PEPTIDES AND COMPOSITIONS FOR TREATMENT OF JOINT DAMAGE

RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 61/775,400, filed on March 8, 2013 and U.S. Provisional Patent Application No. 61/938,123, filed February 10, 2014, each of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Osteoarthritis (OA) represents the most common musculoskeletal disorder. Approximately 40 million Americans are currently affected; a number predicted to increase to 60 million within the next twenty years as a result of aging population and an increase in life expectancy, making it the fourth leading cause of disability. OA is characterized by a slow degenerative breakdown of a joint including both articular cartilage (containing the cells and matrix which produce lubrication and cushioning for the joint) and subchondral bone underlying the articular cartilage. OA can be considered a consequence of various etiologic factors. For example, it can be caused by abnormal biomechanical stress or genetic or acquired abnormalities of articular cartilage or bone. Current OA therapies include pain relief with oral NSAIDs or selective cyclooxygenase 2 (COX-2) inhibitors, intra-articular (IA) injection with agents such as corticosteroids and hyaluronan, and surgical approaches.

[0003] Joint damage, e.g., acute joint injury, such as a meniscal or ligament tear, or an intra-articular fracture can also lead to arthritis, e.g., posttraumatic arthritis. Because articular cartilage has a limited ability to repair, even small undetectable damage can often get worse over time and lead to OA. Current treatments for joint injury can include surgery and other invasive procedures focused on regeneration of damaged joints as well as treatment with agents to reduce pain and inflammation.

[0004] Mesenchymal stem cells (MSCs) are present in adult articular cartilage and upon isolation can be programmed in vitro to undergo differentiation to chondrocytes and other mesenchymal cell lineages, and may be used for cartilage regeneration. In part, the process is regulated by growth factors (TGFβs, BMPs), serum conditions and cell-cell contact. WO2011/008773 describes peptide compositions and use of those compositions for treating or preventing arthritis and joint injury and for inducing differentiation of mesenchymal cells

into chondrocytes. Additionally, WO2012/129562 describes small molecule compounds, compositions and use of those compositions for amelioration of arthritis and joint injury and for inducing differentiation of mesenchymal cells into chondrocytes.

[0005] Though surgical techniques, and regenerative technology have made some progress in restoration of cartilage, slowing degeneration, and improved repair of joint damage, a continued need exists for improvement of compositions and methods for effective cartilage regeneration, treatment of joint damage and amelioration or prevention of OA.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention relates to the identification of new polypeptide and protein variants of angiopoietin-like 3 (ANGPTL3) that have improved pharmaceutical properties, e.g., are more stable, less susceptible to proteolysis and enzymatic degradation than wild-type ANGPTL3. Also provided are pharmaceutical compositions and methods for treatment of joint damage or joint injury, and methods of ameliorating or preventing arthritis, joint damage or joint injury in a mammal.

[0007] Thus, provided are protease-resistant polypeptides comprising an amino acid sequence that has at least 95% amino acid sequence identity, or at least 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence selected from any one or more of the sequences of TABLE 1, and as further described herein. The modified polypeptides of TABLE 1 include an amino acid that is a polar amino acid other than K or R at position 423, as determined with reference to the full length ANGPTL3 polypeptide sequence, SEQ ID NO:1. In some embodiments the amino acid at position 423 as determined with reference to SEQ ID NO:1 is Q or S. In certain embodiments the amino acid at position 423 as determined with reference to SEQ ID NO:1 is Q. In certain embodiments the amino acid at position 423 as determined with reference to SEQ ID NO:1 is S. In certain embodiments the amino acid at position 423 as determined with reference to SEQ ID NO:1 is deleted. In addition, provided polypeptides have chondrogenic activity.

[0008] In some embodiments, the polypeptide comprises a sequence having at least 95% identity or at least 96%, 97%, 98%, 99% or 100% to any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:70. In some embodiments the polypeptide comprises a sequence having at least 95%

identity or at least 96%, 97%, 98%, 99% or 100% to any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In some embodiments, the polypeptide comprises any one of the sequences of TABLE 1. In some embodiments, the polypeptide comprises any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:70. In some embodiments the polypeptide comprises any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In some embodiments, the polypeptide is any one of the sequences of TABLE 1. In some embodiments, the polypeptide is any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:70. In some embodiments the polypeptide is any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64.

[0009] Polypeptides of the invention may incorporate one or more chemical modifications (e.g., PEGylation). In some embodiments, polypeptides of the invention may comprise a heterologous peptide as a fusion protein, which may optionally be fused at the amino-terminal or the carboxy-terminal end of the polypeptide. Also provided are polynucleotides encoding the polypeptides of the invention; vectors containing polynucleotides encoding the polypeptides; and host cells comprising such vectors.

[0010] The present invention also provides pharmaceutical compositions comprising the polypeptides of the invention and a pharmaceutically acceptable carrier. Such compositions can be used in methods provided herein for treating, ameliorating or preventing arthritis or

joint damage in a patient, where the method comprises administering to a joint of a patient a therapeutically effective amount of a pharmaceutical composition of the invention. Examples of conditions that can benefit from such methods include, but are not limited to arthritis (e.g., osteoarthritis, traumatic arthritis), and joint damage (e.g., acute joint injury).

[0011] The present invention further provides methods of treating a subject comprising administering a therapeutically effectively amount of a polypeptide of the invention. Provided methods include treating a subject having or at risk of having joint damage and/or arthritis, comprising administering to the subject a therapeutically effective amount of one or more polypeptides of the invention or a pharmaceutical composition thereof. Still further provided are methods of inducing differentiation of mesenchymal stem cells into chondrocytes, comprising contacting mesenchymal stem cells with an effective amount of a polypeptide of the invention to induce differentiation of the mesenchymal stem cells into chondrocytes.

[0012] These and other aspects of the invention, including additional features, advantages, and embodiments of the invention, will be described and elucidated in further detail in the following detailed description and appended claims of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figure 1** depicts a schematic of hANGPTL3 proteins engineered to improve protein stability and enhance proteolytic resistance. During protein production of wild type protein and peptide sequences, 100% cleavage was observed between Lys423 and Ser424. To mitigate proteolysis, various mutant peptides were generated wherein Lys 423 was mutated to Gln or Ser; or Ser424 was mutated to Thr; or Lys 423 was deleted.

[0014] **Figure 2A and B** depicts graphical representations of expression of cartilage specific proteins in the presence or absence of ANGPTL3 and engineered constructs. Fixed cells were stained for **2A** Pro-collagen Type 2A quantification (PIIANP) or **2B** Type II collagen quantification to determine the % of cells differentiating into chondrocytes following treatment as described in the Exemplification. **Figure 2C** depicts graphical representation of quantification of angiogenesis assays in the presence or absence of ANGPTL3 or engineered construct as compared to a positive control protein, bFGF. Total tube length and number of branch points were quatitative measurements of angiogenesis. Although others have reported angiogenic activity in ANGPTL3, and this study confirms

activity as well as that of FGF; the results indicated no significant activity is retained in a Cterminal ANGPTL3 construct.

[0015] **Figure 3** are graphical representations showing an increase in expression of cartilage specific proteins in the presence of ANGPTL3 or engineered constructs. **3A**. Cells were evaluated ten days following treatment using qRT-PCR to measure RNA expression for cartilage specific proteins following treatment as described. Lubricin, aggrecan and Sox9 represent cartilage related proteins; IGF and IFITM1 represent differentiation potential, and osteocalcin and type X collagen represent bone/fibrosis related proteins. **3B.** Cells were evaluated three days following treatment as described. Increased aggrecan expression was seen following treatment with engineered construct or wild type ANGPTL1 C-terminal region polypeptide.

[0016] **Figure 4** depicts graphical representations of chondro-protective activity of ANGPTL3 and engineered constructs. **4A**: Glycosaminoglycan (GAG) release, an indicator of matrix damage, was inhibited with increasing amount of ANGPTL3 and mutant constructs. *Ex vivo* GAG release (an indicator of matrix damage) inhibition assays were performed using bovine cartilage treated in the presence or absence of constructs as described. **4B** and **4C**: NO release was inhibited with increasing amount of ANGPTL3 and engineered constructs as indicated. Chondrocytes were treated in the presence or absence of constructs as described followed by Greiss reaction assays to determine the inhibition of NO release as an indicator of chondro-protection.

[0017] **Figure 5** depicts a graphical representation showing an inhibition of type X collagen expression (an indicator of fibrotic cartilage formation activity) in the presence of constructs under hypertrophic conditions. Primary chondrocytes were treated in the presence of absence of constructs under hypertrophic conditions as described, followed by determination of type X collagen expression, assessed by immunofluorescence, as a measurement of formation of fibrotic and hypertrophic cartilage/chondrocyte differentiation. **5A** depicts results of wild type C-terminal ANGPTL3 or engineered construct. **5B** depicts results of C-terminal ANGPTL3 (WT) or engineered constructs 242KQ or 242Kdel or C-terminal ANGPTL1.

[0018] **Figure 6** depicts a schematic representation of the dosing paradigm (**6A**), followed by a graphical representation (**6B**) of the improvement in joint severity after treatment with mouse ANGPTL3 (17-460) as measured by cartilage erosion score of the lateral femoral condyle.

[0019] **Figure 7**. is a graphical representation of incapacitance measurements (an indicator of pain) in mice following surgical induction of cartilage damage and subsequent treatment with ANGPTL3 constructs once weekly for three weeks (beginning on day7). **7A** represents incapacitance measurements on day 35 following surgery; and **7B** represents measurements taken on day 56 following surgery.

[0020] **Figure 8**. is a graphical representation of the total joint severity score and improvement in severity to cartilage damage induced by collagenase in mice following 3 once weekly treatments (days 7, 14 and 21) of ANGPTL3 constructs (indicated).

[0021] **Figure 9**. depicts results in a rat meniscal tear model of joint damage following treatment with engineered ANGPTL3 construct. Figure **9A** is a graphical representation of the proteoglycan content in joints five weeks following treatment; Figure **9B** is a graphical representation of the femoral joint severity score five weeks following treatment. Results illustrate improvement to cartilage damage induced by surgical severing of the meniscus in rats following 3 once weekly treatments (days 7, 14 and 21) of ANGPTL3 constructs (indicated).

[0022] **Figure 10** depicts results in a rat meniscal tear model of joint damage following treatment with engineered ANGPTL3 construct. Figure **10A** is a graphical representation of percent of in vivo repair as measured by severity, safranin O intensity, cartilage area and cartilage thickness. Figure **10B** is a graphical representation of of incapacitance measurements (an indicator of pain) in rats following surgical induction of cartilage damage and subsequent treatment.

[0023] **Figure 11** is a graphical representation of the total gross severity score to illustrate improvement of cartilage damage induced by surgical disruption of the medial meniscus in dogs following biweekly dosing beginning on day 4 (each of the 1.5ug/dose or 15ug/dose) or a single 30ug dose) given on day 7 only.

DETAILED DESCRIPTION

[0024] The present invention is based, at least in part, on the identification of Angiopoietin-like 3 (ANGPTL3) polypeptides that stimulate chondrocyte differentiation of mesenchymal stem cells and that are resistant to cleavage by proteases (e.g., trypsin-like proteases). WO2011/008773, describes ANGPTL3 peptide compositions and use of peptide compositions for treating or preventing arthritis and joint injury and for inducing differentiation of mesenchymal cells into chondrocytes. We found that wild type ANGPTL3

proteins are subject to protease clipping and instability and have identified sequence variants to mitigate this effect. The present invention thus provides improved peptide compositions for repairing cartilage. In particular, provided are ANGPTL3 peptides modified in accordance with the present invention to have increased protease-resistance as compared to a wildtype ANGPTL3 polypeptide. Also provided are compositions and methods for administration of ANGPTL3 polypeptides to prevent or ameliorate arthritis or joint injury by administering a polypeptide of the invention into a joint, a cartilage tissue or a cartilage proximal tissue, or systemically. Further, the invention provides compositions and methods for induction of mesenchymal stem cell differentiation into chondrocytes.

Definitions

[0025] The term "protease-resistant" as used herein refers to a polypeptide comprising a modification that renders the polypeptide less susceptible to cleavage by a trypsin-like protease than a corresponding non-modified wildtype polypeptide. In specific embodiments a protease-resistant polypeptide is an ANGPTL3 polypeptide that has an amino acid substitution, relative to a native wildtype peptide sequence, at an R or a K residue.

[0026] "ANGPTL3" refers to a member of the angoipoietin protein family. An amino acid sequence of ANGPTL3 (GenBank Accession No. NP_055310.1) is set forth in SEQ ID NO:1; and the corresponding polynucleotide sequence of which is set forth as SEQ ID NO: 2 (NCBI reference sequence number NM014495.2, wherein the ANGPTL3 coding sequence comprises nt 52-1434 of SEQ ID NO:2). "ANGPTL3 polypeptide" refers to a naturally occurring expressed polypeptide. For the purposes of the present disclosure, the numbering of an amino acid is typically determined with reference to the full-length wildtype human ANGPTL3 polypeptide sequence (SEQ ID NO:1). Thus, in embodiments in which a polypeptide of the invention contains only a C-terminal portion of full-length ANGPTL3, but not the N-terminal portion, although the peptide is less than 460 amino acids in length, the numbering of the positions is based on SEQ ID NO:1. For example, reference to position 423 of an ANGPTL3 polypeptide of the invention refers to position 423 of SEQ ID NO:1, even though the ANGPTL3 polypeptide of the invention itself may only be 200 amino acids in length. In determining an amino acid in a sequence of interest that "corresponds to" a position in a reference sequence, such as SEQ ID NO:1, this is performed by optimally aligning the sequences, *e.g.,* using the default CLUSTAL alignment parameters or default BLAST 2 alignment parameters and comparing the sequences. For example, position 423 in a sequence of interest that is "determined with reference to SEQ ID NO:1", or an amino acid

that "corresponds to" position 423 of SEQ ID NO:1, means the amino acid that aligns with position 423 of SEQ ID NO:1 when the sequence of interest is optimally aligned with SEQ ID NO:1.

[0027] The terms "peptidomimetic" and "mimetic" refer to a synthetic chemical compound that has substantially the same functional characteristics of a naturally or non-naturally occurring polypeptide (e.g., ANGPTL3), but different (though typically similar) structural characteristics. Peptide analogs are commonly used in the field as non-peptide active compounds (e.g., drugs) with properties analogous to those of a template peptide. Such non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. *Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p. 392 (1985); and Evans *et al. J. Med. Chem.* 30:1229 (1987)). Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity), such as found in a polypeptide of interest, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of, e.g., $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, -CH=CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$, and $-CH_2SO-$. A mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. A mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. For example, a mimetic composition is within the scope of the invention if it is capable of chondrogenic activity of an ANGPTL3 polypeptide.

[0028] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. Polypeptides, peptides, and proteins of the invention comprise protease resistant ANGPTL3 peptidomimetics having chondrogenic activity.

[0029] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to naturally occurring amino acids. Naturally occurring amino acids are those encoded by the

genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) as well as pyrrolysine, pyrroline-carboxy-lysine, and selenocysteine.

[0030] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every polypeptide sequence herein which is encoded by a polynucleotide encompasses every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0031] One of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids with reference to an original encoded amino acid sequence results in a "conservatively modified variant" where the alteration produces substitution of an amino acid with a chemically similar amino acid and/or a polypeptide sequence that produces a structurally similar protein having similar functional activity to the

original protein. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0032] The term "conservative amino acid substitutions" refers to the substitution (conceptually or otherwise) of an amino acid from one such group with a different amino acid from the same group. One example of substitutions is based on analyzing the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (see, e.g., Schulz, G. E. and R. H. Schirmer, Principles of Protein Structure, Springer-Verlag). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other and, therefore, resemble each other most in their impact on the overall protein structure (see, e.g., Schulz, G. E. and R. H. Schirmer, Principles of Protein Structure, Springer-Verlag). One example of a set of amino acid groups defined in this manner include: (i) a charged group, consisting of Glu and Asp, Lys, Arg and His; (ii) a positively-charged group, consisting of Lys, Arg and His; (iii) a negatively-charged group, consisting of Glu and Asp; (iv) an aromatic group, consisting of Phe, Tyr and Trp; (v) a nitrogen ring group, consisting of His and Trp; (vi) a large aliphatic nonpolar group, consisting of Val, Leu and Ile; (vii) a slightly-polar group, consisting of Met and Cys; (viii) a small-residue group, consisting of Ser, Thr, Asp, Asn, Gly, Ala, Glu, Gln and Pro; (ix) an aliphatic group consisting of Val, Leu, Ile, Met and Cys; and (x) a small hydroxyl group consisting of Ser and Thr. Other examples of conservative substitutions based on shared physical properties are the substitutions within the following groups :1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (*see*, *e.g.*, Creighton, *Proteins* (1984)).

[0033] "Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the amino acid sequence or polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (e.g., a polypeptide of the invention), which does not comprise additions or deletions, for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number

of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0034] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same sequences. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 95% identity, optionally 96%, 97%, 98%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. The invention provides polypeptides that are substantially identical to the polypeptides, respectively, exemplified herein (*e.g.*, any of SEQ ID NOs: 11-42), as well as uses thereof including but not limited to use for treating or preventing arthritis or joint injury. Optionally, for nucleic acids, the identity exists over a region that is at least about 150 nucleotides in length, or more preferably over a region that is 300 to 450 or 600 or more nucleotides in length, or the entire length of the reference sequence. For amino acid sequence, optionally, identity exists over a region that is at least about 50 amino acids in length, or more preferably over a region that is 100 to 150 or 200 or more amino acids in length, or the entire length of the reference sequence.

[0035] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0036] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 50 to 600, usually about 75 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.*

2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA), or by manual alignment and visual inspection (*see, e.g.,* Ausubel *et al.*, *Current Protocols in Molecular Biology* (1995 supplement)).

[0037] Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0038] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum

probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0039] The term "isolated," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is purified to be essentially free of other cellular components with which it is associated in the natural state. It is often in a homogeneous or nearly homogeneous state. It can be in either a dry or aqueous solution. Purity and homogeneity may be determined using analytical chemistry techniques known and used typically in the art, e.g., polyacrylamide gel electrophoresis, high performance liquid chromatography, etc. A protein that is the predominant species present in a preparation is substantially purified. The term "purified" in some embodiments denotes that a protein gives rise to essentially one band in an electrophoretic gel. Typically, it means that a protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0040] The term "hyaluronic acid" are used herein to include derivatives of hyaluronic acid that include esters of hyaluronic acid, salts of hyaluronic acid and also includes the term hyaluronan. The designation also includes both low and high molecular weight forms of hyaluronans and crosslinked hyaluronans or hylans. Examples of such hyaluronans are Synvisc™ (Genzyme Corp. Cambridge, Mass.), ORTHOVISC™ (Anika Therapeutics, Woburn, Mass.), HYALGAN™ (Sanofi-Synthelabo Inc., Malvern, Pa.), and ProVisc (Alcon/Novartis).

[0041] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

Angiopoietin-like 3 protease-resistant polypeptides

[0042] Angiopoietin-like 3 is a member of the angiopoietin-like family of secreted factors. It is predominantly expressed in the liver, and has the characteristic structure of angiopoietins, consisting of a signal peptide, N-terminal coiled-coil domain (CCD) and the C-terminal fibrinogen (FBN)-like domain. Angiopoietin-like 3 was shown to bind αV/β3 integrins and FBN-like domain alone was sufficient to induce endothelial cell adhesion and in vivo angiogenesis (Camenisch *et al., J. Biol. Chem.* 277: 17281-17290, 2002). Endogenous ANGPTL3 is generally cleaved *in vivo* into amino-terminal and carboxy-terminal fragments.

As summarized above and further described herein, the present invention contemplates use of various protease-resistant ANGPTL3 proteins having chondrogenic activity.

[0043] In some embodiments, an isolated polypeptide comprises an amino acid sequence that has at least 95% identity, or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of the sequences of TABLE 1, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1. The polypeptides of the invention have chondrogenic activity. In some embodiments, a polypeptide comprises the amino acid sequence that has at least 95% identity, or at least or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70. wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In a further embodiment, a polypeptide comprises the amino acid sequence that has at least 95% identity, or at least or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64 wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity.

[0044] In some embodiments, an isolated polypeptide comprises an amino acid sequence selected from any one of the sequences of TABLE 1, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In some embodiments, a polypeptide comprises an amino acid sequence selected from any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37,

SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70 wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In a further embodiment, a polypeptide comprises an amino acid sequence selected from any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64 wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity.

[0045] In some embodiments, an isolated polypeptide has at least 95% identity, or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of the sequences of TABLE 1, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In some embodiments, a polypeptide has at least 95% identity, or at least or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In a further embodiment, a polypeptide has at least 95% identity, or at least or at least 96%, 97%, 98%, or 99% identity, to an amino acid sequence selected from any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64 wherein the polypeptide comprises an amino acid that is a polar

amino acid other than K or R at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity.

[0046] In some embodiments, an isolated polypeptide is an amino acid sequence selected from any one of the sequences of TABLE 1. In some embodiments, a polypeptide is an amino acid sequence selected from any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70. In a further embodiment, a polypeptide is an amino acid sequence selected from any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

TABLE 1: ANGPTL3 variant constructs

SEQ ID	Construct	Sequence
14	207KQ	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPE RRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
15	207KS	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPE RRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
16	225KQ	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE
17	225KS	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE
18	225ST	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAKTKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE

SEQ ID	Construct	Sequence
19	226KQ	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
20	226KS	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
21	228KQ	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
22	228KS	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
23	228ST	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAKTKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
24	233KQ	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTDSESFE
25	233KS	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTDSESFE
26	241KQ	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
27	241KS	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
28	242KQ	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
29	242KS	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE

SEQ ID	Construct	Sequence
30	225-455KQ	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTD
31	225-455KS	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTD
32	226-455KQ	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
33	226-455KS	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
34	228-455KQ	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKST KMLIHPTD
35	228-455KS	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKST KMLIHPTD
36	233-455KQ	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTD
37	233-455KS	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTD
38	241-455KQ	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
39	241-455KS	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
40	242-455KQ	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD

SEQ ID	Construct	Sequence
41	242-455KS	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
58	207Kdel	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPER RRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
59	225Kdel	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
60	226Kdel	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKS TKMLIHPTDSESFE
61	228Kdel	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTK MLIHPTDSESFE
62	233Kdel	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHP TDSESFE
63	241Kdel	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
64	242Kdel	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
65	225-455Kdel	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
66	226-455Kdel	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKS TKMLIHPTD
67	228-455Kdel	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTK MLIHPTD

SEQ ID	Construct	Sequence
68	233-455Kdel	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHP TD
69	241-455Kdel	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
70	242-455Kdel	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD

[0047] Modified ANGPTL3 polypeptides of the invention have at least one substitution in the C-terminal portion of the polypeptide to render the polypeptide protease resistant. The substitution is at an R or K residue so that polypeptides have increased resistance, e.g., to trypsin-like proteases. Any amino acid may be substituted for an R or K in a protease resistant ANGPTL3 polypeptide of the invention. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, a substitution is S or Q. In some embodiments, the substitution is Q. In some embodiments the substitution is S. In some embodiments, a protease-resistant peptide has an amino acid at position 423, with reference to SEQ ID NO:1, that is other than K or R. In some embodiments, a polypeptide of the invention comprises an amino acid at position 423 that is a polar amino acid. For example, the amino acid at position 423 may be Q or S or another polar amino acid. In certain embodiments a polypeptide of the invention has a Q at position 423. In other embodiments a polypeptide of the invention has an S at position 423. In some embodiments, in addition to substitution at 423, the protease-resistant peptide has a substitution of another R or K in the C-terminus of SEQ ID NO:1, or a variant thereof, wherein the substitution is a polar amino acid other than R or K. In some embodiments, the substitution at position 423 as determined with reference to SEQ ID NO:1, is Q or S. In still other embodiments a polypeptide of the invention has a deletion at position 423 as determined with reference to SEQ ID NO:1.

[0048] In some embodiments, a polypeptide of the invention is 250 amino acids or less in length and comprises the amino acid sequence of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:59, SEQ ID NO:60, SEQ ID

NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

[0049] In some embodiments, the invention provides for use of full-length protease-resistant, chondrogenic ANGPTL3 proteins. In some embodiments, the invention provides for protease-resistant ANGPTL3 proteins comprising a C-terminal portion of the ANGPTL3 sequence, or a chondrogenic variant thereof. In certain embodiments ANGPTL3 proteins lack the the amino-terminal end of the native protein. In some embodiments, protease-resistant ANGPTL3 proteins of the invention lack the CCD domain and/or lacks significant CCD activity. Thus, in some embodiments, the protease-resistant ANGPTL3 proteins of the invention comprise at least a fragment (e.g., at least 100, 150, 200, 220 or 215 contiguous amino acids) of a human ANGPTL3 protein carboxy-terminal domain, or a substantially identical sequence to the human carboxy-terminal ANGPTL3 protein sequence, wherein the polypeptide and variants thereof retains chondrogenic activity. In some embodiments, a protease-resistant polypeptide of the invention lacks at least a portion of the C-terminal sequence, e.g., lacks 5, 10, 15, or 20 amino acids from the C-terminal end of SEQ ID NO:1 (i.e., lacks 456-460, 451-460, 446-460 or 441-460 of SEQ ID NO:1) .

[0050] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention comprises contiguous amino acids corresponding to the amino acid regions: amino acids 241-455, or 241-460 of SEQ ID NO:1; amino acids 242-455, or 242-460 of SEQ ID NO:1; amino acids 233-455 or 233-460 of SEQ ID NO:1; amino acids 228-455 or 228-460 of SEQ ID NO:1, amino acids 226-455- or 226-260 or amino acids 225-455- or 225-260 of SEQ ID NO:1 in which an amino acid is substituted for an R or K or a single residue is deleted. In some embodiments, a substitution is at position 423 as determined with reference to SEQ ID NO:1. In some embodiments a deletion is at position 423 as determined with reference to SEQ ID NO:1. In some embodiments, a protease-resistant polypeptide comprises contiguous amino acids corresponding to the amino acid regions 207-455 or 207-460 of SEQ ID NO:1 in which an amino acid is substituted for R or K or a single residue is deleted. In some embodiments, a substitution or deletion is at position 423. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, a substitution is S or Q. In some embodiments, a substitution is Q. In certain embodiments a deletion at position 423 relative to SEQ ID NO:1 is included.

[0051] The invention additionally provides a protease-resistant polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 95% identity, or at least 96%, 97%, 98%, or 99% identity, to amino acids 240-454 of SEQ ID NO:1, amino acids 241-455 of SEQ ID NO:1, or amino acids 242-455 of SEQ ID NO:1 with a substitution or deletion at the amino acid corresponding to position 423 of SEQ ID NO:1, where the substituted amino acid is not R, and wherein the polypeptide has chondrogenic activity. In other embodiments, the polypeptide comprises amino acids 240-454 of SEQ ID NO:1, amino acids 241-455 of SEQ ID NO:1, or amino acids 242-455 of SEQ ID NO:1, each polypeptide with a substitution or deletion at the amino acid corresponding to position 423 of SEQ ID NO:1, where the substituted amino acid is Q or S.

[0052] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention comprises an amino acid sequence having at least 95%, or at least 96%, at least 97%, at least 98%, or at least 99% identity to amino acids amino acids 242-455 or 242-460 of SEQ ID NO:1; 241-455 or 241-460 of SEQ ID NO:1; amino acids 233-455 or 233-460 of SEQ ID NO:1; amino acids 228-455 or 228-460 of SEQ ID NO:1, amino acids 226-455- or 226-260 of SEQ ID NO:1, or amino acids 225-455- or 225-260 of SEQ ID NO:1 in which an amino acid is substituted for an R or K, or an R or K is deleted. In some embodiments, the substitution or deletion is at position 423. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is a Q. In certain embodiments there is a deleted residue at position 423 relative to SEQ ID NO:1.

[0053] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention is 250 or 240 or fewer amino acids in length and comprises the amino acid sequence of SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:70. In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention is 230 or 225 or fewer amino acids in length and comprises the amino acid sequence of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27,

SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

[0054] In some embodiments the protease resistant ANGPTL3 proteins of the invention comprise an amino acid sequence having at least 95% identity, or at least 96%, 97%, 98%, or 99% identity, to the C-terminal canine, bovine, or equine ANGPTL3 protein sequence. In some embodiments, the protease-resistant ANGPTL3 proteins of the invention comprise at least a fragment (e.g., at least 100, 150, 200, 215 contiguous amino acids) of a native canine (SEQ ID NO:4), equine (SEQ ID NO:5), or bovine (SEQ ID NO:6) ANGPTL3 protein sequence, or a substantially identical sequence to the native canine, bovine, or equine ANGPTL3 protein sequence wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In some embodiments, an isolated polypeptide comprises an amino acid sequence having at least 95% identity, or at least 96%, 97%, 98%, or 99% identity, to SEQ ID NO:42 or SEQ ID NO:43, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In some embodiments, a polypeptide has at least 95% identity, or at least or at least 96%, 97%, 98%, or 99% identity, to SEQ ID NO:42, or SEQ ID NO:43 wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423 or the polypeptide comprises a deletion at position 423, as determined with reference to SEQ ID NO:1, and the polypeptide has chondrogenic activity. In certain embodiments a polypeptide comprises SEQ ID NO:42, or SEQ ID NO:43. In a further embodiment, a polypeptide is SEQ ID NO:42, or SEQ ID NO:43.

[0055] In some embodiments, a protease-resistant ANGPTL3 of the invention comprises an amino acid sequence that has at least 95%, or at least 96%, 97%, 98%, or at least 99% identity to amino acids 232-454 of SEQ ID NO:4, amino acids 240-454 of SEQ ID NO:4, amino acids 227-454 of SEQ ID NO:4, or amino acids 224-454 of SEQ ID NO:4 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 422 of SEQ ID NO:4, which corresponds to position 423 of SEQ ID NO:1. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S,

T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is a Q. In some embodiments an amino acid deletion is at position 422 of SEQ ID NO:4.

[0056] In some embodiments, a protease-resistant ANGPTL3 of the invention comprises an amino acid sequence that has at least 95%, or at least 96%, 97%, 98%, or at least 99% identity to amino acids 233-455 of SEQ ID NO:5, amino acids 241-455 of SEQ ID NO:5, amino acids 228-455 of SEQ ID NO:5, or amino acids 225-455 of SEQ ID NO:5 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 423 of SEQ ID NO:5, which corresponds to position 423 of SEQ ID NO:1. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is a Q. In some embodiments an amino acid deletion is at position 423 of SEQ ID NO:5.

[0057] In some embodiments, a protease-resistant ANGPTL3 of the invention comprises an amino acid sequence that has at least 95%, or at least 96%, 97%, 98%, or at least 99% identity to amino acids 233-455 of SEQ ID NO:6, amino acids 241-455 of SEQ ID NO:6, amino acids 228-455 of SEQ ID NO:6, or amino acids 225-455 of SEQ ID NO:6 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 422 of SEQ ID NO:6, which corresponds to position 423 of SEQ ID NO:1. In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is a Q. In some embodiments an amino acid deletion is at position 422 of SEQ ID NO:6.

[0058] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention comprises contiguous amino acids corresponding to the amino acid regions: amino acids 240-454 of SEQ ID NO:4; amino acids 232-454 of SEQ ID NO:4; amino acids 227-454 of SEQ ID NO:4, or amino acids 224-454 of SEQ ID NO:4 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 422 of SEQ ID NO:4 (which is position 423 as determined with reference to SEQ ID NO:1). In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some

embodiments, the substitution is S or Q. In some embodiments, the substitution is Q. In some embodiments an amino acid deletion is at position 422 of SEQ ID NO:4.

[0059] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention comprises contiguous amino acids corresponding to the amino acid regions: amino acids 241-455 of SEQ ID NO:5; amino acids 233-455 of SEQ ID NO:5; amino acids 228-455 of SEQ ID NO:5, or amino acids 225-455 of SEQ ID NO:5 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 423 (which corresponds to position 423 as determined with reference to SEQ ID NO:1). In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is Q. In some embodiments an amino acid deletion is at position 423 of SEQ ID NO:5.

[0060] In some embodiments, a protease-resistant ANGPTL3 polypeptide of the invention comprises contiguous amino acids corresponding to the amino acid regions: amino acids 241-455 of SEQ ID NO:6; amino acids 233-455 of SEQ ID NO:6; amino acids 228-455 of SEQ ID NO:6, or amino acids 225-455 of SEQ ID NO:6 in which an amino acid is substituted for an R or K or there is a deletion of an R or K. In some embodiments, the substitution or deletion is at position 422 of SEQ ID NO:6 (which is position 423 as determined with reference to SEQ ID NO:1). In some embodiments, a substitution is a polar amino acid, *e.g.,* H, N, Q, S, T, A, or Y. In some embodiments, a substitution is H, N, Q, S, T, or Y. In some embodiments, the substitution is S or Q. In some embodiments, the substitution is Q. In some embodiments there is a deletion at position 422 of SEQ ID NO:6.

[0061] The ANGPTL3 proteins of the invention as described above may include native ANGPTL3 protein sequences flanking the regions described above. Alternatively, in some embodiments, the ANGPTL3 proteins of the invention can include non-native ANGPTL3 protein flanking sequences. For example, the chondrogenic active portion of an ANGPTL3 protein can be fused to one or more fusion partners and/or heterologous amino acids to form a fusion protein. Fusion partner sequences can include, but are not limited to, amino acid tags, non-L (e.g., D-) amino acids or other amino acid mimetics to extend *in vivo* half-life and/or protease resistance, targeting sequences or other sequences.

[0062] In some embodiments, a polypeptide of the invention is PEGylated. In some embodiments, a polypeptide of the invention is fused to a heterologous peptide. In certain embodiments a polypeptide is fused to any one of human serum albumin (HSA), an

immunoglobulin heavy chain constant region (Fc), a polyhistidine, a glutathione S transferase (GST), a thioredoxin, a protein A, a protein G, a maltose binding protein (MBP), or a fragment of any of the foregoing heterologous polypeptide(s). In particular embodiments a heterologous polypeptide is fused at the amino-terminal end of the polyptide of the invention. In additional or alternative embodiments a heterologous polypeptide is fused at the carboxy-terminal end of the polypeptide of the invention.

[0063] ANGPTL3 proteins of the invention have chondrogenic activity and are protease-resistant. As defined herein, chondrogenesis or chondrogenic activity refers to the development of chondrocytes from MSCs. Indicators of chondrogenic activity include, but are not limited to, cartilage matrix production. Cartilage matrix production may be measured by various markers, for example, such as Sox9, type II collagen, or glycosaminoglycan (GAG) production. In some embodiments, GAG production is measured as a marker for cartilage matrix production. In some embodiments, a 3-fold increase in GAG production with cartilage specific protein expression indicates positive cartilage matrix production.

[0064] A polypeptide may be evaluated for protease resistance using any known assay that measures cleavage by a serine protease such as trypsin. In some embodiments, the protease employed to evaluate proteolysis susceptibility is the serine protease trypsin. A polypeptide is considered to be protease-resistant if it has reduced sensitivity to trypsin when compared to its wild-type counterpart. An example of an assay is to measure the amount of cleaved product that is generated when a polypeptide is exposed to trypsin over a period of time in comparison to a corresponding native human peptide. Cleavage can be measured using any known assay, *e.g.,* SDS PAGE or LCMS. An illustrative assay is provided in the Examples section.

[0065] In an illustrative assay, limited proteolysis by trypsinolysis is performed by incubating 10 ng of the protein to be evaluated with trypsin at mass ratio of 8000:1 (Protein:Trypsin) for 1 hr at room temperature. The trypsinolysis reaction can then be quenched by addition of acetic acid to bring the reaction to pH 3.0. The quenched samples are then separated analyzed by SDS-PAGE, *e.g.,* on a 4-12% Tris-Bis gel to identify proteins which are resistant to cleavage from those that are cleaved by the appearance of a fragment that is generated by trypsin cleavage. The cleavage product is absent or reduced in the protease-resistant polypeptides in comparison to their wildtype counterparts.

[0066] In some embodiments, the ANGPTL3 polypeptides of the invention will comprise at least one non-naturally encoded amino acid. In some embodiments, a polypeptide

comprises 1, 2, 3, 4, or more unnatural amino acids. Methods of making and introducing a non-naturally-occurring amino acid into a protein are known. *See*, e.g., US Patent Nos. 7,083,970; and 7,524,647. The general principles for the production of orthogonal translation systems that are suitable for making proteins that comprise one or more desired unnatural amino acid are known in the art, as are the general methods for producing orthogonal translation systems. For example, see International Publication Numbers WO 2002/086075, entitled "METHODS AND COMPOSITION FOR THE PRODUCTION OF ORTHOGONAL tRNA-AMINOACYL-tRNA SYNTHETASE PAIRS;" WO 2002/085923, entitled "IN VIVO INCORPORATION OF UNNATURAL AMINO ACIDS;" WO 2004/094593, entitled "EXPANDING THE EUKARYOTIC GENETIC CODE;" WO 2005/019415, filed Jul. 7, 2004; WO 2005/007870, filed Jul. 7, 2004; WO 2005/007624, filed Jul. 7, 2004; WO 2006/110182, filed Oct. 27, 2005, entitled "ORTHOGONAL TRANSLATION COMPONENTS FOR THE VIVO INCORPORATION OF UNNATURAL AMINO ACIDS" and WO 2007/103490, filed Mar. 7, 2007, entitled "SYSTEMS FOR THE EXPRESSION OF ORTHOGONAL TRANSLATION COMPONENTS IN EUBACTERIAL HOST CELLS." For discussion of orthogonal translation systems that incorporate unnatural amino acids, and methods for their production and use, see also, Wang and Schultz, (2005) "Expanding the Genetic Code." Angewandte Chemie Int Ed 44: 34-66; Xie and Schultz, (2005) "An Expanding Genetic Code." Methods 36: 227-238; Xie and Schultz, (2005) "Adding Amino Acids to the Genetic Repertoire." Curr Opinion in Chemical Biology 9: 548-554; and Wang, et al., (2006) "Expanding the Genetic Code." Annu Rev Biophys Biomol Struct 35: 225-249; Deiters, et al, (2005) "In vivo incorporation of an alkyne into proteins in Escherichia coli." Bioorganic & Medicinal Chemistry Letters 15:1521-1524; Chin, et al., (2002) "Addition of p-Azido-L-phenylalanine to the Genetic Code of Escherichia coli." J Am Chem Soc 124: 9026-9027; and International Publication No. WO2006/034332, filed on Sep. 20, 2005. Additional details are found in U.S. Pat. No. 7,045,337; No. 7,083,970; No. 7,238,510; No. 7,129,333; No. 7,262,040; No. 7,183,082; No. 7,199,222; and No. 7,217,809.

[0067] A "non-naturally encoded amino acid" refers to an amino acid that is not one of the common amino acids or pyrolysine, pyrroline-carboxy-lysine, or selenocysteine. Other terms that may be used synonymously with the term "non-naturally encoded amino acid" are "non-natural amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and variously hyphenated and non-hyphenated versions thereof. The term "non-naturally encoded amino acid" also includes, but is not limited to, amino acids that occur by modification (e.g. post-translational modifications) of a naturally encoded amino acid (including but not limited

to, the 20 common amino acids or pyrrolysine, pyrroline-carboxy-lysine, and selenocysteine) but are not themselves naturally incorporated into a growing polypeptide chain by the translation complex. Examples of such non-naturally-occurring amino acids include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine.

[0068] A non-naturally encoded amino acid is typically any structure having any substituent side chain other than one used in the twenty natural amino acids. Because the non-naturally encoded amino acids of the invention typically differ from the natural amino acids only in the structure of the side chain, the non-naturally encoded amino acids form amide bonds with other amino acids, including but not limited to, natural or non-naturally encoded, in the same manner in which they are formed in naturally occurring polypeptides. However, the non-naturally encoded amino acids have side chain groups that distinguish them from the natural amino acids. For example, R optionally comprises an alkyl-, aryl-, acyl-, keto-, azido-, hydroxyl-, hydrazine, cyano-, halo-, hydrazide, alkenyl, alkynl, ether, thiol, seleno-, sulfonyl-, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, ester, thioacid, hydroxylamine, amino group, or the like or any combination thereof. Other non-naturally occurring amino acids of interest that may be suitable for use in the present invention include, but are not limited to, amino acids comprising a photoactivatable cross-linker, spin-labeled amino acids, fluorescent amino acids, metal binding amino acids, metal-containing amino acids, radioactive amino acids, amino acids with novel functional groups, amino acids that covalently or noncovalently interact with other molecules, photocaged and/or photoisomerizable amino acids, amino acids comprising biotin or a biotin analogue, glycosylated amino acids such as a sugar substituted serine, other carbohydrate modified amino acids, keto-containing amino acids, amino acids comprising polyethylene glycol or polyether, heavy atom substituted amino acids, chemically cleavable and/or photocleavable amino acids, amino acids with an elongated side chains as compared to natural amino acids, including but not limited to, polyethers or long chain hydrocarbons, including but not limited to, greater than about 5 or greater than about 10 carbons, carbon-linked sugar-containing amino acids, redox-active amino acids, amino thioacid containing amino acids, and amino acids comprising one or more toxic moiety.

[0069] Exemplary non-naturally encoded amino acids that may be suitable for use in the present invention and that are useful for reactions with water soluble polymers include, but are not limited to, those with carbonyl, aminooxy, hydrazine, hydrazide, semicarbazide, azide

and alkyne reactive groups. In some embodiments, non-naturally encoded amino acids comprise a saccharide moiety. Examples of such amino acids include N-acetyl-L-glucosaminyl-L-serine, N-acetyl-L-galactosaminyl-L-serine, N-acetyl-L-glucosaminyl-L-threonine, N-acetyl-L-glucosaminyl-L-asparagine and O-mannosaminyl-L-serine. Examples of such amino acids also include examples where the naturally-occurring N- or O-linkage between the amino acid and the saccharide is replaced by a covalent linkage not commonly found in nature--including but not limited to, an alkene, an oxime, a thioether, an amide and the like. Examples of such amino acids also include saccharides that are not commonly found in naturally-occurring proteins such as 2-deoxy-glucose, 2-deoxygalactose and the like.

[0070] Another type of modification that can optionally be introduced into the ANGPTL3 proteins of the invention (e.g. within the polypeptide chain or at either the N- or C-terminal), e.g., to extend in vivo half-life, is PEGylation or incorporation of long-chain polyethylene glycol polymers (PEG). Introduction of PEG or long chain polymers of PEG increases the effective molecular weight of the present polypeptides, for example, to prevent rapid filtration into the urine. In some embodiments, a Lysine residue in the ANGPTL3 sequence is conjugated to PEG directly or through a linker. Such linker can be, for example, a Glu residue or an acyl residue containing a thiol functional group for linkage to the appropriately modified PEG chain. An alternative method for introducing a PEG chain is to first introduce a Cys residue at the C-terminus or at solvent exposed residues such as replacements for Arg or Lys residues. This Cys residue is then site-specifically attached to a PEG chain containing, for example, a maleimide function. Methods for incorporating PEG or long chain polymers of PEG are well known in the art (described, for example, in Veronese, F. M., et al., *Drug Disc. Today* 10: 1451-8 (2005); Greenwald, R. B., et al., *Adv. Drug Deliv. Rev.* 55: 217-50 (2003); Roberts, M. J., et al., *Adv. Drug Deliv. Rev.*, 54: 459-76 (2002)), the contents of which is incorporated herein by reference. Other methods of polymer conjugations known in the art can also be used in the present invention. In some embodiments, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) is introduced as a polymer conjugate with the ANGPTL3 proteins of the invention (*see*, e.g., WO2008/098930; Lewis, et al., *Bioconjug Chem.*, 19: 2144-55 (2008)). In some embodiments, a phosphorylcholine-containing polymer conjugate with the ANGPTL3 proteins can be used in the present invention. A person of skill would readily recognize that other biocompatible polymer conjugates can be utilized.

[0071] A more recently reported alternative approach for incorporating PEG or PEG polymers through incorporation of non-natural amino acids (as described above) can be

performed with the present polypeptides. This approach utilizes an evolved tRNA/tRNA synthetase pair and is coded in the expression plasmid by the amber suppressor codon (Deiters, A, et al. (2004). *Bio-org. Med. Chem. Lett.* 14, 5743-5). For example, p-azidophenylalanine can be incorporated into the present polypeptides and then reacted with a PEG polymer having an acetylene moiety in the presence of a reducing agent and copper ions to facilitate an organic reaction known as "Huisgen [3+2]cycloaddition."

[0072] In certain embodiments, the present invention also contemplates specific mutations of the ANGPTL3 proteins so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, including but not limited to, O-linked or N-linked glycosylation sites. In certain embodiments, the ANGPTL3 proteins of the present invention have glycosylation sites and patterns unaltered relative to the naturally-occurring ANGPTL3 proteins. In certain embodiments, a variant of ANGPTL3 proteins includes a glycosylation variant wherein the number and/or type of glycosylation sites have been altered relative to the naturally-occurring ANGPTL3 proteins. In certain embodiments, a variant of a polypeptide comprises a greater or a lesser number of N-linked glycosylation sites relative to a native polypeptide. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. In certain embodiments, a rearrangement of N-linked carbohydrate chains is provided, wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created.

[0073] Exemplary ANGPTL3 proteins variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) relative to the amino acid sequence of the naturally-occurring ANGPTL3 proteins. In certain embodiments, cysteine variants may be useful when ANGPTL3 proteins must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. In certain embodiments, cysteine variants have fewer cysteine residues than the native polypeptide. In certain embodiments, cysteine variants have an even number of cysteine residues to minimize interactions resulting from unpaired cysteines.

[0074] In some embodiments, functional variants or modified forms of the ANGPTL3 proteins include fusion proteins of an ANGPTL3 protein of the invention and one or more fusion domains. Well known examples of fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy chain constant region (Fc), maltose binding protein (MBP), and/or human serum albumin (HSA). A fusion domain or a fragment thereof may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt-conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QLAexpress™ system (Qiagen) useful with (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ANGPTL3 proteins. Examples of such detection domains include the various fluorescent proteins (e.g., GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or Thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In certain embodiments, an ANGPTL3 protein is fused with a domain that stabilizes the ANGPTL3 protein in vivo (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (e.g., dimerizing, tetramerizing) domains and functional domains (that confer an additional biological function, as desired). Fusions may be constructed such that the heterologous peptide is fused at the amino terminus of a polypeptide of the invention and/or at the carboxy terminus of a polypeptide of the invention.

Nucleic acids encoding Angiopoietin-like 3 protease-resistant polypeptides

[0075] The invention also provides nucleic acids encoding protease resistant polypeptides of the invention and expression vectors and host cells for expression of a protease resistant polypeptide. In other aspects, the invention provides a polynucleotide encoding a polypeptide of the invention and expression vectors and host cells comprising such a polynucleotide. In some embodiments, the polynucleotide is optimized for expression in the host cells. In some embodiments, the invention provides a method of ameliorating or preventing arthritis or joint injury in a human patient, the method comprising: administering to a joint of the patient an expression vector encoding a polypeptide of the invention whereupon expression of the polypeptide ameliorates or prevents arthritis or joint injury in the patient. In some embodiments, the patient has arthritis or joint injury. In some embodiments, the individual does not have, but is at risk for, arthritis or joint injury. In some embodiments, the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis.

[0076] Expressing polypeptides of the invention employs routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook and Russell eds. (2001) Molecular Cloning: A Laboratory Manual, 3rd edition; the series Ausubel et al. eds. (2007 with updated through 2010) Current Protocols in Molecular Biology, among others known in the art.

[0077] Expression can employ any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc. Both prokaryotic and eukaryotic expression systems are widely available. In some embodiments, the expression system is a mammalian cell expression, such as a CHO cell expression system. In some embodiments, a nucleic acid may be codon-optimized to facilitate expression in a desired host cell.

[0078] Nonviral vectors and systems include plasmids and episomal vectors, typically comprising an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, e.g., Harrington et al., Nat Genet 15:345, 1997). For example, nonviral vectors useful for expression of the polypeptides of the invention in mammalian (e.g., human) cells include pThioHis A, B & C, pcDNA3. I/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include, but are not limited to, vectors based on adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma

virus, HBP Epstein Barr virus, fowpox vectors, vaccinia virus vectors and Semliki Forest virus (SFV).

[0079] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (e.g., enhancers) that are operably linked to the polynucleotides encoding a polypeptide of the invention. In some embodiments, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, e.g., arabinose, lacZ, a metallothionein promoter, a glucocorticoid promoters or a heat shock promoter. In addition, other regulatory elements may also be incorporated to improve expression of a nucleic acid encoding a polypeptide of the invention, *e.g.,* enhancers, ribosomal binding site, transcription termination sequences, and the like.

[0080] In some embodiments, a nucleic acid encoding an polypeptide of the invention may also include a sequence encoding a secretion signal sequence so that the polypeptide is secreted from the host cell. Such a sequence can be provided by the vector, or as part of the ANGPTL3 nucleic acid that is present in the vector.

[0081] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally *Sambrook et al., supra*). Other methods include, e.g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation: nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22, agent-enhanced uptake of DNA, and ex vivo transduction. For long-term, high- yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express polypeptides of the invention can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene.

[0082] In some embodiments, nucleic acids encoding protease resistant ANGPTL3 polypeptides of the invention can be delivered to a patient for treatment of a joint-related injury or disease. Delivery of such nucleic acids can be achieved using any means known in the art, but is typically performed using direct injection into the affected joint. In some

embodiments, a DNA is delivered as naked DNA using direct injection into the joint. In some embodiments, a viral vector is employed, including, but not limited to, an adenovirus or adenovirus-associated vector, a herpes virus vector, fowlpox virus, or a vaccinia virus vector.

Methods of therapeutic use of polypeptides, and indications

[0083] Provided methods of the invention include a method of treating a subject comprising administering to the subject a therapeutically effective amount of a polypeptide of the invention, wherein the subject has or is at risk of joint damage or arthritis. The invention also provides a method of ameliorating or preventing arthritis or joint injury in a human patient, the method comprising: administering to a joint of the patient a composition comprising an effective amount of a polypeptide of the invention, thereby ameliorating or preventing arthritis or joint injury in the patient. In some embodiments, the patient has arthritis or joint injury. In some embodiments, the individual does not have, but is at risk for, arthritis or joint injury. In some embodiments, the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis. In some embodiments, the composition administered to the further comprises hyaluronic acid.

[0084] In another aspect, the invention provides a method of inducing differentiation of mesenchymal stem cells into chondrocytes, the method comprising, contacting mesenchymal stem cells with a sufficient amount of a polypeptide of the invention to induce differentiation of the stem cells into chondrocytes. In some embodiments, the method is performed *in vivo* and the stem cells are present in a human patient.

[0085] It is contemplated that polypeptides, compositions, and methods of the present invention may be used to treat, ameliorate or prevent any type of articular cartilage damage (e.g., joint damage or injury) including, for example, damage arising from a traumatic event or tendon or ligament tear. In some embodiments, proteins of the invention are administered to prevent or ameliorate arthritis or joint damage, for example where there is a genetic or family history of arthritis or joint damage or joint injury or prior or during joint surgery. In some embodiments polypeptides, compositions and methods are used to treat joint damage. In particular embodiments joint damage is traumatic joint injury. In other embodiments joint damage is damage arising from age or inactivity. In yet other embodiments joint damage is damage arising from an autoimmune disorder. In some embodiments of the invention, polypeptides, compositions, and methods of the present invention may be used to treat, ameliorate or prevent osteoarthritis. In some embodiments, the polypeptides, compositions and methods are used to ameliorate or prevent arthritis in a subject at risk of having or

acquiring arthritis. In some embodiments, the polypeptides, compositions and methods are used to ameliorate or prevent joint damage in a subject at risk of having or acquiring joint damage.

[0086] In some embodiments, polypeptides, compositions, and methods of the present invention provide a method for stimulating chondrocyte proliferation and cartilage production in cartilagenous tissues that have been damaged, e.g., due to traumatic injury or chondropathy. In particular embodiments polypeptides, compositions, and methods of the present invention are useful for treatment of cartilage damage in joints, e.g., at articulated surfaces, e.g., spine, shoulder, elbow, wrist, joints of the fingers, hip, knee, ankle, and joints of the feet. Examples of diseases or disorders that may benefit from treatment include osteoarthritis, rheumatoid arthritis, other autoimmune diseases, or osteochondritis dessicans. In addition, cartilage damage or disruption occurs as a result of certain genetic or metabolic disorders, cartilage malformation is often seen in forms of dwarfism in humans, and/or cartilage damage or disruption is often a result of reconstructive surgery; thus polypeptides, compositions, and methods would be useful therapy in these patients, whether alone or in connection with other approaches.

[0087] It is further contemplated that polypeptides, compositions, and methods of the present invention may be used to treat, ameliorate or prevent various cartilagenous disorders and/or associated symptoms or effects of such conditions. Exemplary conditions or disorders for treatment, amelioration and/or prevention with polypeptides, compositions, and methods of the invention, include, but are not limited to systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, degenerative disc disease, spondyloarthropathies, Ehlers Danlos syndrome, systemic sclerosis (scleroderma) or tendon disease. Other conditions or disorders that may benefit from treatment with polypeptides for amelioration of associated effects include idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barr syndrome, and chronic inflammatory demyelinating

polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease.

[0088] A "patient" as used herein refers to any subject that is administered a therapeutic polypeptide of the invention. It is contemplated that the polypeptides, compositions, and methods of the present invention may be used to treat a mammal. As used herein a "subject" refers to any mammal, including humans, domestic and farm animals, and zoo, sports or pet animals, such as cattle (e.g. cows), horses, dogs, sheep, pigs, rabbits, goats, cats, etc. In some embodiments of the invention, the subject is a human. In certain embodiments, the subject is a horse. In other embodiments the subject is a dog.

[0089] In some embodiments, the polypeptides of the invention can be heterologous to the mammal to be treated. For example, a human ANGPTL3 protein or fragments thereof, a protein or peptide derived from a human ANGPTL3 protein (e.g., a modified human ANGPTL3 protein, a conservative variant of human ANGPTL3 protein, a peptidomimetic derived from a human ANGPTL3 protein) are used in the treatment of an animal such as an equine, bovine or canine. In some embodiments, a heterologous ANGPTL3 protein can be used to expand chondrocyte populations in culture for transplantation. In some embodiments, expanded cultures will then be optionally admixed with polypeptides and compositions homologous to the mammal to be treated, and placed in the joint space or directly into the cartilage defect. Alternatively, polypeptides of the invention are derived from the same species, i.e., a human ANGPTL3 protein or fragments thereof, a protein or peptide derived from a human ANGPTL3 protein (e.g., a modified human ANGPTL3 protein, a conservative variant of human ANGPTL3 protein, a peptidomimetic derived from a human ANGPTL3 protein) is used in the treatment of a human patient. By using a protein derived from the same species of mammal as is being treated, inadvertent immune responses may be avoided.

[0090] In some embodiments, polypeptides and compositions of the present invention are applied by direct injection into the synovial fluid of a joint, systemic administration (oral or intravenously) or directly into a cartilage defect, either alone or complexed with a suitable carrier for extended release of protein. In some embodiments, polypeptides or compositions are administered in a biocompatible matrix or scaffold. Polypeptides, compositions, and methods of the present invention can also be used in conjunction with a surgical procedure at an affected joint. Administration of a polypeptide of the invention may occur prior to, during or in conjunction with, and/or after a surgical procedure. For example, polypeptides, compositions and methods of the invention can be used to expand chondrocyte populations in culture for autologous or allogenic chondrocyte implantation (ACI). Chondrocytes can be optionally implanted with concurrent treatment consisting of administration of polypeptides and compositions of the present invention. In these procedures, for example, chondrocytes can be harvested arthroscopically from an uninjured minor load-bearing area of a damaged joint, and can be cultured *in vitro*, optionally in the presence of polypeptides and compositions of the present invention and/or other growth factors to increase the number of cells prior to transplantation. Expanded cultures are then optionally admixed with polypeptides and compositions of the present invention and/or placed in the joint space or directly into the defect. In certain embodiments, expanded cultures (optionally with polypeptides of the present invention) are placed in the joint space suspended in a matrix or membrane. In other embodiments, polypeptides and compositions of the present invention can be used in combination with one or more periosteal or perichondrial grafts that contain cartilage forming cells and/or help to hold the transplanted chondrocytes or chondrocyte precursor cells in place. In some embodiments, polypeptides and compositions of the present invention are used to repair cartilage damage in conjunction with other procedures, including but not limited to lavage of a joint, stimulation of bone marrow, abrasion arthroplasty, subchondral drilling, or microfracture of proximal subchondral bone. Optionally, following administration of polypeptides and compositions of the present invention and growth of cartilage, additional surgical treatment may be beneficial to suitably contour newly formed cartilage surface(s).

Pharmaceutical Compositions

[0091] Therapeutic compositions comprising provided polypeptides are within the scope of the present invention, and are specifically contemplated in light of the identification of several polypeptide sequences exhibiting enhanced stability and protease resistance. Thus, in

a further aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide of the invention. In certain embodiments, pharmaceutical compositions further comprise a pharmaceutically or physiologically acceptable carrier. In some embodiments, a pharmaceutical composition further comprises a hyaluronic acid or a derivative thereof.

[0092] In addition, the invention provides a method of ameliorating or preventing arthritis or joint injury in a human patient, the method comprising: administering to a joint of the patient a composition comprising an effective amount of a polypeptide of the invention, thereby ameliorating or preventing arthritis or joint injury in the patient. In some embodiments, the patient has arthritis or joint injury. In some embodiments, the individual does not have, but is at risk for, arthritis or joint injury. In some embodiments, the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis. In some embodiments, the composition administered to the further comprises hyaluronic acid.

[0093] In another aspect, the invention provides a method of inducing differentiation of mesenchymal stem cells into chondrocytes, the method comprising, contacting mesenchymal stem cells with a sufficient amount of a polypeptide of the invention to induce differentiation of the stem cells into chondrocytes. In some embodiments, the method is performed *in vivo*, the stem cells are present in a human patient, and the contacting comprises administering to a joint of the patient a composition comprising an effective amount of a polypeptide of the invention, thereby inducing differentiation of stem cells into chondrocytes, and generation of cartilage.

[0094] Therapeutic compositions comprising nucleic acids encoding polypeptides of the invention can be delivered to a patient for treatment of a joint-related injury or disease, and are also within the scope of the present invention. In some embodiments, pharmaceutical compositions comprise naked DNA encoding a polypeptide of the invention. In some embodiments, a viral vector is employed to effect delivery and a pharmaceutical composition comprises a vector encoding a polypeptide of the invention, including, but not limited to, an adenovirus or adenovirus-associated vector, a herpes virus vector, fowlpox virus, or a vaccinia virus vector. Pharmaceutical compositions comprise a therapeutically effective amount of a nucleic acid encoding a polypeptide of the invention with a pharmaceutically or physiologically acceptable carrier.

[0095] In another aspect of the present invention, provided polypeptides for use as a medicament for treatment of joint damage is contemplated. In certain embodiments

polypeptides of the invention for use as a medicament for amelioration of arthritis or joint damage are provided. In some embodiments arthritis is osteoarthritis, trauma arthritis or autoimmune arthritis. In some embodiments joint damage is traumatic joint injury, autoimmune damage, age related damage, or damage related to inactivity. In other embodiments, nucleic acid encoding a polypeptide of the invention for use in a medicament is provided.

[0096] Formulations suitable for administration include excipients, including but not limited to, aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In certain embodiments pharmaceutical compositions comprise a therapeutically effective amount of a peptide in admixture with a pharmaceutically acceptable formulation agent selected for suitability with the mode of administration, delivery format, and desired dosage. See, e.g., Remington's Pharmaceutical Sciences (18^{th} Ed., A.R. Gennaro, ed., Mack Publishing Company 1990), and subsequent editions of the same. The primary vehicle or carrier in a pharmaceutical composition can be aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection can be water, physiological saline solution or artificial cerebrospinal fluid, optionally supplemented with other materials common in compositions for parenteral administration. For example, buffers may be used, e.g., to maintain the composition at physiological pH or at a slightly lower pH, typically within a range of from about pH 5 to about pH 8, and may optionally include sorbitol, serum albumin, detergent, or other additional component. In certain embodiments pharmaceutical compositions comprising polypeptides or a nucleic acid encoding a polypeptide of the invention can be prepared for storage in a lyophilized form using appropriate excipients (e.g., sucrose).

[0097] In yet other embodiments formulation with an agent, such as injectable microshperes, bio-erodable particles, polymeric compounds, beads, or liposomes or other biocompatible matrix that provides for controlled or sustained release of the polypeptide or a nucleic acid encoding a polypeptide of the invention can then be delivered via a depot injection. For example, polypeptides or nucleic acid encoding a polypeptide of the invention may be encapsulated in liposomes, or formulated as microparticles or microcapsules or may be incorporated into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez et al., 1999, Bioconjugate Chem., 10, 1068-1074; Wang et al.,

International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLCA microspheres (see for example U.S. Pat. No. 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722) or by the use of conjugates. Still other suitable delivery mechanisms include implantable delivery devices.

[0098] The dose of a compound of the present invention for treating the above-mentioned diseases or disorders varies depending upon the manner of administration, the age and/or the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. The dose administered to a subject, in the context of the present invention should be sufficient to effect a beneficial response in the subject over time. Such a dose is a "therapeutically effective amount". Accordingly, an appropriate dose may be determined by the efficacy of the particular protein or a nucleic acid encoding a polypeptide of the invention employed and the condition of the subject, as well as the body weight or surface area of the area to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular protein or vector in a particular subject. Administration can be accomplished via single or divided doses, or as a continuous infusion via an implantation device or catheter. Frequency of dosing will depend upon the pharmacokinetic parameters of the polypeptide or a nucleic acid encoding a polypeptide of the invention in the formulation used. A clinician may titer dosage and/or modify administration to achieve the desired therapeutic effects. A typical dosage ranges from about 0.01 µg/kg to about 100mg/kg, depending on the factors. In certain embodiments, a dosage ranges from about 0.1 µg/kg up to about 10mg/kg; or about 0.1 µg/kg; about 0.5 µg/kg; about 1 µg/kg; about 2 µg/kg; about.5 µg/kg; about 10 µg/kg; about 15 µg/kg; about 20 µg/kg; about 25 µg/kg; about 30 µg/kg; about 35 µg/kg; about 40 µg/kg; about 45 µg/kg; about 50 µg/kg; about 55 µg/kg; about 60 µg/kg; about 65 µg/kg; about 75 µg/kg; about 85 µg/kg; about 100 µg/kg. In certain embodiments a dosage is about 50 µg/kg; about 100 µg/kg; about 150 µg/kg; about 200 µg/kg; about 250 µg/kg; about 300 µg/kg; about 350 µg/kg; about 400 µg/kg; about 450 µg/kg; about 500 µg/kg; about 550 µg/kg; about 600 µg/kg; about 650 µg/kg; about 700 µg/kg; about 750 µg/kg; about 800 µg/kg; about 850 µg/kg; about 900 µg/kg; about 950 µg/kg; about 1 mg/kg; about 2 mg/kg; about 3 mg/kg; about4 mg/kg; about 5 mg/kg; about 6 mg/kg; about 7 mg/kg; about 8 mg/kg; about 9 mg/kg; about 10 mg/kg.

Methods of Administration

[0099] Any method for delivering the proteins or a nucleic acid encoding a polypeptide of the invention of the invention to an affected joint can be used. In the practice of this invention, compositions can be parenterally administered, for example injected, e.g., intra-articularly (i.e., into a joint), intravenously, intramuscularly, subcutaneously; infused, or implanted, e.g., in a membrane, matrix, device, etc. When injected, infused or implanted, delivery can be directed into the suitable tissue or joint, and delivery may be direct bolus delivery or continuous delivery. In some embodiments delivery can be in a suitable tissue located in close proximity to an affected joint. In some embodiments delivery may be via diffusion, or via timed release bolus. In some embodiments, a controlled release system (e.g., a pump) can be placed in proximity of the therapeutic target, e.g., the joint to which the polypeptide is administered. In other embodiments, compositions can be selected for ingestion, e.g., inhalation or oral delivery.

[0100] The therapeutic polypeptides or a nucleic acid encoding a polypeptide of the invention of the present invention can also be used effectively in combination with one or more additional active agents (e.g., hyaluronic acid or a derivative or salt thereof, growth factor(e.g., FGF18, BMP7), chondrogenic agent (e.g., oral salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3 (choliecalciferol), collagen hydrolyzate, rusalatide acetate, avocado soy unsaponifiables (ASU), a compound described in WO2012/129562, kartogenin), a steroid, a non-steroidal anti-inflammatory agent (NSAID), etc.) depending on the desired therapy or effect to improve or enhance the therapeutic effect of either. This process can involve administering both agents to the patient at the same time, either as a single composition or pharmacological formulation that includes both agents, or by administering two distinct compositions or formulations, wherein one composition includes a polypeptide or a polynucleotide encoding a polypeptide of the invention and the other includes the second agent(s). Administration of a therapeutic composition comprising a polypeptide or a polynucleotide encoding a polypeptide of the invention can precede or follow administration of the second agent by intervals ranging from minutes to weeks.

[0101] Formulations of compounds can be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. In some embodiments formulations can be presented in single or multi-chambered pre-filled syringes

(e.g., liquid syringes, lysosyringes). Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0102] Also provided are kits comprising the polypeptides or a nucleic acid encoding a polypeptide of the invention of the invention. In one embodiment provided are kits for producing a single dose administration unit. The kit comprises a first container comprising a dried polypeptide or a nucleic acid encoding a polypeptide of the invention and a second container having an aqueous reconstitution formula. In certain embodiments one container comprises a single chamber pre-filled syringe. In other embodiments the containers are encompassed as a multi-chambered pre-filled syringe

Exemplification

[0103] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Protease-resistant Angptl3 peptide constructs

[0104] Various N-terminal truncation mutants were constructed to remove O-linked glycosylations and facilitate biophysical protein characterization. To identify protease-resistant peptides, amino acid substitutions were introduced into various positions of human Angptl3 peptide fragments corresponding to the C-terminal region of the peptide. Figure 1 shows positions of mutations in the human Angptl3. Constructs were initially prepared with His tags. The mutant proteins were: 225-460 K423Q (225KQ), 225-460 S424T(225ST), 226-460 K423Q (226KQ), 226-460 K423S (226KS), 228-460 K423Q (228KQ), 228-460 S424T (228ST), 233-460 K423Q (233KQ), 233-460 K423S (233KS), 241-460 K423Q (241KQ), 241-460 K423S (241KS), 241-460 Kdel (241Kdel), 242-460 K423Q (242KQ), 242-460 K423S (242KS) and 242-460 Kdel (242Kdel).

[0105] His-tagged proteins were expressed in HEK Freestyle™ cells and purified by Ni-NTA column chromatography. Tag-less C-terminal constructs were also cloned, purified by previously described method (Gonzalez R et al *PNAS* 2010). Briefly, target protein with signal sequence (1-16) was cloned in a mammalian expression vector with cytomegalovirus promoter. At 96 h after DNA/PEI transfection in HEK 293 Freestyle (Invitrogen), media containing secreted target protein were harvested and purified by Hi-Trap SP column (GE Healthcare). Protein was eluted between 50 mM MES (pH 6.0), 125 mM NaCl to 50mM MES (pH 6.0), 150 mM NaCl. SDS-PAGE confirmed that the purified protein was at least 95% pure.

[0106] Protease-resistance was assessed as follows. Limited trypsinolysis was performed by incubating 10 ng of each prepared protein with trypsin at mass ratio of 8000:1 (Protein:Trypsin) for 1 h r at room temperature. The trypsinolysis reaction was then quenched by addition of acetic acid to bring the reaction to pH 3.0, and quenched samples were analyzed by LC/MS. A 5 min RP HPLC peak corresponding to the mass of the C-terminal 43 amino acids (S424-E460) was evident for the respective wild type protein constructs. The clip site was at the same site, *i.e.*, between K423 and S424, as observed during full length wild type ANGPLT3 protein production. This peak was absent when the Lys at the clip site was mutated to Gln. Each of peptide constructs 225KQ, 228KQ, 233KQ, 233KS, 241KQ, and 242KQ; and the wildtype 225 peptide were prepared and analyzed. The peak corresponding to the mass of the C-terminal 43 amino acids was absent when the Lys at the clip site was mutated to Gln or Ser for each of the constructs, or when the Lys at position 423 was deleted.

Example 2: Integrin binding assays

[0107] **αVβ3 integrin** Prepared peptides 225KQ, 228KQ, 233KQ, 241KQ and 242KQ were tested *in vitro* for binding to αVβ3 integrin. Briefly, Maxisorp plates were coated with 2 μg/ml Integrin αVß3, and various concentrations of polypeptide construct (indicated) were added. Bound peptide was detected by the addition of Anti-ANGPTL3 mAb followed by horseradish peroxidase-conjugated Goat anti-Mouse IgG antibody. All tested peptides retained or improved integrin binding capacity. EC_{50} for each were determined from the binding data, and results are shown in TABLE 2.

TABLE 2: *In vitro* binding of ANGPTL3 and engineered polypeptide constructs to Integrins

	α5β1 integrin EC_{50}	**αVβ3 integrin EC_{50}**
WT	3.054	3.245
242KQ	1.566	3.076
241KQ	2.693	4.032
233KQ	13.83	6.636
228KQ	4.26	4.051
225KQ	19.89	11.18

[0108] **α5β1 integrin** Prepared peptides 225KQ, 228KQ, 233KQ, 241KQ and 242KQ were tested *in vitro* for binding to α5β1 integrin. Plates were coated with 2 μg/ml as described above but with Integrin α5ß1, and various concentrations of polypeptide construct

(indicated) were added, and detection carried out as described above. All tested peptides retained or improved integrin binding capacity. EC_{50} for each were determined from the binding data, and results are shown in TABLE 2.

Example 3: Functional analysis of constructs

[0109] **Cell culture and differentiation.** Primary human bone marrow derived mesenchymal stem cells (hMSCs) were FACS sorted and proven to be >98% positive for CD29, CD44, CD166 and CD105 and <0.1% positive for CD45; and cells were used from passages 2-8 for experiments. Human cartilage resident MSCs (hCR-MSCs) were derived from human primary articular chondrocytes, which were separated into single cells, clonally grown in MSCGM and validated as MSCs through chondrogenic, osteogenic and adipogenic differentiation. Cells were FACS sorted and proven to be >98% positive for CD166 and CD105. hCR-MSCs were cultured up to 20 passages with no alteration in the cell profile, growth or differentiation rates identified.

[0110] **Chondrogenesis.** Peptide constructs of the invention were evaluated in physical and functional assays to assess chondrogenesis activity.

[0111] Engineered constructs provided herein are derived from ANGPTL3 which belongs to a family of seven identified ANGPTL proteins that have structural similarity to the angiopoietins, but lack the ability to bind the Tie2 receptor and thus have distinct functions. ANGPTL proteins contain an N-terminal coiled-coil domain (CCD) and a C-terminal fibrinogen-like domain (FLD), and are believed to be tightly regulated by their microenvironment and interactions with the extracellular matrix (ECM) such as fibronectin and integrins. Conklin et al., Genomics 62(3): 477-482 (1999); Goh YY, et al., Am J Pathol 177(6): 2791-2803 (2010); Goh YY, et al J Biol Chem 285(43): 32999-33009(2010). Sequences for ANGPTL family members most closely related to ANGPTL3, ANGPTL1 (full length and C-terminal domain) and ANGPTL4 (full length and C-terminal domain) are provided in Table 3; and Table 5B depicts an alignment across the C-terminal domains of these family members. Sequence identities across the extracellular domains and C-terminal domains ANGPTL1, ANGPTL4, as well as other angiopoietin proteins ANGPTL7, ANGPT1 and ANGPT2 are provided in Table 5A. The C-terminal domain (CT) of ANGPTL3 shares 37% sequence identity with CT ANGPTL1 and 40% sequence identity with CT ANGPTL4.

[0112] Cell-based 2D chondrogenesis was induced *in vitro* and assessed as described previously in Johnson, K., et al., (2012) *Science* **336**, 717. Briefly, primary human bone

marrow derived mesenchymal stem cells (hMSCs) were plated in growth media then subsequently changed to a chondrogenic stimulation media with and without constructs.

[0113] To initially image nodule formation, wells were fixed and stained with Rhodamine B where the nodules were easily detected by eye and images captured by light microscopy. To facilitate high throughput imaged-based detection and quantification, chondrogenic nodules were stained with Nile red which binds non-specifically to collagens. Nile Red stained nodules were quantified on an Acumen eX3 (high content imaging device) by excitation with a 488 laser for rapid detection of the nodules.

TABLE 3: ANGPTL Family Sequences

SEQ ID	Construct	Sequence
71	hANGPTL1 1-491	MKTFTWTLGVLFFLLVDTGHCRGGQFKIKKINQRRYPRATDGKEEAKKCA YTFLVPEQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLSRQKREID VLQLVVDVDGNIVNEVKLLRKESRNMNSRVTQLYMQLLHEIIRKRDNSLE LSQLENKILNVTTEMLKMATRYRELEVKYASLTDLVNNQSVMITLLEEQC LRIFSRQDTHVSPPLVQVVPQHIPNSQQYTPGLLGGNEIQRDPGYPRDLM PPPDLATSPTKSPFKIPPVTFINEGPFKDCQQAKEAGHSVSGIYMIKPEN SNGPMQLWCENSLDPGGWTVIQKRTDGSVNFFRNWENYKKGFGNIDGEYW LGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPESEFYRLRLG TYQGNAGDSMMWHNGKQFTTLDRDKDMYAGNCAHFHKGGWWYNACAHSNL NGVWYRGGHYRSKHQDGIFWAEYRGGSYSLRAVQMMIKPID
72	CT hANGPTL1 271-491	FINEGPFKDCQQAKEAGHSVSGIYMIKPENSNGPMQLWCENSLDPGGWTV IQKRTDGSVNFFRNWENYKKGFGNIDGEYWLGLENIYMLSNQDNYKLLIE LEDWSDKKVYAEYSSFRLEPESEFYRLRLGTYQGNAGDSMMWHNGKQFTT LDRDKDMYAGNCAHFHKGGWWYNACAHSNLNGVWYRGGHYRSKHQDGIFW AEYRGGSYSLRAVQMMIKPID
73	hANGPTL4 1-406	MSGAPTAGAALMLCAATAVLLSAQGGPVQSKSPRFASWDEMNVLAHGLLQ LGQGLREHAERTRSQLSALERRLSACGSACQGTEGSTDLPLAPESRVDPE VLHSLQTQLKAQNSRIQQLFHKVAQQQRHLEKQHLRIQHLQSQFGLLDHK HLDHEVAKPARRKRLPEMAQPVDPAHNVSRLHRLPRDCQELFQVGERQSG LFEIQPQGSPPFLVNCKMTSDGGWTVIQRRHDGSVDFNRPWEAYKAGFGD PHGEFWLGLEKVHSITGDRNSRLAVQLRDWDGNAELLQFSVHLGGEDTAY SLQLTAPVAGQLGATTVPPSGLSVPFSTWDQDHDLRRDKNCAKSLSGGWW FGTCSHSNLNGQYFRSIPQQRQKLKKGIFWKTWRGRYYPLQATTMLIQPM AAEAAS
74	CT hANGPTL4 179-406	SRLHRLPRDCQELFQVGERQSGLFEIQPQGSPPFLVNCKMTSDGGWTVIQ RRHDGSVDFNRPWEAYKAGFGDPHGEFWLGLEKVHSITGDRNSRLAVQLR DWDGNAELLQFSVHLGGEDTAYSLQLTAPVAGQLGATTVPPSGLSVPFST WDQDHDLRRDKNCAKSLSGGWWFGTCSHSNLNGQYFRSIPQQRQKLKKGI FWKTWRGRYYPLQATTMLIQPMAAEAAS

TABLE 4: Chondrogenesis of ANGPTL family member proteins

Protein	Nodule Formation activity	Induction Type II collagen	Genbank Accession
Angptl1	Yes	Yes	NP_004664
Angptl2	No	n/a	NP_036230
Angptl3	Yes	Yes	NP_055310
Angptl4	Yes	No	NP_647475

Angptl6	No	No	NP_114123
Angptl7	No	No	NP_066969
Angpt2	No	n/a	NP_001138
Angpt1	No	n/a	NP_004664

[0114] Cell-based 2D chondrogenesis was induced *in vitro* and assessed as described previously in Johnson, K., et al., (2012) *Science* **336**, 717. Briefly, primary human bone marrow derived mesenchymal stem cells (hMSCs) were plated in growth media then subsequently changed to a chondrogenic stimulation media with and without constructs, and cultured for 7 or 14 days. Cells were then fixed with formaldehyde, washed and then stained using standard immuno-cytochemical techniques to detect primary cartilage proteins Pro-collagen Type 2A (PIIANP) (Figure 2A) and Type II collagen (Figure 2B). For detection of type II collagen, cells were digested with 0.2% Collagenase II (Worthington Biochemical, Lakewood, NJ) which was added to the permeabilization solution. Immuno- fluorescence for each protein detected was quantified through high content imaging (Image Express Ultra (Molecular Devices, Sunnyvale, CA)), using multi-wavelength cell scoring script, and as described previously. See Figure 2. Aggrecan expression was monitored by preparing cells as follows: briefly, primary hMSCs (5000 cells) were plated in a Griener 384 well plate. After 24 hours the growth media was removed and replaced with 25µl of DMEM containing 1% FBS. Protein constructs were then added to each well at the indicated dose, and cultures were grown at 37°C for 3 days. The cells were fixed with 10% formalin and subjected to immunocytochemical methods to detect Aggrecan protein expression. Wells were imaged on the ImageXpress Ultra and quantified with the multi-wavelength cell scoring script, n=6 / protein concentration. Results are exemplified in Figure 3B relative to control (cells stimulated without construct, diluent alone) for WT wild type C-terminal (225-460) ANGPTL3, engineered construct 242KQ or 242Kdel or full length ANGPTL1, a related family member of the ANGPTL proteins. Similar results were seen with experiments using each of 225WT, 225KQ, 226KQ, 228KQ, 233KQ, 241KQ and 242KQ constructs.

[0115] Chondrogenesis assays were carried out using assays and methods described previously and herein for additional ANGPTL related family members. Experiments were carried out to examine whether closely related proteins confer chondrogenic activity, and if the activity was retained in the C-terminal end of the protein. ANGPTL1 and ANGPTL4 demonstrated activity in nodule formation assays; however, only ANGPTL1 showed an induction of type II collagen in chondrogenesis assays. See Table 4. Results of nodule formation activity and induction of Type II collagen assays are summarized in Table 4.

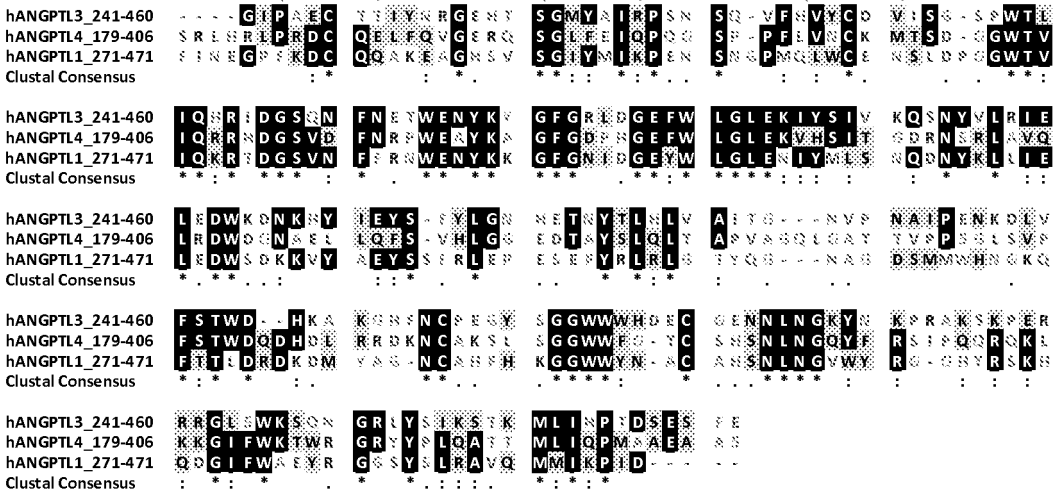
Additional characterization of ANGPTL1 is described herein. See other portions of this Example and Figures 3-5.

TABLE 5: Sequence homology among human angiopoeitin like family members

5A: Sequence identity among human angiopoeitin like family members (ECD or CTD)

Family member	Family member	% Sequence Identity
hANGPTL3_17-460	hANGPTL4_26-406	32.6
hANGPTL3_17-460	hANGPTL1_24-491	25.7
hANGPTL3_17-460	hANGPTL7_27-346	28.1
hANGPTL3_17-460	hANGPT1_23-498	24.1
hANGPTL3_17-460	hANGPT2_19-496	23.4
hANGPTL3_241-460	hANGPTL4_179-406	40.0
hANGPTL3_241-460	hANGPTL1_271-491	36.8
hANGPTL3_241-460	hANGPTL7_122-343	36.4
hANGPTL3_241-460	hANGPT1_277-497	37.3
hANGPTL3_241-460	hANGPT2_275-495	36.4

5B: Sequence alignment of C-terminal domains of human angiopoeitin like family members hANGPTL1(271-491) /hANGPTL3(241-460) /hANGPTL4(179-406)



[0116] RNA expression analysis was also used to evaluate expression of cartilage specific proteins. Briefly, qRT-PCR hMSCs were grown in pellet culture ($1x10^6$ cells/pellet) for 3, 7, 10, 21 days in serum free DMEM, 1X ITS plus constructs (as indicated). Media was replaced every 3 days. Lubricin, Aggrecan, Sox9, IGF1, IFITM1, Osteocalcin and type X collagen mRNA expression were quantified using Roche LightCycler (data pooled from 3 experiments performed in duplicate (n=6)). Figure 3A represents expression data at Day 10 for 242KQ and 225WT. Gene expression data was similar for all genes at days 3, 7 and 21.

[0117] Full length ANGPTL3 had been previously shown to have chondrogenesis activity in both human and mouse mesenchymal stem cells. Constructs were tested for activity in human, mouse, rat and canine mesenchymal stem cells to demonstrate the ability of additional species cross reactivity. CR-MSCs from mouse, rat, human and dog were cultured

with constructs as described above for 18 days. Cultures were fixed and stained using standard imunnocytochemical techniques to detect the chondrocyte specific protein type II collagen, and type II collagen positive cells were quantified using high content imaging. Similar fold increase in the amount of type II collagen quantified was confirmed for each species of cells evaluated.

[0118] **Chondroprotection.** Peptide constructs were evaluated in functional assays to assess chondroprotective activity.

[0119] An *ex vivo* glycosaminoglycan (GAG) release inhibition assay (an indicator of matrix damage) was performed as described in Johnson, K., et al., (2012) *Science* **336**, 717-721. Briefly, bovine cartilage was isolated, punched into symmetric circles and put into organ culture. Slices were treated for 48 hours with 20ng/ml TNFα and 10ng/ml oncostatin M (OSM) (inflammatory mediators) to induce degradation of the cartilage matrix in the presence or absence of protein constructs to identify percent inhibition of glycosaminoglycan(GAG) release. Results shown in Figure 4A depict data pooled from 4 donors, n=12 with the engineered constructs as indicated and WT 225-460.

[0120] An *in vitro* nitric oxide (NO) inhibition assay (an indicator of chondro-protection) was performed as described in Johnson, K., et al., (2012) *Science* **336**, 717-721. Briefly, primary chondrocytes were treated for 48 hrs with protein constructs as indicated. Greiss reaction was performed, to determine the effect of constructs on inhibition of NO release as Results shown in Figure 4B depict results with the engineered constructs as indicated and WT Cterminal fragment 225-460. Results shown in Figure 4C depict results with wild type Cterminal ANGPTL1, engineered ANGPTL3 242KQ or control.

[0121] **Inhibition of fibrotic cartilage formation.** Primary human articular chondrocytes were cultured as described above with the addition of ascorbic acid and the presence or absence of constructs (indicated) for 14 days to induce hypertrophy and type X collagen expression was assessed by immunoflurescence. Results shown in Figure 5A depict data with constructs 225WT or 242KQ as indicated. Results shown in Figure 5B depict data with wild type C-terminal ANGPTL1, engineered ANGPTL3 242KQ or 242Kdel or wild type C-terminal ANGPTL3 fragment 225-460 as indicated. The presence of wild type or active constructs confer an inhibitory effect on formation of fibrotic cartilage under hypertrophic conditions, as detected by expression of type X collagen.

[0122] **Angiogenesis.** The WT C-terminal domain of the ANGPTL3 protein has been reported to have angiogenic activities and properties *in vitro* and *in vivo* in a rat corneal model. See Camenisch et al., J. Biol. Chem. 277(19): 17281-17290 (2002). To address the possible risk of inducing new blood vessels following *in vivo* administration of C-terminal ANGPTL3, *in vitro* angiogenic assays were examined. Briefly, primary human umbilical vein endothelial cells (HUVECs) were serum starved overnight with basal endothelial cell media. Cells were then labeled with cell tracker green and added to pre-coated matrigel plates embedded with protein construct (indicated). Following culture for 18 hours in the presence of full length ANGPTL3 (50ng/mL) or 242KQ (50ng/mL) or bFGF (50ng/mL) which was used as a positive control, the number of branch points and the total tube length formed was quantified using high content imaging as a measure of angiogenic activity. In contrast to the effect seen in the presence of full length ANGPTL3 or positive control, no significant increase in either parameter was detected when cells were incubated with 242KQ. See Figure 2C.

[0123] CR-MSCs exist within hyaline articular cartilage and increase in number in response to injury. Following injury to the cartilage tissue, these cells have the capacity to participate in repair processes, but do not sufficiently lead to proper cartilage repair on their own. Patients are therefore left with articular cartilage that lacks the proper ability to support painless joint movements and often require surgical intervention and/or a joint replacement to maintain their quality of life. We have found ANGPTL3 and in particular engineered protease resistant ANGPTL3 peptides have the ability to direct the differentiation of human CR-MSCs into chondrocytes, specifically secreting hyaline articular cartilage proteins type II collagen and Sox9 while inhibiting the fibrotic cartilage formation noted by expression of type X collagen.

[0124] No expression of ANGPTL3 has been reported to our knowledge nor observed in our studies using western blotting in human chondrocytes, human MSCs or human synovial fibroblasts. In rodent joints, little to no expression was found through immunohistochemistry (IHC). However, in human osteoarthritic synovial fluid (n=2), low level ANGPTL3 (1.3-6.0 ng/mL) was detected by enzyme-linked immunosorbent assay (ELISA), suggesting in a compromised joint, systemically circulating protein can enter the synovial cavity.

Example 4: In vivo analysis of constructs

[0125] **Mouse acute injury surgical model.** Surgical transection of the anterior cruciate ligament (ACL), medial meniscal tibial ligament (MMTL), and medial collateral ligament

(MCL) of the right knee from C57BL/6 mice (n=12 / group) was performed to induce instability in the knee joint and thus lead to an OA phenotype, adapted from the previously described model Glasson, S.S., et al., *Osteoarthritis Cartilage* **15**, 1061 (2007). To evaluate a potential therapeutic benefit of ANGPTL3 treatment, 15 weeks following surgery, mice were dosed intra-articularly as indicated in Figure 6A once / per week on weeks17-19: mANGPTL3 dose = 200ng / knee. Quantitative assessments of the tibial plateau were made on a 0-4 scale, 0 being normal and 5 being severe osteoarthritis (full thickness disruption of the cartilage). Two sections from each mouse were blindly graded by 2 independent observers (Figure 6B).

[0126] Alleviation of osteoarthritis induced pain for animals was measured by incapacitance testing, or determining the percentage of time the mouse stood on a surgically treated leg vs the non-treated leg using an incapacitance monitoring device. Figure 7 depicts results of readouts, representing pain response on days 35 and 56 after surgery were reported as a % weight bearing on the surgical limb versus the non surgical limb. Treatment depicts results of animals dosed as described above with full length murine ANGPTL3 (WT17-460) or C-terminal human ANGPTL3 (WT225-460).

[0127] **Mouse chronic OA model (collagenase VII induced)** Another widely used animal model of osteoarthritis, the collagenase VII-induced chronic joint injury model, was used to evaluate *in vivo* efficacy of constructs. The model and evaluation was performed as previously described. See van der Kraan, P.M., et al., *Am. J. Pathol.* **135**, 1001 (1989); and Johnson, K., et al., *Science* **336**, 717 (2012). Briefly, a three (3) day period of inflammation is followed by collagenase induced destabilization of the joint, resulting in mild to moderate cartilage destruction. Intra-articular administration of constructs was carried out following induction in the knee once / week for three weeks, beginning 3 weeks after addition of collagenase VII. Forty (42) days following treatment, joints were collected and sectioned. Histological joint severity scoring of femoral and tibial plateau allowed quantification of the tissue repair. The severity of the joint score was determined through histological scoring as described above. Figure 8 depicts repair with 225WT, 225KQ, 228KQ, 233KQ, and 241KQ constructs. To confirm the presence of protein in the joint (long term intra-articular retention), tissue was fixed and stained for the presence of the WT protein construct through immunohistochemistry. Analysis confirmed the presence of protein indicating intra-articular retention of ANGPTL3 (with no effects seen on lipid/triglyceride, assessed using a standard metabolic panel, data not shown.)

[0128] Histological analysis and grading on Safranin O stained sections of the medial tibial plateau (for detection of proteoglycan at the injury site, as described above) revealed regeneration in cartilage matrix (data not shown). Qualitative analysis confirmed replacement of proteoglycans similar to levels seen in a naïve mouse, while vehicle controls did not show similar replacement. Tissue sections were also stained as described above for type II collagen 8 weeks following injection of the injury. Qualitative analyses confirmed an increase of type II collagen in joints treated with construct similar to levels seen in a naïve mouse; while vehicle treated controls did not show similar increase (data not shown).

[0129] **Rat meniscal tear model**

[0130] A rat surgical injury model was also used to evaluate *in vivo* efficacy of constructs. The model and evaluation was initially performed as previously described Gerwin N. et al. *Osteoarthritis Cartilage.* **Suppl 3:** S24 (2010). Briefly, skin was shaven over a knee joint and the medial collateral ligament (MCL) was isolated through an incision, and the MCL was stabilized and a distal cut of the meniscus made using a scalpel. On weeks 1, 2 and 3 following surgery protein construct or vehicle control was injected intra-articularly, then joints were collected and sectioned at 4 and 6 weeks after surgery. Histological joint severity scoring of femoral and tibial plateau were performed for quantification of the tissue repair as described above. Data is shown for the 6 weeks analyses.

[0131] Healthy hyaline cartilage replaced damage following treatment. Histological analysis and grading of the lateral tibial plateau of safranin O stained cartilage were performed as described above and quantified Results demonstrated animals treated with 242KQ construct revealed regeneration in cartilage matrix and replacement of proteoglycans similar to levels seen in a naïve rat, while vehicle controls did not show similar replacement. See Figure 9. Similar results were seen with 225WT.

[0132] A slightly altered surgically induced meniscal tear model from that described above was used to initiate cartilage damage in male Lewis rats in order to test the efficacy of 242KQ in promoting cartilage repair *in vivo.* Surgery on rats was performed to completely sever the medial collateral ligament and the medial meniscus to destabilize the joint so that future weight bearing would lead to rapid degeneration of the cartilage. An incision was made to sever the ligament on both sides of the needle, thus ensuring a complete cut. A scalpel blade was then used to slip under the patellar ligament into the synovial space and the pointed tip was used to cut the meniscus. A successful cut was accomplished when the joint

dislocated laterally. One week after surgery, rats were dosed by intra-articular injection of 242KQ or saline in a volume of 25 uL into the intra-articular synovial space.

[0133] Twenty eight days after meniscal tear surgery and twenty one days post intra-articular injection of saline or construct, study animals were euthanized and affected joints were harvested for analysis, fixed in 10% formalin in PBS, decalcified with formic acid, and embedded in paraffin prior to sectioning. Coronal sections were cut and stained for Safranin O or left unstained for future immunohistochemical staining. Analysis revealed that the medial tibial plateau had the greatest amount of cartilage damage and it was decided to evaluate only this area of the joint for efficacy of 242KQ. Using the OARSI scoring system, cartilage severity scores were assigned for six sections across the width of the tibial cartilage for each animal (N=10) in a blinded manner. Scoring was done twice at different time-points and the scores were then averaged to create a score of cartilage damage. Additionally, objective scoring analyses were performed with a custom script generated in Matlab. The algorithm identified the articular cartilage surfaces and objectively quantified additional cartilage parameters (zonal analyses, safranin O intensity, cartilage area, cartilage thickness). Results are depicted in Figure 10A.

[0134] Structural repair of cartilage is not always associated with relief of pain, at least in humans. Although rodent physiology and gait are significantly different than humans, 242KQ was evaluated to determine if there was any improvement in the gait or length of time spent on the surgical limb after treatment. Incapacitance monitoring was performed on rats treated with 242KQ. Rats were subjected to the modified meniscal surgery as described above. One week following the surgery, 242KQ was injected into the synovial space. On day 28, the rats were placed on an incapacitance monitor on their hind limbs and 30 subsequent readings were taken over 10 minutes for each rat to determine the percent of time spent (weight distribution) on each hind limb. These data give an indication of the pain-induced weight redistribution It was determined that in the rat meniscal tear model, treatment with 242KQ one week following surgery led to a partial restoration of the equal weight bearing capacity of the rats. See Figure 10B.

[0135] One of the primary challenges during spontaneous or surgical cartilage repair is the replacement of hyaline articular cartilage with fibrotic cartilage. To explore the type of cartilage repair mediated by ANGPTL3, sections from the rat knees collected from the rat meniscal tear study performed above were stained for the presence of type II collagen (to indicate hyaline articular cartilage) and type X collagen (to indicate fibrotic cartilage). After

a single injection of 20 µg of 242KQ, there was a qualitative reduction in the amount of type X collagen expression.

[0136] Long term retention of 242KQ following intravenous and intra-articular injection into rat knees was determined through ^{124}I labeling of protein and administration followed by PET / uCT imaging to monitor retention. See, Gerwin, N., et al. (2006) *Advanced drug delivery reviews* **58**, 226-242. The mean residence time (MRT) after IA injection of 242KQ into the joint was determined to be ~17.3h which is significantly increased over the standard 2-3h reported (See TABLE 6)

TABLE 6: Persistence of ^{124}I 242KQ

Route	Dose (µg)	C_{max} (µg/mL)	$AUC_{0\text{-}inf}$ (hr*µg/mL)	CL (mL/h)	Vss (mL)	MRT (h)	$T_{1/2}$ (h)
IV	164.2	129.3	22.1	7.4	53.4	7.2	12.4
IA	38.3	0.2	1.9	-	-	17.3	7.2

[0137] **Dog partial menisectomy joint injury model** We also evaluated ANGPTL3 activity in a canine joint injury model. The model was performed and evaluations performed as described in Connor, J.R., et al., *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* **17**, 1236-1243 (2009). Briefly, skin was shaven over a knee joint and the medial collateral ligament (MCL) was isolated through an incision, and the MCL was stabilized and a distal cut of the meniscus made using a scalpel. Four (4) days following surgery, animals received either twice weekly dosing (1.5ug or 15 ug), or a single dose (30ug) of the protein construct (full length canine ANGPTL3) on day 7 or vehicle control (injected intra-articularly). Dogs were euthanized on day 28 and the knees were subjected to histological, sectioning and grading as described above for the rat and mouse experiments. Figure 10 depicts the Total gross score of the repair associated with treatment of canine ANGPTL3. Upon histological grading and evaluation of the dog joint sections stained with Safranin O, areas where the most severe cartilage loss took place in the saline groups was the portion of the joint that had the largest reduction in lesion area following a single 30 µg dose of cANGPTL3.

[0138] It is understood that the examples and embodiments described herein are for illustrative purposes and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

SEQUENCES

SEQ ID	Construct	Sequence
1	Human ANGPTL3	MFTIKLLLFIVPLVISSRIDQDNSSFDSLSPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLQTSEIKEEEKELRRTTYKLQVKN EEVKNMSLELNSKLESLLEEKILLQQKVKYLEEQLTNLIQNQPETPEHPEVTSLK TFVEKQDNSIKDLLQTVEDQYKQLNQQHSQIKEIENQLRRTSIQEPTEISLSSKP RAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCD VISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYV LRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWD HKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGR LYSIKSTKMLIHPTDSESFE
2	Human ANGPTL3 REFSEQ	ttccagaagaaaacagttccacgttgcttgaaattgaaaatcaagataaaaatgt tcacaattaagctccttcttttttattgttcctctagttatttcctccagaattga tcaagacaattcatcatttgattctctatctccagagccaaaatcaagatttgct atgttagacgatgtaaaaattttagccaatggcctccttcagttgggacatggtc ttaaagactttgtccataagacgaagggccaaattaatgacatatttcaaaaact caacatatttgatcagtctttttatgatctatcgctgcaaaccagtgaaatcaaa gaagaagaaaaggaactgagaagaactacatataaactacaagtcaaaaatgaag aggtaaagaatatgtcacttgaactcaactcaaaacttgaaagcctcctagaaga aaaaattctacttcaacaaaaagtgaaatatttagaagagcaactaactaactta attcaaaatcaacctgaaactccagaacacccagaagtaacttcacttaaaactt ttgtagaaaaacaagataatagcatcaaagaccttctccagaccgtggaagacca atataaacaattaaaccaacagcatagtcaaataaaagaaatagaaaatcagctc agaaggactagtattcaagaacccacagaaatttctctatcttccaagccaagag caccaagaactactccctttcttcagttgaatgaaataagaaatgtaaaacatga tggcattcctgctgaatgtaccaccatttataacagaggtgaacatacaagtggc atgtatgccatcagacccagcaactctcaagttttcatgtctactgtgatgtta tatcaggtagtccatggacattaattcaacatcgaatagatggatcacaaaactt caatgaaacgtgggagaactacaaatatggttttgggaggcttgatggagaattt tggttgggcctagagaagatatactccatagtgaagcaatctaattatgttttac gaattgagttggaagactggaaagacaacaaacattatattgaatattcttttta cttgggaaatcacgaaaccaactatacgctacatctagttgcgattactggcaat gtccccaatgcaatcccggaaaacaaagatttggtgttttctacttgggatcaca aagcaaaaggacacttcaactgtccagagggttattcaggaggctggtggtggca tgatgagtgtggagaaaacaacctaaatggtaaatataacaaaccaagagcaaaa tctaagccagagaggagaagaggattatcttggaagtctcaaaatggaaggttat actctataaaatcaaccaaaatgttgatccatccaacagattcagaaagctttga atgaactgaggcaaatttaaaaggcaataatttaaacattaacctcattccaagt taatgtggtctaataatctggtattaaatccttaagagaaagcttgagaaataga ttttttttatcttaaagtcactgtctatttaagattaaacatacaatcacataac cttaaagaataccgtttacatttctcaatcaaaattcttataatactatttgttt taaattttgtgatgtgggaatcaattttagatggtcacaatctagattataatca ataggtgaacttattaaataacttttctaaataaaaaatttagagacttttattt taaaaggcatcatatgagctaatatcacaactttcccagtttaaaaaactagtac tcttgttaaaactctaaacttgactaaatacagaggactggtaattgtacagttc ttaaatgttgtagtattaatttcaaaactaaaaatcgtcagcacagagtatgtgt aaaaatctgtaatacaaatttttaaactgatgcttcattttgctacaaaataatt tggagtaaatgtttgatatgatttatttatgaaacctaatgaagcagaattaaat actgtattaaaataagttcgctgtctttaaacaaatggagatgactactaagtca cattgactttaacatgaggtatcactataccttatt

SEQ ID	Construct	Sequence
3	Murine ANGPTL3	MHTIKLFLFVVPLVIASRVDPDLSSFDSAPSEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLRTNEIKEEEKELRRTTSTLQVKN EEVKNMSVELNSKLESLLEEKTALQHKVRALEEQLTNLILSPAGAQEHPEVTSLK SFVEQQDNSIRELLQSVEEQYKQLSQQHMQIKEIEKQLRKTGIQEPSENSLSSKS RAPRTTPPLQLNETENTEQDDLPADCSAVYNRGEHTSGVYTIKPRNSQGFNVYCD TQSGSPWTLIQHRKDGSQDFNETWENYEKGFGRLDGEFWLGLEKIYAIVQQSNYI LRLELQDWKDSKHYVEYSFHLGSHETNYTLHVAEIAGNIPGALPEHTDLMFSTWN HRAKGQLYCPESYSGGWWWNDICGENNLNGKYNKPRTKSRPERRRGIYWRPQSRK LYAIKSSKMMLQPTT
4	Canine ANGPTL3	MYTIKLFLFIIPLVISSKIDRDYSSYDSVSPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLQTNEIKEEEKELRRTTSKLQVKN EEVKNMSLELNSKVESLLEEKILLQQKVRYLEKQLTSLIKNQPEIQEHPEVTSLK TFVEQQDNSIKDLLQTVEEQYRQLNQQHSQIKEIENQLRNVIQESTENSLSSKPR APRTTPFLHLNETKNVEHNDIPANCTTIYNRGEHTSGIYSIRPSNSQVFNVYCDV KSGSSWTLIQHRIDGSQNFNETWENYRYGFGRLDGEFWLGLEKIYSIVKQSNYIL RIELEDWNDNKHYIEYFFHLGNHETNYTLHLVEITGNILNALPEHKDLVFSTWDH KAKGHVNCPESYSGGWWWHNVCGENNLNGKYNKQRAKTKPERRRGLYWKSQNGRL YSIKSTKMLIHPIDSESSE
5	Equine ANGPTL3	MYTIKLFLVIAPLVISSRIDQDYSSLDSIPPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYALSLQTNEIKEEEKELRRTTSKLQVKN EEVKNMSLELNSKLESLLEEKSLLQQKVKYLEEQLTKLIKNQPEIQEHPEVTSLK TFVEQQDNSIKDLLQTMEEQYRQLNQQHSQIKEIENQLRRTGIQESTENSLSSKP RAPRTTPSFHLNETKDVEHDDFPADCTTIYNRGEHTSGIYSIKPSNSQVFNVYCD VISGSSWILIQRRIDGSQNFNETWQNYKYGFGRLDFEFWLGLEKIYSIVKRSNYI LRIELEDWKDNKHTIEYSFHLGNHETNYTLHLVEITGNVPNALPEHKDLVFSTWD HKAKGQLNCLESYSGGWWWHDVCGGDNPNGKYNKPRSKTKPERRRGICWKSQNGR LYTIKSTKMLIHPIDSESFELRQIKKPMN
6	Bovine ANGPTL3	MYTIKLFLIIAPLVISSRTDQDYTSLDSISPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLQTNEIKEEEKELRRATSKLQVKN EEVKNMSLELDSKLESLLEEKILLQQKVRYLEDQLTDLIKNQPQIQEYLEVTSLK TLVEQQDNSIKDLLQIVEEQYRQLNQQQSQIKEIENQLRRTGIKESTEISLSSKP RAPRTTPSFHSNETKNVEHDDIPADCTIIYNQGKHTSGIYSIRPSNSQVFNVYCD VKSGSSWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVMQSNYI LRIELEDWKDKYYTEYSFHLGDHETNYTLHLAEISGNGPKAFPEHKDLMFSTWDH KAKGHFNCPESNSGGWWYHDVCGENNLNGKYNKPKAKAKPERKEGICWKSQDGRL YSIKATKMLIHPSDSENSE
7	207-455WT	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAKSKPE RRRGLSWKSQNGRLYSIKSTKMLIHPTD
8	225-455WT	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTD
9	228-455WT	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGRLYSIKST KMLIHPTD

SEQ ID	Construct	Sequence
10	233-455WT	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTD
11	241-455WT	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRAKSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
12	ANGPTL3KQ	MFTIKLLLFIVPLVISSRIDQDNSSFDSLSPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLQTSEIKEEEKELRRTTYKLQVKN EEVKNMSLELNSKLESLLEEKILLQQKVKYLEEQLTNLIQNQPETPEHPEVTSLK TFVEKQDNSIKDLLQTVEDQYKQLNQQHSQIKEIENQLRRTSIQEPTEISLSSKP RAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCD VISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYV LRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWD HKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGR LYSIKSTKMLIHPTDSESFE
13	ANGPTL3KS	MFTIKLLLFIVPLVISSRIDQDNSSFDSLSPEPKSRFAMLDDVKILANGLLQLGH GLKDFVHKTKGQINDIFQKLNIFDQSFYDLSLQTSEIKEEEKELRRTTYKLQVKN EEVKNMSLELNSKLESLLEEKILLQQKVKYLEEQLTNLIQNQPETPEHPEVTSLK TFVEKQDNSIKDLLQTVEDQYKQLNQQHSQIKEIENQLRRTSIQEPTEISLSSKP RAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCD VISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYV LRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWD HKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGR LYSIKSTKMLIHPTDSESFE
14	207KQ	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPE RRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
15	207KS	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPE RRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
16	225KQ	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE
17	225KS	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE
18	225ST	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAKTKPERRRGLSWKSQNGRLYSI KSTKMLIHPTDSESFE

SEQ ID	Construct	Sequence
19	226KQ	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
20	226KS	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
21	228KQ	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
22	228KS	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
23	228ST	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAKTKPERRRGLSWKSQNGRLYSIKST KMLIHPTDSESFE
24	233KQ	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTDSESFE
25	233KS	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTDSESFE
26	241KQ	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
27	241KS	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
28	242KQ	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
29	242KS	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE

SEQ ID	Construct	Sequence
30	225-455KQ	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTD
31	225-455KS	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSI KSTKMLIHPTD
32	226-455KQ	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
33	226-455KS	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
34	228-455KQ	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKST KMLIHPTD
35	228-455KS	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKST KMLIHPTD
36	233-455KQ	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTD
37	233-455KS	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIH PTD
38	241-455KQ	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
39	241-455KS	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
40	242-455KQ	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRAQSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD

SEQ ID	Construct	Sequence
41	242-455KS	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
42	Canine 227KQ	FLHLNETKNVEHNDIPANCTTIYNRGEHTSGIYSIRPSNSQVFNVYCDVKSGSSW TLIQHRIDGSQNFNETWENYRYGFGRLDGEFWLGLEKIYSIVKQSNYILRIELED WNDNKHYIEYFFHLGNHETNYTLHLVEITGNILNALPEHKDLVFSTWDHKAKGHV NCPESYSGGWWWHNVCGENNLNGKYNKQRAQTKPERRRGLYWKSQNGRLYSIKST KMLIHPIDSESSE
43	Canine 227KS	FLHLNETKNVEHNDIPANCTTIYNRGEHTSGIYSIRPSNSQVFNVYCDVKSGSSW TLIQHRIDGSQNFNETWENYRYGFGRLDGEFWLGLEKIYSIVKQSNYILRIELED WNDNKHYIEYFFHLGNHETNYTLHLVEITGNILNALPEHKDLVFSTWDHKAKGHV NCPESYSGGWWWHNVCGENNLNGKYNKQRASTKPERRRGLYWKSQNGRLYSIKST KMLIHPIDSESSE
44	Nucleic acid sequence 225WT	ACTACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTC CTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGC CATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGT AGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAA CGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGG CCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAG TTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAA ATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAA TGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAA GGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGT GTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAAATCTAAGCC AGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATA AAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
45	Nucleic acid sequence 225KQ	ACTACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTC CTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGC CATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGT AGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAA CGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGG CCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAG TTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAA ATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAA TGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAA GGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGT GTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCACAATCTAAGCC AGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATA AAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
46	Nucleic acid sequence 225KS	ACTACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTC CTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGC CATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGT AGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAA CGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGG CCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAG TTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAA ATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAA TGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAA GGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGT GTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAGCTCTAAGCC AGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATA AAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA

SEQ ID	Construct	Sequence
47	Nucleic acid sequence 226KQ	ACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTCCTG CTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGCCAT CAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGTAGT CCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAACGT GGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCT AGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTG GAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAAATC ACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAATGC AATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAAGGA CACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGTGTG GAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCACAATCTAAGCCAGA GAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATAAAA TCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
48	Nucleic acid sequence 226KS	ACTCCCTTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTCCTG CTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGCCAT CAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGTAGT CCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAACGT GGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCT AGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTG GAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAAATC ACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAATGC AATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAAGGA CACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGTGTG GAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAGCTCTAAGCCAGA GAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATAAAA TCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
49	Nucleic acid sequence 228KQ	TTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTCCTGCTGAAT GTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGCCATCAGACC CAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGTAGTCCATGG ACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAACGTGGGAGA ACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCTAGAGAA GATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGAC TGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAAATCACGAAA CCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAATGCAATCCC GGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAAGGACACTTC AACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGTGTGGAGAAA ACAACCTAAATGGTAAATATAACAAACCAAGAGCACAATCTAAGCCAGAGAGGAG AAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATAAAATCAACC AAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
50	Nucleic acid sequence 228KS	TTTCTTCAGTTGAATGAAATAAGAAATGTAAAACATGATGGCATTCCTGCTGAAT GTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGTATGCCATCAGACC CAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATCAGGTAGTCCATGG ACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAATGAAACGTGGGAGA ACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCTAGAGAA GATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGAC TGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTGGGAAATCACGAAA CCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCCCCAATGCAATCCC GGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGCAAAAGGACACTTC AACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGATGAGTGTGGAGAAA ACAACCTAAATGGTAAATATAACAAACCAAGAGCAAGCTCTAAGCCAGAGAGGAG AAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTCTATAAAATCAACC AAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA

SEQ ID	Construct	Sequence
51	Nucleic acid sequence 233KQ	GAAATAAGAAATGTAAAACATGATGGCATTCCTGCTGAATGTACCACCATTTATA ACAGAGGTGAACATACAAGTGGCATGTATGCCATCAGACCCAGCAACTCTCAAGT TTTTCATGTCTACTGTGATGTTATATCAGGTAGTCCATGGACATTAATTCAACAT CGAATAGATGGATCACAAAACTTCAATGAAACGTGGGAGAACTACAAATATGGTT TTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCTAGAGAAGATATACTCCATAGT GAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGACTGGAAAGACAACAAA CATTATATTGAATATTCTTTTTACTTGGGAAATCACGAAACCAACTATACGCTAC ATCTAGTTGCGATTACTGGCAATGTCCCCAATGCAATCCCGGAAAACAAAGATTT GGTGTTTTCTACTTGGGATCACAAAGCAAAAGGACACTTCAACTGTCCAGAGGGT TATTCAGGAGGCTGGTGGTGGCATGATGAGTGTGGAGAAAACAACCTAAATGGTA AATATAACAAACCAAGAGCACAATCTAAGCCAGAGAGGAGAAGAGGATTATCTTG GAAGTCTCAAAATGGAAGGTTATACTCTATAAAATCAACCAAAATGTTGATCCAT CCAACAGATTCAGAAAGCTTTGAA
52	Nucleic acid sequence 233KS	GAAATAAGAAATGTAAAACATGATGGCATTCCTGCTGAATGTACCACCATTTATA ACAGAGGTGAACATACAAGTGGCATGTATGCCATCAGACCCAGCAACTCTCAAGT TTTTCATGTCTACTGTGATGTTATATCAGGTAGTCCATGGACATTAATTCAACAT CGAATAGATGGATCACAAAACTTCAATGAAACGTGGGAGAACTACAAATATGGTT TTGGGAGGCTTGATGGAGAATTTTGGTTGGGCCTAGAGAAGATATACTCCATAGT GAAGCAATCTAATTATGTTTTACGAATTGAGTTGGAAGACTGGAAAGACAACAAA CATTATATTGAATATTCTTTTTACTTGGGAAATCACGAAACCAACTATACGCTAC ATCTAGTTGCGATTACTGGCAATGTCCCCAATGCAATCCCGGAAAACAAAGATTT GGTGTTTTCTACTTGGGATCACAAAGCAAAAGGACACTTCAACTGTCCAGAGGGT TATTCAGGAGGCTGGTGGTGGCATGATGAGTGTGGAGAAAACAACCTAAATGGTA AATATAACAAACCAAGAGCAAGCTCTAAGCCAGAGAGGAGAAGAGGATTATCTTG GAAGTCTCAAAATGGAAGGTTATACTCTATAAAATCAACCAAAATGTTGATCCAT CCAACAGATTCAGAAAGCTTTGAA
53	Nucleic acid sequence 241KQ	GGCATTCCTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCA TGTATGCCATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTAT ATCAGGTAGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTC AATGAAACGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTT GGTTGGGCCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACG AATTGAGTTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTAC TTGGGAAATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATG TCCCCAATGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAA AGCAAAAGGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCAT GATGAGTGTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCACAAT CTAAGCCAGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATA CTCTATAAAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
54	Nucleic acid sequence 241KS	GGCATTCCTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCA TGTATGCCATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTAT ATCAGGTAGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTC AATGAAACGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTT GGTTGGGCCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACG AATTGAGTTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTAC TTGGGAAATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATG TCCCCAATGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAA AGCAAAAGGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCAT GATGAGTGTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAGCT CTAAGCCAGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATA CTCTATAAAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA

SEQ ID	Construct	Sequence
55	Nucleic acid sequence 242KQ	ATTCCTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGT ATGCCATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATC AGGTAGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAAT GAAACGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGT TGGGCCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAAT TGAGTTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTG GGAAATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCC CCAATGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGC AAAAGGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGAT GAGTGTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCACAATCTA AGCCAGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTC TATAAAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
56	Nucleic acid sequence 242KS	ATTCCTGCTGAATGTACCACCATTTATAACAGAGGTGAACATACAAGTGGCATGT ATGCCATCAGACCCAGCAACTCTCAAGTTTTTCATGTCTACTGTGATGTTATATC AGGTAGTCCATGGACATTAATTCAACATCGAATAGATGGATCACAAAACTTCAAT GAAACGTGGGAGAACTACAAATATGGTTTTGGGAGGCTTGATGGAGAATTTTGGT TGGGCCTAGAGAAGATATACTCCATAGTGAAGCAATCTAATTATGTTTTACGAAT TGAGTTGGAAGACTGGAAAGACAACAAACATTATATTGAATATTCTTTTTACTTG GGAAATCACGAAACCAACTATACGCTACATCTAGTTGCGATTACTGGCAATGTCC CCAATGCAATCCCGGAAAACAAAGATTTGGTGTTTTCTACTTGGGATCACAAAGC AAAAGGACACTTCAACTGTCCAGAGGGTTATTCAGGAGGCTGGTGGTGGCATGAT GAGTGTGGAGAAAACAACCTAAATGGTAAATATAACAAACCAAGAGCAAGCTCTA AGCCAGAGAGGAGAAGAGGATTATCTTGGAAGTCTCAAAATGGAAGGTTATACTC TATAAAATCAACCAAAATGTTGATCCATCCAACAGATTCAGAAAGCTTTGAA
57	Nucleic acid sequence c227KQ	TTTTTGCATCTCAACGAAACGAAGAATGTCGAACACAACGACATTCCGGCAAATT GCACAACTATCTACAATAGAGGCGAACATACGTCCGGTATCTACTCCATTAGACC TTCAAACAGCCAGGTATTCAATGTGTACTGCGATGTAAAGTCAGGATCGTCATGG ACACTGATCCAGCATAGGATCGACGGGTCCCAGAACTTCAACGAGACATGGGAGA ACTACCGCTATGGATTTGGAAGGCTGGATGGGGAGTTCTGGTTGGGACTTGAGAA AATCTACAGCATTGTGAAGCAGTCGAACTACATTCTCCGGATTGAACTGGAGGAC TGGAATGACAACAAACACTACATCGAGTATTTCTTTCATCTCGGCAACCATGAAA CGAATTACACCTTGCACCTTGTGGAAATCACGGGCAACATTTTGAACGCGCTGCC AGAACACAAAGACCTGGTGTTTTCGACATGGGATCACAAAGCAAAGGGGCACGTG AACTGTCCCGAATCATATAGCGGGGGATGGTGGTGGCACAATGTCTGTGGTGAGA ACAATCTCAACGGGAAATACAATAAGCAGCGAGCTCAGACGAAACCCGAGCGGCG GAGAGGTCTGTATTGGAAGTCGCAGAATGGACGCCTGTATTCGATCAAATCGACG AAAATGCTCATCCACCCCATCGACTCCGAATCGTCGGAG
58	207Kdel	IQEPTEISLSSKPRAPRTTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAI RPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGL EKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNA IPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPER RRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
59	225Kdel	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIK STKMLIHPTDSESFE
60	226Kdel	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKS TKMLIHPTDSESFE

SEQ ID	Construct	Sequence
61	228Kdel	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTK MLIHPTDSESFE
62	233Kdel	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHP TDSESFE
63	241Kdel	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
64	242Kdel	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE
65	225-455Kdel	TTPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISG SPWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIE LEDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAK GHFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIK STKMLIHPTD
66	226-455Kdel	TPFLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGS PWTLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIEL EDWKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKG HFNCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKS TKMLIHPTD
67	228-455Kdel	FLQLNEIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPW TLIQHRIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELED WKDNKHYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHF NCPEGYSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTK MLIHPTD
68	233-455Kdel	EIRNVKHDGIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQH RIDGSQNFNETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNK HYIEYSFYLGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEG YSGGWWWHDECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHP TD
69	241-455Kdel	GIPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNF NETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFY LGNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWH DECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD
70	242-455Kdel	IPAECTTIYNRGEHTSGMYAIRPSNSQVFHVYCDVISGSPWTLIQHRIDGSQNFN ETWENYKYGFGRLDGEFWLGLEKIYSIVKQSNYVLRIELEDWKDNKHYIEYSFYL GNHETNYTLHLVAITGNVPNAIPENKDLVFSTWDHKAKGHFNCPEGYSGGWWWHD ECGENNLNGKYNKPRASKPERRRGLSWKSQNGRLYSIKSTKMLIHPTD

SEQ ID	Construct	Sequence
71	hANGPTL1 1-491	MKTFTWTLGVLFFLLVDTGHCRGGQFKIKKINQRRYPRATDGKEEAKKCAYTFLVP EQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLSRQKREIDVLQLVVDVDGNI VNEVKLLRKESRNMNSRVTQLYMQLLHEIIRKRDNSLELSQLENKILNVTTEMLKM ATRYRELEVKYASLTDLVNNQSVMITLLEEQCLRIFSRQDTHVSPPLVQVVPQHIP NSQQYTPGLLGGNEIQRDPGYPRDLMPPPDLATSPTKSPFKIPPVTFINEGPFKDC QQAKEAGHSVSGIYMIKPENSNGPMQLWCENSLDPGGWTVIQKRTDGSVNFFRNWE NYKKGFGNIDGEYWLGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPES EFYRLRLGTYQGNAGDSMMWHNGKQFTTLDRDKDMYAGNCAHFHKGGWWYNACAHS NLNGVWYRGGHYRSKHQDGIFWAEYRGGSYSLRAVQMMIKPID
72	CT hANGPTL1 271-491	FINEGPFKDCQQAKEAGHSVSGIYMIKPENSNGPMQLWCENSLDPGGWTVIQKRTD GSVNFFRNWENYKKGFGNIDGEYWLGLENIYMLSNQDNYKLLIELEDWSDKKVYAE YSSFRLEPESEFYRLRLGTYQGNAGDSMMWHNGKQFTTLDRDKDMYAGNCAHFHKG GWWYNACAHSNLNGVWYRGGHYRSKHQDGIFWAEYRGGSYSLRAVQMMIKPID
73	hANGPTL4 1-406	MSGAPTAGAALMLCAATAVLLSAQGGPVQSKSPRFASWDEMNVLAHGLLQLGQGLR EHAERTRSQLSALERRLSACGSACQGTEGSTDLPLAPESRVDPEVLHSLQTQLKAQ NSRIQQLFHKVAQQQRHLEKQHLRIQHLQSQFGLLDHKHLDHEVAKPARRKRLPEM AQPVDPAHNVSRLHRLPRDCQELFQVGERQSGLFEIQPQGSPPFLVNCKMTSDGGW TVIQRRHDGSVDFNRPWEAYKAGFGDPHGEFWLGLEKVHSITGDRNSRLAVQLRDW DGNAELLQFSVHLGGEDTAYSLQLTAPVAGQLGATTVPPSGLSVPFSTWDQDHDLR RDKNCAKSLSGGWWFGTCSHSNLNGQYFRSIPQQRQKLKKGIFWKTWRGRYYPLQA TTMLIQPMAAEAAS
74	CT hANGPTL4 179-406	SRLHRLPRDCQELFQVGERQSGLFEIQPQGSPPFLVNCKMTSDGGWTVIQRRHDGS VDFNRPWEAYKAGFGDPHGEFWLGLEKVHSITGDRNSRLAVQLRDWDGNAELLQFS VHLGGEDTAYSLQLTAPVAGQLGATTVPPSGLSVPFSTWDQDHDLRRDKNCAKSLS GGWWFGTCSHSNLNGQYFRSIPQQRQKLKKGIFWKTWRGRYYPLQATTMLIQPMAA EAAS

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence that has at least 95% amino acid sequence identity to an amino acid sequence selected from the group of sequences of TABLE 1, wherein the polypeptide comprises an amino acid that is a polar amino acid other than K or R at position 423, as determined with reference to SEQ ID NO:1, and wherein the polypeptide has chondrogenic activity.

2. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence that has at least 95% sequence identity to an amino acid sequence of any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

3. The polypeptide of claim 2, wherein the polypeptide comprises an amino acid sequence that has at least 95% sequence identity to an amino acid sequence of any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

4. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence of any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

5. The polypeptide of claim 4, wherein the polypeptide comprises an amino acid sequence of any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

6. The polypeptide of claim 1, wherein the polypeptide has at least 95% sequence identity to an amino acid sequence of any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

7. The polypeptide of claim 6, wherein the polypeptide has at least 95% sequence identity to an amino acid sequence of any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

8. The polypeptide of claim 1, wherein the polypeptide is an amino acid sequence selected from the group of sequences of TABLE 1.

9. The polypeptide of claim 8, wherein the polypeptide is any one of SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:70.

10. The polypeptide of claim 9, wherein the polypeptide is any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

11. The polypeptide of any one of claims 1-10, wherein the amino acid at position 423 is Q or S or the amino acid at position 423 is deleted.

12. The polypeptide of any one of claims 1-10, wherein the amino acid at position 423 is Q.

13. The polypeptide of any one of claims 1-10, wherein the amino acid at position 423 is S.

14. The polypeptide of any one of claims 1 to 11, wherein the polypeptide is PEGylated.

15. The polypeptide of any one of claims 1 to 14, wherein the polypeptide is fused to any of human serum albumin (HSA), an immunoglobulin heavy chain constant region (Fc), a polyhistidine, a glutathione S transferase (GST), a thioredoxin, a protein A, a protein G, or a maltose binding protein (MBP), or a fragment thereof.

16. The polypeptide of any claim 15, wherein the heterologous peptide is fused at the amino-terminal end of the polypeptide.

17. The polypeptide of claim 15, wherein the heterologous peptide is fused at the carboxy-terminal end of the polypeptide.

18. A pharmaceutical composition comprising a polypeptide of any one of claims 1 to 17.

19. The pharmaceutical composition of claim 18, further comprising a hyaluronic acid or a derivative thereof.

20. The pharmaceutical composition of claim 18, further comprising an agent selected from the group consisting of oral salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3 (choliecalciferol), collagen hydrolyzate, FGF18, BMP7, rusalatide acetate, avocado soy unsaponifiables (ASU), kartogenin, a steroid, and a non-steroidal anti-inflammatory agent (NSAID).

21. A method of treating, ameliorating or preventing arthritis or joint damage in a patient, the method comprising administering to a joint of the patient a therapeutically effective amount of a pharmaceutical composition of claim 18, thereby treating, ameliorating or preventing arthritis or joint damage in the patient.

22. The method of claim 21, wherein the patient has arthritis or joint damage.

23. The method of claim 21, wherein the patient is at risk for, arthritis or joint damage.

24. The method of claim 22 or claim 23, wherein the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis.

25. The method of any one of claims 21 to 24, wherein the composition further comprises hyaluronic acid.

26. The method of any one of claims 21 to 25, further comprising a surgical procedure to an affected joint.

27. The method of any one of claims 21 to 24 wherein administering the pharmaceutical composition occurs during or after a surgical procedure.

28. The method of claim 26 wherein administering the pharmaceutical composition occurs in conjunction with any one of bone marrow stimulation, cartilage replacement, autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI).

29. The method of any one of claims 21-26 wherein administering the pharmaceutical composition occurs in conjunction with one or more additional chondrogenic factors.

30. The method of any one of claims 21-26 wherein administering the pharmaceutical composition occurs in a matrix or biocompatible scaffold.

31. A method of inducing differentiation of mesenchymal stem cells into chondrocytes, the method comprising contacting mesenchymal stem cells with an effective amount of a polypeptide of any one of claims 1 to 17 to induce differentiation of the mesenchymal stem cells into chondrocytes.

32. The method of claim 31, wherein the method is performed *in vivo* and the stem cells are present in a human subject.

33. The method of claim 32, wherein the subject has arthritis or joint damage.

34. The method of claim 32, wherein the subject is at risk for, arthritis or joint damage.

35. The method of claim 33 or claim 34, wherein the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis.

36. The method of any one of claims 32 to 35, further comprising a surgical procedure to an affected joint in a subject.

37. The method of any one of claims 32 to 35 wherein administering the polypeptide occurs during or after a surgical procedure.

38. The method of any one of claims 32-35 wherein administering the polypeptide occurs in conjunction with one or more additional chondrogenic factors.

39. The method of claim 36 wherein administering the polypeptide occurs in conjunction with any one of bone marrow stimulation, cartilage replacement, autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI).

40. The method of claim 36 wherein administering the polypeptide occurs in a matrix or biocompatible scaffold.

41. A method of treating a subject comprising administering to the subject a therapeutically effective amount of a polypeptide of any one of claims 1 to 17, wherein the subject has or is at risk of joint damage or arthritis.

42. The method of claim 41, wherein the patient has arthritis or joint damage.

43. The method of claim 41, wherein the patient is at risk for, arthritis or joint damage.

44. The method of claim 42 or claim 43, wherein the arthritis is osteoarthritis, trauma arthritis, or autoimmune arthritis.

45. The method of any one of claims 41 to 44, further comprising administering hyaluronic acid.

46. The method of any one of claims 41 to 45, further comprising a surgical procedure to an affected joint.

47. The method of any one of claims 41 to 45 wherein administering the polypeptide occurs during or after a surgical procedure.

48. The method of claim 46 wherein administering the polypeptide occurs in conjunction with any one of bone marrow stimulation, cartilage replacement, autologous chondrocyte implantation (ACI), matrix-induced autologous chondrocyte implantation (MACI).

49. The method of any one of claims 41-46 wherein administering the polypeptide occurs in conjunction with or in addition to one or more additional chondrogenic factors.

50. The method of any one of claims 41-46 wherein administering the polypeptide is in conjunction with or in addition to a matrix or biocompatible scaffold.