SERPIN FUSION POLYPEPTIDES AND METHODS OF USE THEREOF

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 61/502055, filed June 28, 2011; U.S. Provisional Application No. 61/570394, filed December 14, 2011; and U.S. Provisional Application No. 61/577204, filed December 19, 2011; and U.S. Provisional Application No. 61/638168, filed April 25, 2012. The contents of each of these applications are hereby incorporated by reference in their entirety.

Field of the Invention

[0002] This invention relates to molecules, particularly polypeptides, more particularly fusion proteins that include a serpin polypeptide or an amino acid sequence that is derived from a serpin polypeptides and a second polypeptide. Additionally, the invention relates to fusion proteins that include a serpin polypeptide or an amino acid sequence that is derived from serpin polypeptides, a second polypeptide, and a third polypeptide. Specifically, this invention relates to fusion proteins that include at least one serpin polypeptide and a second polypeptide or fusion proteins that include at least one serpin polypeptide, a second polypeptide, and a third polypeptide, where the second and third polypeptides of the fusion proteins of the invention can be at least one the following: an Fc polypeptide or an amino acid sequence that is derived from an Fc polypeptide; a cytokine targeting polypeptide or a sequence derived from a cytokine targeting polypeptide; a WAP domain containing polypeptide or a sequence derived from a WAP containing polypeptide; or an albumin polypeptide or an amino acid sequence that is derived from a serum albumin polypeptide. This invention also relates to methods of using such molecules in a variety of therapeutic and diagnostic indications, as well as methods of producing such molecules.

Background of the Invention

[0003] Aberrant serine protease activity or an imbalance of protease-to-protease inhibitor can lead to protease-mediated tissue destruction and inflammatory responses. Accordingly, there exists a need for therapeutics and therapies that target aberrant serine protease activity and/or imbalance of protease-to-protease inhibitor. Furthermore, enhanced therapeutic effects may be gained through the attenuation of aberrant cytokine signaling and

serine protease activity. In addition, serpin proteins have demonstrated anti-infective activities while targeting inflammatory cytokines has been shown to increase the risk of infection. The fusion proteins of this invention have the potential to dampen inflammatory cytokine activity and limit the risk of infection.

Summary of the Invention

[0004] The fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin polypeptide (Polypeptide 1) and second polypeptide (Polypeptide 2). Additionally, the fusion proteins described herein include a serpin polypeptide or an amino acid sequence that is derived from a serpin polypeptide (Polypeptide 1), a second polypeptide (Polypeptide 2), and a third polypeptide (Polypeptide 3). As used interchangeably herein, the terms "fusion protein" and "fusion polypeptide" refer to a serpin polypeptide or an amino acid sequence derived from a serpin polypeptide operably linked to at least a second polypeptide or an amino acid sequence derived from at least a second polypeptide. The individualized elements of the fusion protein can be linked in any of a variety of ways, including for example, direct attachment, the use of an intermediate or a spacer peptide, the use of a linker region, the use of a hinge region or the use of both a linker and a hinge region. In some embodiments, the linker region may fall within the sequence of the hinge region, or alternatively, the hinge region may fall within the sequence of the linker region. Preferably, the linker region is a peptide sequence. For example, the linker peptide includes anywhere from zero to 40 amino acids, *e.g.*, from zero to 35 amino acids, from zero to 30 amino acids, from zero to 25 amino acids, or from zero to 20 amino acids. Preferably, the hinge region is a peptide sequence. For example, the hinge peptide includes anywhere from zero to 75 amino acids, *e.g.*, from zero to 70 amino acids, from zero to 65 amino acids or from zero to 62 amino acids. In embodiments where the fusion protein includes both a linker region and hinge region, preferably each of the linker region and the hinge region is a peptide sequence. In these embodiments, the hinge peptide and the linker peptide together include anywhere from zero to 90 amino acids, *e.g.*, from zero to 85 amino acids or from zero to 82 amino acids.

[0005] In some embodiments, the serpin polypeptide and the second polypeptide can be linked through an intermediate binding protein. In some embodiments, the serpin-based portion and second polypeptide portion of the fusion protein may be non-covalently linked.

[0006] In some embodiments, fusion proteins according to the invention can have one of the following formulae, in an N-terminus to C-terminus direction or in a C-terminus to N-terminus direction:

Polypeptide $1_{(a)}$ – $hinge_m$ – Polypeptide $2_{(b)}$

Polypeptide $1_{(a)}$ – $linker_n$ – Polypeptide $2_{(b)}$

Polypeptide $1_{(a)}$ – $linker_n$ – $hinge_m$ – Polypeptide $2_{(b)}$

Polypeptide $1_{(a)}$ – $hinge_m$ – $linker_n$ – Polypeptide $2_{(b)}$

Polypeptide $1_{(a)}$ – Polypeptide $2_{(b)}$– Polypeptide $3_{(c)}$

Polypeptide $1_{(a)}$ – $hinge_m$ – Polypeptide $2_{(b)}$– $hinge_m$ – Polypeptide $3_{(c)}$

Polypeptide $1_{(a)}$ – $linker_n$ – Polypeptide $2_{(b)}$– $linker_n$ – Polypeptide $3_{(c)}$

Polypeptide $1_{(a)}$ – $hinge_m$ – $linker_n$ – Polypeptide $2_{(b)}$–$hinge_m$ – $linker_n$ – Polypeptide $3_{(c)}$ Polypeptide $1_{(a)}$ – $linker_n$ – $hinge_m$ – Polypeptide $2_{(b)}$– $linker_n$ – $hinge_m$– Polypeptide $3_{(c)}$

where n is an integer from zero to 20, where m is an integer from 1 to 62 and where a, b, and c integers of at least 1. These embodiments include the above formulations and any variation or combination thereof. For example, the order of polypeptides in the formulae also includes Polypeptide $3_{(c)}$ – Polypeptide $1_{(a)}$– Polypeptide $2_{(b)}$, Polypeptide $2_{(b)}$ – Polypeptide $3_{(c)}$– Polypeptide $1_{(a)}$, or any variation or combination thereof.

[0007] In some embodiments, the Polypeptide 1 sequence includes a serpin polypeptide. Serpins are a group of proteins with similar structures that were first identified as a set of proteins able to inhibit proteases. Serpin proteins suitable for use in the fusion proteins provided herein include, by way of non-limiting example, alpha-1 antitrypsin (AAT), antitrypsin-related protein (SERPINA2), alpha 1-antichymotrypsin (SERPINA3), kallistatin (SERPINA4), monocyte neutrophil elastase inhibitor (SERPINB1), PI-6 (SERPINB6), antithrombin (SERPINC1), plasminogen activator inhibitor 1 (SERPINE1), alpha 2-antiplasmin (SERPINF2), complement 1-inhibitor (SERPING1), and neuroserpin (SERPINI1).

[0008] In some embodiments, the Polypeptide 1 sequence includes an alpha-1 antitrypsin (AAT) polypeptide sequence or an amino acid sequence that is derived from AAT. In some embodiments, the Polypeptide 1 sequence includes a portion of the AAT protein. In some embodiments, the Polypeptide 1 sequence includes at least the reactive site loop portion of the AAT protein. In some embodiments, the reactive site loop portion of the

AAT protein includes at least the amino acid sequence:
GTEAAGAMFLEAIPMSIPPEVKFNK SEQ ID NO:1).

[0009] In a preferred embodiment, the AAT polypeptide sequence or an amino acid sequence that is derived from AAT is or is derived from a human AAT polypeptide sequence.

[00010] In some embodiments, the fusion protein includes a full-length human AAT polypeptide sequence having the following amino acid sequence:

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  1 EDPQGDAAQK TDTSHHDQDH PTFNKITPNL AEFAFSLYRQ LAHQSNSTNI FFSPVSIATA
 61 FAMLSLGTKA DTHDEILEGL NFNLTEIPEA QIHEGFQELL RTLNQPDSQL QLTTGNGLFL
121 SEGLKLVDKF LEDVKKLYHS EAFTVNFGDT EEAKKQINDY VEKGTQGKIV DLVKELDRDT
181 VFALVNYIFF KGKWERPFEV KDTEEEDFHV DQVTTVKVPM MKRLGMFNIQ HCKKLSSWVL
241 LMKYLGNATA IFFLPDEGKL QHLENELTHD IITKFLENED RRSASLHLPK LSITGTYDLK
301 SVLGQLGITK VFSNGADLSG VTEEAPLKLS KAVHKAVLTI DEKGTEAAGA MFLEAIPMSI
361 PPEVKFNKPF VFLMIEQNTK SPLFMGKVVN PTQK (SEQ ID NO: 2)
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[00011] In some embodiments, the fusion protein includes a human AAT polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2.

[00012] In some embodiments, the AAT polypeptide sequence is, or the amino acid sequence derived from an AAT polypeptide is derived from, one or more of the human AAT polypeptide sequences shown in GenBank Accession Nos. AAB59495.1, CAJ15161.1, P01009.3, AAB59375.1, AAA51546.1, CAA25838.1, NP_001002235.1, CAA34982.1, NP_001002236.1, NP_000286.3, NP_001121179.1, NP_001121178.1, NP_001121177.1, NP_001121176.16, NP_001121175.1, NP_001121174.1, NP_001121172.1, and/or AAA51547.1.

[00013] In some embodiments, the fusion proteins contain one or more mutations. For example, the fusion protein contains at least one mutation at a methionine (Met) residue in the serpin portion of the fusion protein. In these Met mutations, the Met residue can be substituted with any amino acid. For example, the Met residue can be substituted with an amino acid with a hydrophobic side chain, such as, for example, leucine (Leu, L). Without wishing to be bound by theory, the Met mutation(s) prevent oxidation and subsequent inactivation of the inhibitory activity of the fusion proteins of the invention. In some embodiments, the Met residue can be substituted with a charged residue, such as, for example, glutamate (Glu, E). In some embodiments, the Met mutation is at position 358 of an AAT polypeptide. For example, the Met mutation is Met358Leu (M358L). In some

embodiments, the Met mutation is at position 351 of an AAT polypeptide. For example, the Met mutation is Met351Glu (M351E). In some embodiments, the Met mutation is at position 351 and at position 358 of an AAT polypeptide, for example, the Met mutation is Met351Glu (M351E) and Met358Leu (M358L). For example, the reactive site loop of this variant of the fusion AAT polypeptide has the following sequence: GTEAAGA**E**FLEAIP**L**SIPPEVKFNK (SEQ ID NO: 32). In some embodiments, the Met mutation is at position 351 and at position 358 of an AAT polypeptide, for example, the Met mutation is Met351Leu (M351L) and Met358Leu (M358L). For example, the reactive site loop of this variant of the fusion AAT polypeptide has the following sequence: GTEAAGA**L**FLEAIP**L**SIPPEVKFNK (SEQ ID NO: 33).

[00014] In some embodiments, the second polypetide (Polypeptide 2) of the serpin fusion protein is an Fc polypeptide or derived from an Fc polypeptide. These embodiments are referred to collectively herein as "serpin-Fc fusion proteins." The serpin-Fc fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin and an Fc polypeptide or an amino acid sequence that is derived from an Fc polypeptide. In some embodiments, the serpin-Fc fusion protein includes a single serpin polypeptide. In other embodiments, the serpin-Fc fusion proteins includes more than one serpin polypeptide, and these embodiments are collectively referred to herein as "serpin$_{(a')}$-Fc fusion protein," wherein (a') is an integer of at least 2. In some embodiments, each serpin polypeptides in a serpin$_{(a')}$-Fc fusion protein includes the same amino acid sequence. In other embodiments, each serpin polypeptide in a serpin$_{(a')}$-Fc fusion protein includes serpin polypeptides with distinct amino acid sequences. The serpin polypeptides of serpin$_{(a')}$-Fc fusion proteins can be located at any position within the fusion protein.

[00015] In some embodiments, the serpin polypeptide of the serpin-Fc fusion protein includes at least the amino acid sequence of the reactive site loop portion of the AAT protein. In some embodiments, the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:1. In some embodiments, the serpin polypeptide of the serpin-Fc fusion protein includes at least the amino acid sequence of a variant of the reactive site loop portion of the AAT protein. In some embodiments, the variant of the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:32 or SEQ ID NO:33. In some embodiments, the serpin

polypeptide of the serpin-Fc fusion protein includes at least the full-length human AAT polypeptide sequence having amino acid sequence of SEQ ID NO: 2. In some embodiments the serpin polypeptide of the serpin-Fc fusion protein includes human AAT polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2 or 32 or 33.

[00016] In some embodiments, the serpin polypeptide of the serpin-Fc fusion protein includes the AAT polypeptide sequence is or the amino acid sequence derived from an AAT polypeptide is derived from one or more of the human AAT polypeptide sequences shown in GenBank Accession Nos. AAB59495.1, CAJ15161.1, P01009.3, AAB59375.1, AAA51546.1, CAA25838.1, NP_001002235.1, CAA34982.1, NP_001002236.1, NP_000286.3, NP_001121179.1, NP_001121178.1, NP_001121177.1, NP_001121176.16, NP_001121175.1, NP_001121174.1, NP_001121172.1, and/or AAA51547.1.

[00017] In some embodiments, the Fc polypeptide of the fusion protein is a human Fc polypeptide, for example, a human IgG Fc polypeptide sequence or an amino acid sequence that is derived from a human IgG Fc polypeptide sequence. For example, in some embodiments, the Fc polypeptide is a human IgG1 Fc polypeptide or an amino acid sequence that is derived from a human IgG1 Fc polypeptide sequence. In some embodiments, the Fc polypeptide is a human IgG2 Fc polypeptide or an amino acid sequence that is derived from a human IgG2 Fc polypeptide sequence. In some embodiments, the Fc polypeptide is a human IgG3 Fc polypeptide or an amino acid sequence that is derived from a human IgG3 Fc polypeptide sequence. In some embodiments, the Fc polypeptide is a human IgG4 Fc polypeptide or an amino acid sequence that is derived from a human IgG4 Fc polypeptide sequence. In some embodiments, the Fc polypeptide is a human IgM Fc polypeptide or an amino acid sequence that is derived from a human IgM Fc polypeptide sequence.

[00018] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG1 Fc polypeptide sequence having the following amino acid sequence:

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  1 APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
 61 PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
121 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
181 TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK (SEQ ID NO: 3)
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[00019] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG1 Fc polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 3.

[00020] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide is mutated or modified to enhance FcRn binding. In these embodiments the mutated or modified Fc polypeptide includes the following mutations: Met252Tyr, Ser254Thr, Thr256Glu (M252Y, S256T, T256E) or Met428Leu and Asn434Ser (M428L, N434S) using the Kabat numbering system. In some embodiments the Fc polypeptide portion is mutated or otherwise modified so as to disrupt Fc-mediated dimerization. In these embodiments, the fusion protein is monomeric in nature.

[00021] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG2 Fc polypeptide sequence having the following amino acid sequence:

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  1 APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP
 61 REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL
121 PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT
181 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK (SEQ ID NO: 4)
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[00022] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG2 Fc polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 4.

[00023] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG3 Fc polypeptide sequence having the following amino acid sequence:

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  1 APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD GVEVHNAKTK
 61 PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKTK GQPREPQVYT
121 LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESSGQPENN YNTTPPMLDS DGSFFLYSKL
181 TVDKSRWQQG NIFSCSVMHE ALHNRFTQKS LSLSPGK (SEQ ID NO: 5)
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[00024] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG3 Fc polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 5.

[00025] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG4 Fc polypeptide sequence having the following amino acid sequence:

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  1 APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK
 61 PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT
121 LPPSQEEMTK NQVSLTCLVK GFYPDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSRLT
181 VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK (SEQ ID NO: 6)
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[00026] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgG4 Fc polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 6.

[00027] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgM Fc polypeptide sequence having the following amino acid sequence:

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  1 IAELPPKVSV FVPPRDGFFG NPRKSKLICQ ATGFSPRQIQ VSWLREGKQV GSGVTTDQVQ
 61 AEAKESGPTT YKVTSTLTIK ESDWLGQSMF TCRVDHRGLT FQQNASSMCV PDQDTAIRVF
121 AIPPSFASIF LTKSTKLTCL VTDLTTYDSV TISWTRQNGE AVKTHTNISE SHPNATFSAV
181 GEASICEDDW NSGERFTCTV THTDLPSPLK QTISRPKG (SEQ ID NO: 7)
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[00028] In some embodiments where the fusion protein of the invention includes an Fc polypeptide, the Fc polypeptide of the fusion protein includes a human IgM Fc polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 7.

[00029] In some embodiments of the fusion proteins provided herein, the second polypeptide (Polypeptide 2) of the serpin fusion protein is a cytokine targeting polypeptide or derived from a cytokine targeting polypeptide. These embodiments are referred to collectively herein as "serpin-cytokine targeting polypeptide fusion proteins." The serpin-cytokine targeting polypeptide fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin polypeptide and a cytokine targeting polypeptide, or derivation thereof. In some embodiments, the serpin-cytokine targeting polypeptide fusion protein includes a single serpin polypeptide. In other embodiments, the serpin-cytokine targeting polypeptide fusion protein includes more than

one serpin polypeptide, and these embodiments are collectively referred to herein as "serpin$_{(a')}$-cytokine targeting polypeptide fusion proteins," wherein (a') is an integer of at least 2. In some embodiments, each serpin polypeptide in a serpin$_{(a')}$-cytokine targeting polypeptide fusion protein includes the same amino acid sequence. In other embodiments, each serpin polypeptide of a serpin$_{(a)}$-cytokine targeting polypeptide fusion protein includes serpin polypeptides with distinct amino acid sequences.

[00030] In some embodiments, the cytokine targeting polypeptide of the serpin-cytokine targeting polypeptide fusion protein is a cytokine receptor or derived from a cytokine receptor. In a preferred embodiment, the cytokine targeting polypeptide or an amino acid sequence that is derived from the cytokine receptor is or is derived from a human cytokine receptor sequence. In other embodiments, the cytokine targeting polypeptide is an antibody or an antibody fragment, for example an anti-cytokine antibody or anti-cytokine antibody fragment. In a preferred embodiment, the cytokine targeting polypeptide or an amino acid sequence that is derived from the antibody or antibody fragment is derived from a chimeric, humanized, or fully human antibody sequence. The term antibody fragment includes single chain, Fab fragment, a F(ab')$_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

[00031] In other embodiments, the cytokine targeting polypeptide binds a cytokine receptor and prevents binding of a cytokine to the receptor. In other embodiments, the cytokine targeting polypeptide is an antibody or an antibody fragment, for example an anti-cytokine receptor antibody or anti-cytokine receptor antibody fragment.

[00032] In some embodiments, the serpin polypeptide of the serpin-cytokine targeting polypeptide fusion proteins includes at least the amino acid sequence of the reactive site loop portion of the AAT protein. In some embodiments, the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:1. In some embodiments, the serpin polypeptide of the serpin-cytokine targeting fusion proteins includes at least the amino acid sequence of a variant of the reactive site loop portion of the AAT protein. In some embodiments, the variant of the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:32 or SEQ ID NO:33. In some embodiments, the serpin polypeptide of the serpin-cytokine targeting fusion protein includes or is derived from at least the full-length human AAT polypeptide sequence having amino acid sequence of SEQ ID NO: 2. In some embodiments the serpin

polypeptide of the serpin-cytokine targeting fusion protein includes human AAT polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2 or 32 or 33.

[00033] In some embodiments, the serpin polypeptide of the serpin-cytokine targeting fusion protein includes an AAT polypeptide sequence or an amino acid sequence derived from an AAT polypeptide that is or is derived from one or more of the human AAT polypeptide sequences shown in GenBank Accession Nos. AAB59495.1, CAJ15161.1, P01009.3, AAB59375.1, AAA51546.1, CAA25838.1, NP_001002235.1, CAA34982.1, NP_001002236.1, NP_000286.3, NP_001121179.1, NP_001121178.1, NP_001121177.1, NP_001121176.16, NP_001121175.1, NP_001121174.1, NP_001121172.1, and/or AAA51547.1.

[00034] The serpin-cytokine targeting polypeptide fusion protein can incorporate a portion of the serpin-Fc fusion protein. For example, an antibody contains an Fc polypeptide. Therefore, in some embodiments where the cytokine targeting polypeptide is a cytokine-targeting antibody, the serpin-cytokine targeting polypeptide fusion protein will incorporate a portion of the serpin-Fc fusion protein. Furthermore, most receptor fusion proteins that are of therapeutic utility are Fc fusion proteins. Thus, in some embodiments, wherein the serpin-cytokine targeting polypeptide fusion protein is a serpin-cytokine receptor fusion protein, the serpin-cytokine targeting polypeptide fusion protein may incorporate an Fc polypeptide in addition to the serpin portion and the cytokine receptor portion.

[00035] In some embodiments, where the serpin-cytokine targeting polypeptide fusion protein includes an Fc polypeptide sequence, the Fc polypeptide sequence includes or is derived from the amino acid sequence of any one of SEQ ID NO: 3, 4, 5, 6, or 7. In some embodiments where the serpin-cytokine targeting fusion protein includes an Fc polypeptide sequence, the Fc polypeptide sequence has at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to any one of the amino acid sequence of SEQ ID NO: 3, 4, 5, 6, or 7. In some embodiments, the serpin polypeptide and the cytokine targeting polypeptide are operably linked via a linker region, for example, a glycine-serine linker or glycine-serine based linker. In some embodiments, the serpin polypeptide and the cytokine targeting polypeptide are operably linked via a hinge region. In some embodiments, the serpin polypeptide and the cytokine targeting polypeptide are

operably linked via a linker region and a hinge region. In other embodiments, the serpin polypeptide and the cytokine targeting polypeptide are directly attached.

[00036] In some embodiments of the fusion proteins provided herein, the second polypeptide (Polypeptide 2) of the serpin fusion protein is a whey acidic protein (WAP) domain containing polypeptide, or an amino acid sequence that is derived from a WAP domain containing polypeptide. These embodiments are referred to collectively herein as "serpin-WAP domain fusion proteins." The serpin-WAP domain fusion proteins include at least a serpin polypeptide or at least an amino acid sequence that is derived from a serpin, a WAP domain-containing polypeptide or an amino acid sequence that is derived from a WAP domain-containing polypeptide. In some embodiments, the serpin-WAP domain fusion protein includes a single serpin polypeptide. In other embodiments, the serpin-WAP targeting polypeptide fusion protein includes more than one serpin polypeptide. These embodiments are collectively referred to herein as "serpin$_{(a')}$-WAP domain fusion proteins," wherein (a') is an integer of at least 2. In some embodiments, serpin polypeptides of the serpin$_{(a')}$-WAP domain fusion protein includes the same amino acid sequence. In other embodiments, the serpin polypeptides of the serpin$_{(a')}$-cytokine targeting polypeptide fusion protein, includes serpin polypeptides with distinct amino acid sequences.

[00037] These serpin-WAP domain fusion proteins include a WAP domain containing polypeptide or polypeptide sequence that is or is derived from a WAP domain containing polypeptide. The WAP domain is an evolutionarily conserved sequence motif of 50 amino acids containing eight cysteines found in a characteristic 4-disulfide core arrangement (also called a four-disulfide core motif). The WAP domain sequence motif is a functional motif characterized by serine protease inhibition activity in a number of proteins.

[00038] WAP domain-containing polypeptides suitable for use in the fusion proteins provided herein include, by way of non-limiting example, secretory leukocyte protease inhibitor (SLPI), Elafin, and Eppin.

[00039] In some embodiments, the WAP domain-containing polypeptide sequence of the fusion protein includes a secretory leukocyte protease inhibitor (SLPI) polypeptide sequence or an amino acid sequence that is derived from SLPI. These embodiments are referred to herein as "serpin-SLPI-derived fusion proteins." In some embodiments, the SLPI polypeptide sequence comprises a portion of the SLPI protein, such as for example, the WAP2 domain or a sub-portion thereof. In a preferred embodiment, the SLPI

polypeptide sequence or an amino acid sequence that is derived from SLPI is or is derived from a human SLPI polypeptide sequence.

[00040] In some embodiments of the serpin-SLPI fusion proteins of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes a full-length human SLPI polypeptide sequence having the following amino acid sequence:

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  1 MKSSGLFPFL VLLALGTLAP WAVEGSGKSF KAGVCPPKKS AQCLRYKKPE CQSDWQCPGK
 61 KRCCPDTCGI KCLDPVDTPN PTRRKPGKCP VTYGQCLMLN PPNFCEMDGQ CKRDLKCCMG
121 MCGKSCVSPV KA (SEQ ID NO:8)
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[00041] In some embodiments of the serpin–SLPI fusion protein of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes a human SLPI polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 8.

[00042] In some embodiments of the serpin–SLPI fusion protein of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes a portion of the full-length human SLPI polypeptide sequence, where the portion has the following amino acid sequence:

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  1 SGKSFKAGVC PPKKSAQCLR YKKPECQSDW QCPGKKRCCP DTCGIKCLDP VDTPNPTRRK
 61 PGKCPVTYGQ CLMLNPPNFC EMDGQCKRDL KCCMGMCGKS CVSPVKA (SEQ ID NO: 9)
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[00043] In some embodiments of the serpin–SLPI fusion protein of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes a human SLPI polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 9.

[00044] In some embodiments of the serpin–SLPI fusion protein of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes the WAP2 domain of the full-length human SLPI polypeptide sequence, where the WAP2 domain has the following amino acid sequence:

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  1 TRRKPGKCPV TYGQCLMLNP PNFCEMDGQC KRDLKCCMGM CGKSCVSPVK A
(SEQ ID NO: 10)
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[00045] In some embodiments of the serpin–SLPI fusion protein of the invention, the SLPI sequence or a SLPI-derived sequence of the fusion protein includes a human SLPI

polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 10.

[00046] In some embodiments of the serpin–SLPI fusion proteins of the invention, the SLPI polypeptide sequence or the amino acid sequence derived from an SLPI polypeptide is or is derived from, one or more of the human SLPI polypeptide sequences shown in GenBank Accession Nos. CAA28187.1, NP_003055.1, EAW75869.1, P03973.2, AAH20708.1, CAB64235.1, CAA28188.1, AAD19661.1, and/or BAG35125.1.

[00047] In some embodiments of the serpin–SLPI fusion proteins of the invention, the SLPI polypeptide sequence or a SLPI-derived sequence of the fusion protein includes a human SLPI polypeptide sequence that is modified at a Methoine (Met) residue. In these Met mutations, the Met residue can be substituted with any amino acid. For example, the Met residue can be substituted with an amino acid with a hydrophobic side chain, such as, for example, leucine (Leu, L) or valine (Val, V). Without wishing to be bound by theory, the Met mutation(s) prevent oxidation and subsequent inactivation of the inhibitory activity of the fusion proteins of the invention. In some embodiments, the Met mutation is at position 98 of an SLPI polypeptide. For example, the modified SLPI polypeptide sequence of the serpin-SLPI includes mutations M98L or M98V in SEQ ID NO: 8.

[00048] In other embodiments, the WAP domain-containing polypeptide sequence of the fusion protein includes an elafin polypeptide sequence or an amino acid sequence that is derived from elafin. These embodiments are referred to herein as "serpin–elafin fusion proteins. In some embodiments, the elafin polypeptide sequence includes a portion of the elafin protein, such as for example, the WAP domain or a sub-portion thereof. In a preferred embodiment, the elafin polypeptide sequence or an amino acid sequence that is derived from elafin is or is derived from a human elafin polypeptide sequence.

[00049] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes a full-length human elafin polypeptide sequence having the following amino acid sequence:

```
1  MRASSFLIVV VFLIAGTLVL EAAVTGVPVK GQDTVKGRVP FNGQDPVKGQ VSVKGQDKVK
61 AQEPVKGPVS TKPGSCPIIL IRCAMLNPPN RCLKDTDCPG IKKCCEGSCG MACFVPQ
(SEQ ID NO: 11)
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[00050] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes a human elafin polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 11.

[00051] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes a portion of the full-length human elafin polypeptide sequence, where the portion has the following amino acid sequence:

```
 1 AVTGVPVKGQ DTVKGRVPFN GQDPVKGQVS VKGQDKVKAQ EPVKGPVSTK PGSCPIILIR
61 CAMLNPPNRC LKDTDCPGIK KCCEGSCGMA CFVPQ
 (SEQ ID NO: 12)
```

[00052] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes a human elafin polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 12.

[00053] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes the WAP domain of the full-length human elafin polypeptide sequence, where the WAP domain has the following amino acid sequence:

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  1 VSTKPGSCPI ILIRCAMLNP PNRCLKDTDC PGIKKCCEGS CGMACFVPQ
(SEQ ID NO: 13)
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[00054] In some embodiments of the serpin–elafin fusion proteins, the fusion protein includes a human elafin polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 13.

[00055] In some embodiments of the serpin–elafin fusion proteins, the elafin polypeptide sequence or the amino acid sequence derived from an elafin polypeptide is derived from one or more of the human elafin polypeptide sequences shown in GenBank Accession Nos. P19957.3, NP_002629.1, BAA02441.1, EAW75814.1, EAW75813.1, Q8IUB2.1, and/or NP_542181.1.

[00056] In other embodiments, the WAP domain-containing polypeptide sequence of the fusion protein includes an Eppin polypeptide sequence or an amino acid sequence that is derived from Eppin. These embodiments are referred to herein as "serpin$_{(a')}$–Eppin fusion proteins. In some embodiments, the Eppin polypeptide sequence of the serpin–Eppin fusion

protein includes a portion of the Eppin protein, such as for example, the WAP domain or a sub-portion thereof. In a preferred embodiment, the Eppin polypeptide sequence or an amino acid sequence that is derived from Eppin is or is derived from a human Eppin polypeptide sequence.

[00057] In some embodiments of the serpin–Eppin fusion proteins, the Eppin polypeptide sequence or amino acid sequence derived from an Eppin polypeptide is or is derived from one or more of the human Eppin polypeptide sequences shown in GenBank Accession Nos. O95925.1, NP_065131.1, AAH44829.2, AAH53369.1, AAG00548.1, AAG00547.1, and/or AAG00546.1.

[00058] In some embodiments, the serpin polypeptide of the serpin-WAP domain fusion protein includes at least the amino acid sequence of the reactive site loop portion of the AAT protein. In some embodiments, the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:1. In some embodiments, the serpin polypeptide of the serpin-WAP fusion protein includes at least the amino acid sequence of a variant of the reactive site loop portion of the AAT protein. In some embodiments, the variant of the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:32 or SEQ ID NO:33. In some embodiments, the serpin polypeptide of the serpin-WAP domain fusion protein includes at least the full-length human AAT polypeptide sequence having amino acid sequence of SEQ ID NO: 2. In some embodiments the serpin polypeptide of the serpin-WAP domain fusion protein includes human AAT polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2 or 32 or 33.

[00059] In some embodiments, the serpin polypeptide of the serpin-WAP domain fusion protein includes the AAT polypeptide sequence is, or the amino acid sequence derived from an AAT polypeptide is derived from, one or more of the human AAT polypeptide sequences shown in GenBank Accession Nos. AAB59495.1, CAJ15161.1, P01009.3, AAB59375.1, AAA51546.1, CAA25838.1, NP_001002235.1, CAA34982.1, NP_001002236.1, NP_000286.3, NP_001121179.1, NP_001121178.1, NP_001121177.1, NP_001121176.16, NP_001121175.1, NP_001121174.1, NP_001121172.1, and/or AAA51547.1.

[00060] In some embodiments, the serpin-WAP domain fusion protein can also include an Fc polypeptide or an amino acid sequence that is derived from an Fc polypeptide.

These embodiments are referred to collectively herein as "serpin-Fc-WAP domain fusion proteins." In these embodiments, no particular order is to be construed by this terminology. For example, the order of the fusion protein can be serpin-Fc-WAP domain, serpin-WAP domain-Fc, or any variation combination thereof. The serpin-Fc-WAP domain fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin, WAP domain-containing polypeptide or an amino acid sequence that is derived from a WAP domain-containing polypeptide, and an Fc polypeptide or an amino acid sequence that is derived from an Fc polypeptide.

[00061] In some embodiments, where the serpin-WAP domain fusion protein includes an Fc polypeptide sequence, the Fc polypeptide sequence can have the amino acid sequence of SEQ ID NO: 3-7. In other embodiments, where the serpin-WAP domain fusion protein includes an Fc polypeptide sequence, the Fc polypeptide sequence can have at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NOs. 3-7. In some embodiments, the serpin-WAP domain fusion protein can also include an albumin polypeptide, or an amino acid sequence that is derived from an albumin polypeptide. These embodiments are referred to collectively herein as "serpin-albumin-WAP domain fusion proteins." In these embodiments, no particular order is to be construed by this terminology. For example, the order of the fusion protein can be serpin-albumin-WAP domain, serpin-WAP domain-albumin, or any variation combination thereof. The serpin-albumin-WAP domain fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin, WAP domain-containing polypeptide, or an amino acid sequence that is derived from a WAP domain-containing polypeptide, and an albumin polypeptide, or an amino acid sequence that is derived from an albumin polypeptide.

[00062] In some embodiments where the serpin-WAP domain fusion protein includes an albumin polypeptide sequence, the albumin polypeptide sequence includes the amino acid sequence of SEQ ID NO: 14-15, described herein. In other embodiments, where the serpin-WAP domain fusion protein includes an albumin polypeptide sequence, the albumin polypeptide sequence has at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the any one of the amino acid sequences having SEQ ID NO: 14 or 15.

[00063] In some embodiments, the second polypeptide (Polypeptide 2) of the serpin fusion protein is an albumin polypeptide or is derived from an albumin polypeptide. These

embodiments are referred to collectively herein as "serpin$_{(a')}$-albumin fusion proteins." The serpin-albumin fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin and an albumin polypeptide or an amino acid sequence that is derived from an albumin polypeptide. In addition this invention relates to serpin albumin binding polypeptide fusion proteins, wherein the albumin is operably linked to the serpin via an intermediate binding molecule. Herein, the serpin is non-covalently or covalently bound to human serum albumin.

[00064] In embodiments where the fusion protein of the invention includes an albumin polypeptide sequence, the albumin polypeptide sequence of the fusion protein is a human serum albumin (HSA) polypeptide or an amino acid sequence derived from HSA. In some embodiments, the fusion protein includes a HSA polypeptide sequence having the following amino acid sequence:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAEN
CDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDV
MCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLD
ELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTE
CCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSL
AADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADP
HECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVS
RNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCF
SALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMD
DFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 14)

[00065] In embodiments where the fusion protein of the invention includes an albumin polypeptide sequence, the albumin polypeptide sequence of the fusion protein includes a human serum albumin polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 14.

[00066] In embodiments where the fusion protein of the invention includes an albumin polypeptide sequence, the albumin polypeptide sequence of the fusion protein fusion protein includes a domain 3 of human serum albumin polypeptide sequence having the following amino acid sequence:

EEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCK
HPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVP
KEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC
KADDKETCFAEEGKKLVA (SEQ ID NO: 15)

[00067] In embodiments where the fusion protein of the invention includes an albumin polypeptide sequence, the albumin polypeptide sequence of the fusion protein includes a human serum albumin polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 15.

[00068] In some embodiments where the fusion protein of the invention includes an albumin polypeptide sequence, the fusion protein is linked to the human serum albumin via an intermediate albumin binding polypeptide. The albumin binding polypeptide can be an antibody or an antibody fragment or derived from an antibody or antibody fragment. In a preferred embodiment, the albumin binding polypeptide or an amino acid sequence that is derived from the antibody or antibody fragment is derived from a chimeric, humanized, or fully human antibody sequence. The term antibody fragment includes single chain, Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In addition, the albumin binding polypeptide can be an albumin binding peptide. Another embodiment of the invention is a serpin albumin binding polypeptide fusion, wherein the albumin binding polypeptide is domain 3 of *Streptococcal* protein G or a sequence derived from domain 3 of *Streptococcal* protein G.

[00069] In some embodiments, the serpin polypeptide of the $serpin_{(a')}$-albumin fusion proteins includes at least the amino acid sequence of the reactive site loop portion of the AAT protein. In some embodiments, the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:1. In some embodiments, the serpin polypeptide of the serpin-albumin fusion protein includes at least the amino acid sequence of a variant of the reactive site loop portion of the AAT protein. In some embodiments, the variant of the reactive site loop portion of the AAT protein includes at least the amino acid sequence of SEQ ID NO:32 or SEQ ID NO:33. In some embodiments, the serpin polypeptide of the serpin-albumin fusion proteins includes at least the full-length human AAT polypeptide sequence having amino acid sequence of SEQ ID NO: 2. In some

embodiments the serpin polypeptide of the serpin-albumin fusion proteins includes human AAT polypeptide sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 2 or 32 or 33.

[00070] In some embodiments, the serpin polypeptide of the serpin-albumin fusion proteins includes the AAT polypeptide sequence or the amino acid sequence derived from an AAT polypeptide is or is derived from one or more of the human AAT polypeptide sequences shown in GenBank Accession Nos. AAB59495.1, CAJ15161.1, P01009.3, AAB59375.1, AAA51546.1, CAA25838.1, NP_001002235.1, CAA34982.1, NP_001002236.1, NP_000286.3, NP_001121179.1, NP_001121178.1, NP_001121177.1, NP_001121176.16, NP_001121175.1, NP_001121174.1, NP_001121172.1, and/or AAA51547.1.

[00071] In some embodiments, the fusion proteins are modified to increase or otherwise inhibit proteolytic cleavage, for example, by mutating one or more proteolytic cleavage sites. In some embodiments, the fusion proteins are modified to alter or otherwise modulate an Fc effector function of the fusion protein, while simultaneously retaining binding and inhibitory function as compared to an unaltered fusion protein. Fc effector functions include, by way of non-limiting examples, Fc receptor binding, prevention of proinflammatory mediator release upon binding to the Fc receptor, phagocytosis, modified antibody-dependent cell-mediated cytotoxicity (ADCC), modified complement-dependent cytotoxicity (CDC), modified glycosylation at Asn297 residue (EU index of Kabat numbering, *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*) of the Fc polypeptide. In some embodiments, the fusion proteins are mutated or otherwise modified to influence Fc receptor binding. In some embodiments, the Fc polypeptide is modified to enhance FcRn binding. Examples of Fc polypeptide mutations that enhance binding to FcRn are Met252Tyr, Ser254Thr, Thr256Glu (M252Y, S256T, T256E) (Kabat numbering, Dall'Acqua *et al* 2006, *J. Biol Chem* Vol 281(33) 23514–23524), or Met428Leu and Asn434Ser (M428L, N434S) (Zalevsky *et al* 2010 *Nature Biotech*, Vol. 28(2) 157-159). (EU index of *Kabat et al* 1991 *Sequences of Proteins of Immunological Interest*). In some embodiments the Fc polypeptide portion is mutated or otherwise modified so as to disrupt Fc-mediated dimerization (Ying *et al* 2012 *J. Biol Chem* 287(23): 19399–19408). In these embodiments, the fusion protein is monomeric in nature.

[00072] The fusion proteins and variants thereof provided herein exhibit inhibitory activity, for example by inhibiting a serine protease such as human neutrophil elastase (NE), a chemotrypsin-fold serine protease that is secreted by neutrophils during an inflammatory response. The fusion proteins provided herein completely or partially reduce or otherwise modulate serine protease expression or activity upon binding to, or otherwise interacting with, a serine protease, *e.g.*, a human serine protease. The reduction or modulation of a biological function of a serine protease is complete or partial upon interaction between the fusion proteins and the human serine protease protein, polypeptide and/or peptide. The fusion proteins are considered to completely inhibit serine protease expression or activity when the level of serine protease expression or activity in the presence of the fusion protein is decreased by at least 95%, *e.g.*, by 96%, 97%, 98%, 99% or 100% as compared to the level of serine protease expression or activity in the absence of interaction, e.g., binding, with a fusion protein described herein. The fusion proteins are considered to partially inhibit serine protease expression or activity when the level of serine protease expression or activity in the presence of the fusion protein is decreased by less than 95%, *e.g.*, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of serine protease expression or activity in the absence of interaction, e.g., binding, with a fusion protein described herein.

[00073] The fusion proteins described herein are useful in a variety of therapeutic, diagnostic and prophylactic indications. For example, the fusion proteins are useful in treating a variety of diseases and disorders in a subject. In some embodiments, the serpin fusion proteins, including, fusion proteins described herein, are useful in treating, alleviating a symptom of, ameliorating and/or delaying the progression of a disease or disorder in a subject suffering from or identified as being at risk for a disease or disorder selected from alpha-1-antitrypsin (AAT) deficiency, emphysema, chronic obstructive pulmonary disease (COPD), acute respiratory distress sydrome (ARDS), allergic asthma, cystic fibrosis, cancers of the lung, ischemia-reperfusion injury, including, *e.g.*, ischemia/reperfusion injury following cardiac transplantation, myocardial infarction, rheumatoid arthritis, septic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, type I and/or type II diabetes, bacterial infections, fungal infections, viral infections, pneumonia, sepsis, graft versus host disease (GVHD), wound healing, Systemic lupus erythematosis, and Multiple sclerosis.

[00074] Pharmaceutical compositions according to the invention include a fusion protein of the invention, including modified fusion proteins and other variants, along with a suitable carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

Brief Description of the Drawings

[00075] Figure 1A is a schematic representation of some embodiments of serpin-Fc fusion proteins according to the invention. The serpin can be located at any position within the fusion protein. Serpin-Fc fusion protein incorporating more than one serpin polypeptide are also represented. Figure 1B is a photograph of a SDS-PAGE gel showing serum derived AAT (lane 1), AAT-Fc1 (lane 2, human IgG1 Fc), and AAT-EL-Fc1 (lane 3, Met351Glu, Met358Leu mutations within AAT, human IgG1 Fc). Figure 1C is a graph showing the inhibition of neutrophil elastase activity by AAT-Fc fusion proteins. Figure 1D is a photograph of a SDS-PAGE gel showing tetravalent AAT-Fc-AAT, having two AAT polypeptides per Fc polypeptide. Figure 1E is a graph showing the inhibition of neutrophil elastase activity by a tetravalent AAT-Fc-AAT fusion protein. Figure 1F is a graphing demonstrating the effect of low pH elution from protein A resin, wherein the NE inhibiting capacity of the AAT-Fc fusion protein eluted at low pH is drastically reduced. Figure 1G is a graph showing that the double mutant, AAT-EL-Fc (Met351Glu, Met358Leu mutations) is resistant to H_2O_2 inactivation (conc.), compared to wild type AAT and the single mutant AAT-EM-Fc (Met351Glu). Figure 1H is a graph depicting the serum clearance rates of serum derived AAT (sdAAT) compared to AAT-Fc in rats dosed with 10mg/kg protein (3 rats/test protein). The half life of AAT-Fc is substantially longer than that of sdAAT.

[00076] Figure 2A is a schematic representation of some embodiments of the serpin-cytokine targeting fusion proteins of the invention. The serpin can be fused to either the heavy chain, the light chain, or both of an antibody. Serpin-cytokine receptor fusion proteins are also depicted. Figure 2B is a photograph of a SDS-PAGE gel showing the D2E7 antibody (lane 1), and the D2E7 antibody with-AAT fused to heavy chain (lane 2). Figure 2C is a graph showing the inhibition of neutrophil elastase activity by a D2E7 antibody fused to AAT. Serum derived AAT is shown as a positive control, whereas the D2E7 antibody alone is shown as a negative control for NE inhibition.

[00077] Figure 3A is a schematic representation of some embodiments of the serpin-Fc-WAP fusion proteins. Figure 3B is a photograph of a SDS-PAGE gel showing AAT-Fc-ELAFIN (lane 1) and AAT-Fc-SLPI (lane 2). Figure 3C is a graph showing the inhibition of neutrophil elastase activity by an AAT-Fc-ELAFIN fusion protein and an AAT-Fc-SLPI fusion protein. An AAT-Fc fusion protein and serum derived AAT are included for comparison.

[00078] Figure 4A is a schematic representation of some embodiments of the AAT-HSA fusion proteins. Figure 4B is a photograph of a SDS-PAGE gel showing an AAT-HSA fusion. Figure 4C is a graph showing the inhibition of neutrophil elastase activity by an AAT-HSA compared to serum derived AAT.

Detailed Description of the Invention

[00079] Human neutrophil elastase (NE) is a chymotrypsin-fold serine protease, secreted by neutrophils during inflammation. Aberrant activity of NE results in a progressive degradation of elastin tissues and the slow destruction of the alveolar structures of the lungs leading to emphysema and lung fibrosis (Lungarella *et al* 2008 *Int. J. Biochem Cell Biol* 40:1287). Often, misguided NE activity is due to an imbalance of the protease with its natural inhibitor, alpha1-antitrypsin (AAT). This imbalance can result from enhanced neutrophil infiltration into the lungs, as observed in the lungs of smokers and patients with Cystic Fibrosis (CF), or Acute Respiratory Distress Syndrome (ARDS). Conversely, a deficiency of AAT, usually as a result of a point mutation that causes ATT to aggregate and accumulate in the liver, leaves the lungs exposed to unchecked NE activity. Individuals with AAT deficiencies are at increased the risk of emphysema, COPD, liver disease, and numerous other conditions.

[00080] AAT deficiency affects approximately 100,000 Americans (according to estimates from the Alpha-1 Foundation), and many of the afflicted people die in their 30's and 40's. There are currently only a few FDA-approved drugs for treatment of ATT deficiency (Prolastin®, Aralast™, Zemaira®, Glassia™). Each drug is the natural AAT derived from pooled human plasma, which appears to be insufficient to meet the anticipated clinical demand. Furthermore, these products have short serum half lives ($T_{1/2}$ of approximately 5 days) and require high dose (60 mg/kg body weight) weekly infusions. The current market for these drugs is estimated at approximately $400 million. The market for

AAT-like drugs is likely substantially larger, based on the estimation that as many as 95% of individuals with AAT-deficiencies go undiagnosed, and the fact that these drugs have the potential to be effective therapies for pathologies characterized by enhanced NE activity in individuals that are not AAT-deficient (*e.g.*, cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), smoking-induced emphysema and/or COPD).

[00081] AAT has been suggested to have broad spectrum anti-inflammatory activity (Tilg *et al* 1993 *J Exp Med* 178:1629–1636, Libert *et al* 1996 *Immunol* 157:5126–5129, Pott *et al*, Journal of Leukocyte Biology 85 2009, Janciauskiene *et al* 2007 *J. Biol Chem* 282(12): 8573–8582, Nita *et al* 2007 *Int J Biochem* Cell Biol 39:1165–1176). Recently, evidence has mounted that AAT may be useful in treating numerous human pathologies, outside of the commonly suggested inflammatory pulmonary conditions. Human AAT has shown to protect mice from clinical and histopathological signs of experimental autoimmune encephalomyelitis (EAE), suggesting it could be a potential treatment of autoimmune diseases, such as multiple sclerosis or systemic lupus erythematosus (SLE) (Subramanian *et al* 2011 Metab Brain Dis 26:107–113). Serum AAT has shown activity in rodent models of Graft Versus Host Disease (GVHD) (Tawara *et al* 2011 *Proc. Natl. Acad. Sci. USA* 109: 564–569, Marcondes *et al* 2011 *Blood* Nov 3;118(18):5031-9), which has lead to a human clinical trial using AAT to treat individuals with Steroid Non-responsive Acute GVHD (NCT01523821). Additionally, AAT has been effective in animal models of type I and type II diabetes, dampening inflammation, protecting islet cells from apoptosis and enabling durable islet cell allograft (Zhang *et al* 2007 *Diabetes* 56:1316–1323, Lewis *et al* 2005 *Proc Natl Acad Sci USA* 102:12153–12158, Lewis *et al* 2008 *Proc Natl Acad Sci USA* 105:16236–16241, Kalis *et al* 2010 *Islets* 2:185–189). Currently, there are numerous early human clinical trials of type I diabetes using serum derived AAT products (NCT01183468, NCT01319331, NCT01304537).

[00082] The current serum-derived AAT products undergo extensive purification and testing to ensure the removal of pathogenic viruses, however, the risk of transmission of infectious agents cannot be completely eliminated. Moreover, serum is limited, which limits the production capacity of serum derived AAT. Attempts to address the concerns of serum derived products and production issues have been aimed at the expression of recombinant AAT. However, after 20 years of work, the generation of a therapeutically viable recombinant AAT has yet to reach the market (Karnaukhova et al 2006 Amino Acids

30: 317). Like the plasma-derived products, recombinant versions of AAT suffer from short serum half-lives, low production yields, and poor lung distribution.

[00083] The fusion proteins of the present invention have enhanced functionalities compared to the unmodified AAT molecule. The fusion of an AAT polypeptide with a second polypeptide that interacts with the neonatal Fc receptor (FcRn), serves to increase the serum half life, providing a much needed dosing benefit for patients. These FcRn interacting polypeptides of the fusion protein include immunoglobulin (Ig) Fc polypeptides derived from human IgG1, IgG2, IgG3, IgG4, or IgM, and derivatives of human albumin. In some embodiments, the fusion protein incorporates mutations with the AAT portion that render the molecule more resistant to inactivation by oxidation. For example Met351Glu, Met358Leu (AAT-EL-Fc), demonstrates resistance inactivation by H_2O_2 oxidation (Figure 1G). While AAT is a natural anti-inflammatory protein, some embodiments of the invention provide enhanced inflammation dampening capacity through the fusion of an AAT polypeptide and a cytokine targeting polypeptide. The coupling of dual anti-inflammatory functionalities from AAT and a second polypeptide, will provide more a potent therapeutic protein than either polypeptide on their own. Additionally, the coupling the anti-infective activity of AAT will mitigate the infection risk of most cytokine targeting biologics. Some embodiments provide for more potent anti-inflammatory and anti-infective proteins through the fusion an AAT-polypeptide and WAP domain contain polypeptide. The fusion proteins of the present invention are expected to be a great therapeutic utility and be superior to the current serum derived AAT products.

[00084] To extend the half life of recombinant AAT, recombinant DNA technology was used to create a AAT gene fusion with the Fc domain of human IgG1, IgG2, IgG3, IgG4, IgM, or HSA, such that the expected protein product would be AAT followed by an Fc domain ((AAT-Fc (IgG1), AAT-Fc (IgG2), AAT-Fc (IgG3), AAT-Fc (IgG4), AAT-Fc (IgM)) or AAT followed by HSA. While it was known that fusion of Fc domains of HSA to some proteins, protein domains or peptides could extend their half-lives (*see e.g.*, Jazayeri *et al.* BioDrugs *22*, 11-26, Huang *et al.* (2009) Curr Opin Biotechnol *20*, 692-699, Kontermann *et al.* (2009) BioDrugs *23*, 93-109, Schmidt *et al.* (2009) Curr Opin Drug Discov Devel *12*, 284-295), it was unknown if an Fc domain or HSA fused to AAT would allow for proper folding and maintenance of NE inhibitory activity, or could extend the half-life of recombinant AAT. The fusion proteins of the present invention are shown to be potent inhibitors of NE, have extended serum half lives, and in some embodiments resistant

to oxidation. In other embodiments, the fusion proteins described herein have distinct properties by the incorporation of other functional polypeptides, including cytokine targeting polypeptides, and WAP domain containing polypeptides.

[00085] The fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin and a second polypeptide. In some embodiments, for example, the invention provides a serpin polypeptide fused to human IgG1-Fc, IgG2-Fc, IgG3-Fc, IgG4-Fc, IgM-Fc, or HSA derivatives. The serpin-fusion described herein are expected to be useful in treating a variety of indications, including, by way of non-limiting example, alpha-1-antitrypsin (AAT) deficiency, emphysema, chronic obstructive pulmonary disease (COPD), acute respiratory distress sydrome (ARDS), allergic asthma, cystic fibrosis, cancers of the lung, ischemia-reperfusion injury, including, *e.g.*, ischemia/reperfusion injury following cardiac transplantation, myocardial infarction, rheumatoid arthritis, septic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, type I and/or type II diabetes, bacterial infections, fungal infections, viral infections, pneumonia, sepsis, graft versus host disease (GVHD), wound healing, Systemic lupus erythematosis, and Multiple sclerosis.

[00086] In some embodiments, the fusion proteins described herein include at least an alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and second polypeptide. For example, the invention provides alpha-1-antitrypsin (AAT) fused to human IgG1-Fc, IgG2-Fc, IgG3-Fc, IgG4-Fc, IgM-Fc, or HSA derivatives.

[00087] In some embodiments, the fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin polypeptide and a cytokine targeting polypeptide or an amino acid sequence that is derived from a cytokine targeting polypeptide. For example, the invention provides serpin polypeptide or a sequence derived from a serpin polypeptide fused to a human cytokine receptor or derivative thereof. Another embodiment of the invention provides serpin polypeptide or a sequence derived from a serpin polypeptide fused to a cytokine targeting antibody, *e.g.*, an anti-cytokine antibody, or a sequence derived from of a cytokine targeting antibody, *e.g.*, an anti-cytokine antibody, or sequence derived from a fragment of cytokine targeting antibody, *e.g.*, a fragment of an anti-cytokine antibody. For example, the invention provides a serpin polypeptide or a sequence derived from a serpin polypeptide fused to a cytokine targeting polypeptide in which the cytokine targeting polypeptide binds any of the following human

cytokines: TNFα, IgE, IL-12, IL-23, IL-6, IL-1α, IL-1β, IL-17, IL-13, IL-4, IL-10, IL-2, IL-18, IL-27, or IL-32.

[00088] For example, in some embodiments, the cytokine targeting polypeptide targets TNFα and includes any of the following TNFα-targeting polypeptide or sequences derived from the following TNFα-targeting polypeptides: Remicade®, Humira®, Simponi®, Cimiza®, Enbrel® or ATN-103 and ATN-192.

[00089] For example, in some embodiments, the cytokine targeting polypeptide targets IgE and includes any of the following IgE-targeting polypeptide or sequences derived from the following IgE-targeting polypeptides: Xolair or FcεRI.

[00090] For example, in some embodiments, the cytokine targeting polypeptide targets the shared p40 subunit of IL-12 and IL-23 and includes the Stelara® polypeptide or sequences derived from the Stelara® polypeptide.

[00091] For example Stelara® the cytokine targeting polypeptide targets IL-13 and includes the CDP7766 polypeptide or sequences derived from the CDP7766 polypeptide.

[00092] In some embodiments, the fusion proteins described herein include at least a alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and a cytokine targeting polypeptide or an amino acid sequence that is derived from a cytokine targeting polypeptide. For example, the invention provides alpha-1-antitrypsin inhibitor (AAT) fused a cytokine targeting polypeptide in which the cytokine targeting polypeptide binds any of the following human cytokines: TNFα, IgE, IL-6, IL-1α, IL-1β, IL-12, IL-17, IL-13, IL-23, IL-4, IL-10, IL-2, IL-18, IL-27, or IL-32.

[00093] In some embodiments the cytokine targeting polypeptide binds a cytokine receptor and prevents binding of the cytokine. For example, the present invention includes a serpin fused to a cytokine receptor targeting antibody. For example, the invention provides alpha-1-antitrypsin inhibitor (AAT) fused a cytokine targeting polypeptide in which the cytokine targeting polypeptide binds the receptor of any of the following human cytokines: TNFα, IgE, IL-6, IL-1α, IL-1β, IL-12, IL-17, IL-13, IL-23, the p40 subunit of IL-12 and IL-23, IL-4, IL-10, IL-2, IL-18, IL-27, or IL-32.

[00094] For example, in some embodiments, the cytokine targeting polypeptide targets the IL-6 receptor and includes the Actemra® polypeptide (as described in patent publication EP0628639), or the ALX-0061 polypeptide (as described in WO2010/115998), or sequences derived from the Actemra® polypeptide, or ALX-0061 polypeptide.

[00095] For example, Actemra® the cytokine targeting polypeptide targets the IL-6 receptor and includes the tocilizumab polypeptide or sequences derived from the tocilizumab polypeptide.

[00096] The targeting of inflammatory cytokines and immune-stimulating agents by protein therapeutics has demonstrated clinical success in numerous inflammatory conditions. The most common proteins used as cytokine targeting agents are the soluble cytokine receptors and monoclonal antibodies and fragments thereof. A significant drawback with targeting cytokines is the increased risk of infection in these patients, as evidenced by the TNFα targeting biologics, Remicade®, Humira®, Simponi®, Cimiza®, and Enbrel®, and the IL-12/23 p40 targeting antibody, Stelara®. This is likely to be a common problem of targeting inflammatory cytokines leading to immune suppression in patients. AAT and other serpin proteins are interesting in that they demonstrate both anti-infective and anti-inflammatory activities. Thus, the serpin-cytokine targeting polypeptide fusion proteins of this invention can dampen aberrant cytokine activities while alleviating the risk of infections.

[00097] In some embodiments, the fusion proteins described herein include a serpin polypeptide or an amino acid sequence that is derived from a serpin, a WAP domain-containing polypeptide or an amino acid sequence that is derived from a WAP domain-containing polypeptide, and an Fc polypeptide or an amino acid sequence that is derived from an Fc polypeptide. For example, the invention provides a serpin polypeptide, a WAP domain-containing polypeptide and human IgG1-Fc, IgG2-Fc, IgG3-Fc, IgG4-Fc or IgM-Fc derivatives operably linked together in any functional combination. In some embodiments, the WAP domain containing protein is human SLPI or derived from human SLPI. In other embodiments, the WAP domain containing protein is human ELAFIN or derived from human ELAFIN. In some embodiments, the fusion proteins described herein include at least an alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and a SLPI polypeptide or an amino acid sequence that is derived from SLPI. In some embodiments, the fusion proteins described herein include at least an AAT polypeptide or an amino acid sequence that is derived from AAT and an ELAFIN polypeptide or an amino acid sequence that is derived from Elafin.

[00098] SPLI and Elafin are WAP domain containing proteins that display serine protease inhibitory activity. Both of these proteins are anti-inflammatory in function. In

addition these proteins possess broad anti-infective capacities toward numerous strains of bacteria, viruses, and fungi.

[00099] In some embodiments, the fusion proteins described herein include at least a serpin polypeptide or an amino acid sequence that is derived from a serpin and a human serum albumin (HSA) polypeptide or an amino acid sequence that is derived from a HSA polypeptide. Further embodiments of invention include serpin-albumin binding polypeptide fusion proteins, wherein the albumin binding polypeptide is responsible for the association of the serpin and HSA. Thereby the invention includes both covalent and non-covalent linkages of the serpin polypeptide and the HSA polypeptide or sequences derived from the serpin polypeptide or a HSA polypeptide. For example, the invention provides a serpin polypeptide fused to human HSA, or HSA derivatives, or HSA binding peptide or polypeptides.

[000100] In some embodiments, the fusion proteins described herein include at least an alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and a HSA polypeptide or an amino acid sequence that is derived from a HSA polypeptide. For example, the invention provides alpha-1-antitrypsin (AAT) fused to HSA or a fragment derived from HSA, or an albumin binding polypeptide.

[000101] In some embodiments, the fusion proteins described herein include a serpin polypeptide or an amino acid sequence that is derived from a serpin, a HSA polypeptide or or an amino acid sequence that is derived from a HSA polypeptide, and a WAP domain-containing polypeptide or an amino acid sequence that is derived from a WAP domain-containing polypeptide. In some embodiments, the fusion proteins described herein include at least an alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and a HSA polypeptide or an amino acid sequence that is derived from a HSA polypeptide, and a SLPI polypeptide or amino acid sequence derived from SLPI. In other embodiments, the fusion proteins described herein include at least an alpha-1-antitrypsin (AAT) polypeptide or an amino acid sequence that is derived from AAT and a HSA polypeptide or an amino acid sequence that is derived from a HSA polypeptide, and an Elafin polypeptide or amino acid sequence derived from Elafin.

[000102] The fusion proteins of the present invention can be readily produced in mammalian cell expression systems. For example Chinese Hamster Ovary (CHO) cells, Human Embryonic Kidney (HEK) 293 cells, COS cells, PER.C6®, NS0 cells, SP2/0,

YB2/0 can readily be used for the expression of the serpin fusion proteins described herein. Importantly, mammalian cell expression systems produce proteins that are generally more optimal for therapeutic use. In contrast to bacterial, insect, or yeast-based expression systems, mammalian cell expression systems yield proteins with glycosylation patterns that are similar or the same as those found in natural human proteins. Proper gylcosylation of a protein can greatly influence serum stability, pharmacokinetics, biodistribution, protein folding, and functionality. Therefore, the ability to produce therapeutic proteins in mammalian expression systems has distinct advantages over other systems. Furthermore, most of the mammalian cell expression systems (e.g., CHO, NS0, PER.C6® cells) can be readily scaled in commercial manufacturing facilities to produce therapeutic proteins to meet clinical demands. The fusion proteins described herein have enhanced functionalities over the natural form of AAT and can be produced in mammalian expression systems for clinical and commercial supply. Some embodiments of the invention include a purification system that enables the isolation of serpin fusion proteins that retain their ability to inhibit NE. Importantly, the purification process of the present invention can be readily incorporated into today's commercial mammalian cell-based manufacturing processes.

[000103] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.,* electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.,* Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures

and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients. The term patient includes human and veterinary subjects.

[000104] It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, buffers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000105] Therapeutic formulations of the invention, which include a fusion protein of the invention, are used to treat or alleviate a symptom associated with a disease or disorder associated with aberrant serine protease activity in a subject. The present invention also provides methods of treating or alleviating a symptom associated with a disease or disorder associated with aberrant serine protease activity in a subject. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient suffering from (or at risk of

developing) a disease or disorder associated with aberrant serine protease activity, using standard methods, including any of a variety of clinical and/or laboratory procedures. The term patient includes human and veterinary subjects. The term subject includes humans and other mammals.

[000106] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular disease or disorder associated with aberrant serine protease activity. Alleviation of one or more symptoms of the disease or disorder associated with aberrant serine protease activity indicates that the fusion protein confers a clinical benefit.

[000107] Methods for the screening of fusion proteins that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA), enzymatic assays, flow cytometry, and other immunologically mediated techniques known within the art.

[000108] The fusion proteins described herein may be used in methods known within the art relating to the localization and/or quantitation of a target such as a serine protease, *e.g.*, for use in measuring levels of these targets within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). The terms "physiological sample" and "biological sample," used interchangeably, herein are intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the terms "physiological sample" and "biological sample", therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph.

[000109] In a given embodiment, fusion proteins specific for a given target, or derivative, fragment, analog or homolog thereof, that contain the target-binding domain, are utilized as pharmacologically active compounds (referred to hereinafter as "Therapeutics").

[000110] A fusion protein of the invention can be used to isolate a particular target using standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. Detection can be facilitated by coupling (*i.e.*, physically linking) the fusion protein to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of

suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[000111] A therapeutically effective amount of a fusion protein of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the fusion protein and its target that, in certain cases, interferes with the functioning of the target. The amount required to be administered will furthermore depend on the binding affinity of the fusion protein for its specific target, and will also depend on the rate at which an administered fusion protein is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an fusion protein or fragment thereof invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 250 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a month.

[000112] Where fusion protein fragments are used, the smallest inhibitory fragment that specifically binds to the target is preferred. For example, peptide molecules can be designed that retain the ability to bind the target. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (*See*, *e.g.*, Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)). The formulation can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, growth-inhibitory agent, an anti-inflammatory agent or anti-infective agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[000113] The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[000114] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[000115] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the fusion protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT ™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

Pharmaceutical compositions

[000116] The fusion proteins of the invention (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the fusion rotein and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[000117] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration

include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.,* topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[000118] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[000119] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of

ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000120] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[000121] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[000122] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[000123] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[000124] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[000125] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[000126] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[000127] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: AAT-Fc Fusion Proteins and Variants

[000128] Exemplary, but non-limiting examples of AAT-Fc fusion proteins according to the invention include the following sequences. While these examples include a hinge sequence and/or a linker sequence, fusion proteins of the invention can be made using any hinge sequence and/or a linker sequence suitable in length and/or flexibility. Alternatively fusion proteins can be made without using a hinge and/or a linker sequence. For example, the polypeptide components can be directly attached.

[000129] An exemplary AAT-Fc fusion protein is the AAT-hFc1 (human IgG1 Fc) described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2) and the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 3).

AAT-hFc1 (human IgG1 Fc)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQK*EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK*
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK (SEQ ID NO:16)

[000130] An exemplary AAT-Fc fusion protein is the AAT-hFc2 (human IgG2 Fc), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2) and the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 4).

AAT-hFc2 (human IgG2 Fc)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQK*ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLM*
ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDW
LNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP

SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO: 17)

[000131] An exemplary AAT-Fc fusion protein is the AAT-MM-EL-hFc1 (human IgG1 Fc, Met351Glu/Met358Leu), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 34), the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 3), and the Met351Glu mutation is boxed, and the Met358Leu mutation is shaded in grey.

AAT-MM-EL-hFc1(human IgG1 Fc, Met351Glu/Met358Leu)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAEFLEAIPSIPPEVKF NKPFVFLMIEQNTKSPLFMGKVVNPTQK*EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK* (SEQ ID NO: 18)

[000132] An exemplary AAT-Fc fusion protein is the AAT-MM-EL-hFc2 (human IgG2 Fc, Met351Glu/Met358Leu), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 34), the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 4), the Met351Glu mutation is boxed, and the Met358Leu mutation is shaded in grey.

AAT-MM-EL-hFc2(human IgG2 Fc, Met351Glu/Met358Leu)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY

LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAEFLEAIPSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQK*ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLM*
ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDW
LNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK (SEQ ID NO: 19)

[000133] An exemplary AAT-Fc fusion protein is the AAT-MM-LL-hFc1(human IgG1 Fc, Met351Leu/Met358Leu), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 35), the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 3), the Met351Leu mutation is shaded in black, and the Met358Leu mutation is shaded in grey.

AAT-MM-LL-hFc1(human IgG1 Fc, Met351Leu/Met358Leu)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGALFLEAIPSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQK*EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK*
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK(SEQ ID NO:36)

[000134] An exemplary AAT-Fc fusion protein is the AAT-MM:LL-hFc2(human IgG2 Fc, Met351Leu/Met358Leu), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 35), the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 4), the Met351Leu mutation is shaded in black, and the Met358Leu mutation is shaded in grey.

AAT-MM:LL-hFc2(human IgG2 Fc, Met351Leu/Met358Leu)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQKERKCCVECPPCP*APPVAGPSVFLFPPKPKDTLM*
ISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDW
LNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPGK (SEQ ID NO:20)

[000135] An exemplary AAT-Fc fusion protein is the AAT-hFc1-AAT (human IgG1), described herein. As shown below, AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), the IgG-Fc polypeptide portion of the fusion protein is italicized (SEQ ID NO: 3).

AAT-hFc1-AAT (human IgG1)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQKEPKSCDKTHTCPPCP*APELLGGPSVFLFPPKPK*
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
*LHNHYTQKSLSLSPGK*ASTGSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQ
LAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLR
TLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVE

KGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKR LGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSA SLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGT EAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK (SEQ ID NO:21)

[000136] These exemplary AAT-Fc fusion proteins were made using the following techniques.

[000137] The gene encoding human AAT was PCR amplified from human liver cDNA (Zyagen). Specific point mutations within the gene encoding AAT or the Fc region were generated by overlapping PCR. The AAT encoding gene was cloned in frame with a gene encoding the hinge region, followed by a CH2 domain, and a CH3 domain of human IgG1, IgG2, IgG3, IgG4, or IgM into a mammalian expression vector, containing a mammalian secretion signal sequence up stream of the AAT gene insertion site. These expression vectors were transfected into mammalian cells (specifically HEK293 or CHO cells) and grown for several days in 8% CO_2 at 37° C. The recombinant AAT-Fc fusion proteins were purified from the expression cell supernatant by protein A chromatography. Importantly, a near neutral pH buffer was used (Gentle Ag/Ab Elution Buffer, Thermo Scientific) to elute the AAT-Fc fusion protein from the protein A resin. The AAT-Fc fusion protein can not be eluted from protein A resin using a standard low pH elution, as the ability of AAT to inhibit NE is compromised following low pH treatment, likely due to a low pH mediated oligomerization of AAT. Figure 1F shows the effects of low pH elution on the ability of AAT to inhibit neutrophil elastase. AAT-Fc fusion protein can be purified either by protein A and a near neutral pH elution buffer, by CaptureSelect® Alpha-1 Antitrypsin affinity matrix (BAC BV).

[000138] The purified AAT-Fc fusion proteins were tested for activity by determining their ability to inhibit neutrophil elastase (NE). Figure 1B and 1D show a reducing SDS-PAGE gel of purified serum derived AAT (sdAAT) and AAT-Fc fusion proteins (Fig 1B-lane 1: sdAAT, lane 2: AAT-Fc (SEQ ID NO: 16), lane 3: AAT-EL-Fc (SEQ ID NO: 18), Fig 1D AAT-Fc-AAT (SEQ ID NO: 20). The proteins were visualized by staining with coomassie blue.

[000139] To monitor human Neutrophil Elastase (NE) activity a fluorescent microplate assay was used. Inhibitory activity was measured by a concomitant decrease in the residual NE activity using the following assay. This assay buffer is composed of 100 mM Tris pH 7.4, 500 mM NaCl, and 0.0005% Triton X-100. Human NE is used at a final concentration of 5 nM (but can also be used from 1-20 nM). The fluorescent peptide substrate AAVP-AMC is used at a final concentration of 100 μM in the assay. The Gemini EM plate reader from Molecular Devices is used to read the assay kinetics using excitation and emission wavelengths of 370 nm and 440 nm respectively, and a cutoff of 420 nm. The assay is read for 10 min at room temperature scanning every 5 to 10 seconds. The Vmax per second corresponds to the residual NE activity, which is plotted for each concentration of inhibitor. The intercept with the x-axis indicates the concentration of inhibitor needed to fully inactivate the starting concentration of NE in the assay. Human serum derived AAT (sdAAT) was used as a positive control in these assays. The AAT-Fc fusion proteins display potent NE inhibitory activity as shown in Figure 1C. The fusion wherein there are two AAT polypeptides fused to single Fc polypetide (AAT-Fc-AAT) displays enhanced potency over both sdAAT and the AAT-Fc fusion protein comprising a single AAT polypeptide (Figure 1E). These findings presented here demonstrate for the first time the AAT can be fused to an Fc region and maintain its ability to inhibit NE. Of particular interest, the AAT-Fc-AAT fusion protein was found to be a more potent NE inhibitor.

[000140] Figure 1F demonstrates the resistance of the AAT-EL-Fc (M351E, M358L) fusion protein to inactivation by oxidation. AAT fusion proteins, AAT-Fc (wt), AAT-EL-Fc (M351E, M358L), and AAT-EM-Fc (M351E), were treated with 33mM H_2O_2 and compared to untreated fusion proteins in the NE inhibition assays. The inhibition of NE by AAT-EL-Fc was not comprised by oxidation, converse to the other proteins tested.

[000141] Furthermore, AAT-Fc fusion protein displayed a longer serum half life in rats compared to serum derived AAT (Figure 1H). In these studies 3 rats per each test protein were injected I.V. with 10mg/kg of sdAAT or AAT-Fc. Serum sample were taken at various time points over a 48 period. The serum ATT concentration was using an ELISA. These findings demonstrate that the fusion proteins of the invention have improved pharmacokinetic properties and are a superior therapeutic format over serum derived AAT, for treating numerous human inflammatory conditions and infectious diseases.

Example 2: AAT-TNFα Targeting Molecule Fusion Proteins

[000142] The studies presented herein describe several, non-limiting examples of recombinant AAT derivatives comprising human AAT fused to an anti-TNFα antibody or a derivative of a TNFα receptor. These examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not and are not intended to limit the claimed invention.

[000143] The fusion proteins below include cytokine targeting polypeptide sequences that are from or are derived from (i) the anti-TNFα antibody D2E7 (also known as Adalimumab or Humira®), or (ii) the extracellular domain of Type 2 TNFα Receptor (TNFR2-ECD). The AAT polypeptide portion of the fusion protein is underlined, the antibody constant regions (CH1-hinge-CH2-CH3, or CL) are italicized, and D2E7-VH, D2E7-VK, and TNFR2-ECD are denoted in bold text. While these examples include a hinge sequence and/or a linker sequence, fusion proteins of the invention can be made using any hinge sequence and/or a linker sequence suitable in length and/or flexibility. Alternatively fusion proteins can be made without using a hinge and/or a linker sequence.

[000144] An exemplary AAT-TNFα fusion protein is D2E7-Light Chain-AAT $(G_3S)_2$ Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), D2E7-VK is denoted in bold text (SEQ ID NO: 37), and the antibody constant regions are italicized (SEQ ID NO: 38)

D2E7-Light Chain-AAT $(G_3S)_2$ Linker

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSR
FSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIK*RTVAAPSVFIFPPSD*
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
*ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*GGGSGGGSEDPQGDAAQKTDTSHHDQDHPT
FNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFN
LTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFT
VNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEE
EDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENE
LTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPL

KLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKV VNPTQK (SEQ ID NO:22)

[000145] An exemplary AAT-TNFα fusion protein is D2E7-Light Chain-AAT ASTGS Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), D2E7-VK is denoted in bold text (SEQ ID NO: 37), and the antibody constant regions is italicized (SEQ ID NO: 38)

D2E7-Light Chain-AAT ASTGS Linker

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQSGVPSR FSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIK*RTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*ASTGSEDPQGDAAQKTDTSHHDQDHPTFNK ITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTE IPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNF GDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDF HVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTH DIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLS KAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNP TQK (SEQ ID NO:23)

[000146] An exemplary AAT-TNFα fusion protein is D2E7-Heavy Chain-AAT $(G_3S)_2$ Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), D2E7-VH is denoted in bold text (SEQ ID NO: 39), and the antibody constant regions is italicized (SEQ ID NO: 40)

D2E7-Heavy Chain-AAT $(G_3S)_2$ Linker

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYA DSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTL*VTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ

VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
*SCSVMHEALHNHYTQKSLSLSPGK*GGGSGGGSEDPQGDAAQKTDTSHHDQDHPTFNKITPN
LAEFAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEA
QIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTE
EAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQ
VTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIIT
KFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVH
KAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK
(SEQ ID NO:24)

[000147] An exemplary AAT-TNFα fusion protein is D2E7-Heavy Chain-AAT ASTGS Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), D2E7-VH is denoted in bold text (SEQ ID NO: 39), and the antibody constant regions is italicized (SEQ ID NO: 40)

D2E7-Heavy Chain-AAT ASTGS Linker

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYA
DSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTL*VTVSSA*
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
*SCSVMHEALHNHYTQKSLSLSPGK*ASTGSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAE
FAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIH
EGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAK
KQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTT
VKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFL
ENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAV
LTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK
(SEQ ID NO:25)

[00149] An exemplary AAT-TNFα fusion protein is TNFR2-ECD-Fc1-AAT$(G_3S)_2$ Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), TNFR2-ECD is denoted in bold text (SEQ ID NO: 41), and the antibody constant regions is italicized (SEQ ID NO: 42)

TNFR2-ECD-Fc1-AAT$(G_3S)_2$ Linker

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTY TQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCR PGFGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSP TRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD*EPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPQVKFNWYVDGVQVH NAKTKPREQQYNSTYRVVSVLTVLHQNWLDGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK***<u>GGGSGGGSEDPQGDAAQKTDT SHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHD EILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVK KLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWER PFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDE GKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADL SGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNT KSPLFMGKVVNPTQK</u>** (SEQ ID NO:26)

[00150] An exemplary AAT-TNFα fusion protein is TNFR2-ECD-Fc1-AAT ASTGS Linker, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), TNFR2-ECD is denoted in bold text (SEQ ID NO: 41), and the antibody constant regions is italicized (SEQ ID NO: 42)

TNFR2-ECD-Fc1-AAT ASTGS Linker

LPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCDSCEDSTY TQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALSKQEGCRLCAPLRKCR PGFGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMDAVCTSTSP TRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGD*EPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPQVKFNWYVDGVQVH***

*NAKTKPREQQYNSTYRVVSVLTVLHQNWLDGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*ASTGSEDPQGDAAQKTDTSHH DQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEIL EGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLY HSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFE VKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKL QHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGV TEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSP LFMGKVVNPTQK (SEQ ID NO:27)

[000148] These exemplary AAT-TNFα targeting molecule fusion proteins were made using the following techniques.

[000149] The genes encoding the variable heavy (VH) and variable kappa (VK) regions of the anti-TNFα antibody, D2E7, were generated by gene synthesis. The D2E7-VH gene was cloned in frame with a gene encoding a human IgG1 antibody heavy chain constant region, consisting of a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain, into a mammalian expression vector, containing a mammalian secretion signal sequence up stream of the VH domain insertion site (D2E7-HC). The D2E7-VK gene was cloned in frame with a human antibody kappa light chain constant (CL) domain, into a mammalian expression vector, containing a mammalian secretion signal sequence up stream of the VK domain insertion site (D2E7-LC). The AAT encoding gene and the adjacent 5' linker sequence were cloned in frame into the 3' end of either, the CH3 domain of the D2E7 heavy chain gene (D2E7-HC-AAT), or the CL domain of the D2E7 light chain gene (D2E7-LC-AAT) coding sequences in the above described mammalian expression vectors. The extracellular domain of the TNFα Receptor 2 (TNFR2-ECD) was generated by gene synthesis and cloned in frame with a gene encoding the hinge region, followed by a CH2 domain and a CH3 domain of human IgG1 (hFc1) into a mammalian expression, containing a mammalian secretion signal sequence up stream of the TNFR2-ECD insertion site. The AAT encoding gene and the adjacent 5' linker sequence were cloned in frame into the 3' end of the gene encoding TNFR2-ECD-hFc1 into a mammalian expression vector (TNFR2-ECD-hFc1-AAT).

[000150] The D2E7-HC-AAT expression vector was co-transfected with either the D2E7-LC or the D2E7-LC-AAT expression vector into mammalian cells (specifically HEK293 or CHO cells) to generate the D2E7 antibody with AAT fused to the C-terminus of the heavy chain or to the C-terminus of both the heavy chain and light chain, respectively. The D2E7-LC-AAT was co-transfected with the D2E7-HC expression vector into mammalian cells to generate the D2E7 antibody with AAT fused to the C-terminus of the light chain. The TNFR2-hFc1-AAT expression vector was transfected into mammalian cells. Transfected cells were grown for several days in 8% CO_2 at 37° C.

[000151] The recombinant AAT-TNFα targeting fusion proteins were purified from the expression cell supernatant by protein A chromatography. A near neutral pH buffer was used (Gentle Ag/Ab Elution Buffer, Thermo Scientific) to elute the AAT-TNFα targeting fusion proteins from the protein A resin.

[000152] Figure 2B shows an SDS-PAGE gel of the D2E7 antibody alone (lane 1) and variant wherein AAT is fused to the heavy chain of D2E7 (lane 2). The proteins were visualized by staining with coomassie blue.

[000153] The purified AAT-TNFα targeting molecule fusion proteins were tested for activity by determining their ability to inhibit neutrophil elastase. Human serum derived AAT (sdAAT) was used as a positive control in these assays. NE inhibitory assay were conducted as described above. Figure 2C demonstrates relative to sdAAT, the AAT-TNFα targeting molecule fusion protein shows similar inhibition of neutrophil elastase, indicating that the inhibitory capacity of AAT has not been compromised by its fusion to an antibody.

Example 3 AAT-Fc-SLPI and AAT-Fc-Elafin

[000154] The studies presented herein describe several, non-limiting examples of recombinant AAT derivatives comprising human AAT fused a WAP domain containing protein. These examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. The AAT polypeptide portion of the fusion protein is underlined, the Fc portion is italicized, and the WAP domain containing polypeptide is in bold font. While these examples include a hinge sequence and/or a linker sequence, fusion proteins of the invention can be made using any hinge sequence and/or a linker sequence suitable in length

and/or flexibility. Alternatively fusion proteins can be made without using a hinge and/or a linker sequence. For example, the polypeptide components can be directly attached.

[000155] An exemplary AAT-Fc-SLPI fusion protein is AAT-hFc1-SLPI (human IgG1 Fc), described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), the Fc portion is italicized (SEQ ID NO: 3), and the WAP domain containing polypeptide is in bold font (SEQ ID NO: 9)

AAT-hFc1-SLPI (human IgG1 Fc)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQK*EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK*
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
*LHNHYTQKSLSLSPGKASTGS***SGKSFKAGVCPPKKSAQCLRYKKPECQSDWQCPGKKRCCP**
DTCGIKCLDPVDTPNPTRRKPGKCPVTYGQCLMLNPPNFCEMDGQCKRDLKCCMGMCGKSC
VSPVKA (SEQ ID NO:28)

[000156] An exemplary AAT-Fc-SLPI fusion protein is AAT-hFc1-SLPI (human IgG1 Fc), described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), the Fc portion is italicized (SEQ ID NO: 3), and the WAP domain containing polypeptide is in bold font (SEQ ID NO: 12)

AAT-hFc1-Elafin (human IgG1 Fc)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ

LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF NKPFVFLMIEQNTKSPLFMGKVVNPTQK*EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGKASTGS***AVTGVPVKGQDTVKGRVPFNGQDPVKGQVSVKGQDKVKAQ EPVKGPVSTKPGSCPIILIRCAMLNPPNRCLKDTDCPGIKKCCEGSCGMACFVPQ*** (SEQ ID NO:29)

[000157] The genes encoding the SLPI and Elafin were PCR amplified from human spleen cDNA (Zyagen). These genes were cloned into the mammalian expression vectors of example 1, wherein the SLPI or Elafin gene was inserted in frame with the AAT-Fc gene. These expression vectors were transfected into mammalian cells (specifically HEK293 or CHO cells) and grown for several days in 8% CO_2 at 37° C. The recombinant AAT-Fc-WAP domain fusion proteins were purified from the expression cell supernatant by protein A chromatography. A near neutral pH buffer was used (Gentle Ag/Ab Elution Buffer, Thermo Scientific) to elute the AAT-Fc-WAP domain fusion protein from the protein A resin.

[000158] Figure 3B shows an SDS-PAGE gel of the AAT-Fc-WAP fusion proteins (lane 1 AAT-Fc-Elafin, lane 2 AAT-Fc-SLPI). The proteins were visualized by staining with coomassie blue. The purified AAT-Fc-WAP domain fusion proteins were tested for activity by determining their ability to inhibit neutrophil elastase. NE inhibitory assays were conducted as described above. Human serum derived AAT (sdAAT) and the AAT-Fc fusion protein were used as a positive control in these assays. Relative to sdAAT, the AAT-Fc-WAP targeting molecule fusion proteins display enhanced potency of NE inhibition of neutrophil elastase (Figure 3C).

Example 4 AAT-Albumin

[000159] The studies presented herein describe several, non-limiting examples of recombinant AAT derivatives comprising human AAT fused an albumin polypeptide. These examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not and are not intended to limit the claimed invention. The AAT portion is

underlined and the albumin portion is italicized. For example, the polypeptide components can be directly attached.

[000160] An exemplary AAT-Albumin fusion protein is AAT-HSA, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), and the albumin polypeptide is italicized (SEQ ID NO: 14)

AAT-HSA

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE
GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQKASTGS*DAHKSEVAHRFKDLGEENFKALVLIAFA*
QYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMAD
CCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFY
APELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAF
KAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSIS
SKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE
YARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCEL
FEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSV
VLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTL
SEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA
ASQAALGL (SEQ ID NO:30)

[000161] An exemplary AAT-Albumin fusion protein is AAT-HSA Domain 3, described herein. As shown below, the AAT polypeptide portion of the fusion protein is underlined (SEQ ID NO: 2), and the albumin polypeptide is italicized (SEQ ID NO: 15)

AAT-HSA Domain 3

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIATAF
AMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNGLFLSE

GLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVKELDRDTVFA
LVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKY
LGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQ
LGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKF
NKPFVFLMIEQNTKSPLFMGKVVNPTQKASTGS*EEPQNLIKQNCELFEQLGEYKFQNALLV*
RYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVS
DRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVE
LVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVA (SEQ ID NO:31)

[000162] The gene encoding human serum albumin (HSA) was PCR amplified from human liver cDNA (Zyagen). A mammalian expression vector was generated, wherein gene encoding HSA or the domain 3 of HSA, was cloned in frame to the 3' end of the AAT encoding gene, containing a mammalian secretion signal sequence up stream of AAT.

[000163] These expression vectors were transfected into mammalian cells (specifically HEK293 or CHO cells) and grown for several days in 8% CO_2 at 37° C. The recombinant AAT-HSA fusion proteins were purified from the expression cell supernatant using the CaptureSelect® Alpha-1 Antitrypsin affinity matrix (BAC BV), wherein the binding buffer consisted of 20mM Tris, 150mM NaCl, pH 7.4 and the elution buffer consisted of 20mM Tris, 2M $MgCl_2$ pH 7.4.

[000164] Figure 4B shows an SDS-PAGE gel of the AAT-HSA fusion protein The proteins were visualized by staining with coomassie blue. The purified AAT-HSA fusion proteins were tested for activity by determining their ability to inhibit neutrophil elastase. NE inhibitory assays were conducted as described above. Human serum derived AAT (sdAAT) was used as a positive control in these assays. Relative to sdAAT, the AAT-HS fusion protein displays similar potency of NE inhibition, demonstrating that the fusion to albumin does not dampen the capacity of AAT to inhibit NE (Figure 4C.)

Other Embodiments

[000165] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. An isolated fusion protein comprising at least one human serpin polypeptide operably linked to a second polypeptide wherein the second polypeptide comprises at least one the following:

an immunoglobulin Fc polypeptide or an amino acid sequence that is derived from an immunoglobulin Fc polypeptide;

a cytokine targeting polypeptide or a sequence derived from a cytokine targeting polypeptide;

a WAP domain containing polypeptide or a sequence derived from a WAP domain containing polypeptide; or

an albumin polypeptide or an amino acid sequence that is derived from a serum albumin polypeptide.

2. An isolated fusion protein comprising at least one alpha-1 antitrypsin (AAT) polypeptide operably linked to a second polypeptide of at least one of the following:

an immunoglobulin Fc polypeptide;

a cytokine targeting peptide;

a human secretory leukocyte proteinase inhibitor (SLPI) polypeptide; and

an immunoglobulin Fc polypeptide and a human SLPI polypeptide, wherein the SLPI polypeptide comprises the WAP2 domain of human SLPI; or

a human serum albumin (HSA) polypeptide.

3. An isolated fusion protein comprising at least one alpha-1 antitrypsin (AAT) polypeptide operably linked to a second polypeptide of at least one of the following:

an immunoglobulin Fc polypeptide;

a cytokine targeting peptide;

a human Elafin polypeptide;

a human Elafin polypeptide and an immunoglobulin Fc polypeptide, wherein the Elafin polypeptide comprises the WAP domain of human Elafin; or

a human serum albumin (HSA) polypeptide.

4. The fusion protein of claim 1 wherein the human serpin polypeptide is a human alpha-1 antitrypsin (AAT) polypeptide or is derived from a human AAT polypeptide.

5. The isolated fusion protein of any one of claims 2-4, wherein AAT polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

6. The isolated fusion protein of any one of claims 2-4, wherein the AAT polypeptide comprises the reactive site loop of AAT comprising the amino acid sequence of SEQ ID NO: 1 or a mutated reactive site loop of AAT comprising the amino acid sequence of SEQ ID NO: 32 or 33.

7. The isolated fusion protein of any one of claims 2-4, wherein the immunoglobulin Fc polypeptide is a human Fc polypeptide.

8. The isolated fusion protein of claim 7, wherein human Fc polypeptide is a human IgM polypeptide or a human IgG Fc polypeptide.

9. The isolated fusion protein of claim 8, wherein the human IgG Fc polypeptide is a human IgG1 polypeptide, a human IgG2 Fc polypeptide, human IgG3 Fc polypeptide, or human IgG4 Fc polypeptide.

10. The isolated fusion protein of any one of claims 1-3, wherein the immunoglobulin Fc polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 4, 5, 6, and 7.

11. The isolated fusion protein of claim 1, wherein the serpin polypeptide and the immunoglobulin Fc polypeptide are operably linked via a hinge region, a linker region, or both a hinge region and linker region.

12. The isolated fusion protein of any one of claims 2-4, wherein the AAT polypeptide and the immunoglobulin Fc polypeptide are operably linked via a hinge region, a linker region, or both a hinge region and linker region.

13. The isolated fusion protein of claim 11 or claim 12, wherein the hinge region, the linker region or both the hinge region and the linker region comprise a peptide sequence.

14. The isolated fusion protein of any one of claims 1-3, wherein the fusion protein comprises an amino acid sequence selected from SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 21.

15. The isolated fusion protein of any one of claims 1-6, wherein the cytokine targeting polypeptide is a human cytokine receptor polypeptide or a derivative of a human cytokine receptor polypeptide, a cytokine targeting polypeptide that binds a cytokine receptor or a cytokine targeting polypeptide binds a human cytokine.

16. The isolated fusion protein of claim 15, wherein the human cytokine receptor polypeptide comprises multiple subunits.

17. The isolated fusion protein of claim 15 or claim 16, wherein the human cytokine receptor is a receptor for TNFα, IgE, IL-12, IL-23, IL-6, IL-1α, IL-1β, IL-17, IL-13, the p40 subunit of IL-12 and IL-23, IL-4, IL-10, IL-2, IL-18, IL-27, or IL-32.

18. The isolated fusion protein of claim 17, wherein the cytokine receptor is a receptor for human TNFα.

19. The isolated fusion protein of claim 15, wherein the human cytokine is TNFα, IgE, IL-12, IL-23, IL-6, IL-1α, IL-1β, IL-17, IL-13, the p40 subunit of IL-12 and IL-23, IL-4, IL-10, IL-2, IL-18, IL-27, or IL-32.

20. The isolated fusion protein of claim 19, wherein the cytokine is human TNFα.

21. The isolated fusion protein of claim 15, wherein the cytokine targeting polypeptide is an antibody or an antibody fragment.

22. The isolated fusion protein of claim 21, wherein the antibody or antibody fragment is chimeric, humanized, or fully human.

23. The isolated fusion protein of claim 1-6, where the fusion protein comprises an amino acid sequence selected from SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27.

24. The isolated fusion protein of claim 1, wherein the WAP domain containing polypeptide sequence comprises a SLPI polypeptide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 9 and 10.

25. The isolated fusion protein of claims 1, wherein the WAP domain containing polypeptide sequence comprises an elafin polypeptide sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 12 and 13.

26. The isolated fusion protein of claim 1, wherein the fusion protein comprises a serpin polypeptide, a WAP domain-containing polypeptide and an immunoglobulin Fc polypeptide such that at least two of the serpin polypeptide, the WAP domain-containing polypeptide and the immunoglobulin Fc polypeptide are operably linked via a hinge region, a linker region, or both a hinge region and linker region.

27. The isolated fusion protein of claim 2, wherein the fusion protein comprises an AAT polypeptide, a SLPI polypeptide and an immunoglobulin Fc polypeptide such that at least two of the AAT polypeptide, the SLPI polypeptide and the immunoglobulin Fc polypeptide are operably linked via a hinge region, a linker region, or both a hinge region and linker region.

28. The isolated fusion protein of claim 3, wherein the fusion protein comprises an AAT polypeptide, an Elafin polypeptide and an immunoglobulin Fc polypeptide such that at least two of the AAT polypeptide, the Elafin polypeptide and the immunoglobulin Fc polypeptide are operably linked via a hinge region, a linker region, or both a hinge region and linker region.

29. The isolated fusion protein of any one of claims 2-4, wherein the fusion protein comprises two AAT polypeptides and an immunoglobulin Fc polypeptide.

30. The isolated fusion protein of claim 29, wherein each of the two AAT polypeptides are operably linked to the immunoglobulin Fc polypeptide via a hinge region, a linker region, or both a hinge region and linker region such that the fusion protein has the structural arrangement from N-terminus to C-terminus as follows: AAT polypeptide-immunoglobulin Fc polypeptide-AAT polypeptide.

31. The isolated fusion protein of any one of claims 26-30, wherein the hinge region, the linker region or both the hinge region and the linker region comprise a peptide sequence.

32. The isolated fusion protein of claim 2, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 28.

33. The isolated fusion protein of claim 2, where the fusion protein comprises the amino acid sequence of SEQ ID NO: 29.

34. The fusion protein of claim 1, wherein the albumin polypeptide is a human serum albumin (HSA) polypeptide or is derived from a HSA polypeptide.

35. The isolated fusion protein of any one of claims 2, 3 or 34, wherein HSA polypeptide comprises the amino acid sequence of SEQ ID NO: 14 or the amino acid sequence of SEQ ID NO: 15.

36. The isolated fusion protein of any one of claims 2, 3 or 34, wherein the HSA polypeptide comprises domain 3 of HSA.

37. The isolated fusion protein of claim 34, wherein the serpin is operably linked to HSA via an albumin binding polypeptide.

38. The isolated fusion protein of claim 2 or claim 3, wherein the AAT polypeptide is operably linked to HSA via an albumin binding polypeptide.

39. The isolated fusion protein of claim 37 or 38, wherein the albumin binding polypeptide is an antibody or antibody fragment or derived from an antibody or antibody fragment.

40. The isolated fusion protein of claim 39, wherein the antibody or antibody fragment or chimeric, humanized, or fully human.

41. The isolated fusion protein of claims 37 or 38, wherein the albumin binding polypeptide is a peptide.

42. The isolated fusion protein of claims 47, wherein the albumin binding polypeptide is Domain 3 of *Streptococcal* Protein G or a sequence derived from Domain 3 of *Streptococcal* Protein G.

43. The isolated fusion protein of claim 1, wherein the serpin is covalently linked to a HSA polypeptide.

44. The isolated fusion protein of claim 1, wherein the serpin is non-covalently linked to a HSA polypeptide.

45. The isolated fusion protein any one of claims 1-3, where the fusion protein comprises the amino acid sequence of SEQ ID NO: 30 or 31.

46. The isolated fusion protein of any one of claims 1-3, wherein the immunoglobulin Fc polypeptide is modified to enhance FcRn binding.

47. The isolated fusion protein of any one of claims 1-3, wherein the immunoglobulin Fc polypeptide comprising at least one of the following mutations: Met252Tyr, Ser254Thr, Thr256Glu, Met428Leu or Asn434Ser

48. A method of treating or alleviating a symptom of a disease or disorder associated with aberrant serine protease expression or activity in a subject in need thereof, the method comprising administering a fusion protein according to any one of the previous claims.

49. A method of treating or alleviating inflammation or a symptom of an inflammatory disease or disorder while reducing the risk of infection, in a subject in need thereof, the method comprising administering a fusion protein according to any one of the previous claims.

50. A method of reducing the risk of infection in a subject in need thereof, the method comprising administering a fusion protein according to any one of the previous claims.

51. The method of any one of claims 48-51, wherein the subject is a human.

52. The method of 49, wherein the inflammatory disease or disorder is selected from the following: emphysema, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), allergic asthma, cystic fibrosis, cancers of the lung, ischemia-reperfusion injury, ischemia/reperfusion injury following cardiac transplantation, myocardial infarction, rheumatoid arthritis, septic arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, type I and/or type II diabetes, pneumonia, sepsis, graft versus host disease (GVHD), wound healing, Systemic lupus erythematosus, and Multiple sclerosis.

53. The method of 50, wherein the infection is selected from bacterial infections, fungal infections, or viral infections.