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(54) Title: ALBUMIN DERIVATIVES AND VARIANTS

(57) Abstract: The application discloses albumin derivatives comprising or consisting of domain III and at least one further do-
main wherein the derivative or variant is not a naturally occurring albumin derivative or variant. The derivatives may be used in
conjugates and fusion polypeptides.



TITLE: ALBUMIN DERIVATIVES AND VARIANTSReference to sequence listing

This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

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FIELD OF THE INVENTION

The invention relates to albumin derivatives and variants and/or fusion polypeptides comprising such albumin derivatives and variants.

The invention further relates to the use of albumin derivatives and variants and/or fusion polypeptides comprising such albumin derivative or variant as a tool for drug delivery, polypeptide stability and *in vivo* half-life extension or modulation.

BACKGROUND OF THE INVENTION

Albumin is a protein naturally found in the blood plasma of mammals, where it is the most abundant protein. It has important roles in maintaining the desired osmotic pressure of the blood and also has a role in the transport of various substances within the blood stream.

Albumin binds *in vivo* to its receptor, the neonatal Fc receptor (FcRn) "Brambell" and this interaction is known to be important for increasing the plasma half-life of albumin. FcRn is a membrane bound protein, expressed in many cell and tissue types, and has been found to reduce the rate of intracellular degradation of albumin (Roopenian, D. C. and Akilesh, S. (2007), *Nat. Rev. Immunol* **7**, 715-725.). FcRn contributes to maintaining both the high level of IgG and albumin in the serum of mammals, such as human beings.

Whilst the FcRn-immunoglobulin G (IgG) interaction has been characterized in the prior art, the FcRn-albumin interaction is less well characterized or understood. The major albumin-FcRn binding-site is localized within domain III (DIII: 381-585) (Andersen *et al* (2010) *Clinical Biochemistry* **43**,367–372). However, it is known within the art that both IgG and albumin bind non-cooperatively to distinct sites on FcRn (Andersen, *et al.* (2006), *Eur. J. Immunol* **36**, 3044-3051; Chaudhury, *et al.* (2006), *Biochemistry* **45**, 4983-4990.).

Human serum albumin (HSA) has been characterized as a polypeptide of 585 amino acids, the sequence of which can be found in Peters, T., Jr. (1996) *All about Albumin: Biochemistry, Genetics and Medical, Applications*, pp10, Academic Press, Inc., Orlando (ISBN 0-12-552110-3). Albumin has a characteristic pH-dependent binding to FcRn, where it binds at an acidic pH, such as pH 6.0 but not at a pH above neutral, such as pH 7.4.

The plasma half-life of HSA has been found to be approximately 19 days (Peters, T., Jr. (1985) *Adv. Protein Chem.* **37**, 161–245; Peters, T., Jr. (1996) *All about Albumin*, Academic Press, Inc., San Diego, CA. (page 245-246)); Benotti P, Blackburn GL: *Crit Care Med* (1979)

7:520-525). A naturally occurring point mutant, having the substitution D494N has a lower plasma half-life (*Biochim Biophys Acta*. 1991, **1097**:49-54). This single substitution creates an N-linked glycosylation site in this variant/mutant, which is not present in wild-type (wt) HSA. It is not known whether the potential glycosylation at this site or the amino acid change itself is responsible for the change in plasma half-life observed.

Albumin is a plasma protein considered to have a long plasma half-life and because of this property it has been suggested to be used in drug delivery. Albumin has been conjugated to pharmaceutically beneficial compounds (WO0069902A). Hence, the resultant plasma half-life of the conjugates has generally been found to be considerably longer than the plasma half-life of the beneficial compounds alone.

Further, albumin has been fused to therapeutically beneficial peptides (WO 01/79271 A and WO 03/59934 A), with the typical result that the fusion polypeptides have the activities of the therapeutically beneficial peptides and a long plasma half-life, which is considerably greater than the plasma half-life of the therapeutically beneficial peptides alone.

Otagiri *et al* (2009), *Biol. Pharm. Bull.* **32**(4), 527-534, discloses that 77 albumin variants are known, of these 25 are found in domain III. A natural variant lacking the last 175 amino acids at the carboxy terminus has been shown to have reduced half-life (Andersen *et al* (2010), *Clinical Biochemistry* **43**, 367-372). Iwao *et al.*(2007) studied the half-life of naturally occurring human albumin variants using a mouse model, and found that K541E and K560E had reduced half-life, E501K and E570K had increased half-life and K573E had almost no effect on half-life (Iwao, *et. al.* (2007) *B.B.A. Proteins and Proteomics* **1774**, 1582-1590).

Galliano *et al* (1993) *Biochim. Biophys. Acta* 1225, 27-32 discloses a natural variant E505K. Minchiotti *et al.*(1990) discloses a natural variant K536E. Minchiotti *et al* (1987) *Biochim. Biophys. Acta* 916, 411-418 discloses a natural variant K574N. Takahashi *et al* (1987) *Proc. Natl. Acad. Sci. USA* 84, 4413-4417, discloses a natural variant D550G. Carlson *et al* (1992). *Proc. Nat. Acad. Sci. USA* 89, 8225- 8229, discloses a natural variant D550A.

WO 2007112940 discloses constructs comprising at least one albumin domain III and at least one therapeutic moiety and the use of such constructs for half-life extension of drugs.

Albumin has the inherent ability to allow the binding of a number of ligands, and these become associated (associates) with albumin. This property has been utilized to extend the plasma half-life of such aforementioned ligands, *e.g.* to extend the plasma half-life of drugs having the ability to non-covalently bind to albumin. This can also be achieved by binding a pharmaceutically beneficial compound, which has little or no albumin binding properties, to a moiety having albumin-binding properties. See review article and reference therein: Kratz (2008). *Journal of Controlled Release* **132**, 171-183.

US7,253,259 discloses a protein produced by gene recombinant technology including at least one domain selected from domains I, II and III of serum albumin but having a different structure from that of native albumin; and a method of producing the protein.

Albumin is used in preparations of pharmaceutically beneficial compounds, in which such a preparation maybe for example, but not limited to, a nano particle or micro particle of albumin. In these examples the delivery of a pharmaceutically beneficial compound or mixture of compounds may benefit from alteration in the albumins affinity to the FcRn receptor where the beneficial compound has been shown to associate with albumin for the means of delivery.

The exact nature or associated properties that influences the extension to the plasma half-life of the formed conjugates or fusion polypeptides is unclear (for example, but not limited to, Levemir®, Kurtzhals P *et al.* Biochem. J. 1995; **312**:725-731), but it appears to be directly related to the albumin moiety and the selected pharmaceutically beneficial compound/peptide they are composed of. It would be desirable to be able to control the plasma half-life of a given albumin domain III derivative, fragments, or variants thereof with a conjugated or fusion or association such that a longer or shorter plasma half-life, than given by the components of the conjugate/fusion alone, can be achieved. This would allow the custom design of a particular drug according to the particulars of the indication intended to be treated.

Albumin is known to accumulate and be catabolised in tumours, it has also been shown to accumulate in inflamed joints of rheumatoid arthritis sufferers. See review article and reference therein, Kratz (2008). Journal of Controlled Release **132**, 171-183. It is envisaged that HSA variants with increased affinity for FcRn would be advantageous for the delivery and/or targeting (such as passive targeting) of pharmaceutically beneficial compounds.

It may be desirable to have variants of albumin that have little or no binding to FcRn in order to provide shorter half-lives or controlled serum pharmacokinetics as described by Vania Kenanova, Tove Olafsen, Felix Bergara and Anna Wu (2009) J Nucl Med.; 50 (Supplement 2):1582).

SUMMARY OF THE INVENTION

The first aspect of the invention provides an albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof, where the albumin derivative or variant or fragment thereof comprises or consists of albumin domain III or derivative or variant thereof, and at least one additional albumin domain, fragment or derivative or variant thereof. Preferably, the first aspect does not include wild-type albumin itself. However, the first aspect may include wild-type albumin modified such that it comprises or consists of wild-type albumin and an alteration such as addition of one or more (several) domains from any albumin, one or more (several) point

mutations, fusion to a beneficial moiety, conjugation to a beneficial moiety and/or association with a beneficial moiety.

In a preferred embodiment the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof comprises or consists of one or more (several) substitutions, insertions or deletions, most preferably substitutions, in positions corresponding to the positions in SEQ ID NO: 31 or SEQ ID NO:1 (HSA) selected from the group consisting of one or more of (several) positions: 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584.

In a second aspect, the invention relates to isolated polynucleotides that encode any of the derivatives or variants of the invention, for example: (i) nucleic acids encoding the albumin derivative, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or fragment thereof, (ii) plasmids comprising said nucleic acids and (iii) host cells comprising said plasmid.

Conjugates comprising an albumin derivative or variant, or fragment thereof, according to the invention and at least one therapeutic or diagnostic moiety form a third aspect of the invention.

A fourth aspect of the invention provides fusion polypeptides comprising or consisting of said albumin derivative or variant or fragment thereof and at least one therapeutic protein or peptide. A fifth aspect of the invention relates to 'associates' of the derivatives or variants of albumin or fragments thereof with another compound bound or associated to the derivative albumin, variant albumin or fragment thereof by non-covalent binding.

The sixth aspect of the invention relates to a composition, preferably a pharmaceutical composition, comprising or consisting of an albumin derivative, fragment, variant thereof or fusion polypeptide comprising or consisting of the albumin derivative, fragment, variant thereof conjugated, fused or associated with a therapeutic, pharmaceutical or other beneficial polypeptide.

A seventh aspect of the invention relates to methods of production of the derivatives or variants.

An eighth aspect of the invention relates to use of the derivatives and/or variants in imaging, for example use of a conjugate, associate or fusion polypeptide for imaging purposes in animals or human beings.

A ninth aspect of the invention relates to a method of treatment and/or use of a polypeptide sequence or nucleotide sequence of the present invention in a method of treatment.

A tenth aspect, the invention relates to compositions comprising the derivative albumin, variant albumin, associates thereof or fragment thereof, derivative or variant albumin fragment

or associates thereof or fusion polypeptide comprising derivative or variant albumin or fragment thereof according to the invention.

5 BRIEF DESCRIPTION OF DRAWINGS

Figure 1, A) Binding of shFcRn-GST to wt HSA and wt HSA derivatives (2000-0.9 nM) at (A) pH 6.0 and (B) pH 7.4. The ELISA values represent the mean of duplicates.

Figure 2. Representative sensorgrams showing binding of 1 μ M of wt HSA and wt HSA derivatives to immobilized shFcRn-GST ($\sim$ 1400 RU) at pH 6.0 and pH 7.4. (A) DI-DII (B) DI-DIII, 10 (C) DII-DIII, (D) DIII, (E) DIII-DIII and (F) wt HSA.

Figure 3, Serial dilutions of wt HSA and wt HSA domain constructs to give final sample concentration at 55nM, 166nM and 500nM, and 500nM and 1500nM, respectively, were pre- 15 incubated shFcRn (50 nM) and injected over immobilized HSA ($\sim$ 2600 RU). Injections were performed at 25°C at a flow rate of 50 μ l/min at pH 6.0.

Figure 4, Multiple alignment of amino acid sequences of (i) full length mature HSA (Hu_1_2_3), (ii) an albumin variant comprising domain I and domain III of HSA (Hu_1_3), (iii) an albumin 20 variant comprising domain II and domain III of HSA (Hu_2_3), (iv) full-length *Macaca mulatta* albumin (Mac_mul), (v) full-length *Rattus norvegicus* albumin (Rat) and (vi) full-length *Mus musculus* albumin (Mouse). Positions 500, 550 and 573 (relative to full length HSA) are indicated by arrows. In Figure 4, Domains I, II and III are referred to as 1, 2 and 3 (respectively).

Figure 5, Multiple alignment of amino acid sequence of mature serum albumin from human, 25 sheep, mouse, rabbit and goat and immature albumins from chimpanzee ("Chimp"), macaque, hamster, guinea pig, rat, cow, horse, donkey, dog, chicken, and pig. The Start and End amino acids of domains 1, 2 and 3 (as defined by Dockal et al (The Journal of Biological Chemistry, 1999, Vol. 274(41): 29303–29310)) are indicated with respect to mature human serum albumin.

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Figure 6, Schematic diagram of plasmid pDB2305

Figure 7, Schematic diagram summarising the generation of expression plasmids *in vivo*. A. 35 PCR was used to generate two PCR fragments. Fragments 1 and 2 share 247 base pair (bp) and 217 bp homology with *Acc65I/BamHI*-digested pDB3936 at their 5' and 3'ends, respectively. Fragments 1 and 2 share 27-30 bp homology with each other at their 3' and 5'

ends, respectively. B Purified-PCR fragments were used, along with *Acc65I/BamHI*-digested pDB3936 to co-transform *S. cerevisiae* BXP10 cir⁰. X = Leader sequence. Y = albumin DI + DII. Z = albumin DIII. Crosses represent *in vivo* recombination.

- 5 **Figure 8**, Representative sensorgrams showing binding of 10 μ M of HSA Domain III and variants thereof to immobilized shFcRn-HIS (~2100RU) at pH 5.5. Figure 8a: DIII wt and DIII K573D, Figure 8b: DIII wt and DIII K573H, Figure 8c: DIII wt and DIII K573N, Figure 8d: DIII wt and DIII K573W, Figure 8e: DIII and DIII Q580K.
- 10 **Figure 9**, Non-reducing SDS-PAGE analysis of albumin and derivatives and variants thereof, some conjugated to HRP: (1) Marker (SeeBlue™), (2) HRP Standard (1 μ g), (3) DI+DIII+DIII:HRP(1 μ g), (4) Albumin Standard(1 μ g), (5) DI+DIII-HRP(1 μ g), (6) DI+DIII K500A-HRP(1 μ g), (7) DI+DIII K573P-HRP(1 μ g), (8) DI+DIII K573Y-HRP(1 μ g), (9) DI+DIII D550N-HRP(1 μ g), (10) DIII+DI-HRP (1 μ g). (11) mixture of HRP and Albumin Standards (1 μ g each),
- 15 (12) mixture of HRP+ and Albumin Standard (2 μ g each).

Figure 10, Representative sensorgrams showing binding of 10 μ M HSA DI+III (and variants thereof) conjugated to HRP to immobilized shFcRn-HIS (~1500 RU) at pH 5.5. (1) DI+DIII K573P-HRP, (2) wt HSA, (3) DI+DIII wt-HRP and (4) DI+DIII K500A -HRP.

- 20 **Figure 11**, Representative sensorgrams showing binding of 10 μ M IL-1ra fused to the N-terminus of HSA DII+III and DII+III K573P to immobilized shFcRn-HIS (~2100 RU) at pH 5.5.

- Figure 12**, Representative sensorgrams showing binding of 10 μ M HSA tandem repeats of DIII,DIII fused and DIII K573P fused to scFv (~1500 RU) at pH 5.5.
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Figure 13, Representative sensorgrams showing binding of 10 μ M of HSA tandem repeats of DIII,DIII fused and DIII D550N fused to scFv (~1500 RU) at pH 5.5

- 30 **Figure 14**, Representative sensorgrams showing binding of 10 μ M HSA tandem repeats of DIII,DIII fused and DIII K573P fused to IL-1ra (~1500 RU) at pH 5.5.

Figure 15, Representative sensorgrams showing binding of 10 μ M of HSA tandem repeats of DIII,DIII fused and DIII D550N fused to IL-1ra (~1500 RU) at pH 5.5

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Figure 16, Visualization of DI+DIII Variant Proteins fused to Fluorescein, with UV Light (A) and a Commercial Protein Stain (B). Non-reducing SDS-PAGE analysis of albumin and derivatives and variants thereof, some conjugated to F5M: (1) Marker (SeeBlue™) (10μL), (2) HSA-F5M control (1μg), (3) Marker (See Blue™) (10μL), (4) DI+DIII+DIII-F5M (1μg), (5) DI+DIII wt-F5M (1μg), (6) DI+DIII K500A-F5M (1μg), (7) DI+DIII K573P-F5M (1μg), (8) DI+DIII K573Y-F5M (1μg), (9) DI+DIII D550N-F5M (1μg), (10) DIII+DI-F5M (1μg). (11) Marker (SeeBlue™) (10μL).

Figure 17, Representative sensorgrams showing binding of 10μM wt HSA, HSA DI+DIII K573P-F5M, HSA DI + DIII K500A-F5M to immobilized shFcRn-HIS (~1500RU) at pH5.5.

Figure 18, Representative sensorgrams showing binding of 10μM wt HSA, HSA DI+DIII+DIII-F5M, HSA DI + DIII-F5M to immobilized shFcRn-HIS (~1500RU) at pH5.5.

DETAILED DESCRIPTION OF THE INVENTION

A first aspect of the invention relates to an albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof, where the albumin derivative or variant or fragment thereof comprises or consists of a first albumin domain III, fragment or derivative or variant thereof, and at least one second albumin domain, fragment or derivative or variant thereof. Preferably, the derivative or variant is not a naturally occurring derivative or variant such as a wild-type albumin from one of human; primate, such as chimpanzee, gorilla or macaque; rabbit; rodent such as mouse, rat and hamster; bovine; equine such as horse or donkey; goat; sheep; dog; guinea pig; chicken and pig. The first aspect may or may not include an albumin derivative or variant comprising or consisting of domains I, II and III (or fragments thereof) of a wild-type albumin and one or more (several) additional domains (or fragments thereof) from any albumin, such as albumin from human or other species disclosed herein.

Some albumin derivatives or variants, fragments thereof or fusion polypeptides according to the first aspect of the invention do not comprise domain I (or fragment thereof) of an albumin. Some albumin derivatives or variants, fragments thereof or fusion polypeptides according to the first aspect of the invention do not comprise domain II (or fragment thereof) of an albumin. Other albumin derivatives or variants, fragments thereof or fusion polypeptides according to the first aspect of the invention comprise domain III of an albumin and one or both of domain I (or fragment thereof) or domain II (or fragment thereof) of an albumin.

The invention is based on the discovery that a polypeptide comprising a first albumin domain III and at least one second albumin domain fragment or derivative or variant thereof,

has a stronger binding to FcRn than the corresponding domain III alone. This is surprising in view of the prior art, in particular WO 2007112940, where it appears that amino residues of domain III are involved in the interaction of albumin with the FcRn receptor which is involved in prolonging the life-span of albumin in circulation, for example in plasma. It is known that domain
5 III is responsible for binding of albumin to FcRn. According to the invention, the term "albumin" means a protein having the same and/or very similar three dimensional structure as HSA and having a long plasma half-life. HSA as disclosed in SEQ ID NO: 31 or SEQ ID NO: 1 or any naturally occurring allele thereof, is the preferred albumin according to the invention. Those skilled in the art would also appreciate that the described characteristics of HSA domain III
10 derivative, fragment, variant thereof would also be probable for other non-human albumins, in accordance to the current invention described.

Thus, the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof according to the invention has the benefit of a long plasma half-life, similar to albumin.

15 The size of the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of the invention may vary depending on the size of the fragment, number of domains, the size of the non-albumin part of the fusion polypeptide etc. In principle, the size may vary from slightly above the size of the domain III and upward but in practise it is preferred that the size is around
20 the size of natural albumin. Without wishing to be bound by any theory it is believed that the size above the kidney threshold value, such as the size of natural albumin, is ideal for high plasma half-life. Thus, it is preferred that the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of the invention and/or a conjugate comprising or consisting of the albumin derivative or
25 variant or fragment thereof has a size in the range of 40-80 kDa, preferably in the range of 50-70 kDa, more preferred in the range of 55-65 kDa and most preferred around 60 kDa.

HSA is a preferred albumin according to the invention and is a protein consisting of 585 amino acid residues and has a molecular weight of 67 kDa. In its natural form it is not glycosylated. The amino acid sequence of HSA is shown in SEQ ID NO: 31 or SEQ ID NO: 1.
30 The skilled person will appreciate that natural alleles may exist having essentially the same properties as HSA but having one or more (several) amino acid changes compared to SEQ ID NO: 31 or SEQ ID NO: 1, and the inventors also contemplate the use of such natural alleles as parent albumin according to the invention.

Albumins have generally a long plasma half-life of approximately 20 days or longer, *e.g.*,
35 HSA has a plasma half-life of 19 days. It is known that the long plasma half-life of HSA is

mediated *via* interaction with its receptor FcRn, however, an understanding or knowledge of the exact mechanism behind the long half-life of HSA is not essential for the invention.

According to the invention the term “albumin” means a protein having the same, or very similar three dimensional structure as HSA and having a long plasma half-life. As examples of albumin proteins according to the invention can be mentioned human serum albumin (e.g. AAA98797 or P02768-1, SEQ ID NO: 31 or SEQ ID NO: 1 (mature), SEQ ID NO: 4 (immature)), primate serum albumin, (such as chimpanzee serum albumin (e.g. predicted sequence XP_517233.2 SEQ ID NO: 5), gorilla serum albumin or macaque serum albumin (e.g. NP_001182578, SEQ ID NO: 6), rodent serum albumin (such as hamster serum albumin (e.g. A6YF56, SEQ ID NO: 7), guinea pig serum albumin (e.g. Q6WDN9-1, SEQ ID NO: 8), mouse serum albumin (e.g. AAH49971 or P07724-1 Version 3, SEQ ID NO: 9) and rat serum albumin (e.g. AAH85359 or P02770-1 Version 2, SEQ ID NO: 10))), bovine serum albumin (e.g. cow serum albumin P02769-1, SEQ ID NO: 11), equine serum albumin such as horse serum albumin (e.g. P35747-1, SEQ ID NO: 12) or donkey serum albumin (e.g. Q5XLE4-1, SEQ ID NO: 13), rabbit serum albumin (e.g. P49065-1 Version 2, SEQ ID NO: 14), goat serum albumin (e.g. ACF10391, SEQ ID NO: 15), sheep serum albumin (e.g. P14639-1, SEQ ID NO: 16), dog serum albumin (e.g. P49822-1, SEQ ID NO: 17), chicken serum albumin (e.g. P19121-1 Version 2, SEQ ID NO: 18) and pig serum albumin (e.g. P08835-1 Version 2, SEQ ID NO: 19). HSA as disclosed in SEQ ID NO: 31 or SEQ ID NO: 1 or any naturally occurring allele thereof, is the preferred albumin according to the invention.

The parent albumin, a fragment thereof, or albumin part of a fusion polypeptide comprising or consisting of albumin or a fragment thereof according to the invention has generally a sequence identity to the sequence of HSA shown in SEQ ID NO: 31 or SEQ ID NO: 1 of at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, more preferred at least 96%, more preferred at least 97%, more preferred at least 98% and most preferred at least 99%.

The parent preferably comprises or consists of at least a part of the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the parent comprises or consists of at least a part of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the parent is an allelic derivative or variant of at least a part of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

The term “isolated derivative” or “isolated variant” means a derivative or variant that is modified by the hand of man. In one embodiment, the derivative or variant is at least 1% pure,

e.g., at least 5% pure, at least 10% pure, at least 20% pure, at least 40% pure, at least 60% pure, at least 80% pure, and at least 90% pure, as determined by SDS-PAGE or GP-HPLC.

The term “substantially pure derivative” or “substantially pure variant” means a preparation that contains at most 10%, at most 8%, at most 6%, at most 5%, at most 4%, at most 3%, at most 2%, at most 1%, and at most 0.5% by weight of other polypeptide material with which it is natively or recombinantly associated. Preferably, the derivative or variant is at least 92% pure, e.g., at least 94% pure, at least 95% pure, at least 96% pure, at least 97% pure, at least 98% pure, at least 99%, at least 99.5% pure, and 100% pure by weight of the total polypeptide material present in the preparation. The derivatives or variants of the present are preferably in a substantially pure form. This can be accomplished, for example, by preparing the derivative or variant by well known recombinant methods or by classical purification methods.

The term “mature polypeptide” means a polypeptide in its final form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one embodiment, the mature polypeptide is amino acids 1 to 585.

The term “mature polypeptide coding sequence” means a polynucleotide that encodes a mature polypeptide. In one embodiment, the mature polypeptide coding sequence is nucleotides 1 to 1758 of SEQ ID NO: 2.

The term “FcRn” means the human neonatal Fc receptor (FcRn). ‘shFcRn’ is a soluble recombinant form of FcRn. The term “smFcRn” is a soluble recombinant form of the mouse neonatal Fc Receptor.

According to the invention the term “albumin derivative, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or fragment thereof” means a non-natural, engineered molecule comprising or consisting of albumin domains as specified. Thus, the term does not include natural full-length albumin or a fusion polypeptide comprising full-length albumin. The term “derivative” includes an albumin polypeptide comprising an alteration, e.g. a substitution, insertion, and/or deletion, at one or more (several) positions. A substitution means a replacement of an amino acid occupying a position with a different amino acid; a deletion means removal of an amino acid occupying a position; and an insertion means adding 1, 2 or 3, or more, amino acids adjacent to an amino acid occupying a position, an insertion can be at the C- or N- side of an amino acid, but is typically at the C-side of an amino acid.

The term “variant” includes an albumin or albumin derivative or fusion protein thereof in which the albumin derivative or fusion is altered by chemical means such as post-translational derivitisation or modification of the polypeptide, e.g. PEGylation and/or conjugation of a desirable moiety (such as a therapeutic moiety) to a thiol group, such as provided by an

unpaired cysteine. The term "variant" also includes a fusion protein of an albumin derivative according to the invention in which the derivative is not altered by chemical means.

The terms "derivative" and "variant" may or may not be used interchangeably.

Albumins are proteins and constitute the most abundant protein in plasma in mammals and albumins from a large and diverse number of mammals have been characterized by biochemical methods and/or by sequence information. Several albumins e.g. HSA, have also been characterized crystallographically and the structure determined, and it has been shown that albumins consists of three distinct domains called Domain I, Domain II and Domain III.

The domains of the invention may in principle be derived from any albumin, however, it is preferred that the domains are derived from human serum albumin, primate serum albumin, such as chimpanzee serum albumin, gorilla serum albumin or or macaque serum albumin, rodent serum albumin such as rabbit serum albumin, mouse serum albumin and rat serum albumin, bovine serum albumin, equine serum albumin, donkey serum albumin, hamster serum albumin, goat serum albumin, sheep serum albumin, dog serum albumin, guinea pig serum albumin, chicken serum albumin and pig serum albumin.

A particularly preferred albumin according to the invention is HSA having the sequence shown in SEQ ID NO: 31 or SEQ ID NO: 1 and preferred albumin domains of the invention are HSA domain I consisting of amino acid residues 1 to 194 $\pm$ 1 to 15 amino acids of SEQ ID NO: 31 or SEQ ID NO: 1; HSA domain II consisting of amino acid residues 192 to 387 $\pm$ 1 to 15 amino acids of SEQ ID NO: 31 or SEQ ID NO: 1 and HSA domain III consisting of amino acid residues 381 to 585 $\pm$ 1 to 15 amino acids of SEQ ID NO: 31 or SEQ ID NO: 1 or a combination of one or more (several) of these domains, e.g. domain 1 and 2, domain 2 and 3 or domain 1 and 3 fused together. No generally accepted convention for the exact borders of the albumin domains exists and the overlap in the above mentioned ranges and the allowance of a varying length of plus or minus 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 from amino acids, preferably from 1 to 15 amino acids, more preferably from 1 to 10 amino acids, most preferably from 1 to 5 amino acids, at the N-terminal and/or C-terminal of the domains, allowing for a total variance in length of up to 30 amino acids, preferably up to 20 amino acids, more preferably up to 10 amino acids for each domain reflects this fact and that there may be some diverging opinions on the amino acid residues in the border between the domains belongs to one or the other domain. For the same reason it may be possible to find references to the amino acid residues of albumin domains that diverge from the numbers above, however, the skilled person will appreciate how to identify the albumin domains based on the teaching in the literature and the teaching above. Corresponding domains of non-human albumins can be identified by alignment with HSA using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package

(EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. Alternative alignment tools can also be used, for example
5 MUSCLE as described herein.

The domains may also be defined according to Dockal or Kjeldsen: Dockal et al (The Journal of Biological Chemistry, 1999, Vol. 274(41): 29303–29310) defines the domains of HSA as: Domain I: amino acids 1 to 197, Domain II: amino acids 189 to 385, Domain III: amino acids 381 to 585. Kjeldsen et al (Protein Expression and Purification, 1998, Vol 13: 163–169) defines
10 the domains as: Domain I: amino acids 1 to 192, Domain II: amino acids 193 to 382, Domain III: amino acids 383 to 585.

Therefore, in this invention, the following domain definitions are preferred. The amino acid numbers correspond to those of SEQ ID NO: 31 or SEQ ID NO: 1 (HSA). However, using these numbers, the skilled person can identify corresponding domains in other albumin
15 sequences.

Domain I may or may not start at amino acid 1 and may or may not end at any of amino acids 192, 193, 194, 195, 196 or 197, preferably any of amino acids 192, 194 or 197.

Domain II may or may not start at amino acid 189, 190, 191, 192 or 193, preferably any of amino acids 189, 192 or 193, and may or may not end at amino acid 382, 383, 384, 385, 386
20 or 387, preferably any of amino acids 382, 385 or 387.

Domain III may or may not start at amino acid 381, 382 or 383, preferably amino acid 381 or 383, and may or may not end at amino acid 585.

Domains in non-human albumins may have the same or different amino acid lengths and/or residue numbers as HSA. For example, a multiple alignment or pair-wise alignment may
25 be prepared using HSA and one or more (several) other albumins, fragments, derivatives, variants and/or fusions in order to identify domains corresponding to domains 1, 2 and/or 3 of HSA. An example of a suitable alignment is given in Fig. 5. Fig. 5 was created using MUSCLE and Boxshade in the same manner as described for Fig. 4. For clarity, the mature sequence of HSA, sheep, mouse, rabbit and goat albumins are provided (*i.e.* numbering starts at the first
30 amino acid after the leader sequence and pro-sequence) and all other albumin sequences provided are immature sequences (*i.e.* include leader sequence, pro-sequence (if present) and mature albumin sequence). Examples of domain coordinates for human, sheep, rabbit and mouse albumins are given in the Examples. These coordinates can be applied to any aspect of the invention. Domain coordinates for all albumins can be identified or extrapolated from a
35 suitable alignment, such as Fig. 5., by identifying amino acids corresponding to one or more (several) of the domain coordinates of HSA (as disclosed herein). Typically, domain coordinates

are designated using the first amino acid of the mature albumin as position 1, therefore the amino acid sequence of a leader sequence and/or a pro-peptide is usually ignored in this respect.

5 The first albumin domain III, fragment or derivative or variant thereof may in principle be any albumin domain III, however, HSA domain III (e.g. SEQ ID NO: 23), fragment or derivatives or variants thereof are preferred.

Throughout this specification, a molecule comprising domain I and domain II may be referred to as 'DI-DII' or as 'DI+DII' or as 'D1+D2' or as 'D1-D2'. Likewise, a molecule comprising domain I and domain III may be referred to as any of 'DI-DIII' or as 'DI+DIII' or as 'D1+D3' or as
10 D1-D3'. Therefore, a molecule comprising domain II and domain III may be referred to as 'DII-DIII' or as 'DII+DIII' or as 'D2+D3' or as 'D2-D3'. Furthermore, a designation such as "HSA 1/2-RSA 3" means that the molecule comprises HSA domain 1, HSA domain 2 and RSA domain 3 which is the same as HSA domain I, HSA domain II and RSA domain III.

The term "derivative" in relation to an albumin domain means a polypeptide having a
15 similar primary and/or tertiary structure to said albumin domain in its natural form, in the following called the parent domain, but which differs from said parent domain with one or more (several) substitutions, insertions, and/or deletions, of one or more (several) amino acid residues at one or more (several) positions. The derivative can be obtained through human intervention by modification of the nucleic acid sequence encoding the parent domain and
20 expression of the modified nucleic acid sequence in a suitable host organism using techniques known in the art.

The term "fragment" means a polypeptide having one or more (several) amino acids deleted from the amino and/or carboxyl terminus of an albumin and/or an internal region of albumin that has retained the ability to bind to FcRn. Fragments may comprise or consist of one
25 uninterrupted sequence derived from HSA or it may comprise or consist of two or more sequences derived from HSA. The fragments according to the invention have a size of at least or more than approximately 20 amino acid residues, preferably at least or more than 30 amino acid residues, more preferred at least or more than 40 amino acid residues, more preferred at least or more than 50 amino acid residues, more preferred at least or more than 75 amino acid
30 residues, more preferred at least or more than 100 amino acid residues, more preferred at least or more than 200 amino acid residues, more preferred at least or more than 300 amino acid residues, even more preferred at least or more than 400 amino acid residues and most preferred at least or more than 500 amino acid residues.

The term "fragment" in relation to the first albumin domain III of this invention means a
35 part of the particular relevant albumin domain having retained the ability to bind FcRn. Fragments may consist of one uninterrupted sequence derived from a parent domain or may

comprise or consist of two or more sequences derived from the parent domain. The fragments according to the invention have a size of at least or more than approximately 20 amino acid residues, preferably at least or more than 30 amino acid residues, more preferred at least or more than 40 amino acid residues, more preferred at least or more than 50 amino acid residues, more preferred at least or more than 75 amino acid residues, and most preferred at least or more than 100 amino acid residues.

The at least one second albumin domain, fragment or derivative or variant thereof may in principle be any albumin domain, fragment or derivative or variant thereof.

The term "fragment" in the relation to the second albumin domain of this invention means a part of the particular relevant albumin domain. Fragments may consist of one uninterrupted sequence derived from the parent domain or it may comprise or consist of two or more sequences derived from the parent domain. The fragments according to the invention have a size of at least or more than approximately 20 amino acid residues, preferably at least or more than 30 amino acid residues, more preferred at least or more than 40 amino acid residues, more preferred at least or more than 50 amino acid residues, more preferred at least or more than 75 amino acid residues, and most preferred at least or more than 100 amino acid residues.

The first albumin domain III, fragment or derivative or variant thereof and the at least one second albumin domain, fragment or derivative or variant thereof may be derived from same species or they may be derived from different species. For example, the first albumin domain III, fragment or derivative or variant thereof may be HSA domain III and the at least one second albumin domain, fragment or derivative or variant thereof be mouse serum albumin domain II, or the first albumin domain III, fragment or derivative or variant thereof may be rabbit serum albumin domain III and the at least one second albumin domain, fragment or derivative or variant thereof be human serum albumin domain I.

The albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof comprises or consists of at least two albumin domains or fragments thereof and it may comprise or consist of more than two domains. In principle there is no upper limit for the number of albumin domains or fragments thereof that may be combined in the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof according to the invention, however, it is preferred that the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof according to the invention comprises or consist of 2 or 3 albumin domains, most preferred 2 domains.

As examples of preferred albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof

according to the invention can be mentioned albumin domain I + albumin domain III (e.g. SEQ ID NO: 22), albumin domain II + albumin domain III (e.g. SEQ ID NO: 21), albumin domain III + albumin domain III (e.g. SEQ ID NO: 24), albumin domain III + albumin domain I and albumin domain III + albumin domain II.

5 Preferred albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant, comprises or consists of an albumin domain III derived from one species and albumin domain I and II derived from a different species. Examples include an albumin derivative consisting of Domain I and II derived from human serum albumin and domain III derived from rabbit serum albumin (e.g. SEQ ID NO: 10 25), a derivative consisting of Domain I and II derived from rabbit serum albumin and domain III derived from human serum albumin (e.g. SEQ ID NO: 26), a derivative consisting of Domain I and II derived from sheep serum albumin and domain III derived from human serum albumin (e.g. SEQ ID NO: 27), and a derivative consisting of domain I and domain II derived from human serum albumin and domain III derived from mouse serum albumin (e.g. SEQ ID NO: 29). These 15 derivatives have surprisingly different binding properties to FcRn than the complete albumin from which the domain III of the derivative is derived.

The derivative consisting of domain I and II derived from human serum albumin and domain III derived from rabbit serum albumin (e.g. SEQ ID NO: 25), the derivative consisting of domain I and II derived from sheep serum albumin and domain III derived from human serum 20 albumin (e.g. SEQ ID NO: 27) and the derivative consisting of the domain I and II derived from human serum albumin and domain III derived from mouse serum albumin (e.g. SEQ ID NO: 29) have stronger binding to FcRn than human serum albumin. The derivative consisting of domain I and II from rabbit serum albumin and domain III from human serum albumin (e.g. SEQ ID NO: 26) has a weaker binding to FcRn than HSA. Variants of the derivatives are also envisaged and 25 included in the invention.

In one embodiment one or more (several) albumin domain III in the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof comprises or consists of one or more (several) 30 alteration(s) such as substitution(s), deletion(s) or insertion(s) in one or more position(s) corresponding to the positions in HSA selected from one or more (several) of: 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584. The inventors have found that these amino acid residues are important for the interaction of serum albumin and the FcRn 35 receptor and alteration in one or more (several) of these positions will alter the binding of the albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of

said albumin derivative or variant or fragment thereof according to the invention to the FcRn receptor and thereby alter the plasma half-life thereof. The alteration at one or more (several) position may be selected independently among substitutions, insertions and deletions. Substitutions are preferred.

5 The derivative or variant albumin, a fragment thereof, or albumin part of a fusion polypeptide comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention has generally a domain III which has an amino acid sequence identity to the sequence of HSA Domain III (as defined herein in relation to SEQ ID NO: 31 or SEQ ID NO: 1) of at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 81%,
10 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%, preferably at least 90 %, more preferred at least 95%, more preferred at least 96%, more preferred at least 97%, more preferred at least 98% and most preferred at least 99%. Even more preferably, the derivative or variant albumin, a fragment thereof, or albumin part of a fusion polypeptide comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention has generally a domain III
15 which has an amino acid sequence identity amino acids 381 to 585 of an albumin, such as any of SEQ ID NO: 31 or SEQ ID NO: 1 or equivalent positions in one or more (several) of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19, SEQ ID NO: 31 or SEQ ID NO: 1 (human serum albumin), of at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%, preferably at least 90
20 %, more preferred at least 95%, more preferred at least 96%, more preferred at least 97%, more preferred at least 98% and most preferred at least 99%.

 In a further embodiment, the derivative or variants of albumin, fragments thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention have a plasma half-life that is longer than the plasma half-life
25 of the parent albumin fragment thereof or fusion polypeptide comprising or consisting of the parent albumin or a fragment thereof. Examples according to this embodiment include derivatives or variants of albumin, fragments thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof comprising or consisting of one or more (several) substitutions in one or more (several) of the position corresponding to the
30 group consisting of E492, N503, D550 and K573 in HSA. Preferably the amino acid residue in the position corresponding to E492 in HSA is substituted with a G residue, the amino acid in the position corresponding to N503 in HSA is substituted with a H or K residues, the amino acid in the position corresponding to D550 in HSA is substituted with a E residue and the amino acid in the position corresponding to K573 in HSA is substituted with an A or a P residue. Also
35 preferred is a derivative or variant where the amino acid corresponding to E492 in HSA is substituted from a G residue and the amino acid corresponding to K573 in HSA is substituted

with an A or a P residue. Other preferred derivative or variant has a number of substitutions corresponding to E492 in HSA with an H residue, E501 in HSA with a P, N503 in HSA with an H, E505 in HSA with a D, T506 in HSA with an S, T540 in HSA with a S, K541 in HSA with a E.

In a further embodiment the derivative or variants of albumin, fragments thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention have a plasma half-life that is shorter than the plasma half-life of the parent albumin fragment thereof or fusion polypeptide comprising or consisting of the parent albumin or a fragment thereof. Examples according to this embodiment include derivatives or variants of albumin, fragments thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof comprising a substitution in the position corresponding to one or more (several) of the group consisting of Q417, H440, H464, D494, E495, T496, P499, K500, E501, H510, H535, K536, P537, K538, K541, D550N, D494+T496 or E492+V493 in HSA. Preferred substitutions include the substitutions corresponding to Q417A, H440A, H464Q, D494E+Q417H, D494N,Q,A, E495Q,A, T496A, D494N+T496A or, P499A, K500A E501A, E501Q, H510Q, H535Q, K536A, P537A, K538A, K541G, K541A K541D or D550N in HSA.

In addition to the one or more (several) substitutions, insertions or deletions (where substitutions are preferred) in one or more (several) positions corresponding to the group consisting of positions 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 of SEQ ID NO: 31 or SEQ ID NO: 1 the derivative or variant albumin, fragments thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention may contain additional substitutions, deletions or insertions in other positions of the molecules. Such additional substitutions, deletions or insertions may be useful in order to alter other properties of the molecules such as but not limited to altered glycosylation; introduction of reactive groups of the surface such a thiol groups, removing/generating a carbamylation site; etc.

Residues that might be altered in order to provide reactive residues on the surface and which advantageously could be applied to the invention has been disclosed in the unpublished patent application EP 2009 152 625 (incorporated herein by reference). Particular preferred residues include the positions corresponding to positions in HSA outside the regions interacting with FcRn.

As examples of alterations that can be made in SEQ ID NO: 31 or SEQ ID NO: 1 or in corresponding positions in other albumins (such as in SEQ ID Numbers 4 to 19) in order to provide a reactive thiol group on the surface includes: L585C, D1C, A2C, D562C, A364C, A504C, E505C, T79C, E86C, D129C, D549C, A581C, D121C, E82C, S270C, A578C, L595LC,

D1DC, A2AC, D562DC, A364AC, A504AC, E505EC, T79TC, E86EC, D129DC, D549DC, A581AC, A581AC, D121DC, E82EC, S270SC, A579AC, C360*, C316*, C75*, C168*, C558*, C361*, C91*, C124*, C169* and C567*. Alternatively a cysteine residue may be added to the N- or C- terminal regions of albumin.

5 In one embodiment, the number of alterations in the derivatives or variants of the invention is 1 to 20 amino acids, e.g., 1 to 10 and 1 to 5, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 alterations.

The derivative or variant albumin, a fragment thereof or fusion polypeptide comprising or consisting of the derivative or variant albumin or fragment thereof has altered plasma half-life
10 compared with the corresponding parent albumin domain(s), fragment thereof, or fusion polypeptide comprising or consisting of the derivative or variant albumin domain(s) or fragment thereof.

In a particularly preferred embodiment, the parent albumin domain(s) are from HSA and the derivative or variant albumin, a fragment thereof or fusion polypeptide comprising or
15 consisting of the derivative or variant albumin domain(s) or fragment thereof has altered plasma half-life compared with the HSA domain(s), the corresponding fragment or fusion polypeptide comprising or consisting of HSA domain(s) or fragment thereof.

One way to determine whether the affinity of a derivative or variant albumin to FcRn is higher or lower than the parent albumin is to use the Surface Plasmon Resonance assay (SPR)
20 as described below. The skilled person will understand that other methods might be useful to determine whether the affinity of a derivative or variant albumin to FcRn is higher or lower than the affinity of the parent albumin to FcRn, e.g., determination and comparison of the binding constants K_D . Thus, according to the invention derivative or variant albumins having a K_D that is lower than the K_D for natural HSA is considered to have a higher plasma half-life than HSA
25 and variant albumins having a K_D that is higher than the K_D for natural HSA is considered to have a lower plasma half-life than HSA.

The derivatives or variants of albumin or fragments thereof or fusion polypeptides comprising or consisting of albumin or fragments thereof comprise or consist of one or more (several) alterations, such as substitutions, deletions or insertions at one or more (several)
30 positions corresponding to the positions in HSA (SEQ ID NO: 31 or SEQ ID NO: 1) selected from the group consisting of 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584. The substitution may be any substitution where the amino acid in the natural albumin sequence is substituted with a different amino acid selected among the remaining 19
35 natural occurring amino acids. For the avoidance of doubt, the skilled person can use the

nomenclature used herein to identify the positions in a domain of HSA or in an albumin, domain or fragment of an alternative albumin.

In one embodiment, a derivative or variant comprises an alteration at one or more (several) positions corresponding to positions 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, a derivative or variant comprises an alteration at two positions corresponding to any of 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, a derivative or variant comprises an alteration at three positions corresponding to any of positions 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, a derivative or variant comprises an alteration at each position corresponding to positions 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises the substitution Q417A,H of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution H440Q of the mature polypeptide of SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution H464Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A490D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution E492G, T,P,H of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution V493P,L of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution D494N,Q,A,E,P of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution E495Q,A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution T496A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution P499A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K500E,G,D,A,S,C,P,H,F,N,W,T,M,Y,V,Q,L,I,R of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution

E501A,P,Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution N503K,D,H of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A504E of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution E505K, D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution T506F, S of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution H510Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution H535Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K536A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution P537A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K538A,H of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution T540S of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K541A,D,G,N,E of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution E542P,D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution D550N of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K573Y,W,P,H,F,V,I,T,N,S,G,M,C,A,E,Q,R,L,D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution K574N of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution Q580K of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution L575F of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A577T,E of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A578R,S of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution S579C,T of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution Q580K of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A581D of the mature polypeptide of SEQ ID NO:

31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution A582T of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1. In another embodiment, the derivative or variant comprises the substitution G584A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

5 In one embodiment, the derivative or variant comprises an alteration at a position corresponding to position 417. In another embodiment, the amino acid at a position corresponding to position 417 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala or His. In another embodiment, the derivative or variant comprises the substitution Q417A, H of the mature
10 polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

 In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 440. In another embodiment, the amino acid at a position corresponding to position 440 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment,
15 the derivative or variant comprises the substitution H440Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

 In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 464. In another embodiment, the amino acid at a position corresponding to position 464 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
20 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution H464Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

 In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 490. In another embodiment, the amino acid at a position
25 corresponding to position 490 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val. In another embodiment, the derivative or variant comprises the substitution A490G of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

 In another embodiment, the derivative or variant comprises an alteration at a position
30 corresponding to position 492. In another embodiment, the amino acid at a position corresponding to position 492 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Gly. In another embodiment, the derivative or variant comprises the substitution E492G of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

35 In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 493. In another aspect, the amino acid at a position corresponding to

position 493 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Pro. In another embodiment, the derivative or variant comprises the substitution V493P of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

5 In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 494. In another embodiment, the amino acid at a position corresponding to position 494 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asn, Gln or Ala. In another embodiment, the derivative or variant comprises the substitution D494N,Q, A of the mature
10 polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 495. In another embodiment, the amino acid at a position corresponding to position 495 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Gln or Ala. In another
15 embodiment, the derivative or variant comprises the substitution E495Q or A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 496. In another embodiment, the amino acid at a position corresponding to position 496 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
20 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution T496A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 499. In another embodiment, the amino acid at a position corresponding to position 499 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
25 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution P499A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 500. In another embodiment, the amino acid at a position corresponding to position 500 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
30 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution K500E,G,D,A,S,C,P,H,F,N,W,T,M,Y,V,Q,L,I,R of the mature polypeptide of SEQ ID NO: 31 or
35 SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 501. In another embodiment, the amino acid at a position corresponding to position 501 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala or Gln to reduce affinity and Pro to increase affinity. In another embodiment, the derivative or variant comprises the substitution E501A, Q, P of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 503. In another embodiment, the amino acid at a position corresponding to position 503 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asp or Lys or His. In another embodiment, the derivative or variant comprises the substitution N503D, K, H of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 504. In another embodiment, the amino acid at a position corresponding to position 504 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val. In another embodiment, the derivative or variant comprises the substitution A504 of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 505. In another embodiment, the amino acid at a position corresponding to position 505 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val. In another embodiment, the derivative or variant comprises the substitution E505D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 506. In another embodiment, the amino acid at a position corresponding to position 506 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val. In another embodiment, the derivative or variant comprises the substitution T506S,F of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 510. In another embodiment, the amino acid at a position corresponding to position 510 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Gln. In another embodiment, the derivative or variant comprises the substitution H510Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 535. In another embodiment, the amino acid at a position corresponding to position 535 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Gln. In another embodiment,
5 the derivative or variant comprises the substitution H535Q of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 536. In another embodiment, the amino acid at a position corresponding to position 536 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
10 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution K536A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 537. In another embodiment, the amino acid at a position corresponding to position 537 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
15 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution P537A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 538. In another embodiment, the amino acid at a position corresponding to position 538 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
20 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution K538H, A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 540. In another embodiment, the amino acid at a position corresponding to position 540 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
25 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val. In another embodiment, the derivative or variant comprises the substitution T540S of the mature polypeptide of SEQ ID NO: 31 or SEQ
30 ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 541. In another embodiment, the amino acid at a position corresponding to position 541 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
35 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Gly, Asp or Ala. In another embodiment, the derivative or variant comprises the substitution K541G, D A, N of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 542. In another embodiment, the amino acid at a position corresponding to position 542 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asp or Pro. In another
5 embodiment, the derivative or variant comprises the substitution E542D, P of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 550. In another embodiment, the amino acid at a position corresponding to position 550 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
10 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asn to reduce affinity, preferably with Glu to increase affinity.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 573. In another embodiment, the amino acid at a position corresponding to position 573 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
15 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Tyr, Trp, Pro, His, Phe, Val, Ile, Thr, Asn, Ser, Gly, Met, Cys, Ala, Glu, Gln, Arg, Leu, Asp. In another embodiment, the derivative or variant comprises the substitution K573Y,W,P,H,F,V,I,T,N,S,G,M,C,A,E,Q,R,L,D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 574. In another embodiment, the amino acid at a position corresponding to position 574 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
20 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asn. In another embodiment, the derivative or variant comprises the substitution K574N of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 575. In another embodiment, the amino acid at a position corresponding to position 575 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
25 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Phe. In another embodiment, the derivative or variant comprises the substitution L575F of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.
30

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 577. In another embodiment, the amino acid at a position corresponding to position 577 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
35 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Thr or Glu. In another embodiment, the derivative or variant comprises the substitution A577TE of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 578. In another embodiment, the amino acid at a position corresponding to position 578 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Arg or Ser. In another
5 embodiment, the derivative or variant comprises the substitution A578R,S of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 579. In another embodiment, the amino acid at a position corresponding to position 579 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
10 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Cys or Thr. In another embodiment, the derivative or variant comprises the substitution S579C,T of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 580. In another embodiment, the amino acid at a position
15 corresponding to position 580 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Lys. In another embodiment, the derivative or variant comprises the substitution Q580K of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position
20 corresponding to position 581. In another embodiment, the amino acid at a position corresponding to position 581 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Asp. In another embodiment, the derivative or variant comprises the substitution A581D of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position
25 corresponding to position 582. In another embodiment, the amino acid at a position corresponding to position 582 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Thr. In another embodiment, the derivative or variant comprises the substitution A582T of the mature polypeptide of SEQ ID
30 NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at a position corresponding to position 584. In another embodiment, the amino acid at a position corresponding to position 584 is substituted with Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile,
35 Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val, preferably with Ala. In another embodiment, the derivative or variant comprises the substitution G584A of the mature polypeptide of SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment, the derivative or variant comprises an alteration at positions corresponding to positions 494 and 496 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

5 In another embodiment, the derivative or variant comprises alterations at positions corresponding to positions 492 and 493 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

In another embodiment, the derivative or variant comprises alterations at positions corresponding to positions 494 and 417 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

10 In another embodiment, the derivative or variant comprises alterations at positions corresponding to positions 492 and 503 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

15 In another embodiment, the derivative or variant comprises alterations at positions corresponding to positions 492 and 573 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

In another embodiment, the derivative or variant comprises alterations at positions corresponding to positions 492, 503, and 573 in SEQ ID NO: 31 or SEQ ID NO: 1, such as those described above.

20 In one embodiment the derivative or variant albumin or fragments thereof, or fusion polypeptides comprising the derivative or variant albumin or fragments thereof according to the invention contains one substitution at a position corresponding to a position in HSA selected from the group consisting of 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1 provided that the derivative or variant
25 albumin is not the derivative or variant consisting of SEQ ID NO: 31 or SEQ ID NO: 1 with the substitution D494N, E501K, K541E, D550G,A, K573E or K574N. The derivative or variant albumin, fragment thereof or fusion polypeptides comprising derivative or variant albumin or a fragment thereof according to the invention may comprise additional substitutions, insertions or deletions at one or more (several) positions corresponding to other positions in HSA.

30 In another embodiment the derivative or variant albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or fragments thereof according to the invention contains two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen fifteen, sixteen, seventeen, eighteen, nineteen twenty or even more substitutions at positions corresponding to positions in HSA selected from the group consisting of 417, 440,
35 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 of SEQ ID NO: 31 or

SEQ ID NO: 1. The derivative or variant albumin or fragments thereof, or fusion polypeptides comprising or consisting of a derivative or variant albumin or fragments thereof according to the invention may comprise additional substitutions, insertions or deletions at positions corresponding to other positions in HSA.

5 In a further embodiment the derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof according to the invention have a plasma half-life that is longer than the plasma half-life of the parent albumin fragment thereof or fusion polypeptide comprising or consisting of the parent albumin or a fragment thereof. Examples according to this embodiment include
10 derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising or consisting of a derivative or variant albumin or a fragment thereof comprising a substitution in the position corresponding to 492, 503, 542, 550, 573, 574, 580, 581, 582 or 584 in SEQ ID NO: 31 or SEQ ID NO: 1. Preferred substitutions according to this embodiment of the invention include the substitution of the amino acid residue in the position corresponding to 492 in SEQ ID
15 NO: 31 or SEQ ID NO: 1 with a G residue, substitution of the amino acid residue in the position corresponding to 503 in SEQ ID NO: 31 or SEQ ID NO: 1 with a H or a K residue, substitution of the amino acid residue in the position corresponding to 550 in SEQ ID NO: 31 or SEQ ID NO: 1 with an E residue, the substitution of the amino acid residue in a position corresponding to 573 in SEQ ID NO: 31 or SEQ ID NO: 1 with an Y, W, P, H, F, V, I, T, N, S, G, M, C, A, E, Q, R, L or a D, the
20 substitution of the amino acid residue in a position corresponding to 574 in SEQ ID NO: 31 or SEQ ID NO: 1 with an N residue, or the substitution of the amino acid residue in the position corresponding to 580 in SEQ ID NO: 31 or SEQ ID NO: 1 with an K residue. Other preferred derivatives or variants have a substitution in the position corresponding to 492 in SEQ ID NO: 31 or SEQ ID NO: 1 with a G residue and a substitution in the position corresponding to 573 in
25 SEQ ID NO: 31 or SEQ ID NO: 1 with an A or a P residue. Other preferred derivative or variant has a number of substitutions corresponding to position 492 in SEQ ID NO: 31 or SEQ ID NO: 1 with an H residue in position 503 in SEQ ID NO: 31 or SEQ ID NO: 1.

Other preferred derivatives or variants have a substitution in the position corresponding to 492 in SEQ ID NO: 31 or SEQ ID NO: 1 with a G residue and a substitution in the position
30 corresponding to position 503 in SEQ ID NO: 31 or SEQ ID NO: 1 corresponding to a H or a K and a substitution in position 573 in SEQ ID NO: 31 or SEQ ID NO: 1 with an A or a P residue.

In a further embodiment the derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising or consisting of a derivative or variant albumin or fragments thereof according to the invention have a plasma half-life that is shorter than the plasma half-life
35 of the parent albumin fragment thereof or fusion polypeptide comprising the parent albumin or a fragment thereof. Examples according to this embodiment include derivatives or variants of

albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or a fragment thereof comprising a substitution in the position corresponding to 417, 440, 494, 495, 496, 499, 500, 501, 536, 537, 538, 541, 494+496 or 492+493 in SEQ ID NO: 31 or SEQ ID NO: 1. Preferred substitutions include the substitutions corresponding to Q417A, H440Q, D494E+Q417H, D494N,Q,A, E495Q,A, T496A, D494N+T496A or, P499A, K500A, E501A, E501Q, K536A, P537A, K538A, K541G, K541A K541D or D550N in SEQ ID NO: 31 or SEQ ID NO: 1.

In another embodiment of the invention the derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or a fragment thereof according to the invention have lost their ability to bind FcRn. In this connection derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or fragments thereof is considered to have lost the ability to bind FcRn if the measured resonance units for the derivative or variant in the SPR assay described below is less than 10% of the measured resonance units for the corresponding parent albumin or fragment thereof. Examples according to this embodiment include derivatives or variants of albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or fragments thereof comprising a substitution at a position corresponding to 464, 500, 510 or 535 in SEQ ID NO: 31 or SEQ ID NO: 1. Preferred substitutions include the substitutions corresponding to H464Q, K500A,P,C,S,A,D.G H510Q or H535Q in SEQ ID NO: 31 or SEQ ID NO: 1.

In addition to the one or more (several) substitutions at one or more (several) positions corresponding to positions 417, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 580 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1 the derivative or variant albumin or fragments thereof, or fusion polypeptides comprising derivative or variant albumin or fragments thereof according to the invention may contain additional substitutions, deletions or insertions in other positions of the molecules. Such additional substitutions, deletions or insertions may be useful in order to alter other properties of the molecules such as but not limited to altered glycosylation; introduction of reactive groups of the surface such a thiol groups, removing/generating a carbamoylation site; etc.

Residues that might be altered in order to provide reactive residues on the surface and which advantageously could be applied to the invention has been disclosed in the unpublished patent application WO 2010/092135 (Included by reference). Particular preferred residues include the positions corresponding to positions in SEQ ID NO: 31 or SEQ ID NO: 1.

As examples of alterations that can be made in SEQ ID NO: 31 or SEQ ID NO: 1 or in corresponding positions in other albumins in order to provide a reactive thiol group on the

surface includes alterations corresponding to following alterations in SEQ ID NO: 31 or SEQ ID NO: 1: L585C, D1C, A2C, D562C, A364C, A504C, E505C, T79C, E86C, D129C, D549C, A581C, D121C, E82C, S270C, A578C, L595LC, D1DC, A2AC, D562DC, A364AC, A504AC, E505EC, T79TC, E86EC, D129DC, D549DC, A581AC, A581AC, D121DC, E82EC, S270SC, A579AC, C360*, C316*, C75*, C168*, C558*, C361*, C91*, C124*, C169* and C567*.

Alternatively a cysteine residue may be added to the N or C terminal of albumin.

In a second aspect, the invention relates to isolated polynucleotides that encode any of the polypeptides of the invention, for example: (i) nucleic acids encoding the albumin derivative or variant, fragment thereof or fusion polypeptide comprising said albumin derivative or variant or fragment thereof, (ii) plasmids comprising said nucleic acids and (iii) host cells comprising said plasmid. The invention also relates to nucleic acid constructs comprising a polynucleotide encoding a derivative or variant of the invention operably linked to one or more (several) control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences. In the second aspect of the invention, preferably, the parent is encoded by a polynucleotide that hybridizes under very low stringency conditions, low stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide-coding sequence of SEQ ID NO: 2 (SEQ ID NO: 3: NCBI Reference Sequence: NM_000477.5, SEQ ID NO: 3 is genomic DNA), (ii) the cDNA sequence encoding the mature polypeptide (cDNA is SEQ ID NO: 2), or (iii) the full-length complementary strand of (i) or (ii) (J. Sambrook, E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning, A Laboratory Manual*, 2d edition, Cold Spring Harbor, New York).

The polynucleotide of SEQ ID NO: 2 or a subsequence thereof, as well as the amino acid sequence of SEQ ID NO: 2 or a fragment thereof, may be used to design nucleic acid probes to identify and clone DNA encoding a parent from strains of different genera or species according to methods well known in the art. In particular, such probes can be used for hybridization with the genomic or cDNA of the genus or species of interest, following standard Southern blotting procedures, in order to identify and isolate the corresponding gene therein. Such probes can be considerably shorter than the entire sequence, but should be at least 14, e.g., at least 25, at least 35, or at least 70 nucleotides in length. Preferably, the nucleic acid probe is at least 100 nucleotides in length, e.g., at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, at least 700 nucleotides, at least 800 nucleotides, or at least 900 nucleotides in length. Both DNA and RNA probes can be used. The probes are typically labeled for detecting the corresponding gene

(for example, with ^{32}P , ^3H , ^{35}S , biotin, or avidin). Such probes are encompassed by the invention.

A genomic DNA or cDNA library prepared from such other organisms may be screened for DNA that hybridizes with the probes described above and encodes a parent. Genomic or
5 other DNA from such other organisms may be separated by agarose or polyacrylamide gel electrophoresis, or other separation techniques. DNA from the libraries or the separated DNA may be transferred to and immobilized on nitrocellulose or other suitable carrier material. In order to identify a clone or DNA that is homologous with SEQ ID NO: 2 or a subsequence thereof, the carrier material is used in a Southern blot.

10 For purposes of the invention, hybridization indicates that the polynucleotide hybridizes to a labeled nucleotide probe corresponding to the polynucleotide shown in SEQ ID NO: 2, its complementary strand, or a subsequence thereof, under low to very high stringency conditions. Molecules to which the probe hybridizes can be detected using, for example, X-ray film or any other detection means known in the art.

15 In one embodiment, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 2. In another embodiment, the nucleic acid probe is nucleotides 1 to 1758 of SEQ ID NO: 2. In another embodiment, the nucleic acid probe is a polynucleotide that encodes the polypeptide of SEQ ID NO: 1 or a fragment thereof. In another embodiment, the nucleic acid probe is SEQ ID NO: 2.

20 For long probes of at least 100 nucleotides in length, very low to very high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and either 25% formamide for very low and low stringencies, 35% formamide for medium and medium-high stringencies, or 50% formamide for high and very high stringencies, following standard Southern blotting
25 procedures for 12 to 24 hours optimally. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C (very low stringency), 50°C (low stringency), 55°C (medium stringency), 60°C (medium-high stringency), 65°C (high stringency), or 70°C (very high stringency).

For short probes that are about 15 nucleotides to about 70 nucleotides in length,
30 stringency conditions are defined as prehybridization and hybridization at about 5°C to about 10°C below the calculated T_m using the calculation according to Bolton and McCarthy (1962, *Proc. Natl. Acad. Sci. USA* 48: 1390) in 0.9 M NaCl, 0.09 M Tris-HCl pH 7.6, 6 mM EDTA, 0.5% NP-40, 1X Denhardt's solution, 1 mM sodium pyrophosphate, 1 mM sodium monobasic phosphate, 0.1 mM ATP, and 0.2 mg of yeast RNA per ml following standard Southern blotting
35 procedures for 12 to 24 hours optimally. The carrier material is finally washed once in 6X SCC

plus 0.1% SDS for 15 minutes and twice each for 15 minutes using 6X SSC at 5°C to 10°C below the calculated T_m .

The parent may be encoded by a polynucleotide with a sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 2 of at least 60%, e.g., at least 65%, at least 70%,
5 at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%,
at least 98%, at least 99%, or 100%, which encodes a polypeptide which is able to function as
an albumin. In an embodiment, the parent is encoded by a polynucleotide comprising or
consisting of SEQ ID NO: 2.

10 According to a third aspect of the invention, the albumin derivative or variant, fragment
thereof or fusion polypeptide comprising said albumin derivative or variant or fragment thereof
according to the invention may be conjugated to a second molecule using techniques well
known within the art. Said second molecule may comprise or consist of a diagnostic moiety, and
in this embodiment the conjugate may be useful as a diagnostic tool such as in imaging; or the
15 second molecule may be a therapeutic compound and in this embodiment the conjugate may
be used for therapeutic purposes where the conjugate will have the therapeutic properties of the
therapeutic compound as well as an altered plasma half-life, derived from its association with
the albumin derivative, fragment, variant thereof. Conjugates of albumin and a therapeutic
molecule are known in the art and it has been verified that such conjugates have long plasma
20 half-lives, compared with the non-conjugated free therapeutic molecule, as such. The
conjugates may conveniently be linked *via* a free thiol group present on the surface of the
albumin derivate, fragment or derivative or variant thereof. The presence of this thiol group
could be either naturally occurring (for example, the free thiol group may be equivalent to amino
acid residue 34 of mature HSA) or engineered into the polypeptide, using techniques well
25 known within the art for genetic engineering and conjugation chemistry. Alternatively the
conjugates may be linked *via* other free thiol groups provided on the surface of the HSA domain
III derivative, fragment, or variant thereof e.g. such as disclosed in the co-pending patent
application (EP 2009 152 625.1, incorporated herein by reference).

The half-life of an albumin conjugation according to the invention may be longer or
30 shorter than the half-life of the conjugation partner molecule alone. The half-life of an albumin
conjugation according to the invention may be longer or shorter than the half-life of the
analogous / equivalent albumin conjugation comprising native HSA (instead of an albumin
variant or derivative according to the invention) and the conjugation molecule.

In one particularly preferred embodiment the derivative or variant albumin or fragment
35 thereof is conjugated to a beneficial therapeutic compound and the conjugate is used for
treatment of a condition in a patient in need thereof, which condition is responsive to the

particular selected therapeutic compound. Techniques for conjugating such a therapeutically compound to the derivative or variant albumin or fragment thereof are known in the art. WO 2009/019314 discloses examples of techniques suitable for conjugating a therapeutically compound to a polypeptide which techniques can also be applied to the invention. Further WO 2009/019314 discloses examples of compounds and moieties that may be conjugated to substituted transferrin and these examples may also be applied to the invention. The teaching of WO 2009/019314 is included herein by reference.

HSA contains in its natural form one free thiol group that conveniently may be used for conjugation. As a particular embodiment within this embodiment the derivative or variant albumin or fragment thereof may comprise further modifications provided to generate additional free thiol groups on the surface. This has the benefit that the payload of the derivative or variant albumin or fragment thereof is increased so that more than one molecule of the therapeutic compound can be conjugated to each molecule of derivative or variant albumin or fragment thereof, or two or more different therapeutic compounds may be conjugated to each molecule of derivative or variant albumin or fragment thereof, *e.g.*, a compound having targeting properties such as an antibody specific for example a tumour; and a cytotoxic drug conjugated to the derivative or variant albumin or fragment thereof thereby creating a highly specific drug against a tumour. Teaching of particular residues that may be modified to provide for further free thiol groups on the surface can be found in co-pending patent application WO 2010/092135, which is incorporated by reference.

According to a fourth aspect of the invention, the albumin derivative, fragment, or variant thereof, according to the invention, may also be fused with a non-albumin polypeptide fusion partner. The fusion partner may in principle be any polypeptide, but generally it is preferred that the fusion partner is a polypeptide having therapeutic or diagnostic properties. Fusion polypeptides comprising albumin are known in the art. It has been found that such a fusion polypeptide comprising or consisting of albumin and a fusion partner polypeptide have a longer plasma half-life compared to the unfused fusion partner polypeptide alone. According to the invention it is possible to alter the plasma half-life of the fusion polypeptides according to the invention compared to the corresponding fusion polypeptides of the prior art. Further teaching regarding albumin fusions to fusion partner polypeptides and examples of suitable fusion partner polypeptides can be found in WO 01/79271 A and WO 03/59934 A. The half-life of an albumin fusion according to the invention may be longer or shorter than the half-life of the fusion partner polypeptide alone. The half-life of an albumin fusion according to the invention may be longer or shorter than the half-life of the analogous / equivalent albumin fusion comprising or

consisting of native HSA (instead of an albumin variant or derivative according to the invention) and the fusion partner.

For all aspects of the invention fusion partner polypeptides and/or conjugates may

5 comprise one or more (several) of: 4-1BB ligand, 5-helix, A human C-C chemokine, A human L105 chemokine, A human L105 chemokine designated huL105_3., A monokine induced by gamma-interferon (MIG), A partial CXCR4B protein, A platelet basic protein (PBP), α 1-antitrypsin, ACRP-30 Homologue; Complement Component C1q C, Adenoid-expressed chemokine (ADEC), aFGF; FGF-1, AGF, AGF Protein, albumin, an etoposide, angiostatin,

10 Anthrax vaccine, Antibodies specific for collapsin, antistasin, Anti-TGF beta family antibodies, antithrombin III, APM-1; ACRP-30; Famoxin, apo-lipoprotein species, Arylsulfatase B, b57 Protein, BCMA, Beta-thromboglobulin protein (beta-TG), bFGF; FGF2, Blood coagulation factors, BMP Processing Enzyme Furin, BMP-10, BMP-12, BMP-15, BMP-17, BMP-18, BMP-2B, BMP-4, BMP-5, BMP-6, BMP-9, Bone Morphogenic Protein-2, calcitonin, Calpain-10a,

15 Calpain-10b, Calpain-10c, Cancer Vaccine, Carboxypeptidase, C-C chemokine, MCP2, CCR5 variant, CCR7, CCR7, CD11a Mab, CD137; 4-1BB Receptor Protein, CD20 Mab, CD27, CD27L, CD30, CD30 ligand, CD33 immunotoxin, CD40, CD40L, CD52 Mab, Cerebus Protein, Chemokine Eotaxin., Chemokine hIL-8, Chemokine hMCP1, Chemokine hMCP1a, Chemokine hMCP1b, Chemokine hMCP2, Chemokine hMCP3, Chemokine hSDF1b, Chemokine MCP-4,

20 chemokine TECK and TECK variant, Chemokine-like protein IL-8M1 Full-Length and Mature, Chemokine-like protein IL-8M10 Full-Length and Mature, Chemokine-like protein IL-8M3, Chemokine-like protein IL-8M8 Full-Length and Mature, Chemokine-like protein IL-8M9 Full-Length and Mature, Chemokine-like protein PF4-414 Full-Length and Mature, Chemokine-like protein PF4-426 Full-Length and Mature, Chemokine-like protein PF4-M2 Full-Length and

25 Mature, Cholera vaccine, Chondromodulin-like protein, c-kit ligand; SCF; Mast cell growth factor; MGF; Fibrosarcoma-derived stem cell factor, CNTF and fragment thereof (such as CNTFAx15` (Axokine™)), coagulation factors in both pre and active forms, collagens, Complement C5 Mab, Connective tissue activating protein-III, CTAA16.88 Mab, CTAP-III, CTLA4-Ig, CTLA-8, CXC3, CXC3, CXCR3; CXC chemokine receptor 3, cyanovirin-N,

30 Darbepoetin, designated exodus, designated huL105_7., DIL-40, Dnase, EDAR, EGF Receptor Mab, ENA-78, Endostatin, Eotaxin, Epithelial neutrophil activating protein-78, EPO receptor; EPOR, erythropoietin (EPO) and EPO mimics, Eutropin, Exodus protein, Factor IX, Factor VII, Factor VIII, Factor X and Factor XIII, FAS Ligand Inhibitory Protein (DcR3), FasL, FasL, FasL, FGF, FGF-12; Fibroblast growth factor homologous factor-1, FGF-15, FGF-16, FGF-18, FGF-3;

35 INT-2, FGF-4; gelonin, HST-1; HBGF-4, FGF-5, FGF-6; Heparin binding secreted transforming factor-2, FGF-8, FGF-9; Glia activating factor, fibrinogen, flt-1, flt-3 ligand, Follicle stimulating

hormone Alpha subunit, Follicle stimulating hormone Beta subunit, Follitropin, Fractalkine, fragment. myofibrillar protein Troponin I, FSH, Galactosidase, Galectin-4, G-CSF, GDF-1, Gene therapy, Glioma-derived growth factor, glucagon, glucagon-like peptides, Glucocerebrosidase, glucose oxidase, Glucosidase, Glycodelin-A; Progesterone-associated endometrial protein, GM-CSF, gonadotropin, Granulocyte chemotactic protein-2 (GCP-2), Granulocyte-macrophage colony stimulating factor, growth hormone, Growth related oncogene-alpha (GRO-alpha), Growth related oncogene-beta (GRO-beta), Growth related oncogene-gamma (GRO-gamma), hAPO-4; TROY, hCG, Hepatitis B surface Antigen, Hepatitis B Vaccine, HER2 Receptor Mab, hirudin, HIV gp120, HIV gp41, HIV Inhibitor Peptide, HIV Inhibitor Peptide, HIV Inhibitor Peptide, HIV protease inhibiting peptides, HIV-1 protease inhibitors, HPV vaccine, Human 6CKine protein, Human Act-2 protein, Human adipogenesis inhibitory factor, human B cell stimulating factor-2 receptor, Human beta-chemokine H1305 (MCP-2), Human C-C chemokine DGWCC, Human CC chemokine ELC protein, Human CC type chemokine interleukin C, Human CCC3 protein, Human CCF18 chemokine, Human CC-type chemokine protein designated SLC (secondary lymphoid chemokine), Human chemokine beta-8 short forms, Human chemokine C10, Human chemokine CC-2, Human chemokine CC-3, Human chemokine CCR-2, Human chemokine Ckbeta-7, Human chemokine ENA-78, Human chemokine eotaxin, Human chemokine GRO alpha, Human chemokine GROalpha, Human chemokine GRObeta, Human chemokine HCC-1, Human chemokine HCC-1, Human chemokine I-309, Human chemokine IP-10, Human chemokine L105_3, Human chemokine L105_7, Human chemokine MIG, Human chemokine MIG-beta protein, Human chemokine MIP-1alpha, Human chemokine MIP1beta, Human chemokine MIP-3alpha, Human chemokine MIP-3beta, Human chemokine PF4, Human chemokine protein 331D5, Human chemokine protein 61164, Human chemokine receptor CXCR3, Human chemokine SDF1alpha, Human chemokine SDF1beta, Human chemokine ZSIG-35, Human Chr19Kine protein, Human CKbeta-9, Human CKbeta-9, Human CX3C 111 amino acid chemokine, Human DNAX interleukin-40, Human DVic-1 C-C chemokine, Human EDIRF I protein sequence, Human EDIRF II protein sequence, Human eosinocyte CC type chemokine eotaxin, Human eosinophil-expressed chemokine (EEC), Human fast twitch skeletal muscle troponin C, Human fast twitch skeletal muscle troponin I, Human fast twitch skeletal muscle Troponin subunit C, Human fast twitch skeletal muscle Troponin subunit I Protein, Human fast twitch skeletal muscle Troponin subunit T, Human fast twitch skeletal muscle troponin T, Human foetal spleen expressed chemokine, FSEC, Human GM-CSF receptor, Human gro-alpha chemokine, Human gro-beta chemokine, Human gro-gamma chemokine, Human IL-16 protein, Human IL-1RD10 protein sequence, Human IL-1RD9, Human IL-5 receptor alpha chain, Human IL-6 receptor, Human IL-8 receptor protein hIL8RA, Human IL-8 receptor protein hIL8RB, Human IL-9 receptor protein, Human IL-9 receptor protein variant #3,

Human IL-9 receptor protein variant fragment, Human IL-9 receptor protein variant fragment#3, Human interleukin 1 delta, Human Interleukin 10, Human Interleukin 10, Human interleukin 18, Human interleukin 18 derivatives, Human interleukin-1 beta precursor, Human interleukin-1 beta precursor., Human interleukin-1 receptor accessory protein, Human interleukin-1 receptor antagonist beta, Human interleukin-1 type-3 receptor, Human Interleukin-10 (precursor), Human Interleukin-10 (precursor), Human interleukin-11 receptor, Human interleukin-12 40 kD subunit, Human interleukin-12 beta-1 receptor, Human interleukin-12 beta-2 receptor, Human Interleukin-12 p35 protein, Human Interleukin-12 p40 protein, Human interleukin-12 receptor, Human interleukin-13 alpha receptor, Human interleukin-13 beta receptor, Human interleukin-15, Human interleukin-15 receptor from clone P1, Human interleukin-17 receptor, Human interleukin-18 protein (IL-18), Human interleukin-3, human interleukin-3 receptor, Human interleukin-3 variant, Human interleukin-4 receptor, Human interleukin-5, Human interleukin-6, Human interleukin-7, Human interleukin-7., Human interleukin-8 (IL-8), Human intracellular IL-1 receptor antagonist, Human IP-10 and HIV-1 gp120 hypervariable region fusion protein, Human IP-10 and human Muc-1 core epitope (VNT) fusion protein, human liver and activation regulated chemokine (LARC), Human Lkn-1 Full-Length and Mature protein, Human mammary associated chemokine (MACK) protein Full-Length and Mature, Human mature chemokine Ckbeta-7, Human mature gro-alpha, Human mature gro-gamma polypeptide used to treat sepsis, Human MCP-3 and human Muc-1 core epitope (VNT) fusion protein, Human MI10 protein, Human MI1A protein, Human monocyte chemoattractant factor hMCP-1, Human monocyte chemoattractant factor hMCP-3, Human monocyte chemotactic proprotein (MCP-3) sequence, Human neurotactin chemokine like domain, Human non-ELR CXC chemokine H174, Human non-ELR CXC chemokine IP10, Human non-ELR CXC chemokine Mig, Human PAI-1 mutants, Human protein with IL-16 activity, Human protein with IL-16 activity, Human secondary lymphoid chemokine (SLC), Human SISD protein, Human STCP-1, Human stromal cell-derived chemokine, SDF-1, Human T cell mixed lymphocyte reaction expressed chemokine (TMEC), Human thymus and activation regulated cytokine (TARC), Human thymus expressed, Human TNF-alpha, Human TNF-alpha, Human TNF-beta (LT-alpha), Human type CC chemokine eotaxin 3 protein sequence, Human type II interleukin-1 receptor, Human wild-type interleukin-4 (hIL-4) protein, Human ZCHEMO-8 protein, Humanized Anti-VEGF Antibodies, and fragments thereof, Humanized Anti-VEGF Antibodies, and fragments thereof, Hyaluronidase, ICE 10 kD subunit., ICE 20 kD subunit., ICE 22 kD subunit., Iduronate-2-sulfatase, Iduronidase, IL-1 alpha, IL-1 beta, IL-1 inhibitor (IL-1i)., IL-1 mature, IL-10 receptor, IL-11, IL-11, IL-12 p40 subunit., IL-13, IL-14, IL-15, IL-15 receptor, IL-17, IL-17 receptor, IL-17 receptor, IL-17 receptor, IL-19, IL-1i fragments, IL1-receptor antagonist, IL-21 (TIF), IL-3 containing fusion protein., IL-3 mutant proteins, IL-3 variants, IL-3 variants, IL-4, IL-4 mutein, IL-4 mutein Y124G, IL-4 mutein Y124X,

IL-4 muteins, IL-5 receptor, IL-6, IL-6 receptor, IL-7 receptor clone, IL-8 receptor, IL-9 mature protein variant (Met117 version), immunoglobulins or immunoglobulin-based molecules or fragment of either (e.g. a Small Modular ImmunoPharmaceutical™ ("SMIP") or dAb, Fab' fragments, F(ab')₂, scAb, scFv or scFv fragment), including but not limited to plasminogen, Influenza Vaccine, Inhibin alpha, Inhibin beta, insulin, insulin-like growth factor, Integrin Mab, inter-alpha trypsin inhibitor, inter-alpha trypsin inhibitor, Interferon gamma-inducible protein (IP-10), interferons (such as interferon alpha species and sub-species, interferon beta species and sub-species, interferon gamma species and sub-species), interferons (such as interferon alpha species and sub-species, interferon beta species and sub-species, interferon gamma species and sub-species), Interleukin 6, Interleukin 8 (IL-8) receptor, Interleukin 8 receptor B, Interleukin-1alpha, Interleukin-2 receptor associated protein p43, interleukin-3, interleukin-4 muteins, Interleukin-8 (IL-8) protein., interleukin-9, Interleukin-9 (IL-9) mature protein (Thr117 version), interleukins (such as IL10, IL11 and IL2), interleukins (such as IL10, IL11 and IL2), Japanese encephalitis vaccine, Kalikrein Inhibitor, Keratinocyte growth factor, Kunitz domain protein (such as aprotinin, amyloid precursor protein and those described in WO 03/066824, with or without albumin fusions), Kunitz domain protein (such as aprotinin, amyloid precursor protein and those described in WO 03/066824, with or without albumin fusions), LACI, lactoferrin, Latent TGF-beta binding protein II, leptin, Liver expressed chemokine-1 (LVEC-1), Liver expressed chemokine-2 (LVEC-2), LT-alpha, LT-beta, Luteinization Hormone, Lyme Vaccine, Lymphotoxin, Macrophage derived chemokine analogue MDC (n+1), Macrophage derived chemokine analogue MDC-eyfy, Macrophage derived chemokine analogue MDC-yl, Macrophage derived chemokine, MDC, Macrophage-derived chemokine (MDC), Maspin; Protease Inhibitor 5, MCP-1 receptor, MCP-1a, MCP-1b, MCP-3, MCP-4 receptor, M-CSF, Melanoma inhibiting protein, Membrane-bound proteins, Met117 human interleukin 9, MIP-3 alpha, MIP-3 beta, MIP-Gamma, MIRAP, Modified Rantes, monoclonal antibody, MP52, Mutant Interleukin 6 S176R, myofibrillar contractile protein Troponin I, Natriuretic Peptide, Nerve Growth Factor-beta, Nerve Growth Factor-beta2, Neuropilin-1, Neuropilin-2, Neurotactin, Neurotrophin-3, Neurotrophin-4, Neurotrophin-4a, Neurotrophin-4b, Neurotrophin-4c, Neurotrophin-4d, Neutrophil activating peptide-2 (NAP-2), NOGO-66 Receptor, NOGO-A, NOGO-B, NOGO-C, Novel beta-chemokine designated PTEC, N-terminal modified chemokine GroHEK/hSDF-1alpha, N-terminal modified chemokine GroHEK/hSDF-1beta., N-terminal modified chemokine met-hSDF-1 alpha, N-terminal modified chemokine met-hSDF-1 beta, OPGL, Osteogenic Protein-1; OP-1; BMP-7, Osteogenic Protein-2, OX40; ACT-4, OX40L, Oxytocin (Neurophysin I), parathyroid hormone, Patched, Patched-2, PDGF-D, Pertussis toxoid, Pituitary expressed chemokine (PGEC), Placental Growth Factor, Placental Growth Factor-2, Plasminogen Activator Inhibitor-1; PAI-1, Plasminogen Activator Inhibitor-2; PAI-2, Plasminogen

Activator Inhibitor-2; PAI-2, Platelet derived growth factor, Platelet derived growth factor Bv-sis, Platelet derived growth factor precursor A, Platelet derived growth factor precursor B, Platelet Mab, platelet-derived endothelial cell growth factor (PD-ECGF), Platelet-Derived Growth Factor A chain, Platelet-Derived Growth Factor B chain, polypeptide used to treat sepsis,
 5 Preproapolipoprotein "milano" variant, Preproapolipoprotein "paris" variant, pre-thrombin, Primate CC chemokine "ILINCK", Primate CXC chemokine "IBICK", proinsulin, Prolactin, Prolactin2, prosaptide, Protease inhibitor peptides, Protein C, Protein S, pro-thrombin, prourokinase, RANTES, RANTES 8-68, RANTES 9-68, RANTES peptide, RANTES receptor, Recombinant interleukin-16, Resistin, restrictocin, Retroviral protease inhibitors, ricin, Rotavirus
 10 Vaccine, RSV Mab, saporin, sarcin, Secreted and Transmembrane polypeptides, Secreted and Transmembrane polypeptides, serum cholinesterase, serum protein (such as a blood clotting factor), Soluble BMP Receptor Kinase Protein-3, Soluble VEGF Receptor, Stem Cell Inhibitory Factor, Straphylococcus Vaccine, Stromal Derived Factor-1 alpha, Stromal Derived Factor-1 beta, Substance P (tachykinin), T1249 peptide, T20 peptide, T4 Endonuclease, TACI, Tarc,
 15 TGF-beta 1, TGF-beta 2, Thr117 human interleukin 9, thrombin, thrombopoietin, Thrombopoietin derivative1, Thrombopoietin derivative2, Thrombopoietin derivative3, Thrombopoietin derivative4, Thrombopoietin derivative5, Thrombopoietin derivative6, Thrombopoietin derivative7, Thymus expressed chemokine (TECK), Thyroid stimulating Hormone, tick anticoagulant peptide, Tim-1 protein, TNF-alpha precursor, TNF-R, TNF-RII; TNF
 20 p75 Receptor; Death Receptor, tPA, transferrin, transforming growth factor beta, Troponin peptides, Truncated monocyte chemotactic protein 2 (6-76), Truncated monocyte chemotactic protein 2 (6-76), Truncated RANTES protein (3-68), tumour necrosis factor, Urate Oxidase, urokinase, Vasopressin (Neurophysin II), VEGF R-3; flt-4, VEGF Receptor; KDR; flk-1, VEGF-110, VEGF-121, VEGF-138, VEGF-145, VEGF-162, VEGF-165, VEGF-182, VEGF-189, VEGF-
 25 206, VEGF-D, VEGF-E; VEGF-X, von Willebrand's factor, Wild type monocyte chemotactic protein 2, Wild type monocyte chemotactic protein 2, ZTGF-beta 9.

Furthermore, conjugates may comprise one or more (several) of chemotherapy drugs such as: 13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacididine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, A, Abraxane, Accutane®, Actinomycin-D,
 30 Adriamycin®, Aducil®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacididine, BCG, BCNU, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide,
 35 BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Carac™, Carboplatin, Carmustine,

Carmustine Wafer, Casodex[®], CC-5013, CCNU, CDDP, CeeNU, Cerubidine[®], Cetuximab,
 Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen[®], CPT-11,
 Cyclophosphamide, Cytadren[®], Cytarabine, Cytarabine Liposomal, Cytosar-U[®], Cytoxan[®],
 Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin, Daunorubicin,
 5 Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome[®], Decadron, Decitabine,
 Delta-Cortef[®], Deltasone[®], Denileukin diftitox, DepoCyt[™], Dexamethasone, Dexamethasone
 acetate, Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex,
 Docetaxel, Doxil[®], Doxorubicin, Doxorubicin liposomal, Droxia[™], DTIC, DTIC-Dome[®],
 Duralone[®], Efudex[®], Eligard[™], Ellence[™], Eloxatin[™], Elspar[®], Emcyt[®], Epirubicin, Epoetin
 10 alfa, Erbitux[™], Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol, Etopophos[®],
 Etoposide, Etoposide Phosphate, Eulexin[®], Evista[®], Exemestane, Fareston[®], Faslodex[®],
 Femara[®], Filgrastim, Floxuridine, Fludara[®], Fludarabine, Fluoroplex[®], Fluorouracil, Fluorouracil
 (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR[®], Fulvestrant, G-CSF, Gefitinib,
 Gemcitabine, Gemtuzumab ozogamicin, Gemzar[®], Gleevec[™], Gliadel[®] Wafer, GM-CSF,
 15 Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony
 Stimulating Factor, Halotestin[®], Herceptin[®], Hexadrol, Hexalen[®], Hexamethylmelamine, HMM,
 Hycamtin[®], Hydrea[®], Hydrocort Acetate[®], Hydrocortisone, Hydrocortisone Sodium Phosphate,
 Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab,
 Ibritumomab Tiuxetan, Idamycin[®], Idarubicin, Ifex[®], IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib
 20 mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate),
 Interleukin - 2, Interleukin-11, Intron A[®] (interferon alfa-2b), Iressa[®], Irinotecan, Isotretinoin,
 Kidrolase[®], Lanacort[®], Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin,
 Leukeran, Leukine[™], Leuprolide, Leurocristine, Leustatin[™], Liposomal Ara-C, Liquid Pred[®],
 Lomustine, L-PAM, L-Sarcosine, Lupron[®], Lupron Depot[®], M, Matulane[®], Maxidex,
 25 Mechlorethamine, Mechlorethamine Hydrochloride, Medralone[®], Medrol[®], Megace[®], Megestrol,
 Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex[™], Methotrexate, Methotrexate
 Sodium, Methylprednisolone, Meticorten[®], Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol[®],
 MTC, MTX, Mustargen[®], Mustine, Mutamycin[®], Myleran[®], Mylocel[™], Mylotarg[®], Navelbine[®],
 Nelarabine, Neosar[®], Neulasta[™], Neumega[®], Neupogen[®], Nexavar[®], Nilandron[®], Nilutamide,
 30 Nipent[®], Nitrogen Mustard, Novaldex[®], Novantrone[®], Octreotide, Octreotide acetate, Oncospar[®],
 Oncovin[®], Ontak[®], Onxal[™], Oprevelkin, Orapred[®], Orasone[®], Oxaliplatin, Paclitaxel, Paclitaxel
 Protein-bound, Pamidronate, Panitumumab, Panretin[®], Paraplatin[®], Pediapred[®], PEG
 Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON[™], PEG-L-asparaginase,
 PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol[®], Platinol-AQ[®], Prednisolone,
 35 Prednisone, Prelone[®], Procarbazine, PROCRIT[®], Proleukin[®], Prolifeprospan 20 with
 Carmustine Implant, Purinethol[®], R, Raloxifene, Revlimid[®], Rheumatrex[®], Rituxan[®], Rituximab,

Roferon-A[®] (Interferon Alfa-2a), Rubex[®], Rubidomycin hydrochloride, Sandostatin[®], Sandostatin LAR[®], Sargramostim, Solu-Cortef[®], Solu-Medrol[®], Sorafenib, SPRYCEL[™], STI-571, Streptozocin, SU11248, Sunitinib, Sutent[®], Tamoxifen, Tarceva[®], Targretin[®], Taxol[®], Taxotere[®], Temodar[®], Temozolomide, Teniposide, TESPAs, Thalidomide, Thalomid[®], TheraCys[®],
 5 Thioguanine, Thioguanine Tabloid[®], Thiophosphoamide, Thioplex[®], Thiotepa, TICE[®], Toposar[®], Topotecan, Toremifene, Tositumomab, Trastuzumab, Tretinoin, Trexall[™], Trisenox[®], TSPA, TYKERB[®], VCR, Vectibix[™], Velban[®], Velcade[®], VePesid[®], Vesanoid[®], Viadur[™], Vidaza[®], Vinblastine, Vinblastine Sulfate, Vincasar Pfs[®], Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon[®], Xeloda[®], Zanosar[®], Zevalin[™], Zinecard[®], Zoladex[®],
 10 Zoledronic acid, Zolinza, Zometa[®]; radiopharmaceuticals such as: Carbon-11, Carbon-14, Chromium-51, Cobalt-57, Cobalt-58, Erbium-169, Fluorine-18, Gallium-67, Gold-198, Indium-111, Indium-113m, Iodine-123, Iodine-125, Iodine-131, Iron-59, Krypton-81m, Nitrogen-13, Oxygen-15, Phosphorous-32, Rhenium-186, Rubidium-82, Samarium-153, Selenium-75, Strontium-89, Technetium-99m, Thallium-201, Tritium, Xenon-127, Xenon-133, Yttrium-90;
 15 imaging agents such as Gadolinium, magnetite, manganese, technetium, I125, I131, P32, TI201, Iopamidol, PET-FDG.

Such polypeptides and chemical compounds may be referred to as diagnostic moieties, therapeutic moieties or beneficial moieties.

20 One or more (several) therapeutic polypeptides may be fused to the N-terminus, the C-terminus of albumin, inserted into a loop in the albumin structure or any combination thereof. It may or it may not comprise linker sequences separating the various components of the fusion polypeptide.

Teachings relating to fusions of albumin or a fragment thereof are known in the art and
 25 the skilled person will appreciate that such teachings can also be applied to the invention. WO 2001/79271 A and WO 2003/59934 A also contain examples of therapeutic polypeptides that may be fused to albumin or fragments thereof, and these examples apply also to the invention.

The albumin derivative, fragment, or variant thereof part of a fused or conjugated
 30 polypeptide according to the invention has generally a sequence identity to the sequence of the corresponding parts of its parent albumin of at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, or 94%, more preferred at least 95%, more preferred at least 96%, more preferred at least 97%, more preferred at least 98% and most preferred at least 99%.

35 In a preferred embodiment the albumin derivative, fragment, or variant thereof part of a fused or conjugated polypeptide according to the invention has generally a sequence identity to

the sequence of the corresponding parts of HSA shown in SEQ ID NO: 31 or SEQ ID NO: 1 of at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 85%, preferably at least 90%, more preferred at least 95%, more preferred at least 96%, more preferred at least 97%, more preferred at least 98% and most preferred at least 99%.

5 The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

For purposes of the invention, the degree of identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: 10 The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends in Genetics* 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

15
$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}).$$

In describing the various derivatives or variants of the invention, the nomenclature described below is adapted for ease of reference. In all cases, the accepted IUPAC single letter or triple letter amino acid abbreviation is employed.

For purposes of the invention, the degree of sequence identity between two 20 deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) 25 substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:
$$(\text{Identical Deoxyribonucleotides} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment}).$$

For purposes of the invention, the mature polypeptide disclosed in SEQ ID NO: 31 or SEQ ID NO: 1 is used to determine the corresponding amino acid residue in another albumin. 30 The amino acid sequence of another albumin is aligned with the mature polypeptide disclosed in SEQ ID NO: 31 or SEQ ID NO: 1, and based on the alignment, the amino acid position number corresponding to any amino acid residue in the mature polypeptide disclosed in SEQ ID NO: 31 or SEQ ID NO: 1 is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the 35 EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 3.0.0 or later.

Identification of the corresponding amino acid residue in another albumin can be confirmed by an alignment of multiple polypeptide sequences using "ClustalW" (Larkin *et al.*, 2007, *Bioinformatics* 23: 2947-2948).

When the other polypeptide (or protein) has diverged from the mature polypeptide of
5 SEQ ID NO: 31 or SEQ ID NO: 1 such that traditional sequence-based comparison fails to detect their relationship (Lindahl and Elofsson, 2000, *J. Mol. Biol.* 295: 613-615), other pairwise sequence comparison algorithms can be used. Greater sensitivity in sequence-based searching can be attained using search programs that utilize probabilistic representations of polypeptide families (profiles) to search databases. For example, the PSI-BLAST program generates profiles
10 through an iterative database search process and is capable of detecting remote homologs (Atschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-3402). Even greater sensitivity can be achieved if the family or superfamily for the polypeptide has one or more representatives in the protein structure databases. Programs such as GenTHREADER (Jones, 1999, *J. Mol. Biol.* 287: 797-815; McGuffin and Jones, 2003, *Bioinformatics* 19: 874-881) utilize information from a
15 variety of sources (PSI-BLAST, secondary structure prediction, structural alignment profiles, and solvation potentials) as inputs to a neural network that predicts the structural fold for a query sequence. Similarly, the method of Gough *et al.*, 2000, *J. Mol. Biol.* 313: 903-919, can be used to align a sequence of unknown structure within the superfamily models present in the SCOP database. These alignments can in turn be used to generate homology models for the
20 polypeptide, and such models can be assessed for accuracy using a variety of tools developed for that purpose.

For proteins of known structure, several tools and resources are available for retrieving and generating structural alignments. For example the SCOP superfamilies of proteins have been structurally aligned, and those alignments are accessible and downloadable. Two or more
25 protein structures can be aligned using a variety of algorithms such as the distance alignment matrix (Holm and Sander, 1998, *Proteins* 33: 88-96) or combinatorial extension (Shindyalov and Bourne, 1998, *Protein Engineering* 11: 739-747), and implementations of these algorithms can additionally be utilized to query structure databases with a structure of interest in order to discover possible structural homologs (*e.g.*, Holm and Park, 2000, *Bioinformatics* 16: 566-567).
30 Another alignment program is MUSCLE (Multiple sequence comparison by log-expectation, Robert C. Edgar, Version 3.6, <http://www.drive5.com/muscle>; Edgar (2004) *Nucleic Acids Research* 32(5), 1792-97 and Edgar (2004) *BMC Bioinformatics*, 5(1):113) which may be used with the default settings as described in the User Guide (Version 3.6, September 2005). Versions of MUSCLE later than 3.6 may also be used for any aspect of the invention.

In describing the albumin derivatives or variants of the invention, the nomenclature described below is adapted for ease of reference. The accepted IUPAC single letter abbreviation or three letter amino acid abbreviation is employed.

Substitutions. For an amino acid substitution, the following nomenclature is used:

5 Original amino acid, position, substituted amino acid. Accordingly, the substitution of threonine with alanine at position 226 is designated as "Thr226Ala" or "T226A". Multiple mutations are separated by addition marks ("+"), e.g., "Gly205Arg + Ser411Phe" or "G205R + S411F", representing mutations at positions 205 and 411 substituting glycine (G) with arginine (R), and serine (S) with phenylalanine (F), respectively. The Figures also use ("/"), e.g., "E492T/N503D"
10 this should be viewed as interchangeable with ("+").

Deletions. For an amino acid deletion, the following nomenclature is used: Original amino acid, position*. Accordingly, the deletion of glycine at position 195 is designated as "Gly195*" or "G195*". Multiple deletions are separated by addition marks ("+"), e.g., "Gly195* + Ser411*" or "G195* + S411*".

15 Insertions. For an amino acid insertion, the following nomenclature is used: Original amino acid, position, original amino acid, new inserted amino acid. Accordingly the insertion of lysine after glycine at position 195 is designated "Gly195GlyLys" or "G195GK". An insertion of multiple amino acids is designated [Original amino acid, position, original amino acid, new inserted amino acid #1, new inserted amino acid #2; etc.]. For example, the insertion of lysine
20 and alanine after glycine at position 195 is indicated as "Gly195GlyLysAla" or "G195GKA".

In such cases the inserted amino acid residue(s) are numbered by the addition of lower case letters to the position number of the amino acid residue preceding the inserted amino acid residue(s). In the above example the sequences would thus be:

<u>Parent:</u>	<u>Variant:</u>
195	195 195a 195b
G	G - K - A

25 Multiple alterations. Variants or derivatives comprising multiple alterations are separated by addition marks ("+"), e.g., "Arg170Tyr+Gly195Glu" or "R170Y+G195E" representing a substitution of tyrosine and glutamic acid for arginine and glycine at positions 170 and 195, respectively.

Different substitutions. Where different substitutions can be introduced at a position, the
30 different substitutions are separated by a comma, e.g., "Arg170Tyr,Glu" represents a substitution of arginine with tyrosine or glutamic acid at position 170. Thus, "Tyr167Gly,Ala + Arg170Gly,Ala" designates the following variants or derivatives:

“Tyr167Gly+Arg170Gly”, “Tyr167Gly+Arg170Ala”, “Tyr167Ala+Arg170Gly”, and “Tyr167Ala+Arg170Ala”. The single letter code may also be used, e.g. “Y167G+R170G”, “Y167G+R170A”, “R167A+R170G”, and “R167A+R170A”.

The expression “amino acid position corresponding to” a position in a reference sequence and similar expression is intended to identify the amino acid residue that in the primary or spatial structure corresponds to the particular position in the reference sequence. The skilled person will appreciate that this can be done by aligning a given sequence with the reference sequence and identifying the amino acid residue that aligns with the particular position in the reference sequence. For example in order to find the amino acid residue in a given albumin sequence that corresponds to position 492 in HSA, the given albumin sequence is aligned with HSA and the amino acid that aligns with position 492 in HSA (SEQ ID NO: 31 or SEQ ID NO: 1) is identified as the amino acid in the given albumin sequence that corresponds to position 492 in HSA.

The expression Xnnn means an amino acid residue X located in a position corresponding to position nnn in HSA and the expression XnnnY means a substitution of any amino acid X located in a position corresponding to position nnn in HSA with the amino acid residue Y.

Throughout this specification amino acid positions are defined in relation to full-length mature human serum albumin (*i.e.* without leader sequence). However, the equivalent positions can be identified in fragments of human serum albumin, in animal albumins and in fragments, fusions and other derivative or variants thereof by comparing amino acid sequences using pairwise (e.g. ClustalW) or multiple (e.g. MUSCLE) alignments. For example, Fig. 4 shows that positions equivalent to 500, 550 and 573 in full length human serum albumin are easily identified in fragments of human serum albumin and in albumins of other species. Positions 500, 550 and 573 are indicated by arrows. Further details are provided in Table 1 below:

Table 1: Albumins from different animals showing positions equivalent to 500, 550 and 573 of HSA.

Organism (accession number of protein)	Albumin			Position equivalent to human serum albumin (native amino acid):		
	Full length or fragment	Fragment details	Total length of mature protein	500 (K)	550 (D)	573 (K)
<i>Homo sapiens</i> (AAA98797)	Full length	-	585	500 (K)	550 (D)	573 (K)
<i>Homo sapiens</i>	Fragment	DI, DIII	399	314 (K)	364 (D)	387 (K)
<i>Homo sapiens</i>	Fragment	DI, DIII	403	318 (K)	368 (D)	391 (K)
<i>Macaca mulatta</i> (NP_001182578)	Full length	-	584	500 (K)	550 (N)	573 (P)
<i>Rattus norvegicus</i> (AAH85359)	Full length	-	584	500 (K)	550 (D)	573 (P)
<i>Mus musculus</i> (AAH49971)	Full length	-	584	500 (K)	550 (D)	573 (P)

Fig. 4 was generated by MUSCLE using the default parameters including output in ClustalW 1.81 format. The raw output data was shaded using BoxShade 3.21 (http://www.ch.embnet.org/software/BOX_form.html) using Output Format: RTF_new; Font Size: 10; Consensus Line: no consensus line; Fraction of sequences (that must agree for shading): 0.5; Input sequence format: ALN. Therefore, throughout this specification amino acid positions defined in human serum albumin also apply to equivalent positions in fragments, derivatives or variants and fusions of human serum albumin, animals from other species and fragments and fusions thereof. Such equivalent positions may have (i) a different residue number in its native protein and/or (ii) a different native amino acid in its native protein.

Plasma half-life is ideally determined using *in vivo* determinations in suitable individuals. However, since it is time consuming and expensive and there inevitably are ethical concerns connected with doing experiments in animals or man it is desirable to use an *in vitro* assay for determining whether plasma half-life is extended or reduced. It is thought that the binding of albumin to its receptor FcRn is important for plasma half-life and the correlation between receptor binding and plasma half-life is that a higher affinity of albumin to its receptor FcRn leads to longer plasma half-life. Thus, for the invention a higher affinity of the albumin derivative,

fragment, or variant thereof to FcRn is considered indicative of an increased (longer) plasma half-life and a lower affinity of the albumin derivative, fragment, or variant thereof to the FcRn receptor is considered indicative of a reduced (shorter) plasma half-life.

In this application the binding of the albumin derivative, fragment, or variant thereof to the receptor FcRn is described using the term affinity (KD) and the expressions “stronger” or “weaker”. Thus, it should be understood that a molecule having a higher affinity to FcRn than HSA is considered to bind stronger to FcRn than HSA and a molecule having a lower affinity to FcRn than HSA is considered to bind weaker to FcRn than HSA.

The terms “longer plasma half-life” or “shorter plasma half-life” and similar expressions are understood to be in relationship to the corresponding parent albumin molecule which maybe a full-length albumin, an albumin fragment or variant or derivative or an albumin fusion protein. Thus, a longer plasma half-life with respect to a variant albumin of the invention means that the variant has longer plasma half-life than the corresponding albumin having the same sequences except for the alteration(s) in positions corresponding to 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574, 575, 577, 578, 579, 580, 581, 582 and 584 in SEQ ID NO: 31 or SEQ ID NO: 1. Therefore, for example, a full-length human serum albumin having a mutation at position 535 must be compared with a full-length human serum albumin not having a mutation at position 535. Likewise, a mouse albumin fragment comprising domains 2 and 3 of mouse albumin and having a mutation at position 582 must be compared to a mouse albumin fragment comprising domains 2 and 3 of mouse albumin but not having a mutation at position 582.

A ‘long’ or ‘short’ plasma half-life, for example in blood, may be relative to (i) serum albumin (or a fragment thereof) and/or (ii) serum albumin (or a fragment thereof) fused to a polypeptide of interest. For example, long plasma half-life may be at least 5% longer than that of serum albumin or a serum albumin fused to a polypeptide of interest, preferably at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% longer. Long plasma half-life includes from at least 5 to 100 days, for example at least 5, 6, 7, 8, 9, 10, 14, 15, 20, 21, 28, 30, 35, 40, 42, 50, 60, 70, 80, 90, 100 days. Short plasma half-life may be at least 5% shorter than that of serum albumin or a serum albumin fused to a polypeptide of interest, more preferably at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% shorter. Short plasma half-life includes at most 5, 4, 3, 2, 1, 0.5, or 0.25 days. Preferably, the half-life of an unfused albumin derivative or variant is compared to an unfused parent or native albumin. Likewise, preferably, the half-life of a fused albumin derivative or variant (“albumin fusion”) is compared to a fused parent or native albumin.

The binding of an albumin derivative, fragment or variant thereof to FcRn may also be described using kinetic factors, in particular “on-rate” (k_a) and “off-rate” (k_d), which describes the reaction rate whereby the albumin derivative, fragment or variant thereof of the invention associated or dissociated with FcRn respectively. The inventors have further realized that the kinetics whereby albumin derivative, fragment or variant thereof interacts with FcRn may have an impact on the plasma half-life, and have realized that an albumin derivative, fragment or variant thereof having a slow off-rate has a higher plasma half-life than a comparable molecule having a faster off-rate.

The correlation between binding of the albumin derivative, fragment, or variant thereof to the FcRn receptor and plasma half-life has been realized by the inventors based on the prior art within the field of this invention.

One way to determine whether the affinity of the albumin derivative, fragment, or variant thereof is higher or lower than wild-type albumins is using the Surface Plasmon Resonance assay (SPR) as described below. The skilled person will understand that other method might be useful to determine whether the affinity of the albumin derivative, fragment, or variant thereof to FcRn is higher or lower than the affinity of the corresponding wild-type albumin to FcRn, e.g. determination and comparison of the binding constants K_D . Thus, according to the invention the albumin derivative, fragment, or variant thereof having a K_D that is lower than the K_D for natural HSA is considered to have a higher plasma half-life than HSA and albumin derivatives, fragments of variants thereof having a K_D that is higher than the K_D for natural HSA is considered to have a lower plasma half-life than HSA.

Preparation of Derivatives and Variants

The albumin derivative, fragment, or variant thereof of the invention can be prepared using techniques well known to the skilled person. One convenient way is by cloning a nucleic acid encoding the parent albumin, fragment thereof or fusion polypeptide comprising the HSA domain III derivative, fragment, or variant thereof. A polynucleotide may be manipulated in a variety of ways to provide for expression of a derivative or variant. Manipulation of the polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides utilizing recombinant DNA methods are well known in the art.

The control sequence may be a promoter sequence, which is recognized by a host cell for expression of the polynucleotide. The promoter sequence contains transcriptional control sequences that mediate the expression of the derivative or variant. The promoter may be any nucleic acid sequence that shows transcriptional activity in the host cell including mutant,

truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (*ENO1*), *Saccharomyces cerevisiae* protease A (*PRA1*), *Saccharomyces cerevisiae* protease B (*PRB1*), *Saccharomyces cerevisiae* translation elongation factor (*TEF1*), *Saccharomyces cerevisiae* translation elongation factor (*TEF2*), *Saccharomyces cerevisiae* galactokinase (*GAL1*), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (*ADH1*, *ADH2/TDH1*), *Saccharomyces cerevisiae* triose phosphate isomerase (*TPI1*), *Saccharomyces cerevisiae* metallothionein (*CUP1*), and *Saccharomyces cerevisiae* 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos *et al.*, 1992, *Yeast* 8: 423-488.

The control sequence may also be a suitable transcription terminator sequence, which is recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3'-terminus of the polynucleotide encoding the derivative or variant. Any terminator that is functional in the host cell may be used.

Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (*CYC1*), *Saccharomyces cerevisiae* alcohol dehydrogenase (*ADH1*) and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase (*TDH1*). Other useful terminators for yeast host cells are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA that is important for translation by the host cell. The leader sequence is operably linked to the 5'-terminus of the polynucleotide encoding the derivative or variant. Any leader sequence that is functional in the host cell may be used.

The albumin derivative, fragment, or variant thereof of the invention may also be connected to a signal sequence (also known as a 'signal peptide' or as a 'leader sequence') in order to have the polypeptide secreted into the growth medium during culturing of the transformed host organism. It is generally advantageous to have the derivative or variant polypeptide secreted into the growth medium in order to ease recovery and purification. Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (*ENO1*), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (*ADH2/TDH1*).

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3'-terminus of the derivative- or variant-encoding sequence and, when transcribed,

is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell may be used.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Mol. Cellular Biol.* **15**: 5983-5990.

5 The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a derivative or variant and directs the derivative or variant into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region that encodes the derivative or variant. Alternatively, the
10 5'-end of the coding sequence may contain a signal peptide coding region that is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the derivative or variant. However, any signal peptide coding
15 region that directs the expressed derivative or variant into the secretory pathway of a host cell may be used.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor (*MATALPHA*) and *Saccharomyces cerevisiae* invertase (*SUC2*). Other useful signal peptide coding sequences are described by Romanos *et al.*, 1992,
20 *supra*.

Where both signal peptide and pro-peptide regions are present at the N-terminus of a derivative or variant, the propeptide region is positioned next to the N-terminus of the derivative or variant and the signal peptide region is positioned next to the N-terminus of the propeptide region.

25 Techniques for preparing derivative or variant polypeptides have also been disclosed in WO 2009019314 and PCT/EP2010/066572 (incorporated herein by reference) and these techniques may also be applied to the invention.

Albumins have been successfully expressed as recombinant proteins in a range of hosts
30 including fungi (including but not limited to *Aspergillus* (WO06066595), *Kluyveromyces* (Fleer 1991, Bio/technology 9, 968-975), *Pichia* (Kobayashi 1998 Therapeutic Apheresis 2, 257-262) and *Saccharomyces* (Sleep 1990, Bio/technology 8, 42-46)), bacteria (Pandjaitab 2000, J. Allergy Clin. Immunol 105, 279-285)), animals (Barash 1993, Transgenic Research 2, 266-276) and plants (including but not limited to potato and tobacco (Sijmons 1990, Bio/technology 8, 217
35 and Farran 2002, Transgenic Research 11, 337-346). The HSA domain III derivative, fragment, or variant thereof of the invention is preferably produced recombinantly in a suitable host cell. In

principle any host cell capable of producing a polypeptide in suitable amounts may be used and it is within the skills of the average practitioner to select a suitable host cell according to the invention. A preferred host organism is yeast, preferably selected among *Saccharomycaceae*, more preferred *Saccharomyces cerevisiae*.

5 The albumin derivative, fragment, or variant thereof of the invention may be recovered and purified from the growth medium using a combination of known separation techniques such as filtrations, centrifugations, chromatography, affinity separation techniques etc. It is within the skills of the average practitioner to purify the albumin derivative, fragment, or variant thereof of the invention using a particular combination of such known separation steps. As an example of
10 purification techniques that may be applied to the derivatives or variants of the invention can be mentioned the teaching of WO0044772.

 The albumin derivative, fragment, or variant thereof of the invention may be used for delivering a therapeutically beneficial compound to an animal or a human individual in need
15 thereof. Such therapeutically beneficial compounds include but are not limited to labels and readily detectable compounds for use in diagnostics, such as various imaging techniques; pharmaceutical active compounds such as drugs, or specifically binding moieties such as antibodies. The albumin derivative, fragment, or variant thereof of the invention may even be connected to two or more different therapeutically beneficial compounds e.g. an antibody and a
20 drug, which gives the combined molecule the ability to bind specifically to a desired target and thereby provide a high concentration of the connected drug at that particular target.

 In one particular preferred embodiment the albumin derivative, fragment, or variant thereof is conjugated to a beneficial therapeutic compound and the conjugate is used for treatment of a condition in a patient in need therefore, which condition is responsive to the
25 particular selected therapeutic compound. Techniques for conjugating such a therapeutically compound to the albumin derivative, fragment, or variant thereof are known in the art. WO2009019314 discloses examples of techniques suitable for conjugating a therapeutically compound to a polypeptide which techniques can also be applied to the invention. Further WO2009019314 discloses examples of compounds and moieties that may be conjugated to
30 substituted transferrin and these examples may also be applied to the invention. The teaching of WO2009019314 and PCT/EP2010/066572 are incorporated herein by reference.

 HSA contains in its natural form one free thiol group in Domain I that conveniently may be used for conjugation provided that the albumin derivative or variant, fragment thereof or fusion polypeptide comprising the albumin derivative or variant, fragment thereof comprises
35 Domain I.

As a particular embodiment within this, the albumin derivative, fragment, or variant thereof may comprise further modifications provided to generate additional free thiol groups on the surface. This has the benefit that the pay load of the albumin derivative, fragment, or variant thereof is increased so that more than one molecule of the therapeutic compound can be conjugated to each albumin derivative, fragment, or variant thereof molecule, or two or more different therapeutic compounds may be conjugated to each molecule of the albumin derivative, fragment, or variant thereof, *e.g.* It may be a compound having targeting properties such as an antibody specific for *e.g.* a tumour; and a cytotoxic drug conjugated to the albumin derivative, fragment, or variant thereof thereby creating a highly specific drug against a tumour. Teaching of particular residues that may be modified to provide for further free thiol groups on the surface can be found in the co-pending patent application (EP 2009 152 625.1, incorporated herein by reference), which is incorporated herein by reference.

In another preferred the fusion polypeptide comprising the albumin derivative, fragment, or variant thereof comprises one or more (several) therapeutic polypeptides. In this the HSA domain III derivative, fragment, or variant thereof and the one or more (several) therapeutic polypeptides is produced as one single polypeptide.

The one or more (several) therapeutic polypeptides may be fused to the N-terminus, the C-terminus of the albumin derivative, fragment, or variant thereof, inserted into a loop in the albumin derivative, fragment, or variant thereof structure or any combination thereof. It may or it may not comprise linker sequences separating the various components of the fusion polypeptide.

Teachings relating to fusions of albumin or a fragment thereof are known in the art and the skilled person will appreciate that such teachings can also be applied to the invention. WO 01/79271 A and WO 03/59934 A also contains examples of therapeutic polypeptides that may be fused to the HSA domain III derivative, fragment, or variant thereof, and these examples applies also for the invention.

The albumin derivative, fragment, or variant thereof or fusion polypeptides comprising the albumin derivative, fragment, or variant thereof according to the invention have the benefit that their plasma half-life is altered compared to the parent albumin, fragments thereof or fusion polypeptides comprising the parent albumin or fragment thereof. This has the advantage that the plasma half-life of conjugates comprising the albumin derivative, fragment, or variant thereof or fusion polypeptide comprising the albumin derivative, fragment, or variant thereof according to the invention can be selected in accordance with the particular therapeutic purpose.

For example for a conjugate or fusion polypeptide used for imaging purposes in animals or human beings, where the imaging moiety has an very short half-life and a conjugate or a fusion polypeptide comprising the albumin derivative, fragment, or variant thereof has a plasma

half-life that is far longer than needed for the imaging purposes it would be advantageous to use an albumin derivative, fragment, or variant thereof of the invention having a shorter plasma half-life than the parent albumin or fragment thereof, to provide conjugates of fusion polypeptides having a plasma half-life that is sufficiently long for the imaging purpose but sufficiently short to be cleared from the body of the particular patient on which it is applied.

In another example for a conjugate or fusion polypeptide comprising a therapeutic compound effective to treat or alleviate a particular condition in a patient in need for such a treatment it would be advantageous to use the albumin derivative, fragment, or variant thereof having longer plasma half-life than the parent albumin or fragment thereof, to provide conjugates or fusion polypeptides having longer plasma half-life which would have the benefit that the administration of the conjugate or fusion polypeptide of the invention would be needed less frequent compared to the situation where the parent albumin or fragment thereof were used.

A fifth aspect of the invention provides 'associates' of the derivatives or variants of albumin or fragments thereof. In this connection the term "associate" means a compound comprising or consisting of a derivative or variant of albumin or a fragment thereof and another compound bound or associated to the derivative or variant albumin or fragment thereof by non-covalent binding. As an example of such an associate can be mentioned an associate consisting of derivative or variant albumin and a lipid associated to albumin by a hydrophobic interaction. Such associates are known in the art and they may be prepared using well known techniques. As an example of a preferred associate according to the invention can be mentioned an associate comprising a derivative or variant albumin and paclitaxel or paclitaxel protein bound. The half-life of an albumin associate according to the invention may be longer or shorter than the half-life of the 'other compound' alone. The half-life of an albumin associate according to the invention may be longer or shorter than the half-life of the analogous / equivalent albumin associate comprising or consisting of native HSA (instead of an albumin variant or derivative according to the invention) and the 'other compound'. Methods for the preparation of associates are well-known to the skilled person, for example, formulation (by association) of HSA with Lipo-compounds is described in Hussain, R. and Siligardi, G. (2006) International Journal of Peptide Research and Therapeutics, Vol. 12, No. 3, pp. 311–315

In a sixth aspect the invention relates to compositions comprising or consisting of the albumin derivative, fragment, or variant thereof or fusion polypeptide comprising the albumin derivative, fragment, or variant thereof according to the invention conjugated, fused or associated with a therapeutic, pharmaceutical or other beneficial polypeptide. The compositions

are preferably pharmaceutical compositions. The composition may be prepared using techniques known in the area such as disclosed in recognized handbooks within the pharmaceutical field.

5 In a particular embodiment the compositions comprise or consist of the albumin derivative, fragment, or variant thereof according to the invention and a compound comprising a pharmaceutically beneficial moiety and an albumin binding domain (ABD). According to the invention ABD means a site, moiety or domain capable of bind to circulating albumin *in vivo* and thereby confer transport in the circulation of the ABD and any compound or moiety bound to said ABD. ABDs are known in the art and it has been shown that ABDs bind very tight to
10 albumin so a compound comprising an ABD bound to albumin will to a certain extent behave as a single molecule. The inventors have realized by using the albumin derivative, fragment, or variant thereof according to the invention together with a compound comprising a pharmaceutically beneficial moiety and an ABD makes it possible to alter the plasma half-life of the compound comprising a pharmaceutically beneficial moiety and an ABD compared to the
15 situation where said compound were injected as such in a patient having need thereof or administered in a formulation comprising natural albumin or a fragment thereof.

Therefore, the invention is directed to the use of a derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising a derivative or variant albumin or fragment thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof, or an
20 associate comprising a derivative or variant of albumin or a fragment thereof for the manufacture of a pharmaceutical composition, where in the derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof, or an associate comprising a derivative or variant of albumin or a fragment thereof has an altered
25 plasma half-life compared with HSA or the corresponding fragment thereof or fusion polypeptide comprising HSA or fragment thereof or conjugate comprising HSA.

In this connection the corresponding fragment of HSA means a fragment of HSA that aligns with and has same number of amino acids as the fragment of the derivative or variant albumin with which it is compared. Similarly the corresponding fusion polypeptide comprising
30 HSA or conjugate comprising HSA means molecules having same size and amino acid sequence as the fusion polypeptide of conjugate comprising derivative or variant albumin, with which it is compared.

Preferably the derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising or consisting of a derivative or variant albumin or fragments thereof, or
35 a conjugate comprising a derivative or variant of albumin or a fragment thereof has a plasma

half-life that is higher than the plasma half-life of HSA or the corresponding fragment thereof or fusion polypeptide comprising HSA or fragment thereof.

Alternatively, this may be expressed as the derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof, fragment thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof has a KD to FcRn that is lower than the corresponding KD for HSA or the corresponding fragment thereof or fusion polypeptide comprising HSA or fragment thereof. Preferably, is KD for the derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof, fragment thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof less than 0.9X KD for HSA, more preferred less than 0.5X KD for HSA, more preferred less than 0.1X KD for HSA, even more preferred less than 0.05X KD for HSA, even more preferred less than 0.02X KD for HSA and most preferred less than 0.01X KD for HSA.

The derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof, fragment thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof, fragment thereof, or a conjugate comprising a derivative or variant of albumin or a fragment thereof according to the invention.

A seventh aspect of the invention relates to methods of production of the derivatives or variants or associates. The derivatives or variants of the invention can be prepared using techniques well known to the skilled person. One convenient way is by cloning nucleic acid encoding the parent albumin or a fragment thereof or fusion polypeptide comprising albumin or a fragment thereof, modifying said nucleic acid to introduce the desired substitution(s) at one or more (several) positions corresponding to positions 417, 464, 490, 492, 493, 494, 495, 496, 499, 500, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 550, 573, 574 and 580 in SEQ ID NO: 31 or SEQ ID NO: 1, where the derivative or variant is not the derivative or variant consisting of SEQ ID NO: 31 or SEQ ID NO:1 with the substitution D494N, E501K, K541E, D550G,A, K573E or K574N., preparing a suitable genetic construct where the modified nucleic acid is placed in operative connection with suitable regulatory genetic elements, such as promoter, terminator, activation sites, ribosome binding sites etc., introducing the genetic construct into a suitable host organism, culturing the transformed host organism under conditions leading to expression of the derivative or variant and recovering the derivative or variant. Optionally the derivative or variant or associate is formulated, for example with a pharmaceutically acceptable excipient. Optionally the derivative or variant or associate is

presented in unit dosage form. All these techniques are known in the art and it is within the skills of the average practitioner to design a suitable method for preparing a particular derivative or variant according to the invention.

The derivative or variant polypeptide of the invention may also be connected to a signal sequence in order to have the derivative or variant polypeptide secreted into the growth medium during culturing of the transformed host organism. It is generally advantageous to have the derivative or variant polypeptide secreted into the growth medium in order to ease recovery and purification.

Techniques for preparing derivative or variant polypeptides have also been disclosed in WO 2009019314 and PCT/EP2010/066572 (incorporated herein by reference) and these techniques may also be applied to the invention.

Albumins have been successfully expressed as recombinant proteins in a range of hosts including fungi (including but not limited to *Aspergillus* (WO06066595), *Kluyveromyces* (Fleer 1991, *Bio/technology* **9**, 968-975), *Pichia* (Kobayashi 1998 *Therapeutic Apheresis* **2**, 257-262) and *Saccharomyces* (Sleep 1990, *Bio/technology* **8**, 42-46)), bacteria (Pandjaitab 2000, *J. Allergy Clin. Immunol.* **105**, 279-285)), animals (Barash 1993, *Transgenic Research* **2**, 266-276) and plants (including but not limited to potato and tobacco (Sijmons 1990, *Bio/technology* **8**, 217 and Farran 2002, *Transgenic Research* **11**, 337-346)). The derivative or variant polypeptide of the invention is preferably produced recombinantly in a suitable host cell. In principle any host cell capable of producing a polypeptide in suitable amounts may be used and it is within the skills of the average practitioner to select a suitable host cell according to the invention. A preferred host organism is yeast, preferably selected among *Saccharomycaceae*, more preferred *Saccharomyces cerevisiae*.

The derivative or variant polypeptides of the invention may be recovered and purified from the growth medium using a combination of known separation techniques such as filtration, centrifugation, chromatography, and affinity separation techniques etc. It is within the skills of the average practitioner to purify the derivative or variants of the invention using a particular combination of such known separation steps. As an example of purification techniques that may be applied to the derivative or variants of the invention can be mentioned the teaching of WO0044772.

The derivative or variant polypeptides of the invention may be used for delivering a therapeutically beneficial compound to an animal or a human individual in need thereof. Such therapeutically beneficial compounds include, but are not limited, to labels and readily detectable compounds for use in diagnostics, such as various imaging techniques; pharmaceutical active compounds such as drugs, or specifically binding moieties such as antibodies. The derivatives or variants of the invention may even be connected to two or more

different therapeutically beneficial compounds, e.g., an antibody and a drug, which gives the combined molecule the ability to bind specifically to a desired target and thereby provide a high concentration of the connected drug at that particular target.

The derivative or variant albumin, fragments thereof or fusion polypeptides comprising derivative or variant albumin or fragments thereof according to the invention have the benefit that their plasma half-life is altered compared to the parent albumin or fragments thereof or fusion polypeptides comprising parent albumin or fragments thereof or the unfused, unconjugated or unassociated therapeutic, diagnostic or other beneficial moiety. This has the advantage that the plasma half-life of conjugates comprising derivative or variant albumin or a fragment thereof or fusion polypeptide comprising derivative or variant albumin or a fragment thereof, or an associate comprising derivative or variant albumin or a fragment thereof according to the invention can be selected in accordance with the particular therapeutic purpose.

An eighth aspect of the invention relates to imaging. For example for a conjugate, associate or fusion polypeptide used for imaging purposes in animals or human beings, where the imaging moiety has an very short half-life and a conjugate or a fusion polypeptide comprising HSA has a plasma half-life that is far longer than needed for the imaging purposes it would be advantageous to use a derivative or variant albumin or fragment thereof of the invention having a shorter plasma half-life than the parent albumin or fragment thereof, to provide conjugates of fusion polypeptides having a plasma half-life that is sufficiently long for the imaging purpose but sufficiently short to be cleared form the body of the particular patient on which it is applied. Examples of imaging agents comprising albumin includes those of WO 2004/071536.

A ninth aspect of the invention relates to a method of treatment and/or use in a method of treatment. The method may comprise use of a therapeutic compound effective to treat or alleviate a particular condition in a patient in need for such a treatment it would be advantageous to use the derivative or variant albumin or fragment thereof having a longer plasma half-life than the parent albumin or fragment thereof, to provide associates or conjugates or fusion polypeptides having longer plasma half-lives, compared to the therapeutic compound alone or the therapeutic compound fused, conjugated or associated with native HSA, which would have the benefit that the administration of the associate or conjugate or fusion polypeptide of the invention would be needed less frequently or reduced dose with less side affects compared to the situation where the parent albumin or associates thereof or fragment thereof was used. The invention also includes methods in which the albumin variant, derivative,

fusion, conjugate or associate has a shorter half-life compared to the therapeutic compound alone or the therapeutic compound fused, conjugated or associated with native HSA.

In a tenth aspect, the invention relates to compositions comprising the derivative or variant albumin, associates thereof or fragment thereof, derivative or variant albumin fragment or associates thereof or fusion polypeptide comprising variant albumin or fragment thereof according to the invention. The compositions are preferably pharmaceutical compositions. The composition may be prepared using techniques known in the area such as disclosed in recognized handbooks within the pharmaceutical field.

In a particular embodiment the compositions comprise or consist of a derivative or variant albumin or a fragment thereof according to the invention and a compound comprising a pharmaceutically beneficial moiety and an albumin binding domain (ABD). According to the invention ABD means a site, moiety or domain capable of binding to circulating albumin *in vivo* and thereby conferring transport in the circulation of the ABD and any compound or moiety bound to said ABD. ABDs are known in the art and have been shown to bind very tight to albumin so a compound comprising an ABD bound to albumin will to a certain extent behave as a single molecule. The inventors have realized by using the derivative or variant albumin or fragment thereof according to the invention together with a compound comprising a pharmaceutically beneficial moiety and an ABD makes it possible to alter the plasma half-life of the compound comprising a pharmaceutically beneficial moiety and an ABD compared to the situation where said compound were injected as such in a patient having need thereof or administered in a formulation comprising natural albumin or a fragment thereof.

The derivative or variant albumin or fragments thereof, conjugates comprising derivative or variant albumin or a fragment thereof or fusion polypeptide comprising derivative or variant albumin or a fragment thereof, or an associate comprising derivative or variant albumin or a fragment thereof according to the invention may also be incorporated into nano- or microparticles using techniques well known within the art. A preferred method for preparing nano- or microparticles that may be applied to the derivative or variant albumins or fragments thereof according to the invention is disclosed in WO 2004/071536, which is incorporated herein by reference.

The following definitions also apply to the invention disclosed herein:

Allelic variant: The term "allelic variant" means any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid

sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

The term "coding sequence" means a polynucleotide, which directly specifies the amino acid sequence of its translated polypeptide product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon or alternative start codons such as GTG and TTG and ends with a stop codon such as TAA, TAG, and TGA. The coding sequence may be a DNA, cDNA, synthetic, or recombinant polynucleotide.

The term "cDNA" means a DNA molecule that can be prepared by reverse transcription from a mature, spliced, mRNA molecule obtained from a eukaryotic cell. cDNA lacks intron sequences that may be present in the corresponding genomic DNA. The initial, primary RNA transcript is a precursor to mRNA that is processed through a series of steps, including splicing, before appearing as mature spliced mRNA.

The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the invention.

The term "control sequences" means all components necessary for the expression of a polynucleotide encoding a derivative or variant of the invention. Each control sequence may be native or foreign to the polynucleotide encoding the derivative or variant or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences within the coding region of the polynucleotide encoding a derivative or variant.

The term "operably linked" means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs the expression of the coding sequence.

The term "expression" includes any step involved in the production of the derivative or variant including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

The term "expression vector" means a linear or circular DNA molecule that comprises a polynucleotide encoding a derivative or variant and is operably linked to additional nucleotides that provide for its expression.

5 The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, and the like with a nucleic acid construct or expression vector comprising a polynucleotide of the invention. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

The term "mutant" means a polynucleotide encoding a derivative or variant.

10 The term "wild-type albumin" means an albumin expressed by a naturally occurring organism, such as a eukaryote, e.g. a mammal such as a human.

The term "parent" or "parent albumin" means an albumin to which an alteration is made to produce the albumin derivatives or variants of the invention. The parent may be a naturally occurring (wild-type) polypeptide or a derivatives or variant thereof.

15 The invention is further described with reference to the following examples that should not be construed as limiting the scope of the invention.

EXAMPLES

Material and methods:

20 (a) ELISA:

Wells were coated with HSA wt or mutants diluted in PBS with concentrations ranging from 100-0.045 µg/ml, incubated over night at 4 °C and then blocked with 4% skimmed milk (Acumedia) for 1 h at room temperature. The wells were then washed four times with phosphate buffered saline (PBS)/0.005% Tween 20 (PBS/T) pH 6.0 before GST-fused shFcRn (0.5 µg/ml
25 (FEBS J. 2008 Aug;275(16):4097-110.)) pre-incubated with an HRP-conjugated polyclonal anti-GST from goat (1:5000; GE Healthcare), diluted in 4% skimmed milk PBS/0.005% Tween 20 (PBS/T) pH 6.0 was added to each well and incubated for 1.5 h at room temperature followed by four times washing with PBS/T pH 6.0. 100 µl of the substrate TMB (Calbiochem) was added to each well and incubated for 45 minutes before 100 µl of 0.25 M HCl was added. The
30 absorbance was measured at 450 nm using a Sunrise TECAN spectrophotometer (TECAN, Maennedorf, Switzerland).

The same ELISA was repeated with PBS/T pH 7.4.

(b) Surface Plasmon Resonance (SPR):

35 SPR experiments were carried out using a Biacore 3000 instrument (GE Healthcare). Flow cells of CM5 sensor chips were coupled with shFcRn-GST (~ 1400-5000 RU) using amine

coupling chemistry as described in the protocol provided by the manufacturer. The coupling was performed by injecting 10 µg/ml of the protein in 10 mM sodium acetate pH 5.0 (GE Healthcare). Phosphate buffer (67 mM phosphate buffer, 0.15 M NaCl, 0.005% Tween 20) at pH 6.0) was used as running buffer and dilution buffer. Regeneration of the surfaces were done using injections of HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) at pH 7.4 (Biacore AB). For binding to immobilized shFcRn-GST, 1.0-0.5 µM of each HSA derivative or variant was injected over the surface at constant flow rate (40 µl/ml) at 25 °C. In all experiments, data was zero adjusted and the reference cell subtracted. Data evaluation was performed using BIAevaluation 4.1 software (Biacore AB).

The same SPR assay was repeated with HBS-EP buffer pH 7.4.

SPR experiments were carried out using a Biacore 3000 instrument (GE Healthcare). Flow cells of CM5 sensor chips were coupled with HSA (~2600 RU) using amine coupling chemistry as described in the protocol provided by the manufacturer. The coupling was performed by injecting 10 µg/ml of the protein in 10 mM sodium acetate pH 5.0 (GE Healthcare). Phosphate buffer (67 mM phosphate buffer, 0.15 M NaCl, 0.005% Tween 20) at pH 6.0) was used as running buffer and dilution buffer. Regeneration of the surfaces were done using injections of HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) at pH 7.4 (Biacore AB). Competitive binding was measured by injecting shFcRn (50 nM) alone or together with different amounts of HSA or RSA domain constructs over immobilized HSA. In all experiments, data were zero adjusted and the reference cell subtracted. Data evaluation was performed using BIAevaluation 4.1 software (Biacore AB).

HSA: Recombinant Human Serum Albumin commercially available under the registered tradename RECOMBUMIN was used for the examples.

Serum albumin from other species: The albumins were produced recombinantly using sequences provided from publicly available databases (data not shown).

FcRn: *PCR and subcloning.* cDNA segments encoding truncated soluble hFcRn (shFcRn) HC and hβ2m were PCR amplified from a U937 cell line (ATCC) cDNA library followed by subcloning of the fragments into the pCDNA3-GST vector, all as previously described (Berntzen et al. (2005) J Immunol Methods 298:93-104). A mouse liver cDNA library (Zyagen) was used to PCR amplify a cDNA encoding a truncated version of the mFcRn HC (encoding the endogenous native leader sequence, α1, α2 and α3 domains; 293 amino acids) using the primers mFcRnForw and mFcRnRev:

mFcRnForw 5-ATT ATG AAT TCA TGG GGA TGC CAC TGC CCT GG-3

mFcRnRev 5-ATA TAC TCG AGT AGG TCC ACA GTG AGA GGC TG -3

Primers were designed to allow in frame ligation of the fragment upstream of a cDNA encoding a GST-tag from *Schistosoma japonicum* into the pcDNA3-GST-hβ2m-oriP vector, which also

contains a cDNA encoding h β 2m and the Epstein Barr virus origin of replication (oriP). The final vector was sequenced and denoted pcDNA3-mFcRn^{wt}-GST-h β 2m-oriP.

Expression and purification of soluble human FcRn variants (shFcRn-GST)- For transient transfections, the hFcRn and mFcRn encoding plasmids were transfected into HEK 293E cells (ATCC) using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. HEK 293E cells were cultured in Dulbecco modified eagle medium (BioWhittaker) using standard conditions. Pooled media were filtrated and applied on a GSTrap FF column 5 ml column (GE Healthcare) connected to a semiautomatic workstation and recorder, and purifications were performed essentially as recommended in the manufacturer's manual. Eluted fractions were pooled, concentrated and analyzed under non-reducing or reducing condition using β -mercaptoethanol (Sigma-Aldrich). Samples of 2 μ g of each receptor were applied on a 12% SDS-PAGE (Bio-Rad). Protein concentrations were determined using a NanoDrop N-1000 spectrophotometer (NanoDrop Technologies).

Methods for the generation of shFcRn expression plasmids, expression and purification of each heterodimer can also be found in Berntzen *et al.* (2005) J. Immunol. Methods 298:93-104) and Andersen *et al.* (2010) J. Biol. Chem., 285:4826-4836.

Alternatively His-tagged shFcRn FcRn heterodimer was produced by GeneArt AG (Germany). Sequences for the two sub units of the heterodimer can be found in SEQ ID NO: 32 (truncated heavy chain of the major histocompatibility complex class I-like Fc receptor (FCGRT)) and SEQ ID NO: 33 (beta-2-microglobulin). Together, SEQ ID NO: 32 and 33 form FcRn. The soluble receptor was expressed in HEK293 cells and purified from culture supernatant using Ni-HiTrap chromatography columns. The His tag is genetically fused to the C-terminus of beta-2-microglobulin.

(c) Construction of plasmids and strains

Standard molecular biology techniques were employed throughout such as those described in Sambrook, J. and D. W. Russell, 2001. Molecular Cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Plasmids containing expression cassettes are listed in Table 2. All expression cassettes comprise the *S. cerevisiae* PRB1 promoter and a modified *S. cerevisiae* ADH1 terminator (mADHt), and encode a leader sequence [either: fusion leader (FL. The same as that in plasmid pDB2244, WO 0044772A), modified fusion leader (mFL. The same as that described in plasmid pDB2305, EP1788084, incorporated herein by reference), the *S. cerevisiae* invertase leader sequence (Suc2p: MLLQAFLFLLAGFAAKISA) or a modified *S. cerevisiae* invertase leader sequence (MLLQAFFIVSIGFAAKISA)], and an albumin-derived protein (*e.g.* full-length, domains, mutants and fusions etc). Figure 6 shows an example of a plasmid map (pDB2305) to

illustrate the components of a typical expression cassette. Unless otherwise stated, final expression plasmids were generated *in vivo* (i.e. via homologous recombination in *S. cerevisiae*; a technique referred to as gap repair or *in vivo* cloning- see Orr-Weaver & Szostak. 1983. *Proc. Natl. Acad. Sci. USA*. 80:4417-4421). Typically, for gap-repair experiments, 100ng of

5 *Acc65I/BamHI* pDB3936 (disclosed in WO 2010/092135) was mixed with equimolar concentrations of DNA fragments containing the expression cassette required to generate the desired final expression plasmid. These were used directly to co-transform *S. cerevisiae* strains using a Yeast Transformation kit (Sigma) described below.

10 Table 2: Summary of plasmid constructs

Construct	Plasmid	SEQ ID
HSA DI + DIII wt	pDB4372	22
HSA DI + DIII K500A	pDB4373	-
HSA DI + DIII D550N	pDB4480	-
HSA DI + DIII K573P	pDB4374	-
HSA DI + DIII K573Y	pDB4375	-
HSA DI + DIII wt + GSL + IL1Ra	pDB4382	-
HSA DI + DIII K500A + GSL + IL1Ra	pDB4383	-
HSA DI + DIII D550N + GSL + IL1Ra	pDB4481	-
HSA DI + DIII K573P + GSL + IL1Ra	pDB4385	-
HSA DI + DIII K573Y + GSL + IL1Ra	pDB4384	-
HSA DII + DIII wt	pDB2217 / pDB4386	21
HSA DII + DIII D550N	pDB4482	-
HSA DII + DIII K573P	pDB4387	-
HSA DII + DIII K573Y	pDB4388	-
HSA DII + DIII wt + GSL + IL1Ra	pDB4483	-
HSA DII + DIII D550N + GSL + IL1Ra	pDB4484	-
HSA DII + DIII K573P + GSL + IL1Ra	pDB4485	-
HSA DII + DIII K573Y + GSL + IL1Ra	pDB4486	-
HSA DIII K573F	pDB4460	-
HSA DIII wt	pDB4461	23
HSA DIII E492G	pDB4462	-
HSA DIII K500A	pDB4463	-
HSA DIII D550N	pDB4464	-
HSA DIII K573A	pDB4465	-

HSA DIII K573D	pDB4466	-
HSA DIII K573H	pDB4467	-
HSA DIII K573P	pDB4468	-
HSA DIII K573W	pDB4469	-
HSA DIII K573Y	pDB4470	-
HSA DIII K574N	pDB4471	-
HSA DIII Q580K	pDB4472	-
HSA DIII E492G/N503K	pDB4473	-
HSA DIII::GSL:: II1Ra	pDB4474	-
HSA DIII K573P::GSL:: II1Ra	pDB4475	-
HSA DIII D550N::GSL:: II1Ra	pDB4476	-
HSA DIII::GSL:: scFv::flag	pDB4477	-
HSA DIII D550N::GSL:: scFv::flag	pDB4478	-
HSA DIII K573P::GSL:: scFv::flag	pDB4479	-
HSA DIII wt + HSA DIII wt	pDB4522	24
HSA DIII E492G + HSA DIII E492G	pDB4112	-
HSA DIII D550N + HSA DIII D550N	pDB4523	-
HSA DIII K573P + HSA DIII K573P	pDB4524	-
HSA DIII wt + HSA DIII K573P	pDB4525	-
HSA DIII K573P + HSA DIII wt	pDB4526	-
HSA DIII wt + HSA DIII wt + GS + IL1Ra	pDB4527	-
HSA DIII wt + HSA DIII wt + GS + scFv + FLAG	pDB4528	-
HSA DIII D550N + HSA DIII D550N + GS + IL1Ra	pDB4529	-
HSA DIII D550N + HSA DIII D550N + scFv + FLAG	pDB4530	-
HSA DIII K573P + HSA DIII K573P + GS + IL1Ra	pDB4531	-
HSA DIII K573P + HSA DIII K573P + scFv + FLAG	pDB4526	-
HSA DIII + DI	pDB4487	-
HSA DIII + DII	pDB4488	-
HSA DIII + DIII + DIII	pDB4534	-
MSA	pDB3257	9
RSA	pDB3442	14
SSA	pDB3994	16

Expression plasmids were also generated using PCR (polymerase chain reactions) fragments and *Acc65I/BamHI* pDB3936 (100 ng) at equimolar concentrations using *in vivo*

cloning. Table 3 lists the constructs generated using PCR fragments and *Acc65I/BamHI* pDB3936. All PCR reactions were performed using Phusion polymerase (New England Biolabs) following the manufacturer's instructions. A typical PCR reaction mixture is: 20µl Buffer HF(5X), 2µl dNTP mix (10mM), 2µl primer (10µM), 2µl primer (10µM), 1µl Phusion polymerase (2U/µl), 1µl plasmid DNA (~5ng) or total DNA (~100ng), 72µl distilled H₂O.

Table 3: Constructs generated in vivo using PCR and *Acc65I/BamHI* pDB3936 (100 ng) at equimolar concentrations

Construct	Reference Number	SEQ ID NO:
IL1Ra + GSL +HSA DI + DIII wt	9506	-
IL1Ra + GSL +HSA DI + DIII K573P	9507	-
IL1Ra + GSL + HSA DII + DIII wt	9508	-
IL1Ra + GSL + HSA DII + DIII K573P	9509	-
IL1Ra + GSL + HSA DIII	9504	-
IL1Ra + GSL + HSA DIII K573P	9505	-
HSA DI + DII + MSA DIII	9226	29
HSA DI + DII + RSA DIII	9009	25
HSA DI + DII + SSA DIII	9114	28
MSA DI + DII + HSA DIII	9225	30
RSA DI + DII + HSA DIII	9010	26
SSA DI + DII + HSA DIII	9113	27
MSA DI + DIII	9008	-
RSA DI + DIII	9007	-

(i) Construction of plasmids for the expression of human/animal chimeras and animal albumin DI +DIII constructs

Plasmids are summarised in Table 2 and Table 3 unless otherwise stated. Plasmids for the expression of full-length rabbit (SEQ ID NO: 14) and mouse albumin (SEQ ID NO: 9) were prepared as follows. *BfrI/SphI* synthetic DNA fragments (2.087kb) containing the 3' region of the *PRB1* promoter, DNA encoding a modified fusion leader sequence, rabbit or mouse albumin and the 5' region of the *mADHt* were generated by gene assembly (GeneArt AG, Germany). An artificial *SphI* site was added directly upstream of the naturally present *BfrI* site to aid subsequent cloning. Synthetic *SphI* DNA fragments were cloned into pCR-script (Agilent Technologies), producing pDB3248 and pDB3429. pDB2541 (a sub-cloning plasmid containing the *PRB1* promoter, DNA-encoding modified fusion leader and HSA, and the *mADHt*) was digested with *BfrI/SphI* to remove the gene encoding HSA and portions of the *PRB1* and *mADHt*

terminator flanking it. This DNA was replaced with the analogous *BfrI/SphI* fragments from pDB3248 and pDB3429 to produce pDB3256 and pDB3435, respectively. pDB3256 and pDB3435 were digested with *NotI* and the 2.989kb products were individually ligated into *NotI*-digested pSAC35 (disclosed in EP-A-286 424 and described by Sleep, D., *et al.* (1991) Bio/Technology 9, 183 – 187, incorporated herein by reference) to produce pDB3257 and pDB3442, respectively.

pDB3257 and pDB3442 were used to directly transform *S. cerevisiae* Strain A (described in WO 2010/092135).

A plasmid for the expression of sheep albumin was prepared as follows. A 2.207kb *PstI/SphI* synthetic DNA fragment (containing 3' region of the *PRB1* promoter, DNA-encoding the fusion leader sequence and sheep albumin (SEQ ID NO: 16), and a portion of the *mADHt*) was generated by gene assembly (GeneArt AG, Germany). The synthetic *PstI/SphI* fragment was cloned into *PstI/SphI*- digested pDB3927 (described in WO 2010/092135) to produce pDB3994.

The final expression plasmid was generated by *in vivo* cloning /gap-repair, that is, pDB3994 was digested with *BstEII/BsrBI*, purified using a Qiagen PCR-Purification kit following the manufacturer's instructions, and 100ng of the digest was combined with 100ng *Acc65I/BamHI*-digested pDB3936 (disclosed in WO 2010/092135) and used to transform *S. cerevisiae* BXP10 cir⁰ (described in WO2001/079480).

Expression plasmids for the various albumin chimeras were generated using PCR and *in vivo* cloning. Table 4 lists the oligonucleotide pairs and template DNA used to generate each construct, Table 5 provides the sequences of the oligonucleotides. That is, PCR was used to amplify two PCR fragments. Fragment 1 contained the *LEU2* ORF and 3' UTR, *PRB1* promoter, the DNA encoding a leader sequence (either modified fusion or fusion leader sequence), DNA-encoding DI+DII of albumin (e.g. from human, mouse rabbit or sheep) and 27-30bp of DNA encoding the 5' sequence of DIII of albumin (e.g. from human, mouse rabbit or sheep). Fragment 2 contained the DNA-encoding DIII of albumin (e.g. from human, mouse rabbit or sheep), the *mADHt* terminator and 217bp flanking sequence homologous with nucleotide sequence in pDB3936 (disclosed in WO 2010/092135).

PCR-products were purified using a Qiagen PCR Purification kit (following the manufacturer's instructions). Figure 7 summarises the generation of expression plasmids by *in vivo* cloning. That is, purified PCR-products were used, along with *Acc65I/BamHI*-digested pDB3936, to co-transform *S. cerevisiae* BXP10 cir⁰.

The expression plasmids for mouse albumin and rabbit albumin DI + DIII were prepared using PCR and *in vivo* cloning using identical methods as those described above for generating the human/animal chimera expression constructs (e.g. HSA DI +DII + mouse DIII, and vice

versa). Oligonucleotide pairs (Table 5) and template DNA used to generate PCR fragments are listed in Table 4.

Table 4: Oligonucleotide pairs and template DNA used to generate PCR fragments.

Construct	SEQ ID NO:	Oligonucleotide pairs used	
		PCR fragment 1	PCR fragment 2
HSA DI + DII + MSA DIII	29	xAP032 / xAP161 (pDB2305)*	xAP160 / xAP033 (pDB3442)
HSA DI + DII + RSA DIII	25	xAP032 / xAP091 (pDB2305)	xAP086 / xAP033 (pDB3257)
HSA DI + DII + SSA DIII	28	xAP032 / xAP121 (pDB2305)	xAP122 / xAP033 (pDB3994)
MSA DI + DII + HSA DIII	30	xAP032 / xAP162 (pDB3442)	xAP093 / xAP033 (pDB2305)
RSA DI + DII + HSA DIII	26	xAP032 / xAP092 (pDB3257)	xAP093 / xAP033 (pDB2305)
SSA DI + DII + HSA DIII	27	xAP032 / xAP120 (pDB3994)	xAP093 / xAP033(pDB2305)
MSA DI + DIII	-	xAP032 / xAP087 (pDB3442)	xAP088 / xAP033 (pDB3442)
RSA DI + DIII	-	xAP032 / xAP085 (pDB3257)	xAP086 / xAP033 (pDB3257)

5 *pDB2305 is shown in Figure 6 and is also described in (disclosed in EP1788084, incorporated herein by reference)

Template DNA is shown in brackets e.g. (pDB2305)

Table 5. Oligonucleotide sequences of oligonucleotides mentioned in Table 4

Oligonucleotide	Sequence (5' – 3')
xAP032	ATGTCTGCCCCTAAGAAGATCGTC
xAP033	AACTTGCATCTAAACTCGACCTCTAC
xAP058	GTTTGATTAAATTCTGAGGCTCTTCCACGGCAGACGAAGCCTTCCCTTCATC
xAP059	GTGGAAGAGCCTCAGAATTTAATCAAAC
xAP075	GACCTGACCATTTGATGGAG
xAP085	GTTTAACCAAATTCTTTGGTTCATCAACAGCAGCAGAAATTAAAGATTTACC

	TTCC
xAP086	GTTGATGAACCAAAGAATTTGGTTAAAC
xAP087	GTTTAAACCAAATTCTTTGGTTCTTCTACAACCTGAAGAAACCAATGCTTTTT CC
xAP088	GTAGAAGAACCAAAGAATTTGGTTAAAC
xAP091	GTTTAAACCAAATTCTTTGGTTCATCAACCAATGGCTTGAATTCATCGAAAAC CTTAG
xAP092	CTTGATCAAGTTTTGTGGTTCTTCGACCAATGGTTGAAATTCATCCAAAAC TTAGC
xAP093	GTCGAAGAACCACAAAACCTTGATCAAG
xAP120	CTTGATCAAGTTTTGTGGTTCTTCGACCAAGTGCTTCAATTTGTCGAAAACA GTAG
xAP121	GTTGACGAACCACAAAACCTTGATCAAGAAG
xAP122	CTTCTTGATCAAGTTTTGTGGTTCGTCAACCAATGGCTTGAATTCATCGAAA ACCTTAG
xAP138	CCTAAGGCAGCTTGACTTG
xAP160	GTAGAAGAACCAAAGAATTTGGTTAAAC
xAP161	GTTTAAACCAAATTCTTTGGTTCTTCTACCAATGGCTTGAATTCATCGAAAA CCTTAG
xAP162	GCTTGATCAAGTTTTGTGGTTCTTCGACCAATGGTTGAAATTCAGCCAAAAC TGTAC
xAP253	AAGCTTATTATAAGCCTAAGGCAGCTTGACTTGCAGCAACAAGTTTAAAACC CTCCTCG
xAP260	GGATTTGCAGCAAAGATCTCCGCTGTGGAAGAGCCTCAGAATTTAATCAAAC AAAATTGTGAGCTTTTTG
xAP290	GTCAAGCTGCCTTAGGCTTAGATGCACACAAGAGTGAGGTTGCTCATC
xAP291	AATTAAGCTTATTAGGCAGACGAAGCCTTCCCTTCATCCCGAAGTTCATC
xAP293	AATTAAGCTTATTAAAGAGGTTTAAATTCATCGAACACTTTGGCATAGCATT CATG
xAP323	AATCTAAGCCTAAGGCAGCTTGGGAAGCAGCGAC
xAP324	CCAAGCTGCCTTAGGCTTAGTGGAAGAGCCTCAGAATTTAATCAAAC
xAP325	AGAATTAAGCTTATTATAAGCCTAAAGCAGCTTGACTTGCAGCAAC
xAP330	GAGAGCGCTATTTTTCTAACAAAGCATC
xAP332	GTGGAAGAGCCTCAGAATTTAATCAAAC
xAP333	CACACAGGAAACAGCTATGACCATG
xAP334	GTTTGATTAAATTCTGAGGCTCTCCACACCACCGGAACCACCAGAACCACC

	GGAACCACCGGATCCAC
xAP337	CGATGAGCAACCTCACTCTTGTGTGCATCACCACCGGAACCACCAGAACCAC CGGAACCACCGGATCCACC
xAP338	TGATGCACACAAGAGTGAGGTTGCTCATC
xAP339	GACGAAGCCTTCCCTTCATCCCGAAGTTCATCACCACCGGAACCACCAGAAC CACCAGGAACCACCGGATCCACC
xAP340	GATGAACTTCGGGATGAAGGGAAGGCTTCG
B01	GCATGCGGCCCGCCGAAGACCTACACAGGGCTTAAGGGC
B02	CCACGCGTCGTACGGGATTGCTGCTTATGAGGATA
B03	ACGCGTGAATTCAAAAAGGGAACCCGTATATTTTCAGC
B04	TATCGATAGTCTTCCTAATATACACATTTTAGCAGATGC
B05	GCATGCATACGCGTCACGCATGTGCCTCAGCGGCCGGCCGGCGCCGGGCCCC GGACCGCCTGCAGGCTCGAGTTAATTAAGTTTAAACGAATTCGCATGCAT
B06	ATGCATGCGAATTCGTTTAACTTAATTAAGTTCGAGCCTGCAGGCGGTCCGG GGCCCGGCGCCGGCCGGCCGCTGAGGCACATGCGTGACGCGTATGCATGC
B07	CTAGAGTTAATTAAGTTTCAATTCAATTCATC
B08	GCCTGAGTTTAAACGTTTTCTTTCCAATTTTT

(ii) Preparation of plasmids encoding HSA DI+DIII mutants and HSA DI+DIII mutants fusions

An initial HSA DI+DIII expression plasmid/yeast strain was generated by PCR and *in vivo* cloning using methods described herein for the mouse and rabbit DI +DIII expression constructs. Oligonucleotides xAP032/xAP058 (Table 5) and xAP059/xAP033 (Table 5) were used to generate PCR fragments 1 and 2, respectively, (using pDB2244 (described in WO 00/44772) as template DNA). The resulting strain was named 8822.

Total DNA (*i.e.* genomic and plasmid DNA) were extracted from yeast 8822 as follows. A 10µL sterile loop was used to scrape yeast cells from an agar plate and the cells were re-suspended in 200µL extraction buffer [50mM Tris-HCl (pH7.5), 10mM EDTA (pH8.0), 100mM NaCl, 2% w/v SDS] in a 1.5mL microfuge tube, before being heated at 80°C for 2 mins. The DNA extraction mixture was centrifuged at 13,000 rpm in a bench-top microfuge for 1 min before the supernatant was removed. Total DNA was precipitated with 3 volumes of ethanol and 0.1 volume of 3M sodium acetate (pH5.4) at -80°C for 10 mins. Precipitated DNA was centrifuged at 13,000 rpm in a bench top microfuge for 20mins, the supernatant was removed, and the DNA pellet was washed with 70% v/v ethanol. The pellet was air-dried briefly before the DNA was re-suspended in 100µL TE buffer.

To generate HSA DI + DIII mutant expression cassettes, oligonucleotide xAP075 and xAP138 (Table 5) were used to PCR-amplify a 2.1kb DNA fragment from the DNA prepared

from yeast strain 8822. The PCR fragment contained the 3' region of the *LEU2* marker, the *PRB1* promoter, DNA-encoding the fusion leader and HSA DI and DIII. The PCR fragment was digested with *NgoMIV/AvrII* and the 1.395 kb product was ligated into *NgoMIV/AvrII*-digested pDB3927 (described in WO 2010/092135), pDB4086, pDB4110 and pDB4184 and pDB4010 (all
 5 described in PCT/EP10/066572, incorporated herein by reference) to produce pDB4372 to pDB4375 and pDB4480, respectively. pDB4372 to pDB4375 and pDB4480 were digested with *NsiI/PvuI*, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions. Final expression plasmids were generated by *in vivo* cloning by co-transforming *S. cerevisiae* BXP10 cir⁰ with each *NsiI/PvuI*-digested DNA and *Acc65I/BamHI*-digested pDB3936.

Plasmids containing the expression cassettes for the production of IL-1ra (SEQ ID NO: 34) genetically fused to the C-terminus of HSA DI + DIII and mutants thereof, were prepared as follows. pDB2588 (described in PCT/EP10/066572, incorporated herein by reference) was digested with *Bsu36I/SphI* and the 705bp DNA fragment encoding the '3 region of the HSA DIII, a GS linker, and human IL1-RA (N84Q) and the 5' region of a modified *S. cerevisiae* *mADHt*,
 15 was purified using a Qiagen PCR-purification kit following the manufacturer's instructions. Purified fragments were ligated into *Bsu36I/SphI*-digested pDB4372, pDB4373, pDB4374, pDB4375 and pDB4480 to generate pDB4382, pDB4383, pDB4385, pDB4384 and pDB4481, respectively. Final expression plasmids were generated by *in vivo* cloning which involved digesting pDB4382 to pDB4385 and pDB4481 with *NsiI/PvuI*, purifying the DNA using a Qiagen
 20 PCR-Purification kit following the manufacturer's instructions, before being used, along with *Acc65I/BamHI*-digested pDB3936 to directly transform *S. cerevisiae* BXP10 cir⁰.

Plasmids containing the expression cassettes for the production of IL-1ra (N84Q) genetically fused to the N-terminus of HSA DI + DIII and HSA DI + DIII K573P were prepared using PCR and *in vivo* cloning using methods described above. Oligonucleotides used to
 25 generate the two PCR fragments are listed in Table 5. PCR fragment 1 was generated using oligonucleotides xAP330/xAP337 and plasmid pDB2590 as the template DNA. Plasmid pDB2590 is identical to pDB2305 (described in WO2006/013859) but contains DNA sequence-encoding the fusion leader, IL-1ra (N84Q), a GS linker followed by HSA. PCR fragment 1 contained 774bp of nucleotide sequence upstream of the *LEU2* ORF, *PRB1* promoter, and
 30 DNA-encoding the fusion leader sequence, IL1-RA (N84Q), a GS-linker, and the first 29bp of HSA DI. PCR fragment 2 (generated using oligonucleotides xAP338/xAP333 using either pDB4372 or pDB4374) contained DNA-encoding HSA DI + DIII (wt or K573P), *mADHt* and 2.031kb flanking sequence, homologous with nucleotide sequence in pDB3936. PCR-fragments were purified using a Qiagen PCR Purification kit following the manufacturer's instructions. Final
 35 expression plasmids were generated *in vivo* which involved co-transformation of *S. cerevisiae* BXP10 cir⁰ with PCR fragments 1 and 2, along with *Acc65I/BamHI*-digested pDB3936.

(iii) Preparation of plasmids encoding HSA DII + DIII mutants and fusions thereof

Expression plasmids for HSA DII+DIII and mutants thereof were generated as follows. pDB2202, containing a HSA DII + DIII expression cassette (*i.e.* *PRB1* promoter, DNA-encoding the fusion leader and HSA DII +DIII, and the *mADHt*), was digested with *NgoMIV*/*AvrII* and a 1.407kb fragment was purified using a Qiagen Gel Extraction kit (following the manufacturer's instructions). The *NgoMIV*/*AvrII* fragment was ligated into *NgoMIV*/*AvrII*-digested pDB3927 (described in WO 2010/092135), pDB4010, pDB4110 and pDB4184 (described in PCT/EP10/066572, incorporated herein by reference) to generate pDB4386, pDB4482, pDB4387, pDB4388, respectively. pDB4386 – pDB4388 and pDB4482 were digested with *NsiI*/*PvuI*, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions. Final expression plasmids were generated *in vivo* which involved co-transformation of *S. cerevisiae* BXP10 cir⁰ with each *NsiI*/*PvuI*-digested plasmid DNA, along with *Acc65I*/*Bam*HI-digested pDB3936.

Plasmids containing the expression cassettes for the production of IL-1ra genetically fused to the C-terminus of HSA DII + DIII and mutants thereof were prepared following the methods described for generating the IL-1ra genetically fused to the C-terminus of HSA DI + DIII mutants constructs. Plasmids generated were named pDB4483, pDB4485, pDB4486 and pDB4484. pDB4483 to pDB4486 *NsiI*/*PvuI*, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions. Final expression plasmids were generated *in vivo* as described for the HSA DI + DIII mutant fusion constructs.

Plasmids containing the expression cassettes for the production of IL-1ra genetically fused to the N-terminus of HSA DII + DIII and HSA DII + DIII K573P were prepared using PCR and *in vivo* cloning, as described for the generation of IL-1ra genetically fused to the N-terminus of HSA DI + DIII and HSA DI + DIII K573P. PCR fragment 1 was generated using oligonucleotides xAP330/xAP339 (Table 5) (pDB2590 was used as template DNA) and shared 32bp of nucleotide sequence homology with that encoding HSA DII (*i.e.* in pDB4386 and pDB4387). PCR fragment 2 was generated using oligonucleotides xAP340/xAP333 (Table 5 (using pDB4386 or pDB4387 as template DNA)) and shared 2.031kb homologous flanking sequence with nucleotide sequence in pDB3936. PCR fragments were purified using a Qiagen PCR-purification kit following the manufacturer's instructions. Final expression plasmids were generated *in vivo* as described for the HSA DI + DIII mutant fusion constructs.

(iv) Preparation of plasmid encoding HSA DIII and mutants and fusions thereof

PCR was used to generate a 649bp fragment encoding the HSA DIII, introduce a change at the codon corresponding to position 573 of the mature albumin protein sequence so

that a phenylalanine would be incorporated instead of a lysine and to tailor the fragment to allow it to be cloned into the expression plasmid pDB4284 (described in PCT/EP10/066572, incorporated herein by reference). Specifically, this was achieved using oligonucleotides xAP260 and xAP253 (Table 5) and the plasmid pDB3927 (described in WO 2010/092135) as
 5 template DNA. The PCR fragment was purified using a Qiagen PCR Purification kit (according to the manufactures instructions), digested with *BglII/Bsu36I*, before the digested DNA was purified using a Qiagen PCR Purification kit. The purified *BglII/Bsu36I* fragment was ligated into *BglII/Bsu36I*-digested pDB4284 to create pDB4460.

A series of expression plasmids encoding HSA DIII mutants was made by the
 10 replacement of the 666bp *AvrII/SphI* fragment from pDB4460 with analogous sequences from plasmids pDB3927 (described in WO 2010/092135), pDB3883, pDB4086, pDB4010, pDB4006, pDB4175, pDB4189, pDB4110, pDB4182, pDB4184, pDB4200, pDB4202 and pDB4094 (described in PCT/EP10/066572, incorporated herein by reference) that contain mutations within the nucleotide sequence encoding HSA DIII to create plasmids pDB4461 to pDB4473.

Plasmids containing the expression cassettes for the production of IL-1ra or scFv (FITC8) (SEQ ID NO: 35) genetically fused to the C-terminus of HSA DIII and DIII variants were prepared as follows. For the IL-1ra fusions, a 1.164kb *AvrII/SphI* fragment from plasmids pDB2588, pDB4288 and pDB4286 (described in PCT/EP10/066572, incorporated herein by reference) [containing the 3' end of the DNA- encoding HSA DIII or mutants thereof, and DNA-
 15 encoding a GS linker and IL-1ra (N84Q), and the 5' region of the *mADHt*) was obtained and subsequently ligated into *AvrII/SphI*-digested pDB4460. This resulted in the plasmids pDB4474 - pDB4476, respectively.

Plasmids containing expression cassettes for the production of the scFv fusions were made by the isolation of the 1.005kb *Bsu36I/SphI* fragment from plasmid pDB3008 (described in,
 25 Evans *et al.*, 2010. Protein Expression and Purification. **73**,113–124) (containing DNA-encoding the C-terminal region of HSA DIII, a GS linker and the scFv and the 5' region of the *mADHt*) and the subsequent cloning of this into *Bsu36I/SphI*-digested pDB4461, pDB4464 and pDB4468. This resulted in the plasmids pDB4477 - pDB4479.

Plasmids pDB4460 - pDB4479 were digested with *NsiI/PvuI* and purified using the Qiagen
 30 PCR Purification kit. Purified DNAs were mixed with *Acc65I/BamH*-digested pDB3936 and used to directly transform *S. cerevisiae* Strain B cir⁰.

S. cerevisiae strain B cir⁰ is a derivative of *S. cerevisiae* strain A cir⁰ (described in WO 2010/092135) and its construction is described below. Following chemical mutagenesis, a *S. cerevisiae* *ura3* mutant (*i.e.* auxotrophic for uracil) was isolated and named *S. cerevisiae* strain

A-ura3. *S. cerevisiae* strain A-ura3 was transformed with pDB3837. pDB3837 was generated as follows: 5' and 3' regions of the *HO* open reading frame were amplified by PCR from *S. cerevisiae* BY4741 (described in *Brachmann, et al.*, (1998) *Yeast* 14, 115) genomic DNA using the primers B01/BO2 and BO3/B04, respectively (Table 5). PCR-products were purified and restriction digested with the enzymes *NotI/MluI* (5' region fragment) and *MluI/ClaI* (3' region fragment). The digested fragments were purified and ligated in a three way ligation with *NotI/ClaI* digested pBST+ [described in Sleep, D. (2001). *Yeast* 18: 403-421]. A polylinker (generated from hybridised oligonucleotides B05 and BO6, Table 5) was cloned at the *MluI* site between the 5' and 3' regions to create pDB3343. The *S. cerevisiae URA3* gene was PCR-amplified from the plasmid YCplac50 [Described in Rose *et al.* (1987). *Gene* 60: 237-243] using primers B07 and B08 (Table 5). The *URA3* product was digested with *PacI* and *PmeI* and ligated into *PacI/PmeI*-digested pDB3343 create pDB3514. A 2.94kb *KpnI* fragment containing the *S. cerevisiae PDI1* gene was obtained from pDB2389 (Finnis *et al.* (2010). *Microbial Cell Factories* 9: 87), blunted using T4 Polymerase, before being ligated into *SfoI*-digested pDB3514. The resulting plasmid, pDB3837, contained two tandem copies of the *PDI1* fragment. pDB3837 was used to directly transform *S. cerevisiae* strain A-ura3 *cir*⁰ to generate strain B *cir*⁰. *S. cerevisiae* strain B contains the native copy of the *PDI1* gene, 2 copies of the *PDI1* gene at the *HO* locus and an additional copy of the *PDI1* gene at an unknown location in the genome of this strain.

(v) Preparation of plasmid encoding HSA DIII and mutants and fusions thereof

Plasmids containing expression cassettes for HSA DIII + DIII, and mutants thereof, were generated as follows. PCR, using oligonucleotides xAP323/xAP324 (Table 5), was used to amplify a 636bp DNA fragments from pDB4282, pDB4283 and pDB4284 (described in PCT/EP10/066572, incorporated herein by reference). PCR fragments were purified using a Qiagen PCR Purification kit following the manufacturer's instructions, digested with *BglII/HindIII*, purified using a Qiagen PCR-purification kit following the manufacturer's instructions, before being ligated into *BglII/HindIII*-digested pDB4284 (described in PCT/EP10/066572, incorporated herein by reference). Resulting plasmids were named pDB4489 - pDB4491.

A second round series of PCRs were performed to amplify DNA-encoding a second HSA DIII and variants thereof. Oligonucleotides xAP324/xAP325 (Table 5) were using to PCR-amplify DNA fragments encoding HSA DIII and variants thereof from pDB3927 (described in WO 2010/092135, incorporated herein by reference), pDB4010 and pDB4110 (described in PCT/EP10/066572, incorporated herein by reference). PCR fragments were purified using a Qiagen PCR-purification kit following the manufacturer's instructions, digested with *Bsu36I*, purified using a Qiagen PCR-purification kit following the manufacturer's instructions, before

being ligated into *Bsu*36I-digested pDB4489 - pDB4491. Resulting plasmids were named pDB4522 - pDB4526. pDB4522 - pDB4526 were digested with *Nsi*I/*Pvu*II, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions, before being used, along with *Acc*65I/*Bam*HI-digested pDB3936 to directly transform *S. cerevisiae* strain B cir⁰.

(vi) Preparation of plasmids encoding HSA DIII E492G + DIII E492G (*i.e.* a tandem domain III)

A plasmid containing a HSA DIII E492G + DIII E492G expression cassette was prepared as follows. A 1.488kb synthetic DNA fragment containing the 3' region of the *PRB1* promoter, DNA-encoding the FIVSI modified invertase leader and HSA DIII E492G + DIII E492G and 5' region of the *mADHt*, was generated by gene assembly (DNA2.0, USA). The synthetic *Pst*II/*Pac*I fragment was ligated into *Pst*II/*Pac*I-digested pDB4181 (described in PCT/EP10/066572, incorporated herein by reference) to generate pDB4112. pDB4112 was digested with *Bst*EII/*Bsr*BI, purified using a Qiagen PCR Purification kit following the manufacturer's instructions, before 100ng was mixed with 100ng *Acc*65I/*Bam*HI-digested pDB3936 (disclosed in WO 2010/092135, incorporated herein by reference) and used to directly transform *S. cerevisiae* strain B cir⁰.

Plasmids containing the expression cassettes for the production of IL-1ra or scFv (FITC8) genetically fused to the C-terminus of HSA DIII + DIII and mutants thereof were prepared as follows. pDB4522, pDB4523 and pDB4524 were digested with *Avr*II/*Sph*I and 7.564kb from each reaction were purified a Qiagen Gel Extraction kit following the manufacturer's instructions. pDB4474 to pDB4479 were digested with *Avr*II/*Sph*I and 1.164kb and 1.464kb fragments (containing DNA-encoding IL-1Ra and scFv, respectively) were purified using a Qiagen Gel Extraction kit following the manufacturer's instructions. Purified *Avr*II/*Sph*I fragments from pDB4474 to pDB4479 were ligated into *Avr*II/*Sph*I-digested pDB4522 to pDB4524 to produce pDB4527 – pDB4531 and pDB4533. pDB4527 – pDB4531 and pDB4533 were digested with *Nsi*I/*Pvu*II, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions, before being used, along with *Acc*65I/*Bam*HI-digested pDB3936 to directly transform *S. cerevisiae* strain B cir⁰.

(vii) Preparation of plasmids encoding DIII+DI and DIII+DII

Plasmids containing expression cassettes for HSA DIII+DI and DIII+DII were generated as follows. Oligonucleotide pairs xAP290/xAP291 and xAP292/xAP293 (Table 5) were used to PCR-amplify the DNA-encoding HSA DI (PCR fragment = 616bp) and HSA DII (PCR-produce = 628bp), respectively, using pDB3927 (described in WO 2010/092135) as template DNA. Both

PCR fragments were digested with *Bsu36I/HindIII*, purified using a Qiagen PCR-purification kit (following the manufacturer's instructions), then ligated into *Bsu36I/HindIII*-digested pDB4478 to produce pDB4487 and pDB4488. pDB4487 and pDB4488 were digested with *NsiI/PvuI*, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions, before being used, along with *Acc65I/BamHI*-digested pDB3936 to directly transform *S. cerevisiae* strain B cir⁰.

(viii) Preparation of plasmids encoding DIII+ DIII + DIII

The expression construct for HSA DIII+ DIII + DIII (*i.e.* triple domain III) was prepared as follows: A 615bp *Bsu36I* DNA fragment, containing DNA-encoding HSA DIII, was obtained from pDB4527 and ligated into *Bsu36I*-digested pDB4522 to create pDB4534. pDB4534 was digested with *NsiI/PvuI*, DNA was purified using a Qiagen PCR-Purification kit following the manufacturer's instructions, before being used, along with *Acc65I/BamHI*-digested pDB3936 to directly transform *S. cerevisiae* strain B cir⁰.

(d) Transformation of *S. cerevisiae*

S. cerevisiae strains were streaked on to YEPD plates (1% (w/v) yeast extract, 2% (w/v) Bactopeptone, 2% (w/v) glucose), 1.5% agar) and allowed to grow for 4 days at 30°C prior to transformation. One µg of whole plasmid (*i.e.* circular plasmid) or, for gap repair, *BstEII/BsrBI*- or *NsiI/PvuI*-digested HSA variant or HSA variant fusion containing plasmid and *Acc65I/BamHI* digested pDB3936 (100 ng) was used (at equimolar concentrations) to transform *S. cerevisiae* using a Sigma Yeast Transformation kit using a modified lithium acetate method (Sigma yeast transformation kit, YEAST-1, protocol 2; Ito *et al.* (1983) *J. Bacteriol.*, 153, 16; Elble, (1992) *Biotechniques*, 13, 18). The protocol was amended slightly by incubating the transformation at room temperature for up to 4 h prior to heat shock. Following heat shock, the cells were briefly centrifuged before being re-suspended in 200µl 1M sorbitol then spread over BMMD agar plates, the composition of BMMD is described by Sleep *et al.*, (2001), *Yeast*, 18, 403. Plates were incubated at 30°C for 4 days before individual colonies were patched on to fresh BMMD plates.

Stocks were prepared for each yeast strain as follows: BMMD broth was inoculated with a heavy loop of each yeast patch and grown for 24h at 30°C with orbital shaking at 200rpm. Cells were harvested by centrifugation at 1900 × g for 5 min in a Sorval RT600 centrifuge, 15mL supernatant was removed and replaced by trehalose 40% (w/v). The cells were re-suspended and transferred to cyrovials (1mL) for storage at -80°C.

(e) Shake flask growth of *S. cerevisiae*

BMMD (recipe 0.17% (w/v) yeast nitrogen base without amino acid and ammonium sulphate (Difco), 37.8mM ammonium sulphate, 29mM citric acid, 142mM disodium hydrogen orthophosphate dehydrate pH6.5, 2% (w/v) glucose) media (10mL) was inoculated with each yeast strain and grown for 24h at 30°C with orbital shaking at 200rpm. An aliquot of each starter culture (4mL) was used to inoculate 2 × 200mL BMMD media and grown for 96h at 30°C with orbital shaking at 200rpm. Cells were harvested by filtration through 0.2µm vacuum filter membranes (Stericup, Millipore) including a GF-D pre-filter (Whatman) and the supernatant retained for purification.

(f) Primary concentration

Retained culture supernatant was concentrated using Tangential Flow Filtration using a Pall Filtron LV system fitted with a Omega 10KD (0.093sq.m2) filter (LV Centramate™ cassette, Pall Filtron) with a transmembrane pressure of 20psi and a recirculation rate of 180mL.min⁻¹.

(g) Purification of albumin derivatives and fusions thereof from shake flask

Albumin derivatives, variants and fusions thereof were purified from shake flask (either culture supernatant or concentrated culture supernatant) using a single chromatographic step using an albumin affinity matrix (AlbuPure™ - ProMetic BioSciences, Inc.). Chromatography was performed at a constant linear velocity of 240cm/h throughout. Culture supernatant was applied to a 6cm bed height, 2.0mL packed bed pre-equilibrated with 50mM sodium acetate pH5.3. Following load the column was washed with 10 column volume (CV) of equilibration buffer, then 50mM ammonium acetate pH8.0 (10CV). Product was eluted with either 50mM ammonium acetate 10mM octanoate pH8.0, 50mM Ammonium Acetate 30mM Sodium Octanoate pH8.0, 50mM Ammonium Acetate 100mM Sodium Octanoate pH8.0 or 200mM Potassium thiocyanate. The column was cleaned with 0.5M NaOH (3cv) and 20mM NaOH (3.5cv). Eluate fraction from each albumin variant were concentrated and diafiltered against 10 volumes of Tris buffered saline (25mM Tris, 150mM NaCl, 2mM KCl, pH7.4) using Vivaspin20 10,000 MWCO PES with optional diafiltration cups (Sartorius). Purified albumin variants were quantified by GP-HPLC as described below (section (j)).

(h) 2 L and 10 L Fermentations**- 10 L Fermentation**

Transformants were cultivated as fed-batch fermentations, carried out in a 10 L Sartorius Biostat C fermenter, at 30°C. The pH was monitored and adjusted by the addition of ammonia

or sulphuric acid, as appropriate. The ammonia also provided the nitrogen source for the cultures. The level of dissolved oxygen was monitored and linked to the stirrer speed, to maintain the level at >20% of saturation. Inocula were grown in shake flasks in buffered minimal media. For the batch-phase, the culture was inoculated into fermenter media (approximately 50% of the fermenter volume) containing 2% (w/v) sucrose. The feed stage was automatically triggered by a sharp rise in the level of dissolved oxygen. Sucrose was kept at growth-limiting concentrations by controlling the rate of feed to a set nominal growth rate. The feed consisted of fermentation media containing 50% (w/v) sucrose, all essentially as described by Collins. (Collins, S.H., (1990) Production of secreted proteins in yeast, in: T.J.R. Harris (Ed.) Protein production by biotechnology, Elsevier, London, pp. 61-77).

- 2 L Fermentation

Transformants were cultivated as fed-batch fermentations. Fed-batch fermentations were carried out in a 2L Pierre Guerin Tryton fermenter, at 30°C. The pH was monitored and adjusted by the addition of ammonia or sulphuric acid, as appropriate. The ammonia also provided the nitrogen source for the cultures. The level of dissolved oxygen was monitored and linked to the stirrer speed, to maintain the level at >20% of saturation. Inocula were grown in shake flasks in buffered minimal media. For the batch-phase, the culture was inoculated into fermenter media (approximately 50% of the fermenter volume) containing 2% (w/v) sucrose. The feed stage was automatically triggered by a sharp decrease in the level of oxygen consumption. Sucrose was kept at growth-limiting concentrations by controlling the rate of feed to a set nominal growth rate. The feed consisted of fermentation media containing 50% (w/v) sucrose, all essentially as described by Collins. (Collins, S.H., (1990) Production of secreted proteins in yeast, in: T.J.R. Harris (Ed.) Protein production by biotechnology, Elsevier, London, pp. 61-77).

(i) Purification of albumin derivatives and fusions thereof from fermentation

Albumin derivatives and fusions therein were purified from high cell density fed batch fermentation supernatants after separation by centrifugation, using a Sorvall RC 3C centrifuge (DuPont). Culture supernatant was chromatographed through an 11cm bed height column 22mL packed bed packed with a custom synthesised albumin affinity matrix (AlbuPure™ - ProMetic BioSciences, Inc.) as described above. Product was eluted using elution buffers describe above at a flow rate of 120cm/h. The eluate fraction(s) was analysed by GP-HPLC (below) and reducing SDS PAGE for purity. Eluate fraction from each albumin variant were evaluated by GP-HPLC (as described below) and reducing SDS-PAGE and if required further separated by preparative gel filtration performed using a 90cm bed height column 488mL packed bed, packed with Sephacryl S200 (GE Healthcare) and run in Tris buffered saline

(25mM Tris, 150mM NaCl, 2mM KCl, pH7.4 at 4 mL.min⁻¹. Eluate fractions from either AlbuPure™ or AlbuPure™ and Sephacryl S200 chromatography were concentrated using either Vivaspin20 10,000 MWCO PES with optional diafiltration cups (Sartorius) or tangential flow filtration using a Pall Filtron LV system fitted with a Omega 10KD (0.093sq.m2) filter (LV Centramate™ cassette, Pall Filtron) with a transmembrane pressure of 20psi and a recirculation rate of 180mL.min⁻¹. For those eluates derived only from AlbuPure™ chromatography, after concentration, diafiltration was then performed against 10 volumes of Tris buffered saline (25mM Tris, 150mM NaCl, 2mM KCl, pH7.4) Purified albumin variants were quantified by GP-HPLC as described below.

All proteins to be assayed for receptor (shFcRn) binding properties and or other analysis were quantified by GP-HPLC as described below corrected for their relative extinction coefficients.

(j) Quantitative analysis of albumin derivatives, variants, fusions and conjugates thereof by GP-HPLC

Purified albumin derivatives, variants, fusions and conjugates thereof were analysed by GP-HPLC and quantification as follows. Injections of 25µL were made onto a 7.8mm id x 300mm length TSK G3000SWXL column (Tosoh Bioscience), with a 6.0mm id x 40mm length TSK SW guard column (Tosoh Bioscience). Samples were chromatographed in 25mM sodium phosphate, 100mM sodium sulphate, 0.05% (w/v) sodium azide, pH 7.0 at 1mL/min, Samples were quantified by UV detection at 280nm, by peak area, relative to a recombinant human albumin standard of known concentration (10mg/mL) and corrected for their relative extinction coefficients.

(k) Conjugation of horse radish peroxidase (HRP) to albumin and derivatives and variants thereof

Albumin and derivatives and variants thereof were purified as described above (sections (g) and (i)), concentrated and subjected to buffer exchange in Tris Buffer Saline (TBS), pH 7.2-7.3.

HRP was conjugated to the molecules by incubating with 2mg/mL EZ-Link® Maleimide Activated Horseradish Peroxidase (HRP, Thermo Scientific) to react with the free sulphhydryl in Domain I. The HRP was dissolved in Phosphate Buffer Saline (PBS), pH 6.5 and incubation provided approximately 2-fold molar excess of HRP relative to the albumin or variant or derivative thereof. This mixture was incubated at 4 °C, for at least 24 hours. The reaction mixtures were then analysed using GP-HPLC to confirm that conjugation had taken place. GP-HPLC analysis is described above (section (j)).

To separate unconjugated species (DI+DIII, DI+DIII+DIII or domain variants and unreacted HRP) from the corresponding conjugated species, the samples were first concentrated (Vivaspin20, 10,000 MWCO PES, Sartorius), and then individually applied to a Tricorn Superdex™ 200, 10/300 GL column (GE Healthcare), and run at a flow rate of 45cm/hr in TBS. The elution peak was fractionated and analysed by GP-HPLC. Fractions containing the conjugated species were pooled, concentrated and subsequently analysed by GP-HPLC and non-reducing SDS PAGE to demonstrate conjugated species.

(I) Conjugation of Fluorescein-5-Maleimide (F5M) to albumin and derivatives and variants thereof

Fluorescein-5-Maleimide, Thermo Scientific (F5M) was dissolved in dimethylformamide, to give a final concentration of 12.5mg/mL. This was then further diluted into 18mls of PBS, pH adjusted to approximately pH 6.5. HSA DI+DIII variants were added to give an approximate 20 fold final molar excess of F5M, in the final mixture. These samples were then incubated and allowed to conjugate overnight at 4°C, in the dark, to allow the maleimide groups on the F5M to react with predominantly the free sulfhydryl, present in the albumin species.

Following overnight incubation aliquots of the reaction mixtures were extensively diafiltered against TBS to remove unconjugated F5M, (Vivaspin20, 10,000 MWCO PES, Sartorius). Conjugation was confirmed by ultraviolet visualization of conjugated Fluorescein::DI+DIII, and variants therein, using standard SDS-PAGE

Example 1. Construction of albumin derivatives or variants

In order to demonstrate the binding of albumin derivatives or variants or fragments, the following constructs were made starting from HSA. The positions cited under the headline “mutation/construct” refer to positions in SEQ ID NO: 31.

Table 6: Albumin derivatives and variants

Sample Details	SEQ ID NO:	Concentration (mg/mL)	Mutation/construct (amino acid residues relative to mature HSA)
a) Domains I+II	20	82.0	1-387
b) Domains II+III	21	33.0	183-585
c) Domains I+III	22	62.4	1-194+381-585
d) Domain III	23	89.7	381-585
e) 2 x Domain III	24	35.5	381-585**

** The nucleotide sequence encoding the N-terminal Domain III was codon optimised, the nucleotide sequence encoding the C-terminal Domain III was non optimised. This was done to

prevent recombination of the nucleotide sequences encoding the identical Domain III amino acid sequences and therefore ensures that a double-Domain III molecule was generated instead of a single Domain III which could arise if the nucleic sequences had recombined.

5 Example 2. Binding of albumin variants to soluble FcRn (shFcRn)

Three established FcRn binding assays were used, ELISA, SPR and a Dynabead binding assay. There are major differences between the assays:

In the ELISA system HSA is coated directly in wells and soluble hFcRn (shFcRn)-GST is added in solution whereas in the SPR assay shFcRn-GST is immobilized to a CM5 chip and HSA injected in solution. The pH can be varied in both systems.

The FcRn Dynabead binding assay is based on site-specific biotinylated shFcRn captured on streptavidin coupled beads. GST-tagged HSA is added in solution and binding is detected using a chicken HRP-conjugated anti-GST Ab.

In addition, competitive binding was measured by injecting shFcRn (50 nM) alone or together with different concentrations of HSA (55-500 nM), HSA DI-DII (500+1500nM), HSA DII-DIII (500+1500nM), HSA DI-DIII (500+1500nM), HSA DIII (500+1500nM) and HSA DIII-DIII (500+1500nM) over immobilized HSA (~2600 RU). Injections were performed at 25°C and at a flow rate of 50µl/min. The variants of Example 2 were analysed for FcRn binding using ELISA (results shown in Figure 1), and using SPR (results shown in Figure 2 and Table 7) and competitive SPR based assay (the results are shown in Figure 3). The SPR and competitive SPR based assays were carried out using shFcRn-GST.

The kinetics of the shFcRn interaction with HSA domains were calculated and shown below (Table 7).

Table 7: Kinetics of the shFcRn-GST interaction with HSA domains

Albumin variant ^a	SEQ ID NO:	ka (10 ³ /Ms)	kd (10 ⁻³ /s)	KD ^b (µM)	KD Req ^c (µM)
HSA wt	31	3.2±0.2	15.5±2.5	4.8	5.4
HSA DII-DIII	21	0.7	7.1	9.8	ND
HSA DI-DIII	22	2.6±0.3	18.3±0.2	7.0±0.2	ND
HSA DIII wt	23	ND	ND	ND	17.6±2.3

a: titrated amounts of albumin or DIII derivatives or variants were injected over immobilized shFcRn (~1500 RU) at pH6.0.

b: The kinetic rate constants were obtained using a simple first-order (1:1) bimolecular interaction model.

c: The steady state affinity constant was obtained using an equilibrium (Req) binding model supplied by the BIAevaluation 4.1 software. The kinetic values represent the average of triplicates.

d: Not determined (ND).

5

The SPR results show that: DI+III, DII+III, DIII, double DIII and full-length albumin binds shFcRn in a pH-dependent manner (Figure 2) In contrast, ELISA data showed that DIII wt, when immobilized, does not interact with shFcRn. The DIII-shFcRn association/dissociation rates are fast, and thus, kinetic values could not be determined directly, however calculation of the steady state affinity constant shows DIII binds less shFcRn than does full-length HSA and there is a 3-fold reduction in binding affinity. This data suggests that other parts of HSA are important for optimal binding to shFcRn of domain III, which is further supported when analyzing DI-DIII and DII-DIII. DII-DIII binds shFcRn, although the binding affinity is reduced compare with that measured for full-length albumin. DI-DIII also binds shFcRn with a higher affinity than DII-DIII, both have an affinity higher than for domain III alone again supporting the observation that other parts of HSA are important for optimal binding to shFcRn receptor. Competitive SPR assay shows that the double domain III construct competes for wt albumin in the assay more effectively than DI+III, DII+III and DIII but not as effectively as wt albumin, *i.e.* HSA.

20 **Example 3. Binding of domain swap derivatives to shFcRn**

The following domain swap derivatives were generated following the methods section (above):

25	HSA 1/2-RSA 3	: HSA (human serum albumin) domain I and II and RSA (rabbit serum albumin) domain III
	RSA 1/2 – HSA3	: RSA domain I and II and HSA domain III
	SSA 1/2 – HSA3	: SSA (sheep serum albumin) domain I and II and HSA domain III
	HSA 1/2 - SSA3	: HSA domain I and II and SSA domain III
	HSA 1/2 – MSA3	: HSA domain I and II and MSA (mouse serum albumin) domain III
	MSA 1/2 – HSA3	: MSA domain I and II and HSA domain III.

30

In the examples, the domains used are shown in Table 8:

Table 8: Domains of human, rabbit, sheep and mouse serum albumins

Albumin		Amino acid residues		
Abbreviation	Source	Domain I	Domain II	Domain III
HSA	Human (SEQ ID NO: 31)	1 – 194	195 – 380	381 – 585
RSA	Rabbit (SEQ ID NO: 14)	1 – 194	195 – 380	381 – 584
SSA	Sheep (SEQ ID NO: 16)	1 – 194	195 – 380	381 – 583
MSA	Mouse (SEQ ID NO: 9)	1 – 194	195 – 380	381 – 584

These are further shown in the alignment of Fig. 5.

The derivatives or variants of Example 3 were analysed for shFcRn binding (shFcRn-GST) using SPR and the results shown below (Table 9).

Table 9: Albumin derivatives and characteristics

Albumin derivative or variant ^a	Source of Domain			SEQ ID NO:	k _a (10 ³ /Ms)	k _d (10 ⁻³ /s)	K ^D ^b (μM)	K ^D Req ^c (μM)
	I	II	III					
HSA	Human	Human	Human	31	3.2±0.2	15.5±2.5	4.8	5.4
MSA	Mouse	Mouse	Mouse	9	2.3±0.0	4.2±0.0	1.8	ND
RSA	Rabbit	Rabbit	Rabbit	14	1.9±0.3	1.7±0.1	0.9	ND
HSA1/2 - RSA3	Human	Human	Rabbit	25	2.2±0.1	1.8±0.0	0.8	ND
RSA1/2 - HSA3	Rabbit	Rabbit	Human	26	1.2±0.1	14.5±0.5	12.1	ND
SSA	Sheep	Sheep	Sheep	16	ND	ND	ND	ND
SSA1/2 - HSA3	Sheep	Sheep	Human	27	3.5±0.0	8.2±0.2	2.3	ND
HSA1/2 – SSA3	Human	Human	Sheep	28	ND	ND	ND	ND
HSA1/2 – MSA3	Human	Human	Mouse	29	6.2±0.0	9.7±0.0	0.2	ND
MSA1/2 – HSA3	Mouse	Mouse	Human	30	ND	ND	ND	ND

a: Dilutions of HSA derivatives or variants were injected over immobilized shFcRn (~1500 RU) at pH6.0.

b: The kinetic rate constants were obtained using a simple first-order (1:1) bimolecular interaction model.

c: The steady state affinity constant was obtained using an equilibrium (Req) binding model supplied by the BIAevaluation 4.1 software. The kinetic values represent the average of triplicates.

d: Not determined (ND) (*i.e.* K^D not obtained).

SSA (SEQ ID NO: 16) and the derivative comprising SSA domain III (SEQ ID NO: 28), and the MSA1/2–HSA3 (SEQ ID NO: 30) did not bind FcRn in this experiment.

HSA DI+DII + RSA DIII and HSA DI+DII + MSA DIII had increased affinity for shFcRn compared to HSA, MSA and RSA. The data also shows that various species albumin DI+DII can modulate the affinity (*i.e.* increase or decrease) of albumin DIII from a different species.

Example 4. Binding of the HSA derivative DI + DIII and fusions thereof to shFcRn

The HSA derivative DI + DIII and fusions thereof (Table 10) were prepared according to the methods section (above).

SPR assays were performed using a Biacore X and Biacore 3000 instrument (G E Healthcare). CM5 chips were coupled with shFcRn-HIS (GeneArt) *via* amine coupling chemistry as per manufacturer's instructions (1600 – 2500RU). The coupling was performed by diluting shFcRn in 10mM sodium acetate pH4.5 (G E Healthcare). Phosphate buffer (67mM phosphate buffer, 0.15M NaCl, 0.005% Tween 20 at pH5.5 ±0.25) was used as running buffer and dilution buffer. Regeneration was performed using HBS-EP buffer pH7.4. For binding to immobilised shFcRn, HSA derivatives or variants were injected for 90s over the active cell surface at a constant flow rate (30µl/min) at 25 °C. For binding assays at pH7.4, HBS-EP (GE Healthcare) was used as dilution and running buffer. In all experiments, data were zero adjusted and the reference cell subtracted. For data interpretation and kinetic value determination, Biaevaluation software was used for kinetic binding modelling.

The kinetic values of shFcRn binding the molecules is provided in Table 10.

Table 10: Kinetic values of shFcRn binding HSA Domain I + III (unfused and fused)

Molecule	KD (µM)
wt DI + DIII	3.0
DI + DIII K500A	ND
DI + DIII K573P	0.86
DI + DIII K573Y	0.45
DI + DIII D550N	8.4
wt DI + DIII::GSL::IL-1ra	5.5
DI + DIII K500A ::GSL::IL-1ra	ND
DI + DIII K573P ::GSL::IL-1ra	0.42
DI + DIII K573Y ::GSL::IL-1ra	0.26
DI + DIII D550N ::GSL::IL-1ra	11

ND: not determined due to weak binding

wt DI + DIII and derivatives thereof bind to shFcRn in a pH-dependent manner (data not shown), *i.e.* binding was detected at pH5.5 but not at pH7.4. The results show that the hierarchy of binding of the DI+DIII derivatives and fusions, thereof to shFcRn was K573Y > K573P > wt > D550N (highest to lowest affinity). Weak binding was detected between DI+DIII K500A (fused and unfused to IL1Ra) and shFcRn at either pH5.5 or pH 7.4 .

Example 5. Binding of HSA derivative DII + DIII and fusions thereof to shFcRn

HSA DII+DIII and fusions thereof (Table 11) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

Table 11: Kinetic values of shFcRn-HIS binding HSA DII + DIII (unfused and fused)

Molecule	KD (μM)
wt DII + DIII	6.8
DII + DIII K573P	0.48
DII + DIII K573Y	0.47
DII + DIII D550N	7.3
wt DII + DIII::GSL::IL-1ra	5.1
DII + DIII K573Y::GSL::IL-1ra	0.5
DII + DIII D550N::GSL::IL-1ra	6.0
DII + DIII K573P::GSL::IL-1ra	0.98

All of the HSA DII + DIII derivatives and fusions thereof interact with shFcRn in a pH-dependent manner (data not shown), *i.e.* binding was detected at pH5.5 but not at pH7.4. The hierarchy of binding of HSA DI + DIII derivatives, fused and unfused, is identical to that described for HSA DI + DIII derivatives and fusions thereof (Example 4), *i.e.* K573Y > K573P > wt > D550N (highest to lowest affinity for shFcRn).

Example 6. Binding of the HSA derivative DIII and derivatives thereof to shFcRn

Unfused DIII derivatives (Table 12a) were prepared according to the methods section (above). SPR analysis and interpretation was carried out according to Example 4.

Table 12a: Kinetic values of shFcRn-HIS binding HSA DIII

Molecule	KD (μ M)
wt DIII	12
DIII K573F	0.42
DIII K500A	21.4
DIII D550N	5.9
DIII K573A	1.1
DIII K573P	0.22
DIII K573Y	0.09
DIII E492G/N503K	14.1
DIII E492G	4.9

All of the HSA DIII derivatives interact with shFcRn in a pH-dependent manner (data not shown), *i.e.* binding was detected at pH5.5 but not at pH7.4. The binding hierarchy to shFcRn is DIII K573Y, DIII K573P, DIII K573F, DIII K573A, DIII E492G, DIII D550N, wt DIII, DIII E492/N503K, DIII K500A (highest to lowest affinity for shFcRn).

DIII derivatives that could not have kinetic values determined, were analysed for binding response (RUs) compared to wt DIII. No binding was detected at pH7.4, only pH5.5. The results are shown in Table 12b. A higher RU value indicates tighter binding. Therefore, compared to wt HSA, all of the mutants tested in Table 12b show weaker binding to shFcRn. wt HSA DIII and derivatives thereof bind to shFcRn in a pH-dependent manner. Representative sensorgrams for HSA DIII K573D, DIII K573H, DIII K573N, DIII K573W and DIII Q580K are shown in Figure 8.

Table 12b: Binding response values of shFcRn interacting with HSA DIII

Molecule	Binding response (RUs)
wt HSA	34.2
wt DIII	7.0
DIII K573D	7.2
DIII K573H	13.3
DIII K573W	16.5
DIII K573N	12.2
DIII Q580K	11.5

Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity.

Example 7. Binding of HSA derivatives genetically fused to IL-1ra or scFv, to shFcRn

Molecules (Table 13) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

- 5 Table 13: Kinetic values of shFcRn-HIS binding HSA DIII derivatives genetically fused to IL-1ra or an scFv

Molecule	KD (μM)
wt DIII::GSL::IL-1ra	10.9
DIII K573P::GSL::IL-1ra	3.0
DIII D550N::GSL::IL-1ra	1.8
wt DIII::GSL::scFv	6.9
DIII K573P::GSL::scFv	1.4
DIII D550N::GSL::scFv	5.9

- Results show that the hierarchy of binding of HSA DIII derivatives genetically fused to IL-1ra to shFcRn is D550N > K573P > wt. In contrast, the hierarchy of binding of HSA DIII derivatives genetically fused to an scFv to shFcRn is K573P > D550N > wt.
- 10

Example 8: Conjugation of horseradish peroxidase to albumin and to albumin derivatives

- HRP was conjugated to albumin derivatives to form albumin variants (Table 14) according to the methods (above). Molecules were analysed by GP-HPLC (results not shown) and non-reducing SDS-PAGE (Figure 9) to confirm that the molecules were of the expected molecular weight and therefore that conjugation had taken place to produce the desired molecules. SPR analysis and interpretation were carried out according to Example 4.
- 15

- Table 14: Binding analysis of shFcRn-HIS interacting with HSA DI + DIII and derivatives thereof conjugated to HRP
- 20

Molecule	Response (RUs)
wt HSA (un-conjugated)	178
wt DI + DIII-HRP	58
DI + DIII K573P -HRP	213
DI + DIII K500A -HRP	ND

ND – Not determined due to weak binding. Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity

Figure 10 confirms an increased binding response for the HSA DI + DIII K573P-HRP variant and shFcRn interaction compared to the binding response for wt HSA DI + DIII and wild-type HSA. Furthermore, Figure 10 shows that the binding interaction of DI + DIII K500A-HRP and shFcRn is reduced compared to wt HSA DI + DIII and wild-type HSA

- 5 DI + DIII wt and variants thereof bind to shFcRn in a pH-dependent manner, *i.e.* binding was detected at pH 5.5 but not at pH7.4. Conjugated DI + DIII variants show the same affinity modulation seen in unconjugated DI + DIII (Example 4).

10 **Example 9: Binding analysis of shFcRn interacting with IL-1ra genetically fused to the N-terminus of HSA DII + DIII**

IL-1ra genetically fused to HSA DII+DIII and HSA DII+DIII K573P (Table 15) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

- 15 Table 15: Binding analysis of shFcRn-HIS interacting with IL-1ra N-terminally fused to HSA DII + DIII

Molecule	Response (RUs)
wt HSA	162
IL-1ra::GSL::DII DIII	145
IL-1ra::GSL::K573P DII DIII	246

Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity

- 20 IL-1ra N-terminally fused to HSA DII + DIII and IL-1ra N-terminally fused to K573P DII DIII bind to shFcRn in a pH-dependent manner, *i.e.* binding was detected at pH 5.5 but not at pH7.4. Figure 11 shows that an N-terminal fusion to DII + DIII lengthens the dissociation time without interfering with the binding of the molecules to shFcRn. Results show an increased binding response between shFcRn and the K573P variant compared to the wild type HSA DII +
25 DIII and HSA.

Example 10: Binding analysis of shFcRn interaction with molecules which are fusions of tandem repeats of DIII and derivatives thereof

- 30 HSA derivatives, and fusions thereof (Table 16) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

Table 16: Binding analysis of shFcRn-HIS interacting with fusions of tandem repeats of DIII

Molecule	Binding response (RUs)
DIII wt + DIII wt::GSL::IL-1ra	30.1
DIII D550N + DIII D550N ::GSL::IL-1ra	82.3
DIII K573P + DIII K573P ::GSL::IL-1ra	48.0
DIII wt + DIII wt::GSL::scFv	151.4
DIII D550N + DIII D550N ::GSL::scFv	167.5
DIII K573P + DIII K573P ::GSL::scFv	181.6

Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity

5 Tandem DIII fusions bind to shFcRn in a pH-dependent manner (*i.e.* binding at pH 5.5 but not at pH 7.4). Binding responses (Table 16) show for the tandem DIII fusions the following order:

(i) IL-1ra fusions: DIII D550N + DIII D550N-IL-1ra, DIII K573P + DIII K573P-IL-1ra, wt DIII-IL-1ra (highest to lowest affinity);

10 (ii) scFv fusions: DIII K573P + DIII K573P-scFv, D550N + DIII D550N-scFv wt DIII-scFv (highest to lowest affinity).

Example 11: Conjugation of Fluorescein-5-Maleimide (F5M) to DI+DIII wt and derivatives thereof.

15 Conjugated HSA derivatives and variants thereof (Table 17) were prepared according to the methods section (above). Molecules were analysed by GP-HPLC (results not shown) and non-reducing SDS-PAGE (Figure 16). Conjugation was confirmed by UV visualisation of conjugated DI + DIII wt and variants. SPR analysis and interpretation were carried out according to Example 4.

20 Table 17: Binding analysis of shFcRn-HIS interacting with HSA DI + DIII and variants thereof conjugated to F5M.

Molecule	Response (RUs)
wt HSA	63.8
DI + DIII wt -F5M	6.5
DI + DIII- K573P F5M	53.5
DI + DIII- K500A F5M	ND

ND – Not determined due to weak binding. Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity

Figure 17 confirms an increased binding response for the HSA DI + DIII K573P-F5M variant and shFcRn interaction compared to the binding response for HSA DI + DIII-F5M but not wild-type HSA. Furthermore, Figure 17 shows that the binding interaction of DI + DIII K500A-F5M and shFcRn is significantly reduced compared to HSA DI + DIII K573P-F5M and wild-type HSA

DI + DIII wt and variants thereof bind to shFcRn in a pH-dependent manner, *i.e.* binding was detected at pH 5.5 but not at pH7.4. Conjugated DI + DIII variants show the same affinity modulation seen in unconjugated DI + DIII variants and full length HSA (Example 4).

Example 12: Conjugation of Fluorescein-5-Maleimide (F5M) to DI+DIII+DIII wt.

Conjugated of the HSA derivative (Table 18) was prepared according to the methods section (above). The molecule was analysed by GP-HPLC (results not shown) and non-reducing SDS-PAGE (Figure 16). Conjugation was confirmed by UV visualisation of conjugated DI + DIII wt +DIII wt . SPR analysis and interpretation was carried out according to Example 4.

Table 18 Binding analysis of shFcRn-HIS interacting with HSA DI + DIII wt + DIII wt conjugated to F5M.

Molecule	Response (RUs)
wt HSA	62.8
DI + DIII wt-F5M	6.5
DI + DIII wt + DIII wt-F5M	31.8

Presented binding responses (RU values) represent relative absolute values recorded at the point the injection has stopped and maximum complexes have formed during the injection period. A higher RU value equates to higher affinity

Figure 18 confirms an increased binding response for the HSA DI + DIII wt + DIII wt - F5M variant and shFcRn interaction compared to the binding response for HSA DI + DIII-F5M but not wt HSA..

Conjugated DI + DIII wt and DI +DIII wt + DIII wt bind to shFcRn in a pH-dependent manner, *i.e.* binding was detected at pH 5.5 but not at pH7.4.

Example 13: Binding analysis of shFcRn interaction with the HSA derivatives DIII + DI and DI + DIII.

HSA derivatives (Table 19) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

Table 19: Binding analysis of shFcRn-HIS interacting with HSA DIII + DI and DI + DIII

Molecule	KD (μM)
DIII wt + DI	8.4
DI + DIII wt	3.0

Table 19 shows that the HSA derivative DIII + DI has reduced affinity for shFcRn compared to HSA DI + DIII.

5

Example 14: Binding analysis of shFcRn-GST interaction with albumin derivatives DI + DIII compared to wild-type albumin.

Species wt albumins and DI + DIII derivatives thereof (Table 20) were prepared according to the methods section (above). SPR analysis and interpretation were carried out according to Example 4.

10

Table 20: Binding analysis of shFcRn–GST interacting with species albumins and DI + DIII derivatives thereof

Molecule ^a	Ka ($10^3/\text{Ms}$)	kd ($10^{-3}/\text{s}$)	KD ^b (μM)
HSA wt	3.2 ± 0.2	15.5 ± 2.5	4.8
MSA wt	3.8 ± 0.0	3.1 ± 0.1	0.8 ± 0.2
RSA wt	1.9 ± 0.3	1.7 ± 0.1	0.9
HSA DI-DIII	2.6 ± 0.3	18.3 ± 0.2	7.0 ± 0.2
RSA DI-DIII	4.8 ± 0.0	3.1 ± 0.1	0.6 ± 0.0
MSA DI-DIII	5.6 ± 0.1	2.5 ± 0.0	0.4 ± 0.0

a: titrated amounts of albumins or DI +DIII derivatives thereof were injected over immobilized shFcRn (~1500 RU).

15

b: The kinetic rate constants were obtained using a simple first-order (1:1) bimolecular interaction model.

The results show that: MSA DI-DIII and RSA DI-DIII both have higher binding affinities to shFcRn than wt MSA, wt HSA and HSA DI-DIII. All DI-DIII variants bound to immobilized shFcRn in a pH-dependent manner (*i.e.* binding at pH6.0 but not at pH7.4). HSA DI-DIII bound shFcRn with a slightly reduced affinity compared with HSA wt, while the RSA and MSA DI-DIII albumin derivatives showed superior binding affinities compared with HSA wt.

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CLAIMS

1. An albumin derivative or variant, fragment thereof or fusion polypeptide comprising said albumin derivative or variant or fragment thereof, where the albumin derivative or variant or
5 fragment thereof comprises or consists of albumin domain III or derivatives or variants thereof, and at least one additional albumin domain, fragment or derivative or variant thereof wherein the derivative or variant is not a naturally occurring albumin derivative or variant.
2. An albumin derivative or variant, fragment thereof or fusion polypeptide according to claim 1 wherein the naturally occurring derivative or variant is wild-type albumin from one of
10 human; primate, such as chimpanzee, gorilla or macaque; rabbit; rodent such as mouse, rat and hamster; bovine; equine such as horse or donkey; goat; sheep; dog; guinea pig; chicken and pig.
3. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of claim 1 or 2, wherein the
15 albumin domain III or derivative or variant thereof is derived from human serum albumin; primate serum albumin, such as chimpanzee serum albumin, gorilla serum albumin or macaque serum albumin; rabbit serum albumin; rodent serum albumin such as mouse albumin, rat serum albumin and hamster serum albumin; bovine serum albumin; equine serum albumin such as horse serum albumin or donkey serum albumin; goat serum albumin; sheep serum albumin; dog
20 serum albumin; guinea pig serum albumin; chicken serum albumin and pig serum albumin.
4. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of any of claims 1 to 3, wherein the albumin domain III or derivative or variant thereof has at least 80% sequence identity to Domain III of SEQ ID NO: 31 or SEQ ID NO: 1, preferably at least 85% sequence
25 identity, more preferred at least 90% sequence identity, even more preferred at least 90% sequence identity and most preferred at least 98% sequence identity.
5. The albumin derivative or variant of claim 4 wherein the Domain III or derivative or variant thereof has at least 80% sequence identity to amino acids 381 to 585 of SEQ ID NO: 31 or SEQ ID NO: 1, preferably at least 85% sequence identity, more preferred at least 90%
30 sequence identity, even more preferred at least 90% sequence identity and most preferred at least 98% sequence identity.
6. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof according to any of claims 1 to 5, wherein the at least one additional albumin domain, fragment or derivative or variant
35 thereof is selected among domain I, domain II and domain III.

7. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of claim 6, wherein the at least one additional albumin domain, fragment or derivative or variant thereof is derived from human serum albumin; primate serum albumin, such as chimpanzee serum albumin, gorilla serum albumin or or macaque serum albumin; rodent serum albumin such as rabbit serum albumin, mouse albumin and rat serum albumin; bovine serum albumin; equine serum albumin; donkey serum albumin; hamster serum albumin; goat serum albumin; sheep serum albumin; dog serum albumin; guinea pig serum albumin; chicken serum albumin and pig serum albumin.

8. The albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of claim 7, wherein the albumin derivative or variant consists of domain I and II derived from human serum albumin and domain III derived from rabbit serum albumin (e.g. SEQ ID NO: 25), domain I and II derived from rabbit serum albumin and domain III derived from human serum albumin (e.g. SEQ ID NO: 26), domain I and II derived from sheep serum albumin and domain III derived from human serum albumin (e.g. SEQ ID NO: 27), domain I and II derived from human serum albumin and domain III derived from mouse serum albumin (e.g. SEQ ID NO: 29), domain I and II derived from human serum albumin and domain III derived from sheep serum albumin (e.g. SEQ ID NO: 28), or domain I and II derived from mouse serum albumin and domain III derived from human serum albumin (e.g. SEQ ID NO: 30).

9. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of claim 7, wherein the at least one additional albumin domain, fragment or derivative or variant thereof have at least 80% sequence identity to the corresponding domain of its parent albumin, preferably at least 85% sequence identity, more preferred at least 90% sequence identity, even more preferred at least 90% sequence identity and most preferred at least 98% sequence identity.

10. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof of claim 8, wherein the at least one additional albumin domain, fragment or derivative or variant thereof have at least 80% sequence identity to the corresponding domain of HSA having SEQ ID NO: 31 or SEQ ID NO: 1, preferably at least 85% sequence identity, more preferred at least 90% sequence identity, even more preferred at least 90% sequence identity and most preferred at least 98% sequence identity

11. The albumin derivative or variant, fragment thereof or fusion polypeptide according to any preceding claim in which the albumin derivative or variant, fragment thereof or fusion polypeptide comprises or consists of an amino acid sequence selected from the group consisting of:

(i) a molecule consisting of human serum albumin domain I and human serum albumin domain II (e.g. SEQ ID NO: 20)

(ii) a molecule consisting of human serum albumin domain II and human serum albumin domain III (e.g. SEQ ID NO: 21),

5 (iii) a molecule consisting of human serum albumin domain I and human serum albumin domain III (e.g. SEQ ID NO: 22), and

(iv) a molecule consisting of two copies of human serum albumin domain III (e.g. SEQ ID NO: 24).

12. The albumin derivative or variant, fragments thereof or fusion polypeptide comprising or
10 consisting of said albumin derivative or variant or fragment thereof according to any of claims 1 to 11, wherein at least one domain III comprises one or more substitutions in positions corresponding to the positions in SEQ ID NO: 31 or SEQ ID NO:1 (HSA) selected among: 573, 500, 550, 417, 440, 464, 490, 492, 493, 494, 495, 496, 499, 501, 503, 504, 505, 506, 510, 535, 536, 537, 538, 540, 541, 542, 574, 575, 577, 578, 579, 580, 581, 582 and 584.

15 13. The albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant or fragment thereof according to claim 12, wherein the one or more substitutions in positions corresponding to the positions in SEQ ID NO:1 (HSA) is selected among: K573Y, W, P, H, F, V, I, T, N, S, G, M, C, A, E, Q, R, L, D, K500E, G, D, A, S, C, P, H, F, N, W, T, M, Y, V, Q, L, I, R Q417A, H440A, H464Q, E492G,
20 D494N,Q,A, E495Q,A, T496A, D494E+Q417H, D494N+T496A, E492G+V493P, P499A, E501A,Q, N503H,K, H510Q, H535Q, K536A, P537A, K538A, K541G,D, D550E,N, E492G+K573P,A, or E492G/N503H/K573P.

14. The albumin derivative or variant, fragment thereof or fusion polypeptide comprising or
25 consisting of said albumin derivative or variant of fragment thereof according to any of the preceding claims, comprising one or more additional mutations that creates one or more thiol groups on the surface of the molecule.

15. The albumin derivative or variant, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or variant of fragment thereof according to claim 14, having a substitution selected from the group of substitutions corresponding to : L585C, D1C, A2C,
30 D562C, A364C, A504C, E505C, T79C, E86C, D129C, D549C, A581C, D121C, E82C, S270C, A578C, L595LC, D1DC, A2AC, D562DC, A364AC, A504AC, E505EC, T79TC, E86EC, D129DC, D549DC, A581AC, A581AC, D121DC, E82EC, S270SC, A579AC, C360*, C316*, C75*, C168*, C558*, C361*, C91*, C124*, C169* and C567* in SEQ ID NO: 31 or SEQ ID NO: 1.

16. A conjugate comprising an albumin derivative or variant or fragment thereof according to any of the claims 1 to 15, and a therapeutic or diagnostic or other beneficial moiety bound to the albumin part.

17. A nucleic acid encoding an albumin derivative, fragment thereof or fusion polypeptide comprising or consisting of said albumin derivative or fragment thereof according to any of the claims 1 to 15, preferably comprised in a plasmid or an expression construct.

18. A host cell comprising a nucleic acid of claim 17.

19. A composition, preferably a pharmaceutical composition, comprising an albumin derivative, fragment thereof or fusion polypeptide or associate comprising said albumin derivative or fragment thereof of claim 1 to 15.

20. An albumin variant or derivative or fusion, conjugate, associate, composition thereof, nucleic acid or host cell encoding the variant, derivative or fusion according to any preceding claim, which albumin variant, derivative, fusion, conjugate, associate, or composition thereof has (i) a longer plasma half-life than native HSA and/or a fusion or conjugate, associate or composition comprising native HSA or (ii) a shorter plasma half-life than native HSA and/or a fusion or conjugate, associate or composition comprising native HSA.

21. A fusion of an albumin variant or derivative conjugate, associate, composition thereof, nucleic acid or host cell encoding the fusion according to any preceding claim, which fusion, conjugate, associate, or composition thereof has (i) a longer plasma half-life than an albumin fusion, conjugate, associate or composition comprising native HSA or (ii) a shorter than a fusion, conjugate, associate or composition comprising native HSA.

22. A fusion of an albumin variant or derivative or a conjugation or associate or composition thereof according to any preceding claim wherein the fusion or conjugation or associate comprises one or more moiety selected from:

(i) 4-1BB ligand, 5-helix, A human C-C chemokine, A human L105 chemokine, A human L105 chemokine designated huL105_3., A monokine induced by gamma-interferon (MIG), A partial CXCR4B protein, A platelet basic protein (PBP), α 1-antitrypsin, ACRP-30 Homologue; Complement Component C1q C, Adenoid-expressed chemokine (ADEC), aFGF; FGF-1, AGF, AGF Protein, albumin, an etoposide, angiostatin, Anthrax vaccine, Antibodies specific for collapsin, antistasin, Anti-TGF beta family antibodies, antithrombin III, APM-1; ACRP-30; Famoxin, apo-lipoprotein species, Arylsulfatase B, b57 Protein, BCMA, Beta-thromboglobulin protein (beta-TG), bFGF; FGF2, Blood coagulation factors, BMP Processing Enzyme Furin, BMP-10, BMP-12, BMP-15, BMP-17, BMP-18, BMP-2B, BMP-4, BMP-5, BMP-6, BMP-9, Bone Morphogenic Protein-2, calcitonin, Calpain-10a, Calpain-10b, Calpain-10c, Cancer Vaccine, Carboxypeptidase, C-C chemokine, MCP2, CCR5 variant, CCR7, CCR7, CD11a Mab, CD137; 4-1BB Receptor Protein, CD20 Mab, CD27, CD27L, CD30, CD30 ligand, CD33 immunotoxin,

CD40, CD40L, CD52 Mab, Cerebus Protein, Chemokine Eotaxin., Chemokine hIL-8, Chemokine hMCP1, Chemokine hMCP1a, Chemokine hMCP1b, Chemokine hMCP2, Chemokine hMCP3, Chemokine hSDF1b, Chemokine MCP-4, chemokine TECK and TECK variant, Chemokine-like protein IL-8M1 Full-Length and Mature, Chemokine-like protein IL-8M10 Full-Length and Mature, Chemokine-like protein IL-8M3, Chemokine-like protein IL-8M8 Full-Length and Mature, Chemokine-like protein IL-8M9 Full-Length and Mature, Chemokine-like protein PF4-414 Full-Length and Mature, Chemokine-like protein PF4-426 Full-Length and Mature, Chemokine-like protein PF4-M2 Full-Length and Mature, Cholera vaccine, Chondromodulin-like protein, c-kit ligand; SCF; Mast cell growth factor; MGF; Fibrosarcoma-derived stem cell factor, CNTF and fragment thereof (such as CNTFAx15` (Axokine™)), coagulation factors in both pre and active forms, collagens, Complement C5 Mab, Connective tissue activating protein-III, CTAA16.88 Mab, CTAP-III, CTLA4-Ig, CTLA-8, CXC3, CXC3, CXCR3; CXC chemokine receptor 3, cyanovirin-N, Darbepoetin, designated exodus, designated huL105_7., DIL-40, Dnase, EDAR, EGF Receptor Mab, ENA-78, Endostatin, Eotaxin, Epithelial neutrophil activating protein-78, EPO receptor; EPOR, erythropoietin (EPO) and EPO mimics, Eutropin, Exodus protein, Factor IX, Factor VII, Factor VIII, Factor X and Factor XIII, FAS Ligand Inhibitory Protein (DcR3), FasL, FasL, FasL, FGF, FGF-12; Fibroblast growth factor homologous factor-1, FGF-15, FGF-16, FGF-18, FGF-3; INT-2, FGF-4; gelonin, HST-1; HBGF-4, FGF-5, FGF-6; Heparin binding secreted transforming factor-2, FGF-8, FGF-9; Glia activating factor, fibrinogen, flt-1, flt-3 ligand, Follicle stimulating hormone Alpha subunit, Follicle stimulating hormone Beta subunit, Follitropin, Fractalkine, fragment. myofibrillar protein Troponin I, FSH, Galactosidase, Galectin-4, G-CSF, GDF-1, Gene therapy, Glioma-derived growth factor, glucagon, glucagon-like peptides, Glucocerebrosidase, glucose oxidase, Glucosidase, Glycodelin-A; Progesterone-associated endometrial protein, GM-CSF, gonadotropin, Granulocyte chemotactic protein-2 (GCP-2), Granulocyte-macrophage colony stimulating factor, growth hormone, Growth related oncogene-alpha (GRO-alpha), Growth related oncogene-beta (GRO-beta), Growth related oncogene-gamma (GRO-gamma), hAPO-4; TROY, hCG, Hepatitis B surface Antigen, Hepatitis B Vaccine, HER2 Receptor Mab, hirudin, HIV gp120, HIV gp41, HIV Inhibitor Peptide, HIV Inhibitor Peptide, HIV Inhibitor Peptide, HIV protease inhibiting peptides, HIV-1 protease inhibitors, HPV vaccine, Human 6CKine protein, Human Act-2 protein, Human adipogenesis inhibitory factor, human B cell stimulating factor-2 receptor, Human beta-chemokine H1305 (MCP-2), Human C-C chemokine DGWCC, Human CC chemokine ELC protein, Human CC type chemokine interleukin C, Human CCC3 protein, Human CCF18 chemokine, Human CC-type chemokine protein designated SLC (secondary lymphoid chemokine), Human chemokine beta-8 short forms, Human chemokine C10, Human chemokine CC-2, Human chemokine CC-3, Human chemokine CCR-2, Human chemokine

Ckbeta-7, Human chemokine ENA-78, Human chemokine eotaxin, Human chemokine GRO alpha, Human chemokine GROalpha, Human chemokine GRObeta, Human chemokine HCC-1, Human chemokine HCC-1, Human chemokine I-309, Human chemokine IP-10, Human chemokine L105_3, Human chemokine L105_7, Human chemokine MIG, Human chemokine

5 MIG-beta protein, Human chemokine MIP-1alpha, Human chemokine MIP1beta, Human chemokine MIP-3alpha, Human chemokine MIP-3beta, Human chemokine PF4, Human chemokine protein 331D5, Human chemokine protein 61164, Human chemokine receptor CXCR3, Human chemokine SDF1alpha, Human chemokine SDF1beta, Human chemokine ZSIG-35, Human Chr19Kine protein, Human CKbeta-9, Human CKbeta-9, Human CX3C 111

10 amino acid chemokine, Human DNAX interleukin-40, Human DVic-1 C-C chemokine, Human EDIRF I protein sequence, Human EDIRF II protein sequence, Human eosinocyte CC type chemokine eotaxin, Human eosinophil-expressed chemokine (EEC), Human fast twitch skeletal muscle troponin C, Human fast twitch skeletal muscle troponin I, Human fast twitch skeletal muscle Troponin subunit C, Human fast twitch skeletal muscle Troponin subunit I Protein,

15 Human fast twitch skeletal muscle Troponin subunit T, Human fast twitch skeletal muscle troponin T, Human foetal spleen expressed chemokine, FSEC, Human GM-CSF receptor, Human gro-alpha chemokine, Human gro-beta chemokine, Human gro-gamma chemokine, Human IL-16 protein, Human IL-1RD10 protein sequence, Human IL-1RD9, Human IL-5 receptor alpha chain, Human IL-6 receptor, Human IL-8 receptor protein hIL8RA, Human IL-8

20 receptor protein hIL8RB, Human IL-9 receptor protein, Human IL-9 receptor protein variant #3, Human IL-9 receptor protein variant fragment, Human IL-9 receptor protein variant fragment#3, Human interleukin 1 delta, Human Interleukin 10, Human Interleukin 10, Human interleukin 18, Human interleukin 18 derivatives, Human interleukin-1 beta precursor, Human interleukin-1 beta precursor., Human interleukin-1 receptor accessory protein, Human interleukin-1 receptor

25 antagonist beta, Human interleukin-1 type-3 receptor, Human Interleukin-10 (precursor), Human Interleukin-10 (precursor), Human interleukin-11 receptor, Human interleukin-12 40 kD subunit, Human interleukin-12 beta-1 receptor, Human interleukin-12 beta-2 receptor, Human Interleukin-12 p35 protein, Human Interleukin-12 p40 protein, Human interleukin-12 receptor, Human interleukin-13 alpha receptor, Human interleukin-13 beta receptor, Human interleukin-

30 15, Human interleukin-15 receptor from clone P1, Human interleukin-17 receptor, Human interleukin-18 protein (IL-18), Human interleukin-3, human interleukin-3 receptor, Human interleukin-3 variant, Human interleukin-4 receptor, Human interleukin-5, Human interleukin-6, Human interleukin-7, Human interleukin-7., Human interleukin-8 (IL-8), Human intracellular IL-1 receptor antagonist, Human IP-10 and HIV-1 gp120 hypervariable region fusion protein, Human

35 IP-10 and human Muc-1 core epitope (VNT) fusion protein, human liver and activation regulated chemokine (LARC), Human Lkn-1 Full-Length and Mature protein, Human mammary associated

chemokine (MACK) protein Full-Length and Mature, Human mature chemokine Ckbeta-7, Human mature gro-alpha, Human mature gro-gamma polypeptide used to treat sepsis, Human MCP-3 and human Muc-1 core epitope (VNT) fusion protein, Human MI10 protein, Human MI1A protein, Human monocyte chemoattractant factor hMCP-1, Human monocyte chemoattractant factor hMCP-3, Human monocyte chemotactic proprotein (MCP) sequence, Human neurotactin chemokine like domain, Human non-ELR CXC chemokine H174, Human non-ELR CXC chemokine IP10, Human non-ELR CXC chemokine Mig, Human PAI-1 mutants, Human protein with IL-16 activity, Human protein with IL-16 activity, Human secondary lymphoid chemokine (SLC), Human SISD protein, Human STCP-1, Human stromal cell-derived chemokine, SDF-1, Human T cell mixed lymphocyte reaction expressed chemokine (TMEC), Human thymus and activation regulated cytokine (TARC), Human thymus expressed, Human TNF-alpha, Human TNF-alpha, Human TNF-beta (LT-alpha), Human type CC chemokine eotaxin 3 protein sequence, Human type II interleukin-1 receptor, Human wild-type interleukin-4 (hIL-4) protein, Human ZCHEMO-8 protein, Humanized Anti-VEGF Antibodies, and fragments thereof, Humanized Anti-VEGF Antibodies, and fragments thereof, Hyaluronidase, ICE 10 kD subunit., ICE 20 kD subunit., ICE 22 kD subunit., Iduronate-2-sulfatase, Iduronidase, IL-1 alpha, IL-1 beta, IL-1 inhibitor (IL-1i)., IL-1 mature, IL-10 receptor, IL-11, IL-11, IL-12 p40 subunit., IL-13, IL-14, IL-15, IL-15 receptor, IL-17, IL-17 receptor, IL-17 receptor, IL-17 receptor, IL-19, IL-1i fragments, IL1-receptor antagonist, IL-21 (TIF), IL-3 containing fusion protein., IL-3 mutant proteins, IL-3 variants, IL-3 variants, IL-4, IL-4 mutein, IL-4 mutein Y124G, IL-4 mutein Y124X, IL-4 muteins, IL-5 receptor, IL-6, IL-6 receptor, IL-7 receptor clone, IL-8 receptor, IL-9 mature protein variant (Met117 version), immunoglobulins or immunoglobulin-based molecules or fragment of either (e.g. a Small Modular ImmunoPharmaceutical™ ("SMIP") or dAb, Fab' fragments, F(ab')₂, scAb, scFv or scFv fragment), including but not limited to plasminogen, Influenza Vaccine, Inhibin alpha, Inhibin beta, insulin, insulin-like growth factor, Integrin Mab, inter-alpha trypsin inhibitor, inter-alpha trypsin inhibitor, Interferon gamma-inducible protein (IP-10), interferons (such as interferon alpha species and sub-species, interferon beta species and sub-species, interferon gamma species and sub-species), interferons (such as interferon alpha species and sub-species, interferon beta species and sub-species, interferon gamma species and sub-species), Interleukin 6, Interleukin 8 (IL-8) receptor, Interleukin 8 receptor B, Interleukin-1alpha, Interleukin-2 receptor associated protein p43, interleukin-3, interleukin-4 muteins, Interleukin-8 (IL-8) protein., interleukin-9, Interleukin-9 (IL-9) mature protein (Thr117 version), interleukins (such as IL10, IL11 and IL2), interleukins (such as IL10, IL11 and IL2), Japanese encephalitis vaccine, Kalikrein Inhibitor, Keratinocyte growth factor, Kunitz domain protein (such as aprotinin, amyloid precursor protein and those described in WO 03/066824, with or without albumin fusions), Kunitz domain protein (such as aprotinin, amyloid precursor

protein and those described in WO 03/066824, with or without albumin fusions), LACI, lactoferrin, Latent TGF-beta binding protein II, leptin, Liver expressed chemokine-1 (LVEC-1), Liver expressed chemokine-2 (LVEC-2), LT-alpha, LT-beta, Luteinization Hormone, Lyme Vaccine, Lymphotactin, Macrophage derived chemokine analogue MDC (n+1), Macrophage
5 derived chemokine analogue MDC-eyfy, Macrophage derived chemokine analogue MDC-yl, Macrophage derived chemokine, MDC, Macrophage-derived chemokine (MDC), Maspin; Protease Inhibitor 5, MCP-1 receptor, MCP-1a, MCP-1b, MCP-3, MCP-4 receptor, M-CSF, Melanoma inhibiting protein, Membrane-bound proteins, Met117 human interleukin 9, MIP-3 alpha, MIP-3 beta, MIP-Gamma, MIRAP, Modified Rantes, monoclonal antibody, MP52, Mutant
10 Interleukin 6 S176R, myofibrillar contractile protein Troponin I, Natriuretic Peptide, Nerve Growth Factor-beta, Nerve Growth Factor-beta2, Neuropilin-1, Neuropilin-2, Neurotactin, Neurotrophin-3, Neurotrophin-4, Neurotrophin-4a, Neurotrophin-4b, Neurotrophin-4c, Neurotrophin-4d, Neutrophil activating peptide-2 (NAP-2), NOGO-66 Receptor, NOGO-A, NOGO-B, NOGO-C, Novel beta-chemokine designated PTEC, N-terminal modified chemokine
15 GroHEK/hSDF-1alpha, N-terminal modified chemokine GroHEK/hSDF-1beta., N-terminal modified chemokine met-hSDF-1 alpha, N-terminal modified chemokine met-hSDF-1 beta, OPGL, Osteogenic Protein-1; OP-1; BMP-7, Osteogenic Protein-2, OX40; ACT-4, OX40L, Oxytocin (Neurophysin I), parathyroid hormone, Patched, Patched-2, PDGF-D, Pertussis toxoid, Pituitary expressed chemokine (PGEC), Placental Growth Factor, Placental Growth Factor-2,
20 Plasminogen Activator Inhibitor-1; PAI-1, Plasminogen Activator Inhibitor-2; PAI-2, Plasminogen Activator Inhibitor-2; PAI-2, Platelet derived growth factor, Platelet derived growth factor Bv-sis, Platelet derived growth factor precursor A, Platelet derived growth factor precursor B, Platelet Mab, platelet-derived endothelial cell growth factor (PD-ECGF), Platelet-Derived Growth Factor A chain, Platelet-Derived Growth Factor B chain, polypeptide used to treat sepsis,
25 Preproapolipoprotein "milano" variant, Preproapolipoprotein "paris" variant, pre-thrombin, Primate CC chemokine "ILINCK", Primate CXC chemokine "IBICK", proinsulin, Prolactin, Prolactin2, prosaptide, Protease inhibitor peptides, Protein C, Protein S, pro-thrombin, prourokinase, RANTES, RANTES 8-68, RANTES 9-68, RANTES peptide, RANTES receptor, Recombinant interleukin-16, Resistin, restrictocin, Retroviral protease inhibitors, ricin, Rotavirus
30 Vaccine, RSV Mab, saporin, sarcin, Secreted and Transmembrane polypeptides, Secreted and Transmembrane polypeptides, serum cholinesterase, serum protein (such as a blood clotting factor), Soluble BMP Receptor Kinase Protein-3, Soluble VEGF Receptor, Stem Cell Inhibitory Factor, Straphylococcus Vaccine, Stromal Derived Factor-1 alpha, Stromal Derived Factor-1 beta, Substance P (tachykinin), T1249 peptide, T20 peptide, T4 Endonuclease, TACI, Tarc,
35 TGF-beta 1, TGF-beta 2, Thr117 human interleukin 9, thrombin, thrombopoietin, Thrombopoietin derivative1, Thrombopoietin derivative2, Thrombopoietin derivative3,

Thrombopoietin derivative4, Thrombopoietin derivative5, Thrombopoietin derivative6, Thrombopoietin derivative7, Thymus expressed chemokine (TECK), Thyroid stimulating Hormone, tick anticoagulant peptide, Tim-1 protein, TNF-alpha precursor, TNF-R, TNF-RII; TNF p75 Receptor; Death Receptor, tPA, transferrin, transforming growth factor beta, Troponin peptides, Truncated monocyte chemotactic protein 2 (6-76), Truncated monocyte chemotactic protein 2 (6-76), Truncated RANTES protein (3-68), tumour necrosis factor, Urate Oxidase, urokinase, Vasopressin (Neurophysin II), VEGF R-3; flt-4, VEGF Receptor; KDR; flk-1, VEGF-110, VEGF-121, VEGF-138, VEGF-145, VEGF-162, VEGF-165, VEGF-182, VEGF-189, VEGF-206, VEGF-D, VEGF-E; VEGF-X, von Willebrand's factor, Wild type monocyte chemotactic protein 2, Wild type monocyte chemotactic protein 2, ZTGF-beta 9;

(ii) chemotherapy drugs such as: 13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, A, Abraxane, Accutane®, Actinomycin-D, Adriamycin®, Aducan®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacitidine, BCG, BCNU, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Campsar®, Camptothecin-11, Capecitabine, Carac TM, Carboplatin, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cydadren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin, Daunorubicin, Daunorubicin Hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin diftitox, DepoCyt TM, Dexamethasone, Dexamethasone acetate, Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex, Docetaxel, Doxil®, Doxorubicin, Doxorubicin liposomal, Droloxia TM, DTIC, DTIC-Dome®, Duralone®, Efudex®, Eligard TM, Ellence TM, Eloxatin TM, Elspar®, Emcyt®, Epirubicin, Epoetin alfa, Erbitux TM, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethylol, Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®, Evista®, Exemestane, Fareston®, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR®, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar®, Gleevec TM, Gliadel® Wafer, GM-CSF, Goserelin, Granulocyte - Colony Stimulating Factor, Granulocyte Macrophage Colony Stimulating Factor, Halotestin®, Herceptin®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®,

Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea, Ibritumomab, Ibritumomab Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, IL-11, IL-2, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate),

5 Interleukin - 2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Kidrolase®, Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™, Liposomal Ara-C, Liquid Pred®, Lomustine, L-PAM, L-Sarcosylsin, Lupron®, Lupron Depot®, M, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®,

10 Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®, MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®, Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®,

15 Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab, Panretin®, Paraplatin®, Padiapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®, Prednisolone, Prednisone, Prelone®,

20 Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant, Purinethol®, R, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin®, Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571, Streptozocin, SU11248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®,

25 Temodar®, Temozolomide, Teniposide, TESPAs, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Tositumomab, Trastuzumab, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoïd®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar Pfs®, Vincristine, Vinorelbine,

30 Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®; radiopharmaceuticals such as: Carbon-11, Carbon-14, Chromium-51, Cobalt-57, Cobalt-58, Erbium-169, Fluorine-18, Gallium-67, Gold-198, Indium-111, Indium-113m, Iodine-123, Iodine-125, Iodine-131, Iron-59, Krypton-81m, Nitrogen-13, Oxygen-15, Phosphorous-32, Rhenium-186, Rubidium-82, Samarium-153,

35 Selenium-75, Strontium-89, Technetium-99m, Thallium-201, Tritium, Xenon-127, Xenon-133, Yttrium-90; and/or

(iii) imaging agents such as Gadolinium, magnetite, manganese, technetium, I125, I131, P32, TI201, Iopamidol, PET-FDG.

Figure 1

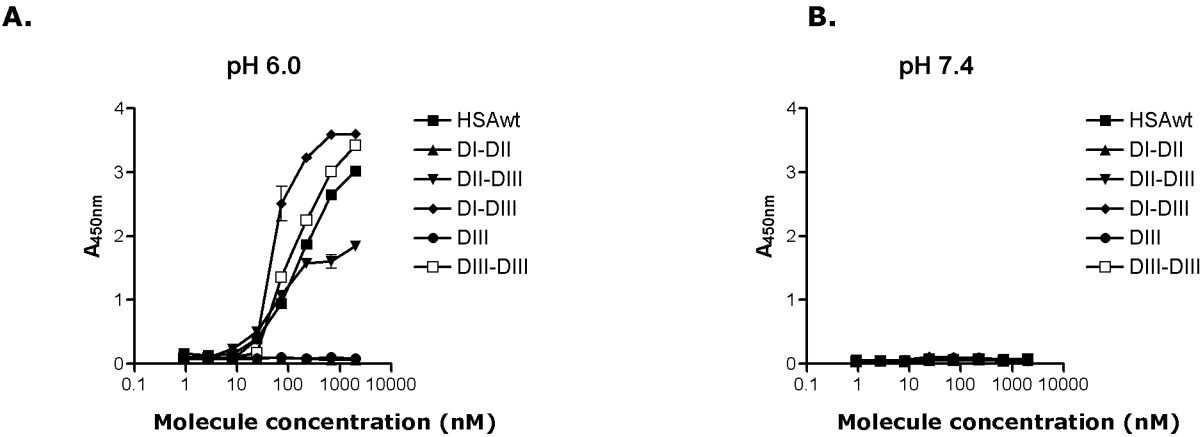


Figure 2

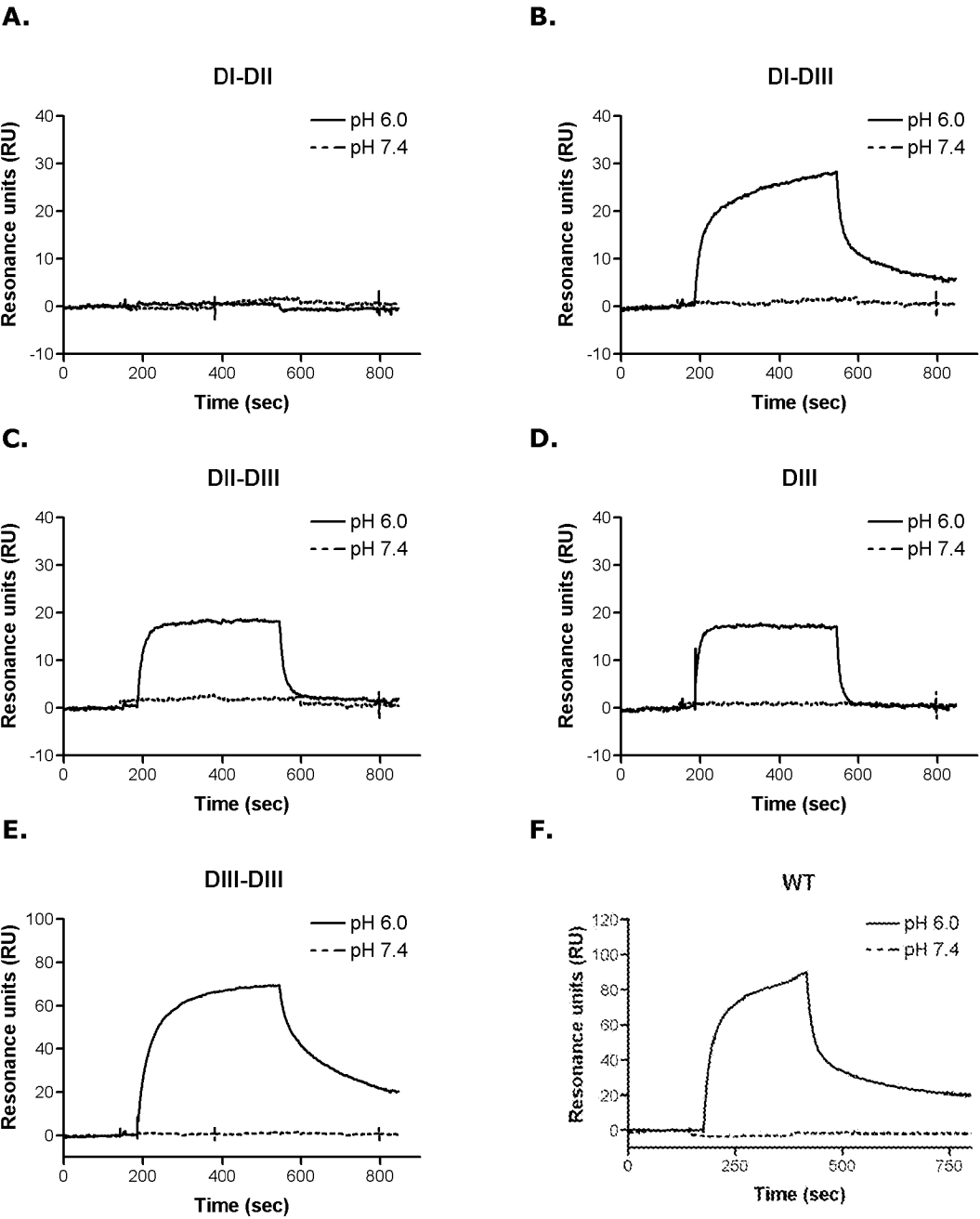


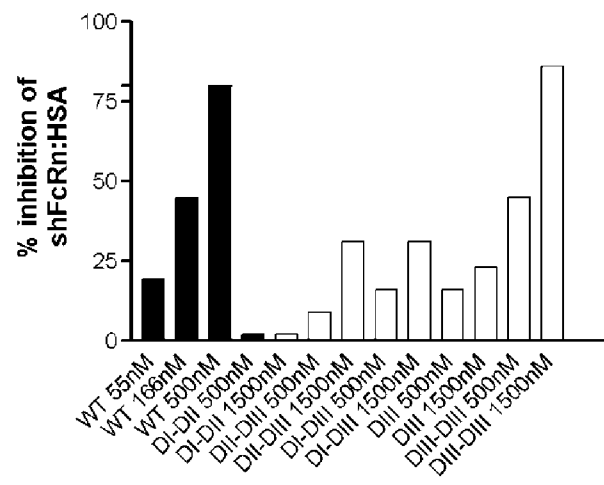
Figure 3

Figure 4

Hu_1_2_3	1	DAHKSEVAHRFKDLGEENFKLVLIAFAQYLLQCCPFEHVKLVNEVTEFAKTCVADESAAE
Hu_1_3	1	DAHKSEVAHRFKDLGEENFKLVLIAFAQYLLQCCPFEHVKLVNEVTEFAKTCVADESAAE
Hu_2_3	1	-----
Mac_mul	1	DTHKSEVAHRFKDLGEEHFKGLVLAFSQQYLLQCCPFEHVKLVNEVTEFAKTCVADESAAE
Rat	1	SAHKSEVAHRFKDLGEHFKGLVLIAFSQQYLQCCPFEHVKLVNEVTEFAKTCVADESAAE
Mouse	1	SAHKSEVAHRNDLGEHFKGLVLIAFSQQYLQCCPFEHVKLVNEVTEFAKTCVADESAAE
Hu_1_2_3	61	NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLOHKDDNPPLPRLVLRPEV
Hu_1_3	61	NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLOHKDDNPPLPRLVLRPEV
Hu_2_3	1	-----
Mac_mul	61	NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLOHKDDNPPLPRLVLRPEV
Rat	61	NCDKSLHTLFGDKLCAIPKLRINRYGEMADCCAKQEPERNECFLOHKDDNPPLPRLVLRPEV
Mouse	61	NCDKSLHTLFGDKLCAIPNLREINRYGEMADCCAKQEPERNECFLOHKDDNPPLPRLVLRPEV
Hu_1_2_3	121	DVMCTAFHDNEETFLKKYLYEVARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
Hu_1_3	121	DVMCTAFHDNEETFLKKYLYEVARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
Hu_2_3	1	-----
Mac_mul	121	DVMCTAFHDNEETFLKKYLYEVARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
Rat	121	AMCTSFQINPTTFGLGHYLHEVARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
Mouse	121	AMCTSFQINPTTFGLGHYLHEVARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
Hu_1_2_3	181	KLDELRLDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKE
Hu_1_3	181	KLDELRLDEGKASSA-----
Hu_2_3	1	--DELRLDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKE
Mac_mul	181	KLDELRLDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKE
Rat	181	KLDGAEKLVAAVQRLKCSSQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKE
Mouse	181	KLDGAEKLVSSVQRLKCSSQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKE
Hu_1_2_3	241	VHTECCHGDLLECADDRADLAKYMCENQDSISSKLKECCDKPLLEKSHCLAEVENDEMPA
Hu_1_3	195	-----
Hu_2_3	59	VHTECCHGDLLECADDRADLAKYMCENQDSISSKLKECCDKPLLEKSHCLAEVENDEMPA
Mac_mul	241	VHTECCHGDLLECADDRADLAKYMCENQDSISSKLKECCDKPLLEKSHCLAEVENDEMPA
Rat	241	VNKECCHGDLLECADDRADLAKYMCENQDSISSKLQACCDKPLLEKSHCLAEVENDEMPA
Mouse	241	VNKECCHGDLLECADDRADLAKYMCENQDSISSKLQACCDKPLLEKSHCLAEVENDEMPA
Hu_1_2_3	301	DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVSLLLRLAKTYETATLEKC
Hu_1_3	195	-----
Hu_2_3	119	DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVSLLLRLAKTYETATLEKC
Mac_mul	301	DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVSLLLRLAKTYETATLEKC
Rat	301	DLPSLAADFVEDKDVCKNYAEAKDVFLGTFLYFYARRHPDYSVSLLLRLAKTYETATLEKC
Mouse	301	DLPSLAADFVEDKDVCKNYAEAKDVFLGTFLYFYARRHPDYSVSLLLRLAKTYETATLEKC
Hu_1_2_3	361	CAAADPHECYAKVFDEFKPLVEEPQNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST
Hu_1_3	195	-----VEEPQNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST
Hu_2_3	179	CAAADPHECYAKVFDEFKPLVEEPQNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST
Mac_mul	361	CAAADPHECYAKVFDEFQPLVEEPQNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST
Rat	361	CAEADPPACYSTVLAEFQPLVEEPKNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST
Mouse	361	CAEADPPACYSTVLAEFQPLVEEPKNLKQNCLEFQQLGEYKFNALLVRYTKKVPQVST

Figure 4 (continued)

Hu_1_2_3	421	PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSSRVTKCCTES
Hu_1_3	235	PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSSRVTKCCTES
Hu_2_3	239	PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSSRVTKCCTES
Mac_mul	421	PTLVEVSRNLGKVGAKCKLPEAKRMPCAEDYLSVVLNRLCVLHEKTPVSSRVTKCCTES
Rat	421	PTLVEAARNLGKVGSKCCTLPEAQFPCVEDYLSALNRLCVLHEKTPVSSRVTKCCGS
Mouse	421	PTLVEAARNLGKVGSKCCTLPEAQFPCVEDYLSALNRLCVLHEKTPVSSRVTKCCGS
		↑
		500
Hu_1_2_3	481	LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKEQIKKQTALVELVKHKPKAT
Hu_1_3	295	LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKEQIKKQTALVELVKHKPKAT
Hu_2_3	299	LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKEQIKKQTALVELVKHKPKAT
Mac_mul	481	LVNRRPCFSALEDEAYVPKAFNAETFTFHADICTLSEKEQIKKQTALVELVKHKPKAT
Rat	481	LVERRPCFSALTIVDETYVPKEFKAETFTFHSDICTLPKEQIKKQTALVELVKHKPKAT
Mouse	481	LVERRPCFSALTIVDETYVPKEFKAETFTFHSDICTLPKEQIKKQTALVELVKHKPKAT
		↑
		500
Hu_1_2_3	541	KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALSL
Hu_1_3	355	KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALSL
Hu_2_3	359	KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALSL
Mac_mul	541	KEQLKAVMDNFDAAFVEKCKADDKEACFAEEGPKFVAASQAALSL
Rat	541	EEQLKTVMGDFAQFVKCKKAADKNTCFATEGPNLVARSKEALSL
Mouse	541	AEQLKTVMDDFDFAQFVKCKKAADKNTCFSTEGPNLVTRCKDALSL
		↑
		550
		↑
		573

Figure 5

Human	1	-----	DA-HKSEIAHREKDLGEENFK
Mouse	1	-----	A-HKSEIAHRENDLGEQHFKG
Sheep	1	-----	DT-HKSEIAHRENDLGEENFOG
Rabbit	1	-----	A-HKSEIAHRENDLGEENFOG
Goat	1	-----	DT-HKSEIAHRENDLGEENFOG
Chimp	1	MNESSCCSTSLPAFGVIVDSGHSSSSAYSARGV--	FRRDA-HKSEIAHREKDLGEENFK
Macaque	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDT-HKSEIAHREKDLGEEHFKG
Hamster	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDA-HKSEIAHREKDLGEEHFKG
Guinea_Pig	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDA-HKSEIAHREKDLGEEHFKG
Rat	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDA-HKSEIAHREKDLGEEHFKG
Cow	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDT-HKSEIAHREKDLGEEHFKG
Horse	1	-----MKWVTFSLFLFSSAYSARGV--	LRRDT-HKSEIAHREKDLGEEHFKG
Donkey	1	-----MKWVTFSLFLFSSAYSARGV--	LRRDT-HKSEIAHREKDLGEEHFKG
Dog	1	-----MKWVTFSLFLFSSAYSARGV--	VRRDA-YKSEIAHREKDLGEEHFKG
Chicken	1	-----MKWVTFSLFLFSSAYSARGV--	QRFARDAHKSEIAHREKDLGEEHFKG
Pig	1	-----MKWVTFSLFLFSSAYSARGV--	FRRDT-YKSEIAHREKDLGEEHFKG

↑
(D1-Start)

Human	22	LVLIAFAQYLLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Mouse	22	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR
Sheep	22	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESHAGCDKSLHTLFGDELCKVATLR
Rabbit	22	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESHAGCDKSLHTLFGDKLCAAPNLR
Goat	22	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESHAGCDKSLHTLFGDELCKVATLR
Chimp	58	LVLIAFAQYLLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Macaque	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Hamster	46	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR
Guinea_Pig	46	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR
Rat	46	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR
Cow	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESHAGCDKSLHTLFGDELCKVATLR
Horse	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Donkey	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Dog	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLR
Chicken	49	LVLIAFSQYLQKCSPEEHAKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR
Pig	46	LVLIAFSQYLQOCPEEHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCAAPNLR

Human	82	ETYGEMADCCAKQEPERNECFLLHKDDNPDL--PRLVRPEVDVCTAF--NEETFLKKYLY
Mouse	82	ENYGE--ADCCTKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NPTTF--GHYLY
Sheep	82	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Rabbit	82	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Goat	82	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Chimp	118	ETYGEMADCCAKQEPERNECFLLHKDDNPDL--PRLVRPEVDVCTAF--NEETFLKKYLY
Macaque	106	ETYGEMADCCAKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Hamster	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Guinea_Pig	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Rat	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Cow	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Horse	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Donkey	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Dog	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Chicken	109	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY
Pig	106	ETYGEMADCCCEKQEPERNECFLLHKDDNPDL--PPLVRPEVDVCTAF--NEETFLKKYLY

Figure 5 (continued)

Human	141	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	A	K	Y	K	A	F	T	E	C	C	Q	A	A	D	K	A	A	C	L	P	K	L	D	E	L	R	E	K	S	S	A	K	Q	R	L	K	C			
Mouse	141	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Q	Y	N	E	L	T	Q	C	A	E	A	D	K	E	S	C	L	T	P	K	L	D	E	K	L	V	S	S	V	Q	R	L	K	C			
Sheep	140	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	N	Y	N	E	F	Q	E	C	C	Q	A	E	D	K	S	A	C	L	P	K	L	D	A	R	E	K	V	L	S	S	A	Q	R	L	K	C		
Rabbit	141	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	Q	Y	K	E	L	T	E	C	C	E	A	A	D	K	S	A	C	L	T	P	K	L	D	A	E	G	K	S	L	I	S	A	Q	E	R	L	K	C
Goat	140	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	N	Y	N	E	F	Q	E	C	C	Q	A	E	D	K	S	A	C	L	P	K	L	D	A	R	E	K	V	L	S	S	A	Q	R	L	K	C		
Chimp	177	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	K	A	F	T	E	C	C	Q	A	A	D	K	A	A	C	L	P	K	L	D	E	L	R	E	K	S	S	A	K	Q	R	L	K	C		
Macaque	165	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	A	Y	K	A	F	A	E	C	C	Q	A	A	D	K	A	A	C	L	P	K	L	D	E	L	R	E	K	S	S	A	K	Q	R	L	K	C		
Hamster	165	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	S	A	F	T	E	C	C	G	E	A	D	K	A	A	C	L	T	P	K	L	D	A	L	E	K	L	S	S	V	N	Q	R	L	K	C	
Guinea_Pig	165	E	V	S	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	K	N	A	L	T	E	C	C	E	A	A	D	K	A	A	C	L	T	P	K	L	D	A	R	E	K	L	V	S	S	A	Q	R	L	K	C
Rat	165	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	N	E	L	T	Q	C	C	T	E	S	D	K	A	A	C	L	T	P	K	L	D	A	R	E	K	L	V	A	A	V	Q	R	L	K	C	
Cow	164	E	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	N	Y	N	E	F	Q	E	C	C	Q	A	E	D	K	S	A	C	L	P	K	L	D	A	R	E	K	V	L	S	S	A	Q	R	L	K	C			
Horse	164	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	K	A	D	F	T	E	C	C	P	A	D	D	K	L	A	C	L	P	K	L	D	A	L	E	I	L	S	S	A	K	E	R	L	K	C	
Donkey	164	E	V	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	E	Y	K	A	D	F	T	E	C	C	P	A	D	D	K	A	C	L	P	K	L	D	A	L	E	I	L	S	S	A	K	E	R	L	K	C		
Dog	165	E	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	Q	Q	Y	K	A	F	A	E	C	C	Q	A	A	D	K	A	A	C	L	G	P	K	L	A	L	R	E	K	V	L	S	S	A	K	E	R	F	K	C
Chicken	169	S	V	A	R	R	H	P	Y	F	Y	A	P	A	L	S	A	V	D	E	H	A	L	Q	S	C	K	E	S	D	V	A	C	L	D	T	K	E	I	V	R	E	K	K	V	S	V	K	Q	Q	Y	F	C			
Pig	164	E	A	R	R	H	P	Y	F	Y	A	P	E	L	L	Y	A	I	I	Y	K	D	E	F	E	C	C	Q	A	A	D	K	A	A	C	L	P	K	L	D	A	R	E	K	V	L	S	A	A	K	Q	R	L	K	C	

<

(D2-Start) (D1-End)

Human	201	A	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	F	P	K	A	F	A	E	V	S	K	V	T	D	L	T	K	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L			
Mouse	201	S	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	T	F	P	N	A	D	F	A	E	K	A	T	D	L	T	K	V	N	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	L				
Sheep	200	A	S	Q	K	F	G	E	R	A	L	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	D	F	T	V	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L			
Rabbit	201	A	S	Q	K	F	G	E	R	A	L	K	A	W	A	V	A	R	L	S	Q	F	P	K	A	D	F	T	V	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L				
Goat	200	A	S	Q	K	F	G	E	R	A	L	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	D	F	T	V	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L			
Chimp	237	A	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	F	P	K	A	F	A	E	V	S	K	V	T	D	L	T	K	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L			
Macaque	225	A	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	K	F	P	K	A	F	A	E	V	S	K	V	T	D	L	T	K	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L		
Hamster	225	S	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	K	F	P	K	A	D	F	A	E	K	A	T	D	L	T	K	V	N	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	L				
Guinea_Pig	225	A	S	Q	K	F	G	E	R	A	F	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	F	A	E	S	T	V	T	S	L	T	K	V	T	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	L				
Rat	225	S	S	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	Q	F	P	N	A	D	F	A	E	K	A	T	D	L	T	K	V	N	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	L					
Cow	224	A	S	Q	K	F	G	E	R	A	L	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	F	A	E	V	S	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L		
Horse	224	S	S	F	Q	N	F	G	E	R	A	V	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	D	F	A	E	V	S	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L
Donkey	224	S	S	F	Q	K	F	G	E	R	A	F	K	A	W	S	V	A	R	L	S	Q	K	F	P	K	A	D	F	A	E	V	S	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L
Dog	225	A	S	Q	K	F	G	E	R	A	F	K	A	W	S	V	A	R	L	S	Q	F	P	K	A	D	F	A	E	S	K	V	T	D	L	T	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L			
Chicken	229	I	I	K	Q	F	G	E	R	V	F	Q	A	R	Q	I	Y	L	S	Q	K	P	A	P	F	S	E	V	S	K	E	V	H	D	S	I	G	V	H	K	E	C	C	E	G	D	E	C	M	D	D	M	A	R						
Pig	224	A	S	Q	K	F	G	E	R	A	F	K	A	W	S	V	A	R	L	S	Q	F	P	K	A	D	F	T	V	K	V	T	D	L	A	K	V	H	K	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L				

Human	261	AKYICENQDSISSKLKECC	KPLLEKSHCIAEVENDE	PADLPSLAADFVESK	VCKNYA	
Mouse	261	AKYICENQASISSKLQ	TCCDKPLLKKAHC	SEVENDE	PADLPSLAADFVEDQ	EVCCKNYA
Sheep	260	AKYICEHODASISSKLKECCDKP	LEKSHCIAEVENDE	PENLPPLTADFAEDKEV	CCKNYQ	
Rabbit	261	AKYICEHODASISSHLKECCDKP	LEKAHCIIYGHNDET	PAGLPAAE	FVEDK	VCKNYE
Goat	260	AKYICEHODASISSKLKECCDKP	LEKSHCIAEVENDE	PENLPPLTADFAEDKEV	CCKNYQ	
Chimp	297	AKYICENQDSISSKLKECC	KPLLEKSHCIAEVENDE	PADLPSLAADFVESK	VEVCCKNYA	
Macaque	285	AKYICENQDSISSKLKECCDKP	LLEKSHCIAEVENDE	PADLPSLAADFVESK	VEVCCKNYA	
Hamster	285	AKYICENQASISSKLQ	ACCDKPLLKSHCIAEVENDE	PADLPSLAADFVEDKEV	CCKNYA	
Guinea_Pig	285	AKYICEHODSISISSKLKECCV	KPTLQKAHCILEQDE	PTLPDLAVDFVEDKEV	CCKNYA	
Rat	285	AKYICENQASISSKLQ	ACCDKPLLKSQCAEVEDN	PADLPSLAADFVEDKEV	CCKNYA	
Cow	284	AKYICENQDSISSKLKECCDKP	LLEKSHCIAEVENDE	PENLPPLTADFAEDK	VEVCCKNYQ	
Horse	284	AKYICEHODSISGKLKACCDK	PLLQKSHCIAEVEDK	PSDLPALAAADFADKE	CKHYK	
Donkey	284	TKYICEHODSISGKLKACCDK	PLLQKSHCIAEVEDK	PSDLPALAAADFADKE	CKHYK	
Dog	285	AKYICENQDSISSKLKECCDKP	LEKSQCAEVEDN	PADLPSLAADFVEDKEV	CCKNYQ	
Chicken	289	MSNICSQDVFSGK	KKCCKPLLEISQ	CIMEAEFDEK	PADLPSLVEK	EDKEVCKSE
Pig	284	AKYICENQDSISSKLKECCDKP	LLEKSHCIAEAKDE	PADLNPLEHDFVEDKEV	CCKNYK	

Figure 5 (continued)

Human	321	EAKDVFLGMFLYEYARRHPDYSVLLRLAKTYETLEKCCAAADPHECYAKVFDEFKPL
Mouse	321	EAKDVFLGTFLYEYSRRHPDYSVLLRLAKKYEATLEKCCAEADNPACYSTVLAEFPL
Sheep	320	EAKDVFLGFLYEYSRRHPYAVSLLRLAKEYEATLEDCCAKEDPHACYATVFDKCLKHL
Rabbit	321	EAKDVLGKFLYEYSRRHPDYSVLLRLAKYEATLEKCCATDDPHACYAKVLDEFPL
Goat	320	EAKDVFLGFLYEYSRRHPYAVSLLRLAKEYEATLEDCCAKEDPHACYATVFDKCLKHL
Chimp	357	EAKDVFLGMFLYEYARRHPDYSVLLRLAKTYETLEKCCAAADPHECYAKVFDEFKPL
Macaque	345	EAKDVFLGMFLYEYARRHPDYSVLLRLAKYEATLEKCCAAADPHECYAKVFDEFPL
Hamster	345	EAKDVFLGTFLYEYARRHPDYSVLLRLAKKYEATLEKCCAEADNPACYKVLDEFPL
Guinea_Pig	345	EAKDVFLGTFLYEYSRRHPYSGLLRKAKGYEAKLEKCCAEADPHACYAKVFDELPL
Rat	345	EAKDVFLGTFLYEYSRRHPDYSVLLRLAKKYEATLEKCCAEADNPACYSTVLAEFPL
Cow	344	EAKDAFLGFLYEYSRRHPYAVSLLRLAKEYEATLECCAKDDPHACYSTVFDKCLKHL
Horse	344	EAKDVFLGTFLYEYSRRHPDYSVLLRLAKTYEATLEKCCAEADNPACYRTVFDQFTPL
Donkey	344	EAKDVFLGTFLYEYSRRHPDYSVLLRLAKTYEATLEKCCAEADNPACYATVFDQFTPL
Dog	345	EAKDVFLGTFLYEYARRHPYSVLLRLAKEYEATLEKCCATDDPPTCYAKVLDEFKPL
Chicken	349	ASHDAFLGFLYEYSRRHPYSGLLRKAKGYESILEKCCCKTDNPAECYANAOQLQH
Pig	344	EAKHVFLGTFLYEYSRRHPDYSVLLRLAKTYEATLEDCCAKEDNPACYATVFDKPL

Human	381	VEEPQNLKQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGKCCCKH
Mouse	381	VEEPKNLVKTNCNLSEKLGEYGFQNALVRYTOKAPQVSTPTLVEAARNLGKVGTKCCCTL
Sheep	380	VEEPQNLKQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGTKCCAK
Rabbit	381	VEEPKNLVKQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGKCCCKH
Goat	380	VEEPQNLKQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGTKCCAK
Chimp	417	VEEPQNLKQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGKCCCKH
Macaque	405	VEEPQNLVQNCSELFQLGGEYKFNALLVRYTKKVPQVSTPTLVESRNLGKVGKCCCKL
Hamster	405	VEEPKNLVKQNCSELFQLGGEYGFQNALVRYTOKAPQVSTPTLVEAARNLGKVGKCCCVL
Guinea_Pig	405	VEEPKNLVQNCSELFQLGGEYGFQNALVRYTOKAPQVSTPTLVEYARKLGKVGKCCCSL
Rat	405	VEEPKNLVKTNCNLSEKLGEYGFQNALVRYTOKAPQVSTPTLVEAARNLGKVGTKCCCTL
Cow	404	VEEPQNLKQNCSELFQLGGEYGFQNALVRYTKKVPQVSTPTLVESRNLGKVGTKCCCK
Horse	404	VEEPKSLVKKNCLFEEGGEYDFQNALVRYTKKAPQVSTPTLVEGRTLKGKVGKCCCKL
Donkey	404	VEEPKSLVKKNCLFEEGGEYDFQNALVRYTKKAPQVSTPTLVEGRTLKGKVGKCCCKL
Dog	405	VEEPQNLVKTNCSELFQLGGEYGFQNALVRYTKKAPQVSTPTLVESRNLGKVGTKCCCKK
Chicken	409	KETQDVKTNCNLHHDHGEADFLKSLRYTKKMPQVPTDLLETGKKTGKCCCKL
Pig	404	VEEPKNLVKQNCSELFQLGGEYGFQNALVRYTKKVPQVSTPTLVEARKLGKVGKCCCKR

↑ ↑
(D3-Start) (D2-End)

Human	441	PEAKRIPCAEDYLSNLNQLCVLHEKTPVSEVTKCCTESLVNRRPCFSALEVDETYVPK
Mouse	441	PEDQRIPCVEDYLSANLNRCVLHEKTPVSEHVTKCCGSLVERRRPCFSALTVDETYVPK
Sheep	440	PESERIPCTEDYLSANLNRLCVLHEKTPVSEKVTKCTESLVNRRPCFSALTDETYVPK
Rabbit	441	PEAERIPCVEDYLSANLNRLCVLHEKTPVSEKVTKCCESLVDRRPCFSALGPDETYVPK
Goat	440	PESERIPCTEDYLSANLNRLCVLHEKTPVSEKVTKCTESLVNRRPCFSALTDETYVPK
Chimp	477	PEAKRIPCAEDYLSNLNQLCVLHEKTPVSEVTKCCTESLVNRRPCFSALEVDETYVPK
Macaque	465	PEAKRIPCAEDYLSANLNRLCVLHEKTPVSEKVTKCTESLVNRRPCFSALEVDETYVPK
Hamster	465	PEAQRIPCVEDYLSANLNRCVLHEKTPVSEVTKCCTGSLVERRRPCFSALPVDETYVPK
Guinea_Pig	465	PETERISCTENYLSANLNRLCVLHEKTPVSEVTKCCTESLVNRRPCFSALHVDETYVPK
Rat	465	PEAQRIPCVEDYLSANLNRLCVLHEKTPVSEKVTKCCGSLVERRRPCFSALTVDETYVPK
Cow	464	PESERIPCTEDYLSANLNRLCVLHEKTPVSEKVTKCTESLVNRRPCFSALTPDETYVPK
Horse	464	PESERIPCSENHLAALNRLCVLHEKTPVSEKVTKCTESLAERRPCFSALEDEGYVPK
Donkey	464	PESERIPCSENHLAALNRLCVLHEKTPVSEKVTKCTESLAERRPCFSALEDEGYVPK
Dog	465	PESERISCAEDYLSANLNRLCVLHEKTPVSEVTKCCESLVNRRPCFSLEVDETYVPK
Chicken	469	GEDRRACSEGYLSANLHDTCKRQETTPNINVOCCQLYANRRPCFANGVDTKYVPP
Pig	464	PEEERISCAEDYLSANLNRLCVLHEKTPVSEKVTKCTESLVNRRPCFSALTPDETYRKP

Figure 5 (continued)

Human	501	EFNAETFTFHADICTLSEKEQI	KKQTALVELVKHKPKATKEQLKAVMDNFAFVKCCCK				
Mouse	501	EFKAETFTFHS	SDICTLPEKEKQI	KKQTALAE	LVKHKPKATAEQLKTVMDNFAFVDKCCCK		
Sheep	500	PFDEKFFTFHADICTLP	TEKQI	KKQTALVELVKHKPKATSEQLKTVMENFAFVDKCCA			
Rabbit	501	EFNAETFTFHADICTLPETE	KI	KKQTALVELVKHKPHATNQLKTVGNFTALDKCCS			
Goat	500	PFD	EFTTFHADICTLP	TEKQI	KKQTALVELVKHKPKATSEQLKTVMENFAFVDKCCA		
Chimp	537	EFNAETFTFHADICTLSEKEQI	KKQTALVELVKHKPKATKEQLKAVMDNFAFVKCCCK				
Macaque	525	AFNAETFTFHADICTLSEKEKQ	KKQTALVELVKHKPKATKEQLKGVMDNFAFVKCCCK				
Hamster	525	EFKAETFTFHADIC	LPEKEKQ	KKQAALVELVKHKPKATGPQLTVGNFTAFDKCCCK			
Guinea_Pig	525	PFHAT	FTFHADICTLPEKEKQ	KKQMALVELVKHKPKATSEQLKTVMDNFAFVKCCCK			
Rat	525	EFKAETFTFHS	SDICTLP	PEKEKQI	KKQTALAE	LVKHKPKATSEQLKTVMDNFAFVDKCCCK	
Cow	524	AFDEKLFTTFHADICTLP	TEKQI	KKQTALVELVKHKPKATSEQLKTVMENFAFVDKCCA			
Horse	524	EFKAETFTFHADICTLP	PEDEKQI	KKQALAE	LVKHKPKATKEQLKTVGNFSAFVAKCCG		
Donkey	524	EFKAETFTFHADICTLP	PEDEKQI	KKQALAE	LVKHKPKATKEQLKTVGNFSAFVAKCCG		
Dog	525	EFNAETFTFHADICTLPEAEKQ	KKQTALVELVKHKPKATSEQLKTVMDNFAFVKCCA				
Chicken	529	PFNPMFTFDEK	CA	PAEE	EG	QMKLLNL	KRKPMTEQLKTVADGFTAMVDKCCCK
Pig	524	EFVEGTFTFHADICTLPEDEKQI	KKQTALVELVKHKPHATSEQLKTVGNFAFVKCCA				

Human	561	AADKETCF	AEEGKKLV	AASQAAL	L---
Mouse	561	AADKETCF	STEGPNLV	TRCKDALA	---
Sheep	560	AADKETCF	VLEGPKLV	ASQAALA	---
Rabbit	561	AADKEACF	AVEGPKLV	ESSKATL	---
Goat	560	AADKETCF	LLEGPKLV	ASQAALA	---
Chimp	597	AADKETCF	AEEGKKLV	AASQAAL	L---
Macaque	585	AADKEACF	AEEGPKFV	AASQAALA	---
Hamster	585	AADKEACF	SEEGPKLV	ASSQAALA	---
Guinea_Pig	585	AADNKEACF	TEEGPKLV	AKQATLA	---
Rat	585	AADKETNC	FATEGPNLV	ARSKEALA	---
Cow	584	AADKEACF	AVEGPKLV	VSSQTALA	---
Horse	584	AADKEACF	AEEGPKLV	ASSQLALA	---
Donkey	584	AADKEACF	AEEGPKLV	ASSQLALA	---
Dog	585	AADNKEACF	SEEGPKLV	AAAQAALV	---
Chicken	589	QSDINTCF	SEEGANLV	QSRATL	IGA
Pig	584	APDHEACF	AVEGPKFV	IEIRILA	---

↑
(D3-End)

Figure 6

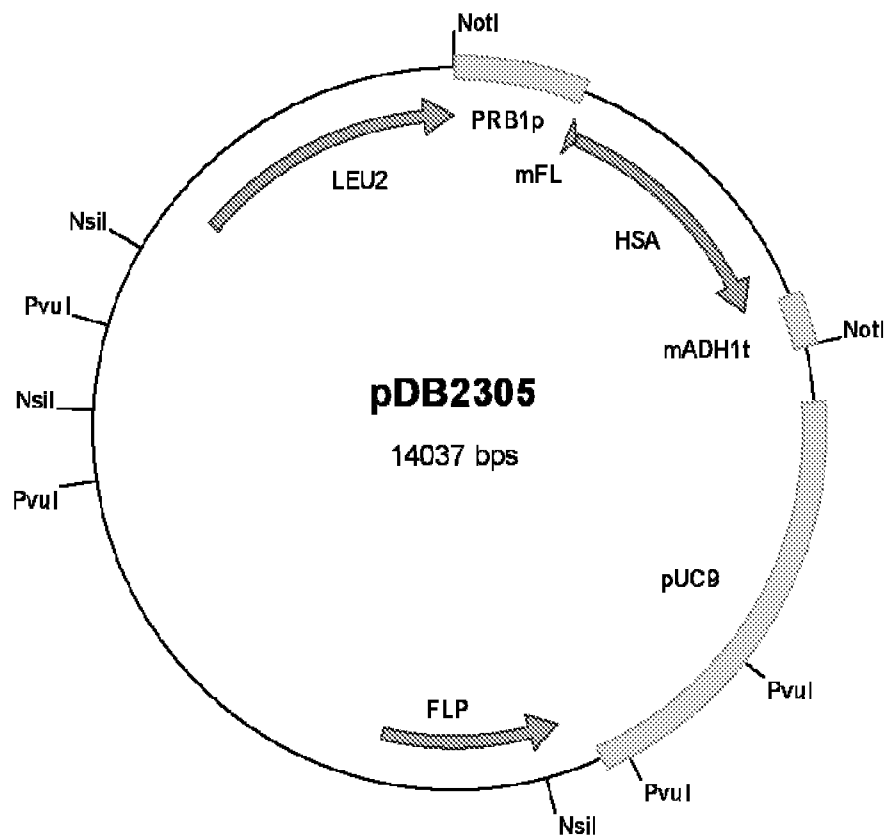


Figure 7

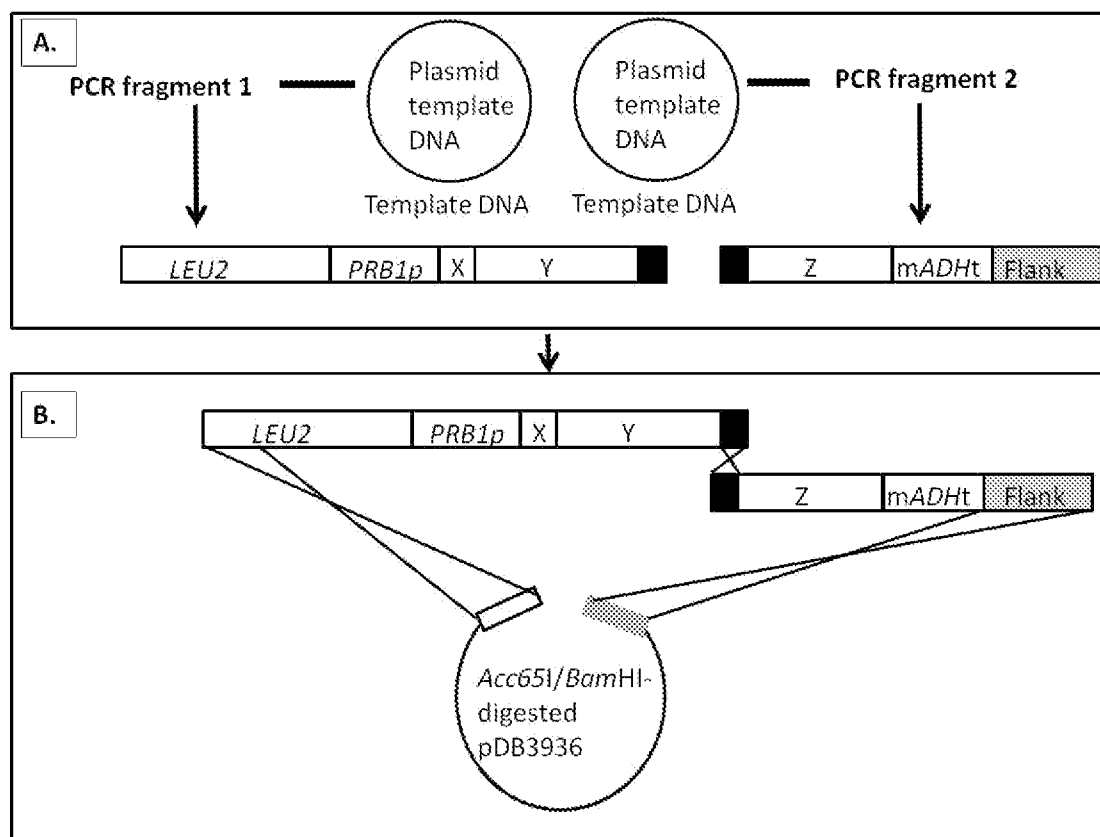


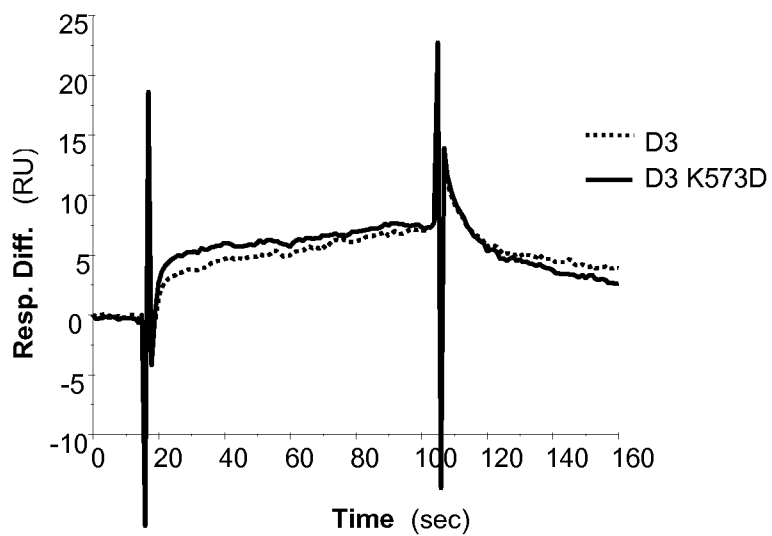
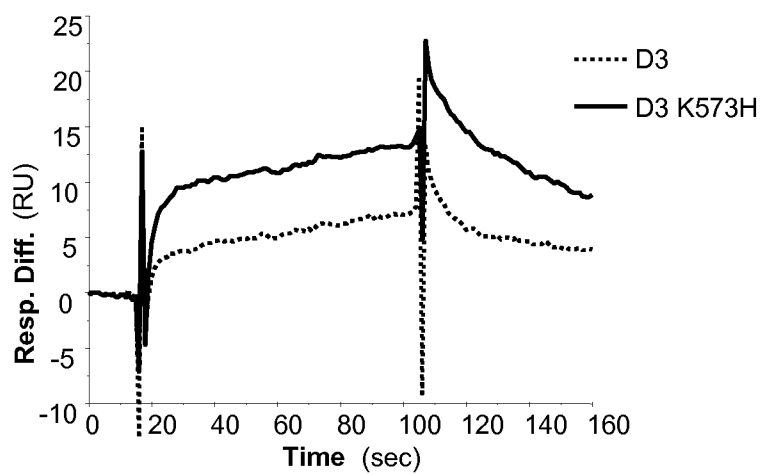
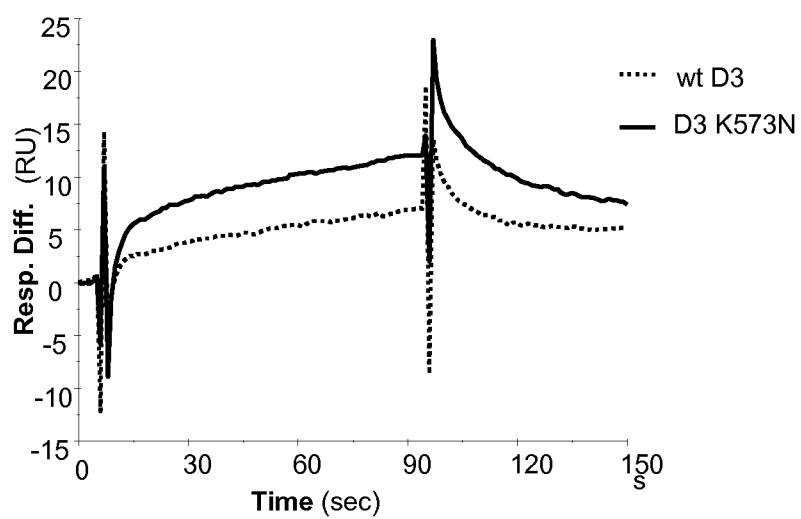
Figure 8a**Figure 8b****Figure 8c**

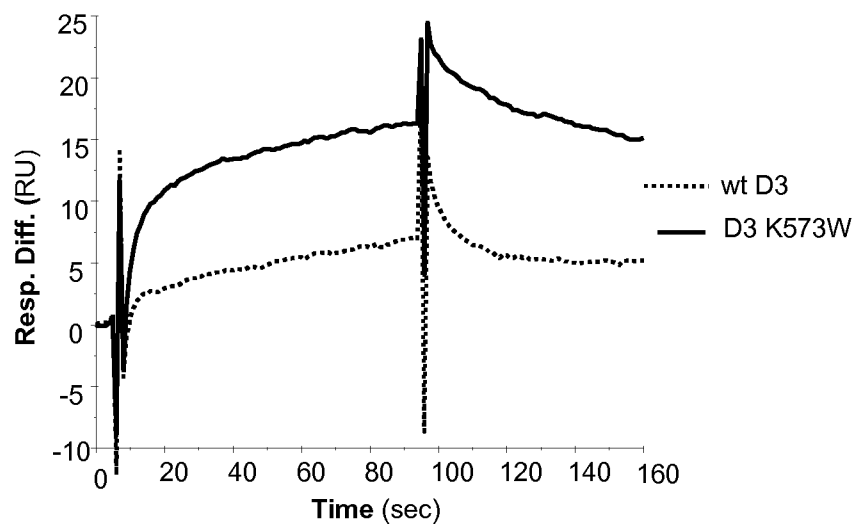
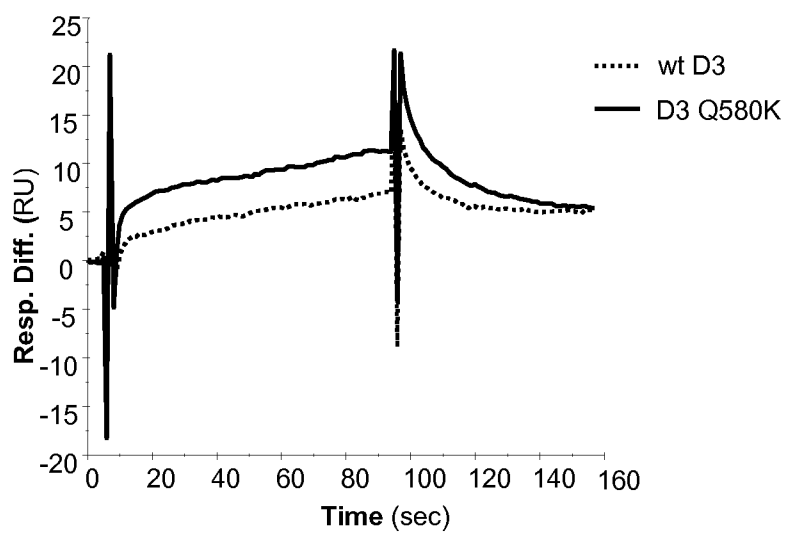
Figure 8d**Figure 8e**

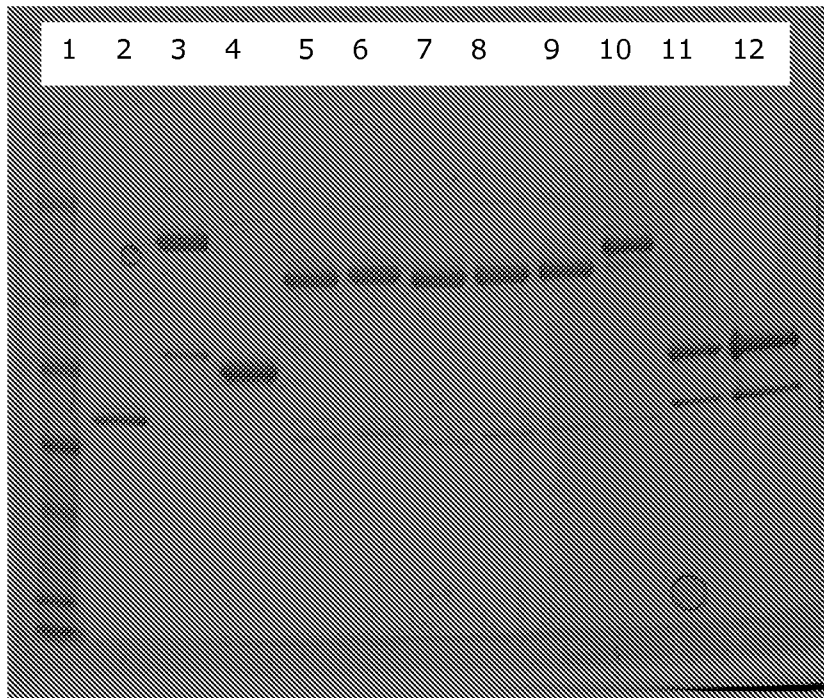
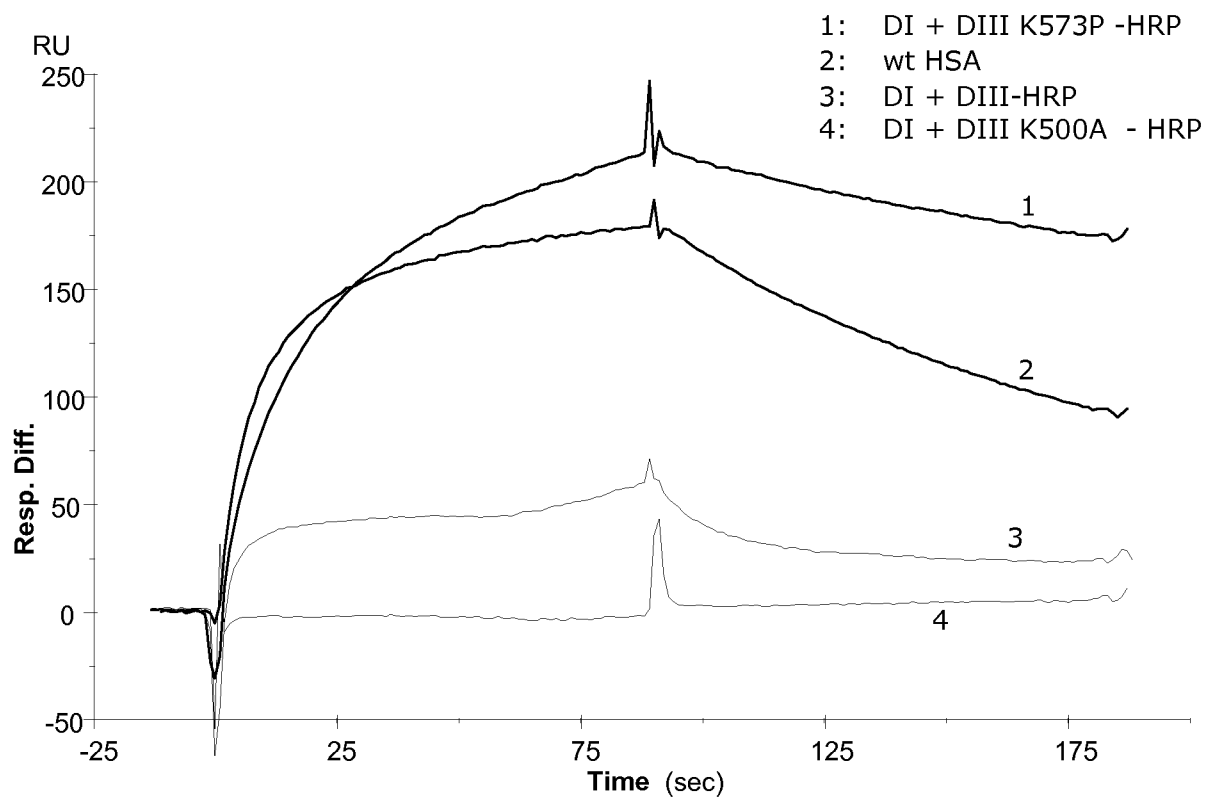
Figure 9**Figure 10**

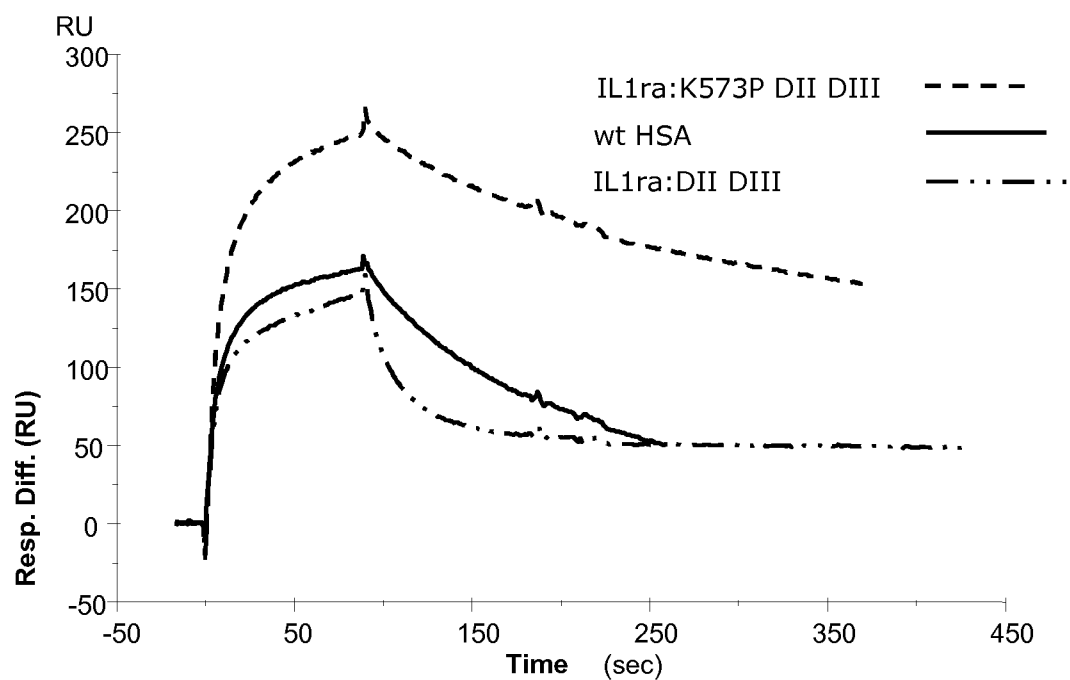
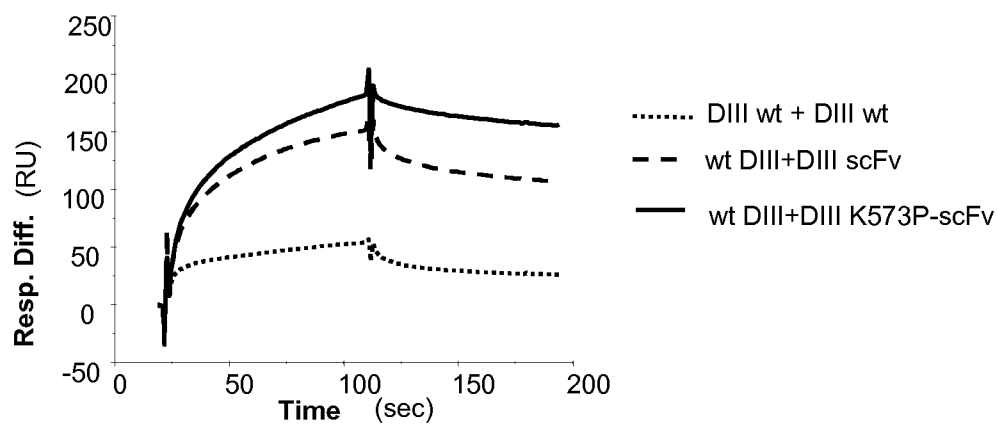
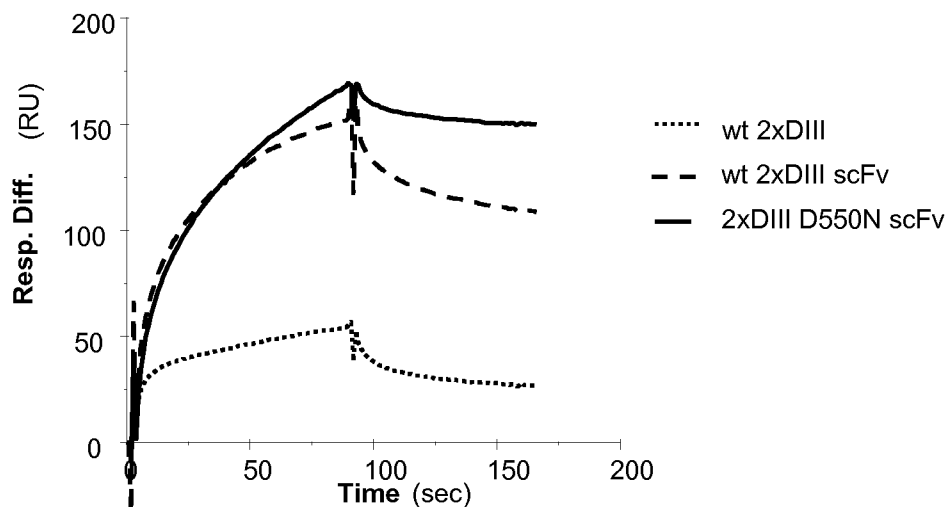
Figure 11**Figure 12****Figure 13**

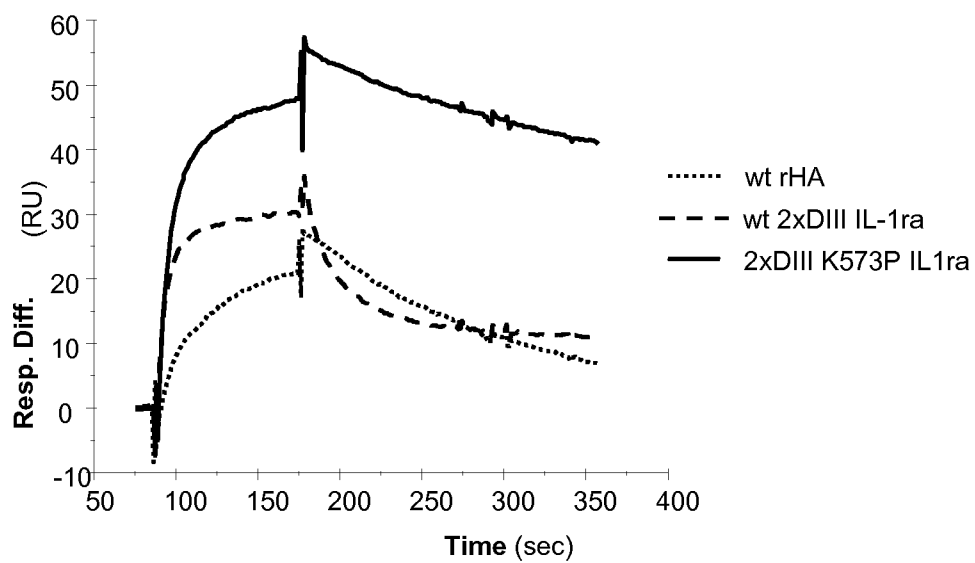
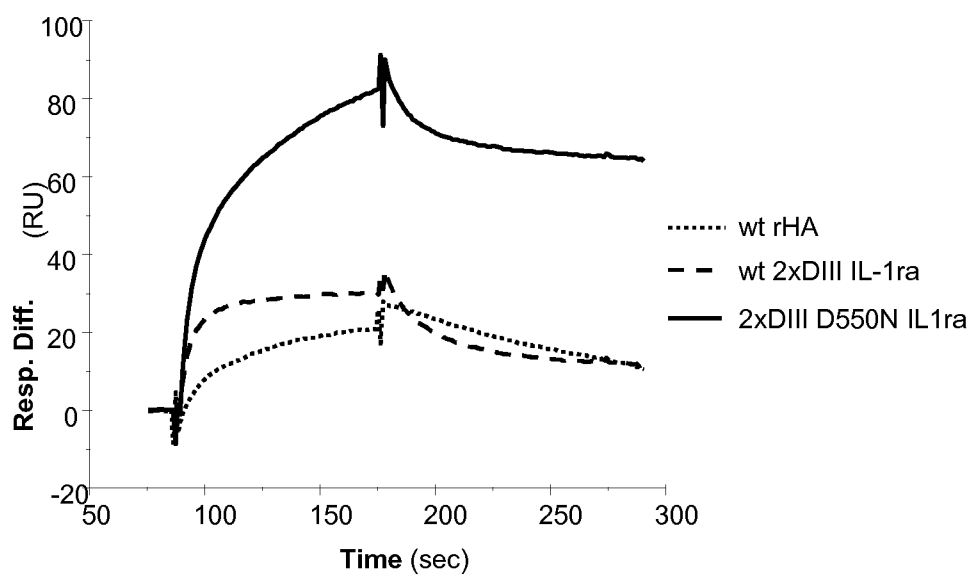
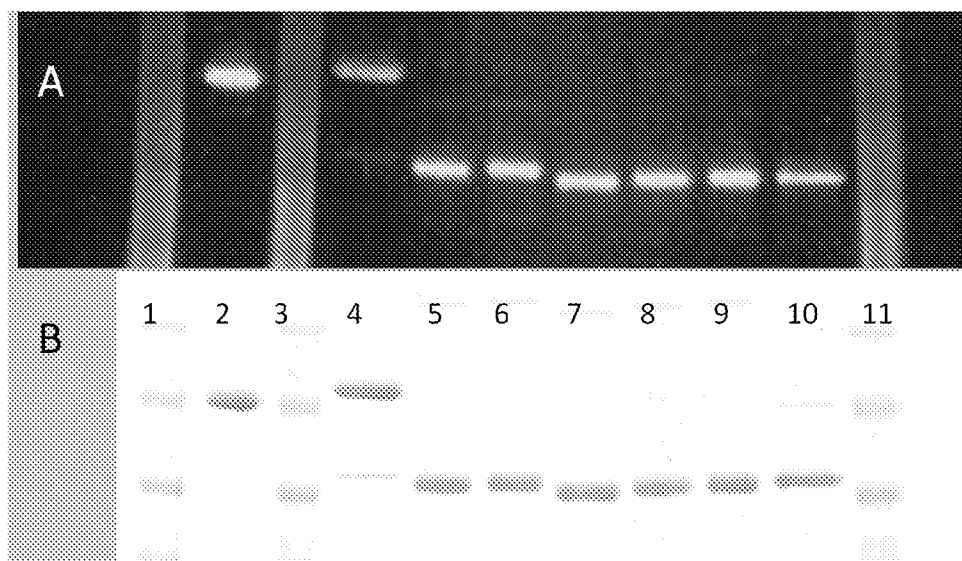
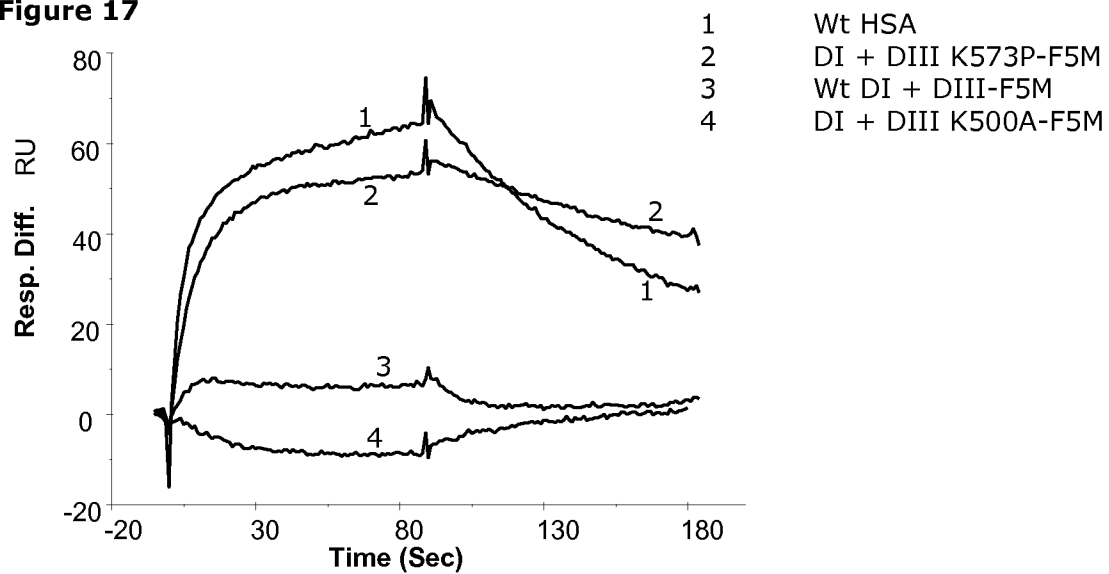
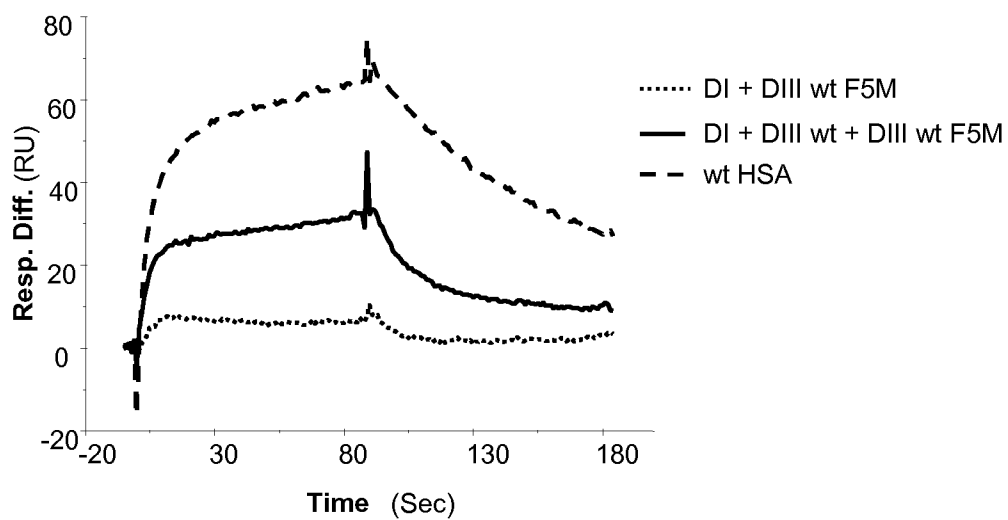
Figure 14**Figure 15**

Figure 16**Figure 17****Figure 18**

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/055577

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/765 C12N15/62
ADD.

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)

C12N C07K

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/060071 A2 (HUMAN GENOME SCIENCES INC [US]; DELTA BIOTECHNOLOGY LTD [GB]; PRINCIPI) 24 July 2003 (2003-07-24) the Figures, paragraphs 0154 and 1245, examples	1-11, 16-22
X	----- WO 2007/112940 A2 (ABLYNX NV [BE]; REVETS HILDE ADI PIERRETTE [BE]; HOOGENBOOM HENDRICUS) 11 October 2007 (2007-10-11) cited in the application the whole document -----	1-22



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search

22 June 2011

Date of mailing of the international search report

04/07/2011

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Authorized officer

Scheffzyk, Irmgard

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2011/055577

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☒ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/055577

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WO 2007112940	A2	11-10-2007	NONE
