGF YAAIEIVTLNLTGNRTAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	326
Z04572	VDNKFNKEGF YAAIEILTLNLTNRQRTAF IDSLGDDPSQ	SANLLAEAKK LNDQAQPK	327
Z04573	VDNKFNKEGF YASMEILSLP NLNGKQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	328
Z04574	VDNKFNKEGF YAAIEILTLNLTNKQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	329
Z04575	VDNKFNKEGF YAAIEIVLP NLNRQRTAF INSLTDDPSQ	SANLLAEAKK LNDQAQPK	330
Z04576	VDNKFNKEGF YAAIEIILP NLTPRQSAF IDSLSDDPQ	SANLLAEAKK LNDQAQPK	331
Z04577	VDNKFNKEGF YAAIEILQLP NLNRQRAAF ISSLPDDPSQ	SANLLAEAKK LNDQAQPK	332
Z04578	VDNKFNKEGF YAAIEIVLP NLNQDRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	333
Z04579	VDNKFNKEGF YAAIEILIP NLNSQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	334
Z04580	VDNKFNKEGF YAAIEILALP NLNTRQHRAE IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	335
Z04581	VDNKFNKEGF YAAIEILVLP NLNHKQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	336
Z04582	VDNKFNKEGF YAAIEILSLP NLTRRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	337
Z04583	VDNKFNKEGF YAAIEIISLP NLNRQRTAF IASLGDDPSQ	SANLLAEAKK LNDQAQPK	338
Z04584	VDNKFNKEGF YAAIEILALP NLNRQRTAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	339
Z04585	VDNKFNKEGF YAAIEIISLP NLNRQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	340
Z04586	VDNKFNKEGF YAAIEILVLP NLNRQRAAF IGSLEDDPSQ	SANLLAEAKK LNDQAQPK	341

FIGURE 1

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Polypeptide	Amino acid sequence		SEQ ID NO:
Z04587	VDNKFNKEGF YAAIEILALP NLNRKQRTAF IQSLDDDDPSQ	SANLLAEAKK LNDQAQPK	342
Z04588	VDNKFNKEGF YAAIEIISLP NLTQRQHEAF IRSLGDDPSQ	SANLLAEAKK LNDQAQPK	343
Z04589	VDNKFNKEGF YAAIEILTLNLTQNQHTAF IRSLGDDPSQ	SANLLAEAKK LNDQAQPK	344
Z04590	VDNKFNKEGF YAAIEILTLNLTQNQHTAF IASLGDDPSQ	SANLLAEAKK LNDQAQPK	345
Z04591	VDNKFNKEGF YAAIEIVSLP NLTQKQRTAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	346
Z04592	VDNKFNKEGF YAAIEILTLNLTNRQRTAF IGSNDDPSQ	SANLLAEAKK LNDQAQPK	347
Z04593	VDNKFNKEGF YAAIEILSLP NLNRQRSAF ISSLGDDPSQ	SANLLAEAKK LNDQAQPK	348
Z04594	VDNKFNKEGF YAAIEILSLP NLTKKQRTAF IRSLGDDPSQ	SANLLAEAKK LNDQAQPK	349
Z04595	VDNKFNKEGF YAAIEILTLNLTNRQRSAF IRSLGDDPSQ	SANLLAEAKK LNDQAQPK	350
Z04596	VDNKFNKEGF YAAIEILTLNLTNSPQRDAF ISSLGDDPSQ	SANLLAEAKK LNDQAQPK	351
Z04597	VDNKFNKEGF YAAIEIITLP NLTQKQRTAF IRSLSDDPSQ	SANLLAEAKK LNDQAQPK	352
Z04598	VDNKFNKEGF YAAIEILTLNLTNRQRSAF IASLGDDPSQ	SANLLAEAKK LNDQAQPK	353
Z04599	VDNKFNKEGF YAAIEILTLNLTNRQRDAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	354
Z04600	VDNKFNKEGF YAAIEILTLNLTNRQRDAF IESLTDDPSQ	SANLLAEAKK LNDQAQPK	355
Z04601	VDNKFNKEGF YAAIEIISLP NLTNRQRDAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	356
Z04602	VDNKFNKEGF YAAIEIVTLNLTNRQRSAF IGS LGDDPSQ	SANLLAEAKK LNDQAQPK	357
Z04604	VDNKFNKEGF YAAIEILTLNLTNRQRDAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	358
Z04605	VDNKFNKEGF YAAIEIITLP NLTNRQRSAF INSLPDDPSQ	SANLLAEAKK LNDQAQPK	359
Z04606	VDNKFNKEGF YAAIEILTLNLTNRQRDAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	360
Z04607	VDNKFNKEGF YAAIEIISLP NLTNRQRDAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	361
Z04608	VDNKFNKEGF YAAIEIVSLP NLTQKQRTAF ITS LTDDPSQ	SANLLAEAKK LNDQAQPK	362
Z04609	VDNKFNKEGF YAAIEIVSLP NLTQKQRTAF ITSLEDDPSQ	SANLLAEAKK LNDQAQPK	363
Z04610	VDNKFNKEGF YAAIEILTLNLTQKQRTAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	364
Z04611	VDNKFNKEGF YAAIEILTLNLTNRQRDAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	365
Z04612	VDNKFNKEGF YAAIEIITLP NLTNRQRDAF INSLTDDPSQ	SANLLAEAKK LNDQAQPK	366
Z04614	VDNKFNKEGF YAAIEILTLNLTNRQRSAF IRSLEDDPSQ	SANLLAEAKK LNDQAQPK	367
Z04615	VDNKFNKEGF YAAIEILVLP NLTQKQRTAF IKSLEDDPSQ	SANLLAEAKK LNDQAQPK	368
Z04616	VDNKFNKEGF YAAIEIVSLP NLTNRQRSAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	369
Z04617	VDNKFNKEGF YAAIEILVLP NLTNRQRDAF IGS LTDDPSQ	SANLLAEAKK LNDQAQPK	370
Z04618	VDNKFNKEGF YAAIEILVLP NLTNRQRDAF INSLGDDPSQ	SANLLAEAKK LNDQAQPK	371
Z04619	VDNKFNKEGF YAAIEILTLNLTNRQRSAF ITS LGDDPSQ	SANLLAEAKK LNDQAQPK	372

FIGURE 1

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Polypeptide	Amino acid sequence		SEQ ID NO:
Z04620	VDNKFNKEGF YAAIEILSLP NLTRQHRAF ITSLEDDPSQ SANLLAEAKK LNDQAAPK		373
Z04621	VDNKFNKEGF YAAIEIIALP NLNRQRSAF IRSLQDDPSQ SANLLAEAKK LNDQAAPK		374
Z04623	VDNKFNKEGF YAAIEILVLP NLTRQRKAF IESLPDDPSQ SANLLAEAKK LNDQAAPK		375
Z04624	VDNKFNKEGF YAAIEILTLP NLNSRQRTAF IGS LGDDPSQ SANLLAEAKK LNDQAAPK		376
Z04625	VDNKFNKEGF YAAIEILVLP NLNPKQRTAF IDSLSDPSQ SANLLAEAKK LNDQAAPK		377
Z04626	VDNKFNKEGF YASIEILVLP NLNGRQRTAF ITS LADDP SQ SANLLAEAKK LNDQAAPK		378
Z04627	VDNKFNKEGF YAAIEILTLP NLNSRQRRAF IGS LQDDPSQ SANLLAEAKK LNDQAAPK		379
Z04628	VDNKFNKEGF YAAMEILTL P NLNRKQRS AF IGS LTDDPSQ SANLLAEAKK LNDQAAPK		380
Z04629	VDNKFNKEGF YAAIEILILP NLNQQRSAF IGS LEDDPSQ SANLLAEAKK LNDQAAPK		381
Z04630	VDNKFNKEGF YAAIEILALP NLNRQRSAF INSLGDDPSQ SANLLAEAKK LNDQAAPK		382
Z04631	VDNKFNKEGF YAAIEIVALP NLNRKQRTAF ISS LTDDPSQ SANLLAEAKK LNDQAAPK		383
Z04632	VDNKFNKEGF YAAMEILGLP NLNRQRRAF ITS LTDDPSQ SANLLAEAKK LNDQAAPK		384
Z04633	VDNKFNKEGF YAAVEILTLP NLTRRQHRAF ITS LGDDPSQ SANLLAEAKK LNDQAAPK		385
Z04634	VDNKFNKEGF YAAIEIVALP NLTTKQRS AF IRS LGDDPSQ SANLLAEAKK LNDQAAPK		386
Z04635	VDNKFNKEGF YAAIEILVLP NLGNQRTAF ITS LTDDPSQ SANLLAEAKK LNDQAAPK		387
Z04636	VDNKFNKEGF YAAIEIIALP NLNQQRS AF IQSLGDDPSQ SANLLAEAKK LNDQAAPK		388
Z04637	VDNKFNKEGF YAAIEILVLP NLNSRQRDAF IRS LSDPSQ SANLLAEAKK LNDQAAPK		389
Z04638	VDNKFNKEGF YAAIEILILP NLNQQRDAF ITS LGDDPSQ SANLLAEAKK LNDQAAPK		390
Z04639	VDNKFNKEGF YAAMEILILP NLTRRQHRAF ITS LEDDPSQ SANLLAEAKK LNDQAAPK		391
Z04640	VDNKFNKEGF YAAIEILSLP NLTPKQRS AF ITS LTDDPSQ SANLLAEAKK LNDQAAPK		392
Z04641	VDNKFNKEGF YAAIEIVVLP NLTPRQRS AF IKS LDDPSQ SANLLAEAKK LNDQAAPK		393
Z04642	VDNKFNKEGF YAAIEILTLP NLNPQRTAF IKS LEDDPSQ SANLLAEAKK LNDQAAPK		394
Z00000	VDNKFNKEQQ NAFYEILHLP NLNEEQRNAF IQSLKDDPSQ SANLLAEAKK LNDQAAPK		395
IGF1R	MKSGSGGSP TSLWGLLFLS AALSLWPTSG EICGPGIDIR NDYQQLKRLE NCTVIEGYLH ILLISKAEDY RSYRFPKLT V ITEYLLLF RV AGLES LGDLF PNLTVIRGWK LFYNYALVIF EMTNLKDIGL YNLRNITRGA IRIEKNADLC YLSTVDWSLI LDAVSNNYIV GNKPPKECGD LCPGTMEKP MCEKTTINNE YNYRCWTTNR CQKMC PSTCG KRAC TENNEC CHPEC IGS CS APDNDTACVA CRHYYYAGVC VPACPPNTYR FEGWRCVDRD FCANILSAES SDSEGFVIHD GECMQECP SG FIRNGSQSMY CIPCEGPCPK VCEEKKTKT IDSVTSAQML QGCTIFKGNL LINIRRGNNI ASELENFMGL IEVVTGYVKI RHSHALVSL S FLKNLRILG EEQLEGNYSF YVLDNQNLQQ LWDWDHRNLT IKAGKMYFAF NPKLCVSEIY RMEEVGTGKG RQSKGDINTR		396

FIGURE 1

Polypeptide	Amino acid sequence	SEQ ID NO:
IGF1r ectodomain	NNGERASCES DVLHFTSTTT SKNRIITWH RYRPPDYRDL ISFTVYYKEA PFKNVTEYDG QDACGSNSWN MVDVDLPPNK DVEPGILLHG LKPWTQYAVY VKAVTLTMVE NDHIRGAKSE ILYIRTNASV PSIPLDVLSA SNSSSQLIVK WNPSPLPNGN LSYIIVRWQR PQDGYLYRH NYCSKDKIPI RKYADGTIDI EEVTENPKTE VCGGEKGPCC ACPKTEAEKQ AEKEEAERYK VFENFLHNSI FVPRPERKRR DVMQVANTTM SSRSRNTTAA DTYNITDPEE LETEYPPFFES RVDNKERTVI SNLRPFITLYR IDIHSCNHEA EKLGCASN FVFARTMPAEG ADDIPGPVTW EPRPENSIFL KWPEPENPNG LILMYEIKYG SQVEDQRECV SRQYRKYGG AKLNRINPGN YTARIQATSL SGNGSWTDVP FFYVQAKTGY ENFIHLIAL PVAVLIIVGG LVIMLYVFHR KRNSRLNG VLYASVNPEY FSAADVVPD EWEVAREKIT MSRELGGSF GMVYEGVAKG VVKDEPETRV AIKTVNEAAS MRERIEFLNE ASVMKEFNCH HVVRLLGVS QGQPTLVIME LMTRGDLKSY LRSLRPEMEN NPVLAPPSLS KMIQAGEIA DGMAYLNANK FVHRDLAARN CMVAEDFTVK IGDFGMTRDI YETDYYRKGG KGLLPVRWMS PESLKDGVFT TYSDVWSFGV VLWEIATLAE QPYQGLSNEQ VLRFVMEGGL LDKPDNCPDM LFELMRMCWQ YNPKMRPSFL EIISSIKEEM EPGFREVSFY YSEENKLPEP EELDLEPENM ESVPLDPSAS SSSLPLPDRH SGHKAENGPG PGVLVLRASF DERQPIAHMN GGRKNERALP LPQSSTC	397
	NNGERASCES DVLHFTSTTT SKNRIITWH RYRPPDYRDL ISFTVYYKEA PFKNVTEYDG QDACGSNSWN MVDVDLPPNK DVEPGILLHG LKPWTQYAVY VKAVTLTMVE NDHIRGAKSE ILYIRTNASV PSIPLDVLSA SNSSSQLIVK WNPSPLPNGN LSYIIVRWQR PQDGYLYRH NYCSKDKIPI RKYADGTIDI EEVTENPKTE VCGGEKGPCC ACPKTEAEKQ AEKEEAERYK VFENFLHNSI FVPRPERKRR DVMQVANTTM SSRSRNTTAA DTYNITDPEE LETEYPPFFES RVDNKERTVI SNLRPFITLYR IDIHSCNHEA EKLGCASN FVFARTMPAEG ADDIPGPVTW EPRPENSIFL KWPEPENPNG LILMYEIKYG SQVEDQRECV SRQYRKYGG AKLNRINPGN YTARIQATSL SGNGSWTDVP FFYVQAKTGY ENFIHLIAL PVAVLIIVGG LVIMLYVFHR KRNSRLNG VLYASVNPEY FSAADVVPD EWEVAREKIT MSRELGGSF GMVYEGVAKG VVKDEPETRV AIKTVNEAAS MRERIEFLNE ASVMKEFNCH HVVRLLGVS QGQPTLVIME LMTRGDLKSY LRSLRPEMEN NPVLAPPSLS KMIQAGEIA DGMAYLNANK FVHRDLAARN CMVAEDFTVK IGDFGMTRDI YETDYYRKGG KGLLPVRWMS PESLKDGVFT TYSDVWSFGV VLWEIATLAE QPYQGLSNEQ VLRFVMEGGL LDKPDNCPDM LFELMRMCWQ YNPKMRPSFL EIISSIKEEM EPGFREVSFY YSEENKLPEP EELDLEPENM ESVPLDPSAS SSSLPLPDRH SGHKAENGPG PGVLVLRASF DERQPIAHMN GGRKNERALP LPQSSTC	

FIGURE 1

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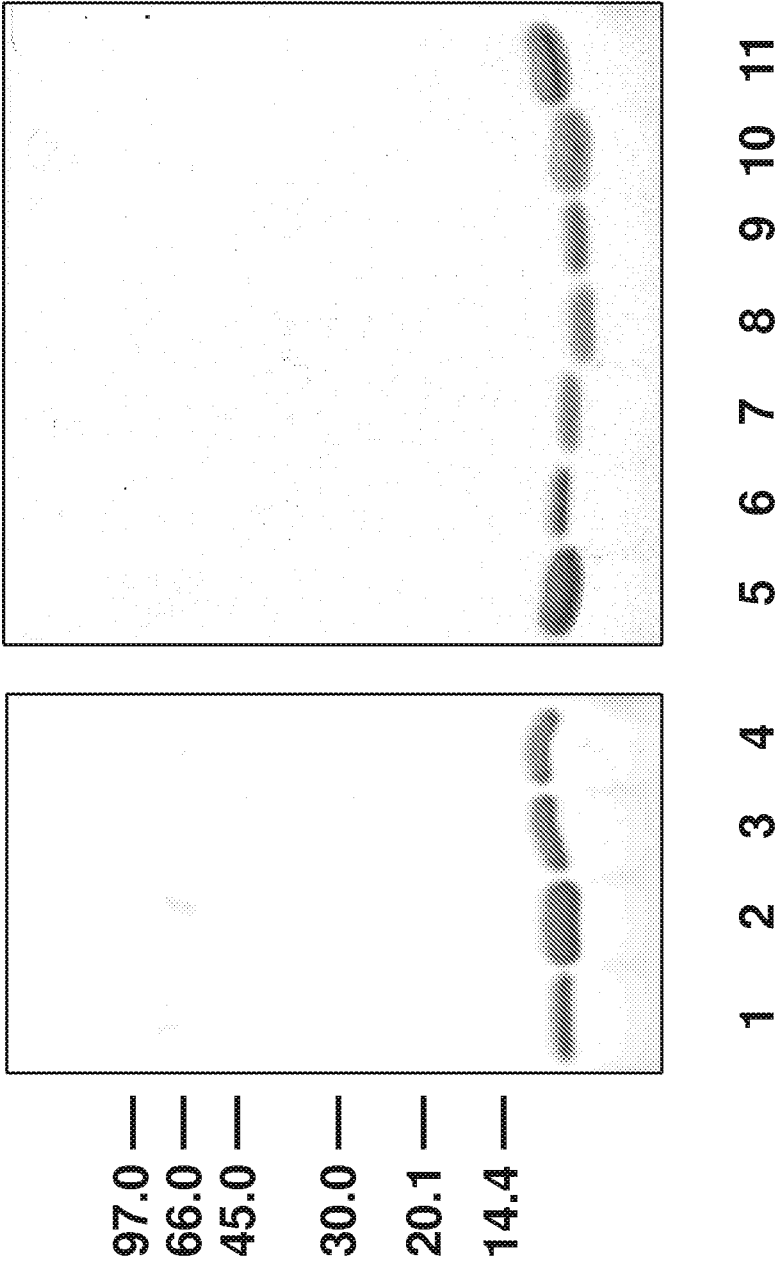
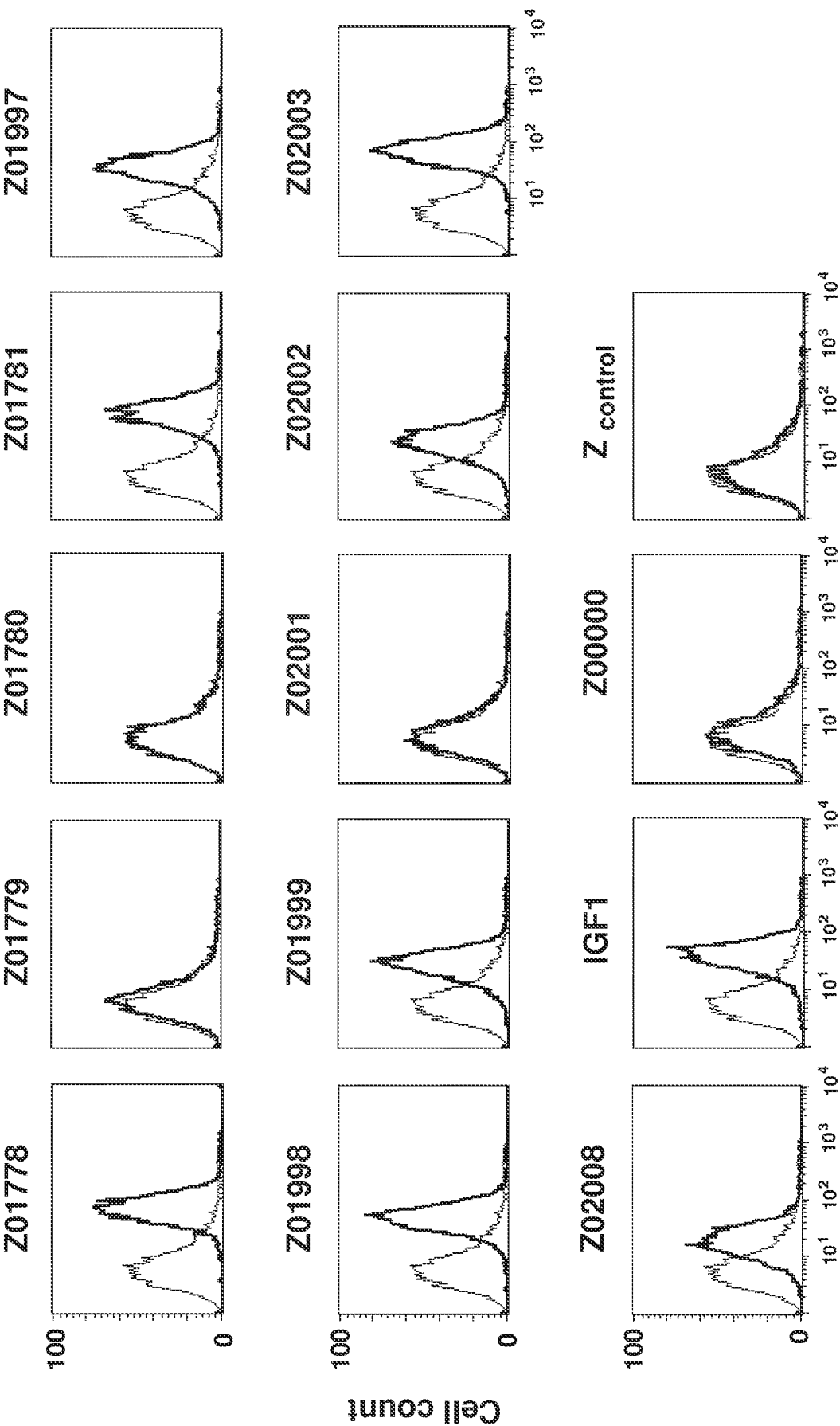


FIGURE 2



Fluorescence intensity

FIGURE 3

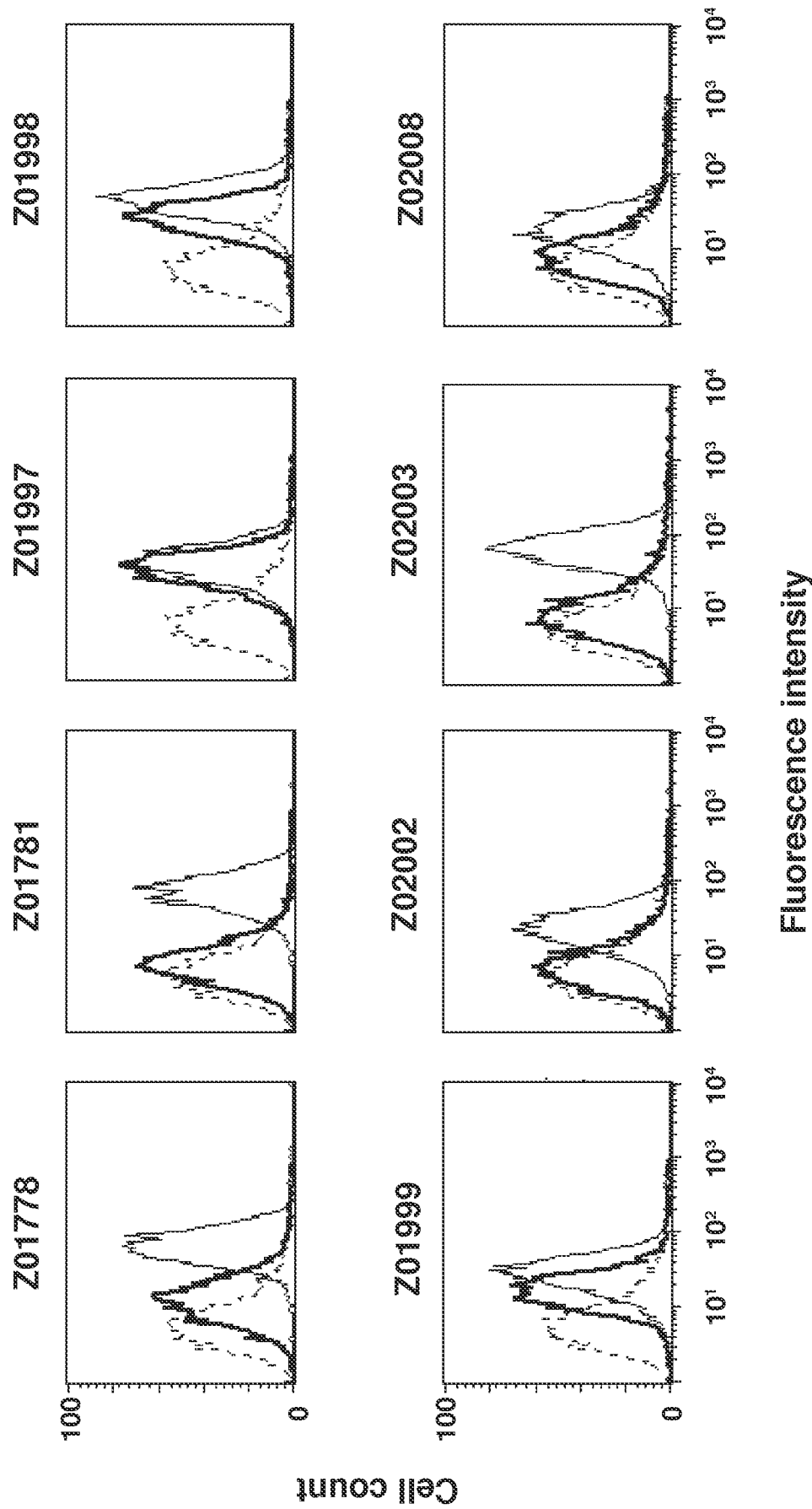


FIGURE 4

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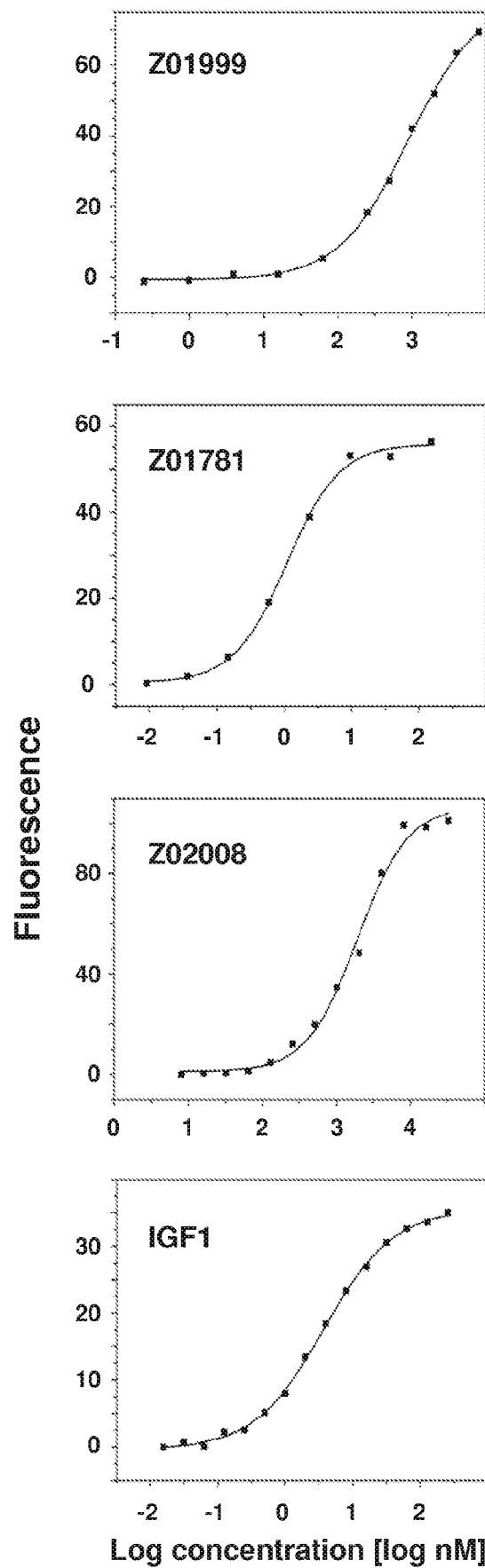


FIGURE 5

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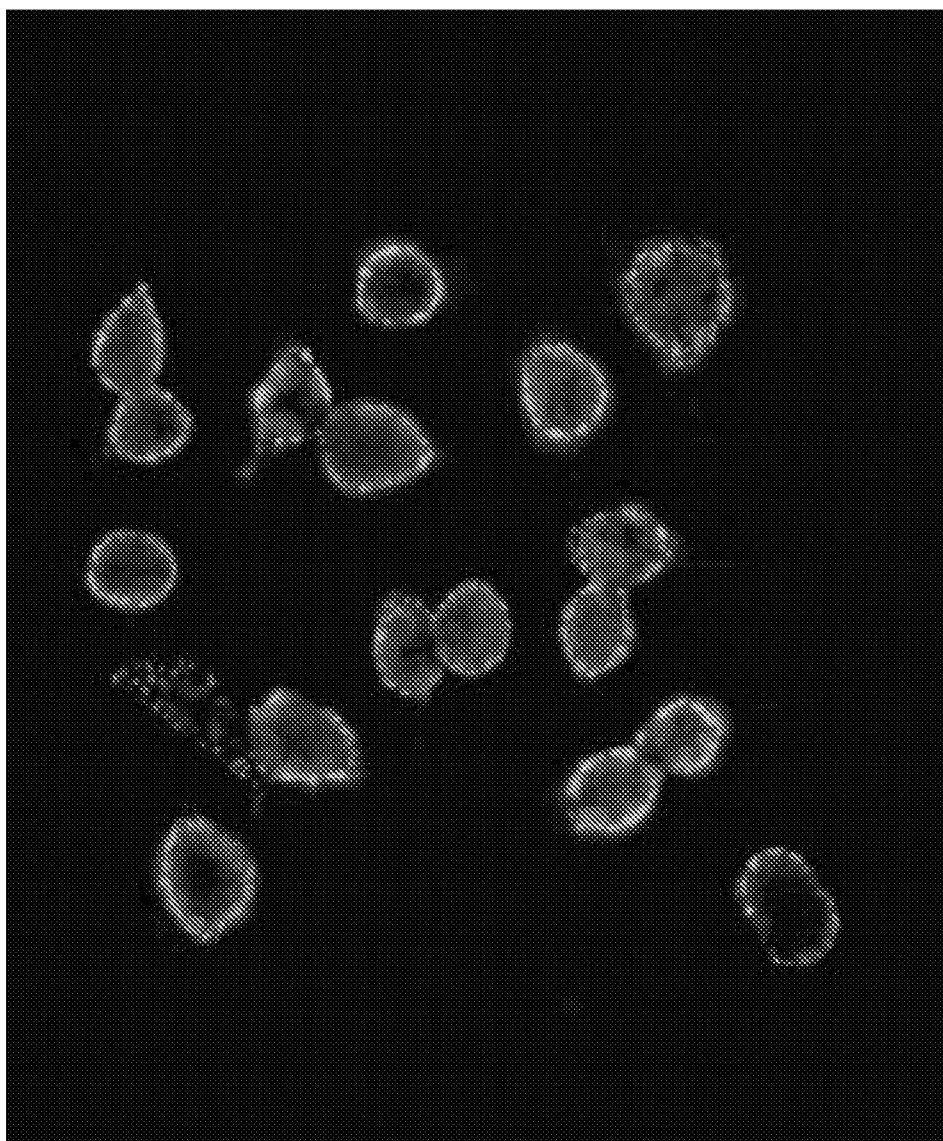


FIGURE 6

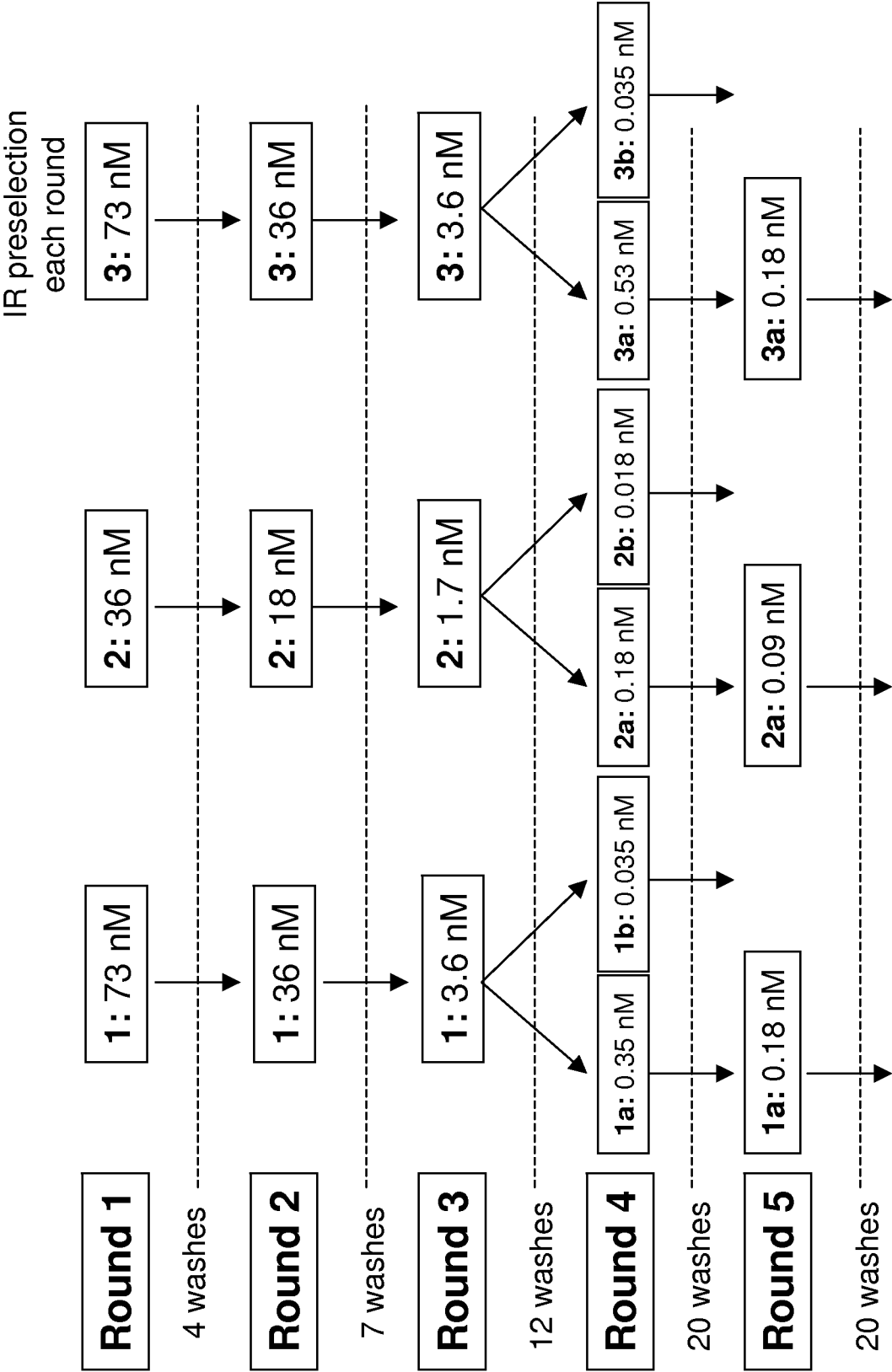
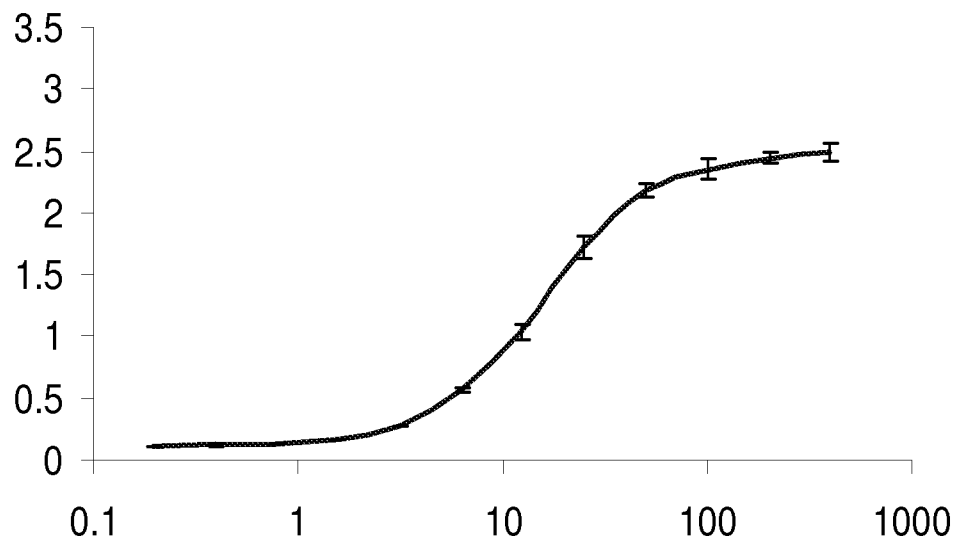


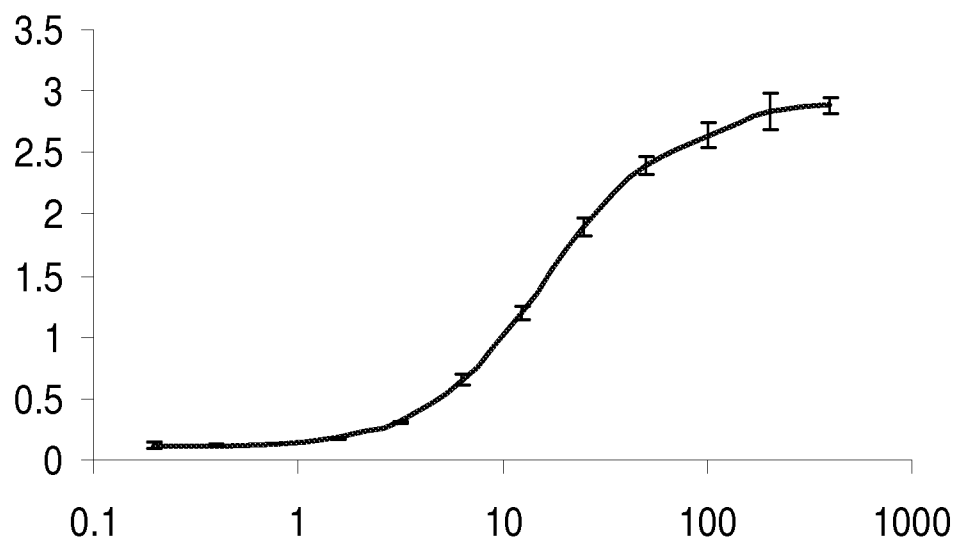
FIGURE 7

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Z01781

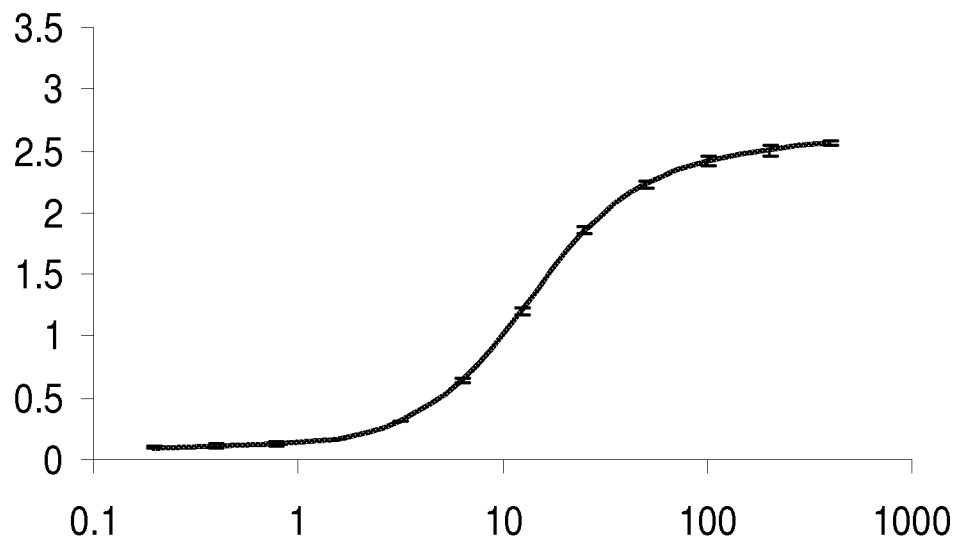


Z04493

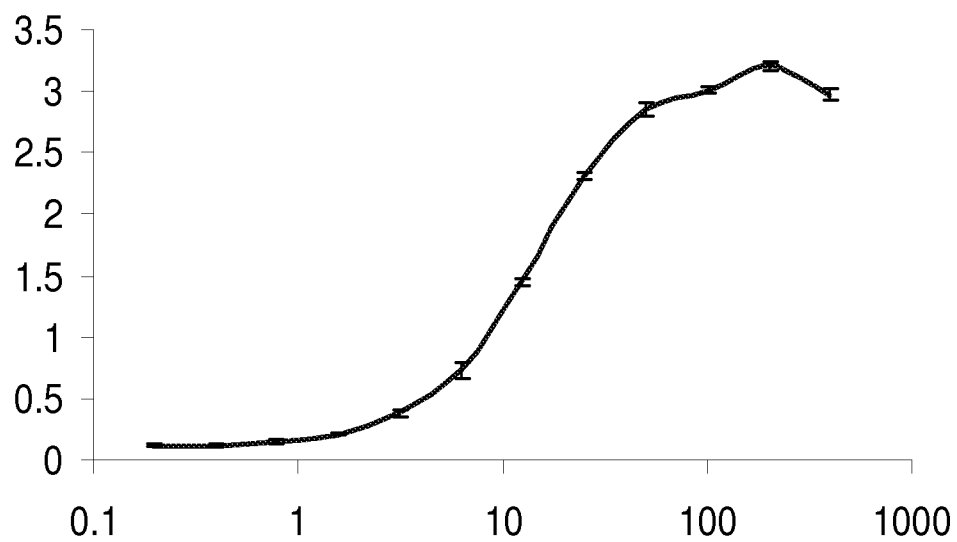
**FIGURE 8A**

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Z04499

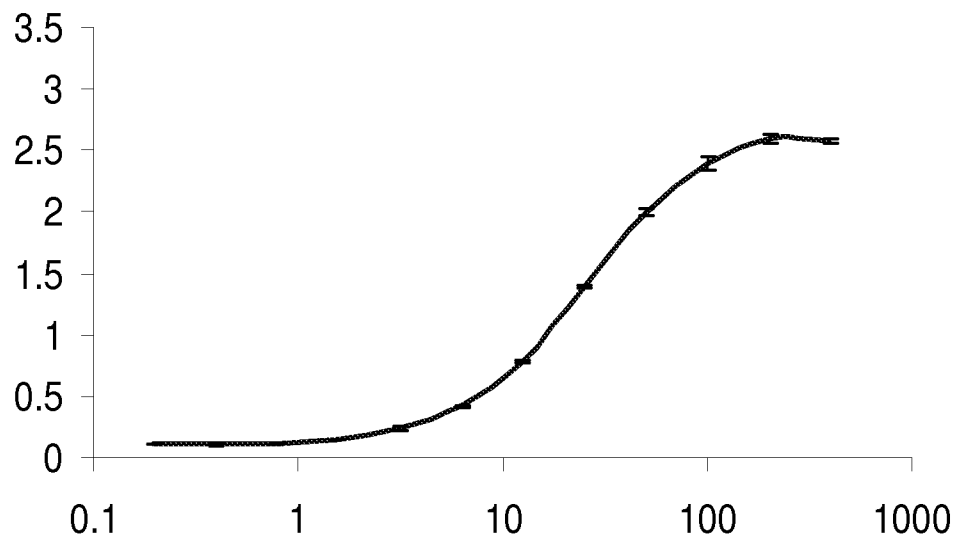


Z04542

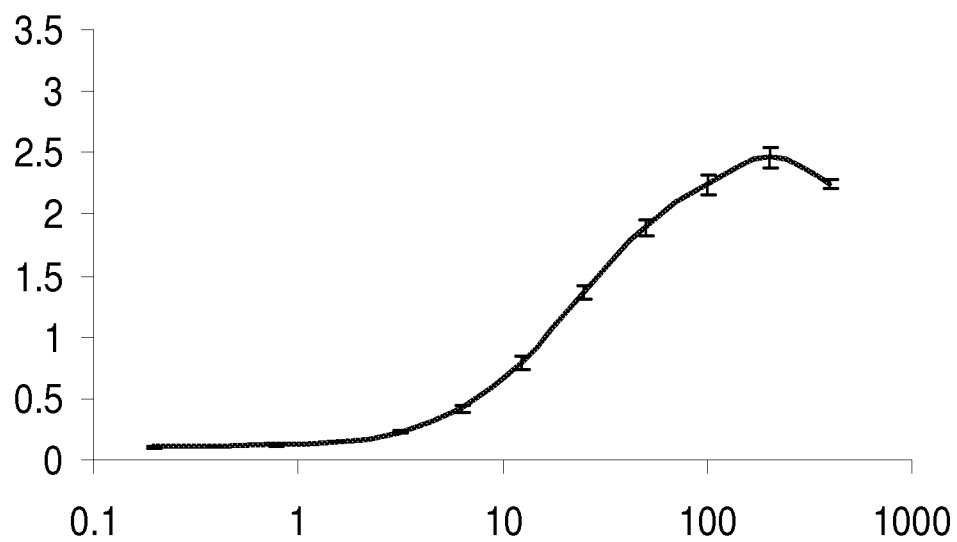
**FIGURE 8B**

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Z04551

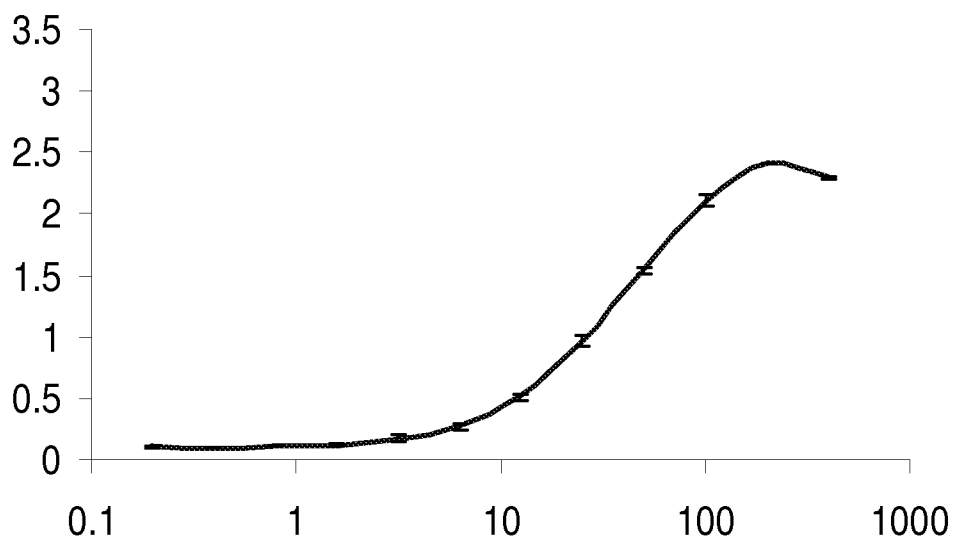


Z04557

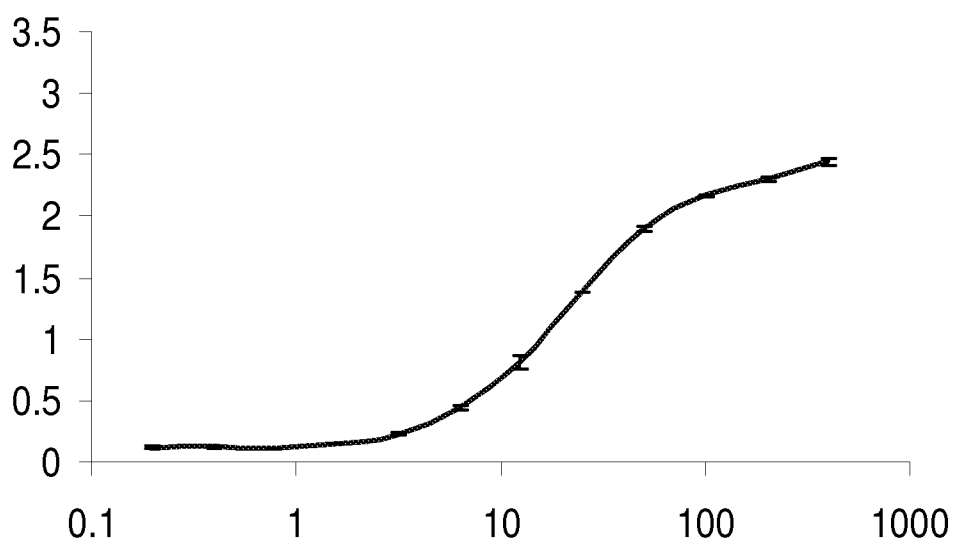
**FIGURE 8C**

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Z04568

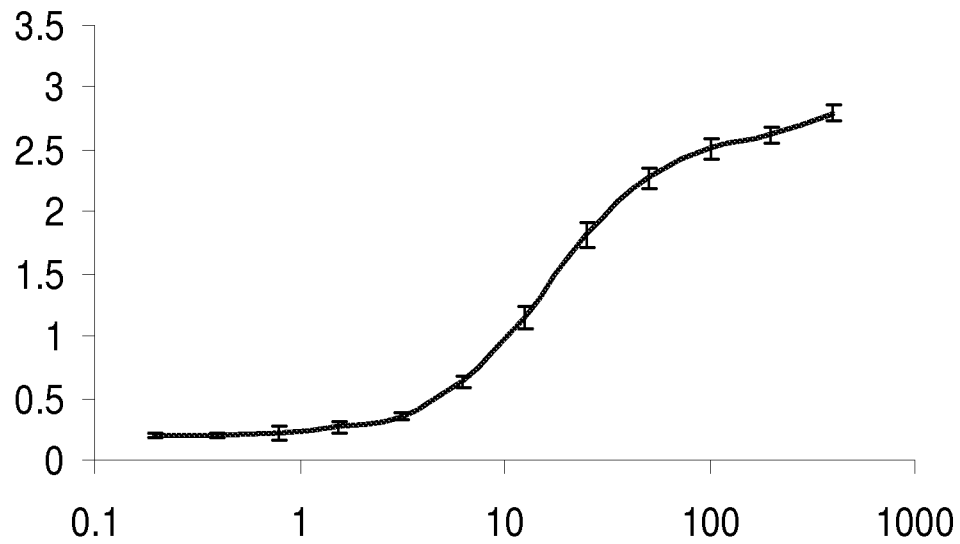


Z04572

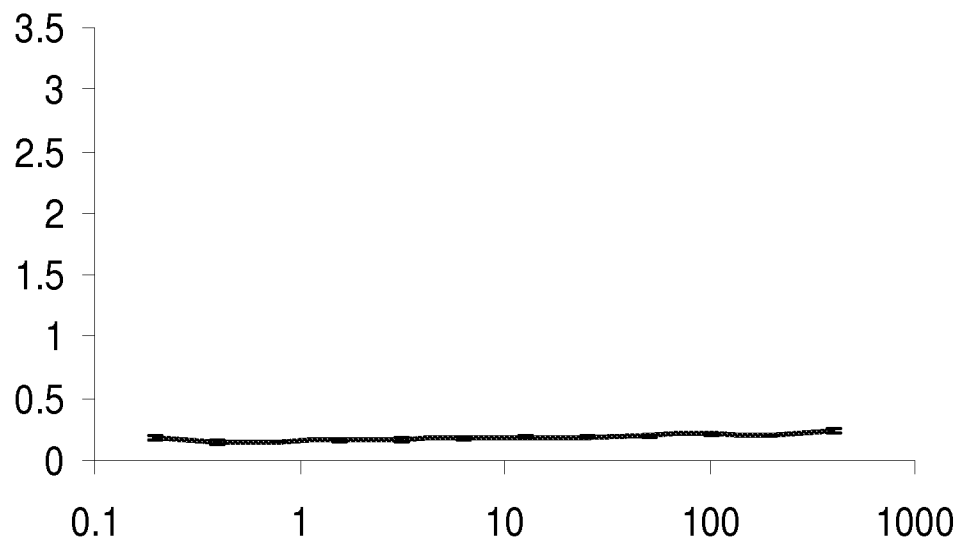
**FIGURE 8D**

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Z04609



Z00810

**FIGURE 8E**

IGF1R binding Z variants on MCF-7 cells

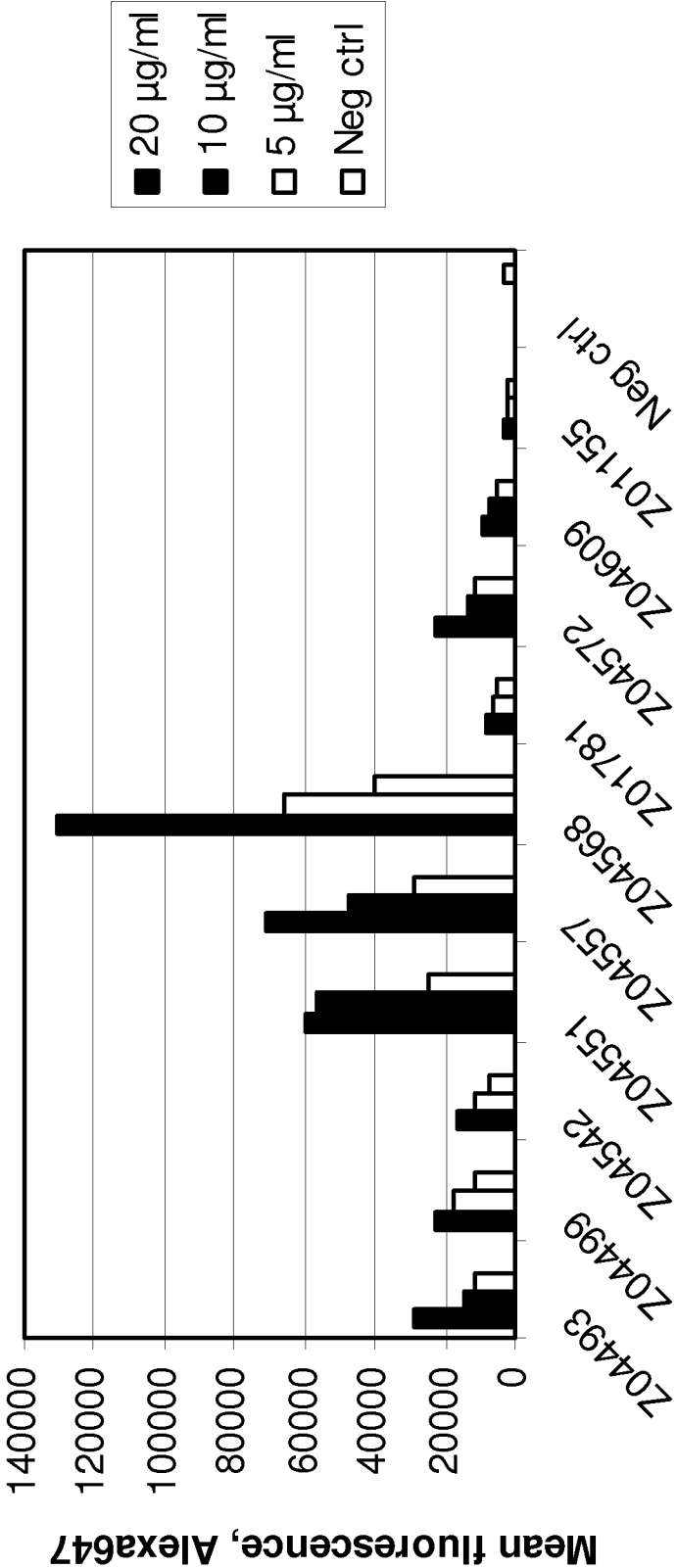


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/059391

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/31 C07K14/65 A61K38/30 G01N33/68 A61K51/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/092338 A (AFFIBODY AB [SE]; HERNE NINA [SE]) 8 September 2006 (2006-09-08)	28-55, 68-70, 73,75
Y	The whole document: see particularly claim 14 and SEQ ID No.58	1-77
Y	US 2003/236190 A1 (PILLUTLA RENUKA [US] ET AL) 25 December 2003 (2003-12-25) the whole document	1-77
Y	WO 95/19374 A (PHARMACIA AB [SE]; NILSSON BJOERN [SE]; NYGREN PER AAKE [SE]; UHLEN MA) 20 July 1995 (1995-07-20) the whole document	1-77
	-/--	



Further documents are listed in the continuation of Box C.



See patent family annex.

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

29 September 2008

Date of mailing of the international search report

07/10/2008

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Authorized officer

Groenendijk, Matti

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/059391

C(Continuation). : DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages.	Relevant to claim No.
Y	WO 00/63243 A (PHARMACIA & UPJOHN AB [SE]; LJUNGQVIST CHARLOTTA [SE]; NORD KARIN [SE]) 26 October 2000 (2000-10-26) the whole document	1-77
Y	WO 2005/000883 A (AFFIBODY AB [SE]; GUNNERIUSSEN ELIN [SE]; FELDWISCH JOACHIM [SE]; NYGR) 6 January 2005 (2005-01-06) the whole document	1-77
A	NILSSON B ET AL: "A SYNTHETIC IGG-BINDING DOMAIN BASED ON STAPHYLOCOCCAL PROTEIN A" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 1, no. 2, 1 February 1987 (1987-02-01), pages 107-113, XP000615228 ISSN: 0269-2139 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/059391

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